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HASHISH—IV[1]

THE ISOLATION AND STRUCTURE OF CANNABINOLIC CANNABIDIOLIC AND CANNABIGEROLIC ACIDS

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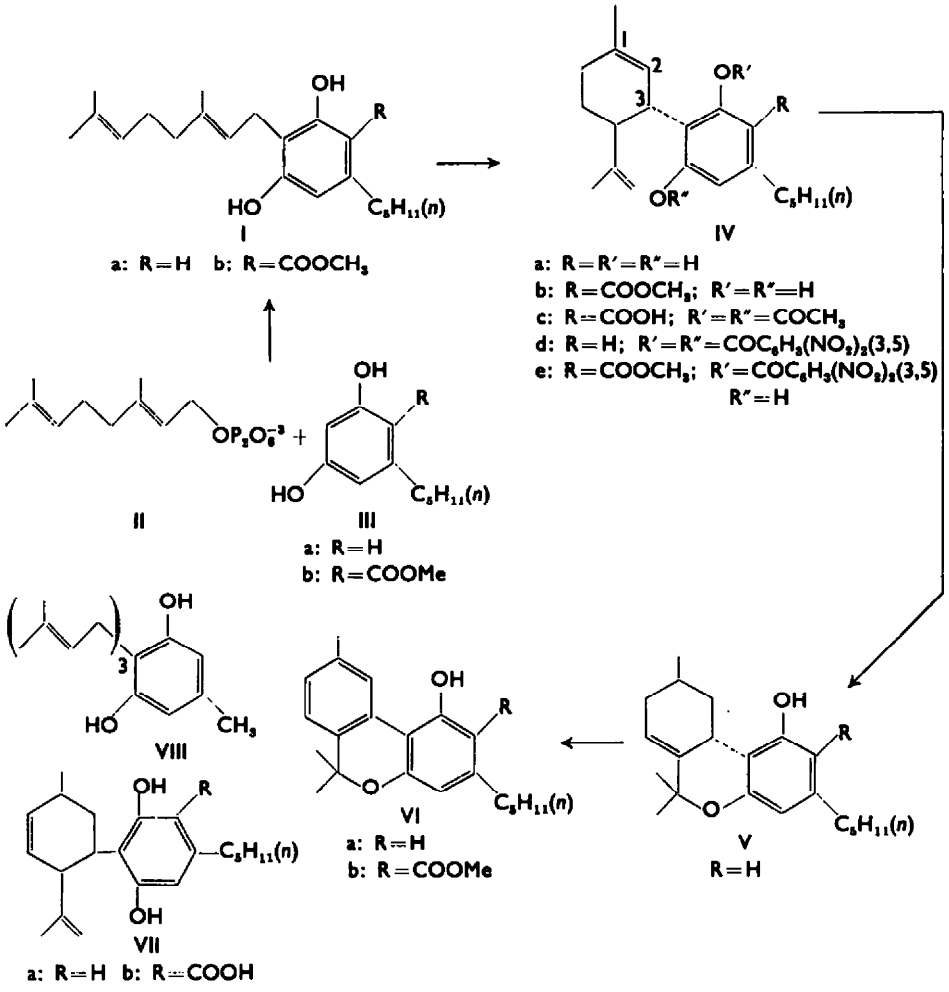
Abstract—The methyl esters of cannabinolic, cannabidiolic and cannabigerolic acids are shown to possess structures VIb, IVb and Ib respectively.

RECENTLY we suggested that in Nature cannabigerol (Ia)[2] is probably formed by condensation of geranyl pyrophosphate (II) with olivetol (IIIa). Conversion into cannabidiol (IVa),[3] tetrahydrocannabinol (V)[1] and cannabinol (VIa) can be visualized to proceed via two successive cyclizations followed by dehydrogenation.[4]

This biogenetic scheme would not explain the formation of cannabidiolic acid, a major constituent. This acid was first isolated from *Cannabis sativa* L. in 1955 by Krejči and Šantavý[5] and was found to be strongly antibiotic.[6,7] Independently Schultz and Haffner[8] isolated it from the same plant and found that it is both sedative and antibiotic. Ǧrlic[9] has shown that it is present in hashish. The amount of cannabidiolic acid in Cannabis samples seems to depend on their geographic origin, those from northern countries (Germany, Sweden, etc.) having a considerably higher content than those from southern ones (Brazil, Ghana etc.).[9,10]

