LXXXVI.—Cannabis Indica *Resin. Part II.*

By ROBERT SIDNEY CAHN.

INVESTIGATIONS of *Cannabis Indica* resin (hashish, etc.), though numerous, are to a large extent contradictory. It is agreed that the active principle is contained in a high-boiling resin and that it is not alkaloidal (T. and H. Smith, *J. Pharm.*, 1857, **21**, 47), but it has never been isolated. From this high-boiling oil four substances, namely, cannabinol, trinitrocannabinol, a hydrocarbon, and nitrocannabinolactone, have been prepared by the use either of solvents or of reagents. The present communication records a preliminary investigation of the first three, concerning which the evidence hitherto available is highly conflicting. The fourth formed the subject of Part I of this series (J., 1930, 986).

Wood, Spivey, and Easterfield (J., 1896, **69**, 539) obtained from five different samples of *Cannabis Indica* resin a fraction boiling apparently constantly at 265°/20 mm. and in four cases giving on analysis results required for the formula $C_{18}H_{24}O_2$; it was not unnaturally assumed to be homogeneous and given the name cannabinol. Later, however, they (J., 1899, **75**, 20) and independently Dunstan and Henry (P., 1898, 44) prepared from this fraction a crystalline acetyl derivative, m. p. 75°. The former authors, who only succeeded in obtaining the crystalline derivative in small yield (20—25%. Dunstan and Henry give no details), concluded correctly that their resin, b. p. 265°/20 mm., was a mixture and transferred the name cannabinol to the resin obtained on hydrolysis of the crystalline acetyl derivative. Later investigators have, however, consistently failed to repeat this work; they have obtained apparently constant-boiling resins and, although these yielded only oily derivatives, have claimed homogeneity for each product, appropriated the name cannabinol, and variously assigned to it the formulæ $C_{20}H_{30}O_2$ (Casparis, *Pharm. Helv. Acta*, 1926, **1**, 210; Bergel, *Annalen*, 1930, **482**, 55) and $C_{21}H_{30}O_2$ (Fränkel, *Arch. exp. Path. Pharm.*, 1903, **49**, 266; Czerkis, *Annalen*, 1907, **351**, 467; also considered possible by Bergel, *loc. cit.*).

The work described below was carried out with several different samples of hashish of uncertain origin, all of which gave similar results, which were confirmed with a *Cannabis Indica* resin of known Indian origin. Attention was directed in the first place to the portion of the ether-soluble material boiling at *ca.* 260—270°/25 mm., which on redistillation very readily gave as main fraction a viscous red oil boiling mostly within 4° and corresponding closely to the supposedly homogeneous resins. Boiling with acetic anhydride or acetyl chloride, the method of acetylation used by Wood, Spivey, and Easterfield and later investigators, gave oily products even after several fractionations; but treatment of a pyridine solution of the once fractionated distillate with acetyl chloride gave a 28% yield of crystalline acetyl cannabinol. After crystals became available for seeding, a 3% yield could be obtained by means of acetic anhydride, although pure cannabinol obtained by hydrolysis of the crystalline acetyl derivative was quantitatively reacetylated by this reagent alone.

It is thus conclusively proved by Wood, Spivey, and Easterfield's investigation and the present one that apparent constancy of boiling point cannot be held to prove the homogeneity of these resins, and it must, by analogy, be concluded that the resins of Fränkel, Czerkis, Casparis and Bergel were mixtures. The name cannabinol will, therefore, in this and succeeding papers be applied only to the substance obtained from the acetyl derivative, m. p. 75°, and the apparently constant-boiling resin will be termed "crude cannabinol."

Crystalline acetyl cannabinol has been given the formulæ $C_{18}H_{23}O_2Ac$ (Dunstan and Henry) and $C_{21}H_{25}O_2Ac$ (Wood, Spivey, and Easterfield), the latter of which was confirmed in the present work.

