131. Cannabis Indica. *Part XI. An Examination of the Alkali-soluble Portion of American Hemp Resin.*

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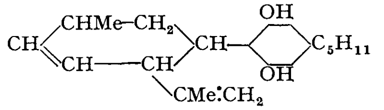
The alkali-soluble portion of the resin extracted from American wild hemp (marihuana) can be separated into two components, one of which can and the other cannot be extracted from alkaline solution with ether. These materials, on boiling with methyl alcohol, or better on standing with alkali, yield alkali-insoluble resins, from which cannabidiol and cannabinol respectively have been isolated. On the evidence presented it is concluded that the alkali-soluble portion of the resin contains esters of cannabidiol and cannabinol with a phenolic acid. This conclusion is supported by the properties of synthetic esters of these compounds with *p*-hydroxybenzoic acid.

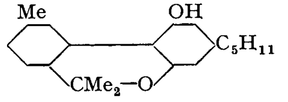
In the latter part of 1939 we were privileged, through the courtesy of Mr. F. Thornton, of the Home Office, and of Mr. H. J. Anslinger of the U.S. Bureau of Narcotics, to read a report submitted to the latter by an American chemist dealing with the alkali-soluble portion of the resin from American hemp (marihuana). The findings in this report were of great interest to us, since, although alkali-solubility had been reported occasionally in the past for a part of the physiologically active resin from *Cannabis* (cf. *e.g.*, Hoffmann-La Roche, D.R.P. 285,829, 1913), no alkali-soluble components other than minor amounts of fatty acids, etc., had been found in any samples of Indian or Egyptian resin examined in these laboratories. This of course may have been due to the fact that our starting materials were always Oriental prepared drugs (ganja or hashish), which might well have undergone various changes after collection from the plant sources. Through the kindness of Mr. H. J. Anslinger, to whom our sincere thanks are due, we were supplied in the early summer of 1940 with a small amount of marihuana; from it we extracted the active resin and examined its properties in the light of the above-mentioned report. Our results obtained at that time we refrained from making public until the American chemist published his original observations; this has now been done [Fulton, *Ind. Eng. Chem.* (*Anal.*), 1942, **14**, 407] and our findings, which amplify them in several important respects, are now recorded.

To make the general position as established by Fulton (*loc. cit.*) clear, it is necessary to summarise his findings briefly. He found that a portion of the undistilled hemp resin could be extracted from light petroleum solution with dilute aqueous sodium hydroxide. The alkali-soluble material could itself be separated into two distinct resinous fractions: (*a*) " Cannabinol IRAB," which could not be extracted from alkaline solution with ether and gave a positive reaction (purple colour) in the alkaline Beam test (4th Report, Wellcome Trop. Res. Lab., Khartoum, 1911, *B*, 25), and (*b*) " cannabinol INRAB," extractable from alkaline solution with ether and giving no coloration in the Beam test. These two resins, on refluxing with methyl alcohol, gradually changed, yielding alkali-insoluble resins whose other properties remained similar to those of the originals. These products were described by him respectively as " cannabinol IIRAB " and " cannabinol IINRAB " and it was tentatively suggested on the basis of colour reactions that they might be identical with cannabidiol (I) and cannabinol (II), both well-known constituents of *Cannabis* resins, although no satisfactory explanation was given of the reactions involved in the various changes.

