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CANNABIS AND CANNABINOIDS

The early history of cannabinoid research

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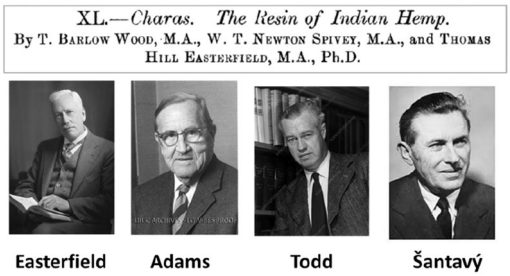
Abstract
Studies on cannabinoids that predate the identification of Δ^9-THC as the intoxicating constituents of recreational cannabis by Raphael Mechoulam in 1964 are reviewed, critically analyzing the controversies and *faux pas* that have characterized the early research in this area. Significant contributions to the elucidation of the signature molecular scaffold of cannabinoids were provided by some of the finest organic chemists of their generation, like Roger Adams and the Nobel laureate Alexander Todd, and important studies of preeminent scientists like Robert Sidney Cahn and František Šantavý also deserve mentioning. The results of these studies include the structure elucidation of cannabinol (**2a**), and the preliminary structure elucidation of cannabidiol (CBD, **3a**) and various semi-synthetic tetrahydrocannabinols (THCs). A comparative analysis of the contributions to the area by Adams and Todd highlights the transition between two generations of organic chemists, and the profound influence that the development of chromatographic methods of purification and of spectroscopic techniques of structure elucidation have played on the development of organic chemistry.

Keywords *Cannabis sativa* L. · Natural products · Cannabinoids · History of chemistry


XL.—*Charas. The Resin of Indian Hemp.*
By T. Barlow Wood, M.A., W. T. Newton Spivey, M.A., and Thomas Hill Easterfield, M.A., Ph.D.

Easterfield Adams Todd Šantavý

With some notable exceptions like the heliocentric theory, radioactivity, and evolution, the generally tortuous and often colorful way in which scientific knowledge can emerge from random observations, *faux pas,* and serendipitous discoveries is largely overlooked in modern discussions, remaining relegated to specialized audiences or becoming summarized in a series of "Eureka moments" of inspiration by a single talented scientist. Raphael Mechoulam is considered the father of modern studies on Cannabis, his "Eureka moments" being the identification of Δ^9-tetrahydrocannabinol (Δ^9-THC, **1a**) as the intoxicating agent of the plant, and the discovery of endocannabinoids, their endogenous analogues. On the other hand, sudden scientific revolutions are rare, and most advancements are the result of incremental developments, whose *fil rouge* through the history of knowledge deserves to be followed since both intellectually stimulating and predictive of future developments in the area. Cannabis (*Cannabis sativa* L.) is a remarkable example of a socially divisive topic confused by too many contrasting contemporary voices. Its ongoing discussion could benefit in terms of clarity from an historical perspective, and there are, indeed, many articles and even books dedicated to the history of the human relationship with Cannabis (Mills 2003; Lee 2016). I will attempt here to describe how research on cannabinoids, the archetypal bioactive constituents of this plant, started and evolved, doing so from the viewpoint of the chemists who worked in the area before the mid Sixties,

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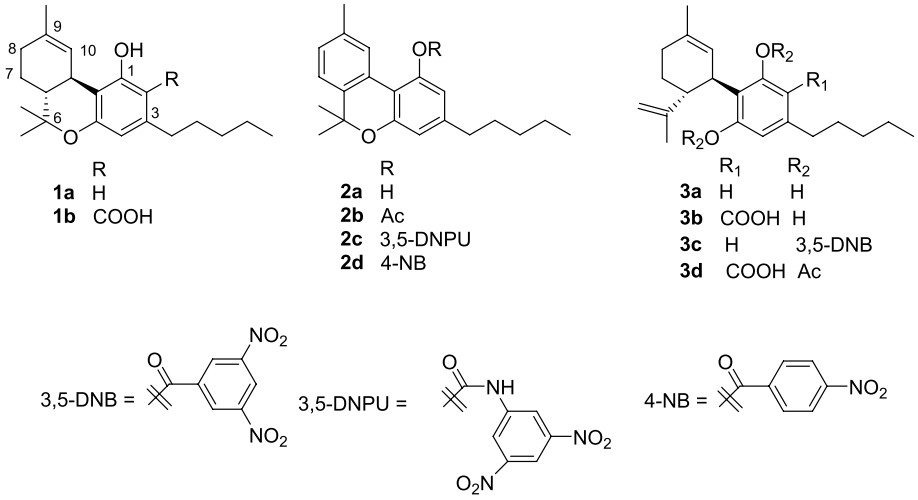
when the seminal contribution of Mechoulam were published (Mechoulam and Shvo 1963; Gaoni and Mechoulam 1964).



