European Monitoring Centre
for Drugs and Drug Addiction

TECHNICAL REPORT

Hexahydrocannabinol (HHC) and related substances

Legal notice

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About the EMCDDA

The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is the central source and confirmed authority on drug-related issues in Europe. For over 25 years, it has been collecting, analysing and disseminating scientifically sound information on drugs and drug addiction and their consequences, providing its audiences with an evidence-based picture of the drug phenomenon at European level.

The EMCDDA's publications are a prime source of information for a wide range of audiences including: policymakers and their advisors; professionals and researchers working in the drugs field; and, more broadly, the media and general public. Based in Lisbon, the EMCDDA is one of the decentralised agencies of the European Union.

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About this report

The markets for hexahydrocannabinol (HHC) and related semi-synthetic cannabinoids (SSC) are rapidly evolving areas with relatively limited information currently available. The European situation presented in Section 1 can only provide an overview and will necessarily be incomplete, it also serves to highlight the need for close monitoring. During 2023, a number of developments, such as reports of important law enforcement seizures and changes in legal status, have already taken place in some countries and, where possible, updates have been included.

Terminology and definitions

The terminology and definitions used to refer to and describe cannabis and cannabinoids can differ substantially based on the context of use, country, as well as over time. For example, differences in use and definitions can be found for the terms ‘cannabis’, ‘hemp’, and ‘marijuana’, or ‘marihuana’, both within the scientific community and between different legislative and regulatory systems (see footnote 3 for further details). Due to this complexity, unless a definition is provided in the publication, it typically reproduces the term verbatim from the original source in order to ensure the original meaning is conveyed.

Finally, Section 1 of the publication uses the definition of low-THC cannabis found in the EMCDDA publication, *Low-THC cannabis products in Europe* (EMCDDA, 2020), namely ‘products being or containing cannabis herb, resin, extracts or oils that claim or appear to have a very low percentage of THC and which would be unlikely to cause intoxication’.

Methods

This publication is primarily based on information from the scientific literature. Searches of the scientific literature on the chemistry and pharmacology of hexahydrocannabinol (HHC) and closely related substances were undertaken using chemical structure searches in SciFinder® for ‘HHC’. In addition, text searches for ‘hexahydrocannabinol’ and ‘HHC’ were conducted in PubMed, Google Scholar, as well as other open sources, including Google. Systematic searches were conducted between 1 November 2022 and 27 February 2023 with occasional subsequent searches undertaken for updates and additional publications. Results from searches were screened for relevance and selected publications were reviewed. Where relevant, the reference list of the publications was then checked for additional relevant references. In addition, regulatory documents, conference proceedings, and books on cannabis were also used for relevant information or older citations not likely to be indexed in other databases.

In addition, information on the current situation in Europe with HHC and other semi-synthetic cannabinoids (SSC) was also provided by the European Union Early Warning System on new psychoactive substances (EWS) and the EMCDDA’s expert networks.

HHC at a glance

Common name: hexahydrocannabinol (abbreviated as HHC)

IUPAC name: 6a,7,8,9,10,10a-hexahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b,d*]pyran-1-ol

What is known:

- HHC was first described in the scientific literature in 1940.
- HHC is chemically similar to Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive substance in cannabis.
- According to laboratory studies *in vitro*, and in several animal species *in vivo*, HHC appears to have broadly similar effects to cannabis and THC products.
- The pharmacology and toxicology of HHC in humans has not been studied.
- HHC does not appear to have documented legitimate uses.

Legal status: HHC is not scheduled under the 1961 and 1971 UN Conventions. In the European Union (EU), HHC is monitored as a new psychoactive substance (NPS) by the EMCDDA through the EWS. At the time of writing this report, HHC was not controlled in most EU Member States.

Classification: for monitoring purposes, the EMCDDA may class NPS according to their chemical structure, pharmacological mode of action, and origin (i.e. natural, semi-synthetic or synthetic).

- Chemically, HHC is categorised as a tricyclic terpenoid derivative with a benzopyran ring (or as an hexahydrobenzochromene). The chemical classification is not unique, as different structural elements and classification algorithms can be used.
- Pharmacologically, HHC is classified as a cannabinoid (i.e. substance that acts on the cannabinoid receptors).
- Based on origin, HHC is classed as a semi-synthetic cannabinoid. This is because the HHC detected on the market is synthesised from cannabidiol (CBD), which in turn is extracted from low-THC cannabis (hemp).

Availability in Europe:

- HHC was first identified in Europe in May 2022. Over an eight month period between May and December 2022, it had been identified in 70 % of the EU Member States.
- HHC is sold openly as a ‘legal’ replacement for cannabis and THC products.
- HHC is sold as low-THC cannabis flower and resin that have been sprayed or mixed with HHC, vapes, and food products (commonly known as ‘edibles’), such as sweets.

- Low-THC cannabis flower and resin containing HHC have a similar look and smell to illicit cannabis. It is therefore possible that HHC may be deliberately or accidentally mis-sold as, or used to adulterate, cannabis, THC, and CBD products.

- Since HHC was first identified, a further two SSCs, HHC acetate and hexahydrocannabiphorol, have been identified on the European drug market.

Responses:

The EMCDDA has responded to potential public health and social risks from this new market with a range of actions, including the production of this technical report that provides authoritative information on HHC and closely related substances. These are intended to strengthen awareness of this emerging issue and to support preparedness and response measures at national and EU levels. In particular, they are intended to support the work of a broad range of practitioners and other experts, including those working in analytical, clinical, and forensic laboratories, early warning systems, public health, health care, law enforcement, risk assessors, risk managers, as well as policymakers, and decision-makers.

Contents

Section 1. HHC and the European drug market

1.1 Purpose

In late 2021, a new semi-synthetic cannabinoid (SSC) called hexahydrocannabinol (HHC) emerged on the drug market in the United States (US). It is synthesised from cannabidiol (CBD), which in turn is extracted from low-THC cannabis (hemp). HHC is sold openly as a 'legal' replacement for cannabis and THC products, and appears to have similar effects. Subsequently, in around May 2022, HHC was identified in Europe for the first time. By 31 March 2023, the EMCDDA had received reports through the EU Early Warning System on new psychoactive substances (EWS) that HHC had been identified in a range of products in 20 European Union (EU) Member States and Norway.

Given the open sale of HHC, its apparent rapid proliferation, potentially large user-base, and no history of use in humans before 2021, the EMCDDA identified the need for authoritative technical information on this new cannabinoid and closely related substances. Together with other responses (see below), this report aims to help bridge this gap by bringing together what is known, and – equally importantly – highlighting what is unknown about this cannabinoid. It is intended to support the work of a broad range of practitioners and other experts. They include: those working in analytical, clinical, and forensic laboratories, early warning systems, public health, health care, law enforcement, risk assessors, risk managers, as well as policymakers, and decision-makers.

The publication is divided into two sections. Section 1 provides a brief background to the appearance of HHC and other SSCs on the drug market and an overview of the situation in Europe up to 31 March 2023. In the latter case, this is largely based on information reported to the EMCDDA through the EWS. It also highlights some priority areas for research to address important information gaps.

Section 2 provides a technical review of HHC. It includes: a brief history of recent hemp regulation in the United States leading to the emergence of HHC and other SSCs on the market; as well as information on the chemistry, physical properties, methods of identification, methods of manufacture, pharmacology and toxicology, as well as legitimate uses of HHC. It also reviews the available scientific information on some closely related cannabinoids that have also recently emerged on the drug market or may do so in the near future.

In addition to this publication, other responses to HHC and SSCs by the EMCDDA include:

- intensive monitoring of HHC within the EWS in order to better understand the potential risks to Europe. This requires Member States to expedite reporting of identifications of HHC to the EMCDDA (EMCDDA, 2019a);
- regular review of signals related to HHC and other SSC, as well as information exchange with the EWS network, including alerting the network to other SSCs identified on the market (EMCDDA, 2019b; Evans-Brown et al., 2021); and,

- an expert technical meeting, held on 16 December 2022, to share information and expertise on this emerging issue with the Member States. This included representatives from those working in national early warning systems, public health agencies, laboratories, law enforcement, as well as other relevant experts (EMCDDA, 2022a)

Together, these responses are intended to strengthen situational awareness of this emerging issue and to support preparedness and response measures at national and EU level related to potential public health and social risks from this new market.

1.2 Background

Hexahydrocannabinol (6a,7,8,9,10,10a-hexahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b,d*]pyran-1-ol; HHC) is a SSC first described in 1940 (section 2.3). According to laboratory studies *in vitro*, and in several animal species *in vivo*, HHC is reported to have broadly similar effects to Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive substance in cannabis (section 2.5). The pharmacological and behavioural effects of HHC in humans have not been studied, though recent anecdotal reports from consumers indicate that its effects are similar to that of cannabis and Δ^9-THC.

HHC is sold openly as a purportedly 'legal' replacement to cannabis and Δ^9-THC. Marketing and advertising often make direct comparison or allusions to similarities in effects between the substances.

HHC is not scheduled under the 1961 and 1971 United Nations (UN) drug conventions. In the EU, HHC is monitored as a NPS since 21 October 2022 under the terms of Regulation (EC) No 1920/2006 and Council Framework Decision 2004/757/JHA (2022b).

HHC appears to have first been sold in the US in around September 2021, although the precise date is unknown. HHC is one of a number of SSCs that have recently been sold openly in the US as 'legal' replacements to cannabis and Δ^9-THC, beginning with Δ^8-tetrahydrocannabinol (Δ^8-THC) in 2019 (see box) (CANN, 2021; US CDC, 2021; Erickson, 2021; US FDA, 2022; Leafly Staff, 2021; Leas et al., 2022; Livingstone et al., 2022). This new market is linked to:

- the legalisation of hemp cultivation in the US in 2018;

- subsequent abundant / surplus supply of hemp and cannabidiol (CBD) derived from hemp that can be used as a precursor for SSCs; and,

- the interpretation by producers that cannabinoids derived from hemp are not controlled under the US Federal Controlled Substances Act, so long as the final product does not contain more than 0.3% Δ^9-THC by dry weight (Leas, 2021; Fruth, 2022).

Box. Δ^8-tetrahydrocannabinol (Δ^8-THC)

The first SSC to emerge on the market in the US as a result of the legalisation of hemp was Δ^8-THC in around September 2019 (CANN, 2021; Erickson, 2021; Leafly Staff, 2021; Leas, 2022). The cannabinoid is typically sold as vapes, hemp leaves that have been sprayed or mixed with the cannabinoid (known as 'flower'), and food products (commonly known as 'edibles'), such as brownies and sweets. Other products, such as tinctures, are sold by some retailers.

Soon after its appearance on the market, reports of poisonings involving adults and children began to increase (US CDC, 2021; US FDA, 2022; Livingston et al., 2022). Typically, the effects of poisoning are similar to those reported for cannabis and Δ^9-THC. Overall, over a 14-month period between 1 January 2021[(1)] and 28 February 2022, US poison centres reported 2 362 suspected Δ^8-THC exposures. Of these, 58 % involved adults and 41 % involved paediatric patients less than 18 years of age. Overall, 70 % required health care facility evaluation, of which 8 % resulted in admission to a critical care unit; 45 % of patients requiring health care facility evaluation were paediatric patients; in part this may reflect caution by health professionals given the young age.

An analysis of search queries in Google search engine suggests that interest in Δ^8-THC is higher in states where cannabis has not been legalised for medical or recreational use (Leas et al., 2022).

While Δ^8-THC has also been identified occasionally in Europe (EMCDDA, 2022b), its availability and use appear to be limited. The reason for this is unclear, however it may be because it is specifically scheduled under the 1971 UN Convention and therefore should be controlled at national level across Europe. However, it is important to note that it is likely that Δ^8-THC is undetected in some circumstances. Identifications to the EMCDDA are also likely to be under-reported.

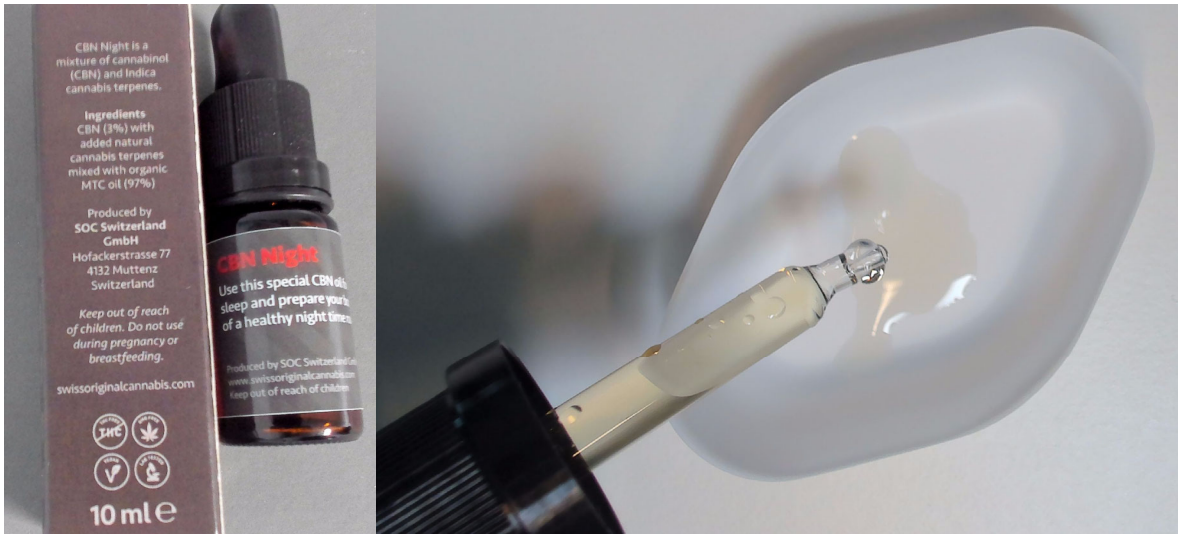
1.3 HHC situation in Europe

Unlike Δ^8-THC, HHC is not specifically controlled under the international drug control system. As of 31 March 2023, it is also not controlled in most EU Member States.

There is limited information on HHC in Europe. The substance was first identified in or around May 2022 in a branded food product sold as a tincture called 'CBN night' that was seized by Danish police. The tincture was marketed as a sleep aid. Laboratory analysis

identified HHC and cannabinol (CBN). HHC was not declared on the packaging. The country of manufacture on the labelling was listed as Switzerland (Figure 1) (EMCDDA, 2022c).

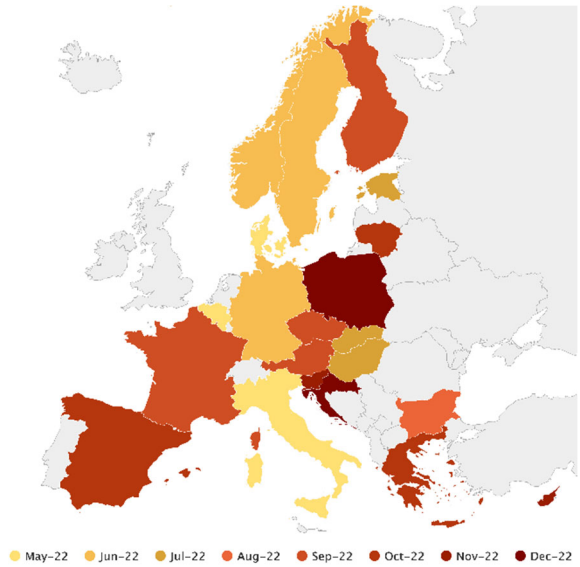
FIGURE 1
'CBN Night' food supplement (left) that contained HHC as a liquid for oral ingestion (right). Seized in Denmark in or around May 2022



Source: Lotte Ask Reitzel, Section of Forensic Chemistry, University of Copenhagen, Denmark.

As of 31 March 2023, identifications of HHC have been reported by 20 Member States (Austria, Belgium, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Lithuania, Poland, Slovakia, Slovenia, Spain, Sweden) and Norway (Figure 2). It has also been identified in Switzerland.

FIGURE 2
European countries reporting identifications of HHC to the EWS, 1 May 2022–31 March 2023



Since it was formally notified as an NPS, the EMCDDA has received reports of approximately 50 seizures through the EWS. Where reported, 25 seizures were from customs, 16 from the police. In total, 70.7 kilograms of products containing HHC have been seized. This includes 28.8 kilograms of low-THC cannabis flower, 25.5 kilograms of resin, 15.5 kilograms of liquid, 0.7 kilograms of sweets, and 0.2 kilograms of unspecified herbal material. In addition, 95.6 litres of liquid have been reported and 809 disposable vapes were seized. Branded products were reported in at least 13 seizures. Where known, the country of origin of these seized products were reported as the United States (10 cases), the Netherlands (4), Spain (3), Austria (1), Germany (1), Italy (1), Poland (1) and Switzerland (1).

Most of the seizures are relatively small-scale, however at least three seizures are indicative of a potentially larger trade including production of finished products in Europe:

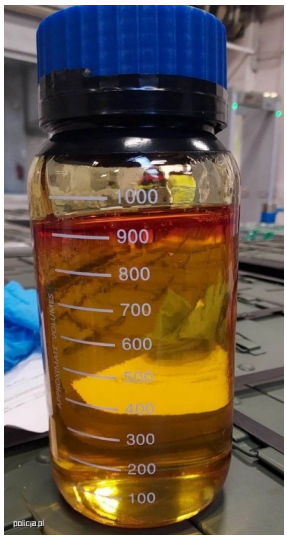
- In August 2022, Italian authorities seized just over 33 kilograms of material containing HHC, comprised of low-THC cannabis flower and resin and bulk quantities of oil/distillate. In addition, 68 kilograms of illicit cannabis containing Δ^9-THC (and no HHC) was also found in this seizure.

- In December 2022, Polish authorities seized 95 litres of HHC oil that originated from the US (Figure 3). The oil was presumably intended to produce consumer products.

- In February 2023, German customs seized 10 kilograms of HHC liquid that were sent from the Netherlands and en route to Italy. The liquid was reported to have originated from the US.

FIGURE 3

A one litre bottle of HHC oil seized at Warsaw Chopin Airport, Warsaw, Poland, December 2022, that was part of a seizure of 95 litres of the cannabinoid. Laboratory analysis identified HHC and Δ^8-THC in the oil. The consignment was shipped from the US. It was seized in a joint operation between Polish Customs and Polish Police



Source: Polish Police, Poland.

Informal reports from some Member States and initial results from internet monitoring of the surface web by the EMCDDA suggests that HHC's availability and use in Europe is likely much greater than suggested by seizures reported so far through the EWS.

A range of branded and unbranded products containing HHC are available in Europe. They include:

- low-THC cannabis flower and resin, onto which HHC has been sprayed or mixed;
- ready-to-use disposable vape pens, e-liquids and e-liquid cartridges for use in electronic cigarettes;
- food products, especially flavoured sweets (gummies and marshmallows) and tinctures resembling food supplements; and,
- HHC oils (distillates).

Low-THC cannabis products containing HHC are marketed in a range of sophisticated, attractive, brightly coloured, designs (Figure 4). In some cases, these are advertised as not for human consumption. In other cases, they may be sold as unbranded products (Figure 5). Low-THC cannabis flower containing HHC are also marketed using the names of popular cannabis strains such as Afghan Kush, Amnesia, BubbleGum Kush, Strawberry Kush, Pineapple Express, and Purple Haze, or mention or allude to the same effects as these strains.

FIGURE 4
'Purple Haze' product (left) of low-THC cannabis flower containing HHC (right)

Source: State General Laboratory, Cyprus.

FIGURE 5
Unbranded products of low-THC cannabis flower containing HHC

Source: Dr Marc Wende, Kriminaltechnisches Institut, Bayerisches Landeskriminalamt (BLKA), Germany.

Importantly, low-THC cannabis flower and resin containing HHC have a similar look and smell to illicit cannabis. Similar to the recent adulteration of low-THC cannabis with synthetic cannabinoids (EMCDDA, 2022), it is possible that HHC may be deliberately or accidentally mis-sold as, or used to adulterate, cannabis, THC, and CBD products. While information is limited, case reports from countries in Europe suggest that HHC is already being mis-sold as illicit cannabis. However, the overall size and scale of this practice is unknown.

Disposable vapes and cartridges are sold in attractive packaging similar to commercial nicotine vapes and cartridges (Figure 6a and Figure 6b).

FIGURE 6a
'Imperial Garden' 1 ml Blueberry flavour HHC vape cartridge. Seized by Swiss Customs in October 2022

Source: Christian Bissig, Zurich Forensic Science Institute, Switzerland.

FIGURE 6b

HHC disposable vapes sold in a vending machine alongside nicotine-containing vaping products in Künzelsau, Baden-Wuerttemberg, Germany, November 2022. The HHC vapes were labelled as 'ACAN Mango Kush' and sold for 40 EUR per pen

Source: State Criminal Police Office Baden-Wuerttemberg, Germany.

HHC sweets are sold openly in a range of flavours and designs, typically in attractive packaging (Figure 7a and Figure 7b).

FIGURE 7a

'HHC Gummies Cola Cola taste' (left) containing teddy bear-shaped HHC-infused gummies (right). Seized by Swiss Customs in October 2022

Source: Christian Bissig, Zurich Forensic Science Institute, Switzerland.

FIGURE 7b

'Strawberry Marshmallows HHC' containing strawberry-shaped HHC-infused sweets. Seized by Swedish Customs in 2022

Source: Swedish Customs.

The size and scale of the retail market is unknown. Products are sold in a range of brick-and-mortar and online shops, particularly those specialised in selling low-THC cannabis and CBD products, as well as vaping products ('smoke shops'). Initial indications from surface web monitoring suggest that retailers can be found in or ship to most Member States. Producers, retailers, and consumers may also buy bulk oils and finished products from suppliers in the US and import them into Europe. In the latter case, this is supported by information from customs seizures (see Figure 3).

Potentially, there could be a large demand for HHC products. These include people who use cannabis as well as Δ^9-THC and CBD products, as well as new consumers attracted to its legal status. In the latter case, this includes young people and others inexperienced with drug use. While there is no specific research on HHC, as noted above, in the United States, interest in Δ^8-THC, at least in terms of Google searches, was higher in states where cannabis has not been legalised for medical or recreational use (Leas et al., 2022).

In some cases, ease of access to products, such as high street shops, may promote use. Consumers may also be attracted to the different effects that HHC is claimed to have compared to cannabis and other THC products. While the accuracy of these claims have not been assessed, it includes assertions that HHC is 'less intoxicating', which has led to it being described sometimes as 'cannabis lite' (not the same as the low-THC 'cannabis light' marketed primarily in Italy since about 2018 ([1])). Vapes and sweets are also an easy and discreet way of using HHC, especially in public settings. Vape cartridges are also supplied using the standard universal 510-thread connector design, fitting many existing e-cigarettes on the market.

([1]) https://apnews.com/article/marijuana-italy-business-courts-international-news-ea9ac614af74488b8977e3dbe54dd795

Information on the retail prices of HHC products is currently limited. However, based on test purchases and internet monitoring, they appear to be comparable with at least some illicit cannabis products. For example, low-THC cannabis flower containing HHC sells for approximately 6-10 EUR/g (depending on the reported strength of the product), which is similar to the average cost of illicit cannabis herb in Europe (EMCDDA, 2020).

As of 31 March 2023, no analytically confirmed serious adverse events involving HHC have been reported to the EMCDDA.

1.4 Other SSCs in Europe

Since HHC was first identified in May 2022, a further two SSCs have been identified on the drug market in Europe: HHC acetate and hexahydrocannabiphorol (HHC-P).

In August 2022, HHC acetate (6,6,9-trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydrobenzo[*c*]chromen-1-yl) acetate was identified for the first time in Europe by Hungary (EMCDDA, 2022d). As of the 31 March 2023, identifications have been reported by 3 Member States: Croatia, Estonia, and Hungary. HHC acetate is closely related to HHC and likely hydrolysed to HHC in the body.

In November 2022, hexahydrocannabiphorol (3-heptyl-6a,7,8,9,10,10a-hexahydro-6,6,9-trimethyl-6*H*-dibenzo[*b,d*]pyran-1-ol) was identified for the first time in Europe by Slovenia (EMCDDA, 2023). As of the 31 March 2023, identifications have been reported by 4 Member States: Bulgaria, Croatia, Estonia, and Slovenia.

Both HHC acetate and HHC-P are sold in the same types of products as HHC and through the same retailers.

Based on products currently available in the US, SSCs described in the scientific literature and discussed on drug user online forums, a number of other SSCs with effects broadly similar to cannabis and Δ^9-THC may also emerge on the European drug market over time (Stone, 2020).

1.5. Priority areas for research

The sudden emergence of HHC and its apparent rapid spread in the US and Europe have posed unusual challenges to laboratories, public health, health professionals, law enforcement, policy and decision-makers as well as to the public.

As for any NPS, many of the questions related to HHC and other SSCs that are posed by the lack of data on the risks to individual health, risks to public health and social risks could be answered through further research. Areas where additional information would be important include studies on epidemiology (frequency and patterns of use, including studies that examine the groups of people who use SSCs and risk behaviours); the market; chemical profiling; extended pharmacological and toxicological profiling; metabolic pathways; behavioural effects; acute and chronic toxicity; the potential interaction between HHC or other SCCs and other substances; the abuse liability and dependence-producing potential; and the public health and social risks associated with their use.

While HHC has been known for more than eight decades in scientific circles, no documented human pharmacological or toxicological studies have been conducted.

From a pharmacological point of view, on one hand, the acute behavioural and psychological effects of HHC are – or appear to be – very similar to those experienced by users of cannabis and other Δ^9-THC products. On the other hand, the novel and currently popular forms of ingestion, such as by vaping or in edibles, might cause unexpected (psycho)toxicity not typical of conventional cannabis products.

Investigating the pharmacokinetics, including the metabolism in humans, should be a priority. Due to its unique chemical and physicochemical properties ([2]) the metabolism and excretion of HHC could differ from that of Δ^9-THC. Identification of the main human urinary metabolite(s) is also important from forensic and clinical points of view.

Comparative pharmacology studies *in vitro* on the mode of action of HHC, including single epimers (stereoisomers) as well as their mixtures, could be readily carried out. Likewise, nonclinical animal studies should shed light on the similarities and differences with regard to Δ^9-THC.

Since HHC and other SSC products often contain other ingredients either as contaminants or intentionally added cannabinoids, as well as diluents, it is important to monitor the market, for example by test purchases, and characterise the composition of these products.

It is important to collect information on the origin of HHC and related substances and products on the market, including identifying sites and the applied methods of the chemical manufacture of HHC.

The emergence of hydrogenated derivatives of other (phyto)cannabinoids (for example tetrahydrocannabigerol) on the drug market is anticipated, so continuous, systematic, monitoring of the market is recommended. This may also include the need for test purchases of new products and substances that emerge on the market.

Research on why such semi-synthetic products are appealing to users and to what extent they compare to other cannabis-based products is also needed.

([2]) For example, the lack of the metabolically vulnerable methylcycloalkene fragment and high lipophilicity.

Section 2. Technical review on HHC and related substances

2.1 Summary

The appearance of the synthetic cannabinoid receptor agonists (synthetic cannabinoids) in 'Spice'- or 'K2'-type 'legal high' products in around 2006 predicated the introduction of products several times more potent than traditional herbal cannabis-based preparations (EMCDDA, 2009). This phenomenon has become global and has been attributed to advances in medicinal chemistry research and low-cost manufacturing typically by Asian chemical companies. In recent years, however, the 'cannabinoid market' responded to changes in legislation, in particular in the US, where, parallel to legalisation of cannabis for recreational use in several jurisdictions, cultivation of industrial hemp re-started ('Green Rush'). As a consequence, hemp producers promptly looked for new hemp-based products, including semi-synthetic cannabinoids made by simple – and low-cost – chemical transformations of hemp extracts to legal or quasi-legal substances. In contrast to synthetic cannabinoids, bioactivity, or potency, appears to have become a non-issue: these semi-synthetic substances do not have to be – and, in fact they are often not – more potent than the natural product Δ^9-tetrahydrocannabinol (Δ^9-THC). Low prices, novel products and forms of consumption, attractive packaging, and aggressive, though often deceptive marketing on the Internet combined with the increasing social acceptance of 'cannabis use', all seem to contribute to this new phenomenon.

For several years, cannabidiol-containing products proliferated not only in the US but globally. Recently, however, cannabidiol (CBD) is no longer simply a 'product' but has also become a 'precursor' to several semi-synthetic cannabinoids, of which hexahydrocannabinol (HHC) is just one of the latest. HHC appeared on the drug market in 2021. It was first identified in Europe in or around May 2022 in Denmark and notified as a new psychoactive substance through the EWS in October 2022 (EMCDDA, 2022b). Since then, a total of 20 Member States and Norway have identified the substances as part of the EWS. It has also been identified in Switzerland.

The synthesis and biological (cannabimimetic) activity of HHC was first reported by research laboratories eight decades ago. The current large-scale manufacturing of HHC is based on low-THC hemp derived CBD-extract which is first transformed into a mixture of Δ^8-THC and Δ^9-THC followed by catalytic hydrogenation of the THC isomer mixture into the final product. Analysis of marketed products and seizures indicate that such substances contain two stereoisomers (epimers) of HHC, the (9*R*)-HHC and (9*S*)-HHC) (or 9β-HHC and 9α-HHC, respectively). Non-clinical studies and anecdotal reports have indicated that HHC, in particular 9β-HHC, indeed has Δ^9-THC-like pharmacological properties, though it appears to have somewhat lower potency. Data on the metabolism of HHC in animals are scarce and inadequate.

The human pharmacology of HHC has not been studied, and (immuno)analytical methods for the rapid and unambiguous detection of HHC or its metabolites in urine are lacking.

Further research would be needed to explore the full biological activity spectrum of this cannabinoid.

2.2. A brief history of recent hemp regulation in the United States

In the recent decade there has been a renewed interest in the therapeutic potential of phytocannabinoids, and CBD in particular (Lewis, 2020; Parker et al., 2022). The appearance in around 2019 on the drug market of semi-synthetic cannabinoids such as Δ^8-tetrahydrocannabinol (Δ^8-THC) (Erickson, 2021; Karschner, 2021; Geci et al., 2022), and subsequently HHC, however, can be attributed to legislative changes in the US.

Eighty-six years ago, the Marihuana Tax Act of 1937 imposed a heavy administrative burden on the cultivation of hemp (*Cannabis sativa* L.) ([3]), and legislations after World War II essentially eliminated hemp production in the US (Cherney and Small, 2016). In the 2014 Farm Bill, the definition of industrial hemp allowed for hemp cultivation under very limited circumstances. The situation however, changed with the enactment in December 2018 of the Agriculture Improvement Act, or the 2018 Farm Bill (as it is more commonly known), which facilitated the wide-scale cultivation of industrial or 'low-THC' ([4]) hemp by removing 'hemp' from the definition of 'marijuana' in the Controlled Substances Act [5] (US Public Law, 2018; USDA, 2023). Such hemp varieties are relatively rich in CBD so the extraction of this phytocannabinoid becomes feasible and thus economically attractive.

The 2018 Farm Bill has made a distinction between THC-rich 'marijuana' and low-THC hemp as defined by the new definition of 'hemp' (7 USC 1639o, SEC. 297A):

> "The term 'hemp' means the plant Cannabis sativa L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta-9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis."

The *Controlled Substances Act* (CSA) was modified accordingly (US DoJ, 2020) and defining the distinction between 'marihuana' and 'hemp':

> 16)(A) Subject to subparagraph (B), the term "marihuana" means all parts of the plant Cannabis sativa L., whether growing or not; the seeds thereof; the resin extracted from any part of such plant; and every compound, manufacture, salt, derivative, mixture, or preparation of such plant, its seeds or resin.
>
> (B) The term "marihuana" does not include (i) hemp, as defined in section 1639o of title 7; or (ii) the mature stalks of such plant, fibre produced from such stalks, oil or cake made from the seeds of such plant, any other compound, manufacture, salt, derivative, mixture, or preparation of such mature stalks (except the resin extracted therefrom), fibre, oil, or cake, or the sterilised seed of such plant which is incapable of germination."

([3]) There are several names used in the scientific and popular literature for this plant. For example, in North America, 'hemp' is commonly used for *C. sativa* varieties used for non-recreational purposes such as fibre production, while the term 'marijuana' (alternative spelling 'marihuana') is used for both the plant and its drug preparations used for recreational purposes. In addition, the word 'cannabis' is also used in a very broad sense worldwide (Small, 2017; see also UNCTAD, 2022). A qualifying word, such as in 'industrial hemp', 'fibre-type hemp', 'hemp oil', 'medical marijuana', and 'medical cannabis', is often added. The indiscriminate use of 'cannabis' may cause confusion since the definition of cannabis in Article 1 of the Single Convention on Narcotic Drugs of 1961 as amended by the 1972 Protocol is as follows: *'"Cannabis" means the flowering or fruiting tops of the cannabis plant (excluding the seeds and leaves when not accompanied by the tops) from which the resin has not been extracted, by whatever name they may be designated.*' In Section 2 of this publication, unless otherwise noted, the term 'hemp' is used for *Cannabis sativa*.
([4]) Meaning low in Δ^9-THC content.
([5]) Congressional Research Service (2019) Defining Hemp: A Fact Sheet https://crsreports.congress.gov/product/pdf/R/R44742

> The CSA was also amended in Schedule I to specifically exclude 'tetrahydrocannabinols in hemp (as defined under section 297A of the Agricultural Marketing Act of 1946)'[6]

As a consequence, the inclusion of the terms 'extract' and 'cannabinoids' in the Farm Bill's definition of 'hemp' and the exclusion of that 'hemp' from the CSA definition of 'marijuana' has been suggested to allow for a wide interpretation of the law but appears to provide a legal authorisation for using hemp extracts for a variety of purposes, including for the manufacture of products containing SSCs.

Responding to a request from the Alabama Board of Pharmacy concerning the control status of Δ^8-THC under the Controlled Substances Act, the US Drug Enforcement Administration stated in September 2021 (US DoJ, 2021):

> "[C]annabinoids extracted from the cannabis plant that have a Δ^9-THC concentration of not more than 0.3 percent on a dry weight basis meet the definition of "hemp" and thus are not controlled under the CSA."

Accordingly, this appears to have been interpreted by manufacturers that hemp-derived Δ^8-THC as well as other hemp-derived non-Δ^9-THC tetrahydrocannabinol isomers are not controlled under the Controlled Substances Act. However, the situation differs in US states that introduced specific control of Δ^8-THC and other semisynthetic cannabinoids.

A recently introduced bill (US House, 2022) now aims to modify the legislation to include all tetrahydrocannabinol isomers in the definition of 'hemp' by placing a limit on their total content:

> "The term 'hemp' means …
>
> (A) the plant Cannabis sativa L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers thereof… with a total tetrahydrocannabinol concentration of not more than 1 percent on a dry weight basis, that is not intended for sale to consumers".
>
> "(B) hemp extract that '(i) is to be used in the making of a hemp product; ''(ii) has not been packaged as a finished product; '(iii) is not intended for sale to consumers; '(iv) has a total tetrahydrocannabinol concentration that exceeds 1 percent on a dry weight basis."

The Bill also introduces the term 'hemp product':

> "The term 'hemp product' means a finished product that … is derived from, or made by, processing hemp; and … has a total tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis.''

Furthermore, the Bill specifically mentions three tetrahydrocannabinol isomers to be covered by the proposed legislation:

(6) https://www.govinfo.gov/content/pkg/PLAW-115publ334/pdf/PLAW-115publ334.pdf

> "The term 'total tetrahydrocannabinol concentration' means the aggregate concentration of delta-8 tetrahydrocannabinol, delta-9 tetrahydrocannabinol, delta-10 tetrahydrocannabinol, and the optical isomers of such substances" ([7]).

The enactment of the Farm Bill, along with the large growth in CBD products, revitalised hemp cultivation, with hemp production increased dramatically. By 2021, open area cultivation of industrial hemp exceeded 21,800 ha of which 6,745 ha was devoted to floral hemp (USDA, 2022) ([8]). Consequently, as supply in hemp products, including CBD, increased, prices dropped. According to a recent special report on hemp (UNCTAD, 2022):

> "The price of crude CBD hemp oil on the European market reached $931 per kg in November 2021. CBD isolate [([9])] was sold at $952 per kg and $1,200 per kg in November 2021 on the European and United States markets, respectively. However, a significant fall in prices due to overproduction of hemp-derived CBD-containing products has been observed since the overheating of the market in late 2019 and early 2020 in the United States. This drop in prices rapidly spread to the European market causing some turmoil in overall industrial hemp production."

Due to the oversupply of CBD extracts and rediscovering that this phytocannabinoid could serve a precursor to a range of semi-synthetic cannabinoids (SSCs), producers diversified their market by converting CBD to other cannabinoids using procedures described in the scientific literature decades ago. The first SSC was Δ^8-THC ([10]) in a variety of forms, such as oils for vaping, tinctures, herbal preparations, or gummy edibles. Soon after, its acetate derivative ('THC-O') appeared on the market. More recently, a novel SSC, hexahydrocannabinol (HHC) has emerged.

While the production and sale of SSC products was until recently considered to be a US phenomenon, by mid-2022 the open sale of HHC products of unknown composition and/or purity became noticeable in other countries as well.

2.3. Chemical and physical properties, methods and precursors used for manufacture or extraction of HHC

2.3.1 Background

To date, 296 cannabinoids have been isolated from various strains of *Cannabis sativa* L. (Lumír Hanuš, personal communication). It must be noted, however, that several of these substances may be artefacts, that is degradation or transformation products formed from genuine phytocannabinoids of biosynthetic origin under environmental conditions (oxygen, heat, light, *etc.*) or during isolation or even analysis.