On extracting the resin from the sample of marihuana at our disposal and performing the alkali separations described by Fulton (*loc. cit.*), we readily obtained the products IRAB and INRAB (for convenience we omit the prefaced " cannabinol," which is in any event misleading) and confirmed their conversion into IIRAB and IINRAB on prolonged boiling with methyl alcohol. This behaviour seemed to us most readily explained on the assumption that IRAB and INRAB contained esters of cannabidiol and cannabinol respectively with some phenolic acid conferring alkali-solubility, and that on heating with methyl alcohol methanolysis occurred, liberating cannabidiol (IIRAB) and cannabinol (IINRAB), which are alkali-insoluble and show the appropriate behaviour in the alkaline Beam test. On this view IRAB and INRAB would conform in type to the depside class of natural products, esters which in many cases undergo fission on heating with methyl alcohol. It is perhaps of some significance in this connection that both cannabidiol and cannabinol may be regarded as derivatives of olivetol (5-*n*-amylresorcinol), a well-known constituent of natural depsides (*e.g.*, olivetoric acid), in which it occurs esterified with phenolic acids. In accordance with this view the strong green ferric chloride reaction of IRAB and INRAB was not given by the alkali-insoluble resins prepared by heating with methyl alcohol, but it remained a property of the alkali-soluble portion of the reaction products. Again, it was found that the conversions IRAB ⟶ IIRAB and INRAB ⟶ IINRAB could be brought about rather more easily by standing with sodium hydroxide. From IIRAB prepared in this way we isolated cannabidiol as its 3 : 5-dinitrobenzoate (Adams, Hunt, and Clark, *J. Amer. Chem. Soc.*, 1940, **62**, 196) and from IINRAB cannabinol as its *p*-nitrobenzoate (Work, Bergel, and Todd, *Biochem. J.*, 1939, **33**, 123), although neither could be isolated from IRAB or INRAB before alkali treatment. The alkali-soluble products of the conversion in each case contained an acid giving a strong green ferric reaction. Unfortunately we were unable with the amount at our disposal to characterise this acid, but it resembled protecatechuic acid closely in its reactions.

(I.)





(II.)

Since lack of marihuana precluded further study of the above phenolic acid, confirmatory evidence for our hypothesis was sought in another way. *Cannabinol* p-*carbomethoxybenzoate* and *cannabidiol bis*-p-*carbomethoxybenzoate* (the latter in a somewhat impure condition) were prepared and partially hydrolysed under conditions designed to remove only the carbomethoxy-group and yield the corresponding *p*-hydroxybenzoates. The products, both resinous, showed, apart of course from the ferric reaction, exactly similar properties to the alkali-soluble fractions of the resin from marihuana. Thus the product from cannabidiol, like IRAB, was soluble in alkali and could not be extracted from alkaline solution with ether, whereas the cannabinol derivative, although also soluble in alkali, could, like INRAB, be extracted from alkaline solution with ether. Like IRAB and INRAB, too, they gave respectively cannabidiol and cannabinol on boiling with methyl alcohol or standing with alkali. In view of these findings there can be little doubt that, in American hemp at least, cannabidiol and cannabinol occur in part esterified with some phenolic acid or acids and that in this form they are alkali-soluble. The extent to which they occur in this form may well vary considerably in different samples of hemp.

In the course of the present investigation we took the opportunity to have the various marihuana fractions examined for hashish activity, using the Gayer test on rabbits. The results, which are recorded in the experimental section, show that the alkali-soluble portion of the resin has at most very little activity, and that the physiologically active material is mainly found, as in the Oriental drugs, in one high-boiling fraction of the alkali-insoluble resin. From this active fraction we isolated a quantity of cannabidiol as its **3** : 5-dinitrobenzoate, but were unable to isolate cannabinol as its *p*-nitrobenzoate, although this procedure is satisfactory in the case of Indian and Egyptian active resins (Work, Bergel, and Todd, *loc. cit.*; Jacob and Todd, J., 1940, 649). Failure to isolate cannabinol from marihuana by this method has already been reported by Adams and his collaborators (*loc. cit.*). The absence of any marked physiological activity in the alkali-soluble portion of the resin explains, of course, the apparent lack of correlation between the proportion of this material and the potency of drugs from different sources. Since the physiological activity of hemp resins appears, on available evidence, to be due to the presence of a mixture of tetrahydrocannabinols, it seems not unreasonable to expect that in some cases the active compounds might also exist in the form of depsides. This might explain the occasional reports of the value of extraction with alkali in concentrating hemp preparations.