The identification of Cannabis as a narcotic plant is associated to the British and French colonial expansion of the nineteenth century, whose penetration in India and North Africa led to the discovery of intoxicating strains of a plant otherwise known in Europe essentially as a source of cordage and cloth (Mills 2003). In those times, the distinction between curative and recreational use was blurred. Abuse of opium was not considered basically different from the one of alcohol, and was associated to social stigma rather than to legal regulation or prohibition. For this reason, interest for Cannabis was, at the outset of the studies, genuinely medicinal, and not forensic, since the therapeutic uses of the plants were also well documented, especially in India (O'Shaugnessy 1843). In the wake of the purification of morphine by Sertuerner in 1817, the first half of the nineteenth century witnessed attempts to replace plants with their active principles, as exemplified by the discovery of quinine, nicotine, emetine, and papaverine (Drobnik and Drobnik 2016). All these compounds show basic properties, and can be obtained from plant material with an acid–base partition scheme, something that was not possible with cannabinoids, at least for the neutral ones contained in the narcotic preparations that were available in Europe from India and Africa. In those times, distillation was another well-established shortcut for the isolation of bioactive plant products, with menthol having been purified as early as 1771 (Drobnik and Drobnik 2016), but cannabinoids are poorly volatile compounds with similar boiling point, and their distillation requires high vacuum and has low resolution power. It is therefore unsurprising that the many nineteenth century investigations on cannabis substantially missed the identification of its intoxicating principles. Paradoxically, cannabinoids in their native acidic form can be obtained from cannabis biomass with a simple acid–base partition scheme (Krejčí and Šantavý 1955), and acids, even complex ones, were the first natural products to be purified from plants (Drobnik and Drobnik 2016). Given the crystalline status of some acidic cannabinoids, included THCA-A (**1b**), cannabinoids in their native form could therefore have been isolated already at the very outset of the studies on Cannabis. However, the only Cannabis of interest in those times was the narcotic/medicinal material that was produced overseas and arrived in Europe variously named (hashish, bhang, charas, ganja) depending on the country of origin and the mode of preparation, and essentially already decarboxylated. The isolation of the native cannabinoids had therefore to wait investigations aimed at a different end-point (antibacterial activity) and based on a locally produced biomass (Krejčí and Šantavý 1955).

In the lack of a suitable animal model for the sedative and narcotic activity of cannabis, the early investigators self-administered the products obtained from the plant, a vivid demonstration of how cannabis was perceived as a very safe medicine. In retrospect, the socially divisive properties of Cannabis were anticipated by the controversies on the nature of its intoxicating principle(s) that dominates the early literature of the plant. The mind-altering compounds known at those times were all alkaloids (morphine, nicotine, scopolamine, cocaine), and it seemed therefore plausible, if not obvious, that also the active principles of Cannabis were nitrogen-containing compounds. The presence of an alkaloid with strychnine-type activity and named tetanocannabin was claimed in hashish, a veritable pharmacological oxymoron given the muscle-relaxant properties of Cannabis, and a crystalline mixture of alkaloids was even commercialized

in 1896 as a safe hypnotic under the name of *Cannabine Alkaloid Merck* by the American subsidiary of the German company (Mechoulam and Hanuš 2000). The seeds of Cannabis contain elevated levels of trigonelline, the crystalline methyl betaine of nicotinic acid, and the Merck cannabine might well have been trigonelline, a compound, in any case, devoid of CNS activity. The isolation of nicotine from Cannabis, another pharmacologic oxymoron in the light of the stimulating activity of this alkaloid on memory and attention, was probably related to the recreational consumption of a mixture of cannabis and tobacco (Mechoulam and Hanuš 2000). Interestingly, this is still the most popular form in which marijuana is consumed in Europe, while in USA (ab) use in purity is preferred.

The non-basic nature of the narcotic principle of Cannabis was suggested as early as in 1847 by the work of the Smith brothers, a couple of Scottish pharmacists (Smith and Smith 1847). After extraction of Cannabis with alcohol, evaporation and purification by sequential depigmentation with lime and removal of any basic compound by washing with sulfuric acid, a resinous material endowed with marked narcotic properties was eventually obtained (Smith and Smith 1847). Nevertheless, duplication of the results of the Smith brothers turned out difficult, and this fueled alternative views on the nature of the intoxicating principle of Cannabis. The early studies were, indeed, plagued by the poor quality of the material investigated, mostly imported directly, or via Egypt, from India, and by the lack of a diagnostic animal model of activity, like the Straub test for morphine. As a matter of fact, the dog catalepsy assay for Cannabis activity was only optimized by Lowe in the 1940s, and the development of the mouse tetrad assay cogently demonstrates that no rodent single experiment can be predictive of "cannabinoid" activity (Pertwee 2006).