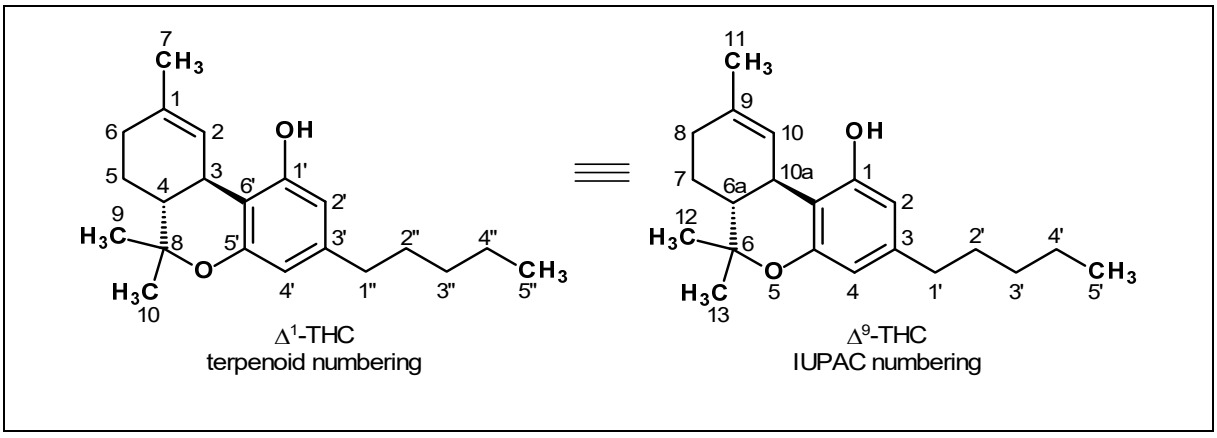
([7]) Note that $\Delta^{6a,10a}$-THC, which is slightly psychoactive as a cannabimimetic (Hollister et al., 1987) is not included. The reason for this omission could be that this isomer is not readily obtainable from CBD.
([8]) For comparison, in Europe hemp was cultivated on ca. 32,000 ha in 2021 (EUROSTAT): https://ec.europa.eu/eurostat/databrowser/view/APRO_CPSH1__custom_4409454/default/table Accessed on 8 January 2023.
([9]) CBD isolate is an extract that contains cannabidiol and essentially no other substances naturally present in hemp.
([10]) Though Δ^8-THC has been isolated from hemp, it might be an artefact resulting from the isomerization of the endocyclic double bond of Δ^9-THC the thermodynamically more stable Δ^8 position (Hanuš et al., 2016).

Hexahydrocannabinol (HHC; 6a,7,8,9,10,10a-hexahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol) is a chemically stable cannabinoid which is not biosynthesised by the plant. It was first prepared in the respective laboratories of Adams (Adams et al., 1940a) and Todd (1940) in 1940. HHC is structurally similar to the well-studied phytocannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC), which is the main psychoactive substance in cannabis, yet its human pharmacology is virtually unexplored. Being a *hexahydro* derivative, HHC is not scheduled by the 1971 UN Convention on Psychotropic Substances, which only controls *tetrahydro*cannabinol and their isomers. From around mid-2021, HHC has been sold openly in a range of products in the US and subsequently Europe and elsewhere. Products include low-THC cannabis (hemp) flower and resin onto which HHC has been sprayed or mixed with HHC, vape products and edibles. This rapid emergence has required a comprehensive appraisal of the available information on this simple and now readily available, yet almost forgotten cannabinoid.

2.3.2 Names and structure

The nomenclature, the atom numbering in particular, of cannabinoids has changed over time. The original numbering system used until the 1970s has a biogenetic background: it reflected monoterpenoid biosynthetic origin of phytocannabinoids. This numbering has by now been superseded by systematic (IUPAC) numbering based on the benzochromene, or dibenzopyran, ring numbering system. This latter nomenclature is now generally accepted and will be used throughout this technical review even when the original publication used the monoterpenoid numbering. Since the present review relies in large part on early publications using the 'old' numbering it is useful to point out the differences between the two systems. For comparison, Figure 8 shows the two numbering systems on the example of Δ^9-THC.

FIGURE 8
The earlier used monoterpenoid numbering (left) and the current systematic (IUPAC) numbering systems (right) for Δ^9-THC

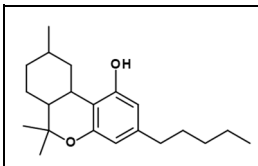
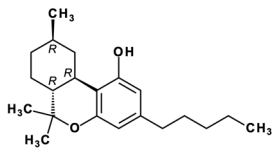
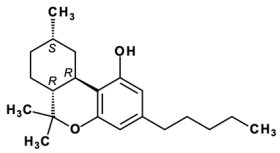


Semi-synthetic HHC is typically a mixture of 9α- and 9β-methyl stereoisomers (epimers) which have different pharmacological properties (Section 2.5). Compared to Δ^9-THC, which is susceptible to chemical (Pars and Razdan, 1971; Miller et al., 1982) and biochemical (Just

et al., 1975) oxidations, HHC, lacking the double bond, is expected to be more resistant to oxidation, though experimental evidence is lacking.

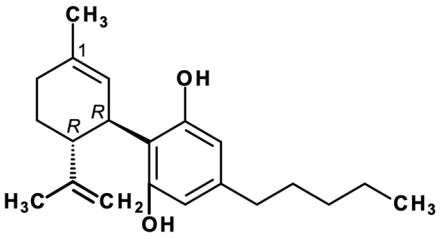
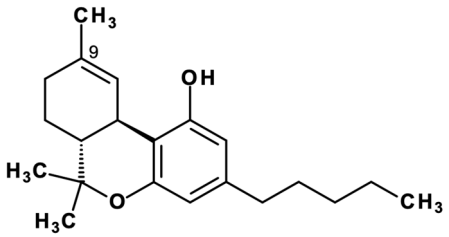
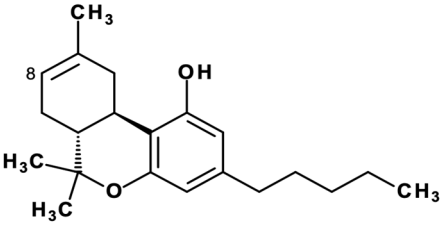
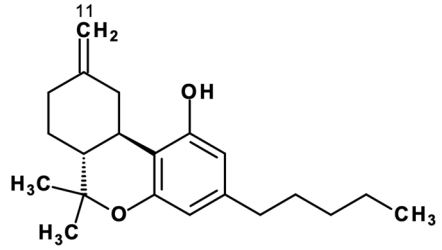
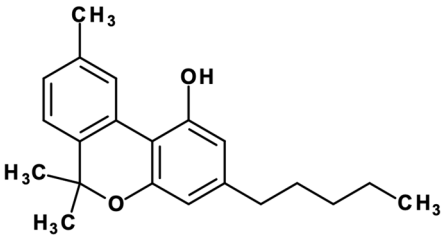
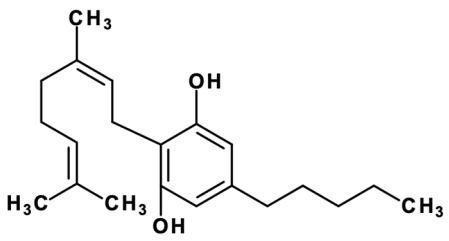
Molecular structures, molecular formulas, and molecular masses of HHC (unspecified stereochemistry) and epimeric HHCs are shown in Figure 9. Since HHC has three stereogenic carbon atom, eight stereoisomers with four pairs of enantiomers are possible. The structures shown in Figure 9 depict HHC of unspecified stereochemistry and the two diastereomers, or epimers, in which the configurations of the 6a and 10a carbon atoms are identical to those of Δ^9-THC. These epimers are the expected main components of HHC-containing products manufactured from CBD-rich low-THC hemp extract.

FIGURE 9
Molecular structure and molecular properties of HHC (unspecified stereochemistry), epimeric (*R*)-HHC and (*S*)-HHC (9β-HHC and 9α-HHC, respectively)

			
	HHC (unspecified stereochemistry)	9β-HHC or (9*R*)-HHC	9α-HHC or (9*S*)-HHC
Molecular formula	$C_{21}H_{32}O_2$	$C_{21}H_{32}O_2$	$C_{21}H_{32}O_2$
Molecular mass	316.48	316.48	316.48
Monoisotopic mass	316.2402	316.2402	316.2402

The molecular structures and molecular properties of related compounds are shown in Figure 10. Information on these related cannabinoids is provided for comparison.

FIGURE 10
Molecular structure and molecular properties of CBD, Δ⁹-THC, Δ⁸-THC, Δ¹¹-THC, CBN and CBG

		
	CBD, cannabidiol	Δ⁹-THC
Molecular formula	$C_{21}H_{30}O_2$	$C_{21}H_{30}O_2$
Molecular mass	314.46	314.46
Monoisotopic mass	314.2245	314.2245
		
	Δ⁸-THC	Δ¹¹-THC
Molecular formula	$C_{21}H_{30}O_2$	$C_{21}H_{30}O_2$
Molecular mass	314.46	314.46
Monoisotopic mass	314.2245	314.2245
		
	CBN, cannabinol	CBG, cannabigerol
Molecular formula	$C_{21}H_{26}O_2$	$C_{21}H_{32}O_2$

Molecular mass	310.43	316.48
Monoisotopic mass	310.1932	316.2402

Common names:

Hexahydrocannabinol

HHC

HXC

Systematic (IUPAC) names:

(6a*R*,9*S*,10a*R*)-6,6,9-trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydrobenzo[*c*]chromen-1-ol (9α isomer)

(6a*R*,9*R*,10a*R*)-6,6,9-trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydrobenzo[*c*]chromen-1-ol (9β isomer)

Chemical Abstract name:

6a,7,8,9,10,10a-Hexahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (unspecified stereochemistry)

Other chemical names:

(6a*R*)-6,6,9-trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-1-ol

(6a*R*,9*S*,10a*R*)-HHC (9α isomer)

(6a*R*,9*R*,10a*R*)-HHC (9β isomer)

9α-HHC

9β-HHC

(*S*)-9α-HHC

(*R*)-9β-HHC

(9*S*)-HHC

(9*R*)-HHC

(*R*/*S*)-HHC (1:1 mixture of the 9α- and 9β-HHC epimers)

(±)-HHC (1:1 mixture of the (6a*R*,9*R*,10a*R*) and (6a*S*,9*S*,10a*S*) enantiomers)

(–)-HHC (6a*R*,9*R*,10a*R*)-HHC

(+)-HHC (6a*S*,9*S*,10a*S*)-HHC

axial HHC (9α-HHC)

equatorial HHC (9β-HHC)

cis-HHC (9β isomer) ([11])

trans-HHC (9α isomer) ([12])

hexahydro-CBN

hexhydrocannabinol

Other names:

(–) NL-106 (9α isomer)

(–) NL-105 (9β isomer)

Cautionary notes: the acronym '*HHC*' has also been used for 9-nor-9-hydroxyhexahydro–cannabinol (*CP-series*) (Johnson et al., 1981; Johnson and Melvin, 1986). In addition, 'HHC' has also been used as an acronym for 'hexahydrocurcumin' ([13]). For related natural and synthetic cannabinoids, see Section 2.3.4.

Chemical Abstract Service (CAS) registry numbers:

6692-85-9 (unspecified stereochemistry)

1972-09-4 (unspecified stereochemistry)

36403-90-4 (6a*R*,9*R*,10a*R*) isomer (9β-HHC)

69855-14-7 *rel*-(6a*R*,9*R*,10a*R*)

36403-91-5 (6a*R*,9*S*,10a*R*) isomer (9α-HHC)

103476-58-0 (6a*S*,9*S*,10a*S*) isomer

23050-51-3 *rel*-(6a*R*,9*R*,10a*S*) 9α-HHC ([14]) *cis*-isomer

58617-32-6 *rel*-(6a*S*,9*R*,10a*S*) 9β-HHC ([15]) *cis*-isomer

59042-47-6 (6a*S*,9*R*,10a*R*) 9β-HHC *cis*-isomer

69855-11-4 (6a*R*,9*R*,10a*S*) 9β-HHC *cis*-isomer

69855-12-5 (6a*R*,9*S*,10a*S*) 9α-HHC *cis*-isomer

69880-65-5 (6a*S*,9*R*,10a*S*) isomer

146338-70-7 *rel*-(6a*R*,9*S*,10a*R*) isomer

946512-74-9 (6a*R**,9ξ,10a*R**) relative configurations at C-6 and C-10 but unknown configuration at C-9

IUPAC International Chemical Identifier Key (InCHI Key):

XKRHRBJLCLXSGE-DJIMGWMZSA-N (9β isomer)

([11]) In some publications '*cis*' refers to the 9-methyl group and the aromatic ring being on the same side of the cyclohexane ring.
([12]) In some publications '*trans*' refers to the 9-methyl group and the aromatic ring being on the opposite side of the cyclohexane ring.
([13]) https://www.ncbi.nlm.nih.gov/pmc/?term=hexahydrocurcumin+hhc
([14]) As indicated in the name and not the structure illustrated in *SciFinder*®.
([15]) As indicated in the name and not the structure illustrated in *SciFinder*®.

XKRHRBJLCLXSGE-USXIJHARSA-N (9α isomer)

XKRHRBJLCLXSGE-UHFFFAOYSA-N (unspecified stereochemistry)

IUPAC International Chemical Identifier String (InCHI String):

InChI=1S/C21H32O2/c1-5-6-7-8-15-12-18(22)20-16-11-14(2)9-10-17(16)21(3,4)23-19(20)13-15/h12-14,16-17,22H,5-11H2,1-4H3/t14-,16-,17-/m1/s1 (9β isomer)

InChI=1S/C21H32O2/c1-5-6-7-8-15-12-18(22)20-16-11-14(2)9-10-17(16)21(3,4)23-19(20)13-15/h12-14,16-17,22H,5-11H2,1-4H3/t14-,16+,17+/m0/s1 (9α isomer)

InChI=1S/C21H32O2/c1-5-6-7-8-15-12-18(22)20-16-11-14(2)9-10-17(16)21(3,4)23-19(20)13-15/h12-14,16-17,22H,5-11H2,1-4H3 (unspecified stereochemistry)

Simplified Molecular-Input Line-Entry System (SMILES):

CCCCCc1cc(O)c2[C@@H]3C[C@H](C)CC[C@H]3C(C)(C)Oc2c1 (9β isomer)

CCCCCc1cc(O)c2[C@@H]3C[C@@H](C)CC[C@H]3C(C)(C)Oc2c1 (9α isomer)

CCCCCc1cc(O)c2C3CC(C)CCC3C(C)(C)Oc2c1 (unspecified stereochemistry)

EC / List No.:

'hexahydrocannabinol' is not listed

Other Identifiers:

DTXSID20985587

NIST Number 6692859

PubChem CID 522237

2.3.3 Physical properties

HHC was first prepared around 1940 during investigations aimed at isolating the psychoactive constituents of 'marihuana' and 'hashish' and to elucidate their chemical structure. The lipophilic nature and chemical instability, the liability to isomerization in particular, of the tetrahydrocannabinol constituents, and the lack of adequate analytical techniques hampered progress. Thus, early studies involving HHC rarely worked with stereochemically pure substances, hence the variations in the reported physicochemical and biological characteristics.

Appearance:

'colorless, highly viscous resin' (Adams et al., 1940a: Adams, 1947)

'colorless, viscous oil' (Adams et al., 1940b)

colorless resin (Hively et al., 1966)

oil (Tietze et al., 1982)

Melting point:

125–127 ºC for the 3,5-dinitrobenzoate of HHC ([16]) (Gaoni and Mechoulam, 1966a)

82–83 ºC for (6a*S**,9ξ,10a*R**)-HHC (*cis*-HHC with unknown configuration at C-9) (Gaoni and Mechoulam, 1968)

Boiling point:

153–155 ºC (0.1 mmHg) (Adams et al., 1940a; Adams, 1947; Hughes et al., 1971)

174–177 ºC (0.1 mmHg (Gaoni and Mechoulam, 1966a)

Refractive index:

n_D^{20} 1.5348 (Adams et al., 1940a)

Optical rotation:

$[a]_D^{27}$ –70º (c 0.016, ethanol) derived from natural cannabidiol (Adams et al., 1940a)

$[a]_D^{26}$ –73º (c 0.020, ethanol) derived from natural cannabidiol (Adams et al., 1941a)

$[\alpha]_D$ –70º (ethanol) (Šantavý, 1964)

$[a]_D^{27}$ –109º (c 0.502, ethanol) from Δ^8-THC (Hively et al., 1966)

$[a]_D^{27}$ –108º (c 0.507, ethanol) from Δ^9-THC (Hively et al., 1966)

$[\alpha]_{578}$ +11º (c 1.12, chloroform) for (6a*R*,9*R*,10a*S*)-HHC (Uliss et al., 1978)

$[\alpha]_{578}$ +26º (c 1.29, chloroform) for (6a*R*,9*S*,10a*S*)-HHC (Uliss et al., 1978)

$[\alpha]_D$ +90º (c 0.16, chloroform) for (6a*S*,9*R*,10a*S*)-HHC (Uliss et al., 1978)

$[\alpha]_D$ -109º (chloroform) for (6a*R*,9*S*,10a*R*)-HHC (Uliss et al., 1978)

$[\alpha]_D$ –109º (chloroform) for 9α-HHC (Gaoni and Mechoulam, 1966a) ([17])

$[\alpha]_D$ –107º (chloroform) for 9β-HHC (Gaoni and Mechoulam, 1966a)

$[a]_D^{25}$ –93.6º (c 0.7, chloroform) for (–)-9β-HHC or (6a*R*,9*R*,10a*R*)-HHC (Tietze et al., 1982)

$[\alpha]_D$ –73.9º (c 0.014) for (–)-9β-HHC or (6a*R*,9*R*,10a*R*)-HHC (Marino and Dax, 1984)

$[\alpha]_D$ +82.9º (c 0.024) for (+)-9α-HHC or (6a*S*,9*S*,10a*S*)-HHC (Marino and Dax, 1984)

$[a]_D^{20}$ –73.2º (c 1, chloroform) for (–)-9β-HHC or (6a*R*,9*R*,10a*R*)-HHC of 76% enantiomeric excess (Casiraghi et al., 1986; Cornia et al., 1989)

([16]) It must be the 9α isomer. In the relevant scheme of the original paper (Gaoni and Mechoulam, 1966a) the graphical notations for the 9β (equatorial C-11 methyl) and 9α (axial C-11 methyl) (VIa and VIb, respectively, in the original publication) appear to be reversed. The text, however, states that "in VIa the methyl group at C_1 [monoterpenoid numbering], is axial and *trans* to the aromatic ring while in VIb it is equatorial". In a subsequent review the correct configuration was given (Mechoulam, 1973).
([17]) See previous footnote.

$[a]_D^{20}$ –74.1º (c 1, chloroform) for (–)-9α-HHC or (6a*R*,9*R*,10a*R*)-HHC of 78% enantiomeric excess (Casiraghi et al., 1986)

$[a]_D^{20}$ +79.5º (c 1, chloroform) for (+)-9β-HHC or (6a*S*,9*S*,10a*S*)-HHC of 84% enantiomeric excess (Casiraghi et al., 1986; Cornia et al., 1989)

$[a]_D^{24}$ –98.6º (c 0.63, chloroform) (Wang et al., 2000)

$[a]_D^{20}$ –98.6º (c 0.45, chloroform) (–)-9β-HHC (Lu et al., 1992; Lu, 2006)

$[\alpha]_D$ –85.4º (c 0.30, chloroform) (–)-9β-HHC or (6a*R*,9*R*,10a*R*)-HHC (Lee and Xia, 2008)

$[\alpha]_D$ +86.9º (c 0.1, chloroform) (+)-9α-HHC or (6a*S*,9*S*,10a*S*)-HHC (Lee and Xia, 2008)

$[a]_D^{20}$ –85.4º (c 0.30, chloroform) (–)-9β-HHC or (6a*R*,9*R*,10a*R*)-HHC (Lee, 2010)

$[a]_D^{20}$ +86.9º (c 0.1, chloroform) (+)-9α-HHC or (6a*S*,9*S*,10a*S*)-HHC (Lee, 2010)

Stability:

According to a producer ([18]), the shelf-life of HHC of 96% purity is 6–12 months. It is also noted that the amber oil "[a]fter exposure to oxygen, HHC may begin to slowly take on a dark orange hue."

2.3.3.1 Other physicochemical properties of pharmacological importance:

In general, phytocannabinoids are non-polar, lipophilic substances thus have low water solubility. There is no specific information on the lipophilicity of HHC, but available data for structurally related phytocannabinoids, such as cannabidiol and benzochromene cannabinoids, including Δ^9-THC, suggest high lipid solubility. Table 1 gives comparative data on calculated and, when available, measured partition coefficients ([19]), affecting pharmacokinetics. (Thomas et al., 1990). Another useful parameter in assessing brain penetration of a substance is the calculated topological polar surface area (TPSA).

([18]) https://coloradochromatography.com/product/hhc. Accessed on 6 January 2023.
([19]) A measure of lipophilicity and usually estimated by measuring the partition of a given substance between *n*-octanol and water phases or estimated by reverse phase HPLC.

TABLE 1
Some measured or calculated physicochemical properties affecting pharmacokinetics of representative cannabinoids

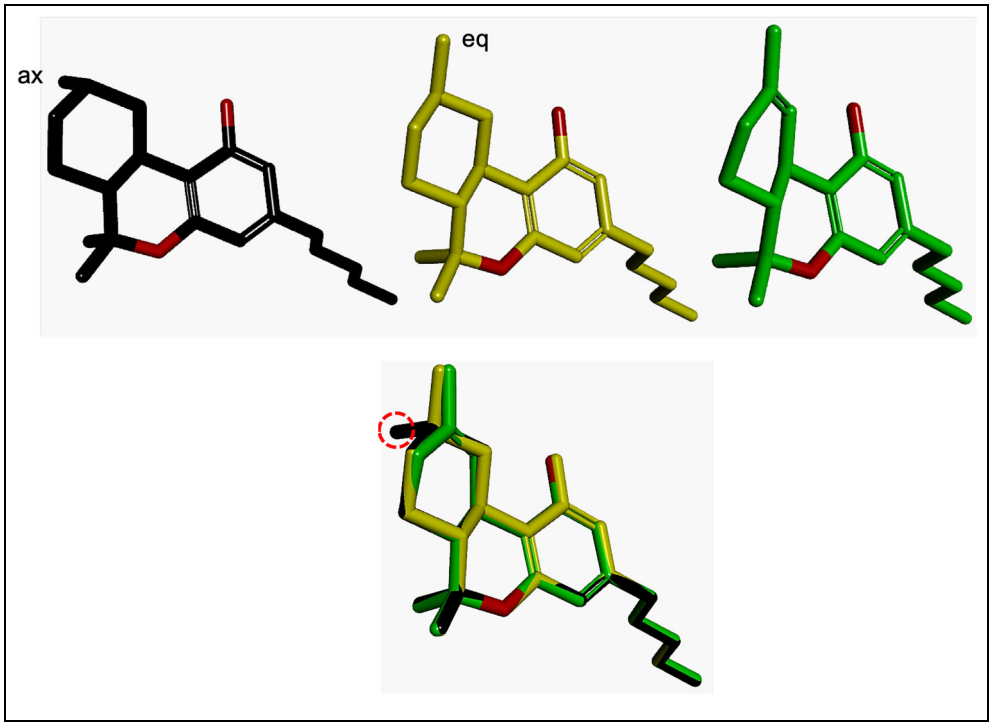
Compound	Lipophilicity				Calculated
	Measured	Calculated			
	$LogP_{ow}$[a]	$CLogP_{ow}$[a]	miLogP[b]	LogP[c]	TPSA,[d] Å^2
Δ^9-THC	6.97	7.02	6.69	7.68	29.46
Δ^8-THC	7.41	7.02	7.64	7.53	29.46
HHC	–	–	6.64	7.93	29.46
Δ^8-THC acetate	–	–	7.21	7.59	35.54
HHC acetate	–	–	6.20	8.00	35.54
11-OH-THC	5.33	5.19	5.45	6.58	49.69
11-OH-HHC	–	–	54.63	5.98	49.69
Cannabidiol	5.79	6.92	7.14	7.03	40.46
Cannabinol	6.23	6.39	6.31	7.35	29.46
11-nor-9-COOH-Δ^8-THC	3.51		6.28	6.06	66.76

[a] $logK_{ow}$ data were computed from reported *n*-octanol–water K_{ow} values obtained by HPLC (Thomas et al., 1990).
[b] miLogP calculations used *Molinspiration* software ([20]).
[c] LogP calculations used *ACD/ChemSketch* software ([21]).
[d] Topological polar surface area (TPSA) ([22]) was calculated using *Molsinspiration* software.

Inspection of the three-dimensional structure of HHC isomers reveals that the 9β epimer, that is (9*R*)-HHC, where the C-11 methyl group is in equatorial position on the cyclohexane ring, is essentially in the same position as the C-11 methyl of Δ^9-THC. As seen in Figure 11, an overlay of the optimised structures of the two HHC epimers and Δ^9-THC indicates a perfect fit for 9β-HHC and the phytocannabinoid, while in the case of the 9α epimer only the core ring systems and the *n*-pentyl side chains can be superimposed, the axial C-11 methyl group protrudes into the bottom face. (For a detailed structural analysis of the three cannabinoids, see Archer et al., 1970; Reggio et al., 1989)

([20]) *Molinspiration* Version 2021.10. https://www.molinspiration.com
([21]) *ACD/ChemSketch* 2015 version (Advanced Chemistry Development Inc., Toronto, Canada)
([22]) TPSA is a calculated measure of the surface area occupied by nitrogen and oxygen atoms and the polar hydrogen atoms attached to them. For drugs acting on the central nervous system the TPSA is typically less than 90 Å^2.

FIGURE 11
Top (from left to right): molecular models of 9α-HHC (axial C-11 HHC; dark grey carbon skeleton), 9β-HHC (equatorial C-11 HHC; yellow carbon skeleton) and Δ^9-THC (green carbon skeleton). Note structural similarity of 9β-HHC (equatorial C-11) and Δ^9-THC. Bottom: overlay of the three cannabinoids with the axial methyl group of 9α-HHC protruding into the bottom face is circled in red. Created by István Ujváry using BIOVIA Studio Visualizer ([23]).



2.3.4 Representative substances containing the hexahydrocannabinol core

The hexahydrobenzochromene scaffold is found in a few natural products and several synthetic cannabinoids. Representative substances related to HHC isolated from hemp and other natural sources are shown in Figure 12. Cannabiripsol (CBR) is the 9,10-dihydroxy derivative of HHC and was isolated from a South African cannabis variety (Boeren et al., 1979); it is most likely formed from Δ^9-THC. Another group of closely related analogues were isolated from the stem bark of the Amazonian liana *Machaerium multiflorum* of which machaeriol A differs from HHC by the stereochemistry at the ring junction and by the side chain (Muhammad et al., 2001).

Of the first synthetic analogues of HHC described in 1942 the *n*-hexyl homologue (Figure 12) showed greatly improved potency in the dog ataxia test (Adams et al., 1942). A group of compounds lacking one of the geminal methyl groups on the hexahydrobenzochromene core but with branched homologous side chains was described in a patent (SmithKline, 1977).

([23]) BIOVIA, Dassault Systèmes, BIOVIA Studio Visualizer, Software Version 21.1.0.20298, San Diego: Dassault Systèmes, 2020.

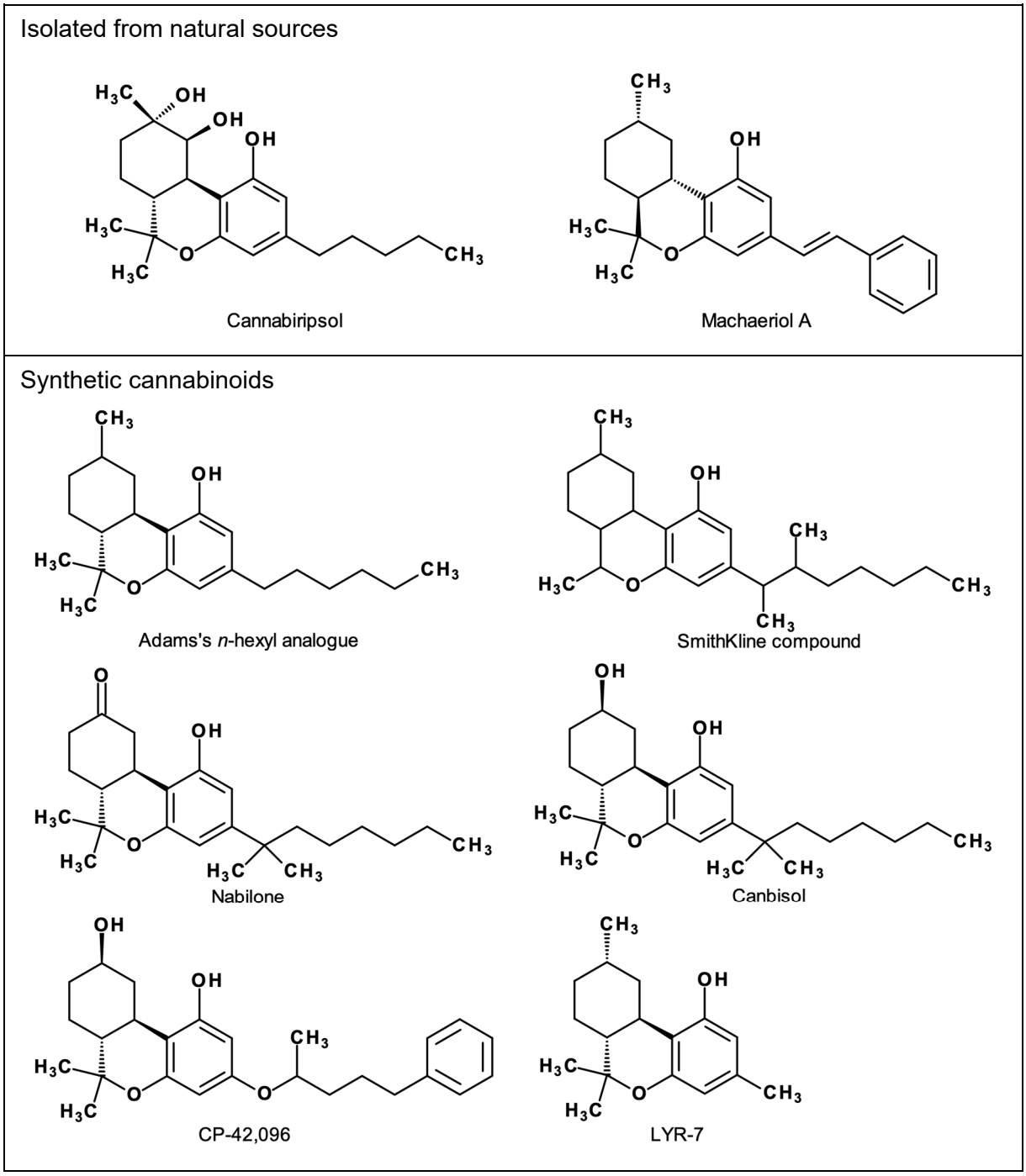
Lilly Research Laboratories prepared and evaluated a series of 9-nor-hexahydrocannabinols including nabilone and canbisol (Figure 12) of which the former eventually became a medicine for the treatment of nausea and vomiting associated with cancer chemotherapy ([24]) (Archer et al., 1986). In search for non-opioid analgesics, Pfizer researchers simultaneously developed but did not commercialise CP-42,096 (Figure 12) (Johnson et al., 1981; Johnson and Melvin, 1986; Compton et al., 1992). Both canbisol and CP-42,096 were ten-fold more potent than morphine and about hundred-fold more potent than Δ^9-THC as antinociceptive drugs in mice and rats (Johnson and Melvin, 1986). Research along this structural type afforded several potent analogues and eventually led to the conceptualisation of a cannabinoid receptor model (Howlett et al., 1988).

Several known and newly synthesised hexahydrocannabinol analogues, including machaeriol A as well as HHC and its lower homologue LYR-7 ([25]) (Figure 12), were investigated as potential anticancer agents (Thapa et al., 2011).

([24]) Cesamet®
([25]) Could also be called hexahydrocannabiorcol, that is the hydrogenated analogue of the phytocannabinoid tetrahydrocannabiorcol (Vree et al., 1972a).

FIGURE 12

Representative substances structurally related to HHC



2.3.5 Methods and chemical precursors used for the manufacture or extraction

Information on the specific manufacturing methods for the HHC currently available on the market is lacking. Two basic approaches have been documented for the synthesis of HHC.

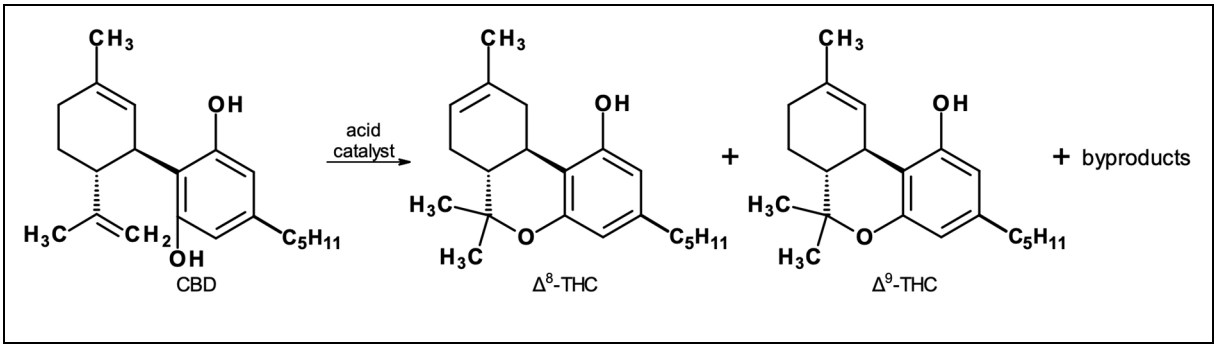
The first relies on chemical precursors obtained from botanical sources, that is hemp, *C. sativa*; while the second possibility is to use total synthesis using other, small molecule natural products or synthetic chemicals as starting materials. The first route is considered to be practical for large-scale production of HHC, while the second has the advantage of providing access to stereoisomeric variants as well as analogues of HHC.

2.3.5.1 Synthesis of HHC from phytocannabinoids obtained from C. sativa (hemp) extract

While details on the actual manufacture method of HHC is unknown, it is thought to rely on industrial hemp-derived CBD, which has now become a commodity chemical (Section 2.2). CBD-based manufacture of HHC is also inextricably connected to the production of Δ^8-THC, which emerged on the US market on its own around 2019 as a 'legal' alternative to its controlled Δ^9-isomer (Erickson, 2021; Karschner, 2021).

Accordingly, HHC is produced in two simple steps from CBD extracts. The first step involves an acid-catalysed cyclisation ([26]),([27]) of CBD at elevated temperature to provide, as the main product, the thermodynamically more stable ([28]) Δ^8-THC, accompanied by isomeric Δ^9-THC, as well as a wide range of byproducts (Figure 13). The cyclisation can be affected by the use of ethanolic solution of hydrogen chloride or phosphoric acid, zinc chloride, sulfamic acid or pyridine hydrochloride as catalyst (Adams et al., 1940a) ([29]). The ratio of the two THC isomers as well as the amount and chemical nature of the by-products depend on the reaction conditions (Section 5.6). In a recently patented method, the use of either *p*-toluenesulfonic acid (*p*-TSA) or boron trifluoride catalyst under optimised conditions yields products highly enriched either in Δ^8-THC or Δ^9-THC, respectively (Webster et al., 2008). A recent in-depth study has investigated product composition of the intramolecular cyclisation of CBD using a range of protic acids and Lewis acids in different solvents (Marzullo et al., 2020). In short, CBD is a 'pre-precursor' with Δ^8-THC being the immediate precursor to HHC.

FIGURE 13
Synthesis of a mixture of Δ^8-THC and Δ^9-THC from CBD. The ratio of the main products and contaminants depends on the nature of catalyst, solvent and temperature



([26]) It is of historical interest that such an acid-catalysed intramolecular ring closure was proposed by Cahn in 1933 (Cahn, 1933), many years before the actual structures of CBD or THC were elucidated.
([27]) This process is colloquially called 'isomerization' and has been described in detail in various popular books already in the early 1970s (Hoye, 1973; Todd, 1974; Starks, 1977).
([28]) The difference in the stability of the two isomeric cannabinoids has been demonstrated by quantum chemical calculations (Reggio et al., 1989) and experiments (Miller et al., 1982).
([29]) For the cyclisation of the CBD homologue cannabidivarinol (CBDV) triisobutylaluminum has also been used (Tesfatsion et al., 2022).

The synthesis of HHC of unspecified stereochemistry by catalytic hydrogenation of '*tetrahydrocannabinol*' at atmospheric pressure was first published in 1940. For the reduction, platinum oxide (Adam's catalyst) (Adams et al., 1940a; Adams, 1947; Gaoni and Mechoulam, 1966a; Hively et al., 1966), or palladium on charcoal catalysts have been used (Adams et al., 1940a; Harvey et al., 1980; Casati et al., 2022; Collins et al., 2022). The method relies on the availability of Δ^8/Δ^9-THC and is suitable for large-scale production of HHC. (Crude Δ^8/Δ^9-THC mixtures are now readily available by acid-catalysed cyclisation of CBD obtained from low-THC industrial hemp, see Figure 13.) Depending on the reaction conditions, one of the epimers may dominate in the final product.

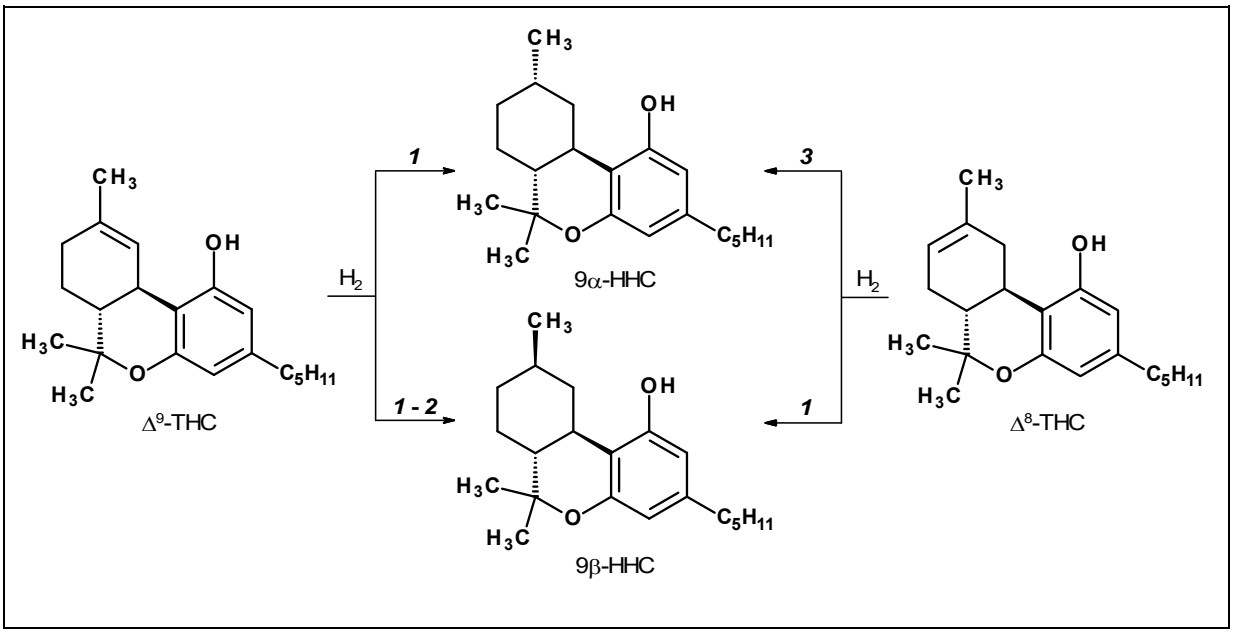
It has been observed that the catalytic hydrogenation of Δ^9-THC using platinum catalyst Adam's catalyst affords the 9α-HHC and 9β-HHC isomers in approximate a 1:2 ratio, while the use of palladium/charcoal catalyst yielded the two epimers in a 1:1 ratio (Skinner et al., 1979) or in a 3:7 ratio (Casati et al., 2022). In contrast, hydrogenation of Δ^8-THC using platinum oxide catalyst favours the production of the axial 9α-HHC isomer over the equatorial 9β-HHC isomer in a 3:1 ratio (Gaoni and Mechoulam, 1966a; Archer et al., 1970; see also Turner at al., 1973) ([30]) (Figure 14).

