32. Cannabis Indica. *Part X. The Essential Oil from Egyptian Hashish.*

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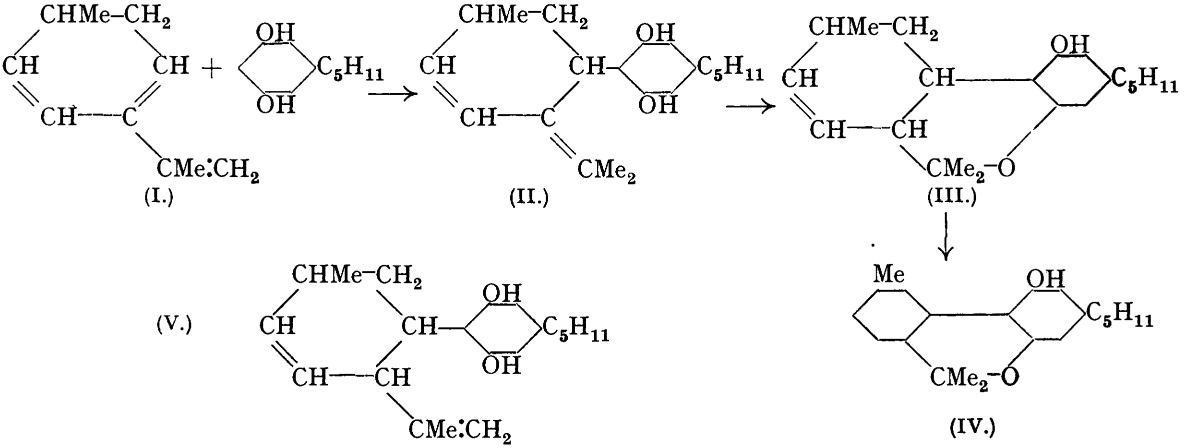
Examination of the essential oil from Egyptian hashish shows that the lower-boiling "terpene fraction" consists mainly of *p*-cymene with small amounts of 1-methyl-4-*iso*propenylbenzene and unidentified optically active material. From the higher-boiling fraction of the oil, humulene (α-caryophyllene) has been isolated. A hypothetical scheme for the biogenesis of cannabinol and related compounds is discussed in the light of these findings.

It has long been known that *Cannabis* resin contains a considerable proportion of material, generally described as the " terpene fraction," which is devoid of hashish activity and boils at considerably lower temperatures than the active " crude cannabinol." The only recorded examination of it was made by Wood, Spivey, and Easterfield (J., 1896, **69**, 539), who reported the separation from it of a terpene, b. p. 165—175°, possibly myrcene, and a sesquiterpene. A renewed examination of the low-boiling constituents of the resin was particularly desirable in view of the suggestion made in earlier papers of this series that some of the typical constituents of hemp resin, *e.g.*, cannabidiol and cannabinol, owe their origin in nature to terpene–phenol condensations. In the course of his investigations on cannabinol (J., 1930, 986; 1931, 630; 1932, 1342; 1933, 1400) Cahn accumulated a quantity of the lower-boiling oils from Egyptian hashish. We have been fortunate in having this material placed at our disposal in the form of two fractions, described as "low boiling" and "high boiling terpenes"; these oils have now been examined with the following results.

The "low boiling terpene" fraction consisted mainly of *p*-cymene in admixture with an unidentified optically active constituent and 1-methyl-4-*iso*propenylbenzene. The last-named substance was identified by oxidation to *p*-tolyl methyl ketone, characterised by preparation of its semicarbazone and 2 : 4-*dinitrophenylhydrazone*. In addition to terephthalic and 4-*iso*propenylbenzoic acids derived from *p*-cymene, oxidation of various fractions of the oil gave succinic and lævulic acids; these were probably derived from the unidentified optically active constituent. Contrary to the findings of Wood, Spivey, and Easterfield (*loc. cit.*) no myrcene was detected. Its absence may have been due to the fact that the oil examined by us was prepared some years ago from Egyptian hashish of uncertain age, since myrcene is known to polymerise readily. 1-Methyl-4-*iso*propenylbenzene does not appear to have been found previously in nature, and its occurrence in hashish is of some interest in connection with the biogenesis of compounds related to cannabinol. A reasonable, if entirely speculative, scheme for the biogenesis of cannabinol is given below in formulæ (I) to (IV). All the reactions involved would appear to be within the normal compass of plant metabolism.