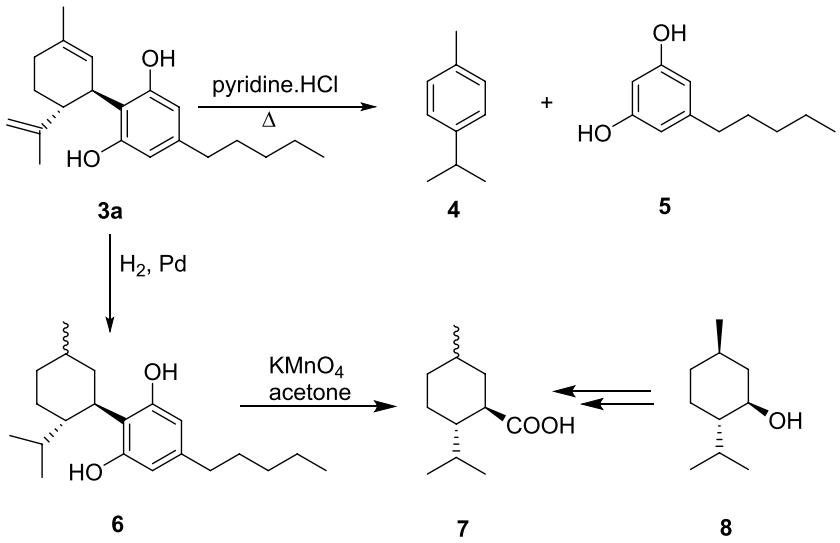
Eventually, a material dubbed "red oil" was found by a Cambridge group (Wood, Spivey and Easterfield) to consistently summarize the intoxicating properties of cannabis (Wood et al. 1896). The red oil, basically a distilled Cannabis resin, was the starting material for all early studies on Cannabis, despite the difficulty of its preparation. It was obtained by distilling under reduced pressure (3 mm) an ethanol or ether Cannabis extract, collecting vapors boiling between 100 and 220 °C, corresponding to a bath temperature of 170–300 °C. The ruby red distillate could be additionally purified by washing with water and by fractionate distillation under reduced pressure. An inactive fraction composed by the crystalline hydrocarbon nonacosane was first obtained, with the purified active fraction (ca. 2% yield) boiling at 175–195 °C and 2 mm pressure. The preparation of the "red oil" was technically demanding, and great care was necessary to keep foaming under control during the distillation (Adams et al. 1940a). Red oil had a relatively narrow boiling point, range, and could be obtained also from various cannabis-based commercial products. It was therefore at first considered a pure compound. The ruby red color developed already during the first distillation step and intensified with light. It could have been related to the formation of quinoid forms of the native cannabinoids during heating (Caprioglio et al. 2020). After acetylation of the red oil, the Cambridge group, led by Thomas Hill Easterfield (1866–1949), obtained an optically inactive crystalline compound (**2b**), whose native phenol was named cannabinol (CBN, **2a**) (Wood et al. 1899), recycling the name previously given to the narcotic red oil (Wood et al. 1896). Easterfield had received his PhD working with Emil Fischer in Wuerzburg with a dissertation on citrazinic acid, a derivative of citric acid, and in those years was holding a lecturer position at Cambridge University. Some dramatic events occurred in the course of these studies, eventually leading to their abrupt end. Thus, Spivey, a collaborator of Easterfield and one of the authors of the cannabinol publications, died in a laboratory accident during a large-scale Etard oxidation in the course of studies on the structural characterization of an elusive "oxycannabine" that a competitor group had reported from the red oil (Wood 1902), while a Cambridge colleague, C. R. Marshall, while taking care of a distillation of diethylzinc, a highly inflammable liquid, ingested a ca. 100 mg dosage of cannabinol to fight the boredom of distillation and to assess if the compound was narcotic.[1] After ca 45 min

[1] In relation to the accident occurred to Marshall, one cannot help quoting this marvellous observations by Primo Levi from the chapter Potassium of *The Periodic Table* (Einaudi, Torino, 1975, p. 56): *Distillare è bello. Prima di tutto, perché è un mestiere lento, filosofico e silenzioso, che ti occupa ma ti lascia tempo di pensare ad altro, un po' come l'andare in bicicletta. Poi perché comporta una metamorfosi: da liquido a vapore (invisibile), e da questo nuovamente a liquido; ma in questo doppio cammino, all'in su ed all'in giu', si raggiunge la purezza, condizione ambigua ed affascinante, che parte dalla chimica ed arriva molto lontano… e finalmente, quando ti accingi a distillare, acquisti la consapevolezza di ripetere un rito ormai consacrato da secoli, quasi un atto religioso, in cui da una materia imperfetta ottieni l'essenza, l'usia, lo spirito, ed in primo luogo l'alcool, che rallegra l'animo e riscalda il cuore*" (Distilling is beautiful. First of all, because it is a slow, philosophic and silent occupation, which keeps you busy but gives you time to think of other things, somewhat like riding a bike. Then, because it involves a metamorphosis: from liquid to vapour (invisible) and from this, once again to liquid, but in this double journey, up and down, purity is attained, an ambiguous and fascinating condition, which starts from chemistry and goes very far. And finally, when you set about distilling, you acquire the consciousness of repeating a ritual consecrated by the centuries, almost a religious act, in which from imperfect material you obtain the essence, the *usia*, the spirit, and in the first place the alcohol, which gladdens the spirit ad warms the heart, Translation by Raymond Rosenthal, Shocken Books, New York, 1984, Chap. Potassium, pp. 60–61). What happened to Marshall is a vivid testimony that "*pensare ad altro*" (think of other things) while distilling can inspire dangerous ideas. In 2006, *The Periodic Table* by Primo Levi was voted "the best science book ever written" at an event organized by the Royal Institution in London.

he was found wondering aimlessly in the lab unable to stop giggling and repeating "this is lovely" while flames were developing around him because oxygen had leaked into the distillation flask and had set fire to diethylzinc. The prompt intervention of colleagues avoided a disaster and Marshall recovered promptly (Mills 2003). These events were exaggerated in biased anti-cannabis publications that appeared in the US during the 1930s, claiming that two of the discoverers of cannabinol had died in laboratory experiments related to these researches, while a third one had suffered severe injuries during these studies.

