

Syntheses of  $\Delta^1$ -Tetrahydrocannabinol and Related Cannabinoids

Raphael Mechoulam,\* Paul Braun, and Yehiel Gaoni

Contribution from the Laboratory of Natural Products,  
Hebrew University Pharmacy School, Jerusalem,  
and the Department of Chemistry, Weizmann Institute of Science,  
Rehovoth, Israel. Received February 16, 1972

**Abstract:** Addition of citral to the lithium derivative of olivetol dimethyl ether, followed by reaction with *p*-toluenesulfonyl chloride, gave ( $\pm$ )-cannabidiol dimethyl ether (**1b**). Demethylation afforded ( $\pm$ )-cannabidiol (**1a**), which was converted into ( $\pm$ )- $\Delta^1$ -tetrahydrocannabinol ( $\Delta^1$ -THC) (**2**) in a low overall yield. An improved synthesis of ( $\pm$ )- $\Delta^1$ -THC was achieved by the reaction of citral with olivetol in the presence of 1% boron trifluoride etherate. Reaction of (+)- or (-)-verbenol with olivetol under the same conditions gives (+)- or (-)- $\Delta^6$ -THC (**3**), respectively. This reaction can be further improved (to 48%) by a stepwise synthesis through the intermediate 4-*trans*-(2-olivetyl)pinene (**4**). Addition of hydrogen chloride to the double bond of (+)- or (-)- $\Delta^6$ -THC, followed by dehydrochlorination, leads to (+)- or (-)- $\Delta^1$ -THC. A method for the preparation of [ $3$ - $^3$ H]- $\Delta^1$ -THC is described.

In a recent publication<sup>1a</sup> we described the isolation and the elucidation of the structures of  $\Delta^1$ -tetrahydrocannabinol ( $\Delta^1$ -THC) (**2**), the major active principle<sup>2</sup> of hashish and of related natural cannabinoids. With the completion of this portion of our research we embarked on a program aimed at facile and practical syntheses of these constituents. Some of the results achieved have been reported in preliminary communications.<sup>3</sup> In the present paper we present the full details of the research leading to the total syntheses of ( $\pm$ )-cannabidiol (**1a**), (-)- $\Delta^1$ -THC (**2**) and (-)- $\Delta^6$ -THC (**3**), as well as to the racemic and (+) modifications of the latter two THC's.

The results of our initial synthetic approach were reported<sup>3a</sup> in 1965, when we completed the first total synthesis of ( $\pm$ )-cannabidiol (**1a**) and ( $\pm$ )- $\Delta^1$ -THC (**2**). The reaction sequence is presented in Scheme I. Reaction of citral (**5**) with the lithium derivative of olivetol dimethyl ether (**6**) afforded a complicated mixture which presumably contained **7a** as one of its components. We were unable to isolate this intermediate, though several careful purifications by column chromatography were attempted. The crude mixture was therefore treated directly with *p*-toluenesulfonyl chloride in pyridine and the reaction product was chromatographed on alumina. The separation was monitored by tlc through comparison with the dimethyl ether of natural cannabidiol (**1b**). Rechromatography on 10% silver nitrate-alumina separated four compounds: 2-isopropyl-5-methyl-2',6'-dimethoxy-4'-pentylbiphenyl (**8**), mp 45–46°; 3-(2-*O,O*-dimethylolivetyl)terpinolene (**9**), mp 51–52°; ( $\pm$ )-cannabidiol dimethyl ether (**1b**); and what appears to be **10**, the 3,4-*cis* isomer of **1b**. The structures of **8**, **9**, and **10** are put forward on the basis of spectroscopic properties and analytical data (see Experimental Section).

\* Address correspondence to this author at the Hebrew University Pharmacy School.

(1) (a) Y. Gaoni and R. Mechoulam, *J. Amer. Chem. Soc.*, **93**, 217 (1971); (b) Y. Gaoni and R. Mechoulam, *ibid.*, **86**, 1646 (1964).

(2) R. Mechoulam, A. Shani, H. Ederly, and Y. Grunfeld, *Science*, **169**, 611 (1970).

(3) (a) R. Mechoulam and Y. Gaoni, *J. Amer. Chem. Soc.*, **87**, 3273 (1965); (b) R. Mechoulam, P. Braun, and Y. Gaoni, *ibid.*, **89**, 4552 (1967); (c) R. Mechoulam, B. Yagnitinsky, and Y. Gaoni, *ibid.*, **90**, 2418 (1968); (d) R. Mechoulam and Z. Ben-Zvi, *Chem. Commun.*, 343 (1969).

The overall yield of **1b** was 7%. The structure was established by comparison of the infrared, nmr, and ultraviolet spectra of ( $\pm$ )-**1b** with those of (-)-**1b** prepared from natural cannabidiol (**1a**).

Heating synthetic **1b** with an excess of dry methylmagnesium iodide<sup>4</sup> at 155–165° for 15 min gave ( $\pm$ )-cannabidiol (**1a**) in an 80% yield. The natural and the synthetic cannabidiols possess identical infrared and nmr spectra. ( $\pm$ )-Cannabidiol ditosylate melts at 138–140°; the ditosylate of natural cannabidiol melts at 81–83°. Their infrared spectra in solution are, however, identical.

The introduction in cannabinoid chemistry of demethylation by a dry Grignard reagent, as described above, has proved to be fruitful. In 1943 Adams<sup>5</sup> reported the preparation of **11** in an attempt to synthesize  $\Delta^6$ -THC (**3**). However, he was unable to demethylate **11**, as it proved too labile to both acidic and basic reagents. This approach was taken up in 1967 by Jen, *et al.*,<sup>6</sup> who were able to demethylate **11** by dry methylmagnesium iodide and complete a total synthesis of  $\Delta^6$ -THC. In 1965 Korte, *et al.*,<sup>7</sup> reported a synthesis of a ( $\pm$ )-cannabidiol dimethyl ether. However, the production of ( $\pm$ )-cannabidiol itself was made possible only later<sup>8</sup> through the use of the above demethylation procedure.

In our preliminary communication<sup>3a</sup> we reported that boiling cannabidiol with 0.05% hydrogen chloride in absolute ethanol for 2 hr gave a mixture of the starting material and ( $\pm$ )- $\Delta^1$ -THC (**2**). The overall yield of **2** in this procedure was 2%. We recently re-

(4) For a review of this reaction see H. Meerwein in "Houben-Weyl, Methoden der Organische Chemie," Vol. VI, E. Muller, Ed., George Thieme Verlag, Stuttgart, 1964, p 160.

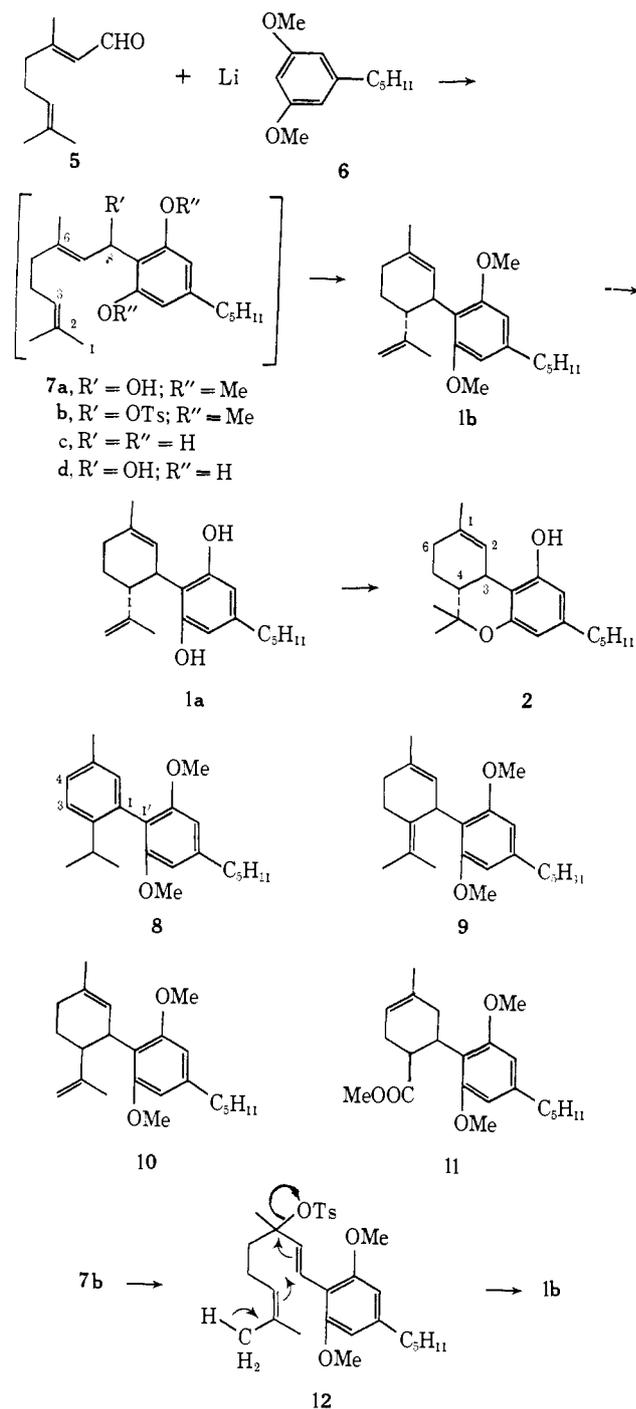
(5) (a) R. Adams and R. B. Carlin, *J. Amer. Chem. Soc.*, **65**, 360 (1943); (b) see also R. Adams and T. E. Bockstahler, *ibid.*, **74**, 5436 (1952). It is of interest to read in Adams' classical review [R. Adams, *Bull. N. Y. Acad. Med.*, **18**, 705 (1942)]: "...repeated attempts to synthesize a tetrahydrocannabinol with a double bond in the  $\gamma,\delta$ -position have failed. Just recently, however, a new approach has appeared and the results have progressed to a point where I am convinced it is merely a matter of time before the goal is reached." In retrospect it is amazing to realize that Adams was indeed one easy step away from a total synthesis of ( $\pm$ )- $\Delta^6$ -THC.

