XIV. THE ACTIVE PRINCIPLES OF *CANNABIS INDICA* RESIN. I

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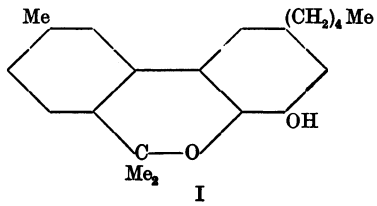
THE resinous exudate of the female flowers of *Cannabis indica* (*C. sativa*) forms the essential constituent of the drug variously known as hashish, bhang, charas, ganja and marihuana according to the country of origin and mode of preparation. Extracts of the flowers have been used in European medicine to some extent but were found to be so variable in activity that *C. indica* has been removed from the *British Pharmacopoeia*. The plant is, however, well known through its use as a drug in oriental countries and recently, under the name of "marihuana", its use has assumed dangerous proportions in America. *Cannabis* resin has been the subject of many investigations in the past but much of the earlier work is contradictory. The active principle is contained in a high-boiling resin and is not an alkaloid [Smith, 1857]. The active fraction isolated from the crude resin by Wood Spivey & Easterfield [1896] and given by them the name cannabinol was later found by the same workers [1899] to be a mixture, and the name cannabinol was transferred to that portion of the active resin yielding a crystalline acetate, M.P. 75°. A good deal of confusion was introduced by later workers, who, although failing to obtain a crystalline acetate, nevertheless applied the name cannabinol to their products. The situation was largely clarified by Cahn [1931], who confirmed the observations of Wood Spivey & Easterfield and established for cannabinol the formula $C_{21}H_{26}O_2$. The constitution of cannabinol has been investigated by Cahn [1930–33] and by Bergel [1932], and for it the former proposed structure (I) in which only the positions of the hydroxyl and *n*-amyl groups are to be regarded as uncertain.



I

In his last communication Cahn states that "cannabinol is not the pharmacologically active principle of *Cannabis indica*" [1933]. It seemed desirable that further investigations on the resin should be made and we have taken up the study of cannabis resin with a view to isolating the substance or substances responsible for its pharmacological action.

The starting point in these investigations was material which corresponded to the high-boiling resin (B.P. 265°/20 mm.) first described by Wood Spivey & Easterfield [1896]. It was found that cannabinol can be removed almost quantitatively as its crystalline p-*nitrobenzoate* (amounting to 25% of the whole) on

p-nitrobenzoylating the resin. On hydrolysis the crystalline ester yields cannabinol as a colourless oil, which gives the above-mentioned crystalline acetate (M.P. 75°) on acetylation. The non-crystalline portion of the *p*-nitrobenzoylated resin gave on hydrolysis a colourless oil from which no crystalline acetate could be obtained.

Active hashish preparations induce a characteristic cataleptic condition in dogs, but this effect, described first by Fraenkel [1903] is not readily made the basis of quantitative assay. Gayer [1928] showed that in various animals—e.g. cats, rabbits, mice—hashish preparations induce corneal anaesthesia and that this effect is characteristic of active fractions of the resin. This Gayer test has been developed by Marx & Eckhardt [1933] using rabbits and has been employed throughout the work described in this paper. The pure cannabinol prepared from the crystalline *p*-nitrobenzoate was found to be highly toxic when injected intravenously into rabbits but, unlike the original resin, did not produce corneal anaesthesia. The cannabinol-free resin on the other hand was much less toxic and always induced corneal anaesthesia in rabbits. This distinction is further emphasized by the fact that an acetone solution of cannabinol was found to be non-toxic after standing for 3 days in contact with air while only slight loss of activity occurred on similar treatment of the cannabinol-free resin.

Attempts to fractionate further the cannabinol-free resin by distillation or crystallization of derivatives failed completely but chromatographic analysis gave promising results. Using activated aluminium oxide as adsorbent it was possible to obtain fairly readily an oil having considerably greater activity in the Gayer test than the starting material. Even better results were obtained by submitting the oily *p*-nitrobenzoate mixture left after separating the cannabinol *p*-nitrobenzoate to chromatographic analysis on activated aluminium oxide. By this procedure remaining traces of cannabinol were removed and an oil was obtained yielding on hydrolysis a product giving a positive result in the Gayer test at a dose of 0·25 mg. per kg. body weight; this material has low toxicity as compared with cannabinol and possesses none of the convulsant action of the latter substance.

Further investigation of this highly active material is in progress and the results will be reported later. We have not as yet obtained any crystalline derivative from the most active preparation and suspect that it does not yet represent the homogeneous active principle.