The death of Spivey abruptly ended research on cannabinoids in Cambridge, and there was no follow-up to the article reporting the isolation of "cannabinol" as red oil and "cannabinol" as a pure compound. In 1899 Easterfield moved to New Zeeland, where he established a natural product school still preeminent today, continuing there his studies on tutin, the toxin of tutu, an issue of topical relevance in New Zeeland because of its involvement in honey poison, a plague for this country. In their first publication (Wood et al. 1896), the Cambridge group claimed that cannabinol, as red oil, was narcotic, but, after recognizing that it was a mixture, and that molecular cannabinol was not narcotic, they clarified the situation in a communication presented at a Meeting of the Chemical Society in 1898 (Mills 2003). This, however, was only mentioned on a note that appeared in the *Pharmaceutical Journal* (Mills 2003) that largely escaped the radars of the chemical community. A confusing period next followed. The first origin of this confusion was, unsurprisingly, associated to the name cannabinol itself that was transferred from the active red oil fraction to its inactive constituent (Wood et al. 1899). In later studies, the name cannabinol was therefore used to refer to both the red oil and to the pure compound contained in it, with obvious and unavoidable confusion, even though bioactivity is not mentioned in the 1899 report on the purification of cannabinol (Wood et al. 1899). A second cause of confusion was related to the cannabis source. CBN (**2a**) is not a genuine natural product, but an artefact formed by the autoxidation of Δ^9-THC (**1a**) and, possibly, other cannabinoids as well (Caprioglio et al. 2019). Its high contents in the red oil investigated by the Cambridge group could have been related to either the use of an old sample of hashish or, alternatively, to a harsh treatment of the resin. It is therefore hardly surprising that many investigators found it difficult to reproduce the isolation of the crystalline acetate of CBN (**2b**) from the red oil, and that confusion arouse also on its optical rotation, that was reported as highly negative by some researchers, who, in hindsight, could have obtained the cannabidiol (CBD, **3a**) rather than cannabinol (CBN, **2b**). An additional confounding factor was the introduction of efficient fractionation columns, like the Widemer Column, that allowed a better purification of the red oil, retaining for instance the polyol quebrachitol into the lower part of the column (Adams et al. 1940a). Clarity was finally done only 3 decades after the original isolation of cannabinol, when Robert Sidney Cahn (1899–1981), of nomenclature and stereochemistry fame, investigated the structure and bioactivity of cannabinol (Cahn 1933). He proposed to refer to the red oil as "crude cannabinol", reserving the name "cannabinol" to the pure compound that he demonstrated was devoid of intoxicating properties. Using degradation studies, the only strategy available that time for structure elucidation, Cahn established a dibenzopyran structure for CBN, with the relative position of the *n*-pentyl and the phenolic hydroxyl on the resorcinol moiety undefined. The uncertainty between the two formulas was eventually independently solved in the early Forties by Roger Adams (1889–1971) at the Illinois State University and by Alexander R. Todd (Baron Todd, 1907–1997) at the University of Manchester.

Adams was the most important American organic chemist of the first half of the past century, and had developed strong ties with govern agencies as well as with private companies, making it possible to assemble a large group of researchers at the University of Illinois at Urbana-Campaign, where he tutored 184 Ph.D. students, countless master's and bachelor's candidates and hired at least 50 post-docs (Tarbell and Tarbell 1981). He had worked extensively on biphenyls and their atropisomerism, and had just completed the study of gossypol, the binaphthyl toxin of cotton (Tarbell and Tarbell 1981). Cannabinol is a biphenyl derivative, and the intoxicating principle of cannabis could have also been a related compound. Therefore, when in the Spring of 1939, less than 2 years after the infamous Marijuana Tax Act that had made de facto cannabis illegal in the USA, Adams was asked to identify the intoxicating principle of marijuana by the Bureau of Narcotics of the US Treasury Department, the choice could not have been better. On the other hand, the origin of the plant material he received (fiber hemp from Minnesota) testifies the confusion reigning in those years on Cannabis in the US regulatory offices. Since fiber hemp is poor in Δ^9-THC (**1a**), the precursor of CBN (**2a**), Adams had significant difficulties in the isolation of cannabinol (CBN). Unable to obtain a direct crystallization of CBN from the red oil by acetylation, Adams tried other acylating reagents, eventually obtaining a crystalline bis-(3,5-dinitrobenzoate) (**3c**), that, after hydrolysis, afforded, however, a cannabinoid different from cannabinol, that Adams named cannabidiol (CBD, **3a**) (Adams et al. 1940b). Adams succeeded to obtain cannabinol only after a laborious purification of the mother liquors from the crystallization of cannabidiol bis-(3,5-dinitrobenzoate) (**3c**). Thus, after hydrolysis of the phenolic esters remaining in the mother liquors, any residual CBD was fragmented by pyrolysis with pyridinium hydrochloride into *p*-cymene (**4**) and olivetol (**5**), a reaction that played a critical role in the structure elucidation of CBD (Scheme 1).



Scheme 1 Key reactions used by Adams for the structure elucidation of cannabidiol (CBD, **3a**)

The residue was next distilled again, and crystallization was induced by treatment with 3,5-dinitrobenzoylazide, eventually obtaining a the crystalline phenylurethane of cannabinol (**2c**) (Adams et al. 1940a). In the meantime, on the other side of the Ocean, Todd, in London and then in Manchester, had continued Cahn's investigations, and, by working initially on an hashish sample from India, he had discovered that *p*-nitrobenzoyl chloride could remove almost all cannabinol from the red oil as the ester **2d** (Work et al. 1939), making it possible the isolation of another cannabinoid from the mother liquors (Jacob and Todd 1940a, b). The material on which Todd was working was a sample of hashish that has been obtained in India by Franz Bergel, an Austrial political refugee and colleague of Todd in London. Bergel had sent the sample to Europe in a diplomatic bag, and had then carried it through the customs in a suitcase. After the publication of the 1939 paper, Todd had to register at the Home Office as a holder of 2.5 kg of hashish, and was requested to provide 25 reprints of any papers on the topic to the Bureau of Drugs and Indecent Publications (!) (Brown and Kornberg 2000). It is surprising how young Todd (32 year old at the publication of his first study on hashish), with a small research group and in the disastrous first years of England during WWII, could successfully compete with Adams, but he had previously already competed with the powerful group of Karrer in Zuerich on the chemistry of vitamin E, and was not, therefore, new to these challenges (Brown and Kornberg 2000).