EXPERIMENTAL.

Preparation of the Resin.—The starting material (1435 g.), consisting of the dried flower tops and leaves of American hemp, was percolated with light petroleum (b. p. 40—60°); the petroleum extract on evaporation yielded a dark brown resin (67 g.).

Alkali-soluble Portion of the Resin: IRAB and INRAB.—The above resin (63 g.), dissolved in light petroleum, was extracted rapidly with sodium hydroxide solution (2%) containing *ca.* 2% of sodium sulphite to prevent oxidation (cf. Fulton, *loc. cit.*). The alkali-insoluble portion was recovered from the petroleum layer by evaporation (41 g.). From the purple alkaline solution, INRAB (3·4 g.) was obtained by ether extraction; this product, a brownish resin, gave a feeble colour in the alkaline Beam test (probably due to traces of IRAB) and a strong green ferric reaction. IRAB (13 g.) was obtained from the residual alkaline solution by acidification and extraction with light petroleum; it showed a strong green ferric reaction and was strongly positive in the alkaline Beam test.

Conversion of IRAB into IIRAB and INRAB into IINRAB.—IRAB (0·25 g.) was refluxed with methyl alcohol (30 c.c.) for 6 hours, the solution evaporated, and the residue extracted with light petroleum. After separation from insoluble matter (0·1 g.) the solution was treated with alkali as in the original separation; the resin (0·12 g.) was now alkali-insoluble (IIRAB). In exactly similar fashion INRAB was converted into IINRAB. In both cases IIRAB and IINRAB gave no coloration with ferric chloride, but the alkali-soluble portion of the reaction products gave a deep green coloration.

Hydrolysis of IRAB with Alkali: Isolation of Cannabidiol.—IRAB (2 g.) was dissolved in the sodium hydroxide–sodium sulphite mixture (18 c.c.) used in the original separation. On standing at room temperature a resin gradually separated; after 2 days this resin was extracted with ether. It had the properties of IIRAB and on treatment with 3 : 5-dinitrobenzoyl chloride it gave cannabidiol bis-3 : 5-dinitrobenzoate, m. p. 105°, undepressed by an authentic sample (m. p. 106—107°) from Egyptian hashish.

The alkaline solution, after removal of IIRAB in this experiment, was acidified and extracted several times with ether. The combined extracts were extracted with sodium bicarbonate solution, the bicarbonate layer being subsequently acidified and again extracted with ether. Evaporation of the ethereal solution gave a small amount of gummy acid which reduced ammoniacal silver nitrate and gave a deep green coloration with ferric chloride. This gum could be made to crystallise by rubbing with a crystal of protocatechuic acid (which shows similar reactions), but the amount obtainable was too small to permit of rigid identification. If the acid in question were indeed protocatechuic acid, most of it would have been lost in the process of working-up owing to high water-solubility.

Hydrolysis of INRAB with Alkali: Isolation of Cannabinol.—The above experiment was repeated with INRAB with similar results. The acid fraction again had the colour reactions of protocatechuic acid. The IINRAB produced was distilled and *p*-nitrobenzoylated. The crude ester, dissolved in methyl alcohol–methyl acetate and allowed to cool, yielded yellowish needles. Recrystallised from alcohol, they had m. p. 161—162°, undepressed by cannabinol *p*-nitrobenzoate (m. p. 161—162°).