It appears probable that the 25% yield of acetyl cannabinol obtained by Wood, Spivey, and Easterfield did not represent the total cannabinol content of their resin, and there is no evidence that the pyridine–acetyl chloride method effects a quantitative separation. In view of this doubt regarding the complete removal of cannabinol, great caution is necessary in drawing conclusions as to the nature of the residual resin. It is also unjustifiable to draw any conclusions as to the constitution of cannabinol from substances prepared from the crude resin only (*vide infra*).

By treating a cold acetic acid solution of "crude cannabinol" with fuming nitric acid, Wood, Spivey, and Easterfield prepared a number of amorphous nitro-derivatives, which were probably mixtures, and a bright yellow, crystalline substance having the formula of a trinitrocannabinol, $C_{21}H_{23}O_2(NO_2)_3$. No great difficulty was experienced by the present author in preparing this substance (see p. 636), and since other workers (Fränkel, Bergel) have failed to prepare either this characteristic derivative * or acetyl cannabinol in a crystalline state, it would appear possible that the composition of *Cannabis* resin may vary considerably, a view in harmony with its variable pharmacological activity. It is important to note

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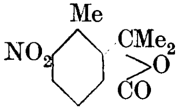
* Compare Czerkis (*Chem. Zentr.*, 1909, ii, 1880), who describes vaguely various products but almost certainly never obtained true trinitrocannabinol.

that the preparation of trinitrocannabinol from pure cannabinol, reported without details by Wood, Spivey, and Easterfield (P., 1898, 153), and consequently the correlation of these two substances, have been confirmed.

Some light is shed upon the nature of trinitrocannabinol by a consideration of its oxidation product, nitrocannabinolactone. This lactone, which was assigned formula (I) in Part I of this series, is obtained on treatment of trinitrocannabinol, " crude " or pure cannabinol (see p. 636) with *hot* nitric acid. Bergel has objected to this formula on the ground that he obtained an acid melting above 300° by reactions (including fusion with alkali) which might have been expected from formula (I) to lead to 6-nitro*iso*phthalic acid (m. p. 245°). However, Bergel's acid was not analysed and Bauer (*Ber.*, 1908, **41**, 503) has shown that nitrophthalides are decomposed by fusion with alkali. On the other hand, the 6-hydroxytoluic acid obtained from hydroxycannabinolactone (*loc. cit.*, p. 990) has now been found to be identical (mixed m. p.) with a synthetic specimen, and the position of the nitro-group is thus definitely proved.

(I.)



Nitrocannabinolactone contains only one nitro-group, leaving two more to be accommodated in the remaining portion of trinitrocannabinol. The latter substance possesses strong acidic properties but no carboxyl group, and Wood, Spivey, and Easterfield (P., 1898, 66) suggested that it was a polynitrophenol. This view is in harmony with the method of preparation of trinitrocannabinol and with the properties now found for its *benzenesulphonyl* compound and *methyl ether*. These are both much paler yellow than the parent nitro-compound. The former is rapidly hydrolysed by alkali and by warm pyridine containing a little water. The methyl ether, which is best prepared in absence of a solvent, is stable to acids and resinified by alcoholic sodium hydroxide solution, but is hydrolysed to trinitrocannabinol by heating with pyridine containing a little water * or with piperidine; methylation is thus proved to have occurred at the hydroxyl group and not at a carbon atom. It was not, however, *a priori* impossible that the acidity was due to (*a*) a secondary or (in view of its mode of formation, less probably) a primary aliphatic nitro-group, or (*b*) an enolic hydroxyl group, the acidity of which was enhanced

* The generality of this reaction is under investigation.

by a neighbouring nitro-group or groups. The alternative (*a*) was eliminated when it was found that trinitrocannabinol reacts neither with alkaline hypobromite solution, either cold or at 40°, nor with nitrous acid. According to (*b*) trinitrocannabinol methyl ether should contain two tertiary nitro-groups and the unsaturated group $-C(OMe){:}C<$. On treatment with potassium permanganate in acetone solution, however, there was no reaction in the cold, and only an exceedingly slow but then total degradation on boiling; whereas trinitrocannabinol itself in acetone solution is rapidly oxidised in the cold. This contrasting behaviour is not consistent with the substances being related as an enol to its *O*-methyl ether.

