Fraction	B. p., °C.	Amt., cc.	n_D^{20}
E 1–10	64–90	470	1.3990–1.4066
11–13	94–104.4	185	1.4096–1.4112
14–22	104.4–112.9	335	1.4122–1.4210

Again, no fraction was obtained having the properties of 2,4,4-trimethylpentene-2. This study is being continued.

Summary

1. Isopropyl-*tert.*-butylcarbinol presents a variety of possible dehydrations.

2. None of the normal dehydration product has been obtained either by dehydration of the carbinol or by thermal decomposition of the Grignard complex from which it is prepared.

3. About 5% of 2,4,4-trimethylpentene-1 has been isolated from the complex olefin mixture.

4. The study of the other dehydration products of the carbinol is being continued.

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[CONTRIBUTION FROM THE MAYO FOUNDATION, ROCHESTER, MINNESOTA]

SOME DERIVATIVES OF DIIODOTYROSINE AND THYROXINE. THE ACTION OF ACETIC ANHYDRIDE ON DIIODOTYROSINE[1]

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The methyl ethers of diiodotyrosine and thyroxine were prepared for a study of their physiologic activity. In general, the procedure used by Karrer[3] for the preparation of O-methyl-*l*-tyrosine was followed. The color reaction with nitrous acid, described by Kendall,[4] which depends on the presence of two iodine atoms ortho to a free hydroxyl group, was conveniently used to determine the completion of the methylation. The spontaneous decomposition of acetylthyroxine, observed by Kendall,[4] which occurs when an aqueous solution of the sodium salt is neutralized, presented some difficulty. This was overcome by performing this step in the procedure in 80% alcohol.

In connection with the synthesis of the methyl ether of diiodotyrosine, it was necessary to establish the identity of N-acetyldiiodotyrosine. The levo form of this derivative was obtained by treating the sodium salt of di-

[1] Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry, 1932.

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[3] Karrer, *Helv. Chim. Acta*, 5, 469 (1922).

[4] Kendall, "Thyroxine," Chemical Catalog Co., New York, 1929.

iodo-*l*-tyrosine with acetic anhydride. In a second preparation, the methyl ester of diiodotyrosine was treated with acetic anhydride and, also, with acetyl chloride. Characterization of the products obtained in each case leaves no doubt that the compound described by Wheeler and Jamieson[5] is not the simple acetyl of diiodotyrosine. The racemic form of the monoacetyl was obtained by racemization of the levo derivative, according to the procedure of Bergmann and Zervas,[6] and was characterized by several of its derivatives.

Wheeler and Jamieson obtained the compound, described by them as the monoacetyl of diiodotyrosine, by boiling diiodotyrosine with a large excess of acetic anhydride. Although this treatment usually produces the azlactone of the amino acid used, instead of the simple acetyl, the insolubility of their product in alcohol and its high decomposition point (225°) are not characteristic of azlactones. The preparation of the azlactones of monoacetyl- and diacetyldiiodotyrosine, described here, showed that their product is not a simple azlactone.

As shown by Bergmann and co-workers[6,7,8] and by Nicolet,[9] amino acids, containing an especially active group other than the amino and carboxyl groups, do not form the corresponding azlactones when treated with excess acetic anhydride. Because of its highly acidic hydroxyl group, the reaction of diiodotyrosine with excess acetic anhydride might, therefore, be expected to be abnormal.

Further investigation of the product obtained by Wheeler and Jamieson showed it to be insoluble in all the usual organic solvents, in dilute and concentrated acids (except concentrated sulfuric acid), readily soluble in aniline, phenol and pyridine, rapidly decomposing in the latter. The compound is optically inactive, and is readily hydrolyzed by dilute sodium hydroxide to N-acetyldiiodo-*d,l*-tyrosine. The iodine content of numerous preparations varied from 53.27 to 54.61%. After further purification, by precipitating the product from phenol with alcohol, the analyses were in good agreement: C, 28.58; H, 2.12; I, 54.59. The decomposition point and other properties remained unchanged after this purification treatment.