(I.) (II.) (III.) (IV.) (V.)

Structure (II), derived from condensation of a menthatriene (I) with olivetol, differs only in the location of one ethenoid linkage from that (V) proposed by Adams, Loewe, Pease, Cain, Wearn, Baker, and Wolff (*J. Amer. Chem. Soc.*, 1940, **62**, 2566) for cannabidiol, and indeed is identical with the structure formerly envisaged by us on the basis of the absorption spectrum of that compound. A compound of structure (V), of course, could hardly be produced by this type of mechanism without some secondary rearrangement. It may, however, perhaps be pertinent to recall here that the *iso*propenyl–*iso*propylidene change can occur under conditions such as would suggest that the two structures may in a sense be regarded as tautomeric (cf. Bradfield, Penfold, and Simonsen, J., 1935, 311). The position of the cyclic ethenoid linkage in cannabidiol (V) is not known with any certainty. Structure (III) probably represents one of the tetrahydrocannabinols produced on cyclisation of cannabidiol by acidic reagents. All available evidence suggests that the active fractions of hashish contain a mixture of tetrahydrocannabinols; these in all probability include the cyclised isomerides of cannabidiol. Dehydrogenation of (III) would yield cannabinol (IV). A menthatriene (I) could, of course, very readily yield by isomerisation *p*-cymene, or by dehydrogenation 1-methyl-4-*iso*propenylbenzene. The fact that these two compounds have been found to make up the bulk of the low-boiling "terpene fraction" of hashish is certainly not inconsistent with the formation of (I) as a phytosynthetic intermediate by the hemp plant, and in that sense lends colour to the speculative scheme outlined above.

From the higher-boiling "terpene fraction" a sesquiterpene was isolated and characterised as its nitrosochloride and nitrolpiperidide. It proved to be humulene (α-caryophyllene), hitherto found only in oil of hops and oil of cloves. Both the hop plant (*Humulus lupulus*) and hemp (*Cannabis sativa*) belong to the same botanical family (*Moraceæ*).

EXPERIMENTAL.

Low-boiling Terpene Fraction.—The pale brown oil used in these experiments had $d_{20^\circ}^{20^\circ}$ 0·8592, $n_D^{20^\circ}$ 1·4863, and $[\alpha]_{5461}$ +17·8°. A quantity of the oil was systematically fractionated (6 times) through a Widmer column. The two final fractionations were made over sodium; more than 50% of the oil distilled between 170° and 185°, the following fractions being obtained:

No.	B. p. (765 mm.).	$d_{20^\circ}^{20^\circ}$.	$n_D^{30^\circ}$.	$[\alpha]_{5461}$.	No.	B. p. (765 mm.).	$d_{20^\circ}^{20^\circ}$.	$n_D^{30^\circ}$.	$[\alpha]_{5461}$.
1	140—150°	0·7894	1·4496	+ 6·3°	5	169—175°	0·8500	1·4782	+27·8°
2	150—157	0·8035	1·4576	+13·1	6	175—178	0·8506	1·4800	+21·9
3	157—165	0·8304	1·4672	+24·4	7	178—180	0·8592	1·4909	+16·2
4	165—169	0·8451	1·4748	+28·5	8	180—185	0·8609	1·4948	+12·2

Crystalline derivatives could not be prepared from any of these fractions, nor did they react with maleic anhydride. From fraction 7 a *monohydrochloride*, b. p. 110—120°/21 mm. (Found: Cl, 20·0. $C_{10}H_{17}Cl$ requires Cl, 20·5%), was prepared. All these fractions were treated with oxidising agents (ozone and potassium permanganate) in attempts to isolate a characteristic derivative of the optically active hydrocarbon (or hydrocarbons) present in the oil. These experiments were all unsuccessful, the results of three being outlined below.