(6) T. Y. Jen, G. A. Hughes, and H. Smith, *J. Amer. Chem. Soc.*, **89**, 4551 (1967).

(7) F. Korte, E. Hackel, and H. Sieper, *Justus Liebigs Ann. Chem.*, **685**, 122 (1965).

(8) F. Korte, E. Dlugosch, and U. Claussen, *ibid.*, **693**, 165 (1966).

Scheme I



ported<sup>1a</sup> a facile conversion with boron trifluoride of cannabidiol to  $\Delta^1$ -THC in 70% yield. At the time the above synthesis of ( $\pm$ )-cannabidiol was completed, this facile formation of  $\Delta^1$ -THC was not known. The use of this procedure increased the overall yield of ( $\pm$ )- $\Delta^1$ -THC to *ca.* 4.5%. As cannabidiol has been converted<sup>9</sup> into  $\Delta^6$ -THC (3), the above synthesis represents formally also the first total synthesis of ( $\pm$ )-3.

The mechanism of the condensation-cyclization reaction (5 + 6  $\rightarrow$  1b) is not entirely clear. If the intermediacy of 7a is postulated, it is possible that the geranyl-tosyl derivative 7b can isomerize, through internal

return,<sup>10</sup> to the linalyl-tosyl derivative 12, which can then undergo cyclization to 1b. The formation of 9, however, excludes the interesting possibility of a fully concerted reaction. This is supported by the observation that 1b or 10 is not converted into 9 with *p*-toluenesulfonyl chloride in pyridine under the exact conditions of the cyclization reaction.

The mechanism outlined parallels the biogenetic pathway proposed,<sup>11</sup> namely that the naturally occurring cannabigerol (7c) is initially oxidized at the C-8 position. The oxidation product, which may but does not necessarily have to be 7d, is rearranged to a linalyl derivative (*cf.* 12). The latter can then stereospecifically cyclize to cannabidiol (1a). In this context it is of interest that the  $\Delta^6$  double bond isomer (*Z* configuration) of 7c derived from nerol has not been detected in nature, while cannabigerol (7c) (*E* configuration), which is obviously formed in the plant from geraniol, is a natural product.<sup>1a</sup>

The above described synthesis suffers from a number of disadvantages: (i) low yield; (ii) difficult separations; and (iii) production of racemic products. These drawbacks prompted us to look for improved routes.

Shortly after our publication, Taylor, *et al.*,<sup>12</sup> reported an independent, though related, synthesis: the reaction of citral (5) with olivetol in the presence of 10% boron trifluoride gave ( $\pm$ )- $\Delta^6$ -THC (3) in 10–20% yield. The ( $\pm$ )- $\Delta^1$  isomer (2) was detected by glc but could not be isolated. Two additional isomers were also obtained:  $\Delta^1$ -*cis*-THC (13) and  $\Delta^{4(8)}$ -*i*-THC (14).<sup>13</sup> In view of the relatively high yields in this synthesis, we reinvestigated it with the hope of establishing conditions under which the  $\Delta^1$  isomer could be isolated. After considerable experimentation we found that citral with olivetol in methylene chloride in the presence of 1% boron trifluoride etherate in the same solvent gave, after 1 hr, a mixture from which ( $\pm$ )- $\Delta^1$ -*trans*-THC (2) and  $\Delta^1$ -*cis*-THC (13) were isolated in 20 and 5% yields, respectively (Scheme II). This modification of Taylor's synthesis represents, in our view, the most facile preparation of ( $\pm$ )- $\Delta^1$ -THC reported so far.

The above described syntheses produce racemic products which are of limited value in psychobiological research for which compounds with the natural modification are preferred. We investigated, therefore, routes leading to optically active products.<sup>3b</sup> In view of our success in obtaining cannabinoids by the condensation of a monoterpene (citral) with olivetol, we decided to employ a modification of this method. A pinane derivative, verbenol (15), was chosen as a starting material for the following reasons. (i) Its bulky dimethylmethylene bridge was expected to provide stereochemical control of the reactions to give exclusively (or mainly) products *trans* to the bridge. The latter when opened would lead to 3,4-*trans*-cannabinoids. (ii) As optically pure verbenol from  $\alpha$ -pinene was available in both the (+) and (–) modifications, an entry into the natural (–) and unnatural (+) cannabinoid series was envisaged. Indeed,  $\Delta^6$ -THC (3),  $[\alpha]_D -245^\circ$ , was isolated

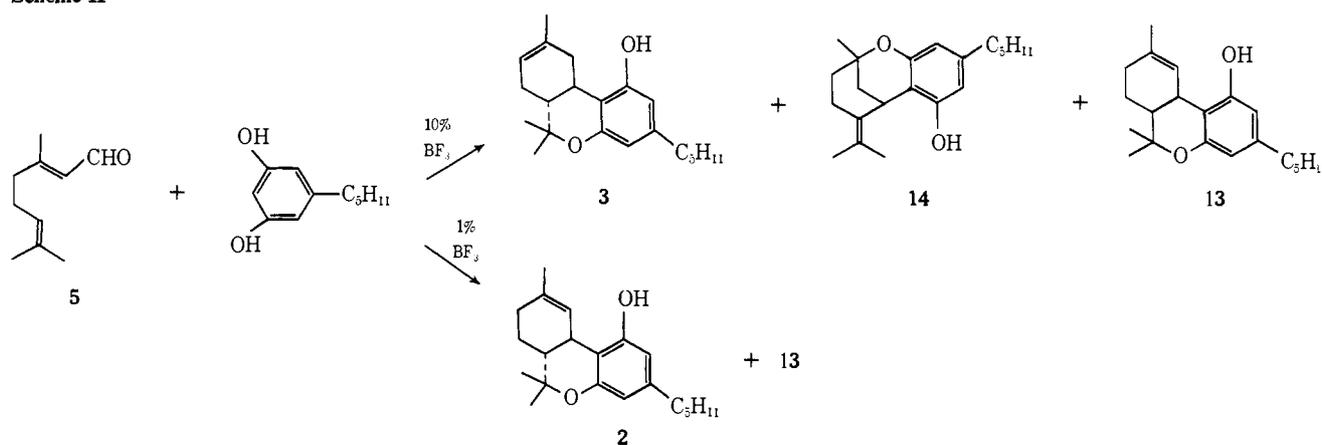
(10) W. G. Young, S. Winstein, and H. L. Goering, *J. Amer. Chem. Soc.*, **73**, 1958 (1951).

(11) R. Mechoulam, *Science*, **168**, 1159 (1970).

(12) E. C. Taylor, K. Lenard, and Y. Shvo, *J. Amer. Chem. Soc.*, **88**, 367 (1966).