Alkali-insoluble Portion of Hemp Resin.—That portion (41 g.) of the original resin which was not extractable from light petroleum solution with alkali was dissolved in warm methyl alcohol (500 c.c.); a crystalline hydrocarbon (12 g.) (assumed to be nonacosane by analogy with other hemp resins) slowly separated. The solution was evaporated, and the residual resin distilled under *ca.* 10^{-4} mm., the following fractions being obtained (all temps. are bath temp.): (i) Up to 108° (3 g.), Beam, $-$ve; (ii) 110—130° (5 g.), Beam, $+$ve; (iii) 130—150° (5 g.), Beam, faintly $+$ve; (iv) residue, 16 g. Fraction (ii) contained virtually all the physiologically active material; it was *p*-nitrobenzoylated and chromatogrammed on aluminium oxide, a solid ester, m. p. *ca.* 70°, being obtained. This on hydrolysis and re-acylation with 3 : 5-dinitrobenzoyl chloride gave cannabidiol bis-3 : 5-dinitrobenzoate, m. p. 105—106°. From the residual oily *p*-nitrobenzoates no cannabinol derivative crystallised; they contained the active material and were practically Beam negative.

Pharmacological Test Results.—We are indebted to Professor A. D. Macdonald of this University for the following results (Gayer test):

1 Total light petroleum extract of marihuana. Active at 5 mg./kg.
2 Alkali-insoluble portion of above extract. Active down to 1 mg./kg.
3 IRAB
4 INRAB } Inactive at 5 mg./kg.
5 IIRAB
6 IINRAB
7 Fraction (ii) of distilled alkali-insoluble resin. Active at 1 mg./kg.

Cannabinol p-*Carbomethoxybenzoate.*—Cannabinol (0·3 g.) was dissolved in pyridine (1 c.c.), *p*-carbomethoxybenzoyl chloride (0·2 g.) (Fischer, *Ber.*, 1908, **41**, 2878) added, and the mixture heated for several hours on the steam-bath and left overnight. On pouring into water and acidifying with hydrochloric acid a brownish precipitate separated; it was washed with sodium bicarbonate solution and water and purified by repeated precipitation from chloroform solution with methyl alcohol. The product was a white powder, m. p. 195°, of doubtful crystalline nature (Found in material dried at 120°: C, 73·5; H, 6·6. $C_{30}H_{32}O_6$ requires C, 73·8; H, 6·6%).

The above *ester* (0·75 g.) was mixed with N-potassium hydroxide (50 c.c.) and left at room temperature for 30 minutes; the mixture was then acidified and extracted with ether, and the extract washed with bicarbonate solution. Evaporation of the dried extract yielded a brownish resin. This was dissolved in light petroleum and the alkali separation of Fulton (*loc. cit.*) applied to the solution. A certain amount of the resin (0·3 g.) was insoluble in alkali (presumably owing to the hydrolysis of the carbomethoxy-ester having in part gone too far, yielding cannabinol), but the remainder (0·2 g.) was, like INRAB, soluble in alkali but extractable from alkaline solution with ether. The latter portion on refluxing with methyl alcohol for 5 hours was largely converted into an alkali-insoluble resin like IINRAB. A small amount remained unaffected at the end of this time; apparently the *p*-hydroxybenzoate of cannabinol is rather more resistant to methanolysis than INRAB.

*Cannabidiol Bis-*p-*carbomethoxybenzoate* (?).—Esterification of cannabidiol (2 g.) with *p*-carbomethoxybenzoyl chloride (2 g.) in pyridine (20 c.c.) was carried out as above. The product was a brownish resin which could not be obtained in solid form. It distilled at *ca.* 130—150° (bath temp.)/10^{-3} mm. but gave unsatisfactory analytical values (Found: C, 65·0; H, 6·4. $C_{39}H_{42}O_{10}$ requires C, 69·9; H, 6·4%). As it probably consisted mainly of the desired ester, it was used directly for further work.

The resin (1 g.) was partially hydrolysed under nitrogen by N-sodium hydroxide (30 c.c.) for 30 minutes. The product (0·5 g.), subjected as above to the alkali separation, gave in addition to some alkali-insoluble material (0·3 g.) a resin (0·1 g.) which, like IRAB, was soluble in alkali, not extractable from alkaline solution with ether, and on prolonged boiling with methyl alcohol was converted into an alkali-insoluble product just as IRAB can be converted into IIRAB.

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