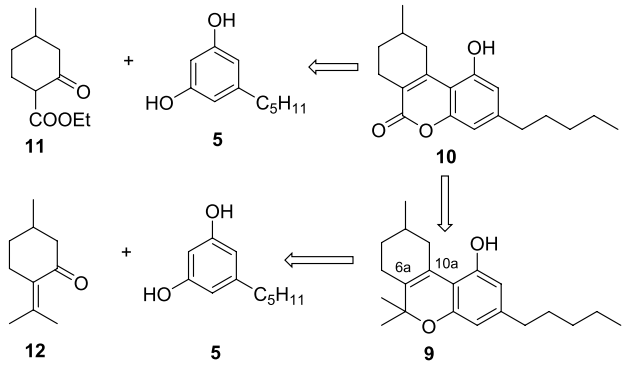
The note by Adams on the isolation of CBD was submitted to the *Journal of America Chemical Society* (*JACS*) on December 4, 1939, (Adams et al. 1940a, b, c, d, e, f, g) and the first one by Todd on this compound was published in *Nature* on March 2, 1940 (Jocob and Todd 1940a), without any detail apart from the positive Beam test of the compound. This curious reaction (the development of a purple color upon treatment of cannabis with ethanolic KOH) had been reported in 1911 and was the standard forensic test to identify hashish and marijuana (incidentally, THC does not produce a color reaction, and the Beam test actually reveals non-narcotic cannabinoids like CBD and CBG) (Beam 1911). Full details of the isolation of CBD from an Egyptian sample of hashish were given by Todd in a full paper that was received by the *Journal of Chemical Society* on March 28th of the same year (Jacob and Todd 1940a, b).

The structure of CBN was independently established by both Adams and Todd, who both capitalized on the work of Cahn, while the structure of CBD was established by Adams. Adams synthesized both proposed formulas for CBN, finally clarifying the structure of this compound (Adams et al. 1940c). The two groups had exchanged information, but clashed when they both discovered that the treatment of CBD with acids affords a narcotic cyclization mixture. Adams submitted a short (three pages long) article to *JACS* on July 23, 1940, to report this finding (Adams et al. 1940a, b, c, d, e, f, g). The note was published in the September issue of the Journal, along with an additional Communication to the Editor where marijuana-like activity was claimed for the cyclization mixture (Adams et al. 1940d). The same observations had also been independently obtained by Todd at Manchester, and their article was submitted in January 1941 and published in the *Journal of the Chemical Society* 3 months later (Ghosh et al. 1941). The appearance of this article prompted a more detailed publication by Adams, who bitterly remarked that "*it was assumed that the discovery and publication especially of this last observation would allow us a certain priority in the study of synthetic analogs and homologs of the tetrahydrocannabinols without competition*" (Adams et al. 1941a). Later on, the two great scientists became close friends, collaborating on the recovery of the German Chemical Society after WWII and the

continuation of the Beilstein and Gmelin, the very important chemical encyclopedias. It is, in any case, unclear how strategic research on cannabis actually was for Todd, who had already started the studies on nucleosides and nucleotides that eventually led him to the Nobel Prize in chemistry. It is remarkable how quickly both groups came to these seminal discoveries on the isolation of cannabinol and its transformation into a narcotic mixture of tetrahydrocannabinols. In particular, Adams started his studies in the spring of 1939, and obtained sufficient data to publish 23 additional articles on the topic in the following years. Todd produced, overall, ten original publications on the chemistry of cannabinoids. Comparison of the contributions by Adams and Todd shows a striking cultural divide, not only in technology, but also in overall thinking. Todd, one generation younger, was making extensive use of chromatography and spectroscopy (UV and IR), while Adams was essentially relying on crystallization and distillation to purify compounds, and on the logic of reactivity for structure elucidation and on UV for structure elucidation. Adams produced five articles on the structure of CBN (numbered I–V), eight articles on the structure of CBD (numbered I–VIII), and eleven articles on the structure–activity relationships of tetrahydrocannabinols (numbered IX–XIX). This production is even more surprising when one considers that 20 out of these 24 articles appeared in 3 years only (1940–1942), along with the "polishing" articles on the structure of gossypol, and the initial articles on the structure of the Senecio alkaloids. Furthermore, Adams was taking care of research at Urbana-Champaign from his office in Washington, where his public service obligations requested his presence. Some of Adams's articles are written in an *Organic Synthesis* style (Adams established this set of volumes): they are a joy to read for the precision and details of the experimental reported, including the detail description of the apparatus used for the preparation of special reagents, like phenyl lithium (Adams et al. 1940g) but lack the discussion on the mechanistic and biogenetic logic that characterize the cannabis articles by Todd. Todd is mentioned to have shared Windaus's view that organic chemistry was not the chemistry of carbon, but the one of natural products (Brown and Kornberg 2000) while Adams, in his lack of interest for chromatography was probably sharing the view expressed by Robinson when paper chromatography started to be used in the Cambridge laboratories (There are no chemists there, just a lot of paper hangers) (Brown and Kornberg 2000).

