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.

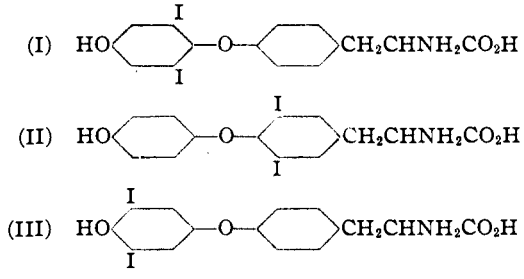
WASHINGTON, D. C. RECEIVED AUGUST 18, 1944

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 976]

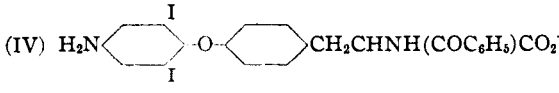
The Synthesis of 2′,6′-Diiodo-*dl*-thyronine[1]

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).

Experimental

N-Benzoyl-*dl*-tyrosine Ethyl Ester (V).—The condensation of 12.2 g. of *p*-hydroxybenzaldehyde and 17.9 g. of hippuric acid according to the procedure of Erlenmeyer and Halsey[9] gave 27.5 g. (90%) of 4-(4'-acetoxybenzal)-2-phenyloxazolone-5 (VI), m. p. 174–176°. The hydrolysis of 157 g. of VI gave after recrystallization, 104 g. (72%) of α-benzamido-β-(4-hydroxyphenyl)-acrylic acid (VII), m. p. 229–230° with decomposition. Catalytic hydrogenation of VII with palladium–charcoal gave a practically quantitative yield of N-benzoyl-*dl*-tyrosine (VIII). The esterification of VIII following the procedure of Curtius[10] led to a practically quantitative yield of V, m. p. 120–122°.

***dl*-α-Benzamido-β-[4-(2',6'-diiodo-4'-nitrophenoxy)-phenyl]-propionic Acid Ethyl Ester (IX).**—A mixture containing 57 g. of V, 80 g. of 3,4,5-tri-iodonitrobenzene,[2] m. p. 162–164°, 18 g. of *anhydrous* potassium carbonate and 300 ml. of dry pentanone-2, b. p. 101–102°, was refluxed for twenty-four hours, filtered, the inorganic residue washed, the filtrate maintained at 5° for sixteen hours, the crystalline product collected, washed thrice with 50-ml. portions of ethanol and finally dried to give 58 g. of IX (53% based on the amount of triiodonitrobenzene used). A sample of IX twice recrystallized from glacial acetic acid melted at 197–198°.

Anal. Calcd. for $C_{24}H_{20}O_6N_2I_2$ (686): C, 42.0; H, 2.9; N, 4.1; I, 37.0. Found: C, 42.4; H, 2.9; N, 4.0; I, 37.3.

***dl*-α-Benzamido-β-[4-(2',6'-diiodo-4'-nitrophenoxy)-phenyl]-propionic Acid (X).**—To a mechanically stirred solution of 5 ml. of 6 *N* sodium hydroxide in 55 ml. of ethanol, heated on a steam-bath, was added 17 g. of IX. The mixture was heated for several minutes after the clear solution initially formed had again turned pasty and was then gradually diluted with 250 ml. of boiling water, the heating being continued until a clear solution resulted. The hot solution was filtered, the filtrate cooled and the nitro-acid precipitated by adding the requisite quantity of 6 *N* hydrochloric acid. The precipitate was collected, washed with water, and dried *in vacuo* at 70° to give a colorless powder which was recrystallized from 70% acetic acid to give 13.6 g. (84%) of X, well-defined colorless needles, m. p. 215–216°.

Anal. Calcd. for $C_{22}H_{16}O_6N_2I_2$ (658): C, 40.1; H, 2.5; N, 4.3; I, 38.6. Found: C, 39.9; H, 2.3; N, 3.9; I, 38.1.