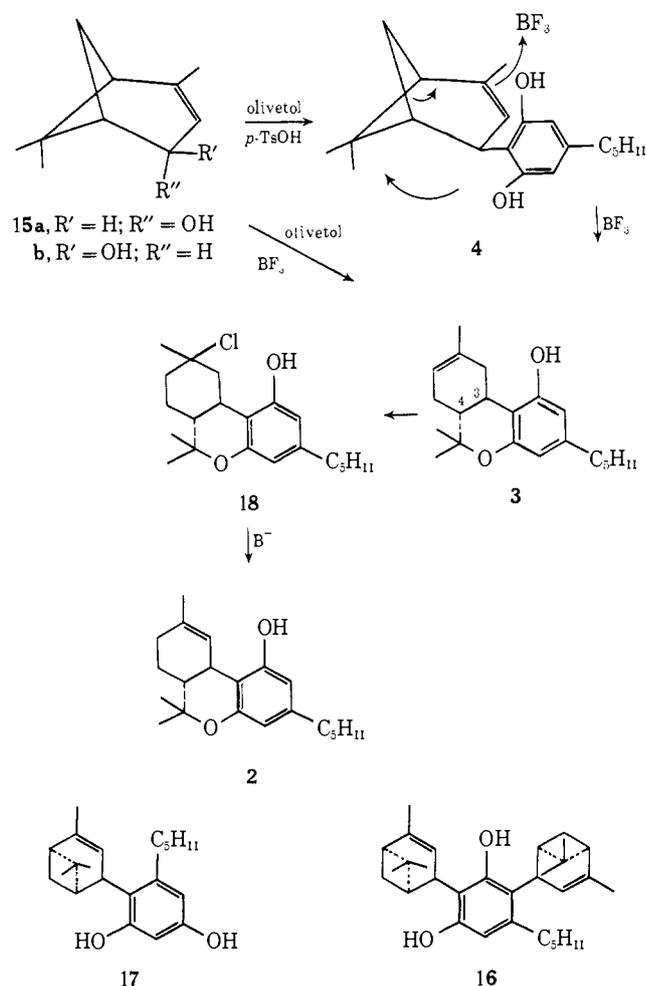
(13) (a) Y. Gaoni and R. Mechoulam, *ibid.*, **88**, 5673 (1966); (b) Y. Gaoni and R. Mechoulam, *Isr. J. Chem.*, **6**, 679 (1968).

Scheme II



directly in a 44% yield from the condensation of either (–)-*cis*- (15a) or (–)-*trans*-verbenol (15b) with olivetol when catalyzed by boron trifluoride etherate (Scheme III). This one-step synthesis of  $\Delta^6$ -THC is experi-

Scheme III



mentally facile and has the advantage of using readily available starting materials. However, the purification of the oily  $\Delta^6$ -THC to a product of *ca.* 93–96% purity requires a tedious column chromatography. This difficulty can be partially overcome by following a two-step reaction sequence. When (–)-*cis*- or (–)-*trans*-verbenol was condensed with olivetol in methylene chloride in the presence of *p*-toluenesulfonic acid, a mix-

ture was obtained which was separated by chromatography. Three products were obtained. Compound 16 (11% yield) was the least polar component. Its structure was established on the basis of its molecular weight (mass spectrum) and nmr spectrum (see Experimental Section). The major product (60% yield) of the reaction was 4-*trans*-(2-olivetyl)pinene (4),  $[\alpha]_D -87^\circ$ . The nmr spectrum of 4 shows the presence of only one olefinic methyl group (at  $\delta$  1.88), one allylic, benzylic proton (at  $\delta$  3.98), a single olefinic proton (at  $\delta$  5.69), and two magnetically equivalent aromatic protons (a singlet at  $\delta$  6.10).

Further elution gave the unstable isomeric olivetylpinene (17) in 15% yield. A stable diacetate could be prepared. The structure of 17 was deduced from its spectral data (see Experimental Section).

When *rac*-verbenol was used in the above reaction, two of the products were obtained in crystalline form, ( $\pm$ )-4, mp 100–101°, and ( $\pm$ )-16, mp 178–180°. The nmr and infrared spectra of these racemates were identical with those of the corresponding noncrystalline compounds obtained from (–)-verbenol.

Treatment of (–)-4 with boron trifluoride etherate in methylene chloride at room temperature for 10 min converted it in 80% yield into  $\Delta^6$ -THC (3) (infrared, nmr, and tlc identical with those of authentic material),  $[\alpha]_D -250^\circ$ , indicating an optical purity of *ca.* 97%. The overall yield in this two-step synthesis was 48%.

In the *p*-toluenesulfonic acid catalyzed condensation, only one 4-(2-olivetyl)pinene isomer (4) is observed, which on isomerization yields exclusively  $\Delta^6$ -THC in which the C-3 and C-4 hydrogens are *trans* (C-3 *R*, C-4 *R*). Since this transformation can hardly involve epimerization at the chiral centers, 4 must also possess the *trans* configuration. Similarly, no *cis*- $\Delta^6$ -THC was observed in the direct condensation, using boron trifluoride. The exclusive formation of *trans*-4 and *trans*- $\Delta^6$ -THC is undoubtedly due to steric factors. These considerations make us assign a *trans* configuration also to compounds 16 and 17.

As mentioned above, the reaction of either *cis*- or *trans*-verbenol (15a and 15b) with olivetol gives the same reaction products. It seems, therefore, that these reactions proceed through an identical allylic cation.

The conversion of  $\Delta^6$ -THC (3) in which the double bond is in the thermodynamically stable position to  $\Delta^1$ -THC whose double bond is easily isomerized proved to be unexpectedly difficult. After considerable experi-

mentation we found that gaseous hydrochloric acid can be added in a quantitative yield to the double bond of  $\Delta^6$ -THC at low temperature with zinc chloride as catalyst. The unstable tertiary chloride **18** obtained had been previously prepared<sup>14</sup> in the ( $\pm$ ) form by a Lucas reaction on ( $\pm$ )-1-hydroxyhexahydrocannabinol. It was also shown<sup>14</sup> that while dehydrochlorination of ( $\pm$ )-**18** with potassium hydroxide in ethanol led to ( $\pm$ )- $\Delta^6$ -THC, elimination with sodium hydride in tetrahydrofuran gave a mixture of ( $\pm$ )- $\Delta^6$ -THC and ( $\pm$ )- $\Delta^1$ -THC. It was suggested that  $\Delta^1$ -THC was produced by internal dehydrochlorination promoted by the initially formed phenolate anion. We were able to reproduce this reaction with (-)-1-chlorohexahydrocannabinol (**18**). (-)- $\Delta^1$ -THC (55% yield in the last two steps) thus obtained was identical with the natural product. After our preliminary communication had been published, Petrzilka, *et al.*,<sup>15</sup> reported an improved procedure for the dehydrochlorination step by the use of potassium *tert*-amylate, which led to a quantitative formation of  $\Delta^1$ -THC from **18**. In the subsequent use of our synthetic route we employed this new modification. In our hands the yield in this step is 90%; hence the overall yield of (-)- $\Delta^1$ -THC from verbenol is *ca.* 43%.

A number of total syntheses leading to optically active natural THC's are now known.<sup>6,16,17</sup> Those by Petrzilka, *et al.*,<sup>16</sup> by Razdan and Handrick,<sup>17</sup> and ours are based on the same principle: condensation of an optically active monoterpene with olivetol. In view of the ready availability of starting materials, the facility of experimental procedures and the reasonable yields of these syntheses make (-)- $\Delta^6$ -THC and (-)- $\Delta^1$ -THC readily available for research. It should be pointed out, however, that both these compounds can be obtained with even greater ease by partial synthesis<sup>18</sup> from cannabidiol, which is available from the decarboxylation of cannabidiolic acid, the major cannabinoid in hemp.

The above described synthesis has been employed by us for the preparation of (+)- $\Delta^6$ -THC and (+)- $\Delta^1$ -THC. The optical rotations obtained for (+)- $\Delta^6$ -THC and (+)- $\Delta^1$ -THC were +248° and +147°, respectively. Both compounds were biologically inactive when tested in monkeys at doses up to 20 times higher than those at which (-)- $\Delta^1$ -THC and (-)- $\Delta^6$ -THC showed activity.<sup>18</sup> This specificity is not surprising as most biologically active compounds show activity in one absolute configuration only.