(1) *Ozonolysis of fraction* 6 (*b. p.* 175—178°). The oil (5 c.c.) in methyl acetate was ozonised at 0°; much formaldehyde was present in the issuing gases (dimedone test). After removal of the solvent under diminished pressure the ozonide was mixed with water (5 c.c.) and decomposed by heating on the water-bath for 1 hour. The aqueous solution was made alkaline with sodium carbonate and kept overnight after the addition of hydrogen peroxide (20 vol.; 1 c.c.). The neutral oxidation product (A) was dissolved in ligroin (b. p. 40—60°), the alkaline solution acidified, and the liquid acid (1·4 g.) extracted with ether.

The neutral oil (A) reacted readily with semicarbazide acetate, yielding a semicarbazone crystallising in silky prismatic needles, m. p. 203—204° alone and in admixture with *p*-tolyl methyl ketone semicarbazone (Found: C, 63·3; H, 7·2. Calc. for $C_{10}H_{13}ON_3$: C, 62·8; H, 6·8%). The 2 : 4-*dinitrophenylhydrazone* prepared from the neutral oil or from *p*-tolyl methyl ketone crystallised from *cyclo*hexyl acetate in scarlet prismatic needles, m. p. 252—253° (Found: N, 17·7. $C_{15}H_{14}O_4N_4$ requires N, 17·8%).

The acidic oxidation product, which was a gum, was not further examined.

(2) *Oxidation of fraction* 5 (*b. p.* 169—175°) *with potassium permanganate.* The oil (13 g.), suspended in water (250 c.c.) containing potassium hydroxide (26 g.), was mixed with ice (750 g.) and solid potassium permanganate (65 g.) and shaken for 20 hours. The excess of permanganate was removed with sulphur dioxide, the neutral oil (A) distilled in steam, and the aqueous solution after removal of the manganese dioxide sludge concentrated, acidified, and extracted with ether. Evaporation of the dried extract gave a gum (3·5 g.), from which a solid slowly separated, identified as terephthalic acid. From the filtrate, after removal of the ether, a crystalline solid slowly separated; after trituration with hydrochloric acid it crystallised from dilute acetic acid in needles, m. p. 156—157°, identified as *p*-*iso*propenylbenzoic acid (Found: C, 74·5; H, 6·2. Calc. for $C_{10}H_{10}O_2$: C, 74·1; H, 6·2%).

The neutral oil (A) (5 c.c.) was distilled over sodium and had b. p. 172—174°/759 mm., $d_{20^\circ}^{20^\circ}$ 0·8556, $n_D^{20^\circ}$ 1·4869, $[\alpha]_{5461}$ + 5·4°. It was oxidised with chromic acid as described by Linstead *et al.* (J., 1940, 1143) and gave terephthalic acid (2·75 g.), identified by the methyl ester, m. p. 140°. The percentage of *p*-cymene in this neutral oil is therefore approximately 72·7%.

(3) *Oxidation of fractions* 7 *and* 8 *with potassium permanganate.* The oil (55 g.) in acetone (200 c.c.), cooled in ice and mechanically stirred, was oxidised with potassium permanganate (150 g.). The manganese dioxide was separated, and the acetone removed from the filtrate, leaving an oil which on distillation at 754 mm. gave the following fractions: (i) b. p. 170—180°, mainly 175—176° (17·3 g.); (ii) b. p. 180—190° (5·8 g.); and (iii) b. p. 190—220° (2·5 g.). Fraction (iii) was practically pure *p*-tolyl methyl ketone, identified by the preparation of the semicarbazone and 2 : 4-dinitrophenylhydrazone. The same ketone was present also in small quantity in the two lower-boiling fractions, which, however, consisted essentially of *p*-cymene.