Ethanol or acetic acid have been the most widely used solvent for such catalytic hydrogenations.

([30]) In the original paper (Gaoni and Mechoulam, 1966a) the notations for the structure of the 9α (axial C-11 methyl) and 9β (equatorial C-11 methyl) appear to be reversed. In the text and a subsequent review the opposite – and correct – configuration is given (Mechoulam, 1973) which is in line with another structural study (Archer et al., 1970).

FIGURE 14

Synthesis of HHC isomer mixtures by catalytic hydrogenation of Δ^9-THC or Δ^8-THC. Numbers in bold italics above the arrows indicate the ratios of the 9α and 9β epimers formed in the hydrogenation of the respective THC isomer

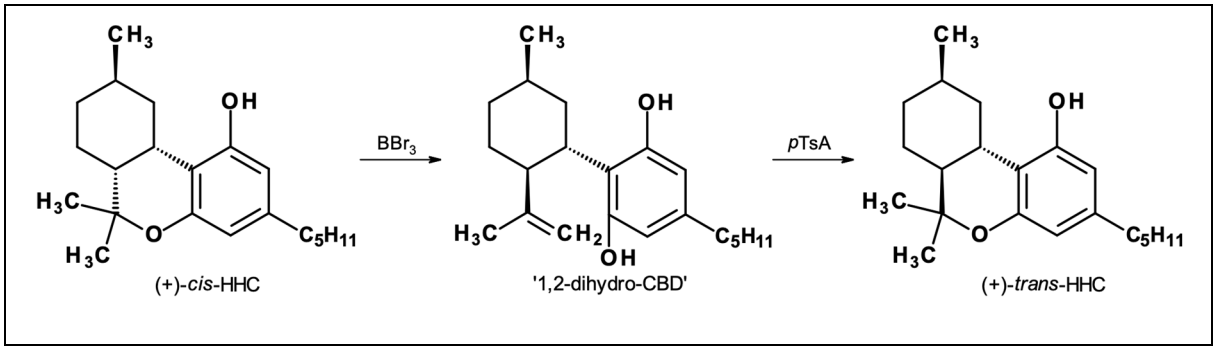


Recent anecdotal information suggests that the large-scale hydrogenation of the THC isomeric mixture is rather unpredictable and the ratio of the 9α and 9β epimers in the product may vary from batch to batch ([31]).

In connection with studies on the isomerizations and transformations of cannabinoids (see also Section 3.6), the synthesis of (+)-HHC ([32]) was accomplished by a *p*-TSA-catalyzed cyclisation of 'unnatural' 1,2-dihydrocannabidiol ('1,2-dihydro-CBD') prepared from boron tribromide-catalyzed rearrangement of (+)-*cis*-HHC ([33]) (Figure 15) (Uliss et al., 1978).

FIGURE 15

Synthesis of (+)-*trans*-HHC from (+)-*cis*-HHC (Uliss et al., 1978)



([31]) https://www.leafly.com/news/strains-products/what-is-hhc. Accessed on 20 December 2022.
([32]) Stereochemical notations in this paragraph reflect those in the original publication.
([33]) Prepared by catalytic hydrogenation of (+)-Δ^9-cis-THC using PtO_2 or Pd/C catalyst.

During studies aimed at the synthesis of Δ^7-THC (Mechoulam et al., 1973), the two C-11 methyl epimers were also prepared from the 8α/β-tosyloxy derivatives of HHC by $LiAlH_4$ reduction while 9β-HHC was obtained from the 8-keto-HHC by Wolff-Kishner reduction ([34]).

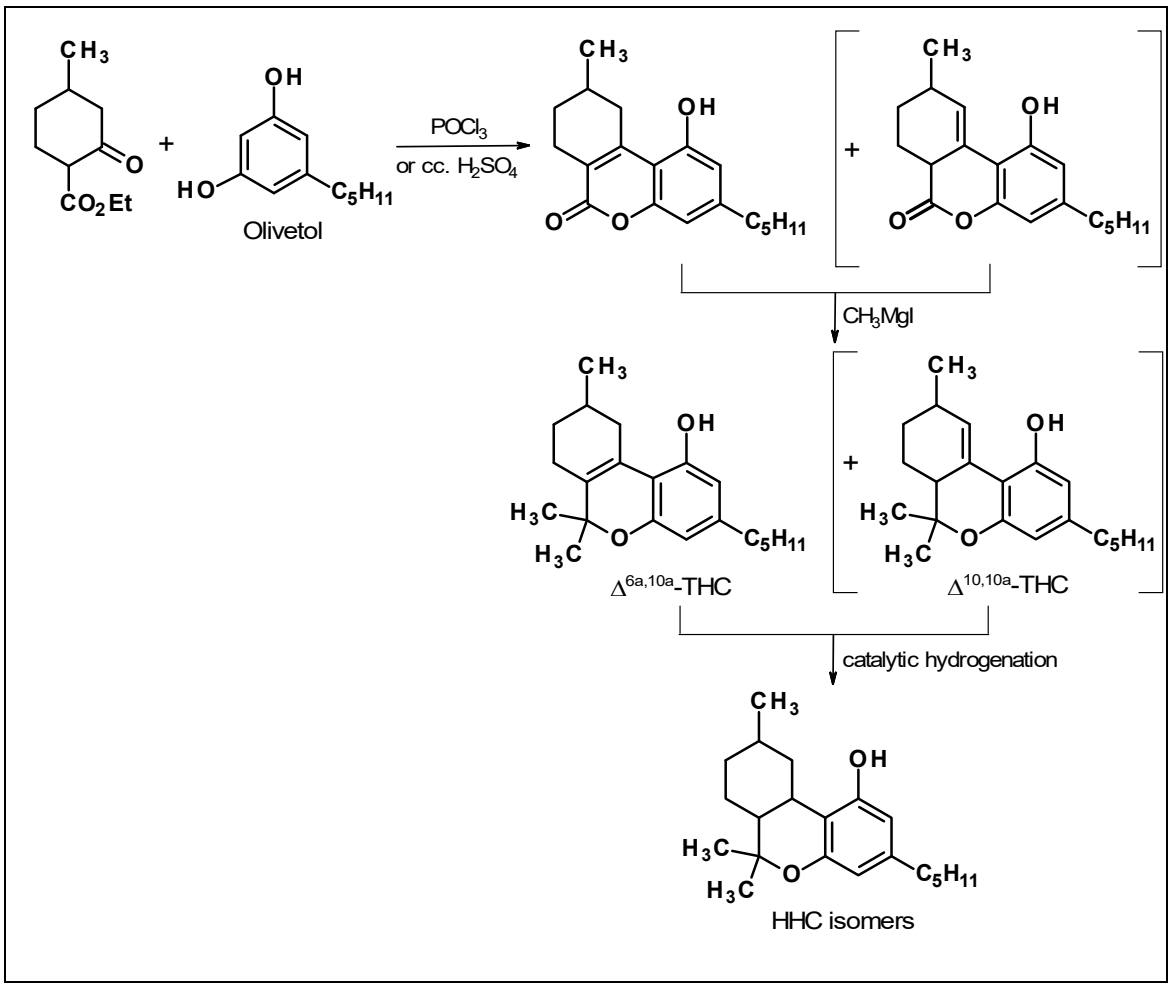
2.3.5.2 Total syntheses of HHC

The first, though non-stereoselective total syntheses, were simultaneously pursued in the research laboratories of Adams in the US and Todd in England around 1940. Both research groups used essentially the same synthetic route as shown in Figure 16. Condensation of racemic ethyl 5-methylcyclohexanone-2-carboxylate with olivetol in the presence of either phosphoryl chloride (Adams et al., 1940b; Adams et al., 1941b; Adams et al., 1941c) or concentrated sulphuric (Ghosh et al., 1940a, Ghosh et al., 1940b; Powell and Bembry, 1940) gave the corresponding tetrahydrobenzochromenone, which upon treatment with excess methyl magnesium iodide yielded – as originally suggested – the corresponding tetrahydrodibenzopyran (*i.e.*, $\Delta^{6a(10a)}$-THC). Catalytic hydrogenation of the latter THC isomer afforded HHC with undefined stereochemistry. However, the intermediates obtained by this procedure were later shown to contain not only the claimed benzochromenone derivative but also its double bond isomer (in square brackets in Figure 16) (Claussen and Korte, 1966a; Claussen and Korte, 1966b). Consequently, the $\Delta^{6a(10a)}$-THC was accompanied by a substantial amount of the $\Delta^{10(10a)}$-THC isomer (in square brackets) in the end product (Claussen and Korte, 1966a; Claussen and Korte, 1966b). Regardless of the position of the C=C double bond, hydrogenation of the two THC isomers afforded HHC albeit with undefined stereoisomeric composition.

([34]) The tosyloxy and keto derivatives of HHC were obtained from Δ^8-THC by a multistep route.

FIGURE 16

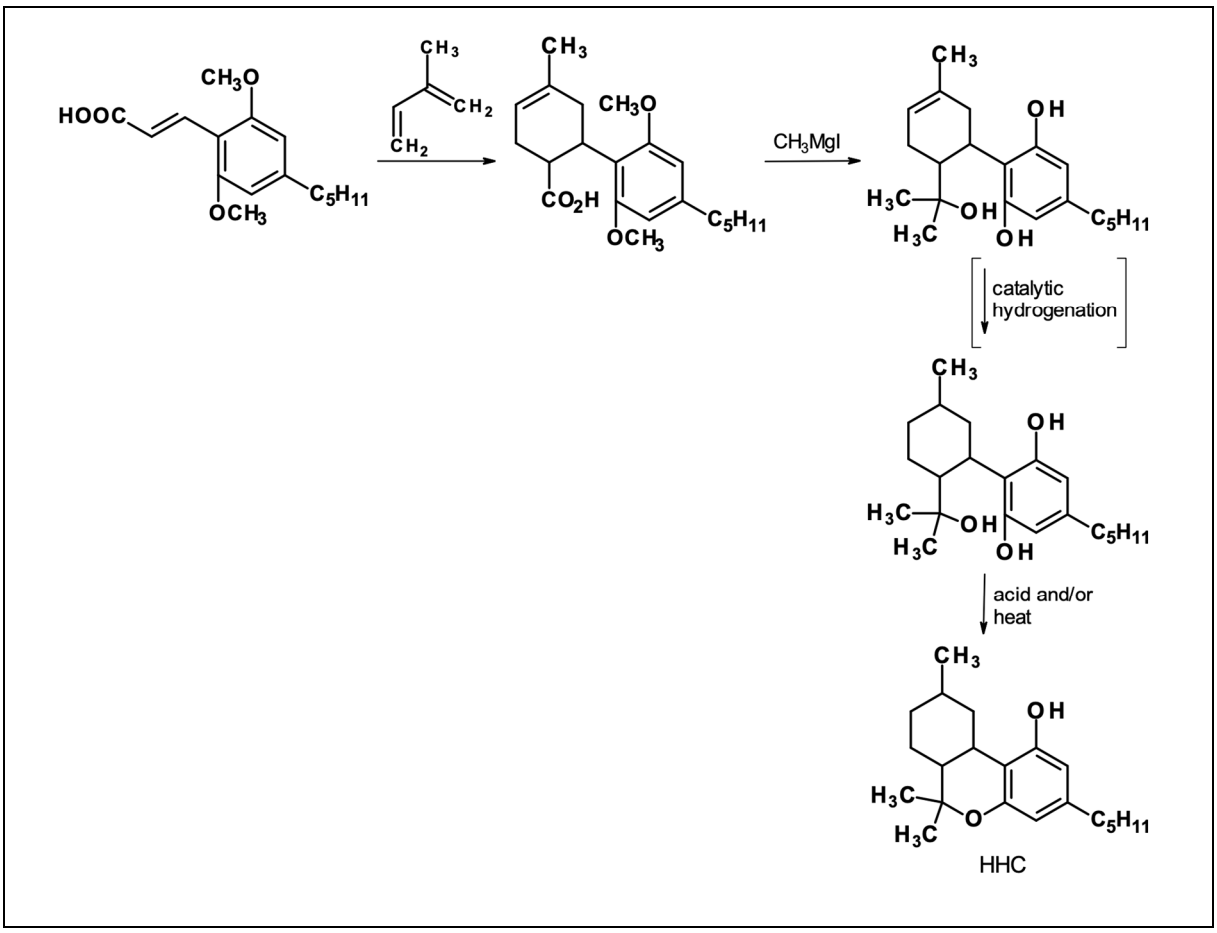
Synthesis of a stereoisomeric mixture of HHC (Adams et al., 1941a; Adams et al., 1941b; Ghosh et al., 1940a)



Following the route which is based on a Diels-Alder reaction of isoprene on an appropriately substituted cinnamic acid derivative (Adams and Carlin, 1943; Jen et al., 1967), a patent described the synthesis of HHC though no experimental details were given (Figure 17) (Hughes et al., 1971). Note that exposure of the cyclohexenecarboxylic acid to an excess of methylmagnesium iodide at 165 °C resulted in the formation of the requisite tertiary alcohol with concomitant demethylation of the two methoxy groups. The racemic cyclohexene–carboxylic intermediate, having the two rings in *trans* configuration, could be resolved into its enantiomers using (+)-1-(2-naphthyl)ethylamine, thus this route, at least in theory, could provide access to stereoenriched HHC isomers and related analogues.

FIGURE 17

Synthesis of HHC using a Diels-Alder reaction. The details of the hydrogenation step (in square brackets) are not reported in the patent (Hughes et al., 1971)



2.3.5.3 Synthesis of non-racemic HHC non-cannabinoid chiral starting materials

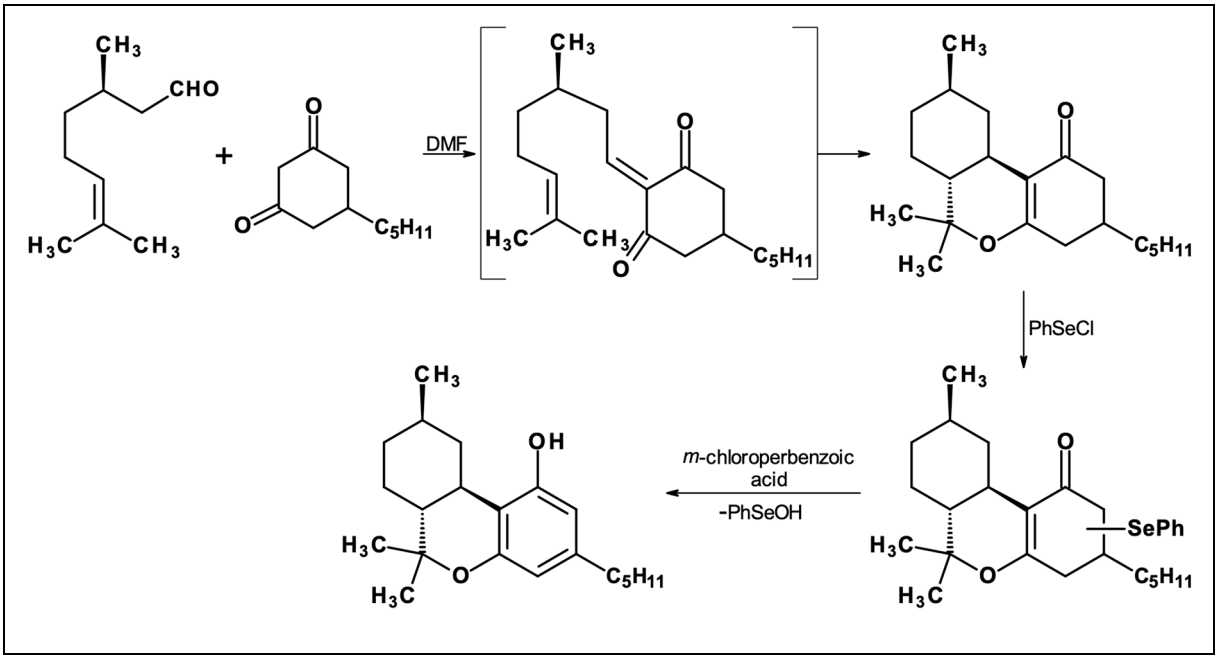
Stereoselective syntheses involving intramolecular hetero-Diels-Alder reaction of transient *o*-quinonemethides have been developed (Tietze et al., 1982; Marino and Dax, 1984; Casiraghi et al., 1986; Cornia et al., 1989; Murphy et al., 1992a; Murphy et al., 1992b; Wang et al., 2000; Lee and Xia, 2008).

The use of the intramolecular hetero-Diels-Alder reaction for the highly efficient stereoselective synthesis of (–)-(6a*R*,9*R*,10a*R*)-HHC, that is 9β-HHC, was first reported by the Tietze-group (Tietze et al., 1982) ([35]). As depicted in Figure 18, condensation of the readily available (*R*)-citronellal with 5-pentylcyclohexane-1,3-dione (Focella et al., 1977) generates a transient *o*-quinonemethide which upon intramolecular cycloaddition affords the appropriately substituted decahydrodibenzopyranone. The tricyclic ketone was then

([35]) Due to the use of toxic selenium reagents this otherwise remarkable stereocontrolled synthesis is impractical for large-scale production of HHC enantiomers.

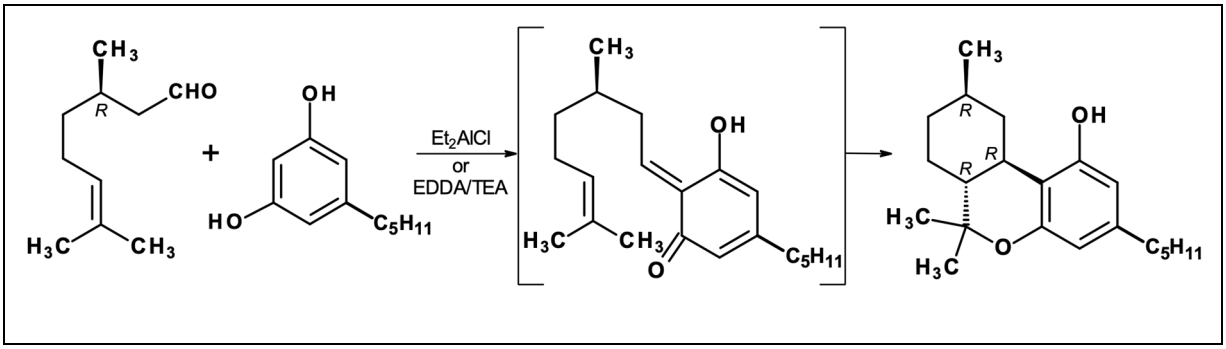
aromatised in two steps *via* selenium intermediates to provide 9β-HHC. The 'unnatural' enantiomer (+)-(6a*S*,9*S*,10a*S*)-HHC can also be synthesised similarly using (*S*)-citronellal.

FIGURE 18
The multistep synthesis of 9β-HHC using an intramolecular Diels-Alder reaction (Tietze et al., 1982). The transient *o*-quinonemethide is shown in square brackets



Improvements on the above method use olivetol derivatives for the intramolecular Diels-Alder reaction thus eliminate the aromatisation step that involves noxious selenium reagents (Marino and Dax, 1984; Casiraghi et al., 1986; Cornia et al., 1989; Lu et al., 1992; Murphy et al., 1992a; Murphy et al., 1992b; Wang et al., 2000; Lee and Xia, 2008). Of these, the most elegant and economic synthetic method provides access to 9β-HHC in a single step (Figure 19). Thus, cyclocondensation of (*R*)-citronellal with olivetol is facilitated by either diethylaluminum chloride (Et_2AlCl) (Casiraghi et al., 1986; Cornia et al., 1989; Andersson et al., 2011) or ethylenediamine diacetate/triethylamine (EDDA/TEA) (Lee and Xia, 2008) leads to 9β-HHC in high chemical yields (>85%) and high stereochemical purity (>76%). It is remarkable that the intramolecular Diels-Alder reaction proceeds with 100% stereocontrol exerted by the single chiral centre of (*R*)-citronellal.

FIGURE 19
A one-step stereoselective synthesis of (6a*R*,9*R*,10a*R*)-HHC (9β-HHC) (Casiraghi et al., 1986; Cornia et al., 1989; Lee and Xia, 2008). The transient *o*-quinonemethide is shown in square brackets



The (6a*S*,9*S*,10a*S*) enantiomer of HHC has also been prepared by the above methods using (*S*)-citronellal as starting material (Marino and Dax, 1984; Casiraghi et al., 1986; Cornia et al., 1989; Lee and Xia, 2008; Lee, 2010; Andersson et al., 2011).

The Et_2AlCl-based method has also been used for the synthesis of HHC homologues such as one lacking the C-11 methyl group (11-nor-HHC), and the C-9 geminal dimethyl analogue of HHC (Andersson et al., 2011).

A related intramolecular hetero-Diels-Alder reaction-based approach to (±)-HHC has also been reported (Inoue et al., 1990).

2.3.6. Other chemical aspects

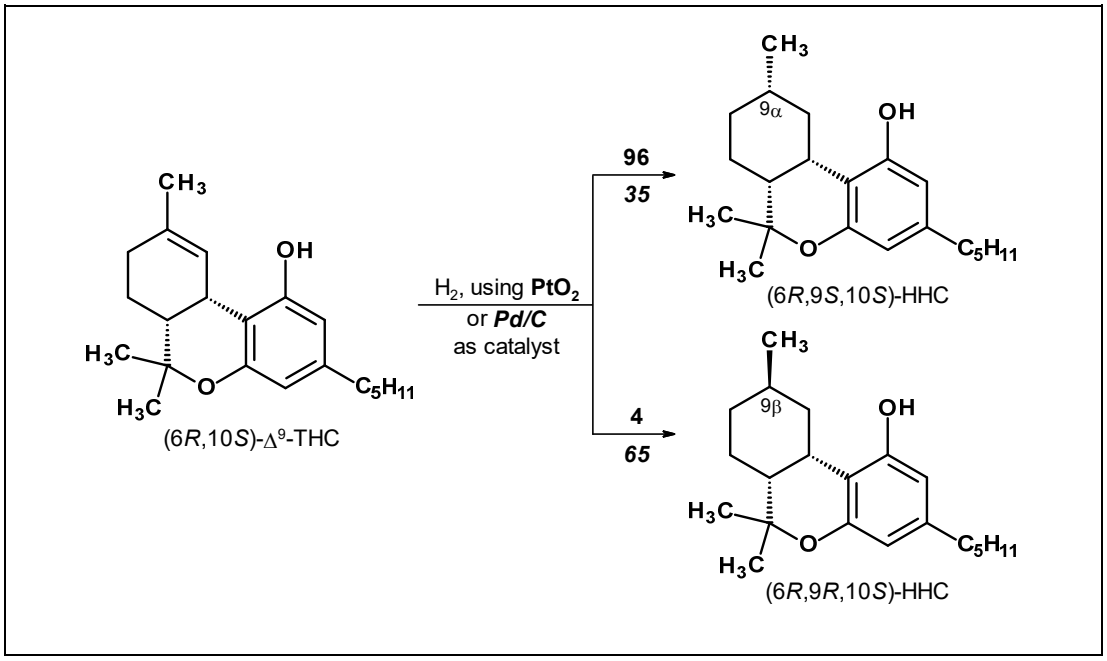
2.3.6.1 Synthesis of cis-9β-HHC

The first isolation of Δ^9-*cis*-tetrahydrocannabinol (*cis*-THC) from marijuana plants seized in the US was reported in 1977 (Smith and Kempfert, 1977). More recently, two enantiomeric *cis*-THCs, that is (6a*S*,10a*R*)-Δ^9-THC and (6a*R*,10a*S*)-Δ^9-THC have been isolated from several hemp varieties cultivated in Europe for fiber production (Schafroth et al., 2021). In both the American and European cases, the plants rich in *cis*-THC were of low-THC but high-CBD containing chemotypes. The biosynthetic precursor of *cis*-THC, that is *cis*-Δ^9-tetra–hydrocannabinolic acid (*cis*-Δ^9-THCA) has also been reported recently (Tolomeo et al., 2022).

The synthesis of *cis*-HHC stereoisomers had already been described before the isolation from hemp the *cis* isomer of its unsaturated counterpart, Δ^9-THC. The *cis*-HHC epimers were prepared by catalytic hydrogenation of the corresponding synthetic *cis*-Δ^9-THC (Gaoni and Mechoulam, 1968; Uliss et al., 1978) or, alternatively, obtained from either $\Delta^{6a,7}$-THC or $\Delta^{6a,10a}$-THC (Arnone et al., 1975a). Once again, it was found that the ratio of the HHC

epimers depended on the nature of the catalyst: hydrogenation of *cis*-Δ^9-THC over PtO_2 gave a 94:6 isomeric mixture of the 9α and 9β epimers, respectively. Hydrogenation using Pd on carbon catalyst reversed the ratio with the 9β epimer predominating as determined by gas chromatography (Figure 20) (Uliss et al., 1978).

FIGURE 20
Synthesis of *cis*-HHC epimers from *cis*-THC (Uliss et al., 1978)



The stereochemistry of the isomerization of *cis*-HHC to *trans*-HHC has been studied in detail (Uliss et al., 1978).

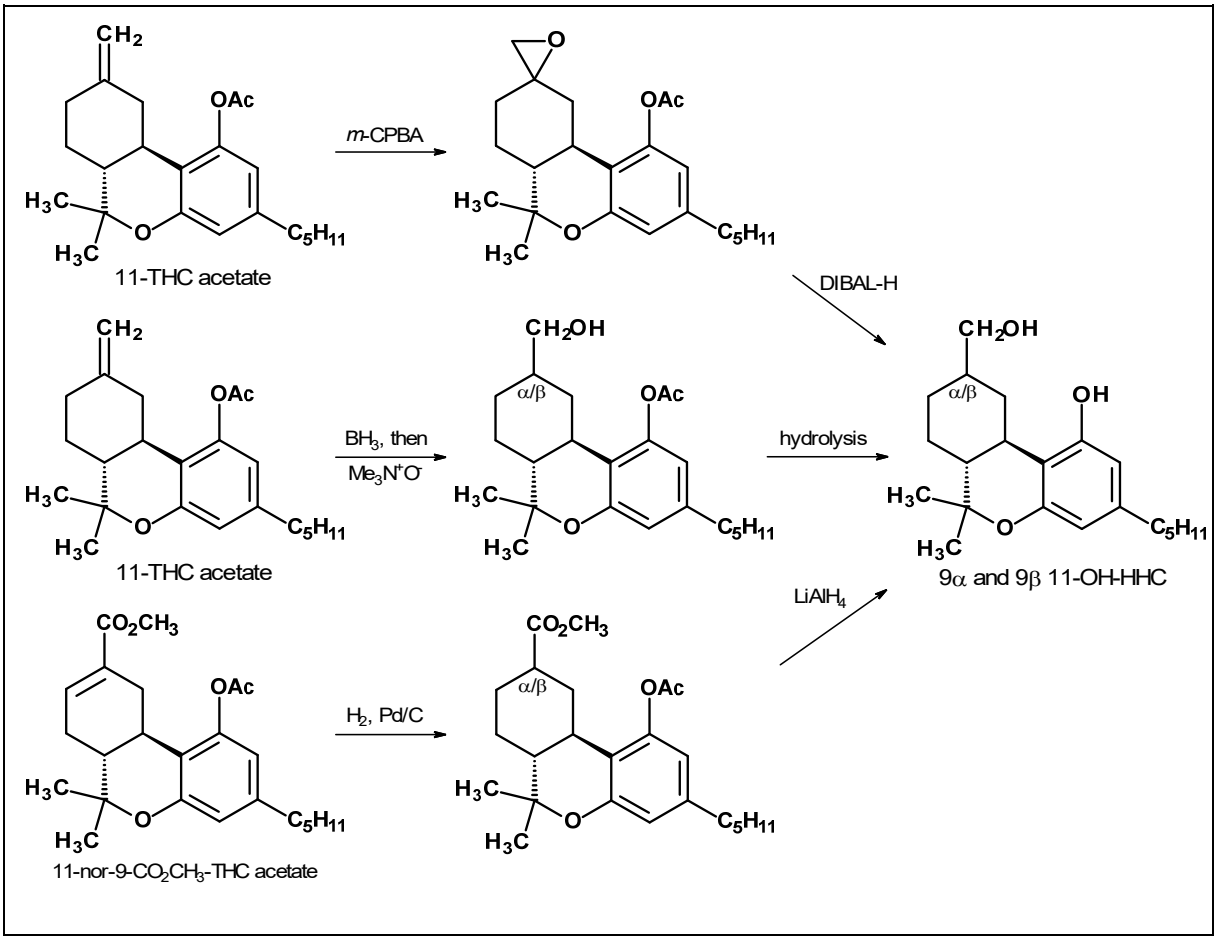
Since CBD, the key precursor for the synthesis of HHC, has become readily available in recent years, the synthetic routes described in the previous paragraphs and shown in Figures 15–18 and 20 are only of theoretical interest, although they may be useful for the synthesis of analogues of HHC, including radiolabelled derivatives, as well as for the preparation of metabolites of HHC. However, the one-step method depicted in Figure 19 offers an excellent route to substances with a hexahydrocannabinol core. For example, the use of readily accessible olivetol homologues, including branched chain ones, may provide hexahydro analogues that are hardly accessible from natural sources.

2.3.6.2 Synthesis of the metabolite 11-OH-HHC and the metabolite-like 11-nor-9-COOH-HHC

Several reports have described the synthesis of established or potential metabolites of HHC either as standards for pharmacokinetic studies or to investigate their biological activity (for metabolism of HHC, see Section 2.5.5).

The three reported multistep syntheses of the active cannabimimetic metabolite 11-OH-HHC rely on known cannabinoids (Figure 21). Epoxidation of Δ^{11}-THC acetate followed by reduction using DIBAL-H yielded 11-OH-Δ^{8}-THC and a mixture of the 9α and 9β epimers of 11-OH-HHC (Razdan et al., 1973). Hydroboration of the same precursor afforded an approximately 1:1 mixture of the two epimers of 11-OH-HHC (Skinner et al., 1979). In the third method, catalytic hydrogenation of the methyl ester of 11-nor-9-COOH-Δ^{8}-THC acetate led to the separable mixture of the 9α-HHC and 9β-HHC epimers in 54% and 34% yields, respectively, which were then reduced by $LiAlH_4$ to the respective 11-OH-9α-HHC and 11-OH-9β-HHC metabolites (Mechoulam et al., 1980).

FIGURE 21
Syntheses of the bioactive metabolite 11-OH-HHC (Razdan et al., 1973; Skinner et al., 1979; Mechoulam et al., 1980)

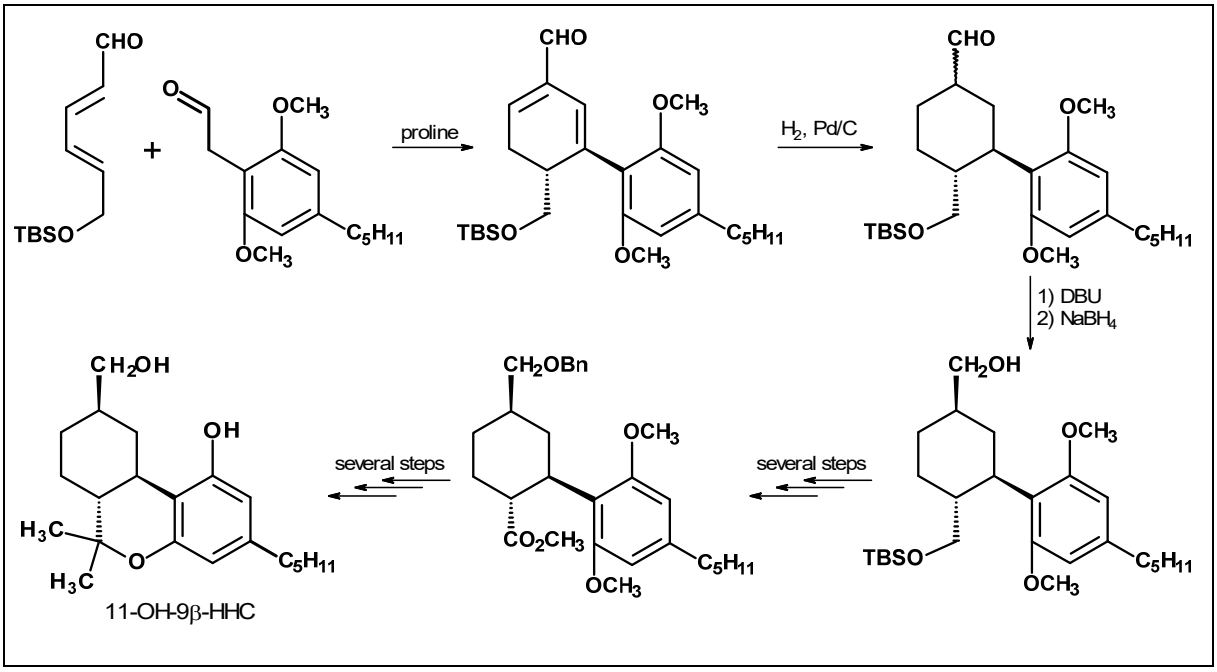


Deuterium-labelled 11-OH-HHC epimers have also been reported (Yang et al., 1991).

Following a route similar to the one shown in Figure 17 (Hughes et al., 1971), an organocatalysis-based asymmetric synthesis of metabolite 11-OH-9β-HHC has recently been reported (Maurya and Appayee, 2020). As outlined in Figure 22, the multistep synthesis is based on a proline-catalysed inverse-electron-demand Diels-Alder reaction and provides

access to 11-OH-9β-HHC as a single diastereomer in high stereochemical purity (97% enantiomeric excess).

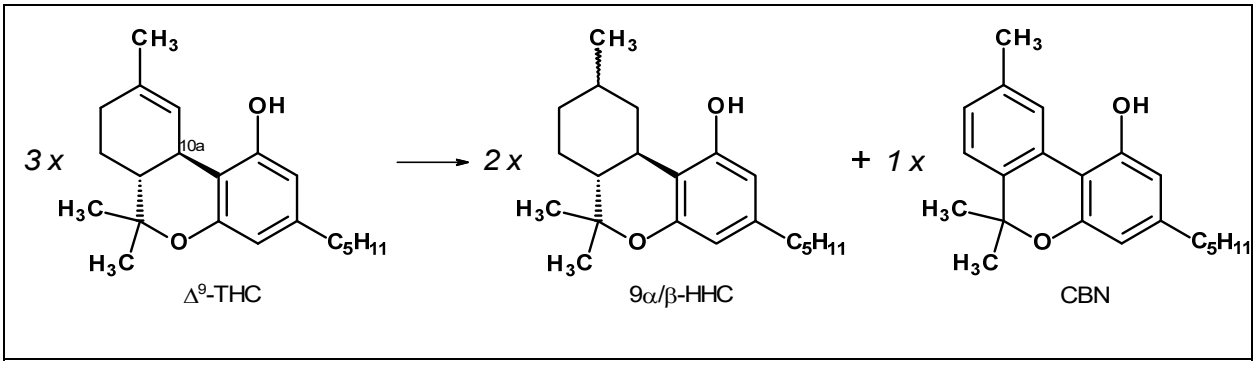
FIGURE 22
Enantioselective total synthesis of 11-OH-9β-HHC (Maurya and Appayee, 2020)



Abbreviations: TBS: *tert*-butyldimethylsilyl; DBU: 1,8-diazabicyclo[5.4.0]undec-6-ene; Bn: benzyl

While HHC is principally a semi-synthetic cannabinoid, it may also co-occur with cannabinol in low amount in cannabis samples (Turner et al., 1973; Garrett et al., 1978; Qureshi et al., 2012; Basas-Jaumandreu et al., 2020). It is thought to be formed from Δ^9-THC by disproportionation as shown in Figure 23. It has been proposed that traces of acids (chloroform solvent, silicic acid in glassware or TLC) catalyse the formation of free radicals at the C-10a benzylic carbon atom leading to disproportionation of the phytocannabinoid into HHC and CBN (Turner et al., 1973; Garrett et al., 1978). Such a free radical initiated redox disproportionation has been described in a 1,2-dihydroquinoline series (Muren and Weissman, 1971).

FIGURE 23
Proposed scheme for the disproportionation of Δ^9-THC to HHC epimers and CBN



In liver extract of mice treated with the shorter ethyl homologue of THC the formations of the ethyl homologues of HHC and CBN have been reported raising the possibility of the occurrence of such a disproportionation also *in vivo* (Brown and Harvey, 1991).

Traces of HHC have also been shown to be formed from CBD by photochemical degradation (Seccamani et al., 2021; Franco et al., 2022).

2.3.7. Methods for identification and analysis

Table 2 summarises the various analytical methods that have been reported for HHC. The paragraphs that follow will only discuss key aspects of selected publications.