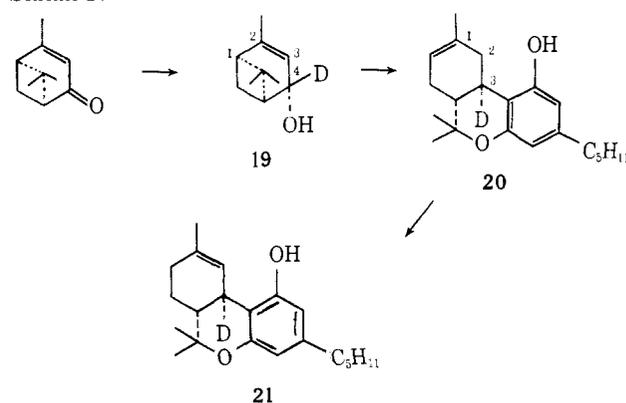
Numerous side chain homologs of (-)- $\Delta^6$ -THC and (-)- $\Delta^1$ -THC have been prepared for biological testing by the above procedure. Their synthesis will be published separately. Their biological properties have been discussed.<sup>15</sup> Recently Gill<sup>19</sup> reported the preparation by this method of the propyl homolog of  $\Delta^1$ -

THC. This active principle, named  $\Delta^1$ -tetrahydrocannabivarol, was found in some hashish samples of Pakistani origin.

Idänpään-Heikkilä, *et al.*,<sup>20</sup> have employed the above synthetic sequence for the preparation of tritium-labeled cannabinoids (at unspecified positions). We have likewise used our synthetic route for the preparation of [3-<sup>3</sup>H]- $\Delta^6$ -THC and [3-<sup>3</sup>H]- $\Delta^1$ -THC. (-)-Verbenone was reduced<sup>21</sup> with lithium aluminum deuteride to give (-)-[4-<sup>2</sup>H]-*cis*-verbenol (**19**), mp 67–69°. (-)-[3-<sup>2</sup>H]- $\Delta^6$ -THC (**20**) was obtained on condensation of **19** with olivetol. The nmr spectrum of this material was identical with that of unlabeled  $\Delta^6$ -THC except for the disappearance of one benzylic proton at  $\delta$  2.70 ppm and changes in the splitting pattern of the  $\alpha$  proton on C-2. (-)- $\Delta^1$ -THC labeled with deuterium at C-3 (**21**) was obtained from (-)-[3-<sup>2</sup>H]- $\Delta^6$ -THC. In the nmr spectrum of **21** the signal assigned to the C-3 proton in  $\Delta^1$ -THC (**2**) is missing. These observations support the nmr assignments put forward by Archer, *et al.*,<sup>22</sup> for the C-2 and C-3 protons in  $\Delta^6$ -THC which had previously been misassigned.<sup>12,13b,14,16</sup>

In the last two years a considerable amount of work has been invested into metabolic studies with the THC's.<sup>23</sup> It has been suggested<sup>11</sup> that THC metabolites are the active species on the molecular level. This suggestion has gained experimental support; the major pathway identified so far involves hydroxylation of the C-7 position leading to the physiologically active 7-hydroxy THC's. This observation will probably increase still further work on THC metabolism and metabolites. As the C-3 position is apparently not involved in metabolic reactions, the above described labeling should prove useful. Indeed, (-)-[3-<sup>3</sup>H]- $\Delta^1$ -

Scheme IV



THC with high specific activity (from reduction of verbenone with lithium aluminum tritride) can be

(20) J. Idänpään-Heikkilä, G. E. Fritchie, L. F. Englert, B. T. Ho, and W. M. McIsaac, *N. Engl. J. Med.*, **281**, 3129 (1969).

(21) M. A. Cooper, J. R. Salmon, D. Whittacker, and U. Scheidegger, *J. Chem. Soc. B*, 1259 (1967).

(22) R. A. Archer, D. B. Boyd, P. V. Demarco, I. J. Tyminsky, and N. L. Allinger, *J. Amer. Chem. Soc.*, **92**, 5200 (1970).

(23) S. H. Burstein, F. Menezes, E. Williamson, and R. Mechoulam, *Nature (London)*, **225**, 87 (1970); Z. Ben-Zvi, R. Mechoulam, and S. Burstein, *J. Amer. Chem. Soc.*, **92**, 3468 (1970); M. E. Wall, D. R. Brine, G. A. Brine, C. G. Pitt, R. I. Freudenthal, and H. D. Christensen, *ibid.*, **92**, 3466 (1970); R. L. Foltz, A. F. Fentiman, E. G. Leighty, J. L. Walter, H. R. Drewes, W. E. Schwarz, T. F. Page, and E. B. Truit, *Science*, **168**, 844 (1970); I. M. Nilsson, S. Agurell, J. L. G. Nilsson, A. Ohlsson, F. Sandberg, and M. Wahlqvist, *ibid.*, **168**, 1228 (1970); L. Lemberger, S. D. Silberstein, J. Axelrod, and I. J. Kopin, *ibid.*, **170**, 1320 (1970). See also Z. Ben-Zvi, R. Mechoulam, H. Ederly, and G. Porath, *ibid.*, **174**, 951 (1971).

(14) K. E. Fahrenholtz, M. Luric, and R. W. Kierstead, *J. Amer. Chem. Soc.*, **89**, 5934 (1967).

(15) T. Petrzilka and C. Sikemeier, *Helv. Chim. Acta*, **50**, 2111 (1967).

(16) T. Petrzilka, W. Haefliger, and C. Sikemeier, *ibid.*, **52**, 1102 (1969).

(17) R. K. Razdan and G. R. Handrick, *J. Amer. Chem. Soc.*, **92**, 6061 (1970).

(18) H. Ederly, Y. Grunfeld, Z. Ben-Zvi, and R. Mechoulam, *Ann. N. Y. Acad. Sci.*, **191**, 40 (1971).

(19) E. W. Gill, *J. Chem. Soc. C*, 679 (1971); see also F. W. H. M. Merkus, *Pharm. Weekbl.*, **106**, 69 (1971); T. B. Vree, D. D. Breimer, C. A. M. Ginneken, J. M. van Rossum, R. A. de Zeeuw, and A. H. White, *Clin. Chim. Acta*, **34**, 365 (1971).

used for radioactive tracing of the metabolites, while (–)-[3-<sup>2</sup>H]-Δ<sup>1</sup>-THC can be used in the same metabolic sample as cold material for structure identification by mass spectrometry.

## Experimental Section

**General.** The instruments, materials, and methods used for the determination of the ir, nmr,<sup>24</sup> uv, and mass spectra, as well as for glc and tlc, have been described.<sup>1a</sup> The monoterpenes used in this study were purchased from Fluka A.G. in Switzerland, or from Aldrich Chemical Co., Milwaukee, Wis., and were distilled. Olivetol was initially synthesized according to Suter and Weston;<sup>25</sup> later it was purchased from Fluka A.G., or received as a gift from Dr. A. Brossi, Hoffmann-La Roche Inc., Nutley, N. J.

(±)-Cannabidiol Dimethyl Ether (**1b**). Freshly distilled citral a (**5**) (4.56 g, 30 mmol) containing ca. 5% citral b (by nmr) in dry ether (50 ml) was added over a period of 5 min to lithium olivetol dimethyl ether (**6**)<sup>25a</sup> (30 mmol) prepared from olivetol dimethyl ether (6.24 g, 30 mmol) in ether (100 ml) and butyllithium (30 mmol) in ether (50 ml). The reaction mixture was stirred for 15 min and poured into ice, and the organic layer was separated, dried over magnesium sulfate, and evaporated. The oily mixture obtained was dissolved in a solution of *p*-toluenesulfonyl chloride (20 g) in pyridine (100 ml) and left at room temperature for 3 days. It was poured onto ice, left for 1 hr and extracted twice with ether (100 ml). The organic layer was washed first with 10% sulfuric acid until all the pyridine was removed, then with 2% sodium hydroxide solution, and finally with water. The ether solution was dried and evaporated, and the mixture obtained was chromatographed on Merck acid-washed alumina (500 g). The fractions eluted with 2% ether–pentane contained a material whose *R<sub>f</sub>* value on tlc was identical with that of (–)-cannabidiol dimethyl ether prepared from the natural product.<sup>26</sup> These fractions were combined and evaporated to give an oil (2.5 g). Half of this material was rechromatographed on Merck acid-washed alumina (250 g) containing 12% silver nitrate. Elution with ether–pentane (1:9) gave the aromatic diether **8** (0.17 g) as an oil which, after 6 years' storage, spontaneously crystallized, mp 45–46° (methanol); mol wt (mass spectrum) 340; λ<sub>max</sub> (C<sub>2</sub>H<sub>5</sub>OH) 220 (ε 18,900), 266 (sh) (ε 2100), and 273 mμ (ε 1970); nmr (CCl<sub>4</sub>) δ 0.90, 1.00 (CH<sub>3</sub>), 2.20 (aromatic CH<sub>3</sub>), 2.32–2.70 (m, 3 H, benzylic), 3.52 (s, two CH<sub>3</sub>, methoxyl), 6.22 (s, 2 H, aromatic, olivetol moiety), 6.60 (br s, C-2 H), and 7.00 (br, C-3 H, C-4 H). The ir and nmr spectra taken from the oily sample<sup>25a</sup> in 1965 and the crystalline one in 1971 were identical.