From the molecular weight determination and elementary analysis, the empirical formula for the compound is approximately $C_{24}H_{18}O_6N_2I_4$. From the evidence at hand the substance is an anhydride of N-acetyldiiodotyrosine with the hydroxyl groups only partially acetylated. The characteristic yellow color of the nitrous acid color reaction, which turns to pink on making alkaline with ammonium hydroxide, may be noted on the particles

[5] Wheeler and Jamieson, *Am. Chem. J.*, **33**, 365 (1905).
[6] Bergmann and Zervas, *Biochem. Z.*, **203**, 280 (1928).
[7] Bergmann, Stern and Witte, *Ann. Chem.*, **449**, 277 (1926).
[8] Bergmann and Köster, *Z. physiol. Chem.*, **159**, 179 (1926).
[9] Nicolet, THIS JOURNAL, **52**, 1192 (1930).

of the compound suspended in water. N-Acetyldiiodotyrosine forms the anhydride at a more rapid rate than does diiodotyrosine.

Of the several structures possible for an anhydride consisting of two molecules of an amino acid, the diketopiperazine structure was most seriously considered. Accordingly, the acetylated levo diketopiperazine of diiodotyrosine was prepared, but was not found to resemble the anhydride under investigation in any way. However, the racemic diketopiperazine, which proved to be identical with the anhydride reported by West,[10] was more promising inasmuch as its physical properties, particularly a high decomposition point and insolubility in acetic anhydride, alcohol and all other ordinary organic solvents, agree well with those of the compound described by Wheeler and Jamieson. Attempts to acetylate this diketopiperazine failed, however, owing to its complete insolubility in acetic anhydride, and suitable solvents. It was not acetylated when heated to 150° in a sealed tube, with excess acetic anhydride.

Further, the anhydride reacts with concentrated ammonium hydroxide, phenol, and sodium ethylate to form the amide, phenyl ester, and ethyl esters, respectively, of diiodotyrosine, which were recovered in yields of 70 to 80%. From these reactions structures II and III, suggested by Levene and Steiger[11] for their double azlactone of acetyl-α-aminoisobutyric acid, might be considered as possibilities for the structure of the diiodotyrosine complex providing both molecules involved in these configurations would react in the same way with the reagents mentioned above.

That the presence of the highly acidic hydroxyl group of diiodotyrosine determines the formation of the anhydride is evidenced by the fact that diacetyldiiodotyrosine follows the normal course of reaction to yield the corresponding azlactone. If the reaction of diiodotyrosine with excess acetic anhydride is carried out in the presence of anhydrous sodium acetate, the hydroxyl groups are completely acetylated and the azlactone formed instead of the condensation product.

It seems probable that the anhydride is formed through the azlactone of acetyldiiodotyrosine, for the azlactone was isolated from the amorphous product obtained when the reaction was stopped just as the condensation product began to separate from the acetic anhydride. When this amorphous soluble substance was dried and further treated with acetic anhydride, the condensation product was formed.

Preliminary experiments have shown that substitution of the hydroxyl group diminishes the effect of diiodotyrosine and thyroxine on amphibian metamorphosis, whereas O-methylthyroxine produces no effect when administered intravenously or subcutaneously to a normal individual, in amounts comparable with the physiologic dose of thyroxine.

[10] West, *Proc. Soc. Expil. Biol. Med.*, **23**, 629 (1926).

[11] Levene and Steiger, *J. Biol. Chem.*, **93**, 581 (1931).

Experimental

O-Acetyl-N-acetyldiiodo-*l*-tyrosine.—Ten grams of pure diiodo-*l*-tyrosine was dissolved in 100 cc. of 2 *N* sodium hydroxide. To this solution, while shaking and externally cooling, 11.0 cc. of acetic anhydride was added slowly. After standing at room temperature for two hours, the solution no longer gave a color reaction with nitrous acid. On acidifying the solution with 35 cc. of 6 *N* hydrochloric acid, the diacetyl was precipitated in an amorphous form. This was filtered out and dissolved in about 100 cc. of hot alcohol. If this solution showed any color, it was treated with a small amount of charcoal and filtered. Hot water, containing a few cubic centimeters of hydrochloric acid, was added until the solution became turbid. On cooling in the refrigerator, the product separated in long colorless blade-like crystals. After drying these in a vacuum over concentrated sulfuric acid, and then in a high vacuum heated to 110°, they melted at 186 to 187° (uncorr.), and gave no color reaction with nitrous acid; yield, 9.3 g.

