119. Cannabis Indica. *Part II. Isolation of Cannabidiol from Egyptian Hashish. Observations on the Structure of Cannabinol.*

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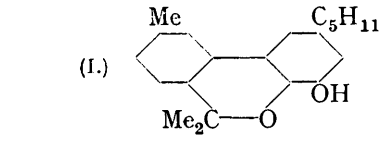
Cannabidiol, $C_{21}H_{30}O_2$, a typical constituent of American hemp resin (Adams, Hunt, and Clark, *J. Amer. Chem. Soc.*, 1940, **62**, 196), has been isolated from hashish of Egyptian origin, where it was accompanied by an approximately equal amount of

cannabinol. The simultaneous occurrence of these substances and their resemblance in many reactions lend support to the view that cannabidiol has a structure of the type suggested by Adams, Hunt, and Clark (*loc. cit.*). Cannabidiol contains two double bonds and its absorption spectrum indicates that neither double bond is conjugated with the aromatic nucleus. The structure of cannabinol is discussed; of four possible structures, (I) and (III) seem most in keeping with the available evidence.

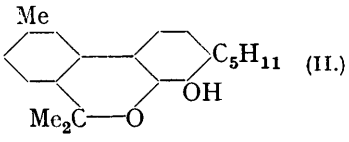
In Part I (Work, Bergel, and Todd, *Biochem. J.*, 1939, **33**, 123) a method was described for the separation of cannabinol from Indian hashish; *p*-nitrobenzoylation of the distilled resin prepared from the drug yielded a mixture of esters, from which the crystalline cannabinol derivative could readily be removed by reason of its lesser solubility in light petroleum. It is known that *Cannabis* resin varies a good deal in composition and physiological potency according to the locality in which the hemp is grown, and it was therefore desirable to apply similar separation methods to other samples of the drug. Through the co-operation of the Home Office (Drugs Branch) we were able to examine a fresh specimen of Egyptian hashish (seized in 1939) and some of our results are now recorded. A preliminary note concerning them has been published elsewhere (*Nature*, 1940, **145**, 350).

The drug was in the form of hard, khaki-coloured, flat slabs containing much sand and other inorganic material; from it the physiologically active resin was extracted with light petroleum. A noteworthy feature of the crude resin thus obtained was that after extraction of its ethereal solution with sodium carbonate, there remained only traces of material which could be extracted with sodium hydroxide. There is apparently much variation in the amount of alkali-extractable material present in the hemp drugs. It has been reported on various occasions that considerable amounts of physiologically active material can be extracted with alkali; neither in the Indian nor in the Egyptian material which we have examined has this been the case. A similar result was obtained by Cahn (J., 1930, 986). Since hashish appears on the illicit market in a variety of forms prepared and stored under very different conditions, it is possible that these anomalies may be due to some alteration of the original constituents of the resin. In order to decide this point absolutely fresh hemp resin ought to be examined; unfortunately through lack of material we have not yet been able to do so. Following our normal procedure, the resin, freed from acidic impurities, was distilled under a pressure of 10^{-3} mm., the main fraction being obtained as a pale yellow resin which became reddish on keeping exposed to light. The distilled resin gave a violet colour with alcoholic potassium hydroxide (Beam test), much more intense than that given by the distilled Indian resin, which it otherwise closely resembled. The resin was *p*-nitrobenzoylated and separated into two fractions according to degree of solubility in light petroleum. The less soluble fraction, which in the case of the Indian resin (Part I, *loc. cit.*) solidified almost completely, in this case remained largely resinous. Fractional crystallisation and chromatographic analysis led to the separation of cannabinol *p*-nitrobenzoate together with another lower-melting ester, which had an indefinite m. p. *ca.* 80° and could not be satisfactorily purified. The low melting ester on warming with alcoholic potassium hydroxide developed a violet colour, differing in this respect from the cannabinol ester. At this juncture the isolation of cannabidiol from the red oil of American wild hemp was reported (Adams, Hunt, and Clark, *J. Amer. Chem. Soc.*, 1940, **62**, 196) and from the behaviour of this substance towards alkali it seemed probable that the low-melting ester mentioned above was a cannabidiol derivative. This was confirmed by hydrolysis, followed by acylation with 3 : 5-dinitrobenzoyl chloride, the ester then obtained corresponding in all its properties to cannabidiol bis-3 : 5-dinitrobenzoate described by the American authors (*loc. cit.*). While an accurate estimate of quantities is difficult on account of inevitable losses in separation, it would appear that cannabinol and cannabidiol are present in approximately equal proportions in Egyptian resin. From resin of Indian origin we have not yet been able to isolate any cannabidiol, although it may be present in traces, since the crude distilled resin gives a positive, if rather weak, Beam test. It is of course also possible that the Indian resin contains not cannabidiol but some other closely related substance. It is interesting to note that in the case of American hemp resin cannabidiol is present in considerable quantities but no cannabinol has yet been isolated (Adams, Hunt, and Clark, *loc. cit.*).