*Anal.* Calcd for C<sub>23</sub>H<sub>32</sub>O<sub>2</sub>: C, 81.13; H, 9.47. Found: C, 80.83; H, 9.37.

The uv spectrum described above differs from that published<sup>1</sup> for the same compound **8** prepared *via* a different route.

Further elution of the chromatography column with ether–pentane (1:4) gave an oil which, on distillation [bp 220° (bath temperature) (0.1 mm)] in a bulb-to-bulb apparatus, yielded **9**: 0.25 g, mp 51–52° (methanol); mol wt (mass spectrum) 342; λ<sub>max</sub> (C<sub>2</sub>H<sub>5</sub>OH) 271 (ε 1110) and 278 mμ (sh) (ε 1020); δ (CCl<sub>4</sub>) 0.9 (paraffinic CH<sub>3</sub>), 1.35 and 1.65 (3 olefinic CH<sub>3</sub>), 3.68 (s, 2 methoxyl CH<sub>3</sub>), 4.55 (br, C-3 H), 5.50 (br, C-2 H), and 6.18 (s, 2 H, aromatic).

*Anal.* Calcd for C<sub>23</sub>H<sub>34</sub>O<sub>2</sub>: C, 80.65; H, 10.01. Found: C, 80.72; H, 9.91.

Further elution with ether–pentane (3:7) gave a mixture of (±)-cannabidiol dimethyl ether (**1b**) and what appears to be the isomeric (±)-3,4-*cis*-cannabidiol dimethyl ether (**10**). This mixture (570 mg) was rechromatographed on acid-washed alumina (70 g) containing 12% silver nitrate. Elution with pentane–ether yielded numerous oily fractions, followed by several fractions containing pure (±)-cannabidiol dimethyl ether (**1b**) (359 mg). The ir, nmr, and uv spectra, as well as the tlc and glc behavior, of the compound obtained were identical with those of (–)-cannabidiol dimethyl ether (**1b**) prepared from the natural product:<sup>25</sup> λ<sub>max</sub> (C<sub>2</sub>H<sub>5</sub>OH) 271 (ε 1160) and 277 mμ (ε 1020); δ (CCl<sub>4</sub>) 0.9 (CH<sub>3</sub>), 1.48, 1.58 (2 olefinic CH<sub>3</sub>), 3.65 (s, 2 methoxyl CH<sub>3</sub>), 4.28 (d, C=CH<sub>2</sub>), 5.1 (br, C-2 H), and 6.18 (s, 2 aromatic H).

(24) Abbreviations used in presenting nmr data: triplet (t), doublet (d), singlet (s), multiplet (m), broad (br).

(25) C. M. Suter and A. W. Weston, *J. Amer. Chem. Soc.*, **61**, 232 (1939).

(26) R. Mechoulam and Y. Shvo, *Tetrahedron*, **19**, 2073 (1963); R. Adams, M. Hunt, and J. M. Clark, *J. Amer. Chem. Soc.*, **62**, 196 (1940).

*Anal.* Calcd for C<sub>23</sub>H<sub>34</sub>O<sub>2</sub>: C, 80.65; H, 10.01. Found: C, 80.92; H, 10.05.

Further elution with the same solvents gave a mixture, followed by a pure oil which was probably (±)-3,4-*cis*-cannabidiol dimethyl ether (**10**) (35 mg): λ<sub>max</sub> (C<sub>2</sub>H<sub>5</sub>OH) 272 (ε 1210) and 278 mμ (ε 1110); δ (CCl<sub>4</sub>) 0.85 (CH<sub>3</sub>), 1.40, 1.58 (2 olefinic CH<sub>3</sub>), 3.60 (s, 2 methoxyl CH<sub>3</sub>), 4.32 (br, C=CH<sub>2</sub>), 5.2 (br, C-2 H), and 6.15 (s, aromatic H).

*Anal.* Calcd for C<sub>23</sub>H<sub>34</sub>O<sub>2</sub>: C, 80.65; H, 10.01. Found: C, 80.72; H, 9.98.

(±)-Cannabidiol (**1a**). (±)-Cannabidiol dimethyl ether (**1b**) (100 mg) in 10 ml of dry ether was added to methylmagnesium iodide prepared from magnesium (120 mg) and methyl iodide (1 g) in 10 ml of dry ether under a nitrogen atmosphere. The ether was evaporated and the temperature within the reaction flask was slowly raised to 155–165°. Gas, presumably methane, was rapidly eliminated from the melted mixture. After 15 min the heating was discontinued. The reaction mixture was cooled to room temperature. Ether (20 ml) was added, followed by a 10% solution of ammonium chloride (2 ml) and a 10% solution of sulfuric acid (5 ml). The organic layer was washed first with a saturated solution of sodium bicarbonate and then with water, dried, and evaporated. The oil obtained was chromatographed on Merck acid-washed alumina (20 g). Elution with ether–pentane (1:10) gave an oil (74 mg) whose ir, nmr, and uv spectra as well as tlc and glc data were identical with those of natural (–)-cannabidiol.<sup>26</sup>

*Anal.* Calcd for C<sub>21</sub>H<sub>30</sub>O<sub>2</sub>: C, 80.21; H, 9.62. Found: C, 80.25; H, 9.45.

A (±)-*di-p*-toluenesulfonate (**1c**) was prepared by dissolving (±)-cannabidiol (100 mg) and *p*-toluenesulfonyl chloride (1.5 g) in 5 ml of pyridine. The solution was left overnight and then ice (20 g) was added. After 0.5 hr the mixture was extracted with ether (twice, 50 ml). The organic layer was washed twice with a saturated solution of sodium bicarbonate (25 ml), then twice with 5% hydrochloric acid (25 ml), again with sodium bicarbonate (10 ml), and water (25 ml), dried over sodium sulfate, and evaporated. The oil obtained was crystallized from ether–pentane: mp 138–140°; δ (CCl<sub>4</sub>) 0.88 (CH<sub>3</sub>), 1.50, 1.64 (olefinic CH<sub>3</sub>), 2.41 (s, 2 aromatic CH<sub>3</sub>), 3.42 (br d, C-3 H), 4.32 (br 3 H, C=CH<sub>2</sub> and vinylic C-2 H), 6.83 (s, 2 aromatic olivetol hydrogens), 7.28 and 7.72 (AB pattern, *J* = 8 Hz, 7 aromatic tosyl protons).

*Anal.* Calcd for C<sub>37</sub>H<sub>42</sub>O<sub>6</sub>S<sub>2</sub>: C, 67.51; H, 6.80. Found: C, 67.52; H, 6.75.

(–)-Cannabidiol Di-*p*-toluenesulfonate (**1c**). This solid derivative of (–)-cannabidiol was prepared as described above for the same derivative of (±)-cannabidiol. The compound thus obtained, mp 81–83°, [α]<sub>D</sub> (C<sub>2</sub>H<sub>5</sub>OH) –107°, has ir (in CCl<sub>4</sub>) and nmr spectra identical with those of (±)-cannabidiol di-*p*-toluenesulfonate. In KBr pellets the ir spectra of the (±) and (–) compounds are different.

*Anal.* Calcd for C<sub>23</sub>H<sub>42</sub>O<sub>6</sub>S<sub>2</sub>: C, 67.51; H, 6.80. Found: C, 67.62; H, 6.75.