All the known facts concerning these compounds are, however, in harmony with the assumption that trinitrocannabinol contains two benzene nuclei, one in the nitrocannabinolactone residue, the other carrying two nitro-groups and a phenolic hydroxyl group. On this view the hydroxyl group in cannabinol itself must be phenolic, although this substance does not give a coloration with ferric chloride and is insoluble in alkali. The former property is, however, not unusual and some phenols are insoluble in alkali (*e.g.*, dihydrodeoxytetrahydro-α-methylmorphimethine; Cahn, J., 1926, 2567).

Cannabinol appears to contain neither a second hydroxyl group nor a carbonyl group, and no volatile halide is obtained by the action of hydriodic acid under the conditions used for the micro-determination of the methylimino-group. Further, trinitrocannabinol is unaffected by heating with piperidine, which hydrolyses polynitrodiphenyl ethers (Le Fevre, Saunders, and Turner, J., 1927, 1168). The second oxygen atom is thus presumably in the form of an internal ether linkage. Further consideration of the structure of cannabinol is, however, deferred until more experimental evidence is available, but it may be noted that the formulæ proposed by Bergel (*a*) are not in accord with the correct molecular formula for cannabinol, (*b*) do not allow of ready formation of a trinitro-derivative, and (*c*) are based on products isolated in small yield from " crude cannabinol."

Personne (*J. Pharm.*, 1857, **31**, 47) first isolated from *Cannabis Indica* resin a hydrocarbon, for which the following data were subsequently recorded: m. p. 63—64°, b. p. 285—286°/15 mm., probably *n*-$C_{29}H_{60}$ (Wood, Spivey, and Easterfield); m. p. 70°, $C_{28}H_{58}$ (Fränkel); m. p. 65° (Bergel). The author is indebted to Professor A. C. Chibnall for a comparison of a specimen of the hydrocarbon obtained in this investigation (m. p. 63°) with synthetic *n*-nonacosane (m. p. 63·6°) and to Dr. S. H. Piper for *X*-ray analysis. It appears highly probable that the main constituent is *n*-nonacosane, but some other substance is also present, and the material is being further investigated by Professor Chibnall.

EXPERIMENTAL.

Hashish.—The material used in this investigation was unadulterated hashish of good quality, seized from smugglers in Egypt and, therefore, probably of Eastern Mediterranean origin. The dark brown ethereal extract (400 g.; *ca.* 33%), on distillation substantially according to the directions of Wood, Spivey, and Easterfield, gave the following fractions, the yields recorded being representative of several batches:—(A) atmospheric distillate, 90—110 g.; the portion volatile in steam will form the subject of a future communication; the non-volatile portion gave *ca.* 0·2 g. of hydrocarbon (*vide infra*) and then boiled at 150—270°/25 mm.; (B) vacuum distillate, 147—156 g.; (C) undistillable pitch, 90—130 g. B gave 1·4—2·0 g. of hydrocarbon and on fractionation:—(D) 35—42 g., b. p. 190—250°/25 mm., (E) 95—110 g., b. p. 250—285°/25 mm., and residue, 5—15 g. Fraction E simulated a pure substance in that already three-quarters of it boiled at 263—268°/25 mm., but it was impossible by repeated distillation either to eliminate the small but definite head and tail fractions or further to separate the mixture.

Distillation in a high vacuum gave substantially similar results.

Indian Resin.—The green resin (supplied by The British Drug Houses Ltd.) obtained by evaporation of an alcoholic extract of *Cannabis Indica* of Indian origin gave similar results on distillation, except that (*a*) low-boiling fractions had been partially lost (probably during removal of the alcohol) and (*b*) the hydrocarbon, being sparingly soluble in alcohol, was present only in traces. Prolonged refractionation had the same negative results as with hashish.