Cannabidiolic acid was assigned[11] structure VIIb on the basis of its conversion, through decarboxylation, to cannabidiol, whose structure had earlier been assumed

[1] Part III: Y. Gaoni and R. Mechoulam, *J. Amer. Chem. Soc.* **86**, 1646 (1964).
[2] Y. Gaoni and R. Mechoulam, *Proc. Chem. Soc.* 82 (1964).
[3] R. Mechoulam and Y. Shvo, *Tetrahedron*, **19**, 2073 (1963).
[4] The cyclizations are probably enzymatic. The dehydrogenation of, what was presumably, V to VIa has been observed to take place spontaneously. J. Levine, *J. Amer. Chem. Soc.* **66**, 1868 (1944).
[5] Z. Krejci and F. Šantavý, *Acta Univ. Palackianea Olomuc.* **6**, 59 (1955); *Chem. Abstr.* **50**, 12080 (1956).
[6] For a review on the antibacterial properties and clinical application of Cannabis see J. Kabelik, Z. Krejči and F. Šantavý, *Bull. Narcotics* **12**, 5 (1960).
[7] A Ukranian group has published a number of papers on the antibacterial and nematocidal properties of different extracts of *Cannabis sativa*, which have been named Cansantin 1, 2, 3 and 4. No definite compounds have been reported however. For leading references see S. I. Zelepukha, A. S. Rabinovich, P. Y. Pochinok, A. K. Negrash and V. A. Kudryavtsev, *Mikrob. Zh. Akad. Nauk Ukr. RSR.* **25**, 42 (1963); *Chem. Abstr.* **59**, 5656b (1963).
[8] O. E. Schultz and G. Haffner, *Arch. Pharm.* **291/63**, 391 (1958).
[9] L. Ǧrlic, *Bull. Narcotics* **14**, 37 (1962).
[10] The reverse relationship exists as regards tetrahydrocannabinol (V), the psychotomimetic principle in *Cannabis sativa*. Usually European hemp has very low content of V, while in the Middle-East or India it becomes one of the major components of the resin.
[11a] Z. Krejči, M. Horák and F. Šantavý, *Acta Univ. Palackianae Olomuc.* **16**, 9 (1958); *Chem. Abstr.* **54**, 12054 (1960); [b] Z. Krejči, M. Horák and F. Šantavý, *Pharmazie* **14**, 349 (1959); [c] O. E. Schultz and G. Haffner, *Z. Naturfor.* **14b**, 98 (1959).



to be VIIa.[12] Recently[3] the structure of cannabidiol was modified to IVa. The constitution of cannabidiolic acid (as its methyl ester) is, therefore, represented by IVb.

We decided to reinvestigate the acidic fraction of hashish[13] with the expectation that the presence of any additional acids would throw light on the biogenetic pathway. Our first hashish samples had very low acidic content which precluded their analysis. However a hashish sole[14] recently supplied to us by the Israeli Police was found to

[12] R. Adams, S. Loewe, D. C. Pease, C. K. Cain, R. B. Wearn, R. B. Baker and H. Wolff, *J. Amer. Chem. Soc.*, 62, 2566 (1940); R. Adams, C. K. Cain, W. D. McPhee and R. B. Wearn, *ibid.*, 63, 2209 (1941).

[13] Prior to the work reported by Šantavý[5,6] and Schultz[8] a number of publications had appeared, dealing with the acidic fraction of hashish. No well defined compounds were isolated. Todd showed, however, that cannabinol and cannabidiol are present in the acidic fraction in some kind of base soluble form presumably as esters of phenolic acids, such as protocatechuic acid. A. Madinaveitia, P. B. Russel and A. R. Todd, *J. Chem. Soc.*, 628 (1942); C. C. Fulton, *Ind. Eng. Chem.* (Annal.) 14, 407 (1942).