***dl*-α-Benzamido-β-[4-(2',6'-diiodo-4'-aminophenoxy)-phenyl]-propionic Acid (IV).**—To a mechanically stirred suspension of ferrous hydroxide, prepared by adding a hot solution of 13.1 g. of ferrous sulfate heptahydrate in 100 ml. of water to a solution of 16.6 g. of barium hydroxide octahydrate in 475 ml. of boiling water, was added a solution of 5.1 g. of X in 120 ml. of hot water containing the requisite amount of sodium hydroxide. After heating the mixture at 90–100° for ten to fifteen minutes, and finally boiling twenty to thirty seconds, a slight excess of sodium sulfate was added. The hot mixture was filtered and the residue thoroughly washed with boiling water. The combined filtrates were cooled, acidified with acetic acid, and heated briefly to 60–80° to induce coagulation. The precipitate was collected, washed with water, and dried to give 3.8 g. of crude IV, m. p. below 140°. The crude dry product was dissolved in ethanol and the solution passed through a short column of alumina. On evaporating the filtrate, the residue (3.4 g.) was completely colorless. Recrystallization of this residue from 70% ethanol gave 2.5 g. (52%) of IV, m. p. 186–187°. A second recrystallization from the same solvent raised the m. p. to the maximum value of 193–194°.

Anal. Calcd. for $C_{22}H_{18}O_4N_2I_2$ (628): C, 42.1; H, 2.9. Found: C, 42.0; H, 2.7.

***dl*-α-Benzamido-β-[4-(2',6'-diiodo-4'-acetoxyphenoxy)-phenyl]-propionic Acid (XI).**—A solution of fluoroboric acid was prepared by adding 100 ml. of 12 *N* hydrochloric acid to 50 g. of recrystallized technical sodium fluoborate in 100 ml. of water and removing the precipitated sodium chloride from the chilled reaction mixture by filtration. Nine grams of IV was dissolved in 180 ml. of hot glacial acetic acid and the solution cooled to 20°. To this solution was added with mechanical stirring 15 ml. of a 2 *N* solution of anhydrous hydrogen chloride in glacial acetic acid followed by the dropwise addition, within three minutes, of 2.4 ml. of freshly distilled butyl nitrite.[11] The reaction mixture was stirred for an additional ten minutes, diluted with 250 ml. of ice water and immediately filtered. To the clear yellow filtrate was added 120 ml. of the fluoroboric acid solution, then 150 ml. of ice water and the mixture chilled to −20°. After fifteen minutes the precipitated diazonium fluoroborate was recovered and in rapid succession washed with ice-cold fluoroboric acid solution, sucked dry, washed with ice-cold ether, and dried *in vacuo* to give 9.3 g. of a light yellow powder. The above dried diazonium fluoroborate was dissolved in 36 ml. of glacial acetic acid by heating on a steam-bath and after fifteen minutes, when nitrogen evolution had subsided, the solution was refluxed for ten minutes. After cooling, the reaction mixture was poured into 4–5 volumes of water, the precipitate collected, triturated with water, filtered and dried to give 7.5 g. (78%) of crude XI. This product, a cream-colored powder, m. p. 105–120°, could not be induced to crystallize but analysis indicated it to be a reasonably pure preparation of XI. The product gave a negative Folin–Denis reaction.

Anal. Calcd. for $C_{24}H_{19}O_6NI_2$ (671): C, 42.9; H, 2.9; I, 37.8. Found: C, 43.0; H, 3.0; I, 37.7.

2',6'-Diiodo-*dl*-thyronine (I).—To 23 ml. of colorless 7 *N* hydriodic acid, d. 1.7, was added an equal volume of acetic anhydride, 2 g. of red phosphorus, and 4.6 g. of XI. The mixture was refluxed for six hours, filtered and the residue washed with a small amount of acetic acid. The yellow filtrate was evaporated to dryness *in vacuo*, 25 ml. of water added to the residue, and the evaporation repeated. The residue was dissolved in 25–35 ml. of hot water containing a small amount of hydrochloric acid, filtered, cooled, and extracted several times with peroxide-free ether to remove the benzoic acid. The aqueous solution was freed of traces of iodine by adding the requisite amount of solid sodium metabisulfite. Saturated aqueous sodium acetate was then added dropwise until no further precipitation of the amino acid was observed. The colorless precipitate was collected, washed, and dried to give 2.4 g. (67%) of crude I. The crude amino acid was dissolved in hot 80% ethanol with the aid of the requisite amount of sodium hydroxide and the solution suddenly acidified with acetic acid to give I, needles, m. p. 220–221°.