(±)-Δ<sup>1</sup>-THC (**2**) from (±)-Cannabidiol (**1a**). The experimental details of this reaction in the (–) series have been described.<sup>1a</sup> (±)-Δ<sup>1</sup>-THC was obtained in 70% yield from (±)-cannabidiol by the same procedure. The ir and nmr spectra and the tlc and glc data of (±)-Δ<sup>1</sup>-THC thus obtained were identical with those of (–)-Δ<sup>1</sup>-THC.

In our original synthesis (±)-Δ<sup>1</sup>-THC was obtained as an oil, but more recently<sup>14</sup> this compound has been obtained in crystalline form, mp 64.5–65.5°.

*Anal.* Calcd for C<sub>21</sub>H<sub>30</sub>O<sub>2</sub>: C, 80.21; H, 9.62. Found: C, 80.26; H, 9.52.

(±)-Δ<sup>1</sup>-Tetrahydrocannabinol (**2**) and (±)-Δ<sup>1</sup>-3,4-*cis*-Tetrahydrocannabinol (**13**) from Citral and Olivetol. Boron trifluoride etherate (0.6 ml; distilled over calcium hydride) was added to a solution of olivetol (2.2 g) in methylene chloride (40 ml). The solution was cooled with ice water and citral (2.4 g) in 20 ml of methylene chloride was added to it rapidly, with stirring, under nitrogen atmosphere. After 1 hr at room temperature the solution was washed with a saturated sodium bicarbonate solution (50 ml), dried, evaporated, and chromatographed on Florisil (200 g). Pentane and then 2% ether–pentane eluted first an unidentified oily mixture (1.05 g). Monitoring by glc and tlc then showed mixtures of Δ<sup>8</sup>-*l*-THC (**14**)<sup>13</sup> and Δ<sup>1</sup>-3,4-*cis*-THC (**13**) (0.1 g) which were followed by mixtures of **13** and **2** (totaling 1.37 g, 35.5%), richer first in **13** and changing gradually to almost pure **2**. These were followed again by unidentified mixtures, eluted with 10 and 20% ether–pentane (0.82 g), and finally by olivetol (0.46 g; eluted with ether–pentane 1:1). Further chromatographies of the mixed fractions yielded pure (±)-Δ<sup>1</sup>-3,4-

*cis*-THC (13) (190 mg), which after two crystallizations from pentane had mp 57–58° (87 mg). The nmr spectrum was identical with that reported<sup>12</sup> for 13 when it was obtained as an oil.

*Anal.* Calcd for C<sub>21</sub>H<sub>30</sub>O<sub>2</sub>: C, 80.21; H, 9.62. Found: C, 80.48; H, 9.78.

The preparation and spectra of the acetate of (±)-Δ<sup>1</sup>-3,4-*cis*-THC have been described.<sup>1,15b</sup> Its nmr spectrum closely resembles that of Δ<sup>1</sup>-THC acetate.<sup>13b</sup> The main difference lies in the chemical shift of one of the methyl groups on C-8 which, in the *cis* compound, is deshielded by 0.20 ppm as compared to that of the *trans* compound. The same deshielding effect is also observed in the nmr spectrum of Δ<sup>1</sup>-*cis*-THC (13) as compared to that of Δ<sup>1</sup>-*trans*-THC (2).

Pure (±)-Δ<sup>1</sup>-THC (2) was obtained by a further chromatography on Florisil of mixed fractions from the chromatography described above. Elution with 1% ether–pentane yielded a few fractions containing a mixture of 13 and 2. Further elution gave (±)-Δ<sup>1</sup>-THC (2) (a total of 770 mg) which was identical in all respects (nmr, ir, tlc, glc) with the material obtained from the previous synthesis.

The original reaction mixture contained (by glc) ca. 35% (±)-Δ<sup>1</sup>-THC (2) and ca. 15% (±)-Δ<sup>1</sup>-*cis*-THC (13), although only 20% of 2 and 5% of 13 were actually obtained in pure form. The separation of these two isomers was tedious and in view of the syntheses of (–)-Δ<sup>1</sup>-THC described below no further attempts to improve the isolation procedures were made.

**Condensation of Verbenol (15) with Olivetol. I. With *p*-Toluenesulfonic Acid.** Either (–)-*cis*-verbenol (15a), mp 65–67°, or (–)-*trans*-verbenol (15b), an oil, was used. The *cis* isomer 15a, [α]<sub>D</sub> –15° (CHCl<sub>3</sub>), was prepared by reduction of verbenone;<sup>21</sup> the *trans* isomer 15b, [α]<sub>D</sub> –117° (CHCl<sub>3</sub>), containing 10% of the *cis* isomer (15a), was prepared<sup>21, 27, 28</sup> by oxidation of pinene, [α]<sub>D</sub> –51.5° (CHCl<sub>3</sub>), with lead tetraacetate to *cis*-2-acetoxypinene which was then rearranged by glacial acetic acid to 15b. The use of 15a is preferable, as it is a crystalline, stable compound, while 15b, an oil, slowly decomposes at room temperature. Olivetol (2.70 g, 15 mmol) and 300 mg of dry *p*-toluenesulfonic acid were dissolved in 600 ml of CHCl<sub>3</sub> (freshly distilled over CaH<sub>2</sub>). Verbenol (2.28 g, 15 mmol) in 50 ml of CHCl<sub>3</sub>, similarly purified, was added over a period of 30 min. The solution was left at room temperature for a further 30 min, washed with a saturated solution of sodium bicarbonate, dried, and evaporated. The oil obtained was chromatographed on Merck silica gel (270 g). Elution with 1% ether–petroleum ether (30–60°) gave compound 16 (740 mg): mol wt (mass spectrum) 448; δ (CCl<sub>4</sub>) 0.93 and 1.30 (four bridge CH<sub>3</sub>); the higher field signal overlaps the ω CH<sub>3</sub> band), 1.85 (2 olefinic CH<sub>3</sub>), 3.65 and 3.86 (br s, 2 C-4 H), 5.65 (two vinylic protons), and 6.05 (s, one aromatic proton).

*Anal.* Calcd for C<sub>31</sub>H<sub>44</sub>O<sub>2</sub>: C, 82.98; H, 9.88. Found: C, 83.38; H, 9.83.

Further elution with 5% ether–petroleum ether gave 4-*trans*-(2-olivetyl)pinene (4) (2.83 g), [α]<sub>D</sub> –87°, on chromatographically pure, undistilled material, in CHCl<sub>3</sub>: δ (CCl<sub>4</sub>) 0.88 (ω-CH<sub>3</sub>), 0.96 and 1.32 (2 bridge CH<sub>3</sub>), 1.88 (C-2 CH<sub>3</sub>), 3.98 (s br, C-4 H), 5.69 (s br, C-3 H), and 6.10 (s, 2 aromatic H); mol wt (mass spectrum) 314.

*Anal.* Calcd for C<sub>21</sub>H<sub>30</sub>O<sub>2</sub>: C, 80.21; H, 9.62. Found: C, 80.60; H, 9.44.

Further elution with 7% ether–petroleum ether gave the unstable isomeric olivetylpinene 17 (706 mg), [α]<sub>D</sub> –48°, on chromatographically pure, undistilled material, in CHCl<sub>3</sub>: mol wt (mass spectrum) 314; δ (CCl<sub>4</sub>) 0.88, 0.93, 1.29 and 1.82 (four CH<sub>3</sub> groups as in 4), 3.67 (C-4 H), 5.58 (C-3 H), 6.01 and 6.15 (2 aromatic H).

A stable diacetate of 17 was prepared with acetic anhydride in pyridine. It is a viscous oil: [α]<sub>D</sub> –70° (CHCl<sub>3</sub>); δ (CCl<sub>4</sub>) 0.88 (ω-CH<sub>3</sub>), 0.95 (s) and 1.30 (s, 2 bridge CH<sub>3</sub>), 1.76 (s, br, vinylic CH<sub>3</sub>), 2.08 (s) and 2.17 (s, 2 acetyl CH<sub>3</sub>), 2.33–2.80 (br, 2 benzylic H), 3.80 (s, br, C-3 H), 5.33 (s, br, C-2 H), 6.50 (d), and 6.73 (d, *J* = 2.5 Hz, 2 aromatic H).

*Anal.* Calcd for C<sub>23</sub>H<sub>34</sub>O<sub>4</sub>: C, 74.34; H, 8.60. Found: C, 75.58; H, 8.68.

