[CONTRIBUTION FROM THE BUREAU OF NARCOTICS LABORATORY, U. S. TREASURY DEPARTMENT]

Origin of Cannabinol

BY JOSEPH LEVINE

The first significant compound to be isolated from the resin of *Cannabis sativa L.* was cannabinol, which was obtained in the form of the crystalline acetate by Dunstan and Henry[1] in 1898 and by Wood, Spivey and Easterfield[2] in 1899. Attempts by a number of workers to repeat this separation were unsuccessful[3] until Cahn, in 1931,[4] was able to confirm the older findings. Since then Work, *et al.*,[5] isolated cannabinol as its *p*-nitrobenzoate from Indian hashish, and Adams, *et al.*,[6] isolated it as its 3,5-dinitrophenyl urethan from American hemp. No reason has hitherto been ascribed for the failure to repeat the isolation of cannabinol during the 32-year interval.

The purpose of the present communication is to report the formation of cannabinol through the spontaneous degradation of tetrahydrocannabinol in the crude state; an obvious explanation of this anomaly is thereby provided.

Crude source material for work performed in this Laboratory, culminating in the isolation of tetrahydrocannabinol,[7] was a 100-lb. lump of charas received from India in 1939. The material, wrapped in goatskin, was a soft, pliant, gummy mass, light brown in color, with a strong characteristic terpene-like odor. After the removal of a portion of the lump, the remainder was rewrapped and stored in an unventilated vault, which was opened occasionally.

After three years of storage, the charas lump was reëxamined. The outer surface had turned to a very hard crust one to two inches thick. The color had darkened considerably, and the characteristic odor had to a considerable extent disappeared. The interior of the lump, after an intermediate layer, remained unchanged in appearance from its original condition.

Fluid extracts made of both the crust and the inside lump were examined for "marihuana potency."[8] The potency of the interior portion was found to be essentially unchanged from its value when first received. The potency of the outer crust had fallen, however, to one-twentieth of its former value. Loss of physiological potency of cannabis preparations has previously been reported, but there is disagreement as to the extent of the deterioration. Eckler and Miller[9] reported that the crude drug lost its activity completely in fifty months, after a slow initial rate of loss. Hamilton,[10] on the other hand, found no substantial loss in activity of similar material after ten years. Marshall[11] found a tenfold loss of activity of charas which had been exposed for ten years. Chopra[12] states that in the hot climates of India, complete loss of activity of ganja takes place within one year.

The crusty material yielded upon percolation with alcohol at room temperature 40% of its weight of resin, comparable in amount to that obtained from the soft inner material, but much more viscous than the latter. The resin was acetylated and then distilled in a continuous cascade-type vacuum still. A 40% yield of distillate was obtained from the crude acetate; a comparable preparation from the soft inner material yielded 60% of distillate.

On standing at room temperature, several crystals appeared in the crude distillate; after several days the entire distillate became permeated with the crystals. A portion of the material was dissolved in alcohol and cooled. The separated crystals were found to be cannabinol acetate, by melting point and mixed melting points with an authentic sample. The yield obtained from the crude distillate was 18%.

In order to obtain a comparison between the properties of the present material and analogous material prepared from the undeteriorated charas, a charge was subjected to fractional distillation in a molecular still.[13] The refractive indices of the fractions, shown in Fig. 1, differ significantly from those of comparable fractions prepared from fresh charas. Fractions 1 and 2 were terpene-like fluids; fractions 3 to 7 were viscous oils, and correspond to the "red oil" of the literature.[14] Each of these fractions was dissolved in ethanol, cooled to 0°, and the crystals filtered and washed. The cannabinol acetate (yields shown in Table I) was obtained quite pure directly; that obtained from Fractions 4 to 7 melted at 75.5–76° without recrystallization. The total yield of acetate was 20% on a basis of the still charge, or 33% on a basis of "red oil."

A portion of the soft inner material of the charas lump was treated in the manner previously

(1) Dunstan and Henry, *Proc. Chem. Soc.*, 44 (1898).

(2) Wood, Spivey and Easterfield, *J. Chem. Soc.*, **75**, 20 (1899).