Anal. Calcd. for $C_{13}H_{12}O_6NI_2$: C, 30.17; H, 2.51; I, 49.12; neut. equiv. (150 mg. in alcohol) 2.9 cc. of 0.1 *N* NaOH (phenolphthalein). Found: C, 30.15; H, 2.55; I, 49.02; neut. equiv., 2.84 cc. of 0.1 *N* NaOH. *Rotation.* $[\alpha]_{546}^{30°}$ +21.52; (1.44% in absolute alcohol).

O-Acetyl-N-acetyldiiodo-*d,l*-tyrosine.—Three grams of O-acetyl-N-acetyldiiodo-*l*-tyrosine was dissolved in 30 cc. of glacial acetic acid containing 0.6 g. (mol. equiv.) of acetic anhydride. After refluxing the solution for one hour, the greater part of the acetic acid was removed under diminished pressure and the residue taken up with water. The undissolved material was filtered out, and recrystallized from hot 25% alcohol. The optically inactive diacetyl was thus obtained in long colorless blade-like crystals which melted at 186°.

Anal. Found: C, 30.19; H, 2.60; I, 48.99; neut. equiv., 150 mg. required 2.84 cc. of 0.1 *N* NaOH.

N-Acetyldiiodo-*l*-tyrosine.—Two grams of the diacetyldiiodo-*l*-tyrosine was dissolved in 150 cc. of alcohol containing 20 cc. of 5 *N* sodium hydroxide. The alcohol was replaced on the steam-bath with water, maintaining a volume of about 100 cc. After standing at room temperature for several hours, the solution was slowly poured into 200 cc. of hot water containing 10 cc. of 20% hydrochloric acid. On cooling, the slightly yellow product separated. This was filtered out, its alcoholic solution treated with a small amount of charcoal and filtered. On adding hot water to the filtrate to the point of turbidity, and then cooling in the refrigerator, the product separated in crystalline form as irregular or hexagonal plates. These were readily soluble in alcohol, ethyl acetate and acetone, but insoluble in ether and cold water. After drying in a high vacuum, heated to about 80°, the compound turned yellow at 185° and decomposed, with frothing, at 198 to 200°; yield, 1.6 g. Both monoacetyl and diacetyldiiodo-*l*-tyrosine hold tenaciously to one-half molecule of water as determined by iodine analyses before and after heating in a high vacuum.

Anal. Calcd. for $C_{11}H_{10}O_4NI_2$: C, 27.78; H, 2.32; I, 53.45; neut. equiv. (220.8 mg. in alcohol), 9.28 cc. of 0.1 *N* NaOH (phenolphthalein). Found: C, 27.70; H, 2.34; I, 53.42; neut. equiv., 9.3 cc. of 0.1 *N* NaOH. *Rotation.* $[\alpha]_{546}^{30°}$ +34.54° (1.44% in absolute alcohol).

This derivative was also prepared by treating the methyl ester of diiodo-*l*-tyrosine with both acetyl chloride and acetic anhydride. The products thus obtained were identical with that described above.

The methyl ester of N-acetyldiiodo-*l*-tyrosine was prepared by treating the solution of the above product in absolute methyl alcohol with dry hydrochloric acid. The solution was neutralized and poured into water to precipitate the product. On recrystalliza-

tion from hot dilute alcohol, the methyl ester was obtained in long, colorless, needle-like crystals which melted at 152 to 153.5°, and proved to be identical with the products obtained from the reaction of the methyl ester of diiodo-*l*-tyrosine with acetyl chloride or acetic anhydride.

Anal. Calcd. for $C_{12}H_{13}O_4NI_2$: C, 29.44; H, 2.65; I, 51.94; neut. equiv. (150 mg. in alcohol), 3.07 cc. of 0.1 *N* NaOH (phenolphthalein). Found: C, 29.40; H, 2.62; I, 51.82; neut. equiv., 3.03 cc. of 0.1 *N* NaOH.

N-Acetyldiiodo-*d,l*-tyrosine.—This compound was obtained by hydrolysis of the racemic diacetyl of diiodotyrosine under the same conditions as was the levo isomer obtained from the optically active diacetyl. The compound crystallized from hot dilute alcohol in microscopic hexagonal plates which turned brown at about 185°, and decomposed, with frothing, at 205 to 206°, when heated at the same rate as was the levo isomer. It is less soluble in alcohol and ethyl acetate than is the levo form. The methyl ester of the inactive acetyl was obtained as long meshed, needle-like crystals which melted at 136 to 137°.