**Conversion of (–)-4 into (–)-Δ<sup>6</sup>-TH C(3).** The olivetylpinene 4 (1.2 g) was dissolved in 30 ml of methylene chloride (freshly distilled over CaH<sub>2</sub>). Boron trifluoride etherate (1.2 ml) (freshly distilled over CaH<sub>2</sub>) was added while the solution was stirred under a nitrogen atmosphere. After 10 min an aqueous solution of 5% sodium bicarbonate (20 ml) was added, and the organic layer was washed with water, dried, and evaporated. The oil obtained was chromatographed on Florisil (230 g). Elution with ether–petroleum ether (0.5:99.5) gave (–)-Δ<sup>6</sup>-THC (960 mg), [α]<sub>D</sub> –250° (CHCl<sub>3</sub>), indicating an optical purity of ca. 97%. The ir and nmr spectra as well as the tlc and glc data were identical with those of authentic material.<sup>9b, 12, 29</sup>

**Condensation of Verbenol (15) with Olivetol. II. With Boron Trifluoride Etherate.** Either (–)-*cis*-verbenol (15a), mp 67–69°, or (–)-*trans*-verbenol (15b) was used. They were prepared as described above. (–)-*cis*-Verbenol (15a) (300 mg) and olivetol (150 mg) were dissolved in 25 ml of methylene chloride (distilled over calcium chloride). Boron trifluoride etherate (0.25 ml) was added. The reaction mixture was kept under nitrogen at –10° for 2 hr and then at room temperature for 0.5 hr, after which it was washed with a 5% sodium bicarbonate solution and then with water, dried, and evaporated. The oil obtained was chromatographed on Florisil (45 g). Elution with ether–petroleum ether (0.5:99.5) gave a mixture containing products formed by the condensation of 2 mol of verbenol with 1 mol of olivetol (molecular weights by mass spectrum). They were not characterized. Further chromatography with 1% ether–petroleum ether gave, after some mixed fractions, (–)-Δ<sup>6</sup>-THC (3) which was distilled in a bulb-to-bulb distillation (bp ca. 220°, bath temperature (0.1 mm)). Pure (–)-Δ<sup>6</sup>-THC (115 mg, 44% based on olivetol) was obtained, [α]<sub>D</sub> –245° (CHCl<sub>3</sub>), identical with authentic material (ir, nmr, tlc, glc).

**1-Chlorohexahydrocannabinol (18).** (–)-Δ<sup>6</sup>-THC (3) (2.95 g) was dissolved in dry toluene (250 ml) containing 0.5 g of anhydrous zinc chloride. The mixture was cooled to –15° and vigorously stirred. Dry gaseous hydrogen chloride was bubbled through the mixture for 6 hr. The toluene solution was then repeatedly washed with water until the washings were neutral and dried, and the toluene was evaporated. The oily 1-chlorohexahydrocannabinol (18) could not be analyzed for purity by tlc or glc as by both methods 18 was converted into Δ<sup>6</sup>-THC (3). The oily 18 had [α]<sub>D</sub> –82° (CHCl<sub>3</sub>); λ<sub>max</sub> (C<sub>2</sub>H<sub>5</sub>OH) 230 (sh) (ε 10,200), 276 (ε 1250), 279 mμ (ε 1280); δ (CDCl<sub>3</sub>) 0.89, 1.14, 1.40 (CH<sub>3</sub> groups), 1.68 (CH<sub>2</sub> α to Cl), 3.40 (br, d, C-2, α-H), and 6.10, 6.28 (aromatic H). Compound (–)-18 thus obtained had nmr and ir spectra identical with those of (±)-18 obtained previously by Fahrenholtz, *et al.*<sup>14</sup>

*Anal.* Calcd for C<sub>21</sub>H<sub>31</sub>ClO<sub>2</sub>: C, 71.88; H, 8.90. Found: C, 72.21; H, 9.15.

**Dehydrochlorination of 18 to (–)-Δ<sup>1</sup>-THC (2).** Initially this reaction was performed as described by Fahrenholtz, *et al.*,<sup>14</sup> for the conversion of (±)-18 to (±)-Δ<sup>1</sup>-THC. With the introduction of the improved method by Petrzilka, *et al.*,<sup>15</sup> we have employed this procedure. In our hands the yields are somewhat lower than in the published procedure, namely 85–90%. (±)-Δ<sup>1</sup>-THC (2) thus obtained was purified by chromatography on Florisil. Elution with 0.5% ether–petroleum ether gave (–)-Δ<sup>1</sup>-THC (2) of 95–98% purity, [α]<sub>D</sub> –152° (C<sub>2</sub>H<sub>5</sub>OH), ir, nmr, tlc, and glc identical with those of the natural material.<sup>1</sup>

*Anal.* Calcd for C<sub>21</sub>H<sub>30</sub>O<sub>2</sub>: C, 80.21; H, 9.62. Found: C, 80.60; H, 9.44.

**(+)-Δ<sup>6</sup>-THC (3a) and (+)-Δ<sup>1</sup>-THC (2a).** These compounds were obtained from (+)-*trans*-verbenol, [α]<sub>D</sub> +112° (CHCl<sub>3</sub>), *via* the procedures described above. (+)-Δ<sup>6</sup>-THC, [α]<sub>D</sub> +248° (CHCl<sub>3</sub>), and (+)-Δ<sup>1</sup>-THC, [α]<sub>D</sub> +147° (CHCl<sub>3</sub>), have ir, uv, and nmr spectra identical with those of (–)-Δ<sup>6</sup>-THC and (–)-Δ<sup>1</sup>-THC. Their chromatographic behavior (tlc and glc) was also the same.

**(–)-[3-<sup>2</sup>H]-Δ<sup>6</sup>-THC (20) and (–)-[3-<sup>2</sup>H]-Δ<sup>1</sup>-THC (21).** Verbenone (3 g) was reduced with lithium aluminum deuteride (0.25 g) in 50 ml of dry ether. Recrystallization from pentane gave 2.5 g of (–)-[4-<sup>2</sup>H]-*cis*-verbenol (19): mp 67–69°; mol wt (mass spectrum) 153; nmr spectrum identical with the published<sup>21</sup> one of *cis*-verbenol (15a) except that the signal for the C-4 H is completely missing. This deuterated *cis*-verbenol (19) was condensed with olivetol in the presence of boron trifluoride etherate as described above to give (–)-[3-<sup>2</sup>H]-Δ<sup>6</sup>-THC (20): mol wt (mass spectrum) 315; [α]<sub>D</sub> –248° (CHCl<sub>3</sub>); nmr spectrum identical with that of Δ<sup>6</sup>-THC (3) except that the broad signal for the C-3 proton in Δ<sup>6</sup>-THC at δ 2.70 has disappeared and the signal of the C-2 proton appears as a doublet at δ 3.20 (*J* = 18 Hz) instead of as a very broad doublet.

The conversion of (–)-[3-<sup>2</sup>H]-Δ<sup>6</sup>-THC (20) into (–)-[3-<sup>2</sup>H]-Δ<sup>1</sup>-THC (21) was done as described above for the nonlabeled compounds. (–)-[3-<sup>2</sup>H]-Δ<sup>1</sup>-THC (21) thus obtained has: mol wt (mass spectrum) 315; [α]<sub>D</sub> –157° (CHCl<sub>3</sub>); nmr spectrum identical with that of (–)-Δ<sup>1</sup>-THC (2) except that the signal of the C-3 proton in 2 (at δ

(27) G. Whitham, *J. Chem. Soc.*, 2232 (1961).

(28) Y. Chretien-Bessiere, *Bull. Soc. Chim. Fr.*, 2182 (1964).

(29) R. L. Hively, W. A. Mosher, and F. W. Hoffmann, *J. Amer. Chem. Soc.*, 88, 1832 (1966).

3.14) has completely disappeared in **21** and the signal of the C-2 olefinic proton which in **2** appears as a broad singlet<sup>1</sup>(or as a triplet,<sup>22</sup>  $J = 1.7$  Hz) in **21** is a singlet.

**Acknowledgments.** We thank Dr. B. Yagen and Mr. Y. Samueloff for repeating and improving the yields of some of the reactions, Miss I. Ramati and Mrs. N. Shoef

for their technical help, Dr. K. E. Fahrenholtz of Hoffmann-La Roche, Nutley, N. J., for a sample and spectra of ( $\pm$ )-**18**, Dr. A. Brossi of Hoffmann-La Roche for olivetol, and the NIMH (Grant No. MH-13180) for financial support of the work in Jerusalem during its last stages.