(3) *E. g.* (a) Frankel, *Arch. exp. Path. Pharm.*, **49**, 266 (1903); (b) Casparis, *Pharm. Helv. Acta*, **1**, 210 (1926); (c) Bergel, *Ann.*, **482**, 55 (1930); and others.

(4) Cahn, *J. Chem. Soc.*, 630 (1931).

(5) Work, Bergel and Todd, *Biochem. J.*, **33**, 123 (1939).

(6) Adams, Pease and Clark, THIS JOURNAL, **62**, 2194 (1940).

(7) Wollner, Matchett, Levine and Loewe, *ibid.*, **64**, 26 (1942).

(8) Bioassays were performed by Dr. W. S. Loewe, Cornell University Medical College. Dogs were used as test animals.

(9) Eckler and Miller, *J. Am. Pharm. Assoc.*, **6**, 872 (1917).

(10) Hamilton, *ibid.*, **6**, 875 (1917).

(11) Marshall, *Pharm. J.*, **82**, 418 (1909).

(12) Chopra, "Indian Medical Research Memoirs" (Memoir No. 31, July, 1939).

(13) Wollner, Matchett and Levine, *Ind. Eng. Chem., Anal. Ed.*, **16**, 529 (1944).

(14) Blatt, *J. Wash. Acad. Sci.*, **28**, 465 (1938).

TABLE I

Fraction	Weight, g.	Cannabinol acetate, g.
1	13.9	0.0
2	5.1	.0
3	13.6	.3
4	15.5	4.9
5	14.7	6.1
6	13.8	6.4
7	11.0	4.7
8	2.9	0.0

described,[7] with the exception that, in order to conform more closely to the procedure followed with the outer crusty material, the resin was acetylated prior to, rather than subsequent to, the initial distillation. As would be expected from the physiological potency of the fluid extract, tetrahydrocannabinol, agreeing in physical constants and in physiological potency with that obtained from fresh charas,[7] was obtained, and in comparable yield. No cannabinol could be isolated. Like results were obtained from a portion of distilled charas resin prepared from the fresh material in 1939 and stored since then in a loosely-covered flask. This stability of both the protected and the partially purified material would suggest that the degradation of the tetrahydrocannabinol results from an enzymatic oxidation.

During the degradation of the crude charas, ring closure of cannabidiol (or its analogs) apparently does not occur. The response of the resin derived from the outer crust to the alkaline Beam test, which depends upon cannabidiol, was undiminished; and cannabidiol was isolated as the bis-3,5-dinitrobenzoate[15] in appreciable yields from the mother liquors of the cannabinol acetate. Marshall and Wood[16] showed that the acetyl number of resin derived from old charas was essentially the same as that from the fresh product. Since ring closure of cannabidiol eliminates one of the two phenolic hydroxyl groups with formation of a pyran ring, any ring closure to form tetrahydrocannabinol[17] or cannabinol would be reflected in the acetyl number. It is uncertain whether dehydrogenation of cannabidiol occurred, since the dehydrogenation product or its derivatives are not at present known; if any did occur, however, it was not to the same extent as in the case of the tetrahydrocannabinol.

Experimental

Preparation of Crude Distillate.—One kilogram of the hard outer crust of charas was continuously percolated with 95% alcohol at room temperature. Two weeks were required for complete extraction of the resin. The alcohol was removed under diminished pressure; weight of resin, 392 g.

The resin was acetylated by refluxing with 900 ml. of acetic anhydride for eight hours. The excess acetic anhydride was removed *in vacuo;* the crude acetate was dissolved in benzene and the solution washed with sodium bicarbonate solution and then with water. After removal of the solvent under reduced pressure, a yield of 435 g. of crude acetate, less viscous than the original resin but more viscous than a comparable preparation from fresh charas, was obtained.

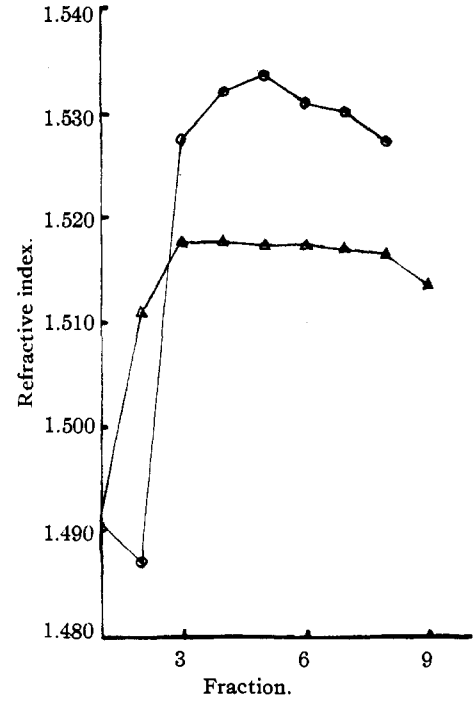

Fig. 1.—△, Fresh charas; O, hard outer crust of charas.