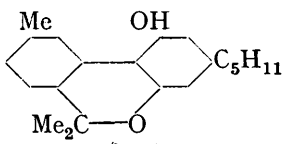
Cannabinol is a cryptophenolic substance, $C_{21}H_{26}O_2$, and for it Cahn (J., 1932, 1342) proposed structure (I), in which only the positions of the hydroxyl and the *n*-amyl group remained uncertain. We have been for some time engaged on synthetic experiments designed to clear up the structure of cannabinol, but it is also possible to draw some conclusions from the colour reactions of the compound. Cannabinol gives an intense blue colour with 2 : 6-dichloroquinonechloroimide, indicating that the *p*-position to the phenolic hydroxyl is unsubstituted (cf. Gibbs, *J. Biol. Chem.*, 1927, **72**, 649). This rules out positions 4′ and 5′ for the hydroxyl group, leaving four possible structures, (I), (II), (III), and (IV), for cannabinol.



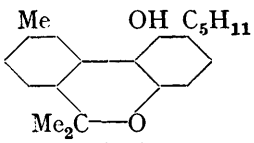
(I.)



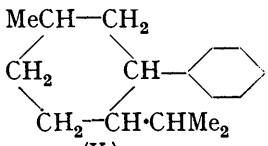
(II.)



(III.)



(IV.)



(V.)

Of these, (I) and (III) are the more probable, as they afford a better explanation of the results of nitration of cannabinol and its methyl ether recorded by Cahn (J., 1932, 1342). The absorption spectrum of cannabinol in alcoholic solution shows a maximum at 2850 A. whose intensity (ε mol. = 16,790) agrees with the presence of a diphenyl chromophoric system (cf. Gillam and Hey, J., 1939, 1170), although of course it throws no further light on the location of substituents.

Cannabidiol prepared by hydrolysis of its bis-3 : 5-dinitrobenzoate is a yellowish resin distilling unchanged in a high vacuum. Analyses of the free phenol and its ester are in agreement with the formula $C_{21}H_{30}O_2$ suggested by the American authors. On microhydrogenation cannabidiol absorbs 2 mols. of hydrogen, indicating the presence of two double bonds. Its absorption spectrum in alcohol has a maximum at 2775 A. whose low intensity (ε mol. = 1350), while in accordance with the presence in the molecule of one aromatic nucleus, indicates that neither of the double bonds can be conjugated with that nucleus. The colour reactions of cannabidiol recorded by Adams, Hunt, and Clark (*loc. cit.*) are very similar indeed to those given by cannabinol, save that the latter gives no coloration with alcoholic potassium hydroxide; probably the presence of two free hydroxyls is necessary for the production of colour. These facts together with the simultaneous presence of cannabinol and cannabidiol in Egyptian hashish support the view of the American authors that cannabidiol is to be regarded as a doubly unsaturated derivative of menthylbenzene (V) containing two hydroxyl groups and one *n*-amyl group located at positions in the benzene ring corresponding to those substituted in cannabinol. In a private communication just received by one of us Prof. Adams (Illinois) states that it has been shown that cannabidiol is a resorcinol derivative. This being so, then structure (III) would seem more likely than (I) for cannabinol, although we have hitherto been inclined to the latter structure on account of the reducing properties shown by cannabinol. A decision on this point is being sought by synthetic methods.

It may be observed that on the above view of the structure of cannabidiol the substance might arise in the plant by condensation of a monocyclic terpene with a dihydric phenol and that it may be converted into cannabinol by cyclisation and dehydrogenation. Attempts to realise such a conversion experimentally are being made.

Both cannabinol and cannabidiol appear to be inactive in the Gayer test (Gayer, *Arch. Exp. Path. Pharm.*, 1928, **129**, 312) in rabbits. Details of biological tests and of the further fractionation of the active portions of Egyptian and Indian hashish will be published in further communications.

Experimental.

Extraction of Egyptian Hashish.—The drug (EH 39; 500 g.) was in the form of hard, khaki-coloured, flat slabs and was broken into small pieces and extracted at room temperature with light petroleum (3 l., b. p. 40—60°). The crude extract was washed with sodium carbonate solution (5%), then with water, and dried over sodium sulphate. Evaporation gave a thick brown oil (70 g.) still containing traces of solvent. This oil was dissolved in hot methyl alcohol (*ca.* 350 c.c.), and the solution left overnight in the ice-chest. The crystalline hydrocarbon (3 g.) which separated was filtered off, and the mother-liquor concentrated to half its volume; as no more crystals separated, the whole solution was evaporated to dryness, and the oil (60 g.) subjected to slow distillation in a molecular still under a pressure of 10^{-3} mm. After a considerable amount of terpene derivatives had come over below 110° the main resin fraction (28·4 g.) distilled between 110° and 130°, leaving a dark-coloured tar (15 g.) behind. The distilled resin was pale yellow and extremely viscous; it reddened slowly on keeping and gave an intense violet colour with alcoholic potassium hydroxide.

p-Nitrobenzoylation of the Distilled Resin.—The above resin (28·4 g.) was acylated in two portions with *p*-nitrobenzoyl chloride in pyridine (cf. Work, Bergel, and Todd, *loc. cit.*). The crude nitrobenzoate mixture (34 g.) was dissolved in light petroleum (b. p. 80—100°) and concentrated to 300 c.c., and the solution left overnight in the ice-chest. A thick oil (20 g.) containing some crystals separated and from it the light petroleum solution was decanted. The decanted solution contained a brownish resin (14 g.) containing all the material giving a positive Gayer test in rabbits. It was worked up separately and will be described elsewhere.