TABLE 2
Methods documented in the literature for the analytical characterisation of HHC in physical and biological samples

Analytical method	Reference	Comment
Presumptive colour test[a]	Dr. Tamás Csesztregi, personal communication	Fast Blue B salt test
Thin layer chromatography	Gaoni and Mechoulam, 1966a Tietze et al., 1982 Stothard et al., 2022	 Visualisation by ceric ammonium molybdate

Gas chromatography	Vree et al., 1972b Fenimore et al., 1973 Waller et al., 1976 Rosenthal et al., 1978 Harvey and Brown, 1990 Harvey and Brown, 1991c Information reported to EMCDDA via EWS Sams, 2022 Smith et al., 2022 Stothard et al., 2022 Collins et al., 2023	Includes HHCV Heptafluorobutyryl derivative GC-MS GC-MS; TMS[b] derivative GC-MS; TMS[b] derivative GC-MS GC-MS GC-MS GC-MS GC-MS
High-performance liquid chromatography	Williams et al., 1978 Moffat et al., 1982 Hill et al., 1987 Information reported to EMCDDA via EWS Karin et al., 2022 Sams, 2022 Stothard et al., 2022 Tesfatsion et al., 2022 Collins et al., 2023	HPLC-radioimmunoassay HPLC-photodiode array UV HPLC-MS/MS HPLC-MS/MS HPLC-MS HPLC-MS
Capillary column supercritical fluid chromatography	Later et al., 1986 Collins et al., 2023	Chiral separation of epimers
Ultraviolet spectroscopy	Gaoni and Mechoulam, 1966a Arnone et al., 1975a Arnone et al., 1975b Cornia et al., 1989 Wang et al., 2000	*cis*-(6a*S*,9*R*/*S*,10a*R*)-HHC epimers
Infrared spectroscopy	Cornia et al., 1989 Marino and Dax, 1984 Wang et al., 2000 Lee. 2010 Information reported to EMCDDA via EWS	9β-HHC GC-solid phase IR; both epimers
Raman spectroscopy	none	

^{1}H NMR spectroscopy	Archer et al., 1970	
	Arnone et al., 1975a	*cis*-(6a*S*,9*R*/*S*,10a*R*)-HHC
	Arnone et al., 1975b	epimers
	Tietze et al., 1982	9β-HHC
	Marino and Dax, 1984	
	Wang et al., 2000	9β-HHC
	Lee, 2010	9β-HHC
	Casati et al., 2022	both epimers
	Information reported to EMCDDA via EWS	9β-HHC
	Stothard et al., 2022	Epimers
	Tesfatsion et al., 2022	both epimers
	Collins et al., 2023	
^{13}C NMR spectroscopy	Zetta et al., 1987	HHC analog lacking side-chain
	Wang et al., 2000	9β-HHC
	Lee, 2010	9β-HHC
	Casati et al., 2022	both epimers
	Information reported to EMCDDA via EWS	epimers
	Tesfatsion et al., 2022	both epimers
	Collins et al., 2023	
Mass spectrometry	Budzikiewicz et al., 1965	
	Rosenthal et al., 1978	GC-MS
	Harvey, 1981	GC-MS; TMS[b] derivative
	Harvey and Brown, 1990	GC-MS; TMS[b] derivative
	Harvey and Brown, 1991c	GC-MS; TMS[b] derivative
	Wang et al., 2000	
	NIST, 2021	
	Casati et al., 2022	
	Cayman Chemicals, 2022a,b	both epimers
	Information reported to EMCDDA via EWS	GC-MS; both epimers
	Karin et al., 2022	HPLC-MS/MS
	Sams, 2022	GC-MS; HPLC-MS
	Smith et al., 2022	DART TOF-MS
	Stothard et al., 2022	GC-MS
	Collins et al., 2023	GC-MS; both epimer

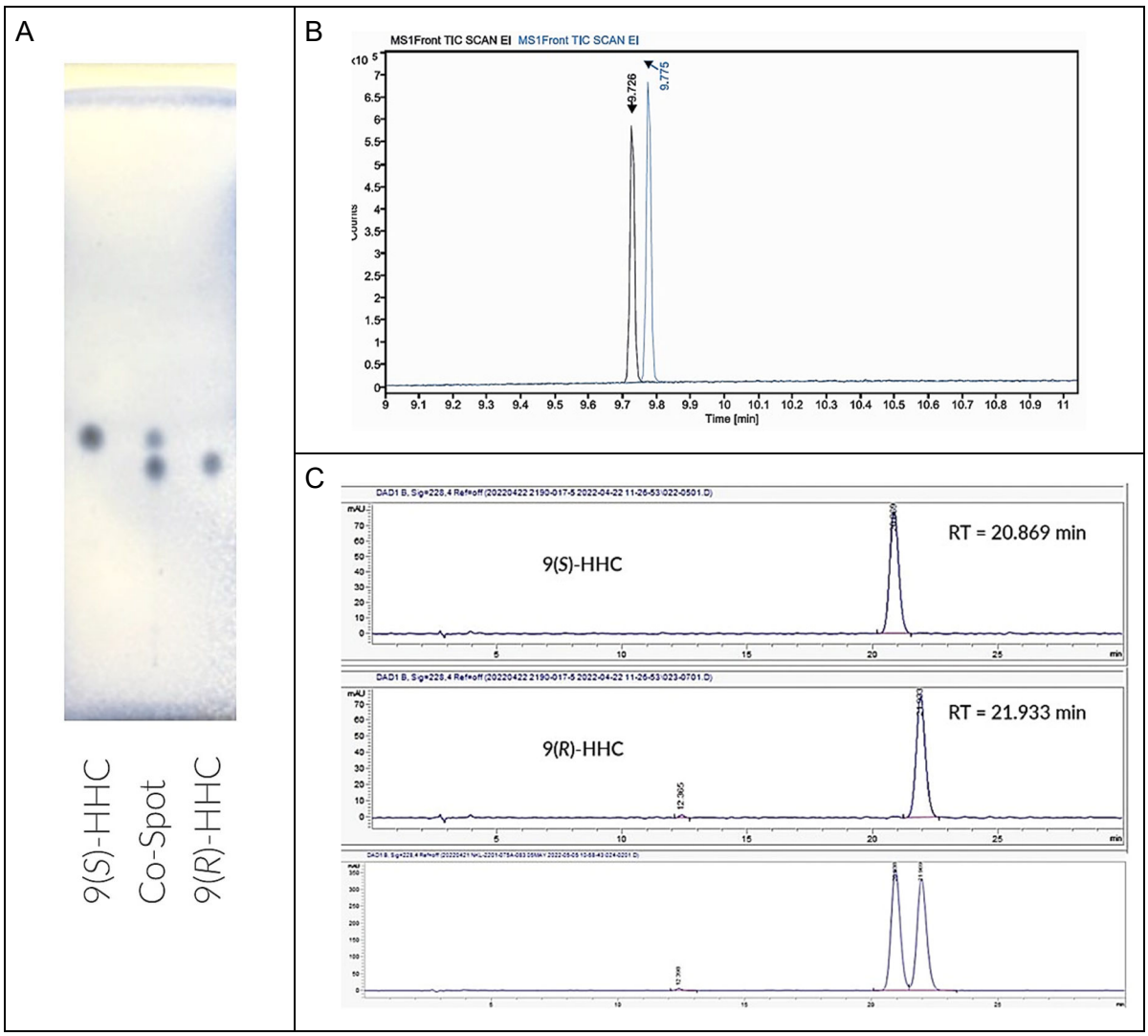
Radioimmunoassay[c]	Teale et al., 1975 Cook et al., 1976 Williams et al., 1978 Moffat et al., 1982 Jones et al., 1984 Jones et al., 1985 ElSohly et al., 1990 Wolf et al., 2022	8α-hydroxy-HHC[d] HPLC-radioimmunoassay See text 11-nor-9β-carboxy-HHC <40% cross-reactivity HHC-O[e] not detected

[a] For general discussion, see: UNODC (2022).
[b] Trimethylsilyl
[c] Based on hapten(s) designed for the carboxylic acid metabolite of THC.
[d] 8α-Hydroxyhexahydrocannabinol is a potential human metabolite.
[e] The acetate derivative of HHC did not cross-react in neither of the six immunoassays.

In general, all chromatographic methods developed have proven to be useful for the separation of the two epimers of HHC. For example, chromatograms published in a recent technical poster demonstrate the excellent analytical separation of the two epimers by three different techniques (Figure 24) (Stothard et al., 2022).

FIGURE 24

Separation of 9α-HHC and 9β-HHC by A) thin layer chromatography,[a] B) gas chromatography[b] and C) reverse phase liquid chromatography[c] (Stothard et al., 2022)



[a] Silica gel plates developed with 10% *tert*-butyl methyl ether (MTBE) in heptane, visualisation by ceric ammonium molybdate.

[b] Detection by MS.

[c] Detection by UV (228 nm).

The results of an exhaustive analysis of the relationship between the chemical structure and GC retention time of 25 common cannabis constituents and 45 synthetic cannabinoids, including HHC and its *n*-propyl homologue have been published (HHCV) (Vree et al., 1972b).

The infrared spectra of the two epimers are similar but not superimposable and some characteristic differences can be seen in the fingerprint region. For example, gas chromatography-coupled solid phase infrared spectroscopy analysis of a sample reported to

the EMCDDA that was seized in Germany of the separable 'HHC I' and 'HHC II' isomers (ca.1:1 mixture; stereochemistry not assigned), displayed the following characteristic bands:

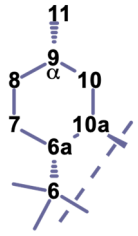
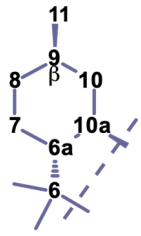
HHC I: IR v 1626, 1581, 1513, 1457, 1427, 1384, 1357, 1335, 1270, 1251, 1221, 1189, 1152, 1139, 1116, 1086, 1056, 1037, 1021, 1005, 920, 905, 878, 866, 828, 798, 732 cm^{-1}.

HHC II: IR v 1626, 1581, 1512, 1464, 1426, 1381, 1358, 1342, 1265,1245, 1205, 1189, 1151, 1140, 1116, 1068, 1047, 1036, 1006, 920, 886, 855, 826, 801, 727 cm^{-1}.

The fragmentation pattern in the mass spectra of the 9α and 9β epimers of HHC are virtually identical (Cayman, 2022a, Cayman, 2022b); GC-MS or HPLC-MS methods require analytical standards for stereochemical analysis of an actual sample.

Proton NMR spectroscopy might also be useful in the discriminating between the epimers. Solvent effects have been noted for the chemical shift of the C-10 and C-10a protons adjacent to the phenolic moiety (Archer et al., 1970). Also useful are ^{13}C NMR spectra in distinguishing between the 9α and 9β stereoisomers since chemical shifts of the the carbon atoms of the cycloalkane fragment are different for the two epimers (Collins et al., 2023) (Table 3). With the exception of C6a, the chemical shift of all carbon atoms of the cycloalkane moiety 9β epimer are shifted downfield.

TABLE 3
Chemical shifts for the carbon atoms of the cycloalkane fragment of 9α- and 9β-HHC (Collins et al., 2023)

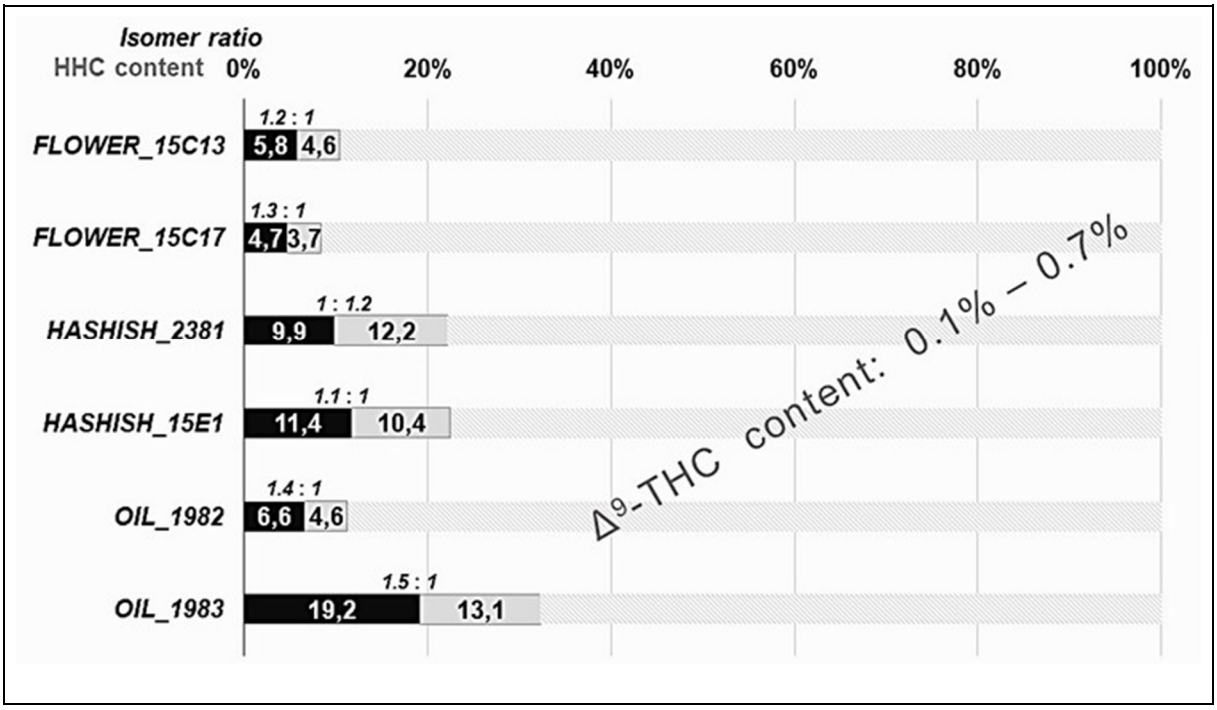
HHC fragment	C6	C6a	C7	C8	C9	C10	C10a	C11
	77.8	51.5	24.2	33.5	29.3	37.3	30.7	19.6
	77.9	50.6	29.2	36.8	34.0	40.2	36.8	23.4

In addition to mass spectrometric characterisation of HHC by several laboratories (Table 2), the fragmentation patterns of the trimethylsilyl derivatives of both 11-OH-HHC, a metabolite detected in microsomal preparations obtained from animal species (*vide infra*) (Harvey and Brown, 1991c), and 11-nor-9-carboxy-HHC have been reported (Harvey, 1992).

Recent analyses of three different large-scale law enforcement seizures, namely low-THC cannabis flower, hashish-type resins and oils, demonstrate the use of GC-MS analysis in the estimation of HHC epimer ratio in the three different matrices. The seizures were made in Italy in August 2022 and reported to the EMCDDA in November 2022. As the graph in Figure 25 indicates, the combined concentrations of the two HHC epimers ranged from 8.4% (herbal material) to 32.3% (oil). The ratio of the two isomers in the samples varied from 1:1.2 (HASHISH_2381) to 1.5:1 (OIL_1983), suggesting that the 9α and 9β epimers occurred in roughly equal amounts in the samples (the actual GC peaks were not assigned to the epimers).

FIGURE 25

Analysis by GC-MS of six HHC-containing seizures reported by Italy to the EMCDDA. The numbers within the bar indicate the concentration of the epimers in the samples, while the figures in italics above each bar indicate the ratio of the epimers. The graph was created from data reported by Italy



Information of the detection of HHC or its putative human metabolites by immunoassay is scarce and originate from decades-old studies.

A radioimmunoassay (RIA) method for the specific detection of Δ^9-THC and other cannabinoids and some of their metabolites was developed using antibodies raised in sheep against Δ^9-THC *O*-hemisuccinate covalently bound via the free carboxylic group to bovine serum albumin (Williams et al., 1978). In the cross-reactivity assay, Δ^9-THC, Δ^8-THC, $\Delta^{9,11}$-THC, CBN, 11-OH-Δ^9-THC, 8α-OH-Δ^9-THC, 11-OH-CBN, 11-nor-9-COOH-Δ^9-THC, 11-nor-9-COOH-CBN, as well as HHC ([36]) at the same 0.6 ng/mL blood plasma concentration equally inhibited tritium-labelled Δ^9-THC binding by 50%. Likewise, a similar RIA method

([36]) The ratio of the 9α and 9β epimers was not stated.

developed for the detection of tricyclic cannabinoids did not discriminate between Δ^9-THC, Δ^8-THC, 11-OH-Δ^8-THC, CBN, and 8α-OH-HHC, a metabolite of HHC (Teale et al., 1975).

Studies carried out in the 1980s indicate substantial cross-reactivity of 11-nor-9β-carboxy-HHC, a potential but hitherto not reported metabolite of HHC, in an immunoassay developed for the detection for the primary THC metabolite 11-nor-9-COOH-THC (Jones et al., 1984; Jones et al., 1985). Specifically, in a cannabinoid radioimmunoassay designed for the urinary detection of the carboxylic acid metabolite of Δ^9-THC (Abuscreen®) the metabolite-like synthetic 11-nor-9β-COOH-HHC was found to show substantial cross-reactivity while the 9α-carboxy epimer was less reactive in the assay; of the two 'intact' HHC epimers only the 9β isomer exhibited appreciable though low cross-reactivity (Jones et al., 1984).

Another early study reported on the cross-reactivity of three commercial immunoassays developed for the detection the carboxylic acid metabolite of THC in urine (Jones et al., 1985). Table 4 shows the cross-reactivity observed for representative cannabinoids and their known or presumed metabolites in a homogeneous enzyme multiplied immunoassay (EMIT) and two radioimmunoassays (RIA-I and RIA-II). Notably, the metabolite-like carboxylic acid derivatives of HHC exhibit high cross-reactivities in all three immunoassays developed for the urinary detection of the THC metabolite 11-nor-9-COOH-Δ^9-THC. This indicates that current immunoassays designed for THC metabolites detect the carboxylic acid metabolite of HHC only if it is formed in the human body (hitherto only monohydroxylated metabolites of HHC have been identified; see Section 2.5.5). The higher, though still moderate cross-reactivity of the 9β- HHC compared to the reactivity of the 9α epimer in these assays is conspicuous. Furthermore, though relevant human data are lacking, the moderate-to-high cross-reactivity of the 11-hydroxylated metabolites of Δ^8/Δ^9-THC suggests that the structurally related 11-OH-HHC, an established metabolite of HHC in the mouse (*vide infra*) and probably occurs in humans who consumed HHC, might show positive reaction in such immunoassays.

TABLE 4

Cross-reactivities of cannabinoids in an enzyme immunoassay (EIA) and two radio–immunoassays (RIAs) (Jones et al., 1985) ([37]). Compounds of particular interest are in bold

Test compound	Percent cross-reactivity		
	EIA[a]	RIA-I[b]	RIA-II[c]
11-nor-9-COOH-Δ^9-THC	**84**	**95**	**97**
11-nor-9-COOH-Δ^8-THC	84	135	93
11-OH-Δ^9-THC	71	161	39
11-OH-Δ^8-THC	88	179	62
Δ^9-THC	6.0	13	4.0
Δ^8-THC	8.2	33	9.8
Δ^8-THC-acetate	**<4.7**	**6.9**	**3.9**
11-nor-9-COOH-CBN	15	6.1	1.8
11-nor-9β-COOH-HHC	**125**	**125**	**152**
11-nor-9α-COOH-HHC	**47**	**41**	**31**
9β-HHC	11	13	14
9α-HHC	4.5	8.3	6.4

[a] EMIT® Cannabinoid Assay (Syva Corporation) based on antibody raised in sheep against 11-nor-9-oxo-hexahydrocannabinol covalently coupled via its oxime to bovine gamma-globulin.

[b] Abuscreen® Radioimmunoassay for Cannabinoids (Roche Diagnostic Systems) based on antibody raised in goats against 11-nor-9-carboxy-Δ^8-THC covalently coupled via the carboxylic acid group to an amino moiety on bovine serum albumin.

[c] Abuscreen® Radioimmunoassay for Cannabinoids (Roche Diagnostic Systems) developed on an antibody which was “raised in goats against hexahydrocannabinol (HHC) ([38]) derivatised from the 9 position with a one carbon spacer terminating in a carboxyl moiety. The derivative was then covalently coupled to BSA via the added carboxyl moiety”.

([37]) From Table 1 of the publication.
([38]) The term ‘HHC’ that has also been used as an acronym for 9-nor-9-hydroxyhexahydrocannabinol, and the subsequent description of the synthesis of the hapten for RIA-II raises some uncertainties regarding the cross-reactivity data for 9α-HHC and 9β-HHC in the assays involving RIA-II.

2.3.8 Dosage regimens

2.3.8.1 Pharmaceutical information and route of administration and dosage

Based on the marketed products, law enforcement seizures, collected samples, and anecdotal reports in Internet forums, the typical forms of administration are smoking low-THC cannabis on which HHC is sprayed, vaping e-liquids for electronic cigarettes or other vaping devices, and eating food products, such as tinctures and edibles (e.g. sweets). If known, the actual composition and HHC-content of these products varies. HHC products are often flavoured.

There are no relevant dose-response studies in humans. It may be assumed that HHC preparations containing predominantly the more active 9β epimer elicit similar activity as Δ^9-THC preparations albeit somewhat larger dosages may need to be ingested. Due to the lack of pharmacokinetic studies, it is not possible to discern the contribution of the epimeric bioactive 11-OH-HHC metabolites to the overall pharmacological effects caused by the consumption of HHC-containing products.

2.4. Legitimate use of HHC

Based on available information in the literature, HHC does not appear to be an active ingredient in any human or veterinary medicinal product.

It appears that the current legitimate use of HHC is restricted to clinical and forensic casework as well as scientific research. For example, HHC has been used as an internal standard in Δ^9-THC pharmacokinetic studies by GC/MS analysis (Fenimore et al., 1973; Wall and Brine, 1979).

However, it is possible that further research with HHC, having similar but not identical pharmacodynamics and pharmacokinetics to Δ^9-THC, may discover unique and therapeutically useful pharmacological properties.

2.5. Pharmacological and toxicological properties of HHC

2.5.1 Summary

Information from scientific research on human pharmacology, including behavioural effects of HHC is currently unavailable. The biological activity of HHC has been studied in several animal species since the 1940s but, due to the unknown purity or composition of the substance used in early investigations, the results of some of these studies is difficult to interpret. Inter-laboratory variations in the bioassays – some of which later turned out to be inadequate for cannabimimetic activity – also complicates the assessment of the results of early studies. Nevertheless, later work with chemically well-characterised substances have consistently revealed that the cannabimimetic effects observed both *in vivo* and *in vitro* resides mainly in the 9β-HHC isomer.

2.5.2 Pharmacodynamics

There have been several publications on the biological activity of HHC since the first synthesis in 1940 (Adams et al., 1940a). Most of the reported studies examined the cannabimimetic effects of HHC in animals. A chronological summary of animal studies is shown in Table 5. There is a paucity of relevant studies *in vitro*, and the results of various investigations *in vitro* will be given in Table 8.

For a discussion of structure-activity relationships in a series of homologues, see Section 2.6.1.

2.5.2.1 Data from animal experiments in vivo

Several animal assays have been developed to study the 'marijuana-like' or cannabimimetic effects of cannabinoids. Studies with HHC have used the following test: corneal reflex in rabbit eye, dog ataxia, monkey behaviour, mouse tetrad test, and discriminative stimulus in rats and pigeons. Additional studies investigated the therapeutic potential of HHC. Early investigations used the readily available isomeric mixtures of HHC, but recent studies focused on the more potent 9β isomer. Table 5 provides a summary of the results of these nonclinical laboratory studies in chronological order. Data for the monohydroxylated 11-OH-HHC metabolite are also shown.

TABLE 5
Chronological list of biological studies *in vivo* of HHC and its metabolite-like derivatives. When available, potency relative to Δ^9-THC is shown as reported or was calculated from dose data when given in the publication

Bioassay	Isomer	Reported dose	Relative potency	Reference
Hexahydrocannabinol				
Rabbit corneal reflex	mixture	5 mg/kg	~0.2[a]	Russell et al., 1941
Dog ataxia	mixture	no data no data	~0.32 ~0.51	Adams et al., 1940c Adams et al., 1942; Loewe, 1944
Rhesus monkey behaviour	9β 9α	1.0 mg/kg 5.0 mg/kg	~0.5 0.02–0.05	Edery et al., 1971
Mouse locomotion	mixture	ED_{50} = 8.58 mg/kg[b]	0.03	Skinner et al., 1979
Mouse postural arrest	mixture	ED_{50} = 6.65 mg/kg[b]	0.06	Skinner et al., 1979

Mouse body temperature	mixture	ED_{50} = 4.02 mg/kg[b]	0.13	Skinner et al., 1979
Mouse antinociception	mixture	no data	inactive	Skinner et al., 1979
Rabbit, convulsion		~0.1 mg/kg	0.5	Consroe et al., 1982
Rabbit intraocular pressure	9β 9α	1 mg/kg 1 mg/kg	inactive inactive	ElSohly et al., 1984
11-Hydroxyhexahydrocannabinol				
Rat discrimination study training drug: Δ^9-THC	9β 9α	ED_{50} = 0.44 mg/kg[c] ED_{50} = 2.16 mg/kg[c]	3.5 0.5	Järbe et al., 1986
Pigeon discrimination study training drug: Δ^9-THC	9β 9α	ED_{50} = 0.02 mg/kg[d] ED_{50} = 1.72 mg/kg[d]	8.0 0.1	Järbe et al., 1986

[a] The purity of the reference ”tetrahydrocannabinol” (optical rotation: –165º) is not known.

[b] Calculated from respective molar doses given as 27.1, 21.0, and 12.7 µmol/kg in the original publication.

[c] Test onset 0.5 hours after injection.

[d] Test onset 1.5 hours after injection.

One of the first assays used to evaluate the marijuana-like properties of cannabinoids was testing for the loss of corneal reflex in rabbits (*Gayer* test) [(39)] (Russell et al., 1941). Intravenous injection of an acetone solution of 5 mg/kg of HHC of unknown isomeric composition provoked corneal areflexia of the same degree as injection of 1 mg/kg ”tetrahydrocannabinol” (obtained from CBD and having an optical rotation of –165º).

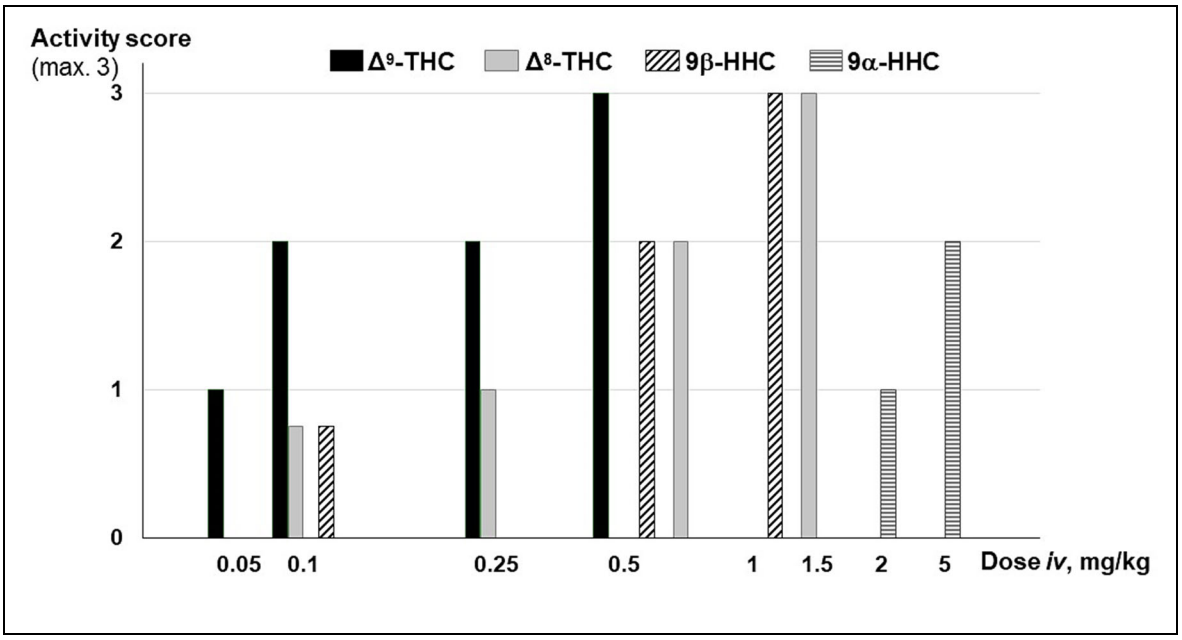
Comparative assessments using the dog ataxia assay ([40]) of ‘tetrahydrocannabinol’ (‘THC’) preparations administered via intravenous injection have been reported in the early 1940s (Adams et al., 1940c; Adams et al., 1942; Loewe, 1944). An initial publication reported that the mean value of potency of HHC (optical rotation –70º) was 0.32 relative to a ‘THC’ preparation obtained from CBD by cyclisation under mild acidic conditions, and ~0.4 relative to a ‘THC’ preparation obtained from CBD by cyclisation under harsh acidic conditions

([39]) In this test the corneal surface is touched lightly with the tip of a pen; upon intravenous pre-treatment with a cannabinoid causing corneal analgesia / anaesthesia the wink reflex of the animal partially or completely abolished (corneal areflexia). However, this test is not considered to be relevant to the psychotomimetic effects of cannabis observed in humans thus has been abandoned.
([40]) The dog ataxia test is a simple yet sensitive semi-quantitative assay that has been used for a century to predict cannabimimetic activity of a substance in humans.

(Adams et al., 1940c). (Based on the respective optical rotations of the two 'tetrahydrocannabinol' starting materials as –165º and –240º, it is reasonable to assume that the former 'THC' preparation consisted mainly of Δ^9-THC, while the latter was mainly $\Delta^{8,}$-THC ([41]). Subsequently, the same group reported that the potency of HHC was 0.52 relative to 'THC' (Adams et al., 1942; Loewe, 1944).

An extensive structure-activity relationship study carried out with rhesus monkeys as suitable model animals included both the 9α and 9β epimers of HHC (Edery et al., 1971; Mechoulam et al., 1980). The drugs were administered intravenously, and the behavioural pattern of the monkeys was assessed using Norton's scoring sheet (Norton, 1957). The data, represented as a graph in Figure 26, indicate that 9β-HHC with the C-11 methyl group in equatorial position is considerably more active than the 9α (axial) isomer. Specifically, the 1 mg/kg dose of 9β-HHC caused severe stupor and ataxia, full ptosis, immobility, crouched posture lasting for more than three hours and absence of reaction to external stimuli, while the 9α epimer was less potent even at the 5.0 mg/kg dose, the highest dose tested. For comparison, a 0.5 mg/kg dose of Δ^9-THC elicited behavioural and somatic changes with similar high scores as observed for the 1 mg/kg dose of 9β-HHC. Furthermore, the psychopharmacological activity of Δ^8-THC and 9β-HHC appeared to be comparable in this investigation. These behavioural experiments with rhesus monkeys revealed that the cannabimimetic activity resides mainly, if not solely, in the laevorotatory 9β-HHC, that is (–)-(6a*R*,9*R*,10a*R*)-HHC, being about half as active as Δ^9-THC.

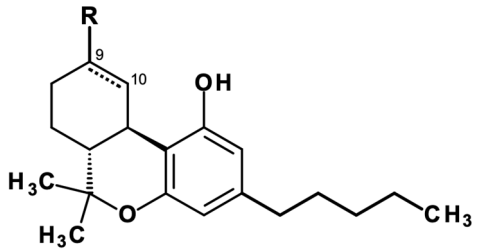
FIGURE 26
Activity of THC and HHC isomers on rhesus monkeys scored according to Norton's sheet. The graph was constructed on published semi-quantitative data (Edery et al., 1971)



([41]) An authoritative review, however, designates the 'tetrahydrocannabinol' tested by the Adams group as $\Delta^{6a,10a}$-THC, the racemic form of which was shown to be several times less potent than Δ^9-THC in rhesus monkeys (Mechoulam and Edery, 1973).

A series of tetrahydrocannabinol and hexahydrocannabinol derivatives were evaluated in mice for their effects on spontaneous locomotor activity, postural arrest, body temperature as measured by rectal hypothermia, and pain sensitivity in the hot plate assay (Skinner et al., 1979). As the estimated ED_{50} values shown in Table 6 indicate HHC was the least potent substance being by an order of magnitude less active than any of the compounds in three of the assays. Unlike Δ^9-THC, the only drug exhibiting antinociception in the hot plate assay, none of the hexahydrocannabinol analogues affected pain sensation. Curiously, 11-OH-HHC, an established metabolite of HHC in the mouse, was essentially as potent as Δ^9-THC in the other three assays.

TABLE 6
Activity profile of Δ9-THC and four hexahydrocannabinol analogues in a battery of tests in mice upon intraperitoneal injection (Skinner et al., 1979)



			ED_{50}, mg/kg[a]			
Compound	R	Double bond	Locomotor activity	Postural arrest	Rectal temperature	Pain sensitivity
Δ^9-THC	CH_3	9,10	0.25	0.41	0.53	1.00
HHC[b]	CH_3	none	8.58	6.65	4.02	inactive
11-nor-HHC	H	none	0.12	0.45	0.27	inactive
11- OH-HHC[b]	CH_2OH	none	0.27	0.33	0.66	inactive
11-nor-9-OH-HHC[b]	OH	none	0.13	0.35	0.22	inactive

[a] Calculated from µmol/kg values given in the publication.
[b] Approximately 1:1 mixture of the 9α and 9β epimers.

Based on the serendipitous findings that a population of New Zealand White rabbits exhibited behavioural convulsions when given low intravenous doses of Δ^9-THC, the cannabimimetic property of a series of cannabinoids was studied using this model (Consroe et al., 1982).

Compared to Δ^9-THC at the 0.05 mg/kg dose, both Δ^8-THC and HHC were half as active as the genuine natural product; neither cannabidiol nor olivetol were active in this model.

In a study examining the therapeutic potential of a series of cannabinoids in the treatment of glaucoma neither 9α-HHC nor 9β-HHC lowered the intraocular pressure (IOP) in the rabbit at the 1.0 mg/kg intravenous dose; in comparison, Δ^9-THC at the same dose significantly lowered IOP, for example, by 24% four hours after treatment (ElSohly et al., 1984).

The 9α and 9β epimers of 11-OH-HHC, the monohydroxylated metabolites of HHC, were tested for cannabimimetic activity by drug discrimination studies ([42]) in rats and pigeons (Järbe et al., 1986). The rats and pigeons were trained to discriminate between the presence or absence of Δ^9-THC in water or grain, respectively. The results of substitution tests with the 11-OH-HHC epimers are shown in Table 7. As the ED_{50} values indicate, 11-OH-9β-HHC, code-name '(–) NL-105', was severalfold more potent in rats than Δ^9-THC, while the 9α epimer, code-name '(–) NL-106', was inferior to the 9β epimer. These differences were more marked in pigeons where, for example, the 9β epimer of the HHC metabolite was about 17 times as potent as Δ^9-THC when assessed 0.5 h post-injection. The results of this study confirm the superiority of the 9β metabolite epimer over the 9α epimer as a cannabimimetic drug. The results are also in line with earlier studies indicating that the 11-OH-Δ^9-THC metabolite of Δ^9-THC is more potent than its parent cannabinoid in rat and pigeon discriminatory models (Järbe and McMillan, 1980).

TABLE 7

Median dose, ED_{50}, values calculated from dose-response data in rats and pigeons trained to discriminate between the presence or the absence of Δ^9-THC (3 mg/kg for rats, 0.5 mg/kg for pigeons) (Järbe et al., 1986). Hours reflect the passage of time since injection until testing

Compound	ED_{50}, mg/kg			
	Rats		Pigeons	
	0.5 h post-injection	1.5 h post-injection	0.5 h post-injection	1.5 h post-injection
Δ^9-THC	0.85	1.07	0.53	0.16
11-OH-9α-HHC	1.58	2.16	2.60	1.72
11-OH-9β-HHC	0.24	0.44	0.03	0.02

([42]) In the drug discrimination paradigm, animals are trained to discriminate between a drug and placebo control in a two-choice situation where the correct choice is rewarded.

When ascertained, the formation of the bioactive 11-hydroxylated metabolite of HHC in humans is of pharmacological as well as of forensic importance.

Although HHC has not been investigated in such a discriminatory paradigm, the above results suggest that the human behavioural (cannabimimetic) effects of its 9β epimer would be qualitatively and quantitatively similar to those of Δ^9-THC.

As mentioned before, scientific publications on the effect of HHC in humans could not be identified.

2.5.2.2 Data in vitro

Prior to the identification of the cannabinoid receptor, a study reported the discovery of a high-affinity cannabinoid binding protein in rat brain membrane preparations using the hydrophilic 5'-trimethylammonium Δ^8-tetrahydrocannabinol (TMA) and its radiolabelled derivative [^{3}H]TMA (Nye et al., 1985). The most potent inhibitor of the specific [^{3}H]TMA binding, as characterised by the binding affinity constants K_i, was the behaviourally inactive cannabigerol (K_i = 8.3 nM), while for 9β-HHC the K_i value was 19 nM. In comparison, Δ^9-THC and Δ^8-THC were somewhat less potent (respective K_i values were 27.3 and 32.9 nM). However, it was noted that some behaviourally inactive cannabinoids, such as enantiomeric THCs and CBD, also inhibited [^{3}H]TMA binding indicating that this particular binding site do not mediate characteristic effects of psychoactive cannabinoids.

An investigation studying the interaction of a series of cannabinoids with cloned human CB_1 and CB_2 cannabinoid receptors reported only semiquantitative data for 9α-HHC and 9β-HHC (Andersson et al., 2011). The cannabinoid receptor activation by the test compounds were presented as a percentage of the forskolin-induced cAMP accumulation in the presence of vehicle control (5% ethanol). At the 100 µM screening concentration 9α-HHC was found to activate the *h*CB_1 and *h*CB_2 receptors by about 60% and about 15%, respectively. At the same large concentration, 9β-HHC was found to activate *h*CB_1 and *h*CB_2 receptors by about 55% and about 30%, respectively, thus no substantial difference between the two HHC epimers were observed in this assay. No data were reported for Δ^9-THC, but Δ^8-THC activated *h*CB1 and *h*CB_2 receptors by about 57% and 40%, respectively, at the screening concentration.

A very recent investigation reported by the Swedish National Board of Forensic Medicine to the EMCDDA ([43]) *in vitro* used transfected cells expressing the CB_1-receptor to compare (9*R*)-HHC (9β-HHC) and JWH-018, a well-studied synthetic cannabinoid receptor agonist ([44]). In this receptor activation assay (9*R*)-HHC was shown to be a partial agonist with an EC_{50} ([45]) of 144 nM with a moderate efficacy (E_{max} = 37% relative to JWH-018); JWH-018 was about three-times more potent (EC_{50} = 44.0 nM).

([43]) '*Rapport angående aktivering av CB_1-receptorn för 9(R)-Hexahydrocannabinol. 2022-11-07*', Rättsmedicialverkert Linköping Rapport No. 106. Personal communication to the EMCDDA.
([44]) For a recent human clinical trial in which the effect of a 75 mg/kg vaporised dose of JWH-018 was used by volunteers, see Theunissen et al., 2022.
([45]) The half maximal effective concentration producing 50% of the response elicited by JWH-018.

A study discussed above also investigated the effect of the two HHC epimers on *h*TRPA1 ion channel activation ([46]) (Andersson et al., 2011). However, only area-under-the-curve (AUC) values were calculated from concentration–response curves for most of the test substances. A comparison of the AUC values indicates that 9β-HHC is about 0.85 times as potent as Δ^9-THC, while 9α epimer is less than half as active; Δ^8-THC is about 0.4 times as potent as Δ^9-THC in activating TRPA1 channels (see also Table 8).