Anal. Calcd. for $C_{15}H_{13}O_4NI_2$ (525): C, 34.3; H, 2.5. Found: C, 34.4; H, 2.5.

Attempted Preparation of 2',3',5',6'-Tetraiodothyronine.—Iodination of I was first attempted by the method of Datta and Prosad.[12] When this procedure failed the following methods were tried but again without success: iodine monochloride in glacial acetic acid; iodine–potassium iodide in dilute methanolic ammonium hydroxide; iodine–potassium iodide in *N* sodium hydroxide at 0°; reaction of a mercurated intermediate with iodine–potassium iodide[13]; and addition of a methanolic solution of iodine to a solution of I in absolute methanol saturated with anhydrous ammonia.[13a] With the last two methods there was some evidence that iodine was introduced but in either case no pure product could be isolated.

N-Acetyl-*dl*-tyrosine Ethyl Ester (XII).—Sixty ml. of acetic anhydride was added, in portions, to a solution of 15

(9) E. Erlenmeyer, Jr., and J. T. Halsey, *Ann.*, **307**, 141 (1889).

(10) T. Curtius, *J. prakt. Chem.*, **95**, 356 (1917).

(11) W. A. Noyes, "Organic Syntheses," **16**, 7 (1936).

(12) R. L. Datta and N. Prosad, THIS JOURNAL, **39**, 441 (1917).

(13) (a) C. Bordeianu, *Ann. Sci. Univ. Jassy*, **20**, 131 (1935); *C. A.*, **30**, 1760 (1936); (b) H. Bauer and E. Strauss, *Ber.*, **69**, 245 (1936); (c) Y. Nagase, *J. Pharm. Soc. Japan*, **58**, 185 (1938); *C. A.*, **32**, 4153 (1938).

g. of *l*-tyrosine in 139 ml. of water and 28 ml. of 6 *N* sodium hydroxide, the temperature of the reaction mixture being maintained at 30–40°. The clear yellow solution was kept at 40° for four hours and then 98% of the theoretical amount of 6 *N* sulfuric acid was added to the solution. The sirupy residue obtained upon evaporation of the solvent[14] was taken up in 200 ml. of absolute ethanol, the solution filtered, evaporated, the residue dissolved in 200 ml. of absolute ethanol and the solution again evaporated. The residue was then dissolved in 100 ml. of absolute ethanol, the solution saturated with anhydrous hydrogen chloride and refluxed for two hours. The reaction mixture was evaporated *in vacuo* to a thick sirup which upon treatment with *M* sodium carbonate crystallized to give, after washing and drying, 11.4 g. (55%) of XII, m. p. 130–132°. On recrystallization from water XII was obtained as well-defined colorless rhombic or hexagonal prisms, m. p. 133–134°. The recrystallized product was insoluble in dilute hydrochloric acid and in dilute sodium carbonate and soluble in dilute sodium hydroxide. It was optically inactive and gave a positive Folin–Denis reaction.

Anal. Calcd. for $C_{18}H_{17}O_4N$ (251): C, 62.1; H, 6.8; N, 5.6. Found: C, 62.4; H, 6.9; N, 5.7.

***dl*-α-Acetamido-β-[4-(2′,6′-diiodo-4′-nitrophenoxy)-phenyl]-propionic Acid Ethyl Ester (XIII).**—The procedure for the preparation of XIII was identical with that used for the preparation of IX. From 59 g. of XIII and 34 g. of 3,4,5-triiodonitrobenzene there was obtained, after one recrystallization from 70% acetic acid, 42.5 g. (58%) of XIII, m. p. 192–193°.

Anal. Calcd. for $C_{19}H_{19}O_6N_2I_2$ (624): C, 36.6; H, 2.9. Found: C, 36.6; H, 2.9.

***dl*-α-Acetamido-β-[4-(2′,6′-diiodo-4′-nitrophenoxy)-phenyl]-propionic Acid (XIV).**—The hydrolysis of 16 g. of XIV conducted as previously described for the preparation of X gave after recrystallization from Cellosolve 9.6 g. of XIV, m. p. 257° with decomposition.