The manganese dioxide sludge was thoroughly extracted with hot water, and the aqueous solution concentrated, acidified, and extracted with ether in a constant extraction apparatus. This gave a liquid acid (20 g.), a considerable quantity of a resinous acid being insoluble in ether. The acid, which contained much acetic acid, reacted readily with carbonyl reagents and gave bromoform on mixing with sodium hypobromite. No crystalline derivatives could be prepared, the calcium and barium salts being soluble in water. The acid was esterified with methyl alcohol and sulphuric acid in the usual manner; the ester, on distillation under 18 mm., yielded the following fractions: (i) b. p. up to 100° (0·6 g.); (ii) b. p. 100—120° (1·7 g.); (iii) b. p. 120—140° (1·7 g.); (iv) b. p. 140—160°, mainly 155° (3·3 g.); and (v) b. p. 160—200° (2·5 g.).

Fractions (i) and (ii) reacted readily with 2 : 4-dinitrophenylhydrazine sulphate to yield a crystalline 2 : 4-dinitrophenylhydrazone, which separated from methyl alcohol in yellow needles, m. p. 131° alone and in admixture with methyl lævulate 2 : 4-dinitrophenylhydrazone (Found: C, 46·7; H, 4·2. Calc. for $C_{12}H_{14}O_6N_4$: C, 46·6; H, 4·2%).

The ester (2 g.) was hydrolysed with methyl-alcoholic potassium hydroxide and yielded by extraction with ether a liquid acid which partially crystallised. The solid was collected and recrystallised from hydrochloric acid; it then had m. p. 182—183° and was identified as succinic acid. No crystalline acid could be separated from the filtrate. Oxidation of this crude acid with sodium hypobromite gave a further small amount of succinic acid.

Fraction (iii) gave a liquid 2 : 4-dinitrophenylhydrazone. Hydrolysis of the ester (1·6 g.) with methyl-alcoholic potassium hydroxide gave an acid (1·4 g.), from which a small quantity of *p*-*iso*propenylbenzoic acid was isolated, formed presumably by loss of water from the *tert*.-hydroxy-acid. The liquid acid was oxidised with sodium hypobromite in the usual manner, the acid so obtained (1·1 g.) depositing an amorphous solid identified as terephthalic acid by the preparation of the methyl ester, m. p. 140°.

Fractions (iv) and (v) behaved similarly to fraction (iii). The only oxidation acid which could be identified was terephthalic acid.

High-boiling " Terpene " Fraction.—This oil distilled at 110—130°/20 mm. as a faintly yellow oil which reacted with Brady's reagent to yield in small amount a red liquid 2 : 4-dinitrophenylhydrazone.

A quantity of the oil (66 g.) was digested with alcoholic potassium hydroxide (KOH, 10 g.) to remove any esters present and after recovery of the neutral oil in the usual manner this was distilled under diminished pressure (18 mm.), the following fractions being obtained : (i) b. p. up to 100° (11·7 g.); (ii) b. p. 100—110° (11·5 g.); (iii) b. p. 115—125° (10·9 g.); (iv) b. p. 125—145° (22·4 g.); and (v) b. p. above 145° (6·2 g.). The lower-boiling fractions were yellow; fractions (iv) and (v) were blue and evidently contained some azulene. Redistillation (at 25 mm.) gave (*a*) b. p. up to 100°, mainly 80° (14·8 g.); (*b*) b. p. 110—115° (7·9 g.); (*c*) b. p. 115—125° (6·2 g.); (*d*) b. p. 125—140°, mainly 133—135° (21 g.); and (*e*) above 140° (10 g.).

Fraction (*d*) was redistilled twice over sodium and obtained as a faintly yellow oil, b. p. 130—132°/20 mm., $d_{20^\circ}^{20^\circ}$ 0·8958, $n_D^{20^\circ}$ 1·4962, $[\alpha]_{5461}$ −8·2°, consisting apparently essentially of humulene (α-caryophyllene). The nitrosochloride, decomp. 168° after softening at 155°, crystallised from chloroform–alcohol in cubes, decomp. 174°, and yielded a nitrolpiperidide, crystallising from alcohol in plates, m. p. 145—146°, unchanged in admixture with humulene nitrolpiperidide. The oil gave a liquid hydrochloride and on dehydrogenation with selenium an azulene resulted which was not further examined. No naphthalene hydrocarbon was formed.

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