A 167-gram charge of the acetate was distilled in a continuous cascade-type vacuum still. Sixty-seven grams (40%) of a brown oily distillate was obtained; the undistillable portion was an intractable black tar, which was brittle when cold. The remainder of the crude material was distilled in two runs, with more rapid passage over the heater units; a yield of 33% was obtained in these runs.

Fractionation of Crude Distillate.—A charge of 111 g. of the above distillate was redistilled in a fractionating molecular still.[18] The temperature of the main charge during the distillation was 139–170°; the pressure was maintained at 1×10^{-4} to 5×10^{-4} mm. Eight fractions were collected; the first two were green terpene-like fluids, while the remaining fractions were thick yellow-orange oils.

Cannabinol Acetate.—(a) On standing, crystals formed in the crude distillate. Twenty grams of the material was dissolved in alcohol and cooled; a yield of 3.5 g. of cannabinol acetate was obtained; m. p. after recrystallization from alcohol, 76°. The melting point was not depressed upon admixture with an authentic specimen of cannabinol acetate, prepared by the dehydrogenation of tetrahydrocannabinol acetate.[7]

(b) Each of fractions 3 to 8 was dissolved in 50 ml. of 95% alcohol and cooled at 0°. The crystals obtained were snowy white rods; those from Fractions 4 to 7 melted at 75.5–76°; recrystallization or admixture with authentic cannabinol acetate caused no change in melting point. The material obtained from Fraction 3 melted at 67–72°.

Cannabidiol Bis-3,5-dinitrobenzoate.—A portion of the mother liquor from Fraction 5 was refluxed with 30 ml. of 2% alcoholic potassium hydroxide solution for one-half hour in a stream of nitrogen. After cooling, the solution was acidified with hydrochloric acid, diluted with 4 volumes of water, and extracted with petroleum ether. This was in turn washed with sodium bicarbonate solution,

(15) Adams, Hunt and Clark, THIS JOURNAL, **62**, 196 (1940).

(16) Marshall and Wood, *British Med. J.*, 1234 (1912).

(17) Adams, Pease, Cain, Baker, Clark, Wolff and Wearn, THIS JOURNAL, **62**, 2245 (1940).

then with water; the solvent was then removed *in vacuo*. The weight of resin was 4.9 g. Ten grams of 3,5-dinitrobenzoyl chloride was dissolved in 50 ml. benzene, and 5 ml. of pyridine added. The resin, dissolved in benzene, was added to this, and the mixture refluxed for four hours. After cooling, 5 ml. of concentrated hydrochloric acid was added. The precipitated excess 3,5-dinitrobenzoic acid was filtered off, and the benzene washed with dilute hydrochloric acid, sodium carbonate solution and finally with water. After removal of the solvent, cannabidiol bis-3,5-dinitrobenzoate was isolated as described by Adams[15]; yield, 4.2 g. (37%); m. p. 105–107°. This was unchanged when admixed with an authentic sample, kindly furnished by Dr. Roger Adams. Fractions 3, 4 and 7 yielded 4, 25 and 21%, respectively, of cannabidiol.

Summary

Tetrahydrocannabinol, the active principle of *Cannabis sativa L.*, is dehydrogenated spontaneously in the crude drug to form cannabinol, with attendant loss of physiological potency of the drug. The variable results obtained in efforts to repeat the earlier isolation of cannabinol are ascribable to this transmutation in the crude drug.

A correlation between chemical changes and loss of physiological potency of cannabis preparations during storage is provided.