The binding of selected cannabinoids including HHC, to opioid receptor preparations isolated from rat brain has also been investigated (Vaysse et al., 1987). Of the compounds, Δ^9-THC, Δ^8-THC, CBD and 9β-HHC were found to be the most potent cannabinoids in inhibiting the binding of the mu receptor selective opioid [^{3}H]dihydromorphine with IC_{50} values of 7, 20, 7, and 10 µM, respectively. It was suggested that the binding of the active cannabinoids was allosteric in nature.

The human voltage-sensitive K^+ channel or hERG ([47]) channel, a voltage-gated potassium channel expressed in the heart and the nervous system, is responsible for the repolarisation during the action potential. Blocking of the hERG channel can lead to life-threatening cardiac arrhythmias and sudden death thus preclinical guidelines require testing drugs under development for hERG liability. In patch clamp assay an approximately 1:1 mixture of the 9α and 9β epimers of HHC did not significantly block cloned hERG ion channels (EC_{50} > 50 µM;) indicating no cardiac safety issues related to hERG; for the positive control cisaprid EC_{50} < 0.05 µM (Collins et al., 2022).

The potential of a series of synthetic hexahydrocannabinol analogues (Lee and Xia, 2008) in the treatment of cancer has also been examined (Thapa et al., 2011). In exploratory experiments, 9β-HHC (LYR-1) and all its seven analogues inhibited human umbilical vein endothelial cell ([48]) proliferation at the 5 µM screening concentration. Of the potent homologues of 9α-HHC, LYR-7 (Figure 12) and a keto analogue (LYR-8), both lacking CB receptor affinity, were selected for further investigations. Δ^9-THC was not included in these studies.

([46]) Transient receptor potential channels of the ankyrin type-1 (TRPA1, the 'mustard oil' receptor) are a group of membrane proteins mediating the sensation of noxious stimuli including, among others, temperature, pressure and pain perception. Drugs interacting with TRPA1 appear to have analgesic properties.
([47]) The channel protein is coded by the ***h***uman ***E***ther-a-go-go ***R***elated ***G***ene, hence the acronym.
([48]) Human umbilical vein endothelial cells (HUVEC) are cells derived from endothelium of veins of the umbilical cord and are commonly used to investigate macromolecule transport, blood coagulation, angiogenesis, and fibrinolysis.

TABLE 8
Chronological list of biological studies *in vitro* of HHC. Potency relative to Δ^9-THC is shown as reported or was calculated from concentration data when given in the publication

Pharmacological assay	Isomer	Reported value	Relative potency	Reference
Rat brain cannabinoid binding site	9β	K_i = 19 nM	1.4	Nye et al., 1985
IC_{50} μ-opioid receptor	9β	IC_{50} = 10 μM	0.7	Vaysse et al., 1987
hCB_1 receptor activation, 0.1 mM	9α 9β	~60% ~55%	–[a] –[a]	Andersson et al., 2011
hCB_2 receptor activation, 0.1 mM	9α 9β	~15% ~30%	–[a] –[a]	Andersson et al., 2011
TRPA1 ion channel activation	9α	~0.65 AUC[b]	~0.35	Andersson et al., 2011
hTRPA1 ion channel activation	9β	~1.5 AUC[b]	~0.85	Andersson et al., 2011
HUVEC proliferation inhibition	9β	active at 5 μM	–[a]	Thapa et al., 2011
hERG ion channel inhibition	9α/9β, ~1:1 mixture	IC_{50} > 50 μM	–[a]	Collins et al., 2022
Cytotoxicity to human lung fibroblasts	9α/9β, ~1:1 mixture	IC_{50} = 14.4 μM	–[a]	Collins et al., 2022
Cytotoxicity to human hepatocytes	9α/9β, ~1:1 mixture	8.9% at 50 μM	–[a]	Collins et al., 2022
PANC-1 pancreatic cancer cells	9α 9β	IC_{50} = 18.9 μM IC_{50} = 10.3 μM	–[a] –[a]	Tesfatsion et al., 2022
HPAF-II pancreatic cancer cells	9α 9β	IC_{50} = 11.7 μM IC_{50} = 10.8 μM	–[a] –[a]	Tesfatsion et al., 2022
AsPC-1 pancreatic cancer cells	9α 9β	IC_{50} = 13.9 μM IC_{50} = 19.6 μM	–[a] –[a]	Tesfatsion et al., 2022
MIA-PaCa2 pancreatic cancer cells	9α 9β	IC_{50} = 27.2 μM IC_{50} = 12.7 μM	–[a] –[a]	Tesfatsion et al., 2022

[a] No data.
[b] Respective AUC values were calculated from concentration–response curves for HHC and Δ^9-THC and used for comparison of their TRPA1 activity. The AUC data for this table were extracted from the graph in the publication.

A recent preprint reported on preliminary results of the anticancer properties of the two HHC epimers in four pancreatic cancer cell lines (Table 8) (Tesfatsion et al., 2022) ([49]). Both the 9α and 9β epimers similarly inhibited the proliferation of cancer cells with IC_{50} values ranging from 10.3 to 27.2 µM; these values are comparable to the IC_{50} values of the anticancer agents olaparib or veliparib. While data for Δ^9-THC were not reported, hexahydro–cannabivarin also inhibited the proliferation of pancreatic cancer cells with similar micromolar IC_{50} values in two of these assays.

Finally, computational approaches have recently been attempted to study the binding features and selectivity of a series of cannabinoids at CB_1 and CB_2 receptors (Aviz-Amador et al., 2021). Molecular docking experiments *in silico* indicated that HHC, Δ^8-THC and Δ^9-THC had similar high calculated binding affinities to the CB_2 receptor, and this binding was facilitated by hydrophobic interactions involving identical amino acid residues of the receptor protein for the three cannabinoids. For the CB_1 receptor, HHC and Δ^9-THC displayed similar high calculated binding affinities, while Δ^8-THC bound to this receptor with lower affinity.

In summary, studies conducted since 1940 in animals *in vivo* and receptor preparations *in vitro* indicate that the pharmacology of HHC is similar to that of Δ^9-THC and in some assays the 9β-epimer of the semi-synthetic substance appears to only slightly less potent than its natural counterpart Δ^9-THC. It may also be noted that the observed potency differences between the two HHC epimers parallel the results of related structural and pharmacological studies with Δ^7-THC epimers showing that the 9β-Δ^7-THC (C-11 methyl in quasi-equatorial orientation) has significant cannabinoid activity while the 9α epimer is weakly active both *in vitro* and *in vivo* (Reggio et al., 1989; Huffman et al., 1995).

While no study examined the (psycho)pharmacology of HHC in humans, recent users' reports indicate that HHC products have Δ^9-THC-like effects reflecting the results of preclinical investigations.

2.5.3 Psychological and behavioural effects

There appear to be no studies on the psychological and behavioural effects of HHC in humans.

Apart from the investigations involving dog ataxia, mouse spontaneous locomotion, and rat and pigeon drug discrimination tests described in Section 2.5.2.1 no other animal behavioural studies with HHC could be identified.

2.5.4 Safety pharmacology

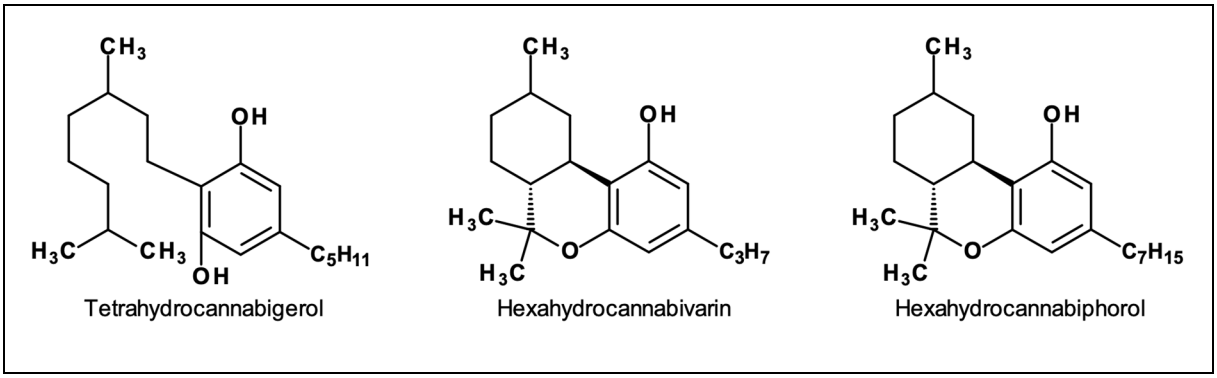
The toxicology and/or safety pharmacology of HHC have not been investigated until recently. A recent study (Collins et al., 2022) assessed the cytotoxicity of an approximately 1:1 epimeric mixture of HHC to human lung fibroblasts and plated human hepatocytes (see Table 8). While potential cytotoxic effects comparable to the positive control 'Cropromazine' [sic] were observed in the fibroblast assay at concentrations above 10 µM, essentially no

([49]) Pancreatic cancer is the seventh leading cause of cancer death worldwide and is one of the most difficult tumours to treat either by surgery or chemotherapy.

hepatotoxic effects were observed at concentrations below 50 µM (for the terfenadine control IC_{50} = 15.8 µM). Furthermore, no mutagenicity was observed in the Ames test with and without metabolic activation up to 100 µM concentration.

Since the production of HHC does not necessarily comply with 'Good Manufacturing Practices', contaminations either with extraction residues or synthetic by-products could pose unforeseen risks. Also, traces of heavy metals originating from the catalyst used for the hydrogenation might also be present. Furthermore, hydrogenation of the acid-treated extract either before or after decarboxylation may contain derivatives of other cannabinoids that were originally present in the crude hemp extract. Among these, cannabigerol as well as tetrahydrocannabivarin and tetrahydrocannabiphorol (THCP) (Section 2.6.1), the respective 3-*n*-propyl and 3-*n*-heptyl homologues of THC, have attracted interest recently; the chemical structure of their hydrogenated derivatives are shown in (Figure 27). There is only scant scientific literature available on the hydrogenated derivatives of these three cannabinoids and their pharmacology or toxicology is unknown. The recent findings in hemp of the bioactive ([50]) *cis*-Δ^9-THC (Section 2.3.6.1) may also pose additional health risk either due to their own pharmacological effects or from drug-drug interactions (Nasrin et al., 2021; Lopera et al., 2022). Since these phytocannabinoids and/or their transformed derivatives could be present in hemp extracts that are used as feedstocks for the production of HHC complete analytical characterisation of any hemp-derived product is important from a safety point of view.

FIGURE 27
The chemical structures of hydrogenated derivatives of cannabigerol (CBG), tetrahydrocannabivarin (THCV) and tetrahydrocannabiphorol (THCP)



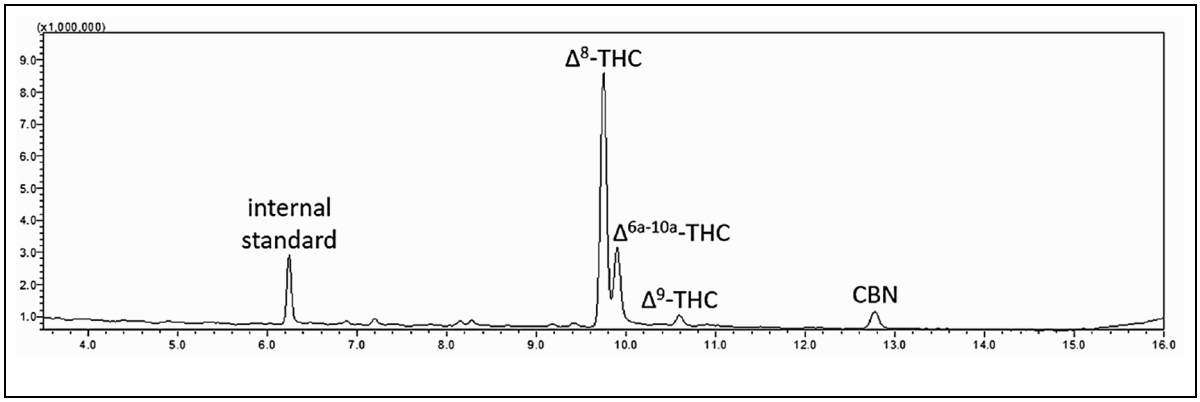
No information is available on any potential drug-drug interaction for HHC.

While suppliers in their marketing advertisements on the Internet often indicate product purity above 95%, it should also be kept in mind that unregulated products might not contain what their label indicates even when there is an 'analytical certificate' provided. For example, it has recently been reported that GC-MS analysis of a product sold in the US and submitted to

([50]) The synthetic *cis*-isomers of THC were at least tenfold less active than Δ^9-THC in animal behavioural studies (Martin et al., 1981). Recently (–)-*cis*-Δ^9-THC was found to be a sub-micromolar partial agonist of CB_1 and CB_2 receptors and an inhibitor at high micromolar concentrations of endocannabinoid processing enzymes; it also displayed cannabimimetic properties in the mouse tetrad test (Schafroth et al., 2021).

the laboratory for analysis as an alleged 'HHC' sample indicated the presence of Δ^8-THC, Δ^9-THC, and $\Delta^{6a,10a}$-THC but not any HHC (Figure 28) (Sams, 2022). In general, such mislabelling might either be intentional or due to the use of inadequate analytical methodology or negligence.

FIGURE 28
Results of GC-MS analysis of a product sample submitted to KCA Laboratories for analysis as "HHC" (Sams, 2022)



2.5.5 Pharmacokinetics

In general cannabinoids are highly lipophilic substances and thus accumulate in fatty tissues. They also highly bound to blood plasma proteins (Widman et al., 1974; Giroud et al., 2001; Schwilke et al., 2009). Though experimental data are not available, HHC is expected to share these properties. The measured and the calculated logP values of representative cannabinoids are shown in Table 1 (Section 2.3.3.1).

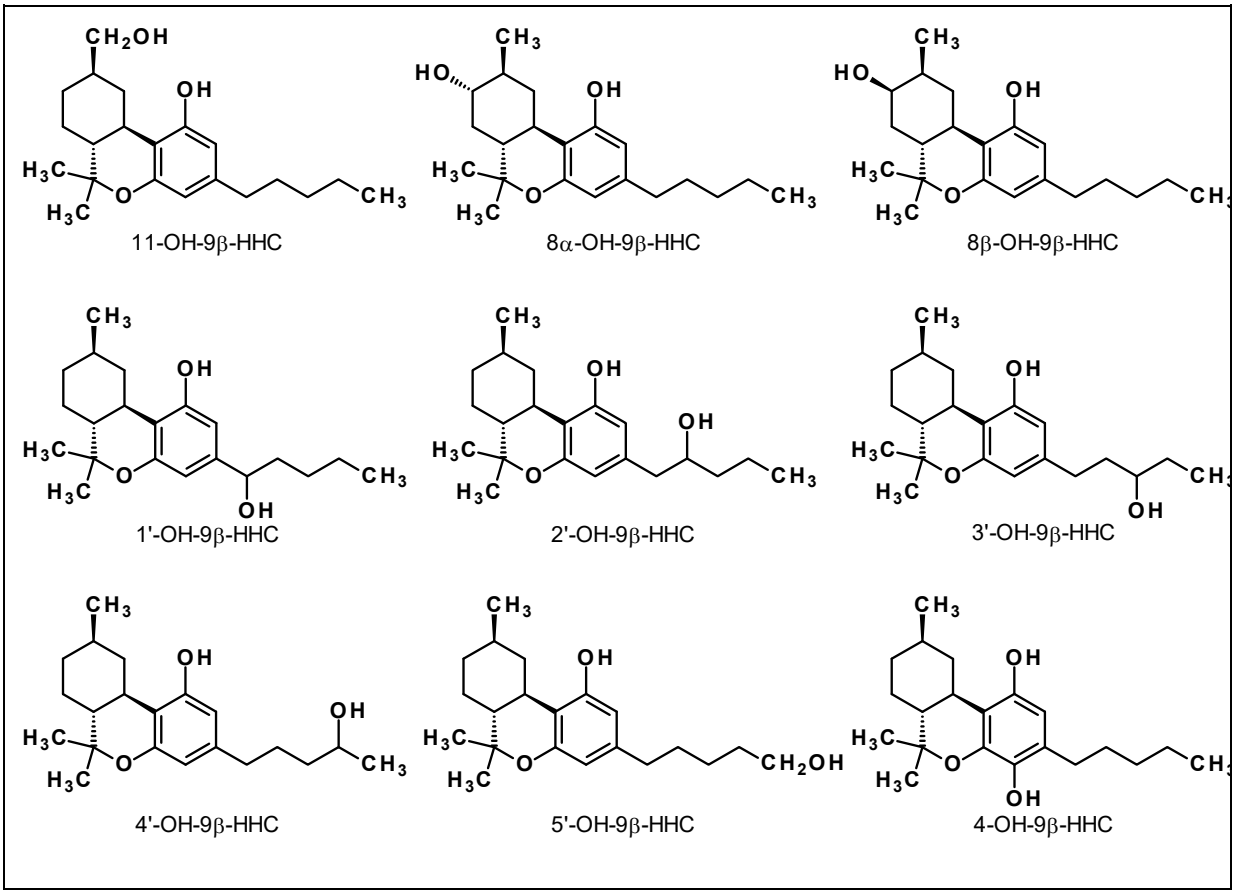
The pharmacokinetics of HHC in humans is not known.

There is no information on the pharmacokinetics of HHC in animals *in vivo*. However, the metabolism of the 9β-HHC epimer has been studied in microsomal preparations obtained from five animal species (Harvey, 1991; Harvey and Brown, 1991a; Harvey and Brown, 1991b; see also Harvey and Brown, 1991c). The hydroxylation pattern *in vitro* appeared to be broadly similar to that of Δ^9-THC even though the Δ^9 double bond was missing. Thus, the main hydroxylation sites were: 1) exocyclic C-11 methyl group, 2) C-8 methylene group, 3) *n*-pentyl side chain, and 4) C-4 of the aromatic ring. The nine monohydroxylated metabolites identified are shown in Figure 29. The species differences were as follows: in the rat, guinea pig and rabbit the 11-C hydroxylated product was the dominant metabolite, but in the mouse and hamster the CYP450-catalyzed oxidation preferred the C-8 atom with the mouse producing the 8α-OH-9β-HHC epimer in abundance while in the hamster microsomal preparation the 8β-OH-9β-HHC was the dominant epimer. The formation of a unique aromatic hydroxylation product, that is 4-OH-9β-HHC, was noted in microsomes obtained from guinea pigs, hamsters and rabbits, albeit in low amounts.

This study appears to have focused on monohydroxylated metabolites and did not report carboxylic acid species, such as 11-nor-9-COOH-HHC ([51]). It is not known whether 11-OH-HHC would undergo further oxidative biotransformation to afford a water-soluble and, presumably, non-psychoactive carboxylic acid, which is common for other cannabinoids such as CBD and Δ^9-THC and is a suitable marker for the rapid urinary detection of cannabis consumption. Though experimental evidence is lacking, it is possible that only the allylic alcohol moiety present in phytocannabinoids can undergo metabolic oxidation ([52]) while the corresponding saturated alcohol is metabolically intact. Since 11-OH-HHC appears to be nearly equipotent to THC (Skinner et al., 1979; Järbe et al., 1986), its accumulation in the body would in this case result in longer-lasting (psycho)pharmacological effect compared to THC.

FIGURE 29

Monohydroxy metabolites formed by incubation of 9β-HHC with hepatic microsomes of several animal species (Harvey, 1991; Harvey and Brown, 1991a)

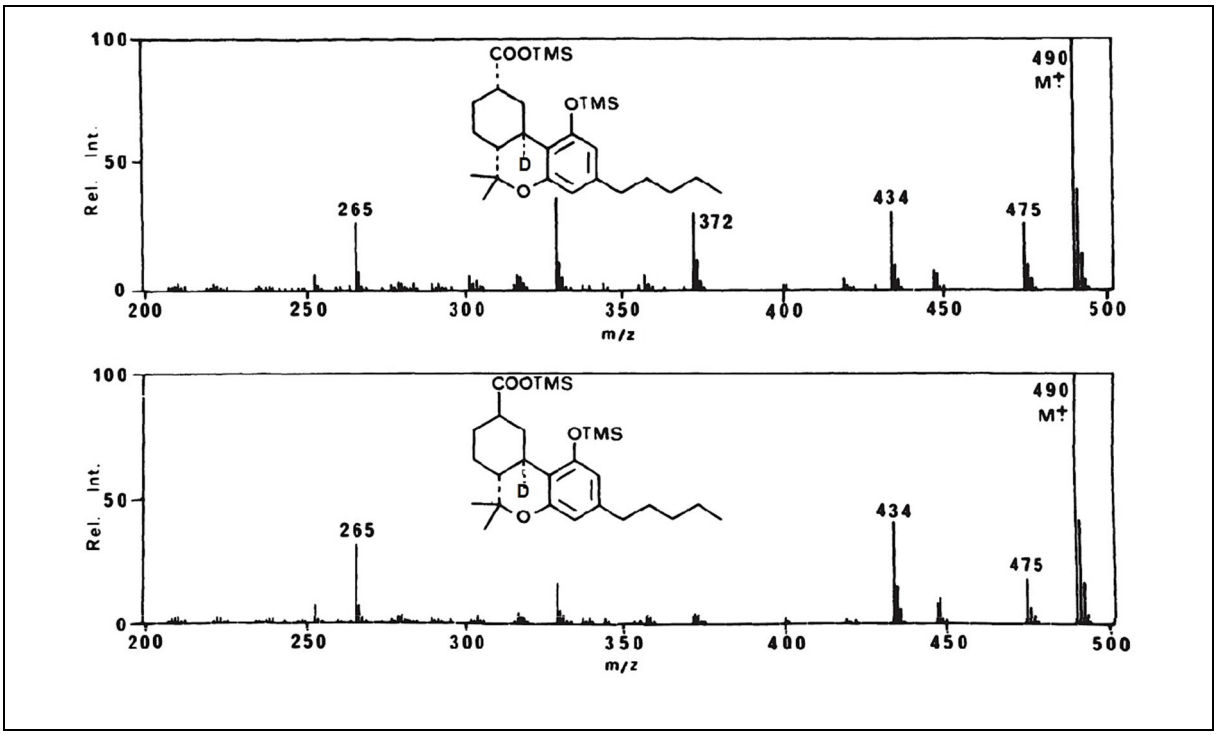


Interestingly, both the 11-OH metabolite and 11-nor-9-COOH-HHC have been detected in the mouse treated with Δ^8- or Δ^9-THC (Harvey et al., 1977) but the carboxylic acid has not

([51]) Analytical standards of the two epimeric metabolites have recently become available. https://www.caymanchem.com/product/36355/11-nor-9(r)-carboxy-hexahydrocannabinol and https://www.caymanchem.com/product/36356/11-nor-9(s)-carboxy-hexahydrocannabinol. Accessed on 7 January 2023.
([52]) The three-step oxidative conversions of Δ^9-THC and CBD into their respective carboxylic acid metabolites are mediated by cytochrome P450 enzymes, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 in particular (Watanabe et al., 2002; Sachse-Seeboth et al., 2009; Ujváry and Grotenhermen, 2014; Ujváry and Hanuš, 2016; Patilea-Vrana et al., 2019; Gasse et al., 2020).

been reported as a metabolite of HHC. Both epimers of 11-nor-9-COOH-HHC have been prepared for analytical studies (Harvey et al., 1977; Harvey and Paton, 1979; Harvey, 1981), and the electron-ionisation (EI) mass spectral fragmentations of their deuterated derivatives are somewhat different at the low ionisation energy (25 eV instead of the common 70 eV) as shown in Figure 30 (adapted from Harvey, 1992). The main difference between the fragmentation patterns of the two bis-trimethylsilylated epimers is the abundance of the *m/z* 372 fragment in the MS spectrum of the 9α epimer. This fragment is produced by the loss of the CO_2TMS group together the benzylic deuterium both in axial configuration. Such an elimination is not favoured in the 9β epimer in which the carboxy group is equatorial.

FIGURE 30
Mass spectra (EI, 25 eV) of the trimethylsilyl derivatives of synthetic metabolite-like carboxylic acid derivatives of deuterated 9α-HHC (top) and 9β-HHC epimers (bottom) (Harvey, 1992)

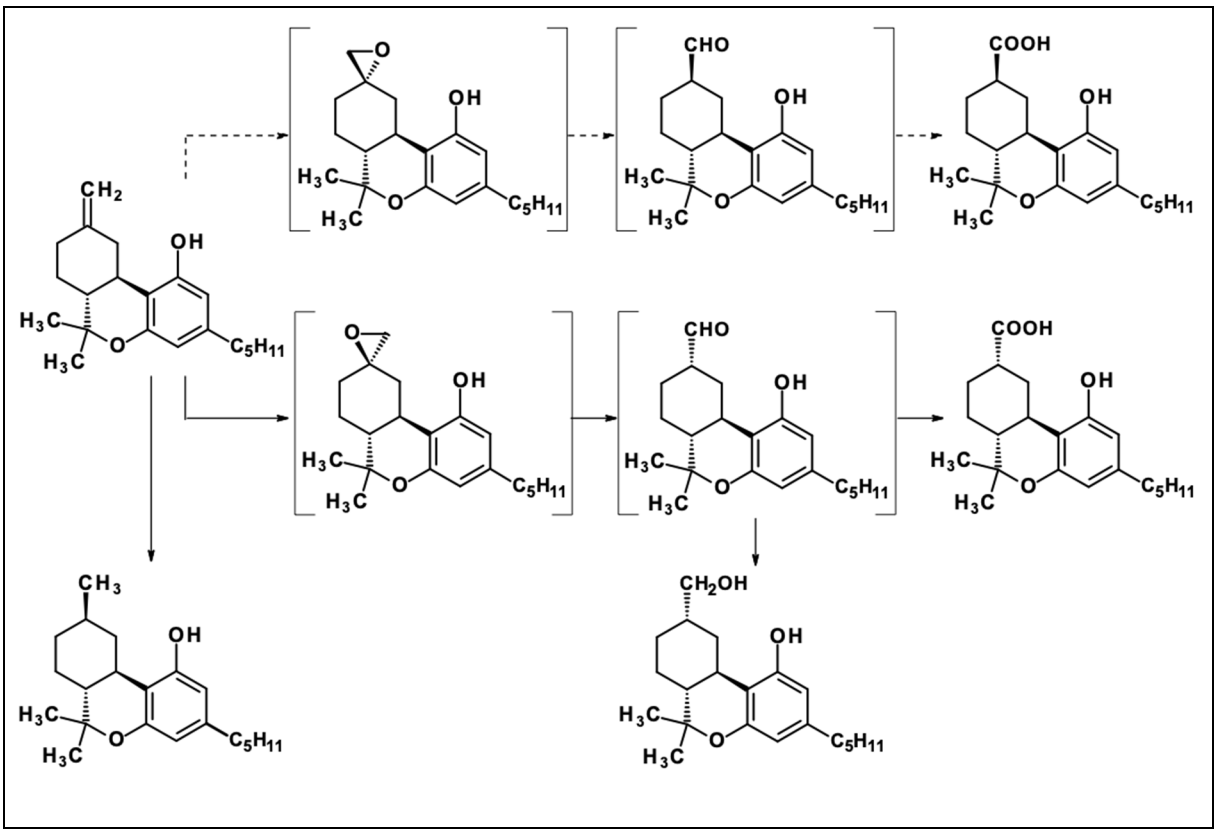


GC/MS analysis of the livers of mice treated with Δ^{11}-THC revealed 26 metabolites including the 9α epimers of 11-OH-HHC and 11-nor-9-COOH-HHC (Harvey et al., 1980). The metabolism of the parent cannabinoid to these compounds is presumed to involve sequential oxidation and reduction steps and the partial metabolic scheme is outlined in Figure 31. Oxidation of the exocyclic double bond would give epoxide isomers ([53]) which then rearrange to aldehyde intermediates (none of them are detectable as indicated by square brackets). In addition, enzymatic reduction of the exocyclic double bond would afford 9β-HHC while reduction of the transient 9α-aldehyde would lead to the corresponding 9α alcohol.

([53]) Chemical reduction diisobutylaluminum hydride of the synthetic epoxide leads to the bioactive 11-OH-HHC and 11-OH-Δ^8-THC (Razdan et al., 1973).

FIGURE 31

Metabolic routes to 9β-HHC, 11α-OH-HHC and the two 11-nor-9α/β-COOH-HHC epimers from Δ^{11}-THC in the mouse (Harvey et al., 1980). Non-detectable intermediates are shown in square brackets, major routes are shown by full arrows, minor routes by dashed arrows



The tritium-labelled, metabolite-like 11-nor-(7,8-$^3H_2$)hexahydrocannabinol-9-carboxylic acid has also been prepared to investigate adsorption phenomena to glass and plastic tools commonly used for cannabinoid immunoassay calibration (Blanc et al., 1993).

In summary, the human metabolism of HHC has not yet been studied. The limited studies on the metabolism of HHC in animal species *in vitro* and *in vivo* in of HHC indicate that the initial oxidative steps are similar to those observed for THC. However, these studies were restricted to the identification of monohydroxylated metabolites and no other Phase I or Phase II metabolites were reported. So far, there has been no evidence for the existence of the carboxylic acid metabolite 11-nor-9-COOH-HHC, which would be the hexahydro analogue of the principal carboxylic acid metabolite of THC for which several urine immunoassays have been developed. It is interesting to note, however, that in the liver of mice treated with isomeric THCs, HHC, and/or 11-nor-9-COOH-HHC were identified (Harvey et al., 1977; Harvey et al., 1980; see also Brown and Harvey, 1988; Brown and Harvey, 1991).

2.5.6 Toxicology

To date, only one study *in vitro* has examined the toxicological properties of HHC (Collins et al., 2022) (section 2.5.4).

Since CBD has been found to be a 'contaminant' in some HHC products, it is worth mentioning an ongoing debate on its transformation in the body into psychotropic THC species.

The report of CBD being converted to Δ^9-THC in artificial gastric juice *in vitro* (Watanabe et al., 2007) has generated scientific debate on whether the oral consumption of the non-psychotropic CBD would undergo such a transformation into psychotropic cannabinoids in the acidic environment of the human gut. While this controversy has not been fully resolved, it is now generally accepted that conversion of CBD to Δ^8-THC and/or Δ^9-THC *in vivo* in amount sufficient to elicit any pharmacological action is unlikely (Golombek et al., 2020).

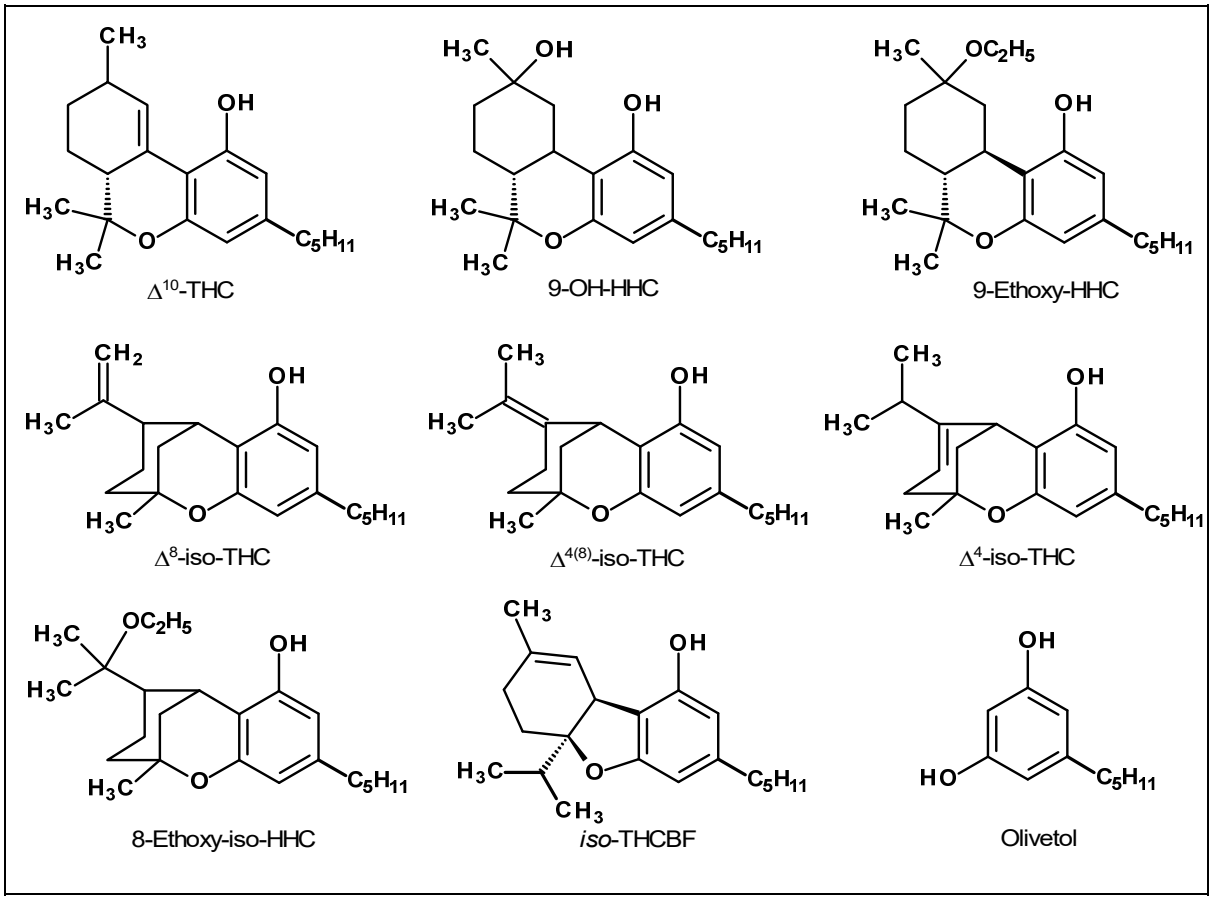
In addition, controversial observations regarding e-cigarettes or vaping tools in this regard should be noted. A recent study aimed to model e-cigarettes using pyrolysis-GC/MS observed the formation of Δ^9-THC and other cannabinoid degradants as pyrolysis products originating from CBD (Czégény et al., 2021; see also: Daniels et al., 2022). Yet, a realistic study using e-cigarettes failed to detect in the vapour condensate and no elevated THC levels were found in the smoke of low-THC cannabis joints (Hindelang et al., 2022). Building upon the role of acid catalysis for the CBD-to-THC conversion, the use of zeolite catalysts for the heterogenous cyclisation of CBD into mixtures of Δ^8-THC and Δ^9-THC have recently been patented (Adair and Geiling, 2022; Gindelberger, 2022). Ostensibly, devices equipped with such a conversion system are intended for vaping and their commercialisation may potentially raise legal issues. However, no handheld hydrogenation device for the conversion of THCs into HHC has been described.

Contaminants present in HHC products may also pose health risk. Several byproducts formed during the intramolecular cyclisation of CBD into THCs have been reported (Kiselak et al., 2020; Marzullo et al., 2020; Meehan-Atrash and Rahman, 2022; Ray et al., 2022; Takashina et al., 2022; Tsujikawa et al., 2022; Twohig et al., 2022; see also Mechoulam, 1973; Garrett et al., 1978). The occurrences and amounts of the actual byproducts appear to depend on the reaction condition, such as the acid, solvent and the temperature used for the transformation of CBD-rich hemp into Δ^8-THC. Furthermore, during the subsequent hydrogenation, the metal catalyst may also induce further transformations. Nevertheless, there is scarce information on the ingredients other than Δ^8-THC or Δ^9-THC or any residual CBD in commercial Δ^8-THC products, which are also utilised in HHC manufacture. Figure 32 depicts the chemical structures of representative byproducts arising either from acid-catalysed cyclisation studies of CBD (Mechoulam, 1973; Kiselak et al., 2020; Marzullo et al., 2020; Meehan-Atrash and Rahman, 2022; Takashina et al., 2022; Tsujikawa et al., 2022), from analysis of Δ^8-THC distillates (Twohig et al., 2022; Radwan et al., 2023), and/or from detections in 27 commercial vaping products (Meehan-Atrash and Rahman, 2022); The latter study reported also the presence of traces of heavy metals and 'silicon' in some of the products. A range of additional THC isomers as well as mono- and dihydroxylated cannabinoids have recently been observed in experiments using either sulphuric acid,

hydrochloric acid or acetic acid as catalyst for the 'isomerization' of CBD (Kiselak et al., 2020).

FIGURE 32

Representative byproducts of cannabidiol-based acid-catalysed syntheses of Δ^8-THC. Additional hydroxylated and chlorinated byproducts have also been reported in model studies (Kiselak et al., 2020)



No analytical study appears to have been reported in the scientific literature for products sold as 'HHC' ([54]). Nevertheless, when Δ^8-THC preparations are used for the manufacture of HHC, the presence of the byproducts or their derivatives formed during the catalytic hydrogenation is to be expected (for representative examples, see Figure 32). Unless the final product is adequately purified after hydrogenation, the users of contaminated HHC preparations are unwittingly expose themselves to a range of pharmacologically and toxicologically uncharacterised chemicals.

While the above contaminations detected in cannabinoid oils or vaping products are the results of chemical transformations, additional impurities may originate from plant-derived minor cannabinoids, such as cannabidivarin or tetrahydrocannabihexol, carried over from the hemp plant during extraction (see, for example Ray et al., 2022). Interestingly, some

([54]) See, however, for example, a forum discussion with analytical certificates: https://future4200.com/t/hhc-distillate-kilos-1-100-kg-hhc-1-200-hhco/158716. Accessed on 8 January 2023.

analytical certificates posted on the Internet for HHC products reported non-hydrogenated cannabinoids, such as CBD, CBG, and CBGA as minor components ([55]).

HHC is often sold in vaping products. In connection with the *E-cigarette or Vaping product use Associated Lung Injury (EVALI)* outbreak in the US in 2019–2020, a study determined by GC/MS analysis the major ingredients and additives in twelve cannabis vape oil cartridge samples obtained through the California state surveillance program from September 2018 to December 2019 (Guo et al., 2021). In addition to naturally occurring cannabinoids, such as Δ^9-THC, cannabicitran, CBN, cannabigerol and cannabivarinol that were present essentially in all liquids, HHC was found, albeit in low level, in two of the liquid as well as in the vapour generated from one of them.

2.5.7 Abuse liability and dependence producing potential

The abuse liability and dependence potential of HHC have not been studied. Data from pharmacological and behavioural experiments with several animal species as well as *in vitro* studies indicate that HHC shares the pharmacological mechanism of action of the structurally related THC suggesting that it may have abuse liability and dependence potential in humans.