[14] The hashish illegally traded in the Middle East usually comes in small bags in the form of shoe soles.

contain ca. 5% acidic constituents and was therefore investigated. A portion of the total acidic fraction was acetylated,[11] yielding an oily material from which crystals precipitated after a few weeks. The obtained cannabidiolic acid diacetate (IVc) m.p. 126–8° was identical with an authentic sample.[11] The NMR spectrum of IVc (Table) is consistent with the structure suggested above. The spectrum is remarkably similar to that of the *bis*-3,5-dinitrobenzoate of cannabidiol[3] (IVd). The NMR data exclude the possibility that the double bond occupies the alternative Δ^5 or $\Delta^{1(6)}$ position. A double bond in any one of these two positions would have been reflected in the NMR pattern, especially as regards the chemical shift of the proton at C(3) which would have moved upfield relative to the shift of the proton at the same position in the bisdinitrobenzoate of cannabidiol (IVd).

The total crude acidic fraction was esterified with diazomethane and chromatographed. Three constituents were found to be present. The two less polar ones had to be rechromatographed twice in order to achieve full separation. The least polar component, which constitutes ca. 10% of the total acidic fraction, slowly crystallized[15] giving needles melting at 86–87°. The NMR spectrum (Table) shows that this component is the methyl ester of the hitherto unknown cannabinolic acid (VIb). The spectrum was essentially identical to that of cannabinol (VIa), except for the presence of only one aromatic hydrogen on the olivetol moiety. The benzylic hydrogens of the amyl chain in VIb are deshielded considerably more than those in VIa, because of the adjacent carbomethoxyl group. Boiling VIb with a solution of potassium hydroxide in methanol–water gave cannabinol (VIa) which was identified by direct comparison of its acetate with authentic cannabinol acetate. The carbomethoxyl group is in the *ortho* position to the phenolic group rather than to the etheric one. This is deduced from the chemical shift of the phenolic proton, which is very strongly deshielded (ca. 13 ppm). This effect is due to hydrogen bonding between the phenolic and the carbomethoxyl groups.[16] By comparison in cannabidiol acid methyl ester (IVb) one of the phenolic protons appears in the 6·5 ppm region, while the second one is in the 12 ppm region.

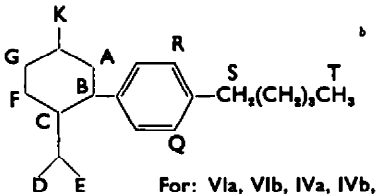
Cannabidiolic acid methyl ester (IVb) is eluted from the chromatographic column immediately after VIb. It is the major component of the esterified acidic fraction, comprising ca. 75% of it. It gives a mixture of *mono*-3,5-dinitrobenzoate, m.p. 173–175° (IVe) and *bis*-3,5-dinitrobenzoate on esterification with 3,5-dinitro benzoyl chloride. The structure of IVb is deduced on the basis of its NMR spectrum, which is very similar to that of IVa (Table). In IVb, however, all the benzylic protons are deshielded more than those in IVa, and only one aromatic proton is present. Cannabidiolic acid methyl ester is readily converted into cannabidiol (IVa) on boiling with dilute base.

The most polar of the three components was found to be the methyl ester of the hitherto unknown cannabigeroilc acid. It comprises nearly 15% of the esterified acidic fraction. It is an oil from which a crystalline *bis*-3,5-dinitrobenzoate, m.p. 110–112°, was prepared. The structure of cannabigerolic acid methyl ester (Ib)

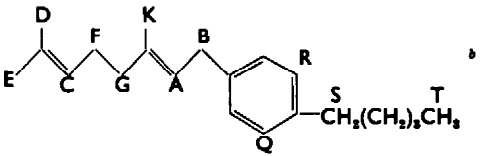
[15] All constituents of the cannabis group and most of their derivatives crystallize with great difficulty. This is probably one of the reasons for the slow progress in this field so far.

[16] Cf. L. M. Jackman, *Applications of N.M.R. Spectroscopy in Organic Chemistry*, p. 69. Pergamon Press, New York, N. Y. (1959).