The portion insoluble in 300 c.c. of light petroleum was dissolved in a mixture of light petroleum (70%, b. p. 40—60°) and benzene (30%) and chromatogrammed in two portions on towers of aluminium oxide (Merck and Birmingham Electric Furnaces Co. products were equally effective), the columns being washed with the same solvent until practically nothing further came through. The combined washings gave on evaporation a yellow oil (A) (*ca.* 10 g.), and the rest of the material (B), which gave several ill-defined bands in the columns, was eluted with acetone.

From the oil (A) pure cannabinol *p*-nitrobenzoate was obtained by repeated crystallisation from alcohol. It formed nearly colourless needles (5 g.), m. p. 159—160°, undepressed on admixture with an authentic specimen. From the mother-liquors of the cannabinol *p*-nitrobenzoate and from alcoholic solutions of (B) quantities of nearly colourless crystals slowly separated; these had rather an indefinite m. p. 70—80° and could not be satisfactorily purified. Unlike cannabinol *p*-nitrobenzoate, this solid material gave an intense violet colour on warming for a short time with alcoholic potassium hydroxide.

Cannabidiol Bis-3 : 5-dinitrobenzoate.—The material (B) above was combined with the product from the mother-liquors of the cannabinol *p*-nitrobenzoate crystallisation and hydrolysed by refluxing in a nitrogen atmosphere with 4% alcoholic potassium hydroxide during 1 hour. When the deep violet solution was acidified, the colour changed to yellow; the hydrolysis product was extracted with ether, freed from *p*-nitrobenzoic acid, dried, and evaporated. The resulting oil (6 g.) was treated with 3 : 5-dinitrobenzoyl chloride (9 g.) in pyridine (100 c.c.), and the product worked up in a manner similar to that described by Adams, Hunt, and Clark (*loc. cit.*). Cannabidiol bis-3 : 5-dinitrobenzoate was obtained as colourless needles (4 g.), m. p. 106—107°, after three recrystallisations from methyl alcohol–methyl acetate (2 : 1); in acetone solution ($c = 1{\cdot}876$; $l = 1$) it had $[\alpha]_D^{13°} - 76{\cdot}2°$ [Found: C, 59·7; H, 5·0; N, 7·9. Calc. for $C_{21}H_{28}(O{\cdot}CO{\cdot}C_6H_3O_4N_2)_2$: C, 59·8; H, 4·9; N, 8·0%]. Adams, Hunt, and Clark (*loc. cit.*) give m. p. 106—107°, $[\alpha]_D^{27°} -76°$. Prof. Adams, to whom we express our thanks, confirmed the identity of our product by direct comparison with material from American hemp; a mixed m. p. showed no depression.

The mother-liquors from the cannabidiol bis-3 : 5-dinitrobenzoate gave on evaporation a thick reddish oil (*ca.* 4 g.) which could not be crystallised; it developed a violet colour on warming with alkali.

Cannabidiol.—(a) A sample of the above dinitrobenzoate was hydrolysed by refluxing for 1 hour with methyl-alcoholic potassium hydroxide (4%) in a nitrogen atmosphere. The alkaline hydrolysis solution had an intense violet colour. From it the product was isolated by acidification, extraction with ether, removal of dinitrobenzoic acid, and distillation in a high vacuum. Cannabidiol distilled smoothly at 160—180° (bath temp.)/10^{-3} mm. as a pale yellow resin (Found: C, 79·7; H, 9·6. Calc. for $C_{21}H_{30}O_2$: C, 80·2; H, 9·6%). In alcoholic solution ($c = 2{\cdot}16$; $l = 1$) it had $[\alpha]_D^{18°} -126{\cdot}6°$. Quantitative micro-hydrogenation in glacial acetic acid solution with a platinum oxide catalyst caused absorption of 2·05 mols. of hydrogen.

Cannabidiol, like cannabinol, gave a deep blue colour with 2 : 6-dichloroquinonechloroimide. (b) Another sample of the dinitrobenzoate was hydrolysed with liquid ammonia in the manner described by Adams, Hunt, and Clark (*loc. cit.*). The distilled product was rather less coloured than that obtained by hydrolysis with potassium hydroxide (Found : C, 79·9; H, 9·7%).

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