2.6 Related substances

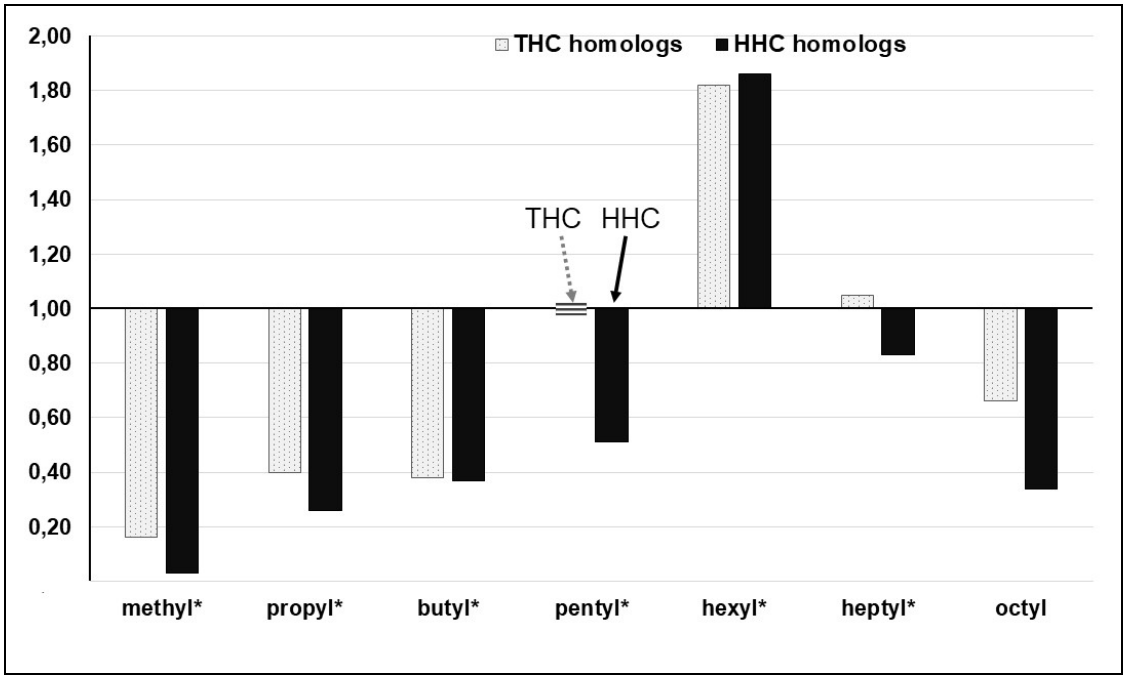
2.6.1 Homologues of HHC and other hydrogenated cannabinoid analogues of HHC

The ‘marihuana activity’ of homologous series of ‘tetrahydrocannabinol’ and hexahydrocannabinol were compared in the dog ataxia assay (Adams et al., 1942; Loewe, 1944; Loewe, 1950). In those early studies the possible variations in the actual purity, including isomer composition, of the test materials demand prudence in the interpretation of the published data. Nevertheless, based on the description of the acid-catalysed transformation of cannabidiol into two isomeric ‘tetrahydrocannabinols’ (Adams et al., 1940c) it can be inferred that the most potent substance having an optical rotation of –165º is a preparation enriched in what is now considered to be Δ^9-THC ([56]). However, the ratio of the epimers of HHC is unknown (for a discussion of the stereochemical outcome of hydrogenation under various conditions, see Section 2.3.5.1). Figure 33 depicts the activities of the two homologous series relative to the potent THC isomer. According to this comparative structure-activity relationship study, the *n*-hexyl homologues in both series are more potent than ‘tetrahydrocannabinol’ in this particular bioassay.

([55]) See, for example, https://irp.cdn-website.com/fcedb9ad/files/uploaded/thcp%20moonrocks.pdf. Accessed on 8 January 2023.
([56]) This substance must have been contaminated with Δ^8-THC the optical rotation of which in a pure form is $[a]_D$ –250º (ethanol) (Mechoulam et al., 1967). The optical rotation of Δ^9-THC isolated from hashish was reported as $[a]_D$ –150º ($CHCl_3$) (Gaoni and Mechoulam, 1964).

FIGURE 33

Biological activity of homologous 'tetrahydrocannabinols' ('THCs') and hexahydrocannabinols relative to a semi-synthetic THC preparation in the dog ataxia assay (Adams et al., 1942; Loewe, 1944; Loewe, 1950)[a]



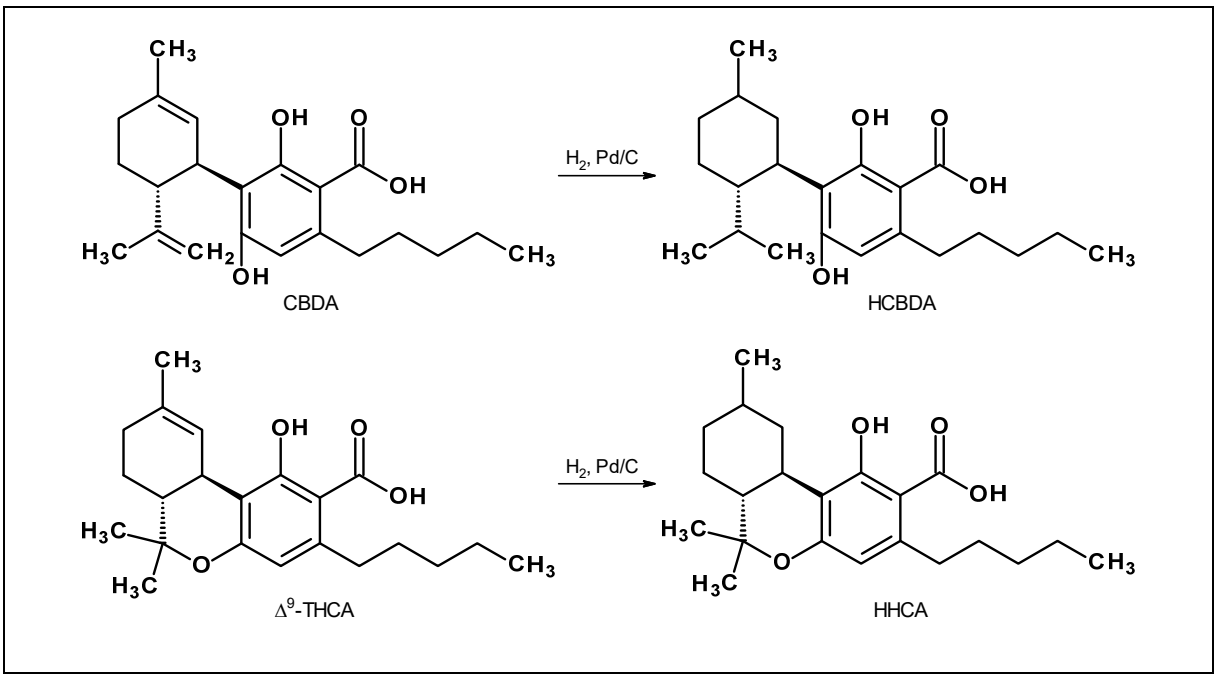
[a] In addition to 'THC' (C_5 side chain), homologous tetrahydrocannabinols marked with an asterisk (*) were subsequently isolated from various parts of *Cannabis* varieties: tetrahydocannabiorcol (C_1), tetrahydocannabivarin (C_3), tetrahydrocannabutol (C_4) tetrahydrocannabihexol (C_6) and tetrahydocannabiphorol (C7). The names for these substances reflect current terminology.

The genuine biosynthetic cannabinoids in *C. sativa* are carboxylated species (Hanuš et al., 2016) that are present in crude, unheated hemp extract. A recent patent has described the catalytic hydrogenation of such a phytocannabinoid acid mixture ([57]) to produce the corresponding hexahydro carboxylic acid species, including hexahydrocannabinolic acid (HHCA), with anti-tumour properties in mice (Figure 34) (Scialdone, 2017). Though not discussed in the patent, heat treatment of the hydrogenated mixture gives the respective decarboxylated cannabinoids, including tetrahydrocannabidiol (HCBD or H4CBD) and HHC ([58]).

([57]) There are no chemical analytical data for the hydrogenated products in the patent.
([58]) It was, however, mentioned in 2017 at a conference by M. Scialdone: https://www.youtube.com/watch?v=ngH3ONY_EI0. Accessed on 9 January 2023.

FIGURE 34

Hydrogenation of phytocannabinoid acids CBDA and THCA in unheated cannabis extract into hexahydrocannabinoic acids HCBDA and HHCA (Scialdone, 2017)



Recently, products with a brand name 'HHC-P' has been offered on the Internet by several suppliers and these allegedly contain the hexahydro derivative of tetrahydrocannabiphorol (THCP). The first identification of hexahydrocannabiphorol[59] (HHC-P) in Europe was reported to the EMCDDA by Slovenia. It was identified in a dark red/brown resinous substance that was analysed by the Customs Laboratory of Slovenia, seized in November 2022 (EMCDDA, 2023).

THCP has recently been isolated from *C. sativa*, albeit in trace amounts (Citti et al., 2019; Linciano et al., 2021). Pharmacological characterisation indicated that in terms of CB_1 receptor affinity THCP is about 30-times as potent as THC; its cannabimimetic properties in three of the tests of the mouse tetrad assay appeared to be comparable to that of THC at half-dose, that is 5 mg/kg intraperitoneally (Citti et al., 2019). The results obtained *in vivo* are similar to those obtained in the early dog ataxia assay (Figure 33) showing comparable potencies for the C_5 and C_7 THC homologues.

A recent analysis by HPLC-MS/MS of the heat-treated ('decarboxylated') flower of 14 plants of different *C. sativa* varieties found THCP content ranging from 0.0023 to 0.0136 percent by weight (0.023 to 0.136 mg/g range) with Δ^9-THC dominant chemotypes being rich in the homologue (Bueno and Greenbaum, 2021). It may be noted that distillation ([60]) of one of the plant extracts yielded fractions enriched in THCP over three-fold. Such an enriched extract could possibly be used to produce by hydrogenation the corresponding 'HHC-P'. Yet, using

([59]) 3-Heptyl-6a,7,8,9,10,10a-hexahydro-6,6,9-trimethyl-6*H*-dibenzo[*b,d*]pyran-1-ol
([60]) The distillation used a spinning band distillation equipment operating at ca. 0.04 mmHg, the vapour temperature was ca. 180 °C.

the one-step synthetic method outlined in Figure 19 could also be appealing for 'illicit' production of HHC-P. Apart from the analytical data provided to support the recent identification reported by Slovenia, no other analytical data for the marketed 'HHC-P' products are available at this time, so their actual chemical composition and the touted high potency remains to be verified by further investigations.

In a battery of screening tests in mice the 1:1 mixture of the 9α and 9β isomers of the metabolite-type 11-OH-HHC was nearly equipotent to THC (Table 6) (Skinner et al., 1979). However, in a rhesus monkey behavioural test, the 1,11-diacetyl derivative of the 11-OH-9β-HHC epimer was about one-fourth as potent as THC (Mechoulam et al., 1980). Apart from species differences and the bioassays used, this suggests that the 11-acetoxy group more resistant to hydrolysis than the acetylated phenolic moiety (see also Section 2.6.2).

The fully hydrogenated derivative of CBD, also known as H4CBD, was first prepared in 1940 by hydrogenation of cannabidiol in acetic acid using platinum oxide catalyst (Jacob and Todd, 1940). Analytical characterisations of the 1α and 1β epimers of H4CBD by NMR, HPLC and GC-MS has recently been published (Collins et al., 2023). H4CBD has been found to have moderate receptor affinity with a K_i = 143 nM (in contrast, CBD has insignificant affinity to cannabinoid receptors), and to have anti-inflammatory properties *in vitro* by suppressing the generation of reactive oxygen intermediates and nitric acid, and by suppressing tumour necrosis factor (Ben-Shabat et al., 2006).

2.6.2 Acetate esters of HHC, and representative tetrahydrocannabinol isomers

2.6.2.1 Chemistry

Cannabinoids acylated at the phenolic group do not occur naturally, they are synthetic derivatives of plant-derived compounds. Acetylation, or acylation in general, of the phenolic groups of tetrahydrocannabinols results in a slight increase in their lipophilicity relative to the parent compound, thereby facilitating penetration into the central nervous system. At the same time, the phenolic groups are, at least temporarily, protected from metabolic inactivation by conjugation or oxidation albeit relevant experimental evidence for the cannabinoids is lacking ([61]). Spontaneous or enzymatic hydrolysis by carboxylesterases of the ester liberates the free bioactive phenol (see Watanabe et al., 2005). Such esters are typically inactive *in vitro*, but serve as prodrugs for the bioactive phenolic species ([62]). Figure 35 shows the molecular structure, molecular formula, and molecular mass of representative cannabinoid acetate derivatives discussed in this section.

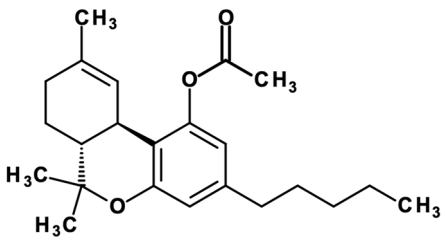
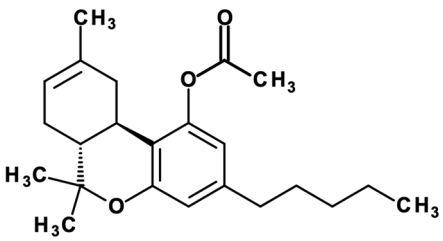
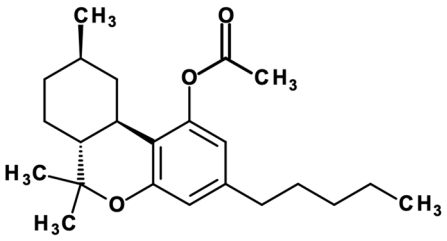
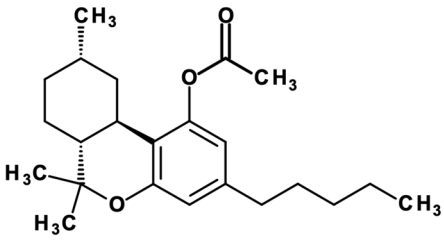
The acetate esters of Δ^8-THC and Δ^9-THC have been known for decades ([63]) (Adams et al., 1940a; Adams et al., 1945; Edery et al., 1972; Inayama et al., 1974; Petrzilka, 1975). In fact, a popular booklet on 'cannabis alchemy' provides a detailed description of the synthesis of 'THC acetate' (Hoye, 1973). The acid-catalysed isomerization of Δ^9-THC acetate (Δ^9-THC-O)

([61]) A notable exception is levanonatradol and its desacetyl derivative (Johnson and Melvin, 1986; Howlett et al., 1988).
([62]) The well-known example is diacetylmorphine, or heroin, which is a prodrug of 6-monoacetylmorphine and morphine.
([63]) The acetylated 'Red Oil', containing the crystalline acetate of cannabinol when purified, is only of historical importance (Dunstan and Henry, 1898; Wood et al., 1899).

into Δ^8-THC acetate (Δ^8-THC-O) has also been demonstrated (Gaoni and Mechoulam, 1966b; Gaoni and Mechoulam, 1968).

The first report on illicit acetylation of a cannabis extract appeared in 1988 and concerns an earlier case encountered in the Jacksonville, Florida area, US, in 1978 (Cooper, 1988). An excess of acetic acid anhydride was used for the acetylation of the extract; no forensic analysis results were given in this publication. Later, the New Zealand Police seized a dark brown/yellow oil which, after analysis by TLC and GC-MS and relying on a reference sample prepared in the laboratory, contained Δ^9-THC acetate as the major product along with cannabinol acetate, a minor component (Valentine, 1996). However, the acetylated derivative of HHC appears to have been reported only once (Wollner et al., 1942). After decades of hiatus, acetylated HHC or HHC acetate, colloquially known as 'HHC-O', emerged on the drug market possibly in late 2021.

FIGURE 35
Molecular structure, molecular formula, and molecular mass of cannabinoid acetate derivatives

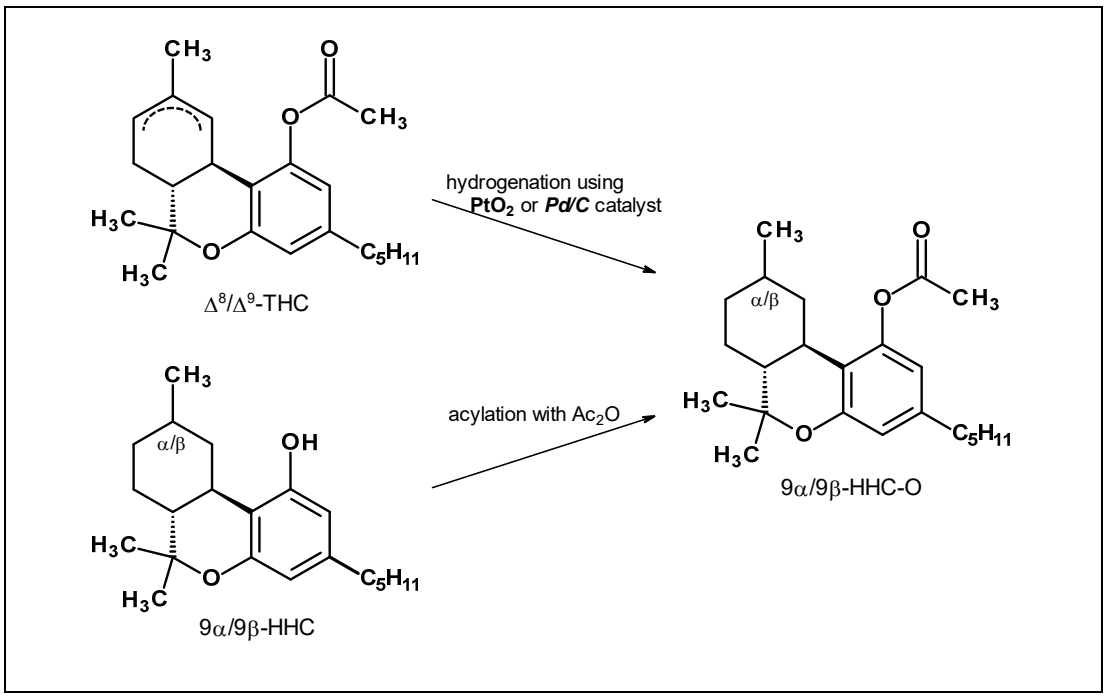
		
	Δ^9-THC-O	Δ^8-THC-O
Molecular formula	$C_{23}H_{32}O_3$	$C_{23}H_{32}O_3$
Molecular Mass	356.50	356.50
Monoisotopic mass	356.2351	356.2351
		
	9β-HHC-O	9α-HHC-O
Molecular formula	$C_{23}H_{34}O_3$	$C_{23}H_{34}O_3$
Molecular Mass	358.51	358.51

Monoisotopic mass	358.2507	358.2507

Synthesis

HHC-O can readily be synthesised either from the acetates of Δ^8-THC or Δ^9-THC, or their mixtures by catalytic hydrogenation as described (Wollner et al., 1940). It was characterised as viscous oil having an optical rotation of $[\alpha]^{21}$ –119º (Wollner et al., 1942). HHC-O can also be conveniently prepared by acylation with acetic anhydride or acetyl chloride similar to the method used for the preparation of THC acetates (Figure 36). Esters of HHC-O or any other phenolic cannabinoid acylated by a desired carboxylic acid can also be readily prepared using similar procedures.

FIGURE 36
Reported (top) and potential (bottom) synthetic routes to 9α/9β-HHC-O



Only the acetate analogues of Δ^8-THC, and Δ^9-THC, and, recently, of HHC appear to have been analytically confirmed in products offered on the drug market (but see below) ([64]).

The first identification of HHC acetate (HHC-O) on the drug market in Europe was in Hungary on the 14 August 2022 (EMCDDA, 2022d). HHC-O was identified in a seizure of 0.88 grams of green plant material. GC-MS analysis revealed that the substance contained an approximately 2:1 mixture of the two HHC-O epimers as well as large amount of CBD as the

([64]) Since December 2022, Cayman Chemical has been offering the acetate ester of (9*R*)-hexahydrocannabiphorol as analytical standard for forensic studies. https://www.caymanchem.com/product/37847/9(r)-hexahydrocannabiphorol-acetate.

three major components. Additional cannabinoids identified as minor components were Δ^9-THC, CBN, CBN-O, CBN and CBG. The presence of the phytocannabinoids Δ^9-THC, CBG and CBD in particular, all containing an unsaturated terpenoid moiety, indicate that for the low-THC herbal cannabis product was sprayed / mixed with semi-synthetic HHC-O obtained from commercial sources. Characteristic spectral data as reported by the Hungarian Institute for Forensic Sciences are given below. (Note: NMR data are for the unseparated mixture hence peak doubling at critical positions. For MS analysis, the two epimers were separated by TLC using toluene as eluent, yet their fragmentation patterns were virtually identical.)

^{1}H-NMR (400 MHz, DMSO-d_6) δ 0.85 (5'-CH_3), 0.89 and 1.06 (two epimeric 11-CH_3), 0.97 and 0.99 (two 13-CH_3) ([65]),1.26 (4'-CH_2), 1.301 and 1.306 (two 12-CH_3) ([66]), 2.24 and 2.25 (CH_3CO of the epimers), 2.43 (1'-CH_2), 6.37 (4-CH), and 6.45 (2-CH) ([67]).

GC-MS (EI): 358.3 *m/z*, 316.1 (M–CH_2CO) ([68]), 299, 273 (100%), 257, 231, 193, and 43.

Six commercially available immunoassays ([69]) developed for the urinary detection of Δ^9-THC have been tested for the detectability of 13 *O*-acetyl cannabinoids, namely the acetate derivatives of HHC ([70]), Δ^9-THC, Δ^9-THCB, Δ^9-THCH, Δ^9-THCP, exo-Δ^9-THC, Δ^8-THC, Δ^8-THCP, Δ^{10}-THC, $\Delta^{6a,10a}$-THC, CBN, or of the diacetates of CBD and CBG (Wolf et al., 2022). According to the published poster abstract: 'The six homogeneous immunoassays were not able to detect all 13 acetate analogues'. Furthermore, it was stated the Δ9-HHC-O [sic], Δ^9-THCH-O, Δ^9-THCP-O, Δ^8-THCP-O, CBN-O, and CBD-di-O could not be detected by any of the six immunoassays.

Recently, HPLC-MS/MS and GC-MS methods have been developed for the identification and quantitation of major phytocannabinoids as well as Δ^8-THC-O, Δ^9-THC-O and CBD-di-acetate for the analysis of a commercial gummy edible product labelled to contain 10 mg 'THC-O' per 100 mg per gummy. Analyses revealed that the amount of cannabinoids in one piece of gummy were 0.18 mg Δ^8-THC, 0.058 mg Δ^9-THC, 0.30 mg Δ^8-THC-O, 0.45 mg Δ^9-THC-O and 0.19 CBD-di-acetate (Holt et al., 2022). It was also noted that the concentrations of the cannabinoids were approximately 30 times larger in the outer section of the gummies than in the inner portions of the gummy. Additional ingredients identified by GC-MS in the gummy were '2-furanmethanol', L-lactic acid, 5-hydroxymethylfurfural, palmitic acid and (10*E*,12*Z*)-linoleic acid. No terpenes were found indicating that the products had been made using CBD isolate rather than full hemp extract.

2.6.2.2 Pharmacology

No data on the pharmacodynamics or pharmacokinetics HHC-O are available.

([65]) Based on previous studies (Archer et al., 1970; Stothard et al., 2022), assigned as α-methyl (I.U.).
([66]) Based on previous studies (Archer et al., 1970; Stothard et al., 2022), assigned as β-methyl (I.U.).
([67]) For numbering system, see Figure 1.
([68]) Loss of ketene fragment (–42) as observed for other cannabinoid acetates (Inayama et al., 1976; Munger et al., 2022).
([69]) Abbott Cannabinoids (enzyme immunoassay), CEDIA™ Multi-Level THC (cloned enzyme donor immunoassay), DRI® Cannabinoid Assay (enzyme immunoassay), ONLINE DAT Cannabinoid II (kinetic interaction of microparticles in a solution), LZI Cannabinoids (cTHC) Enzyme Immunoassay (enzyme immunoassay), and Syva EMIT® II Plus (enzyme immunoassay).
([70]) In the published abstract it is mentioned as 'Δ9-HHC-O', which is an obvious naming error.

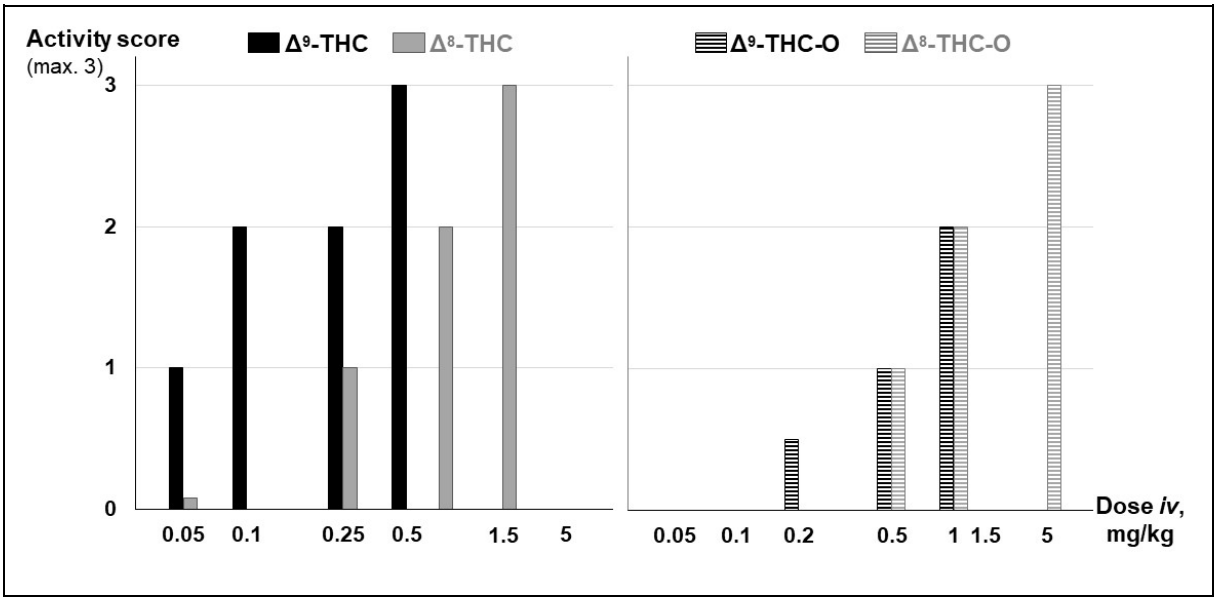
The cannabinoid-like pharmacology of the acetates of Δ^8-THC and Δ^9-THC (Δ^8-THCO and Δ^9-THC-O, respectively) has been studied both *in vitro* and *in vivo*. Only the most relevant publications are discussed next.

Early studies assessed the potency of derivatives 'tetrahydrocannabinol' preparations in the dog ataxia test and reported that the acetate of 'tetrahydrocannabinol' had about twice the potency of the parent phenolic 'tetrahydrocannabinol' (Wollner et al., 1942; Adams et al., 1945) ([71]) but the material used in these studies might have contained predominantly the semi-synthetic $\Delta^{6a,10a}$-THC isomer, which is less potent than the natural Δ^9 isomer (see Table II in Mechoulam and Edery, 1973).

In rhesus monkey behavioural assay, both isomeric THC acetate prodrugs at 1 mg/kg intravenous dose elicited behavioural changes to a similar degree as those caused by the phenolic parents, that is Δ^8-THC or Δ^9-THC at the lower 0.5–0.9 mg/kg or 0.1–0.25 mg/kg doses, respectively (Edery et al., 1971; Edery et al., 1972). Figure 37, in which the graph is based on the numerical scores in the original publications, indicates that Δ^8-THC ([72]) is about half as potent as Δ^9-THC ([73]), but this potency difference between the acetate derivatives disappears in this assay. Yet both esters are severalfold less active than their parent phenols upon intravenous administration. It was noted that the activity of the acetates was slightly delayed (10–15 minutes versus 15–30 minutes) and might be prolonged (4–4.5 hours versus 5–6 hours) suggesting that the acetates act as prodrugs which need to undergo hydrolysis to release the active phenolic species.

([71]) In spite of uncertainties regarding the actual identity of the test materials (Mechoulam and Edery, 1973), the results of these early studies have often been misleadingly quoted on the Internet.
([72]) For a comprehensive review on the pharmacology of Δ^8-THC, see Tagen and Klumpers, 2022; on the dependence potential of Δ^8-THC in the mouse, see Vanegas et al., 2022.
([73]) In a human trial, the potency of Δ^8-THC was found to be two-thirds that of Δ^9-THC either upon oral or intravenous administration (Hollister and Gillespie, 1973).

FIGURE 37
Activity of THC isomers and their acetates on rhesus monkeys scored according to Norton's sheet. The graph was constructed on published semi-quantitative data (Edery et al., 1971; Edery et al., 1972)



Finally, it should be mentioned that the known cannabidiol diacetate (Edery et al., 1972), as 'CBD-O', as well as cannabigerol diacetate, as 'CBG-O' ([74]), have recently been offered by online vendors. While the anticonvulsant properties of CBD diacetate have been demonstrated (Consroe et al., 1981), it is likely that – similar to the parent cannabidiol – it is devoid of any cannabimimetic activity.

2.6.3. Esters of related tetrahydrocannabinoids

2.6.3.1 Chemistry and pharmacology

As with the acetate esters of Δ^8-THC and Δ^9-THC, other acyl derivatives of cannabinoid phenols are expected to be prodrugs of the parent cannabinoid. The kinetics of the release of the parent phenol from its *O*-acyl derivative might be manipulated by the nature of the acylating group, albeit relevant information in the cannabinoid series is limited to a few studies ([75]).

The acetates of both Δ^8-THC and Δ^9-THC have been shown to metabolise *in vitro* by liver microsomes of several animals into the parent cannabinoid and the biotransformation is catalysed by the serine hydrolase carboxylesterases (Watanabe et al., 2005). Such hydrolysis is plausible for HHC-O as well.

([74]) Note that 'CBGO' is also an acronym for the shorter CBG homologue cannabigerorcin.
([75]) Interestingly, the 4-hydroxybutanoate derivatives of THC-type cannabinoids ('mutual prodrugs') have not been described in the literature in spite of their likely facile synthesis from GBL (gamma-butyrolactone) and a phenolic cannabinoid.

The pharmacological profile of the hemisuccinate ester of Δ^9-THC (R = $C(O)CH_2CH_2COOH$ in Figure 38) upon intravenous injection in mice was similar to that of the parent cannabinoid but it was less potent (Martin et al., 1987). It depressed spontaneous activity (SA), caused hypothermia (HT) and produced antinociception as indicated in the tail-flick (TF) assay but was 0.15, 0.30 and 0.34, respectively, as potent as Δ^9-THC, for which the ED_{50} values were 2.3 and 1,9 mg/kg for SA and TF, respectively, and caused a 1.6 ºC drop at 3 mg/kg. Conceivably, the cannabimimetic activity is due to *in vivo* hydrolysis of the ester prodrug to Δ^9-THC.

Suppository formulation of several ester derivatives, including the hemisuccinate, the methoxyacetyl derivative and a carbamate derivative of Δ^9-THC were prepared and tested in monkeys and dogs (Figure 38) (ElSohly et al., 1991).

Among several water-soluble prodrugs of Δ^9-THC esterified with piperazine- or morpholine-containing acyl moieties, the 4-morpholinobutyric acid ester (SP-111) (Figure 38) had cannabimimetic properties similar to the parent cannabinoid (Zitko et al., 1972; Järbe and McMillan, 1980).

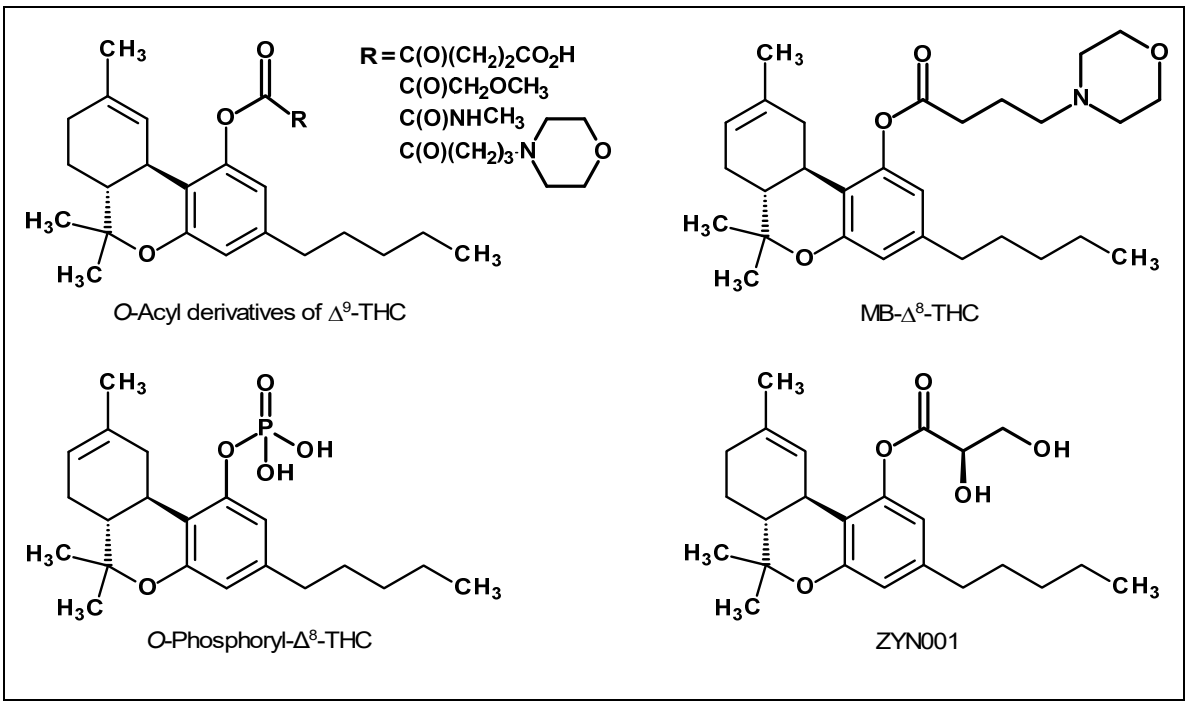
Evaluation of the cannabimimetic activity of MB-Δ^8-THC (Figure 38) in the mouse tetrad test indicated that this analogue was approximately equipotent to the phenolic Δ^8- and Δ^9-THC in reducing locomotor activity, but of intermediate potency in producing hypothermia relative to the phenolic cannabinoids; this water-soluble derivative was less potent than the phenolic cannabinoids as an antinociceptive drug in the mouse tail-flick assay (Compton and Martin, 1990).

The water-soluble phosphate ester prodrug of Δ^8-THC showed cannabimimetic activities with favourable toxicological properties in mice (Figure 38) (Yoshimura et al., 1978).

A transdermal formulation of the D-(–)-glyceric acid ester prodrug of Δ^9-THC, ZYN001 (Figure 38), was developed as potential treatment of neuropsychiatric disorders (Banks et al., 2015) but after phase 1 clinical trials its development was discontinued ([76]).

([76]) https://www.zynerba.com/zynerba-pharmaceuticals-announces-top-line-results-from-zyn001-thc-prodrug-patch-phase-1-study. Accessed on 20 December 2022.

FIGURE 38
Chemical structures of some biologically active *O*-acylated cannabinoid derivatives (for references and relevant biological activity data, see text)



2.6.3.2 Analysis

Mass spectrometry analysis of the acetyl derivatives of Δ^8-THC and Δ^9-THC as well as CBN has been reported (Inayama et al., 1976).

Radioimmunoassays of Δ^8-THC acetate using either the Roche Abuscreen® or the Abbott TDx® cannabinoid test kits did not show appreciable cross-reactivity (Jones et al., 1984; Jones et al., 1985; ElSohly et al., 1990).

2.6.3.3 Toxicology

HHC-O products are often sold in cartridges and as refill liquids intended for vaping. In 2019 US health officials reported an outbreak of e-cigarette vaping associated acute lung injury (EVALI) associated with vaping products containing vitamin E acetate (VEA) ([77]) diluent added to the active ingredients, such as nicotine, THC or synthetic cannabinoids (US CDC, 2020). By February 2020, the epidemic resulted in 2,807 hospitalisation and 68 deaths associated with EVALI in the US (Rebuli et al., 2023).

The aetiology of the serious lung injury is yet to be clarified. It has been suggested that ketene ([78]) generated upon heating VEA is the causative agents of EVALI although other

([77]) Commercial vitamin E acetate is a mixture of stereoisomeric tocopheryl acetates.
([78]) Ketene is a reactive, colourless gas with a penetrating odour; its boiling point is −56 °C (https://pubchem.ncbi.nlm.nih.gov/compound/ketene; see also: http://www.cdc.gov/niosh/idlh/463514.html. Accessed on 20 December 2022.

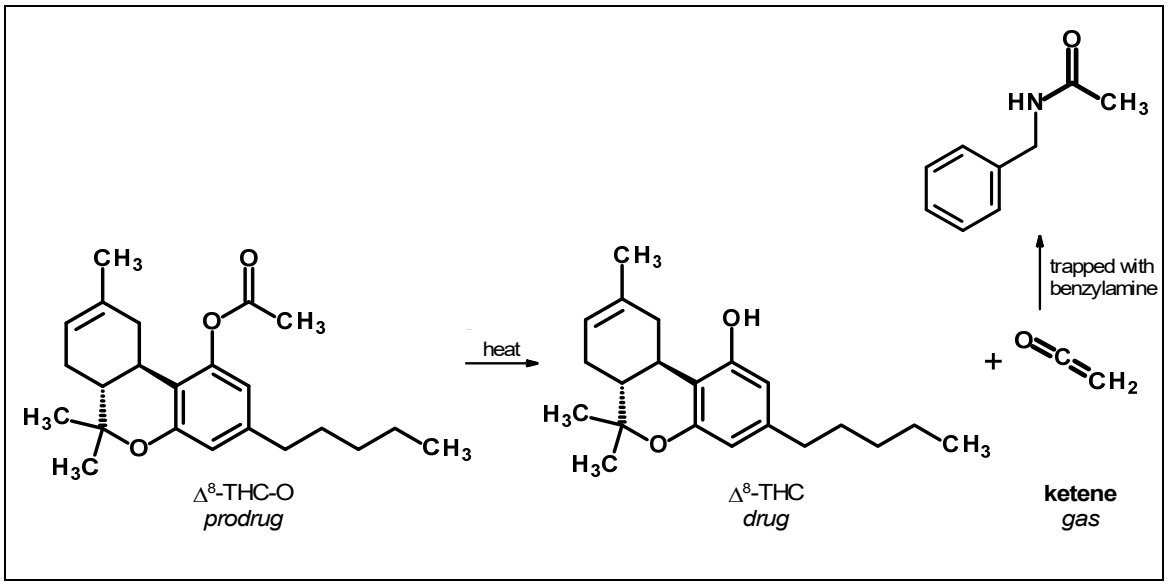
toxic or sensitising components ([79]) of the hitherto poorly characterised mixture of chemicals in the vapour could also be involved in EVALI (Narimani and da Silva, 2020; Wu and O'Shea, 2020; Feldman et al., 2021; Xantus et al., 2021; Marrocco et al., 2022).