TABLE 1. NMR SPECTRA OF HASHISH CONSTITUENTS AND DERIVATIVES[a]



For: VIa, VIb, IVa, IVb, IVd, IVc



For: Ia, Ib

Compound	Solvent	C_A—H	C_B—H	C_C—H	C_D—H	C_E—H	C_{F+G}—H	C_K—H	C_Q—H	C_R—H	C_S—H	C_T—H	C_R—$COOCH_3$
Cannabinol VIa	CCl_4	8·20 (1)(br)(s)	—	—	C_{D+E}—H 1·58 (6)(s)		7·10 (2)(s)	2·35 (3)(s)	C_{Q+R}—H 6·25 (1)(d) J = 2cps 6·40(1)(d) J = 2cps		2·20–2·60 (m)	0·88 (3)(t)	—
Cannabinolic acid, methyl ester[c] (VIb)	CCl_4	8·5 (1)(br)(s)	—	—	C_{D+E}—H 1·50 (6)(s)		7·00 (2)(s)	2·32 (3)(s)	6·22 (1)(s)	—	2·62–2·90 (m)	0·88 (3)(t)	3·85 (3)(s)
Cannabidiol (IVa)	$CDCl_3$	5·59 (1)(br)(s)	3·81 (1)(d)(br)		C_{D+K}—H 1·68 1·80 (6)	4·66 4·58 (2)		see C_{D+K}	C_{Q+R}—H 6·22 (2)(s)		2·20–2·65 (m)	0·88 (3)(t)	—
Cannabidiolic acid, methyl ester[d] (IVb)	$CDCl_3$	5·60 (1)(br)(s)	4·0–4·3 (1)(br)		C_{D+K}—H 1·70 1·82 (6)	4·45 4·55 (2)		see C_{D+K}	6·25 (1)(s)	—	2·50–2·92 (m)	0·88 (3)(t)	3·88 (3)(s)

Cannabidiol *bis* 3,5-dinitro benzoate (IVd)	$CDCl_3$	5·33 (1)(br)(s)	3·58 (1)(br)(d) J = 11 cps		C_{D+E}—H 1·59 4·55 1·22 4·73 (6) (2)	see C_{D+E}—H	C_{Q+R}·–H 6·95 (2)(s)	—	2·40– 2·80 (m)	0·92 (3)(t)	—
Cannabidiolic acid, diacetate[c] (IVc)	$CDCl_3$	5·30 (1)(br)(s)	3·50 (1)(br)(d)		C_{D+E}—H 1·60 4·50 1·70 4·58 (6) (2)	see C_{D+E}—H	6·90 (1)(s)	—	2·58– 3·00 (m)	0·90 (3)(t)	—
Cannabigerol (Ia)	CCl_4	C_{A+C}—H 4·8–5·3 (2)(m)	3·35 (2)(d) J = 7 cps	see C_{A+C}—H	C_{D+E+K}—H 1·60(3) 1·69(3) 1·82(3)	see C_{D+E}—H	C_{Q+R}—H 6·18 (2)(s)		2·25– 2·60 (m)	0·95 (3)(t)	—
Cannabigerolic acid, methyl ester (Ib)	CCl_4	C_{A+C}—H 4·8–5·3 (2)(m)	3·37 (2)(d) J = 7 cps	see C_{A+C}—H	C_{D+E+K}—H 1·59(3) 1·66(3) 1·80(3)	see C_{D+E}	6·15 (1)(s)	—	2·40– 2·95 (m)	0·91 (3)(t)	3·92 (3)(s)

[a] Spectra were determined on a Varian A-60 spectrometer. Values given in ppm relative to tetramethylsilane as internal standard (frequency zero). Numbers in parentheses denotes number of protons, determined by integration of areas. Letters in parentheses denote singlet (s); doublet (d); triplet (t); broad (br) and multiplet (m).

[b] All carbons in this Table are designated by capital letters, as in the above formulae. Thus C_A—H, for example, means proton(s) on carbon A.

[c] Phenolic proton at 12·8 ppm (1)(br)

[d] Phenolic protons at 6·5 ppm (1)(s) and 12·0 ppm (1)(br).