Adams elucidated only partially the structure of CBD, since location of the olefin double bond and the configuration at the two stereogenic centers remained unassessed (Adams et al. 1940f). The key reactions that led to the gross clarification of the structure of CBD were (Scheme 1) the pyrolytic β-elimination to *p*-cymene (**4**) and olivetol (**5**), that deconstructed in biogenetic terms the compound into its terpene and ketide moieties, and the permanganate degradation of the tetrahydroderivative of CBD (**6**) to *p*-menthane-3-carboxylic acid (**7**), a compound that could also be prepared from menthol (**8**). This clarified the site of junction of the two biogenetic moieties, while the position of the two alkyl residues of the resorcinol moiety was established as para by comparison of the UV spectra of two model compounds prepared from the addition of a lithiated resorcinol to menthone (Adams et al. 1940g).

Having fully elucidated the structure of CBN and partially the one of CBD, Adams went on to address the issue of the identification of the narcotic principle of Cannabis, collaborating with the pharmacologist Walter S. Loewe, a European refugee based at the Cornell Medical School. The dog catalepsy assay, an assay requiring far more material than the one used by Todd (disappearance of the corneal reflex in rabbit, the Gayer assay) was used as biological end-point. The Gayer assay was found unreliable by Adams, who found a different bioactivity profile for the compounds prepared by Todd when the biological end-point was changed to the dog ataxia test (Adams et al. 1942), and the lack of a reliable bioassay could be an additional reason as to why Todd did not systematically pursued the structure–activity relationships of $\Delta^{6a,10a}$-THC (**9**). Adams, just like Todd, did not manage to identify the intoxicating principle of cannabis that would have required more advanced chromatographic and spectroscopic techniques than those available in the Forties. Nevertheless, both researcher suggested that the active principle of cannabis could have been a mixture of the two compounds formed in the acidic degradation of CBD. Neither of them was crystalline, but Adams could obtain them as single compounds by using different reaction conditions, distinguishing a "high rotating" ($[\alpha]_D = -240$) and a "low rotating" ($[\alpha]_D = -165$) isomer, corresponding to what are now known, respectively, as Δ^8- and Δ^9-THC (**1a**) (Adams et al. 1940d). The structure of the two tetrahydrocannabinols obtained from CBD by Adams suffered from the same structural uncertainties of their starting material, that is, the location of the endocyclic double bond and the configuration of the two stereogenic centers. Adams made some confused suggestions on the location of the double bond in these compounds, curiously locating it at all possible endocyclic position except the correct one (Adams et al. 1940f). Adams was, this time correctly, convinced that the cyclized derivatives of CBD were the narcotic principles of cannabis, and its failure to isolate it could be, technical reasons aside, also related to the origin of his plant biomass, fiber hemp rather than marijuana. Eventually, Adams decided to focus on the tetrahydrocannabinols from the cyclization of CBD as lead structures to study the structure–activity relationship of narcotic cannabinoids. Capitalizing on his previous work on the synthesis of cannabinol, he could easily prepare an isomer of the semi-synthetic mixture of active tetrahydrocannabinol. This compound (**9**) had a double bond at the terpenyl-pyrane

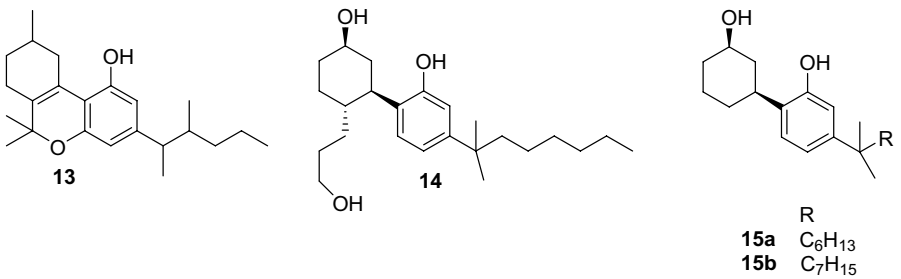


Scheme 2 Synthesis of $\Delta^{6a,10a}$-tetrahydrocannabinol by Adams and by Todd

junction ($\Delta^{6a,\ 10a}$ in the current nomenclature) and was devoid of the configurational issues that plagued the tetrahydrocannabinols obtained from CBD. As to the endocyclic double bond of uncertain location, the lead tetrahydrocannabinol structure devised by Adams was devoid of it, and was a racemate rather than a possible mixture of regioisomeric olefins. The synthesis used by Adams capitalized on the Pechmann coumarin synthesis, and offered the possibility of modulating the nature of the alkyl substituent of the resorcinol moiety, and starts (Scheme 2) with the condensation of olivetol (**5**) with the β-ketoester **11**. The racemic coumarin obtained in this way (**10**) was then treated with an excess methyl magnesium bromide to obtain racemic $\Delta^{6a,10a}$-tetrahydrocannabinol (**9**). This compound retained 10% of the activity of the CBD-derived tetrahydrocannabinols in the dog ataxia assay, the biological end-point used by Loewe (Adams et al. 1941b). The same tetrahydrocannabinol was prepared by Todd in a more direct way, condensing olivetol with pulegone (**12**) (Ghosh et al. 1941), but Todd did not exploit the reaction for systematic structure–activity studies.

Conversely, Adams prepared various derivatives where the *n*-pentyl group of the natural product was replaced by linear chains of different length as well as by branched alkyl residues (Adams et al. 1942). In this way, Adams identified in six carbons the ideal length for a linear alkyl group, while branching at the benzyl carbon led to a significant increase of potency, with modifications of the methyls at C-6 and C-9 being all detrimental for activity (Adams et al. 1948a, b).