Anal. Found: C, 29.43; H, 2.63; I, 51.75; neut. equiv. (150 mg. in alcohol), 3.08 cc. of 0.1 *N* NaOH (phenolphthalein).

The ethyl ester crystallized from hot dilute alcohol in clusters or rosets of short needle-like crystals which melted at 140 to 141°.

Anal. Calcd. for $C_{13}H_{15}O_4NI_2$: C, 31.01; H, 2.98; I, 50.49; neut. equiv. (150 mg. in alcohol), 2.97 cc. of 0.1 *N* NaOH (phenolphthalein). Found: C, 30.89; H, 3.00; I, 50.38; neut. equiv., 2.93 cc. of 0.1 *N* NaOH.

O-Methyl-N-acetyldiiodo-*l*-tyrosine.—To a solution of 3.0 g. of N-acetyldiiodo-*l*-tyrosine in 15 cc. of alcohol containing 1.0 cc. of 5 *N* sodium hydroxide were added 0.5-cc. portions of dimethyl sulfate, alternately, with 1.0-cc. portions of 5 *N* sodium hydroxide, while shaking vigorously and allowing the heat of reaction to subside between each addition of the reagents. A total of 5 cc. of alkali and 2.5 cc. of dimethyl sulfate was added before the reaction solution gave a negative nitrous acid color reaction. About 15 cc. of alcohol was then added and the solution acidified with 20% hydrochloric acid. On pouring this solution into 100 cc. of hot water containing 2 cc. of hydrochloric acid, and cooling, the product separated in crystalline form. It was redissolved in alcohol, and treated with charcoal. The filtrate from the charcoal was poured slowly into about 300 cc. of hot water containing 1 cc. of concentrated hydrochloric acid. The methyl ether separated as colorless, microscopic leaves, which, after drying in a desiccator over concentrated sulfuric acid, melted at 207 to 208°, without decomposition. The product gave no color reaction with nitrous acid and was readily soluble in methyl alcohol, ethyl alcohol, ethyl acetate and acetone, sparingly soluble in cold water and insoluble in ether. The yield was 2.54 g.

Anal. Found for $C_{12}H_{13}O_4NI_2$: C, 29.40; H, 2.72; I, 51.83; neut. equiv. (150 mg. in alcohol), 3.03 cc. of 0.1 *N* NaOH (phenolphthalein). *Rotation.* $[\alpha]_{5461}^{28.5}$ +14.5° (1.44% in absolute alcohol).

O-Methyldiiodo-*l*-tyrosine.—Three grams of O-methyl-N-acetyldiiodo-*l*-tyrosine was dissolved in 40 cc. of alcohol containing 30 cc. of 10% sulfuric acid. The alcohol was evaporated on the steam-bath and the aqueous solution heated at 90 to 100° for sixteen hours. On removing the alcohol, much of the material separated from solution but gradually redissolved after several hours of heating. The acid solution was evaporated to dryness under diminished pressure, and the residue dissolved in the smallest possible amount of alcohol. About 100 cc. of water was added and the methyl ether precipitated by making the solution neutral to litmus with sodium bicarbonate. The product was filtered out, dissolved in hot water with the aid of a few drops of acetic acid,

and this solution was treated with charcoal. On filtering and cooling, the methyl ether was obtained as colorless, meshed, needle-like crystals which gave a negative nitrous acid color reaction, decomposed at 204 to 206° and were insoluble in water, sparingly soluble in ethyl alcohol.

Anal. Calcd. for $C_{10}H_{11}O_3NI_2$: C, 26.85; H, 2.48; I, 56.82; neut. equiv. (150 mg. in alcohol), 3.35 cc. of 0.1 *N* NaOH (phenolphthalein). Found: C, 26.78; H, 2.49; I, 56.58. *Rotation.* $[\alpha]_{546}^{28.5}$ −3.2 (2.5% in 5% NaOH).

The sodium salt of O-methyldiiodo-*l*-tyrosine was obtained from 15% sodium hydroxide in the form of large rectangular plates.