Polymerisation.—As the above distillations involve high temperatures for about 1·5 hours, and Wood, Spivey, and Easterfield's hydrocarbon $C_{10}H_{18}$ polymerised very readily, portions of all fractions except that volatile in steam were separately heated at 200° for 4·5 hours in an atmosphere of carbon dioxide and then distilled. No evidence of polymerisation was obtained.

Extraction with Alkali.—It has been reported (Hofmann-La Roche & Co., G.P. 285,829, 1913; Casparis, *loc. cit.*) that shaking a solution of the crude extract with alkali removes 50% of pharmacologically inactive material. The hashish-resin used in this investigation yielded only about 5% to alkali, and the B.D.H. resin even less. The proportion of undistillable pitch obtained after the treatment was somewhat smaller, but the distillates were similar in quantity and properties to those obtained without alkali treatment. The difficulty of separating the dark brown ethereal and the aqueous layer destroyed the utility of this modification.

Acetyl Cannabinol.—Acetyl chloride (150 c.c.) was run during 90 minutes into a solution of the resin E (185 g.) in dry pyridine (500 c.c.), mechanically stirred and cooled in ice, crystals separating during the addition. After standing for 2 hours at room temperature, the mixture was poured on ice and dilute acid, the oil taken up in ether, shaken successively with dilute acid, sodium carbonate solution, and water, and dried over calcium chloride. The residue after removal of the solvent boiled almost entirely at 240—270°/20 mm. On solution in alcohol (500 c.c.) and standing for 4 days at 4°, colourless crystals of acetyl cannabinol, m. p. 70°, gradually separated. The residue remaining on evaporation of the mother-liquors gave on fractionation (i) 93 g., b. p. 230—255°/20 mm., and (ii) 58 g., b. p. 255—270°/20 mm. The latter on solution in alcohol deposited a further small quantity (varying in different experiments) of acetyl cannabinol (total yield, 27—28·4%). Acetyl cannabinol [Found : C, 78·2, 78·1; H, 8·0, 8·0; $CH_3{\cdot}CO$,* 12·1; *M* (in camphor; mean of 4 results), 312. Calc. for $C_{21}H_{25}O_2{\cdot}CO{\cdot}CH_3$: C, 78·4; H, 8·0; $CH_3{\cdot}CO$, 12·2%; *M*, 352] crystallised in irregular plates, m. p. 75°, from alcohol, in which it was considerably more soluble at room temperature than at 4°.

A similar yield (26%) of the same substance (mixed m. p.) was obtained from the corresponding fraction of the B.D.H. resin.

Substitution of quinoline for pyridine greatly reduced the yield of acetyl cannabinol. By refluxing the resin E (3 g.) with acetic anhydride (10 c.c.) and fused sodium acetate (3 g.) for 1 hour, 0·1 g. of crystalline acetyl cannabinol was obtained after, but not before, crystals for seeding became available.

Acetyl cannabinol was unchanged by refluxing with acetic anhydride; addition of fused zinc chloride caused decomposition. Bromination and nitration took place readily, but crystalline derivatives have not been isolated.

The oily residues left after separation of the crystalline acetyl cannabinol were much more mobile than the resin E. Their colour, pale yellow when freshly distilled, darkened rapidly on standing, and they were rapidly transformed into coloured products by alcoholic potassium hydroxide solution. Reacetylation with acetyl chloride and pyridine gave no further yield of crystalline material. On oxidation of the higher-boiling fraction with nitric acid according to Wood, Spivey, and Easterfield's directions (*loc. cit.*) nitro-

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* The author is indebted to Naguib Abdulla El Azizi Eff. for this determination.

cannabinolactone (6·2 g., m. p. 176—177°, from 110 g. of residues) was obtained. Similar treatment of pure cannabinol (2 g.) yielded the same substance (0·45 g., m. p. 172°). Oxidation under these conditions of lower-boiling fractions of the crude resin was very violent and nitrocannabinolactone could not be isolated.