The remarkable increase of potency observed with the methyl branches at the benzyl carbon, eventually resulting in extensive investigation of the 1,2-dimethylheptyl analogue of $\Delta^{6a,10a}$-tetrahydrocannabinol (pyrahexyl, **13**) that was several hundreds of times more potent than the pentyl analogue (Adams et al. 1948a, b). This compound has three stereogenic centers, and was prepared and assayed by Adams as a mixture. A vivid record on the extraordinary potency of pyrahexyl was provided by one of Adams post-docs (E. H. Volwiler), who described what occurred to him 1 day in 1943 after ingesting a 15 mg dose of pyrahexyl (Tarbell and Tarbell 1981). *…At 4.45 pm took one capsule, went home on train.. at 7.00 rose from the (dinner) table. Very suddenly my legs felt numb: it was 7.03. My mind felt fuzzy, disoriented… My wife talked to me. Occasionally she asked me a question but by the time she has said the last word, I had forgotten the question before I could answer. Time stood still: after what seemed like the passing of hours, the clock showed that only a minutes or two had gone by. My mouth was intensely dry; my tongue felt several times its usual size. I found myself eating several pieces of candy, but had no recollection of having picked them up. By 7.15, I began to feel that my sanity might have been impaired, and questioned whether I had really taken a capsule at all. Doubt entered my head that I would recover; my anxiety increased. Finally, at about 8.00 pm, when the symptomes were at their worst, I asked my wife to phone Dr. Carter to determine whether I had actually taken a capsule in his presence…*



For reasons probably related to the progressive involvement of Adams into war-related research themes and his growing governmental tasks, research on cannabinoids ceased in the Urbana-Champaign laboratories in 1943, with data on the ultrapotent analogues reported later, in 1948 (Adams et al. 1948a, b). CBD was found inactive in the dog ataxia assay, but Loewe made the interesting observation that it could significantly prolong barbiturate-induced sleep in mice (Loewe 1944). The mechanism of this activity was clarified 3 decades later, when it was discovered that CBD, but not THC or CBN, is a powerful inhibitor of several classes of liver microsomes (Paton and Pertwee 1972).

The research of Adams was interrupted by the evolving commitment of the USA in WWII, with research on antimalarial compounds and synthetic rubber taking over in his laboratories. Furthermore, at the end of the war, Adams was involved with the US efforts to rebuild German and Japanese science, a commitment that delayed its return to the chemical research that in the late Forties was then growingly dominated by the younger generation of organic chemists exemplified by Woodward. Curiously, when WWII broke, Adams, despite these official commitments, was suspected by the FBI of anti-patriotic activities because of his studies on cannabinoids and because the anti-Nazi organization *Lincoln's Birthday Committee for Democracy and Intellectual Freedom*, to which Adams was effectively associated, was confused with the communist group *Lincoln's Birthday Committee*, to whom Adams was completely unrelated. Although the confusion was clarified, Adams was seemingly never fully trusted by FBI (Tarbell and Tarbell 1981). The correspondence between Adams and Loewe on their research on cannabinoids has been presented, but this hefty (several hundred pages) documentation has not yet been analyzed. As a member of a family who had given a Presidents to US, Adams' political views were definitely conservative. He did not approve the student protests of the 1960s, but appeared as a defense witness of a student charged for the possession of marijuana. His declaration that he doubted marijuana could be considered really narcotic seemingly helped the acquaintance of the student, but Adams always refused to testify for the defense in cases where the charge was a trade of marijuana (Tarbell and Tarbell 1981).

With the USA entering the Cold War era, the potent activity of pyrahexyl (**13**) did not go unnoticed by the Army that included this compound in a development program for incapacitating chemical weapons (Williams and Himmelsbach 1946). The aim of this program was to develop compounds endowed with a "couch lock" effect, that is, non-lethal agents that could be sprayed from airplanes beyond enemy lines to incapacitate soldiers. For this reason, pyrahexyl, renamed dimethyl heptylpyran (DMHP) and assigned code number EA-2233 as the mixture of its eight stereoisomers, was included in the project on chemical weapons that run from 1948 to 1975 at the Edgewood Arsenal in Maryland. In a remarkable effort of resolution and asymmetric synthesis, all eight stereoisomers of DMPH were synthesized, given individual codes EA-2233-1 through EA-2233-8, and investigated for bioactivity. EA-2233-2 was the most potent isomer, and could already induce confusion, sedation and hallucinogenic effects at a dosage of 0.5–2.8 μg/kg, corresponding to 35–200 μg for a 70 kg adult. In general, an oral dosage of EA-2233 of 1–2 mg was sufficient to make all volunteers incapable of performing coordinate activities, like those requested for military action, for as long as 2–3 days (Ketchum 2006a, b). In terms of acute toxicity, pyrahexyl was relatively safe, with a 2000 therapeutic index in laboratory animals, but could occasionally induce severe hypotensive crises and hypothermia, and was not eventually weaponized, also because of the discovery of more efficacious and safer anticholinergic agents from the quinuclidinyl benzilate series, like the infamous BZ (3-quinuclidyl benzylate) (Ketchum 2006a, b).