Experimental

Starting material. The starting material was the resin (B.P. 185–190°/0·6 mm.) obtained on working up hashish of Indian origin in the manner described by Bergel [1930]. The resin was nearly colourless when freshly distilled. It corresponds to the "crude cannabinol" of Wood Spivey & Easterfield and possessed the characteristic pharmacological properties of the original extract.

Isolation of cannabinol. The above resin (25 g.) was dissolved in pyridine (110 ml.) and *p*-nitrobenzoyl chloride (36 g.) added. The mixture was refluxed for 4 hr., then poured on a mixture of ice and sufficient H_2SO_4 to make the resulting suspension acid to Congo red. The precipitate was collected, washed with water, dried and refluxed with light petroleum (750 ml., B.P. 80–100°) for 1 hr. and filtered hot, the filter residue being treated in the same way with a further quantity of light petroleum (250 ml.). The combined filtrates were washed with aqueous Na_2CO_3, dried and concentrated to about 150 ml. On standing *cannabinol* p-*nitrobenzoate* separated. Recrystallized first from alcohol

then from light petroleum (B.P. 80–100°) it formed pale yellow needles M.P. 160° (yield, *ca.* 6·5 g.). (Found: C, 73·0; H, 6·2; N, 3·2 %. $C_{28}H_{29}O_5N$ requires C, 73·2; H, 6·3; N, 3·2 %.) Since cannabinol *p*-nitrobenzoate is very sparingly soluble in methyl alcohol the oil left on evaporating the original light petroleum mother liquors may be largely freed of cannabinol by fractionation with this solvent.

Cannabinol p-*aminobenzoate.* Cannabinol *p*-nitrobenzoate (1 g.) dissolved in alcohol (80 ml.) was hydrogenated using a platinum oxide catalyst. Absorption of hydrogen ceased when 200 ml. had been absorbed (theoretical 190 ml.). The resulting p-*aminobenzoate* crystallized from methyl alcohol in colourless needles M.P. 149–150°. (Found: C, 78·2; H, 7·4 %. $C_{28}H_{31}O_3N$ requires C, 78·3; H, 7·2 %.)

Cannabinol. Cannabinol *p*-nitrobenzoate hydrolysed by refluxing with methyl alcoholic KOH (5 %) during 1½ hr. gave cannabinol as an almost colourless oil which, with acetic anhydride-pyridine gave in quantitative yield a crystalline acetate M.P. 75° not depressed on admixture with a sample of cannabinol acetate kindly supplied by Dr R. S. Cahn.

Pharmacological tests

The Gayer test was carried out on rabbits as described by Marx & Eckhardt [1933], the substances being injected in acetone solution (0·5 % wt./vol.).

(*a*) *Cannabinol.* Pure cannabinol prepared by hydrolysing the *p*-nitrobenzoate and subsequent distillation in a high vacuum was used. In doses less than 2 mg. per kg. body weight the material had no visible effect and the corneal reflex remained normal. At any higher dosage the following sequence of events was observed. For about 1 min. the animal behaved normally, but at the end of this time it lay down and in a few seconds rolled over on its side and became rigid, the corneal reflex remaining normal. In any time from a few seconds to 1 min. later, depending on the size of the dose, the rabbit went into violent convulsions terminating in death within about 30 sec.

(*b*) *Material from non-crystalline* p-*nitrobenzoates.* After separation of the crystalline *p*-nitrobenzoate the mixture of oily esters from the resin was hydrolysed and the product distilled in a high vacuum. The nearly colourless oil obtained had no effect when injected into rabbits in doses less than 1 mg. per kg. body weight. In a dose of 1 mg. per kg. body weight the drug had no effect for about 3 min., after which time the animal's head began to nod gently and it subsided to its normal sleeping position and remained so. During this period the corneal reflex slowly disappeared until no response could be elicited. The animal could be roused for a few seconds by violent shaking but when left undisturbed quickly relapsed. In doses up to 5 mg. per kg. body weight exactly the same effect was observed, the period of sleep or stupor extending from 30 min. up to 6 or 7 hr. after which the rabbit recovered completely. In doses of 5 mg. or more the animal, although giving a positive Gayer test, died with convulsions within the first 2 hr. after injection. The death after convulsions was presumably due to the presence of some cannabinol in the injected material (cf. below).