[e] Acetyl protons at 2·22 (6)(s).

was likewise elucidated by comparison of its NMR spectrum with that of cannabigerol (Ia)[8] (Table) and through decarboxylation to this latter compound.

in vitro Both cannabigerolic acid methyl ester and cannabigerol show considerable antibacterial activity[17] against gram positive bacteria. Both compounds are, however, inactivated by serum. In structure and antibacterial properties both Ia and Ib (which are geranyl-resorcinol derivatives) are closely related to grifolin[18] (VIII; which is a farnesyl-resorcinol derivative), an antibiotic from the basidomycete *Grifola confluens*. It is intriguing that these natural products of a rather rare type have been found up till now in a single plant and in a single fungus only.

The presence of two parallel lines of compounds in hashish, one derived from olivetol (IIIa) and the other from olivetol-carboxylic acid (IIIb) poses a problem of biogenesis. With the data in hand it is impossible to decide whether cannabigerol and cannabigerolic acid are formed independently and each is the starting point of a chain of compounds or whether the olivetolic acid chain is the only one and the other compounds (Ia, IVa, Va and VIa) are formed by decarboxylation in the plant.

EXPERIMENTAL

IR spectra were recorded on a Perkin Elmer Infracord 137-B(I). UV spectra were recorded on Cary 14 in EtOH. Thin layer chromatoplates, made of Kieselgel-G Merck, were eluted with pentane–ether (88:12) and sprayed with a $KMnO_4$ solution. Microanalyses were performed by the microanalytical department of the Weizmann Institute.

Isolation. Powdered hashish (100 g) was stirred with high boiling pet. ether (41) for 3 hr at room temp under N_2. The mixture was filtered and the filtrate was concentrated to ca. 500 cc. This solution was extracted 3 times with a solution of 2% NaOHaq and 2% Na_2SO_3aq. The aqueous solution was rapidly acidified with an ice-cold solution of 5% H_2SO_4aq and extracted with ether. The ether extract was washed with a sat, NaClaq, dried and evaporated under red. press., giving a mixture (5 g) of acids. A solution of 2·8 g of this mixture in ether was esterified with excess diazomethane at room temp. After 0·5 hr, the solvents were evaporated. On TLC three components were shown to be present. Separation was achieved by chromatography on 300 g acid-washed Merck alumina. Pentane–ether (98:2) eluted *cannabinolic acid methyl ester* (VIb) followed by a mixture of VIb and *cannabidiolic acid methyl ester* (IVb) and then pure IVb. In order to elute all the IVb present the polarity of the solvent mixture had to be increased (pentane–ether, 95:5). Elution with 15% ether in pentane gave pure *cannabigerolic acid methyl ester* (Ib; 420 mg). In order to effect full separation of VIb and IVb the mixed fractions were rechromatographed twice. The total yield of VIb was 260 mg, and that of IVb—2·0 g. The remaining material was a mixture of VIb and IVb. The purity of all three compounds was established by chromatoplate.

Cannabinolic acid methyl ester (VIb) m.p. 86–87° (MeOH); UV spectrum: λ_{max} 220 mμ (shoulder) (ε, 28,300), 266 mμ (ε, 41,300), 295 mμ (shoulder) (ε, 11,200), 326 mμ (ε, 6100). (Found: C, 74·63; H, 7·82. $C_{22}H_{28}O_4$ requires: C, 74·97; H, 7·66%).

Cannabidiolic acid methyl ester (IVb) UV spectrum : λ_{max} 224 mμ (ε, 23,100), 271 mμ (ε, 12,000), 304 mμ (ε, 4530). IR spectrum (in $CHCl_3$): 892 cm^{-1} (=CH_2).

Esterification of IVb *with* 3,5-*dinitrobenzoyl chloride.* A solution of 0·5 g IVb in 5 cc pyridine was added to 1·5 g 3,5-dinitrobenzoyl chloride in 30 cc pyridine and was left overnight. It was poured over ice and extracted with benzene. The benzene solution was washed successively with 5% H_2SO_4aq, 5% $NaHCO_3$aq and water, dried over Na_2SO_4 and evaporated to dryness. The foam obtained was chromatographed on 50 g acid washed alumina. The crystalline fractions eluted with pentane–ether (4:1) were recrystallized from acetone–hexane, giving 150 mg *mono* 3,5-*dinitrobenzoate of cannabidiolic acid methyl ester* (IVe) m.p. 173–175° $[\alpha]_D$-20°, δ^{19} (in $CDCl_3$), 0·88 (3) (t) (C_T − H);

[17] The tests were performed by Drs. M. Aronson and J. Markenson of the Israel Institute for Biological Research.