The seminal discovery by Adams that branched alkyl substituents magnify the narcotic potency of cannabinoids played a critical role in the identification of cannabinoid receptors. Due to its high lipophilicity, natural THC interacts strongly with biological membranes, making it difficult to detect specific binding. In the Seventies, by capitalizing on the observations of Adams, researches at Pfizer developed ultrapotent analogues of Δ^9-THC, one of which, CP-55-940 (**14**), was used in a tritiated form, to proof the existence of specific recognition sites for THC, and played a seminal role also for the discovery of endocannabinoids (Di Marzo 2018). These compounds are known with the code name of CP-*xx*, *xxx* (*x* = number), CP referring to C. P. Pharmaceuticals, a subsidiary of Pfizer. One of the most popular compound of this series is cannabicyclohexanol (CP-47,497, **15a**) (Mechoulam and Hanuš 2000). In 2009, its dimethyl octyl analogue [(C8) CP-47–497, **15b**] was detected, as a mixture of *cis*- and *trans*-stereoisomers, in a sample of an herbal incense (spice) in Germany, and was the first synthetic cannabinoid discovered in the illegal market (Appendino et al. 2014). The pharmacological evaluation of this compound showed that it outperformed CP-47,497, a surprising observation since within methyl cannabinoids, potency peaks at the 1,1-dimethylheptyl derivatives (Appendino et al. 2014).

With cannabis and THC officially enlisted in the narcotic drugs, scientific interest for cannabinoids ceased almost completely in Western countries. On the other hand, studies on hemp led to remarkable new findings. The most significant studies on cannabinoids in the years 1945–1960 were carried out at the Palacký Olomouc University (Czechoslovakia), by Zdeněk Krejčí (1923–1992), and František Šantavý (1915–1983). The context in which this research developed was the one of the Cold War. The Forties and the Fifties were the golden age for the discovery of antibiotics,

whose introduction in therapy was mainly related to industrial and not academic research. In Socialist countries, to by-pass patents related to antibiotics, a systematic search for plant sources of antibacterial agents was pursued. In the course of the studies carried out at Olomouc University, more than 3000 indigenous plant species were investigated for antibacterial activity, identifying extracts from fiber hemp as most promising candidates for clinical use, with remarkable efficacy against tuberculosis lesions. The active principle was purified from crude extracts of the plant by treatment with Na_2CO_3, and was obtained as an amorphous gum from which a crystalline diacetate was obtained (Krejčí and Šantavý 1955). The amorphous compound was identified as cannabidiolic acid (CBDA, **3b**), a carboxylated version of CBD (**3a**) that afford a crystalline diacetate (**3d**). Its structure was assigned based on a detailed analysis of the IR and UV spectra, correctly locating the carboxylate group on the resorcinolic moiety. Cannabidiolic acid showed antibacterial activity comparable, or even better, than the one of penicillin and streptomycin against various strains of Gram positive bacteria, including *Mycobacterium tuberculosis*, but was almost inactive against Gram negative microorganisms. CBDA (**3b**) also showed powerful analgesic and anti-inflammatory activity, and a non-narcotic hemp extract named IRC was developed and clinically investigated for various otorhinolaryngology and obstetric conditions in affections resistant to standard antibiotic treatment, as well as for the management of infected wounds (Kabelic et al. 1960). Of particular relevance were the results observed with dental caries and pulp infection, in otitis, and with skin tuberculotic lesions (Kabelic et al. 1960). In a pioneering study, Šantavý established the location of the double bond and the absolute configuration of natural CBD and of Adams' THC (Šantavý 1964). Even more remarkable, these conclusions were based exclusively on data from optical rotation and IR spectroscopy, and from published reactivity data, without the need of any additional experimental result. It is a pity that this remarkable exercise of chemical logic was published in a journal of limited distribution (*Acta Universitatis Palackianae Olomucensis*) that is still substantially overlooked in the Cannabis literature. Šantavý assigned a resubstituted nature to the endocyclic double bond of CBD based on the presence IR band at 800 cm^{-1} in the IR nujol spectrum of the natural product, as independently also proposed by Mechoulam based on the NMR data (Mechoulam and Shvo 1963). He then went on to locate the double bond and assign a *trans* configuration to the two stereogenic centers capitalized on data available for natural menthol and on the trend of optical rotations in cyclohexene derivatives compared to their corresponding saturated and/or additionally cyclized derivatives. Using a similar reasoning, he then correctly assigned a *trans*-Δ^8 and *trans*-Δ^9 configuration to Adams's semi-synthetic tetrahydrocannabinols, with the Δ^9-derivative being described as a natural product the same year, albeit with an incorrect absolute configuration, by Mechoulam and Shvo (1963). One cannot help wondering why Šantavý published his studies on the structure of CBD and Δ^9-THC in a journal of limited distribution, and not, for instance, in *Collection of Czechoslovak Chemical Communication*, the flagship journal of the Czechoslovak chemical community, and a leading chemical journal well known in Western countries.

Our story terminates in 1964, when Raphael Mechoulam in Jerusalem isolated and characterized the native intoxicating principle of hashish (Gaoni and Mechoulam 1964), identifying it as the "low-rotation" semi-synthetic THC described by Adams 2 decades earlier (**1a**). The work by Mechoulam ended a century-long period of uncertainties, and by the identification of additional cannabinoids and their first synthesis, it led the foundation of what in the following decades became a vibrant area of biomedical research. On the other hand, other researcher should not be neglected for their contributions to the area. Adams and Todd were among the finest organic chemists of their generation, and Cahn and Šantavý left remarkable contributions to organic chemistry and natural products chemistry, with Easterfield almost single-handed establishing chemical research in New Zeeland. The tragic death in a laboratory accident of Spivey, one of the discoverers of cannabinol, should not be forgotten, while the Guinea-pig experiences with cannabinoids of Marshal, wondering smiling in the lab surrounded by the flames of his diethyl zinc distillation, and Leonard, unable to answer the question of his wife because it could not remember the word of her questions, vividly testifies the potent bioactivity of cannabinoids.

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Availability of data and material/data transparency Not applicable.

Compliance with ethical standards

Conflict of interest None.

Code availability ChemDraw 11 for the chemical formulas.

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