It has been proposed that not only VEA but cannabinoid acetates may also release ketene when heated (Meehan-Atrash and Rahman, 2021; Benowitz et al., 2023). In fact, ketene formation has recently been noted in model experiments with chemically pure acetates CBN-O, Δ^8-THC-O and the diacetate of CBD (Munger et al., 2022) using the method devised for the vapour analysis of heated VEA (Wu and O'Shea, 2020). For example, the gaseous ketene released from a heated dab of Δ^8-THC acetate was trapped with benzylamine to yield *N*-benzylacetamide (Figure 39), which was readily characterised by proton NMR and GC-MS. It was estimated that this amount would correspond to 0.022 mg ketene per puff, which is below the 0.042 mg NISH IDHL ([80]) threshold value calculated from the 5.0 ppm established for occupational exposure to the gas ([81]). Similar experiments with commercial vape pen cartridges also confirmed ketene formation (Munger et al., 2022; Benowitz et al., 2023). Thus, formation of ketene is to be expected when smoking or vaping other acetylated phenolic cannabinoids, including HHC-O.

([79]) In addition to ketene, reactive oxygen species and quinonemethides, as well as other pyrolytic degradation products not originally present in the e-liquid but formed at high temperature in the vaping device may act in a synergistic manner to cause lung damage when inhaled.
([80]) For the IDLH (Immediately Dangerous to Life or Health) limit for ketene, see https://www.cdc.gov/niosh/npg/npgd0367.html. Accessed on 20 December 2022.
([81]) Based upon model experiments, at vaping temperature above 700 ºC, in-lung ketene concentration as high as 30 ppm has been predicted (Narimani and da Silva, 2020).

FIGURE 39

Ketene formation from Δ8-THC acetate and subsequent trapping with benzylamine yields N-benzylacetamide for analytical characterisation (Munger et al., 2022)



Ketene may also be similarly formed from heated heroin, which also contains a labile acetylated phenol moiety, and such a degradation can be expected when inhaling the vapours of the heated drug (‘chasing the dragon’). However, this has not been experimentally demonstrated (Klous et al., 2006; for a review on the pyrolysis of psychoactive substances, see Bell and Nida, 2015).

References

Adair, C. and Geiling, B. (2022), 'Cartridge for vapor-phase cannabinoid reactions within a device', US Patent 2022/0218653 A1 (Jul. 14, 2022).

Adams, R. (1942), 'Marihuana', *Bulletin of the New York Academy of Medicine*, 18(11), pp. 705-730.

Adams, R. (1947), 'Marihuana active compounds', US patent 2,419,937 (May 6, 1947).

Adams, R. and Carlin, R. B. (1943), 'The addition of dienes to certain di-*o*-methoxy-substituted cinnamic acids. II.' *Journal of the American Chemical Society*, 65(3), pp. 360-363 (https://doi.org/10.1021/ja01243a017).

Adams, R., Cain, C. K., McPhee, W. D. and Wearn, R. B. (1941a), 'Structure of cannabidiol. XII. Isomerization to tetrahydrocannabinols', *Journal of the American Chemical Society*, 63(8), pp. 2209-2213 (https://doi.org/10.1021/ja01853a052).

Adams, R., Loewe, S., Jelinek, C. and Wolff, H. (1941b), 'Tetrahydrocannabinol homologs with marihuana activity. IX', *Journal of the American Chemical Society*, 63(7), pp. 1971-1973 (https://doi.org/10.1021/ja01852a052)

Adams, R., Smith, C. M. and Loewe, S. (1941c), 'Tetrahydrocannabinol homologs and analogs with marihuana activity. X', *Journal of the American Chemical Society*, 63(7), pp. 1973-1976 (https://doi.org/10.1021/ja01852a053).

Adams, R., Pease, D. C., Cain, C. K. and Clark, J. H. (1940a), 'Structure of cannabidiol. VI. Isomerization of cannabidiol to tetrahydrocannabinol, a physiologically active product. Conversion of cannabidiol to cannabinol', *Journal of the American Chemical Society*, 62(9), pp. 2402-2405 (https://doi.org/10.1021/ja01866a0407).

Adams, R., Pease, D. C., Cain, C. K., Baker, B. R., Clark, J. H., Wolff, H. and Wearn, R. B. (1940b), 'Conversion of cannabidiol to a product with marihuana activity. A type reaction for synthesis of analogous substances. Conversion of cannabidiol to cannabinol', *Journal of the American Chemical Society*, 62(8), pp. 2245-2246 (https://doi.org/10.1021/ja01865a508).

Adams, R., Loewe, S., Pease, D. C., Cain, C. K., Wearn, R. B., Baker, R. B. and Wolff, H. (1940c), 'Structure of cannabidiol. VIII. Position of the double bonds in cannabidiol. Marihuana activity of tetrahydrocannabinols', *Journal of the American Chemical Society*, 62(9), pp. 2566-2567 (https://doi.org/10.1021/ja01866a510).

Adams, R., Loewe, S., Smith, C. M. and McPhee, W. D. (1942), 'Tetrahydrocannabinol homologs and analogs with marihuana activity. XIII', *Journal of the American Chemical Society*, 64(3), pp. 694-697 (https://doi.org/10.1021/ja01255a061).

Adams, R., Chen, K. H. and Loewe, S. (1945), 'Tetrahydrocannabinol homologs with a *s*-alkyl group in the 3-position. XVI', *Journal of the American Chemical Society*, 67(9), pp. 1534-1537 (https://doi.org/10.1021/ja01225a040).

Andersson, D. A., Gentry, C., Alenmyr, L., Killander, D., Lewis. S. E., Andersson, A., Bucher, B., Galzi, J.-L., Sterner, O., Bevan, S., Högestätt, E. D. and Zygmunt, P. M. (2011), 'TRPA1 mediates spinal antinociception induced by acetaminophen and the cannabinoid Δ^9-tetrahydrocannabiorcol', *Nature Communications*, 2, 551 (https://doi.org/10.1038/ncomms1559).

Archer, R. A., Boyd, D. B., Demarco, P. V., Tyminski, I. J. and Allinger, N. L. (1970), 'Structural studies of cannabinoids. A theoretical and proton magnetic resonance analysis', *Journal of the American Chemical Society*, 92(17), pp. 5200-5206 (https://doi.org/10.1021/ja00720a033).

Archer, R. A., Stark, P. and Lemberger, L. (1986), 'Nabilone', in: *Cannabinoids as therapeutic agents*. ed. R. Mechoulam. CRC Press, Inc., Boca Raton, Florida, pp. 85-103.

Arnone, A., Merlini, L. and Servi, S. (1975a), 'Hashish. Synthesis of (+)-Δ^4-tetrahydrocannabinol', *Tetrahedron*, 31(24), pp. 3093-3096 (https://doi.org/10.1016/0040-4020(75)80154-2).

Arnone, A., Bernardi, R., Merlini, L. and Servi, S. (1975b), 'Spectroscopic methods for distinguishing tricyclic cannabinoids from "abnormal" synthetic isomers', *Gazzetta Chimica Italiana*, 105(9-10), pp. 1127-1131.

Aviz-Amador, A., Contreras-Puentes, N. and Mercado-Camargo, J. (2021), 'Virtual screening using docking and molecular dynamics of cannabinoid analogs against CB_1 and CB_2 receptors', *Computational Biology and Chemistry*, 95, pp. 107590 (https://doi.org/10.1016/j.compbiolchem.2021.107590).

Banks, S., O'Neill, C. and Sebree, T. (2015). 'Pharmacokinetic evaluation of subcutaneously administered ZYN001 in male Sprague-Dawley rats', Poster #8 presented at PAINWeek 2015. 8–12 September, 2015, Las Vegas, NV. https://www.zynerba.com/wp-content/uploads/2015/09/PK-Evaluation-of-ZYN001.pdf, accessed on 20 December 2022.

Basas-Jaumandreu, J. and de las Heras, F. X. C. (2020), 'GC-MS metabolite profile and identification of unusual homologous cannabinoids in high potency *Cannabis sativa*', *Planta Medica*, 86(5), pp. 338-347 (https://doi.org/10.1055/a-1110-1045).

Bell, S. and Nida, C. (2015), 'Pyrolysis of drugs of abuse: a comprehensive review', *Drug Testing and Analysis*, 7(6), pp. 445-456 (https://doi.org/10.1002/dta1794).

Ben-Shabat, S., Hanuš, L. O., Katzavian, G. and Gallily, R. (2006), 'New cannabidiol derivatives: synthesis, binding to cannabinoid receptor, and evaluation of their antiinflammatory activity', *Journal of Medicinal Chemistry*, 49(3), pp. 1113-1117 (https://doi.org/10.1021/jm050709m).

Benowitz, N. L., Havel, C., Jacob, P., O'Shea, D. F., Wu, D. and Fowles, J. (2023), 'Vaping THC-O acetate: Potential for another EVALI epidemic', *Journal of Medical Toxicology*, 19(1), pp. 37-39 (https://doi.org/10.1007/s13181-022-00921-3).

Blanc, J. A., Manneh, V. A., Ernst, R., Berger, D. E., de Keczer, S. A., Chase, C., Centofanti, J. M. and DeLizza, A. J. (1993), 'Adsorption losses from urine-based cannabinoid calibrators during routine use', *Clinical Chemistry*, 39(8), pp. 1705–1712 (https://doi.org/10.1093/clinchem/39.8.1705).

Boeren, E. G., Elsohly, M. A. and Turner, C. E. (1979), 'Cannabiripsol: A novel *Cannabis* constituent', *Experientia*, 35(10), pp. 1278-1279 (https://doi.org/10.1007/BF01963954).

Brown, N. K. and Harvey, D. J. (1988), '*In vivo* metabolism of the methyl homologues of delta-8-tetrahydrocannabinol, delta-9-tetrahydrocannabinol and delta-8-tetrahydrocannabinol in the mouse', *Biomedical and Environmental Mass Spectrometry*;15(7), pp. 389-398 (https://doi.org/10.1002/bms.1200150706).

Brown, N. K. and Harvey, D. J. (1991), '*In vivo* metabolism of the ethyl homologues of delta-8-tetrahydrocannabinol and delta-9-tetrahydrocannabinol in the mouse', *Biological Mass Spectrometry*, 20(5), pp. 324-328 (https://doi.org/10.1002/bms.1200200514).

Budzikiewicz, H., Alpin, R. T., Lightner, D. A., Djerassi, C., Mechoulam, R. and Gaoni, Y. (1965), 'Massenspektroskopie und ihre Anwendung auf strukturelle und stereochemische Probleme–LXVIII. Massenspektroskopische Untersuchung der Inhaltstoffe von Haschisch', *Tetrahedron*, 21(7), pp. 1881-1888 (https://doi.org/10.1016/0040-4020(01)98657-0).

Bueno, J. and Greenbaum, E. A. (2021), '(−)-*trans*-Δ^9-Tetrahydrocannabiphorol content of *Cannabis sativa* inflorescence from various chemotypes', *Journal of Natural Products*, 84(2), pp. 531-536 (https://doi.org/10.1021/acs.jnatprod.0c01034).

Cahn, R. S. (1933), '*Cannabis indica* resin. Part IV. The synthesis of some 2:2-dimethyldibenzopyrans, and confirmation of the structure of cannabinol', *Journal of the Chemical Society*, pp. 1400-1405 (https://doi.org/10.1039/JR9330001400).

CANN - Cannabis Chemistry Subdivision of the American Chemical Society (2021), 'A delta-8 discussion', July 29, 2021, https://www.youtube.com/watch?v=rdbQXLfKREg, accessed on 20 December 2022.

Casati, S., Rota, P., Bergamaschi, R. F., Palmisano, E., La Rocca, P., Ravelli, A., Angeli, I., Minoli, M., Roda, G. and Orioli, M. (2022), 'Hexahydrocannabinol on the light cannabis market: the latest "new" entry', *Cannabis and Cannabinoid Research*, Online ahead of print (https://doi.org/10.1089/can.2022.0253).

Casiraghi, G., Cornia, M., Casnati, G., Gasparri Fava, G. and Ferrari Belicchi, M. (1986), 'A one-step highly stereocontrolled synthesis of (–)- and (+)-hexahydrocannabinol and related compounds', *Journal of the Chemical Society, Chemical Communications*, (3), pp. 271-273 (https://doi.org/10.1039/C39860000271).

Cayman Chemicals (2022a), 'Cayman Spectral Library: 9(R)-Hexahydrocannabinol', https://cdn.caymanchem.com/cdn/gcms/27500-0646274-GCMS.pdf, accessed on 20 December 2022.

Cayman Chemicals (2022b), 'Cayman Spectral Library: 9(R)-Hexahydrocannabinol'. https://cdn.caymanchem.com/cdn/gcms/27501-0645988-GCMS.pdf, accessed on 20 December 2022.

Cherney, J. H. and Small, E. (2016), 'Industrial hemp in North America: production, politics and potential', *Agronomy*, 6(4), 58 (https://doi.org/10.3390/agronomy6040058).

Citti, C., Linciano, P., Russo, F., Luongo, L., Iannotta, M., Maione, S., Laganá, L., Capriotti, A. L., Forni, F., Vandelli, M. A., Gigli, G. and Cannazza, G. (2019), 'A novel phytocannabinoid isolated from *Cannabis sativa* L. with an *in vivo* cannabimimetic activity higher than Δ^9-tetrahydrocannabinol: Δ^9-tetrahydrocannabiphorol', *Scientific Reports*, 9, pp. 20335 (https://doi.org/10.1038/s41598-019-56785-1).

Claussen, U. and Korte, F. (1966a), 'Haschisch XII. Die Struktur zweier synthetischer Isomerer des Tetrahydrocannabinol', *Zeitschrift für Naturforschung*, 21B(6), pp. 594-595. (https://doi.org/10.1515/znb-1966-0626).

Claussen, U. and Korte, F. (1966b), 'Herkunft, Wirkung und Synthese der Inhaltsstoffe des Haschisch', *Die Naturwissenschaften*, 53(21), pp. 541-546. (https://doi.org/10.1007/BF00602952).

Collins, A. C., Tesfatsion, T. T., Ramirez, G. A., Ray, K. P. and Cruces, W. (2022), 'Nonclinical in vitro safety assessment summary of hemp derived (R/S)-hexahydrocannabinol ((R/S)-HHC)', *Cannabis Science and Technology*, 5(7), pp. 23-27 (https://doi.org/10.21203/rs.3.rs-2299264/v1).

Collins, A., Ramirez, G., Tesfatsion, T., Ray, K. P., Caudill, S., Cruces, W. (2023), 'Synthesis and characterization of the diastereomers of HHC and H4CBD', *Natural Product Communications*, 18(3). (https://doi.org/10.1177/1934578X231158910).

Compton, D. R. and Martin, B. R. (1990), 'Pharmacological evaluation of water soluble cannabinoids and related analogs', *Life Sciences*, 46(22), pp. 1575-1785 (https://doi.org/10.1016/0024-3205(90)90391-4).

Compton, D. R., Johnson, M. R., Melvin, L. S. and Martin, B. R. (1992), 'Pharmacological profile of a series of bicyclic cannabinoid analogs: classification as cannabimimetic agents', *The Journal of Pharmacology and Experimental Therapeutics*, 260(1), pp. 201-209 (https://jpet.aspetjournals.org/content/260/1/201.long).

Consroe, P., Martin, A. and Singh, V. (1981), 'Antiepileptic potential of cannabidiol analogs', *Journal of Clinical Pharmacology*, 21(Supplement), pp. 428S-436S (https://doi.org/10.1002/j.1552-4604.1981.tb02623.x).

Consroe, P., Martin, A. R. and Schneiderman Fish, B. (1982), 'Use of a potential rabbit model for structure–behavioral activity studies of cannabinoids', *Journal of Medicinal Chemistry*, 25(5), pp. 596-599 (https://doi.org/10.1021/jm00347a021).

Cook, C. E., Hawes, M. L., Amerson, E. W., Pitt, C. G. and Williams, D. (1976), 'Radioimmunoassay of Δ^9-tetrahydrocannabinol', *NIDA Research Monograph*, 7, pp. 15-27.

Cooper, D. A. (1988), 'Future synthetic drugs of abuse', in: *Proceedings of the International Symposium on the Forensic Aspects of Controlled Substances*, March 28-April 1, 1988, Quantico, Virginia, The Laboratory Division, Federal Bureau of Investigation, US Department of Justice, Washington, D. C., pp. 79-103 (https://catalog.hathitrust.org/Record/002444757).

Cornia, M., Casiraghi, G., Casnati, G. and Zetta, L. (1989), 'A concise stereocontrolled route to hexahydrocannabinol and relatives', *Gazzetta Chimica Italiana*, 119(6), pp. 329-333.

Czégény, Z., Nagy, G., Babinszki, B., Bajtel, Á., Sebestyén, Z., Kiss, T., Csupor-Löffler, B., Tóth, B. and Csupor, D. (2021), 'CBD, a precursor of THC in e-cigarettes', *Scientific Reports*, 11, pp. 8951 (https://doi.org/10.1038/s41598-021-88389-z).

Daniels, R., Yassin, O. A., Toribio, J. M., Gascón, J. A. and Sotzing, G. (2022), 'Re-examining cannabidiol: conversion to tetrahydrocannabinol using only heat', *Cannabis and Cannabinoid Research* (https://doi.org/10.1089/can.2022.0235).

Dunstan, W. R. and Henry, T. A. (1898), 'On oxycannabin from Indian hemp', *Proceedings of the Chemical Society*, (189), 44-45 (https://doi.org/10,1039/PL8981400033).

Edery, H., Grunfeld, Y., Ben-Zvi, Z. and Mechoulam, R. (1971), 'Structural requirements for cannabinoid activity', *Annals of the New York Academy of Sciences*, 191, pp. 40-53 (https://doi.org/10.1111/j.1749-6632.1971.tb13985.x).

Edery, H., Grunfeld, Y., Porath, G., Ben-Zvi, Z., Shani, A. and Mechoulam, R. (1972), 'Structure-activity relationships in the tetrahydrocannabinol series: Modifications on the aromatic ring and in the side-chain', *Arzneimittelforschung*, 22(11), pp. 1995-2003.

ElSohly, M. A., Harland, E. C., Benigni, D. A. and Waller, C. W. (1984), 'Cannabinoids in glaucoma II: The effect of different cannabinoids on intraocular pressure of the rabbit', *Current Eye Research*, 3(6), pp. 841-850 (https://doi.org/10.3109/02713688409000797).

ElSohly, M. A., Jones, A. B. and ElSohly, H. N. (1990), 'Cross-reactivity of selected compounds in the Abbott TDx® cannabinoid assay', *Journal of Analytical Toxicology*, 14(5), pp. 277-279 (https://doi.org/10.1093/jat/14.5.277).

ElSohly, M. A., Little, T. L., Jr, Hikal, A., Harland, E., Stanford, D. F. and Walker, L. (1991), 'Rectal bioavailability of delta-9-tetrahydrocannabinol from various esters', *Pharrnacology Biochemistry & Behavior*, 40(3), pp. 497-502 (https://doi.org/10.1016/0091-3057(91)90353-4).

EMCDDA (European Monitoring Centre for Drugs and Drug Addiction) (2009), *Understanding the 'Spice' phenomenon*, Office for Official Publications of the European Communities, Luxembourg. https://www.emcdda.europa.eu/publications/thematic-papers/understanding-spice-phenomenon_en

EMCDDA (2019a), *Guidance note 6: Intensive monitoring. EMCDDA operating guidelines for the European Union Early Warning System on new psychoactive substances*, EMCDDA, Lisbon. https://www.emcdda.europa.eu/publications/guidelines/operating-guidelines-for-the-european-union-early-warning-system-on-new-psychoactive-substances_en

EMCDDA (2019b), *Guidance note 2: Formal notification of a new psychoactive substance. EMCDDA operating guidelines for the European Union Early Warning System on new psychoactive substances*, EMCDDA, Lisbon. https://www.emcdda.europa.eu/publications/guidelines/operating-guidelines-for-the-european-union-early-warning-system-on-new-psychoactive-substances_en

EMCDDA (2020), *Low-THC cannabis products in Europe*, Publications Office of the European Union, Luxembourg. https://www.emcdda.europa.eu/publications/ad-hoc-publication/low-thc-cannabis-products-europe_en

EMCDDA (2022a), *EMCDDA technical expert meeting on hexahydrocannabinol (HHC) and related cannabinoids*, EMCDDA, Lisbon, 19 December 2022. https://www.emcdda.europa.eu/news/2022/emcdda-technical-expert-meeting-hexahydrocannabinol-hhc-and-related-cannabinoids_en

EMCDDA (2022b), *New psychoactive substances: 25 years of early warning and response in Europe — an update from the EU Early Warning System*, Publications Office of the European Union, Luxembourg. https://www.emcdda.europa.eu/publications/rapid-communication/update-eu-early-warning-system-2022_en

EMCDDA (2022c), *Formal notification of 6a,7,8,9,10,10a-hexahydro-6,6,9-trimethyl-3-pentyl-6H[1]dibenzo[b,d]pyran-1-ol (hexahydrocannabinol; HHC) by Denmark as a new psychoactive substance under the terms of Regulation (EC) No 1920/2006 and Council Framework Decision 2004/757/JHA. EU-EWS-RCS-FN-2022-0031*, EMCDDA, Lisbon, 21 October 2022.

EMCDDA (2022d), *Formal notification of (6,6,9-trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydrobenzo[c]chromen-1-yl) acetate (hexahydrocannabinol acetate; HHC acetate) by Hungary as a new psychoactive substance under the terms of Regulation (EC) No 1920/2006 and Council Framework Decision 2004/757/JHA. EU-EWS-RCS-FN-2022-0035*, EMCDDA Lisbon, 5 December 2022.

EMCDDA (2023), *Formal notification of 3-heptyl-6a,7,8,9,10,10a-hexahydro-6,6,9-trimethyl-6H- dibenzo[b,d]pyran-1-ol (hexahydrocannabiphorol; HHC-P) by Slovenia as a new psychoactive substance under the terms of Regulation (EC) No 1920/2006 and Council Framework Decision 2004/757/JHA. EU-EWS-RCS-FN-2023-0001*, EMCDDA, Lisbon, 6 January 2023.

Erickson, B. E. (2021), 'Delta-8-THC craze concerns chemists', *Chemical and Engineering News*, 99(31), pp. 24-28 (https://cen.acs.org/biological-chemistry/natural-products/Delta-8-THC-craze-concerns/99/i31).

Evans-Brown, M., Hughes, B., and Sedefov, R. (2021), 'Legal classification and international systems for monitoring and responding to novel psychoactive substances', in Dargan, P., and Wood, D., (eds.) *Novel psychoactive substances: classification, pharmacology and toxicology*, pp. 3-56 (https://doi.org/10.1016/B978-0-12-818788-3.00016-4).

Feldman, R., Stanton, M. and Suelzer, E. M. (2021), 'Compiling evidence for EVALI: a scoping review of in vivo pulmonary effects after inhaling vitamin E or vitamin E acetate', *Journal of Medical Toxicology*, 17(3), pp. 278-288 (https://doi.org/10.1007/s13181-021-00823-w).

Fenimore, D. C., Freeman, R. R. and Loy, P. R. (1973), 'Determination of Δ^9-tetrahydrocannabinol in blood by electron capture gas chromatography', *Analytical Chemistry*, 45(14), pp. 2331-2335 (https://doi.org/10.1021/ac60336a041).

Focella, A., Teitel, S. and Brossi, A. (1977), 'A simple and practical synthesis of olivetol', *Journal of Organic Chemistry*, 42(21), pp. 3456-3457 (https://doi.org/10.1021/jo00441a036. Correction: *idem ibid* (1978) 43(26), pp. 5027 (https://doi.org/10.1021/jo00420a612).

Franco, C., Protti, S., Porta, A., Pollastro, F., Profumo, A., Mannucci, B. and Merli, D. (2022), 'Stability of cannabidiol (CBD) in solvents and formulations: A GC-MS approach', *Results in Chemistry*, 4, pp. 100465 (https://doi.org/10.1016/j.reschem.2022.100465).

Fruth J. (2022), 'The state of delta 8', *Cannabis Law Journal*. https://journal.cannabislaw.report/alexander-hymowitz-the-state-of-delta-8/, accessed 14 February 2023.

Gaoni, Y. and Mechoulam, R. (1964), 'Isolation, structure, and partial synthesis of an active constituent of hashish', *Journal of the American Chemical Society*, 86(8), pp. 1646-1647 (https://doi.org/10.1021/ja01062a046).

Gaoni, Y. and Mechoulam, R. (1966a), 'Hashish–VII. The isomerization of cannabidiol to tetrahydrocannabinols', *Tetrahedron*, 22(4), pp. 1481-1488 (https://doi.org/10.1016/S0040-4020(01)99446-3).

Gaoni, Y. and Mechoulam, R. (1966b), 'Concerning the isomerization of Δ^1- to $\Delta^{1(6)}$-tetrahydrocannabinol', *Journal of the American Chemical Society*, 88(23), pp. 5673-5675 (https://doi.org/10.1021/ja00975a071).

Gaoni, Y. and Mechoulam, R. (1968), 'The *iso*-tetrahydrocannabinols', *Israel Journal of Chemistry*, 6(5), pp. 679-690 (https://doi.org/10.1002/ijch.196800086).

Garrett, E. R., Gouyette, A. J. and Roseboom, H. (1978), 'Stability of tetrahydrocannabinols II', *Journal of Pharmaceutical Sciences*, 67(1), pp. 27-32 (https://doi.org/10.1002/jps.2600670108).

Gasse, A., Vennemann, M., Köhler, H. and Schürenkamp, J. (2020), 'Toxicogenetic analysis of Δ9-THC-metabolizing enzymes', *International Journal of Legal Medicine*, 134(6), pp. 2095-2103 (https://doi.org/10.1007/s00414-020-02380-3).

Geci, M., Scialdone, M. and Tishler, J. (2022), 'The dark side of cannabidiol: the unanticipated social and clinical implications of synthetic Δ^8-THC', *Cannabis and Cannabinoid Research*, Online ahead of print (https://doi.org/10.1089/can.2022.0126).

Ghosh, R., Todd, A. R. and Wilkinson, S. (1940a), '*Cannabis indica*. Part IV. The synthesis of some tetrahydrodibenzopyran derivatives', *Journal of the Chemical Society*, pp. 1121-1125 (https://doi.org/10.1039/JR9400001121).

Ghosh, R., Todd, A. R. and Wilkinson, S. (1940b), '*Cannabis indica*. Part V. The synthesis of cannabinol', *Journal of the Chemical Society*, pp. 1393-1396 (https://doi.org/10.1039/JR9400001393).

Gindelberger, D. (2022), 'Zeolite catalyst and method for preparation of aromatic tricyclic pyrans', US Patent 11,352,337 (Jun. 7, 2022).

Giroud, C., Ménétrey, A., Augsburger, M., Buclin, T., Sanchez-Mazas, P. and Mangin, P. (2001), 'Δ^9-THC, 11-OH-Δ^9-THC and Δ^9-THCCOOH plasma or serum to whole blood concentrations distribution ratios in blood samples taken from living and dead people', *Forensic Science International*, 123, pp. 159-164 (https://doi.org/10.1016/s0379-0738(01)00538-2).

Golombek, P., Müller, M., Barthlott, I., Sproll, C. and Lachenmeier, D. W. (2020), 'Conversion of cannabidiol (CBD) into psychotropic cannabinoids including tetrahydrocannabinol (THC): A controversy in the scientific literature', *Toxics*, 8, Article No. 41 (https://doi.org/10.3390/toxics8020041).

Guo, W., Vrdoljak, G., Liao, V.-C. and Moezzi, B. (2021), 'Major constituents of cannabis vape oil liquid, vapor and aerosol in California vape oil cartridge samples', *Frontiers in Chemistry*, 9(June), pp. 694905 (https://doi.org/10.3389/fchem.2021.694905).

Hanuš, L. O., Meyer, S. M., Muñoz, E., Taglialatela-Scafati, O. and Appendino, G. (2016), 'Phytocannabinoids: a unified critical inventory', *Natural Products Reports*, 33(12), pp. 1357-1392 (https://doi.org/10.1039/c6np00074f).

Harvey, D. J. (1981), 'The mass spectra of the trimethylsilyl derivatives of *cis*- and *trans*-hexahydrocannabinol and their hydroxy and acid analogues', *Biomedical Mass Spectrometry*, 8(8), pp. 366-372 (https://doi.org/10.1002/bms.1200080810).

Harvey, D. J. (1991), 'Metabolism and pharmacokinetics of the cannabinoids', in: *Biochemistry and physiology of substance abuse*. ed. R. R. Watson. CRC Press, Boca Raton, pp. 279-365.

Harvey, D. J. (1992), 'Cannabinoids', in: *Mass spectrometry: clinical and biomedical applications*. ed. D. M. Desiderio. Plenum Press, New York, Volume 1, pp. 207-257 (https://doi.org/10.1007/978-1-4899-1173-5_6).

Harvey, D. J. and Brown, N. K. (1990), 'A method based on catalytic hydrogenation for the identification of monohydroxy metabolites of isomeric tetrahydrocannabinols', *Rapid*

Communications in Mass Spectrometry, 4(2), pp. 67-68 (https://doi.org/10.1002/rcm.1290040209).

Harvey, D. J. and Brown, N. K. (1991a), '*In vitro* metabolism of the equatorial C_{11}-methyl isomer of hexahydrocannabinol in several mammalian species', *Drug Metabolism and Disposition*, 19(3), pp. 714-716 (https://dmd.aspetjournals.org/content/19/3/714.long).

Harvey, D. J. and Brown, N. K. (1991b), 'Comparative in vitro metabolism of the cannabinoids', *Pharmacology, Biochemistry and Behavior*, 40(3), pp. 533-540 (https://doi.org/10.1016/0091-3057(91)90359-A).

Harvey, D. J. and Brown, N. K. (1991c), 'Electron impact-induced fragmentation of the trimethylsilyl derivatives of monohydroxy-hexahydrocannabinols', *Biological Mass Spectrometry*, 20(8), pp. 292-302 (https://doi.org/10.1002/bms.1200200510).

Harvey, D. J. and Paton, W. D. M. (1979), 'The metabolism of deuterium-labelled analogues of Δ^1-,Δ^6-, and Δ^7-tetrahydrocannabinol and the use of deuterium labelling', in: *Recent developments in mass spectrometry in biochemistry and medicine*. ed. A. Frigerio. Plenum Press, New York, Volume 2, pp. 127-147.

Harvey, D. J., Gill, E. W., Slater, M. and Paton, W. D. M. (1980), 'Identification of *in vivo* liver metabolites of (–)-Δ^7-tetrahydrocannabinol produced by the mouse', *Drug Metabolism and Disposition*, 8(6), pp. 439-445 (https://dmd.aspetjournals.org/content/8/6/439.long).

Harvey, D. J., Martin, B. R. and Paton, W. D. M. (1977), 'Identification of metabolites of Δ^1- and $\Delta^{1(6)}$-tetrahydrocannabinol containing a reduced double bond', *Journal of Pharmacy and Pharmacology*, 29(8), pp. 495-497 (https://doi.org/10.1111/j.2042-7158.1977.tb11376.x).

Hill, D. W. and Langner, K. J. (1987), 'HPLC photodiode array UV detection for toxicological drug analysis', *Journal of Liquid Chromatography*, 10(2-3), pp. 377-409 (https://doi.org/10.1080/01483918708066724).

Hindelang, P., Scharinger, A., Golombek, P., Liable, M., Tamosaite, S., Walch, S. G. and Lachenmeier, D. W. (2022), 'Absence of relevant thermal conversion of cannabidiol to tetrahydrocannabinol in e-cigarette vapor and low-tetrahydrocannabinol cannabis smoke', *Cannabis and Cannabinoid Research*, 7(5), pp. 616-620 (https://doi.org/10.1089/can.2022.0163).

Hively, R. L., Mosher, W. A. and Hoffmann, F. W. (1966), 'Isolation of *trans*-Δ^6-tetrahydrocannabinol from marijuana', *Journal of the American Chemical Society*, 88(8), pp. 1832-1833 (https://doi.org/10.1021/ja00960a056).

Hollister, L. E. and Gillespie, H. K. (1973), 'Delta-8- and delta-9-tetrahydrocannabinol comparison in man by oral and intravenous administration', *Clinical Pharmacology and Therapeutics*, 14(3), 353-357 (https://doi.org/10.1002/cpt1973143353).

Hollister, L. E., Gillespie, H. K., Mechoulam, R. and Srebnik, M. (1987), 'Human pharmacology of 1S and 1R enantiomers of delta-3-tetrahydrocannabinol', *Psychopharmacology*, 92(4), pp. 505-507 (https://doi.org/10.1007/BF001176485).

Holt, A. K., Poklis, J. L. and Peace, M. R. (2022), 'Δ^{8}-THC, THC-O acetates and CBD-di-O acetate: emerging synthetic cannabinoids found in commercially sold plant material and gummy edibles', *Journal of Analytical Toxicology*, 46(8), pp. 940–948 (https://doi.org/10.1093/jat/bkac036).

Howlett, A. C., Johnson, M. R., Melvin, L. S. and Milne, G. M. (1988), 'Nonclassical cannabinoid analgetics inhibit adenylate cyclase: development of a cannabinoid receptor model', *Molecular Pharmacology*, 33(3), pp. 297-302 (https://molpharm.aspetjournals.org/content/33/3/297.long).

Hoye, D. (1973), *Cannabis alchemy: The art of modern hashmaking.* Level Press, San Francisco. pp. 17-24. Republished: Gold, D. (1989), *Cannabis alchemy: The art of modern hashmaking. Methods for preparation of extremely potent cannabis products*. Ronin Publishing Inc., Berkeley, CA. pp. 24-36.

Huffman, J. W., Banner, W. K., Zoorob, G. K., Joyner, H. H., Reggio, P. H., Martin, B. R. and Compton, D. R. (1995), 'Stereoselective synthesis of the epimeric Δ^{7}-tetrahydrocannabinols', *Tetrahedron*, 51(4), pp. 1017-1032 (https://doi.org/10.1016/0040-4020(94)00995-7).

Hughes, G. A., Jen, T. Y. and Smith, H. (1971), 'Process for the preparation of oxaphenanthrenes and intermediates therefor', US patent 3,576,887 (April 27, 1971).

Inayama, S., Sawa, A. and Hosoya, E. (1974), 'The oxidation of Δ^{1}- and Δ^{6}-tetrahydrocannabinol with selenium dioxide', *Chemical and Pharmaceutical Bulletin*, 22(7), pp. 1519-1525 (https://doi.org/10.1248/cpb.22.1519).

Inayama, S., Sawa, A. and Hosoya, E. (1976), 'Mass spectrometry of oxidation products of Δ^{1}- and Δ^{6}-tetrahydrocannabinols', *Chemical and Pharmaceutical Bulletin*, 24(9), pp. 2209-2218 (https://doi.org/10.1248/cpb.24.2209).

Inoue, T., Inoue, S. and Sato, K. (1990), 'Lewis acid catalyzed reaction of *o*-[1-(alkylthio)alkyl]phenols: the generation and reaction of *o*-quinonemethides and applications to cannabinoid synthesis', *Bulletin of the Chemical Society of Japan*, 63(6), pp. 1647-1652 (https://doi.org/10.1246/bcsj.63.1647).

Jacob, A. and Todd, A. R. (1940), '*Cannabis indica*. Part II. Isolation of cannabidiol from Egyptian hashish. Observations on the structure of cannabinol', *Journal of the Chemical Society*, pp. 649-653 (https://doi.org/10.1039/JR9400000649).

Järbe, T. U. C. and McMillan, D. E. (1980), 'Δ^{9}-THC as a discriminative stimulus in rats and pigeons: generalization to THC metabolites and SP-111', *Psychopharmacology*, 71(3), pp. 281-289 (https://doi.org/10.1007/BF00433063).

Järbe, T. U. C., Hiltunen, A. J., Lander, N. and Mechoulam, R. (1986), 'Cannabimimetic activity (Δ^1-THC cue) of cannabidiol monomethyl ether and two stereoisomeric hexahydrocannabinols in rats and pigeons', *Pharmacology, Biochemistry and Behavior*, 25(2), pp. 393-399 (https://doi.org/10.1016/0091-3057(86)90015-8).

Jen, T. Y., Hughes, G. A. and Smith, H. (1967), 'Total synthesis of Δ^8-($\Delta^{1(6)}$)-tetrahydrocannabinol, a biologically active constituent of hashish (marijuana)', *Journal of the American Chemical Society*, 89(17), pp. 4551-4552 (https://doi.org/10.1021/ja0093a071).

Johnson, M. R. and Melvin, L. S. (1986), 'The discovery of nonclassical cannabinoid analgetics', in: *Cannabinoids as therapeutic agents*. ed. R. Mechoulam. CRC Press, Inc., Boca Raton, Florida, pp. 121-145.

Johnson, M. R., Melvin, L. S., Althuis, T. H., Bindra, J. S., Harbert, C. A., Milne, G. M. and Weissman, A. (1981), 'Selective and potent analgetics derived from cannabinoids', *Journal of Clinical Pharmacology*, 21(S1), pp. S271-S282 (https://doi.org/10.1002/j.1552-4604.1981.tb02605.x).

Jones, A. B., ElSohly, H. N. and ElSohly, M. A. (1984), 'Analysis of the major metabolite of Δ^9-tetrahydrocannabinol in urine. V. Cross-reactivity of selected compounds in a radioimmunoassay', *Journal of Analytical Chemistry*, 8(6), pp. 252-254 (https://doi.org/10.1093/jat/8.6.252).

Jones, A. B., ElSohly, H. N. and ElSohly, M. A. (1985), 'Cross-reactivity of selected compounds in urine immunoassays for the major metabolite of delta-9-tetrahydrocannabinol', in: *Marihuana '84: Proceedings of the Oxford Symposium on Cannabis*. ed. D. J. Harvey, LRI Press Ltd., Oxford. pp. 169-176.

Just, W. W., Erdmann, G., Thel, S., Werner, G. and Wiechmann, M. (1975), 'Metabolism and autoradiographic distribution of Δ^8- and Δ^9-tetrahydrocannabinol in some organs of the monkey Callithrix jacchus', *Naunyn-Schmiedeberg's Archives of Pharmacology*, 287(2), pp. 219–225 (https://doi.org/10.1007/BF00510453).