Chromatographic analysis of hydrolysate of non-crystalline p-*nitrobenzoates*

A sample of the oil (2 g.) prepared by hydrolysis of the non-crystalline fraction of the *p*-nitrobenzoylated resin and subsequent distillation in a high vacuum was dissolved in light petroleum (300 ml.; B.P. 60–80°) and allowed to percolate through a column of activated aluminium oxide (Merck), the chromatogram being developed first with light petroleum (750 ml.; B.P. 60–80°), then with a mixture (1 litre) of equal parts of light petroleum (B.P. 60–80°) and ether.

When the column was viewed in ultraviolet light four distinct bands were visible; from the top downwards these were: (1) yellow 6 cm., (2) colourless 8 cm., (3) yellow 8 cm., (4) blue fluorescent 6 cm. On elution with a mixture of ether and methyl alcohol (4 : 1) the oils from sections (1), (2) and (3) were found to be inactive when tested on rabbits while that from section (4) gave a positive Gayer test in a dose of 3 mg. per kg. body weight.

The oil from section (4) was therefore combined with that obtained by evaporating the filtrate from the chromatogram, dissolved in light petroleum (B.P. 60–80°) containing 5% ether and re-adsorbed on a column (2 × 30 cm.) of activated aluminium oxide (Merck), the chromatogram being developed with the same solvent mixture. From the top downwards the column showed in ultra-violet light the following bands: (1) purple 3 cm., (2) yellowish blue 10 cm., (3) deep blue 10 cm., (4) strongly blue fluorescent 2 cm., (5) yellow 3 cm. Tests on rabbits showed that the oil from section (4) (50 mg.) was lethal in a dose of 2·5 mg. per kg. body weight and had the typical action of cannabinol. The oil from section (2) (60 mg.) was active in the Gayer test in a dose of 1 mg. per kg. body weight and when injected in a dose of 5 mg. per kg. body weight the animal survived for 10 hr. without any trace of muscular rigidity and died in sleep without any convulsions. The oil from section (3), like the starting material, possessed both convulsant and sleep-producing properties.

Chromatographic analysis of non-crystalline p-*nitrobenzoates.* The oily residue (8 g.) left on removing the crystalline cannabinol derivative from the *p*-nitrobenzoylation product of the distilled resin, was dissolved in light petroleum (B.P. 40–60°) and subjected to adsorption on a column (5 × 45 cm.) of activated aluminium oxide (Merck) previously washed with a solution of phenol in light petroleum to reduce alkalinity. After developing with light petroleum (4 litres) the chromatogram showed six distinct bands when viewed in ultra-violet light. Each of these was separately eluted, hydrolysed, distilled and tested, the filtrate from the column being evaporated and the residue similarly treated and tested. The following table shows the results obtained, the bands in the chromatogram being numbered from the top downwards.

	Colour	Length cm.	Wt. of eluate g.	Millon test	Gayer test	M.L.D. mg.
1	Yellow	3	0·3	–	–	–
2	Colourless	15	1·2	–	–	–
3	Bright blue	3	0·25	+	–	6
4	Colourless	10	2·0	+	–	5·5
5	Yellow	5	0·9	–	–	–
6	Colourless	10	1·2	+	+(2 mg.)	9
7	Colourless	Filtrate	1·5	+	+(0·25 mg.)	5

The biological test results were reproducible in different animals, there being very little variation either in the minimum active dose or in the minimum lethal dose (M.L.D.). Sections (3) and (4) possessed the typical convulsion-producing properties of cannabinol while sections (6) and (7) had no such action. It is clear then from the table that the remaining traces of cannabinol were concentrated in sections (3) and (4), while the material producing corneal anaesthesia was concentrated in sections (6) and (7). The oil from sections (6) and (7) gave, like cannabinol, a precipitate with Millon's reagent.

Relative stabilities of cannabinol and resin from fraction 7 (*above*). A solution (0·5%) of cannabinol in acetone was exposed to the air for 3 days. When tested on rabbits at the end of this time it was found to be non-toxic. A solution of the

resin from fraction 7 (above) after standing for 6 months under the same conditions retained about 25 % of its activity in the Gayer test.

SUMMARY

p-Nitrobenzoylation of the high-boiling pharmacologically active resin from the female flowers of *Cannabis indica* yields crystalline cannabinol *p*-nitrobenzoate and a mixture of resinous esters. Cannabinol is highly toxic and gives a completely negative reaction in the Gayer hashish test on rabbits, while the hydrolysis product of the resinous esters gives a strong positive reaction and is less toxic than cannabinol. The material giving a positive Gayer test has been fractionated by adsorption methods and a product obtained showing a positive Gayer test in rabbits in a dose of 0·25 mg. per kg. body weight.

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