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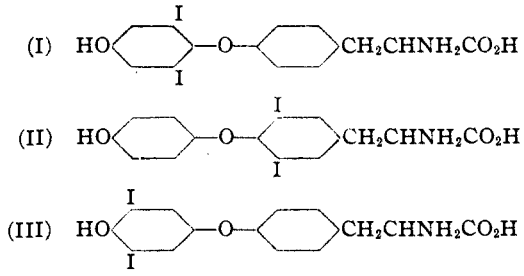
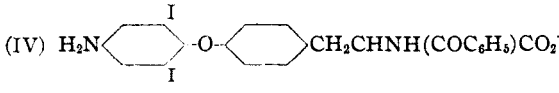
The Synthesis of 2′,6′-Diiodo-*dl*-thyronine[1]

BY CARL NIEMANN AND G. E. MCCASLAND

In continuation of our studies on compounds related to thyroxine[2] we report in this communication the synthesis of 2′,6′-diiodo-*dl*-thyronine (I). This is the third isomeric diiodo-*dl*-thyronine that has been prepared, the 3,5-diiodo-*dl*-thyronine (II) previously having been described by Harington and Barger,[3] and 3′,5′-diiodo-*dl*-thyronine (III) by Block and Powell.[4]



The most convenient starting point for the synthesis of 2′,6′-diiodo-*dl*-thyronine appeared to be the compound α-benzamido-β-[4-(2′,6′-diiodo-4′-aminophenoxy)-phenyl]-propionic acid (IV), the *l*-antipode of which had been prepared by



Canzanelli, Harington and Randall[5] from N-benzoyl-*l*-tyrosine ethyl ester and 3,4,5-triiodonitrobenzene. Proceeding in a similar manner the *dl*-compound was prepared from the intermediate N-benzoyl-*dl*-tyrosine but when the *dl*-amine (IV) was diazotized, and the diazonium salt decomposed in the usual manner, a very poor yield of the desired phenol was obtained. We therefore turned to a method described by Smith and Haller,[6] in which the diazonium fluoroborate[7] is isolated, and converted to the acetoxy compound by heating with glacial acetic acid. Hydrolysis of the acetoxy compound with a mixture of hydriodic and acetic acids resulted in the simultaneous removal of the acetyl and benzoyl groups and in the formation of the desired 2′,6′-diiodo-*dl*-thyronine (I). An attempted synthesis of 2′,6′-diiodo-*dl*-thyronine via the previously unreported N-acetyl-*dl*-tyrosine ethyl ester failed principally because of difficulties encountered in the preparation of the intermediate α-acetamido-β-[4-(2′,6′-diiodo-4′-aminophenoxy)-phenyl]-propionic acid.

It was our original intention to convert the 2′,6′-diiodo-*dl*-thyronine into 2′,3′,5′,6′-tetraiodo-*dl*-thyronine, an isomer of thyroxine. Unfortunately, the customary method of introducing the two final iodine atoms into thyroxine-like compounds failed completely in this case and, although a number of other methods were tried, it was not possible in any instance to isolate from the reaction mixture any pure compound other than the starting material. In view of the above experience it is of interest to recall that Harington and McCartney[8] were unable to prepare tetraiodo derivatives of thyronine other than thyroxine itself.

A comparison of the physiological activities of the three known isomeric diiodo-*dl*-thyronines was contemplated when this work was undertaken but unfortunately these studies have had to be deferred until a later date.

(1) Taken in part from the Ph.D. Thesis of G. E. McCasland, California Institute of Technology, March, 1944.

(2) For previous papers from these Laboratories, see THIS JOURNAL, **63**, 609, 1549, 2204, 2685 (1941).

(3) C. R. Harington and G. Barger, *Biochem. J.*, **21**, 169 (1927).

(4) P. Block, Jr., and G. Powell, THIS JOURNAL, **64**, 1070 (1942).

(5) A. Canzanelli, C. R. Harington and S. Randall, *Biochem. J.*, **28**, 68 (1934).

(6) L. E. Smith and H. L. Haller, THIS JOURNAL, **61**, 145 (1939).

(7) Although it has been claimed by G. Schiemann, *J. prakt. Chem.*, **140**, 97 (1934), that a free phenolic or carboxyl group in the molecules interferes with the precipitation of diazonium fluoroborates, no interference was found in this instance.

(8) C. R. Harington and W. McCartney, *J. Chem. Soc.*, 982 (1929).