Cannabinol.—By heating acetyl cannabinol (20 g.) with a solution of potassium hydroxide (6·4 g.) in 90% alcohol (120 c.c.) under reflux for 2 hours and isolating the product in the usual manner, cannabinol (yield, 90% of the theoretical) was obtained as a very pale yellow oil, setting to a transparent varnish when cold, which very slowly became reddish on standing. It boiled at 263—264°/20 mm. and could not be crystallised. On heating under reflux for 15 minutes with 2 parts of acetic anhydride it was quantitatively converted into crystalline acetyl cannabinol. Cannabinol rapidly decolorises potassium permanganate in hot acetone solution, but no crystalline derivatives have yet been obtained.

Trinitrocannabinol.—Nitration according to Wood, Spivey, and Easterfield's directions of the resin E (177 g.) and of pure cannabinol (5 g.) gave trinitrocannabinol (19·5 g. and 3·3 g. respectively) which, once recrystallised, never melted above 150°. Although the purity and yield of the product thus obtained leave much to be desired, they could not be improved by modification of the method. Much material is lost in *complete* purification, which is best effected by two recrystallisations from acetic acid, transformation into the sodium or ammonium salt, and two further crystallisations of the liberated phenol from acetic acid. Thus purified, it had all the properties described by Wood, Spivey, and Easterfield (Found: C, 56·5; H, 5·2; N, 9·0. Calc. for $C_{21}H_{23}O_8N_3$: C, 56·6; H, 5·2; N, 9·4%. Found in silver salt: Ag, 19·4. Calc. for $C_{21}H_{22}O_8N_3Ag$: Ag, 19·5%), except that (*a*) the silver salt was slowly decomposed by alcohol, and (*b*) the sodium salt appeared to exist in a bright orange and a paler yellow form, the former passing into the latter in contact with water slowly in the cold, rapidly on heating. This difference, which may be due to water of crystallisation or be similar to other not entirely explained differences in colour of salts of nitrophenols, was not investigated owing to lack of material. A specimen of trinitrocannabinol melting at 158° (m. p., when pure, 160°) still contained an appreciable amount of lower nitration products or other impurities (Found: C, 57·0; H, 5·2; N, 8·6%), a factor of considerable importance which will be dealt with in a later communication.

Trinitrocannabinol Methyl Ether.—Methylation, which Wood, Spivey, and Easterfield were unable to effect, was eventually carried out by the following methods, of which the first is the most convenient. (i) On addition of dry methyl iodide (7·5 c.c.) to trinitrocannabinol (5·0 g.) and dry silver oxide (freshly precipitated from 3 g. of silver nitrate) cooled in ice, a bright red colour (considerably deeper than that of a solution of trinitrocannabinol in methyl iodide and probably due to transient formation of the *aci*-methyl ether) developed, which changed to a pale yellow in 15 minutes, the whole then having set to a stiff crystalline paste. Methyl iodide (2·5 c.c.) was added, and the whole kept at room temperature for 2 hours. After addition of ether, filtration and evaporation of the solvent, the residue was warmed with very dilute sodium hydroxide solution (sodium ions greatly reduce the solubility of trinitrocannabinol sodium salt in water). The residual *trinitrocannabinol methyl ether* (5 g.), crystallised from methyl or ethyl alcohol, in which it was sparingly soluble, in very pale greenish-yellow needles m. p. 150° [Found: C, 57·4; H, 5·3; N, 9·2; MeO (determined as for NMe), 8·6. $C_{21}H_{22}O_7N_3(OMe)$ requires C, 57·5; H, 5·5; N, 9·2; MeO, 7·25%].