## Synthesis of a Hydrophobic Potassium Binding Peptide<sup>1</sup>

B. F. Gisin\* and R. B. Merrifield

Contribution from The Rockefeller University, New York, New York 10021, and from the Department of Physiology, Duke University Medical Center, Durham, North Carolina 27710. Received March 22, 1972

**Abstract:** Based on the known structures of alkali ion complexing agents the design of a homodetic cyclopeptide that would be able to bind the potassium ion was undertaken. A molecular model of the peptide *cyclo*-[*L*-Val-D-Pro-D-Val-*L*-Pro]<sub>3</sub> exhibited that property as judged by the oxygen-lined cavity it could provide in one of its most probable conformations. The linear peptide was synthesized by the solid-phase method starting with *L*-proline at the C terminus. After cleavage from the resin and cyclization the neutral cyclododecapeptide was found to form a crystalline, hydrophobic 1:1 complex with potassium picrate.

Among the ligands for metal ions the so-called "ion carriers" constitute a group of compounds which are able to complex with alkali ions and make them soluble in nonpolar media. This quality has generated great interest mainly for three reasons. First, some of the ion carriers exhibit antibiotic activity<sup>2-9</sup> and they show dramatic effects on the ionic balance in mitochondria<sup>10,11</sup> and in red blood cells.<sup>12,13</sup> Second, they have been found to produce similar effects in lipid membranes<sup>13,14</sup> (lipid bilayers) and in other

artificial systems related to membranes.<sup>3,4,11,15-18</sup> All of these properties appear to be a consequence of the ion complexing ability which is thought to be a necessary though not sufficient condition for activity in natural membranes.<sup>4</sup> Third, spectroscopic investigation of some of these compounds has contributed significantly to the understanding of conformational principles in molecules of biological origin.<sup>3-5,15,19,20</sup>

Alkali ion carriers of known structure from natural sources are depsipeptides (valinomycin,<sup>21</sup> the en-

(1) This investigation was supported by NIH Grant HE 12 157, U. S. Public Health Service Grant AM 1260, and by the Hoffman-La Roche Foundation. It was presented at the 163rd National Meeting of the American Chemical Society, Boston, Mass., April 1972.

(2) H. Brockmann and G. Schmidt-Kastner, *Chem. Ber.*, **88**, 57 (1955); C. E. Meyer and F. Reusser, *Experientia*, **23**, 85 (1967); K. Bevan, J. S. Davies, C. H. Hassall, R. B. Morton, and D. A. S. Phillips, *J. Chem. Soc. C*, 514 (1971).

(3) M. M. Shemyakin, Yu. A. Ovchinnikov, and V. T. Ivanov, *Angew. Chem.*, **81**, 53 (1969).

(4) M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, V. K. Antonov, E. I. Vinogradova, A. M. Shkrob, G. G. Malenkov, A. V. Evstratov, I. A. Laine, E. I. Melnik, and I. D. Ryabova, *J. Membrane Biol.*, **1**, 402 (1969).

(5) Yu. A. Ovchinnikov, V. T. Ivanov, and I. I. Mikhaleva, *Tetrahedron Lett.*, 159 (1971).

(6) W. Mechlinski, C. P. Schaffner, P. Ganis, and G. Avitabile, *ibid.*, 3873 (1970).

(7) A. Agtarap, J. W. Chamberlin, M. Pinkerton, and L. K. Steinrauf, *J. Amer. Chem. Soc.*, **89**, 5737 (1967).

(8) L. K. Steinrauf, M. Pinkerton, and J. W. Chamberlin, *Biochem. Biophys. Res. Commun.*, **33**, 29 (1968).

(9) C. A. Maier and I. C. Paul, *Chem. Commun.*, 181 (1971).

(10) W. C. Murray and R. W. Begg, *Arch. Biochem. Biophys.*, **84**, 546 (1959); C. Moore and B. C. Pressman, *Biochem. Biophys. Res. Commun.*, **15**, 562 (1964); R. S. Cockrell, E. J. Harris, and B. C. Pressman, *Biochemistry*, **5**, 2326 (1966); H. Lardy, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **27**, 1278 (1968).

(11) B. C. Pressman, *Antimicrob. Ag. Chemother.*, 28 (1969).

(12) D. C. Tosteson, P. Cook, T. Andreoli, and M. Tieffenberg, *J. Gen. Physiol.*, **50**, 2513 (1967).

(13) D. C. Tosteson, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **27**, 1269 (1968).

(14) A. A. Lev and E. P. Buzhinsky, *Tsitologiya*, **9**, 102 (1967); P. Mueller and D. O. Rudin, *Biochem. Biophys. Res. Commun.*, **26**, 398 (1967); T. E. Andreoli, M. Tieffenberg, and D. C. Tosteson, *J. Gen. Physiol.*, **50**, 2527 (1967); P. Mueller and D. O. Rudin, *Nature (London)*, **217**, 713 (1968); A. Finkelstein and A. Cass, *J. Gen. Physiol.*, **51**, 145

(1968); G. Eisenman, S. M. Ciani, and G. Szabo, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **27**, 1289 (1968); P. Lauger and G. Stark, *Biochim. Biophys. Acta*, **211**, 458 (1970).

(15) M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, V. K. Antonov, A. M. Shkrob, I. I. Mikhaleva, A. V. Estratov, and G. G. Malenkov, *Biochem. Biophys. Res. Commun.*, **29**, 834 (1967).

(16) M. Pinkerton, L. K. Steinrauf, and P. Dawkins, *ibid.*, **35**, 512 (1969).

(17) B. Dietrich, J. M. Lehn, and J. P. Sauvage, *Tetrahedron Lett.*, 2885, 2889 (1969); *Chem. Commun.*, 1055 (1970).

(18) D. C. Tosteson, T. E. Andreoli, M. Tieffenberg, and P. Cook, *J. Gen. Physiol.*, **51**, 373 (1968); Z. Stefanac and W. Simon, *Microchem. J.*, **12**, 125 (1967); L. A. R. Pioda, H. A. Wachter, R. E. Dohner, and W. Simon, *Helv. Chim. Acta*, **50**, 1373 (1967); H. K. Wipf, L. A. R. Pioda, Z. Stefanac, and W. Simon, *ibid.*, **51**, 377 (1968); H. K. Wipf, W. Pache, P. Jordan, H. Zahner, W. Keller-Schierlein, and W. Simon, *Biochem. Biophys. Res. Commun.*, **36**, 387, 1969; L. A. R. Pioda, H. K. Wipf, and W. Simon, *Chimia*, **22**, 189 (1968); R. P. Scholer and W. Simon, *ibid.*, **24**, 372 (1970); R. M. Izatt, J. H. Rytting, D. P. Nelson, B. L. Haymore, and J. J. Christensen, *Science*, **164**, 433 (1969); M. J. Hall, *Biochem. Biophys. Res. Commun.*, **38**, 590 (1970); M. S. Frant and J. W. Ross, Jr., *Science*, **167**, 987 (1970).

(19) V. T. Ivanov, I. A. Laine, N. D. Abdullaev, L. B. Senyavina, E. M. Popov, Yu. A. Ovchinnikov, and M. M. Shemyakin, *Biochem. Biophys. Res. Commun.*, **34**, 803 (1969); M. Ohnishi and D. W. Urry, *ibid.*, **36**, 194 (1969); D. F. Mayers and D. W. Urry, *J. Amer. Chem. Soc.*, **94**, 77 (1972).

(20) H. Diebler, M. Eigen, G. Ilgenfritz, G. Maas, and R. Winkler, *Pure Appl. Chem.*, **20**, 93 (1969); A. I. McMullen, *Biochem. J.*, **119**, 10 (1970); Th. Wieland, H. Faulstich, W. Burgermeister, W. Otting, W. Mohle, M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and G. G. Malenkov, *FEBS Lett.*, **9**, 89 (1970); V. T. Ivanov, A. I. Miroshnikov, N. D. Abdullaev, L. B. Senyavina, S. F. Arkhipova, N. N. Uvarova, K. Kh. Khalilulina, V. F. Bystrov, and Yu. A. Ovchinnikov, *Biochem. Biophys. Res. Commun.*, **42**, 654 (1971).

(21) M. M. Shemyakin, N. A. Aldanova, E. I. Vinogradova, and M. Yu. Feigina, *Tetrahedron Lett.*, **28**, 1921 (1963).