Anal. Calcd. for $C_{17}H_{14}O_6N_2I_2$ (596): C, 34.3; H, 2.4. Found: C, 34.4; H, 2.5.

***dl*-α-Acetamido-β-[4-(2′,6′-diiodo-4′-aminophenoxy)-phenyl]-propionic Acid (XV).**—The procedure which proved to be successful for the preparation of IV failed to give satisfactory yields of XV nor was any other method found to be suitable. A sample of XV recrystallized from 70% acetic acid melted at 226° with decomposition.

Anal. Calcd. for $C_{17}H_{16}O_4N_2I_2$ (566): C, 36.1; H, 2.9. Found: C, 36.1; H, 2.9.

(14) See V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, **98**, 295 (1932).

Summary

The synthesis of 2′,6′-diiodo-*dl*-thyronine and of N-acetyl-*dl*-tyrosine ethyl ester has been described.

PASADENA, CALIF. RECEIVED JULY 18, 1944

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE STATE UNIVERSITY OF IOWA]

The Nitration of Brominated Fluorophenols by the Zincke Method[1]

BY L. CHAS. RAIFORD AND ARTHUR L. LEROSEN

In earlier work Raiford and Heyl[2] have shown that iodine or bromine, but not chlorine, atoms situated in *o*- or *p*-positions to the hydroxyl group of a phenol can be replaced by the nitro group in the Zincke method of nitration.[3] It was found that when two bromine atoms were present in *o*- and *p*-positions to the phenolic group, either could be removed, yielding isomeric nitrobromophenols. Later Raiford and Miller[4] studied chlorobromophenols and found that when both halogens occurred in the favorable positions mentioned, only the bromine was replaced.

The present work shows that when bromofluorophenols are treated with sodium nitrite in glacial acetic acid the fluorine is never displaced while, with one exception, any of the favorably situated bromine atoms was removed. Hodgson and Nixon[5] have found that the nitro group in 2-nitro-3-fluorophenol migrates to the 6-position during bromination of this compound and that on treatment with nitric acid the 6-bromine atom of 2,4,6-tribromo-3-fluorophenol is replaced by the nitro group. In agreement with this behavior we have found that the 2-bromine atom of the latter compound is in no case replaced in the Zincke procedure while the 4- and 6-bromine atoms react easily.

When 2,6-dibromo-4-fluorophenol was treated with sodium nitrite in glacial acetic acid, 2-bromo-4-fluoro-6-nitrophenol was the only product isolated. The isomeric 2-fluoro-4,6-dibromophenol gave both of the expected isomers, *viz.*, 2-fluoro-4-nitro-6-bromophenol, and 2-fluoro-4-bromo-6-nitrophenol. These compounds were separated on the basis of the greater solubility of the para compound in dilute acetic acid and its non-volatility with steam.[6] The *o*- and *p*-positions were assigned to the nitro group in these compounds on the basis of their color, melting points, volatility with steam, and their solubility.

Experimental[7]

Starting Materials.—The preparation of the three fluorobromophenols used for this study will be described later.[8]

(1) From a thesis submitted by Arthur L. LeRosen in partial fulfillment of the requirements for the degree of Doctor of Philosophy to the Graduate College of the State University of Iowa, June, 1940.

(2) L. C. Raiford and F. W. Heyl, *Am. Chem. J.*, **43**, 393 (1910); **44**, 209 (1911).

(3) T. Zincke, *J. prakt. Chem.*, [2] **61**, 563 (1900), first used nitrous acid to replace a bromine atom by the nitro group in bromine substituted phenols.

(4) L. C. Raiford and G. R. Miller, THIS JOURNAL, **55**, 2125 (1933).

(5) H. H. Hodgson and J. Nixon, *J. Chem. Soc.*, 273 (1932).

(6) L. C. Raiford, *Am. Chem. J.*, **46**, 417 (1911); THIS JOURNAL, **44**, 158 (1922); Sidgwick, "The Electronic Theory of Valency," Oxford University Press, London, 1929, p. 148.

(7) Melting points are uncorrected. Samples for analyses were decomposed in the Parr bomb and the bromine was determined by the Volhard method.

(8) L. C. Raiford and A. L. LeRosen, submitted for publication.