(ii) The dry silver salt (1·4 g.) of trinitrocannabinol was kept for 24 hours at 4° with an excess of dry methyl iodide. Worked up as in (i), the product gave 1·04 g. (95·5%) of methyl ether, m. p. 150°.

(iii) When the silver salt of trinitrocannabinol in partial solution in methyl alcohol and excess of methyl iodide was kept for 24 hours, 35% of methyl ether, m. p. 147°, and 60% of trinitrocannabinol were obtained.

No depression of the melting point was observed on mixing the products obtained by the three methods.

Trinitrocannabinol methyl ether was unchanged after being refluxed with a mixture of alcohol and concentrated hydrochloric acid, and boiled for 2 minutes with concentrated nitric acid (in which it dissolved); it was unaffected by hydriodic acid under macro- or micro-Zeisel conditions (being insoluble), but was hydrolysed under the conditions for the estimation of NMe (*vide supra*). It was unaffected by ketonic reagents and by refluxing with acetic anhydride and a drop of pyridine. It was not hydrolysed by aqueous alkali, presumably because of its insolubility, and was rapidly resinified by alcoholic sodium hydroxide solution. When its solution in (*a*) pyridine to which a few drops of water had been added or (*b*) piperidine was heated on the water-bath, it rapidly assumed the reddish-orange colour of solutions of trinitrocannabinol salts; after 30 minutes the solution, worked up in the usual manner, gave quantitatively trinitrocannabinol, which, after recrystallisation from acetic acid, melted, alone or mixed with an authentic specimen, in both experiments at 160°. Addition of water to the piperidine solution precipitated the piperidine salt of trinitrocannabinol, which crystallised from water in bright yellow needles, m. p. 207° (decomp.); it was very sparingly soluble in water, readily soluble in alcohol, and crystallised readily from aqueous alcohol but then had an indefinite melting point.

Benzenesulphonyltrinitrocannabinol.—On addition of benzenesulphonyl chloride (1 c.c.) to a cooled solution of trinitrocannabinol (0·8 g.) in dry pyridine (4 c.c.), the red colour of the solution changed instantaneously to pale yellow with slight evolution of heat, and crystals separated on scratching. After 1 minute the whole was poured into ice-water and the precipitated oil, which rapidly solidified, was filtered off, dried, and recrystallised from benzene–alcohol. *Benzenesulphonyltrinitrocannabinol* formed rhombic needles, m. p. 196—197° (Found: C, 55·9; H, 4·7; S, 5·5. $C_{27}H_{27}O_{10}N_3S$ requires C, 55·35; H, 4·6; S, 5·5%), easily soluble in benzene, acetone and acetic acid, sparingly soluble in alcohol. It was stable to short heating with hydrochloric acid in acetone or acetic acid solution, but was very readily hydrolysed in the cold by addition of a drop of aqueous sodium hydroxide to the acetone solution; on addition of a few drops of water to the pyridine solution hydrolysis occurred fairly slowly in the cold, but instantaneously in the hot solution and trinitrocannabinol was obtained.

Hydrocarbon.—The hydrocarbon obtained from distillates A and B was purified for analysis by two crystallisations from ethyl acetate, in which it was readily soluble when hot but sparingly soluble when cold, and one from ethyl alcohol (Found: C, 85·3, 85·4; H, 14·7, 14·55; *M*, in camphor, 395, 387. Calc. for $C_{29}H_{60}$: C, 85·3; H, 14·7%; *M*, 408). Addition of alcohol, in which the higher paraffins and their derived ketones and alcohols are sparingly soluble, to the ethyl acetate mother-liquors precipitated a mixture melting indefinitely at *ca.* 52°. The small quantity of this material (*ca.* 5%) together with the above analyses indicates that ketones or alcohols, if present at all, formed only a minor constituent (contrast Channon and Chibnall, *Biochem. J.*, 1929, **23**, 168).

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EGYPTIAN UNIVERSITY, ABBASSIA, CAIRO, AND
UNIVERSITY COLLEGE OF N. WALES, BANGOR.

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