[18] H. Kubo and T. Mizuno, *Bot. Mag. Japan* **61**, 64 (1948); Y. Hirata and K. Nakanishi, *J. Biol. Chem.* **184**, 135 (1950); T. Goto, H. Kakisawa and Y. Hirata, *Tetrahedron* **19**, 2079 (1963).

[19] The abbreviations and symbols used are the same as in the Table.

2·40–2·85 (m) (C_8—H); 3·70 (3) (s) (C_E—$COOCH_3$); 3·55–3·70 (1) (C_B—H); 4·45, 4·60 (2) (C_E—H); 5·60 (1) (br) (s) (C_A—H); 6·70 (1) (s) (C_Q—H); 9·30 (3) (s) (aromatic protons of the 3,5-dinitrophenyl ring); 6·2–6·5 (1) (br) (phenolic H) (exchanges with D_2O). (Found: C, 63·99; H, 6·20; N, 4·63. $C_{30}H_{34}O_8N_2$ requires: C, 63·59; H, 6·05; N, 4·94%). Pentane–ether (3:1) eluted a number of fractions containing mixtures followed by the amorphous *bis-3,5-dinitrobenzoate of cannabidiolic acid methyl ester* (IVd) (150 mg), δ^{19} (in $CDCl_3$), 0·88 (3) (t) (C_T—H); 2·50–2·80 (m) (C_S—H); 3·75 (3) (s) (C_E—$COOCH_3$); ca. 3·60–3·75 (1) (C_B—H); 4·55, 4·75 (2) (C_E—H); 5·40 (1) (br) (C_A—H); 7·10 (1) (s) (C_Q—H); 9·30 (6) (aromatic protons of the 3,5-dinitrophenyl ring).

Cannabigerolic acid methyl ester (Ib) UV spectrum: λ_{max} 223 mμ (ε, 22,650), 271 mμ (ε, 12,580), 302 mμ (ε, 4080).

Esterification of Ib *with 3,5-dinitrobenzoyl chloride.* This reaction was done as described for the parallel one of IVb. A mixture of the *bis* with, probably, the *mono*, 3,5-dinitrobenzoate was obtained. Chromatography on silica gel (elution with pentane: ether, 8:2) gave a white powder which on crystallization from hexane gave crystals, m.p. 110–112°. (Found: C, 58·60; H, 5·22; N, 6·90. $C_{37}H_{38}N_4O_{14}$ requires: C, 58·30; H, 5·02; N, 7·35%).

Cannabidiolic acid diacetate (IVc) was prepared according to Šantavý.[11] It melts at 126–128°. Direct comparison with a sample kindly supplied by Prof. Šantavý showed that the compounds are identical. Their IR spectra are superimposable and on mixing no lowering of their m.p. was observed.

Decarboxylation of VIb. A 20 mg portion of VIb was boiled in 5 cc MeOH containing 3 cc 5% NaOH solution for 1 hr. The cooled solution was extracted with ether. On evaporation 18 mg cannabinol (VIa) were obtained. On TLC, VIa and authentic cannabinol showed identical R_f values. As cannabinol acetate is crystallized with greater ease than cannabinol, VIa was acetylated with pyridine (1 cc) and acetic anhydride (100 mg). Ten mg crystals, m.p. 69–72°, were obtained after the usual work up followed by filtration through acid washed alumina and crystallization from MeOH. The compound moved as a single spot on TLC with R_f equivalent to that of an authentic sample of cannabinol acetate. Identification was further established on the basis of mixture m.p. as well as IR comparisons.

Decarboxylation of Ib *and* IVb. These two compounds were decarboxylated as described above for VIb. Ib gave cannabigerol (Ia), identified on the basis of identical R_f value on TLC, mixture m.p. and IR comparison with an authentic sample.[3] IVb gave cannabidiol (IVa) identified on the basis of identical R_f value on TLC, and IR comparison with an authentic sample.[3] A mixture m.p. of the *bis*-3,5-dinitrobenzoate of IVa with an authentic sample gave no depression.

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