Karin, K. N., Holt, A. K., Smith, E. R., Orlowicz, S., Taylor, A. M., Prengaman, L., Wolf, C. E., Williams, G. R., Peace, M. R. and Poklis, J. L. (2022), 'Separation and quantitation of natural and unnatural THC isomers and analogues by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS)', Poster #65, presented at the SOFT 2022 Annual Meeting in Cleveland, OH, October 30 – November 4, 2022. https://soft.memberclicks.net/assets/Programs/2022%20Program%20Book%20for%20Website%20101922.pdf, accessed on 7 January 2023.

Karschner, E. L. (2021), 'Delta dawn: The rise of Δ8-THC in the United States', *TIAFT Bulletin* 51(2), pp. 19-24.

Kiselak, T. D., Koerber, R. and Verbeck, G. F. (2020), 'Synthetic route sourcing of illicit at home cannabidiol (CBD) isomerization to psychoactive cannabinoids using ion mobility-coupled-LC–MS/MS', *Forensic Science International*, 308, Article No. 110173 (https://doi.org/10.1016/j.forsciint.2020.110173).

Klous, M.G., Lee, W., Hillebrand, M. J. X., van den Brink, W., van Ree, J. M. and Beijnen. J. H. (2006), 'Analysis of diacetylmorphine, caffeine, and degradation products after volatilization of pharmaceutical heroin for inhalation', *Journal of Analytical Toxicology*, 30(1), pp. 6-13 (https://doi.org/10.1093/jat/30.1.6).

Later, D. W., Richter, B. E., Knowles, D. E. and Andersen, M. R. (1986), 'Analysis of various classes of drugs by capillary supercritical fluid chromatography', *Journal of Chromatographic Science*, 24(6), pp. 249-253 (https://doi.org/10.1093/chromsci/24.6.249).

Leafly Staff. (2021), 'What is delta-8', https://www.leafly.com/news/science-tech/what-is-delta8-thc, accessed 14 February 2023.

Leas E. C. (2021), 'The hemp loophole: A need to clarify the legality of delta-8-THC and other hemp-derived tetrahydrocannabinol compounds', *American Journal of Public Health*, 111(11), pp. 1927–1931 (https://doi.org/10.2105/AJPH.2021.306499).

Leas, E. C., Nobles, A. L., Shi, Y., & Hendrickson, E. (2022), 'Public interest in ∆8-tetrahydrocannabinol (delta-8-THC) increased in US states that restricted ∆9-tetrahydrocannabinol (delta-9-THC) use', *International Journal on Drug Policy*, 101, 103557 (https://doi.org/10.1016/j.drugpo.2021.103557).

Lee, Y. R. (2010), '[A novel method for the preparation of hexahydrocannabinol derivatives]' Korean Patent KR20100010793A (in Korean), https://worldwide.espacenet.com/patent/search/family/042085387/publication/KR20100010793A?q=KR20100010793, accessed on 30 December2022.

Lee, Y. R. and Xia, L. (2008), 'Efficient one-pot synthetic approaches for cannabinoid analogues and their application to biologically interesting (–)-hexahydrocannabinol and (+)-hexahydrocannabinol', *Tetrahedron Letters*, 49(20), pp. 3283-3287 (https://doi.org/10.1016/j.tetlet.2008.03.075).

Lewis, A. C. (2020), 'A hidden origin story of the CBD craze', *The New York Times*, May 23, 2020, ahttps://www.nytimes.com/2020/05/23/sunday-review/coronavirus-cbd-oil.html, accessed on 20 December 2022.

Linciano, P., Russo, F., Citti, C., Tolomeo, F., Paris, R., Fulvio, F., Pecchioni, N., Vandelli, M. A., Laganà, A., Capriotti, A. L., Biagini, G., Carbone, L., Gigli, G. and Cannazza, G. (2021), 'The novel heptyl phorolic acid cannabinoids content in different *Cannabis sativa* L. accessions', *Talanta*, 235, pp. 112704 (https://doi.org/10.1016/j.talanta.2021.122704).

Livingston, M. D., Walker, A., Cannell, M. B., & Rossheim, M. E. (2022), 'Popularity of delta-8 THC on the internet across US States, 2021', *American Journal of Public Health*, 112(2), pp. 296–299 (https://doi.org/10.2105/AJPH.2021.306586).

Loewe S. (1944), 'Pharmacological study', In: *The Marihuana Problem in the City of New York.* Mayor LaGardia's Committee on Marihuana. The Jacques Cattell Press, Lancester, PA, pp. 149-212.

Loewe, S. (1950), 'Cannabiswirkstoffe und Pharmakologie der Cannabinole', *Archiv für Experimentelle Pathologie und Pharmakologie*, 211(2), pp. 175-193 (https://doi.org/10.1007/BF00249872).

Lopera, V., Rodríguez, A. and Amariles, P. (2022), 'Clinical relevance of drug interactions with cannabis: a systematic review', *Journal of Clinical Medicine*, 11(5), pp. 1154 (https://doi.org/10.3390/jcm11051154).

Lu, Z.-G. (2006), '[A facile synthesis of *trans*-hexahydrocannabinol via an *o*-quinonemethide]', *Acta Chimica Sinica*, 64(17), pp. 1843-1846 (in Chinese) (http://sioc-journal.cn/Jwk_hxxb/EN/Y2006/V64/I17/1843).

Lu, Z. G., Sato, N., Inoue, S. and Sato, K. (1992), 'Generation and intramolecular cycloaddition of *o*-quinonemethides in protic solvent. An efficient synthesis of (–)-*trans*-hexahydrocannabinol', *Chemistry Letters*, (7), pp. 1237-1238 (https://doi.org/10.1246/cl.1992.1237).

Marino, J. P. and Dax, S. L. (1984), 'An efficient desilylation method for the generation of *o*-quinone methides: application to the synthesis of (+)- and (–)-hexahydrocannabinol', *Journal of Organic Chemistry*, 49(19), pp. 3671-3672 (https://doi.org/10.1021/jo00193a051).

Marrocco, M., Singh, D., Christiani, D. C. and Demokritou, P. (2022), 'E-cigarette vaping associated acute lung injury (EVALI): state of science and future research needs', *Critical Reviews in Toxicology*, 52(3), pp. 188-220 (https://doi.org/10.1080/10408444.2022.2082918).

Martin, B. R., Balster, R. L., Razdan, R. K., Harris, L. S. and Dewey, W. L. (1981), 'Behavioral comparisons of the stereoisomers of tetrahydrocannabinols', *Life Sciences*, 29(6), pp. 565-574 (https://doi.org/10.1016/0024-3205(81)90434-3).

Martin, B. R., Compton, D. R., Little, P. J., Martin, T. J. and Beardsley, P. M. (1987), 'Pharmacological evaluation of agonistic and antagonistic activity of cannabinoids', *NIDA Research Monograph*, 79, pp. 48-58.

Marzullo, P., Foschi, F., Coppini, D. A., Fanchini, F., Magnani, L., Rusconi, S., Luzzani, M. and Passarella, D. (2020), 'Cannabidiol as the substrate in acid-catalyzed intramolecular cyclization', *Journal of Natural Products*, 83(10), pp. 2894-2901 (https://doi.org/10.1021/acs.jnatprod.0c00436).

Maurya, V. and Appayee, C. (2020), 'Enantioselective total synthesis of potent 9β-11-hydroxyhexahydrocannabinol', *Journal of Organic Chemistry*, 85(2), pp. 1291-1297 (https://doi.org/10.1021/cas.joc.9b02962).

Mechoulam, R. (1973), 'Cannabinoid chemistry'. In: *Marijuana: chemistry, pharmacology, metabolism and clinical effects*. ed. R. Mechoulam. Academic Press, New York. pp. 1-99.

Mechoulam, R. and Edery, H. (1973), 'Structure–activity relationships in the cannabinoid series'. In: *Marijuana: chemistry, pharmacology, metabolism and clinical effects*. ed. R. Mechoulam. Academic Press, New York. pp. 101-136.

Mechoulam, R., Braun, P. and Gaoni, Y. (1967), 'A stereospecific synthesis of (–)-Δ^{1}- and (–)-$\Delta^{1(6)}$-tetrahydrocannabinols', *Journal of the American Chemical Society*, 89(17), pp. 4552-4554 (https://doi.org/10.1021/ja00993a072).

Mechoulam, R., Ben-Zvi, Z., Varconi, H. and Samuelov, Y. (1973), 'Cannabinoid rearrangements. Synthesis of Δ^{5}-tetrahydrocannabinol', *Tetrahedron*, 29(11), pp. 1615-1619 (https://doi.org/10.1016/S0040-04020(01)83406-2).

Mechoulam, R., Lander, N., Varkony, T. H., Kimmel, I., Becker, O., Ben-Zvi, Z., Edery, H. and Porath, G. (1980), 'Stereochemical requirements for cannabinoid activity', *Journal of Medicinal Chemistry*, 23(10), pp. 1068-1072 (https://doi.org/10.1021/jm00184a002).

Meehan-Atrash, J. and Rahman, I. (2021), 'Cannabis vaping: existing and emerging modalities, chemistry, and pulmonary toxicology', *Chemical Research in Toxicology*, 34(10), pp. 2169-2179 (https://doi.org/10.1021/acs.chemrestox.1c00290).

Meehan-Atrash, J. and Rahman, I. (2022), 'Novel Δ^{8}-tetrahydrocannabinol vaporizers contain unlabeled adulterants, unintended byproducts of chemical synthesis, and heavy metals', *Chemical Research in Toxicology*, 35(1), pp. 73-76 (https://doi.org/10.1021/acs.chemrestox.1c00388).

Miller, I. J., McCallum, N. K., Kirk, C. M. and Peake, B. M. (1982), 'The free radical oxidation of the tetrahydrocannabinols', *Experientia*, 38(2), pp. 230-231 (https://doi.org/10.1007/BF01945081).

Moffat, A. C., Williams, P. L. and King, L. J. (1982), 'Combined high-performance liquid chromatography and radioimmunoassay method for the analysis of Δ^{9}-tetrahydrocannabinol and its metabolites in plasma and urine', in: *The analysis of cannabinoids in biological fluids*. ed. R. L. Hawks. National Institute of Drug Abuse, Rockville, MD, *NIDA Research Monograph*, 42, pp. 56-68.

Muhammad, I., Li, X.-C., Dunbar, D. C., ElSohly, M. A. and Khan, I. A. (2001), 'Antimalarial (+)-*trans*-hexahydrodibenzopyran derivatives from *Machaerium multiflorum*', *Journal of Natural Products*, 64(10), pp. 1322-1325 (https://doi.org/10.1021/np0102861).

Munger, K. R., Jensen, R. P. and Strongin, R. M. (2022), 'Vaping cannabinoid acetates leads to ketene formation', *Chemical Research in Toxicology*, 35(7), pp. 1202-1205 (https://doi.org/10.1021/acs.chemrestox.2c00170).

Muren, J. F. and Weissman, A. (1971), 'Depressant 1,2-dihydroquinolines and related derivatives', *Journal of Medicinal Chemistry*, 14(1), pp. 49-53 (https://doi.org/10.1021/jm00283a013).

Murphy, W. S., Culhane, A., Duffy, B. and Tuladhar, S. M. (1992a), 'Base-catalyzed reaction of citronellal with phenols', *Journal of the Chemical Society, Perkin Transactions 1*, (24), pp. 3397-3400 (https://doi.org/10.1039/P19920003397).

Murphy, W. S., Tuladhar, S. M. and Duffy, B. (1992b), 'Simple synthesis of hexahydrocannabinoids using phenylboronic acid catalyst', *Journal of the Chemical Society, Perkin Transactions 1*, (5), pp. 605-609 (https://doi.org/10.1039/P19920000605).

Narimani, M. and da Silva, G. (2020), 'Does 'Dry Hit' vaping of vitamin E acetate contribute to EVALI? Simulating toxic ketene formation during e-cigarette use', *PLoS One*,15(9), e0238140 (https://doi.org/10.1371/journal.pone.0238140).

Nasrin, S., Watson, C. J. W., Perez-Paramo, Y. X. and Lazarus, P. (2021), 'Cannabinoid metabolites as inhibitors of major hepatic CYP450 enzymes, with implications for cannabis-drug interactions', *Drug Metabolism and Disposition*, 49(12), pp. 1070-1080 (https://doi.org/10.1124/dmd.121.000442).

NIST Standard Reference Database 69: NIST Chemistry WebBook (2021), 'Hexahydrocannabinol', Eds. P.J. Linstrom and W.G. Mallard, https://webbook.nist.gov/cgi/cbook.cgi?ID=6692-85-9, accessed on 7 January 2023.

Norton, S. (1957), 'Behavioral patterns as a technique for studying psychotropic drugs', in *Psychotropic drugs*. eds. S. Garattini and V. Ghetti, Elsevier, Amsterdam. pp. 73–82.

Nye, J. S., Seltzman, H. H., Pitt, C. G. and Snyder, S. H. (1985), 'High-affinity cannabinoid binding sites in brain membranes labeled with [^{3}H]-5'-trimethylammonium Δ^8-tetrahydrocannabinol', *The Journal of Pharmacology and Experimental Therapeutics*, 234(3), pp. 784-791 (https://jpet.aspetjournals.org/content/234/3/784.long).

Parker, L. A., Rock, E. M. and Mechoulam, R. (2022), *CBD: what does the science say?* MIT Press, Cambridge, MA.

Pars, H. G. and Razdan, R. K. (1971), 'Tetrahydrocannabinols and synthetic analogs', *Annals of the New York Academy of Sciences*, 191(1), pp. 15-22 (https://doi.org/10.1111/j.1749-6632.1971.tb13983.x).

Patilea-Vrana, G. I., Anoshchenko, O. and Unadkat, J. D. (2019), 'Hepatic enzymes relevant to the disposition of (–)-Δ^9-tetrahydrocannabinol (THC) and its psychoactive metabolite, 11-OH-THC', *Drug Metabolism and Disposition*, 47(3), pp. 249-256 (https://doi.org/10.1124/dmd.118.085548).

Petrzilka, T. (1975), 'Tetrahydrodibenzopyrans', US patent 3,873,576 (Mar. 25, 1975).

Powell, G. and Bembry, T. H. (1940), 'Synthesis of cannabinol', *Journal of the American Chemical Society*, 62(9), pp. 2568-2569 (https://doi.org/10.1021/ja01866a513).

Qureshi, M. N., Kanwal, F., Siddique, M., Inayat-ur-Rahman and Akram, M. (2012), 'Estimation of biologically active cannabinoids in *Cannabis indica* by gas chromatography-

mass spectrometry (GC-MS)', *World Applied Sciences Journal*, 19(7), pp. 918-923 (https://doi.org/10.5829/idosi.wasj.2012.19.07.192).

Radwan, M. M., Wanas, A. S., Gul, W., Ibrahim, E. A. and ElSohly, M. A. (2023), 'Isolation and characterization of impurities in commercially marketed Δ^8-THC products', *Journal of Natural Products* (https://doi.org/10.1021/acs.jnatprod.2c01008).

Ray, C. L., Bylo, M. P., Pescaglia, J., Gawenis, J. A. and Greenlief, C. M. (2022), 'Delta-8 tetrahydrocannabinol product impurities.' *Molecules* 27(20), 6924 (https://doi.org/10.3390/molecules27206924).

Razdan, R. K., Uliss, D. B. and Dalzell, H. C. (1973), 'Hashish. Synthesis of 7-hydroxy-Δ^1-tetrahydrocannabinol (THC). An important active metabolite of Δ^1-THC in man', *Journal of the American Chemical Society*, 95(7), pp. 2361-2362 (https://doi.org/10,1021/ja00788a043).

Rebuli, M. E., Rose, J.J., Noël, A., Croft, D. P., Benowitz, N. L., Cohen, A. H., Goniewicz, M. L., Larsen, B. T., Leigh, N., McGraw, M. D., Melzer, A. C., Penn, A. L., Rahman, I., Upson, D., Crotty Alexander, L. E., Ewart, G., Jaspers. I., Jordt, S. E., Kligerman, S., Loughlin, C. E., McConnell, R., Neptune, E. R., Nguyen, T. B., Pinkerton, K. E. and Witek, T. J., Jr. (2023), 'The e-cigarette or vaping product use-associated lung injury epidemic: pathogenesis, management, and future directions. An official American Thoracic Society workshop report', *Annals of the American Thoracic Society*, 20(1), pp. 1-17 (https://doi.org/10.1513/AnnalsATS.202209-796ST).

Reggio, P. H., Greer, K. V. and Cox, S. M. (1989), 'The importance of the orientation of the C9 substituent to cannabinoid activity', *Journal of Medicinal Chemistry*, 32(7), pp. 1630-1635 (https://doi.org/10.1021/jm00127a038).

Rosenthal, D., Harvey, T. M., Bursey, J. T., Brine, D. R. and Wall, M. E. (1978), 'Comparison of gas chromatography mass spectrometry methods for the determination of Δ^9-tetrahydrocannabinol in plasma', *Biomedical Mass Spectrometry*, 5(4), pp. 312-316 (https://doi.org/10.1002/bms.1200050407).

Russell, P. B., Todd, A. R., Wilkinson, S., Macdonald, A. D. and Woolfe, G. (1941), '*Cannabis indica*. Part VII. The relation between chemical constitution and hashish activity', *Journal of the Chemical Society*, pp. 169-172 (https://doi.org/10.1039/jr9410000169).

Sachse-Seeboth, C., Pfeil, J., Sehrt, D., Meineke, I., Tzvetkov, M., Bruns, E., Poser, W., Vormfelde, S. V. and Brockmöller, J. (2009), 'Interindividual variation in the pharmacokinetics of Δ9-tetrahydrocannabinol as related to genetic polymorphisms in *CYP2C9*', *Clinical Pharmacology & Therapeutics*, 85(3), pp. 273-276 (https://doi.org/10.1038/clpt.2008.213).

Sams, R. A. (2022), 'Analysis of hexahydrocannabinols: eliminating uncertainty in its identification', https://kcalabs.com, accessed on 20 December 2022.

Šantavý, F. (1964), 'Notes on the structure of cannabidiol compounds', *Acta Universitatis Palackianae Olomucensis, Facultatis Medicae*, 35, pp. 5-9.

Schafroth, M. A., Mazzoccanti, G., Reynoso-Moreno, I., Erni, R., Pollastro, F., Caprioglio, D., Botta, B., Allegrone, G., Grassi, G., Chicca, A., Gasparrini, F., Gertsch, J., Carreira, E. M. and Appendino, G. (2021), 'Δ^9-*cis*-Tetrahydrocannabinol: natural occurrence, chirality, and pharmacology', *Journal of Natural Products*, 84(9), pp. 2502-2510 (https://doi.org/10.1021/acs.jnatprod.1c00513).

Schwilke, E. W., Schwope, D. M., Karschner, E. L., Lowe, R. H., Darwin, W. D., Kelly, D. L., Goodwin, R. S., Gorelick, D. A. and Huestis, M. A. (2009), 'Δ^9-Tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-9-carboxy-THC plasma pharmacokinetics during and after continuous high-dose oral THC', *Clinical Chemistry*, 55(12), pp. 2180-2189 (https://doi.org/10.1373/clinchem.2008.122119).

Scialdone, M. A. (2017), 'Hydrogenation of cannabis oil', US Patent 9,694,040 (Jul. 4, 2017).

Seccamani, P., Franco, C., Protti, S., Porta, A., Profumo, A., Caprioglio, D., Salamone, S., Mannucci, B. and Merli, D. (2021), 'Photochemistry of cannabidiol (CBD) revised. A combined preparative and spectrometric investigation', *Journal of Natural Products*, 84(11), pp. 2858-2865 (https://doi.org/10.1021/acs.jnatprod.1c00567).

Skinner, W. A., Rackur, G. and Uyeno, E. (1979), 'Structure–activity studies on tetrahydro- and hexahydrocannabinol derivatives', *Journal of Pharmaceutical Sciences*, 68(3), pp. 330-332 (https://doi.org/10.1002/jps.2600680319).

Small, E. (2017), 'Classification of *Cannabis sativa* L. in relation to agricultural, biotechnological, medical and recreational utilization', in: Cannabis sativa *L. - Botany and biotechnology*. ed. S. Chandra, Lata, H. and ElSohly, M. A. Springer, Cham, Switzerland, pp. 1-62.

Smith, E. R., Karin, K. N., Poklis, J. L. and Peace, M. R. (2022), 'A qualitative analysis of THC isomers and derivatives in e-liquids', Poster #12, presented at the SOFT 2022 Annual Meeting in Cleveland, OH, October 30 – November 4, 2022. https://soft.memberclicks.net/assets/Programs/2022%20Program%20Book%20for%20Website%20101922.pdf, accessed on 7 January 2023.

Smith, R. M. and Kempfert, K. D. (1977), 'Δ^1-3,4-*cis*-Tetrahydrocannabinol in *Cannabis sativa*', *Phytochemistry*, 16(7), pp. 1088-1089 (https://doi.org/10.1016/S0031-9422(00)86745-X).

SmithKline Corporation (1977), 'Dibenzo[b,d]pyrans and pharmaceutical compositions thereof', Great Britain patent 1 464 695 (16 Feb 1977)

Starks, M. (1977), *Marijuana Potency*. And/Or Press, Inc., Berkeley, CA. Republished: Starks, M. (1990), *Marijuana Chemistry. Genetics, Processing, & Potency*. Ronin Publishing, Inc., Berkeley, CA.

Stone E. (2020), 'Meet THCP and CBDP: Study reveals the identification of two new cannabinoids', Leafly. https://www.leafly.com/news/science-tech/thcp-cbdp-study-reveals-identification-two-new-cannabinoids, accessed on 14 February 2023.

Stothard, A. I., Layle, N. K., Martin, M. J., Liu, J., Bassman, J. R., Williams, J. B. and Hering, K. W. (2022), 'Characterization of Hexahydrocannabinol Diastereomers by NMR, HPLC, GC-MS, and TLC', Scientific Poster. https://cdn2.caymanchem.com/cdn/cms/caymanchem/LiteratureCMS/Characterization%20of%20HHC%20Diastereomers.pdf, accessed on 20 December 2022.

Tagen, M. and Klumpers, L. E. (2022), 'Review of delta-8-tetrahydrocannabinol (Δ^8-THC): Comparative pharmacology with Δ^9-THC', *British Journal of Pharmacology*, 179(15), pp. 3915-3933 (https://doi.org/10.1111/bph.15865).

Takashina, S., Takahashi, M., Morimoto, K. and Inoue, K. (2022). 'LC-MS/MS assay for the measurement of cannabidiol profiling in CBD oil from Japanese market and application for convertible tetrahydrocannabinol in acetic acid condition', *Chemical and Pharmaceutical Bulletin* 70(2), pp. 169-174 (https://doi.org/10.1248/cpb.c21-00901).

Teale, J. D., Forman, E. J., King, L. J., Piall, E. M. and Marks, V. (1975), 'The development of a radioimmunoassay for cannabinoids in blood and urine', *Journal of Pharmacy and Pharmacology*, 27(7), pp. 465-472 (https://doi.org/10.1111/j.2042-7158.1975.tb09486.x).

Tesfatsion, T. T., Collins, A. C., Ramirez, G. A., Mzannar, Y., Khan, H. Y., Aboukameel, O., Azmi, A. S., Jagtap, P. G., Ray, K. P. and Cruces, W. (2022), 'Antineoplastic properties of THCV, HHC and their anti-proliferative effects on HPAF-II, MIA-paca2, Aspc-1, and PANC-1 PDAC pancreatic cell lines', *ChemRxiv*, Not peer-reviewed preprint. (https://doi.org/10.26434/chemrxiv-2022-v4zqc-v2. https://chemrxiv.org/engage/chemrxiv/article-details/63a37aeb81e4bac11355ee92), accessed on 15 January 2023.

Thapa, D., Lee, J. S., Heo, S.-W., Lee, Y. R., Kang, K. W., Kwak, M.-K., Choi, H. G. and Kim, J.-A. (2011), 'Novel hexahydrocannabinol analogs as potential anti-cancer agents inhibit cell proliferation and tumor angiogenesis', *European Journal of Pharmacology*, 650(1), pp. 64-71 (https://doi.org/10.1016/j.ejphar.2010.09.073).

Theunissen, E. L., Reckweg, J. T., Hutten, N. R. P. W., Kuypers, K. P. C., Toennes, S. W., Neukamm, M. A., Halter, S. and Ramaekers, J. G. (2022), 'Psychotomimetic symptoms after a moderate dose of a synthetic cannabinoid (JWH-018): implications for psychosis', *Psychopharmacology*, 239(5), pp. 1251-1261 (https://doi.org/10.1007/s00213-021-05768-0).

Thomas, B. F., Compton, D. R. and Martin, B. R. (1990), 'Characterization of the lipophilicity of natural and synthetic analogs of Δ^9-tetrahydrocannabinol and its relationship to pharmacological potency', *The Journal of Pharmacology and Experimental Therapeutics*, 255(2), pp. 624-630 (https://jpet.aspetjournals.org/content/255/2/624.long).

Tietze, L.-F., von Kiedrowski, G. and Berger, B. B. (1982), 'Stereo- and regioselective synthesis of enantiomerically pure (+)- and (–)-hexahydrocannabinol by intramolecular cycloaddition', *Angewandte Chemie, International Edition*, 21(3), pp. 221-222 (https://doi.org/10.1002/anie.198202212).

Todd, A. R. (1940), 'Chemistry of the hemp drugs', *Nature*, 146(3713), pp. 829-830 (https://doi.org/10.1038/146829a0).

Todd, L. S. (1974), *Dr. Atomic's marijuana multiplier*. Republished: Todd, L. S. (1998), *Dr. Atomic's marijuana multiplier*. Ronin Publishing Inc., Oakland, CA.

Tolomeo, F., Russo, F., Kaczorova, D., Vandelli, M. A., Biagini, G., Laganà, A., Capriotti, A. L., Paris, R., Fulvio, F., Carbone, L., Perrone, E., Gigli, G., Cannazza, G. and Citti, C. (2022), '*Cis*-Δ^9-tetrahydrocannabinolic acid occurrence in *Cannabis sativa* L.', *Journal of Pharmaceutical and Biomedical Analysis*, 219, 114958 (https://doi.org/10.1016/j.jpba.2022.114958).

Tsujikawa, K., Okada, Y., Segawa, H., Yamamuro, T., Kuwayama, K., Kanamori, T. and Iwata, Y. T. (2022), 'Thin-layer chromatography on silver nitrate-impregnated silica gel for analysis of homemade tetrahydrocannabinol mixtures', *Forensic Toxicology*, 40(1), pp. 125-131 (https://doi.org/10.1007/s11419-021-00592-9).

Turner, C. E., Hadley, K. W., Fetterman, P. S., Doorenbos, N. J., Quimby, M. W. and Waller, C. (1973), 'Constituents of *Cannabis sativa* L. IV: Stability of cannabinoids in stored plant material', *Journal of Pharmaceutical Sciences*, 62(10), pp. 1601-1605 (https://doi.org/10.1002/jps.2600621005).

Twohig, M., Aubin, A. J. and Hudalla, C. J. (2022), 'Chromatography, detection, and software tools to aid in the exploration of delta-8 THC distillates', Available at: https://www.waters.com/content/dam/waters/en/app-notes/2022/720007692/720007692-en.pdf, accessed on 20 December 2022.

UNCTAD (United Nations Conference on Trade and Development) (2022), '*Commodities at a glance: Special issue on industrial hemp*', UNCTAD/DITC/COM/2022/1. United Nations, Geneva. https://unctad.org/system/files/official-document/ditccom2022d1_en.pdf, accessed 8 January 2023.

US CDC (US Centers for Disease Control and Prevention) (2020), 'Outbreak of lung injury associated with the use of e-cigarette, or vaping, products', https://www.cdc.gov/tobacco/basic_information/e-cigarettes/severe-lung-disease, accessed on 20 December 2022.

US CDC (2021), 'Increases in availability of cannabis products containing delta-8 THC and reported cases of adverse events. CDCHAN-00451', https://emergency.cdc.gov/han/2021/han00451.asp, accessed 14 February 2023

USDA (US Department of Agriculture) (2022), *National Hemp Report*. USDA. https://release.nass.usda.gov/reports/hempan22.pdf, accessed 20 December 2022.

USDA (2023), 'Hemp production', https://www.ams.usda.gov/rules-regulations/hemp, accessed 24 January 2023.

US DoJ (US Department of Justice) (2020). 'Implementation of the Agriculture Improvement Act of 2018', *Federal Register*, 85(163), pp. 51639-51645. https://www.govinfo.gov/content/pkg/FR-2020-08-21/pdf/2020-17356.pdf, accessed on 20 December 2022.

US DoJ (2021), [DEA Letter] https://albop.com/oodoardu/2021/10/ALBOP-synthetic-delta8-THC-21-7520-signed.pdf, accessed on 20 December 2022.

US FDA (US Food and Drug Administration. (2022), '5 things to know about delta-8-tetrahydrocannabinol - delta-8-THC' https://www.fda.gov/consumers/consumer-updates/5-things-know-about-delta-8-tetrahydrocannabinol-delta-8-thc, accessed 14 February 2023

US Public Law (2018), *Agriculture Improvement Act of 2018*. Public Law 115-334. (December 20, 2018) 115th Congress. https://www.govinfo.gov/app/details/PLAW-115publ334/summary, accessed on 20 December 2022.

US House (2022), *To promote the production of hemp and hemp products, and for other purposes (Hemp Advancement Act of 2022).* 117th Congress, 2d Session (February 8, 2022), H.R. 6645. https://www.congress.gov/bill/117th-congress/house-bill/6645, accessed on 20 December 2022.

Ujváry, I. and Grotenhermen, F. (2014), '11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol – a ubiquitous yet underresearched cannabinoid. A review of the literature', *Cannabinoids*, 9(1), pp. 1-8 (http://www.cannabis-med.org/data/pdf/en_2014_01_1.pdf).

Ujváry, I. and Hanuš, L. (2016), 'Human metabolites of cannabidiol: a review on their formation, biological activity, and relevance in therapy', *Cannabis and Cannabinoid Research*, 1(1), pp. 90-101 (https://doi.org/10.1089/can.2015.0012).

Uliss, D. B., Handrick, G. R., Dalzell, H. C. and Razdan, R. K. (1978), 'The conversion of 3,4-*cis*- to 3,4-*trans*-cannabinoids', *Tetrahedron*, 34(13), pp. 1885-1888 (https://doi.org/10.1016/0040-4020(78)80092-1).

United Nations Office on Drugs and Crime (2022), *Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products*. United Nations, Vienna.

Valentine, M. D. (1996), 'Δ-9-Tetrahydrocannabinol acetate from acetylation of cannabis oil', *Science & Justice*, 36(3), pp. 195-197 (https://doi.org/10.1016/S1355-0306(96)72595-9).

Vanegas, S. O., Reck, A. M., Rodriguez, C. E., Marusich, J. A., Yassin, O., Sotzing, G., Wiley, J. L. and Kinsey, S. G. (2022), 'Assessment of dependence potential and abuse liability of Δ^8-tetrahydrocannabinol in mice', *Drug and Alcohol Dependence*, 240, pp. 109640 (https://doi.org/10.1016/j.drugalcdep.2022.109640).

Vaysse, P. J.-J., Gardner, E. L. and Zukin, R. S. (1987), 'Modulation of rat brain opioid receptors by cannabinoids', *The Journal of Pharmacology and Experimental Therapeutics*, 241(2), pp. 534-539 (https://jpet.aspetjournals.org/content/241/2/534.long).

Vree, T. B., Breimer, D. D., van Ginneken, C. A. M. and van Rossum, J. M. (1972a), 'Identification in hashish of tetrahydrocannabinol, cannabidiol and cannabinol analogues with a methyl side-chain', *Journal of Pharmacy and Pharmacology*, 24(1), pp. 7-12 (https://doi.org/10.1111/j.2042-7158.1972.tb08857.x).

Vree, T. R., Breimer, D. D., van Ginneken, C. A. M. and van Rossum, J. M. (1972b), 'Gas chromatography of cannabis constituents and their synthetic derivatives'. *Journal of Chromatography*, 74(2), 209-224 (https://doi.org/10.1016/s0021-9673(01)86151-3).

Wall, M. E. and Brine, D. R. (1979), 'Applications of mass spectrometry in cannabinoid research', in: *Marihuana biological effects: analysis, metabolism, cellular responses, reproduction and brain*. eds. G. G. Nahas, Paton, W. D. M., Braude, M., Jardillier, J. C. and Harvey, D. J. Pergamon Press, Oxford, pp. 15-43.

Waller, C. W., Hadley, K. W. and Turner, C. E. (1976), 'Detection and identification of compounds in cannabis', in: *Marihuana: chemistry, biochemistry, and cellular effects*. eds. G. G. Nahas, Paton, W. D. M. and Idäpään-Heikkilä, J. E. Springer-Verlag, New York, pp. 15-30.

Wang, T., Burgess, J. P., Reggio, P. H. and Seltzman, H. H. (2000), 'Mild generation of *o*-quinone methides. Synthesis of (-)-hexahydrocannabinol and dihydrocannabidiol', *Synthetic Communications*, 30(8), pp. 1431-1435 (https://doi.org/10.1080/00397910008087171).

Watanabe, K., Matsunaga, T., Kimura, T., Funahashi, T., Funae, Y., Ohshima, T. and Yamamoto, I. (2002), 'Major cytochrome P450 enzymes responsible for microsomal aldehyde oxygenation of 11-oxo-Δ^8-tetrahydrocannabinol and 9-anthraldehyde in human liver', *Drug Metabolism and Pharmacokinetics*, 17(6), pp. 516-521 (https://doi.org/10.2133/dmpk.17.516).

Watanabe, K., Matsunaga, T., Kimura, T., Funahashi, T., Yamaori, S., Shoyama, Y. and Yamamoto, I. (2005), 'Stereospecific and regioselective hydrolysis of cannabinoid esters by ES46.5K, an esterase from mouse hepatic microsomes, and its differences from carboxylesterases of rabbit and porcine liver', *Biological and Pharmaceutical Bulletin*, 28(9), pp. 1743-1747 (https://doi.org/10.1248/bpb.28.1743).

Watanabe, K., Itokawa, Y., Yamaori, S., Funahashi, T., Kimura, T., Kaji, T., Usami, N. and Yamamoto, I. (2007), 'Conversion of cannabidiol to Δ^9-tetrahydrocannabinol and related cannabinoids in artifical gastric juice, and their pharmacological effects in mice', *Forensic Toxicology*, 25(1), pp. 16-21 (https://doi.org/10.1007/s11419-007-0021-y).

Webster, G. R. B., Sarna, L. P. and Mechoulam, R. (2008), 'Conversion of CBD to Δ^8-THC and Δ^9-THC', US patent 7,399,872 (July 15, 2008)

Widman, M., Agurell, S., Ehrnebo, M. and Jones, G. (1974), 'Binding of (+)- and (–)-Δ^1-tetrahydrocannabinols and (–)-7-hydroxy-Δ^1-tetrahydrocannabinol to blood cells and plasma proteins in man', *Journal of Pharmacy and Pharmacology*, 26(11), pp. 914-916 (https://doi.org/10.1111/j.2042-7158.1974.tb09207.x).

Williams, P. L., Moffat, A. C. and King, L. J. (1978), 'Combined high-pressure liquid chromatography and radioimmunoassay method for the quantitation of Δ^9-tetrahydrocannabinol and some of its metabolites in human plasma', *Journal of Chromatography A*, 155(2), pp. 273-283 (https://doi.org/10.1016/S0021-9673(00)87988-1).

Wolf, C. E., Holt, A. K., Karin, K. N., Pokhai, A. A., Poklis, J. L., Williams, G. R. and Peace, M. R. (2022), 'The potential to detect 13 cannabinoid acetate analogs in urine using 6 commercially available cannabinoid homogeneous screening kits', Poster #94, presented at the SOFT 2022 Annual Meeting in Cleveland, OH, October 30 – November 4, 2022. https://soft.memberclicks.net/assets/Programs/2022%20Program%20Book%20for%20Website%20101922.pdf, accessed on 7 January 2023.

Wollner, H. J., Matchett, J. R., Levine, J. and Loewe, S. (1942), 'Isolation of a physiologically active tetrahydrocannabinol from Cannabis sativa resin', *Journal of the American Chemical Society*, 64(1), pp. 26-29 (https://doi.org/10.1021/ja01253a008).

Wood, T. B., Spivey, W. T. N. and Easterfield, T. H. (1899), 'Cannabinol. Part I.' *Journal of the Chemical Society*, 75, pp. 20-36 (https://doi.org/10.1039/CT8997500020).

Wu, D. and O'Shea, D. F. (2020), 'Potential for release of pulmonary toxic ketene from vaping pyrolysis of vitamin E acetate', *Proceedings of the National Academy of Sciences of the USA*, 117(12), pp. 6349-6355 (https://doi.org/10.1073/pnas.1920925117).

Xantus, G., Gyarmathy, V. A., Johnson, C. A., Sanghera, P., Zavori, L. and Kanizsai, P. L. (2021), 'The role of vitamin E acetate (VEA) and its derivatives in the vaping associated lung injury: systematic review of evidence', *Critical Reviews in Toxicology*, 51(1), pp. 15-23 (https://doi.org/10.1080/10408444.2020.1858754).

Yang, D.-P., Banijamali, A., Charalambous, A., Marciniak, G. and Makriyannis, A. (1991), 'Solid state ^{2}H-NMR as a method for determining the orientation of cannabinoid analogs in membranes', *Pharmacology, Biochemistry and Behavior*, 40(3), pp. 553-557 (https://doi.org/10.1016/0091-3057(91)90362-6).

Yoshimura, H., Watanabe, K., Oguri, K., Fujiwara, M. and Ueki, S. (1978), 'Synthesis and pharmacological activity of a phosphate ester of Δ^8-tetrahydrocannabinol', *Journal of Medicinal Chemistry*, 21(10), pp. 1079-1081 (https://doi.org/10.1021/jm00208a014).

Zetta, L., De Marco, A., Anklin, C., Cornia, M. and Casiraghi, G. (1987), 'Structure elucidation of a hexahydrocannabinol derivative via ^{13}C–^{13}C homonuclear correlation and ^{1}H–^{13}C heteronuclear correlation with proton detection', *Magnetic Resonance in Chemistry*, 25(12), pp. 1081-1083 (https://doi.org/10.1002/mrc.1260251213).

Zitko, B. A., Howes, J. F., Razdan, R. K., Dalzell, B. C., Dalzell, H. C., Sheehan, J. C., Pars, H. G., Dewey, W. L. and Harris, L. S. (1972), 'Water-soluble derivatives of Δ^1-tetrahydrocannabinol', *Science*, 177(4047), pp. 442-444 (https://doi.org/10.1126/science.177.4047.442).