Reaction of the Condensation Product of Acetyldiiodotyrosine: with Sodium Hydroxide.—One gram of the anhydride was allowed to stand at room temperature with 25 cc. of 0.5 *N* sodium hydroxide. The suspension was occasionally shaken and kept in the dark. The material was completely dissolved at the end of twenty hours and was precipitated by acidifying the solution with dilute hydrochloric acid. The precipitate, dissolved in alcohol, was treated with charcoal. The alcoholic solution was then poured into 100 cc. of hot acidulated water. On cooling, the product crystallized out in irregular or hexagonal plates which turned brown at 185° and decomposed with frothing at 206°; yield, 0.85 g. From the elementary analysis, neutral equivalent, "mixed" melting point, preparation of its methyl ester, and its general physical properties, this compound proved to be N-acetyldiiodo-*d,l*-tyrosine, described above. On heating with a large excess of acetic anhydride, the condensation product was again obtained.

With Sodium Ethylate.—To 10 cc. of absolute ethyl alcohol, to which had been added 75 mg. of metallic sodium, 1.5 g. of the anhydride was added. After shaking for fifteen minutes, the substance was completely dissolved to form a clear, yellow solution. This was acidified with a few drops of concentrated hydrochloric acid and then poured into several volumes of hot water. On cooling, 1.2 g. of the ethyl ester of acetyldiiodo-*d,l*-tyrosine separated in sheaves and rosets of short needles.

With Concentrated Ammonium Hydroxide.—A suspension of 2.0 g. of the anhydride in 30 cc. of concentrated ammonium hydroxide was allowed to stand at room temperature for seventy-two hours with occasional shaking. The resulting solution was filtered, aerated and then acidified with dilute hydrochloric acid. The precipitate obtained crystallized from alcohol in microscopic plates which darkened at 195° and decomposed at 204 to 206°; yield, 1.45 g. The substance gave a positive color reaction with nitrous acid, and readily yielded ammonia when gently warmed with dilute sodium hydroxide. From the latter reaction and the following analyses, this was identified as the amide of N-acetyldiiodo-*d,l*-tyrosine. It is only moderately soluble in cold alcohol, readily soluble in hot alcohol and insoluble in ether, chloroform, benzene and water.

Anal. Calcd. for $C_{11}H_{12}O_3N_2I_2$: C, 27.84: H, 2.74; N, 5.90; I, 53.58; neut. equiv. (150 mg. in alcohol), 3.15 cc. of 0.1 *N* NaOH (phenolphthalein). Found: C, 27.79; H, 2.70; N, 5.82; I, 53.48; neut. equiv., 3.15 cc. of 0.1 *N* NaOH.

With Phenol.—A solution of 3.0 g. of the acetyl anhydride in 15 g. of phenol was heated at the boiling point of the solvent for five minutes. The excess phenol was removed by distillation in a vacuum. The residual oil was washed with a small amount of cold ether and dissolved in alcohol. The alcoholic solution was treated with charcoal. After filtering out the charcoal, the product was precipitated from the alcohol with water, and was thus obtained as a white, non-crystalline powder which would not crystallize from dilute alcohol nor on spontaneous evaporation of its alcoholic solution. It melted indefinitely between 95 and 115°, and gave a positive nitrous acid color reaction. On allowing a solution of this substance in normal sodium hydroxide to stand at room temperature for a short time, N-acetyldiiodo-*d,l*-tyrosine and phenol were recovered.

The substance was undoubtedly a mixture of the phenyl esters of monoacetyl and diacetyldiiodotyrosine, containing approximately 65% of the latter, as calculated from the following analyses.

Anal. Calcd. for $C_{17}H_{15}O_4NI_2$: C, 38.44; H, 2.72; I, 46.18; neut. equiv. (200 mg. in alcohol), 3.37 cc. of 0.1 *N* NaOH (phenolphthalein). Calcd. for $C_{19}H_{17}O_5NI_2$: C, 37.09; H, 2.86; I, 42.76. Found: C, 37.95; H, 2.77; I, 43.82; neut. equiv., 1.20 cc. of 0.1 *N* NaOH.

Azlactone of N-Acetyldiiodo-*d,l*-tyrosine.—Three grams of diiodotyrosine was gently heated with 15 cc. of acetic anhydride. As soon as the resulting solution showed any turbidity due to the separation of the acetyl anhydride of diiodotyrosine, the solution was quickly cooled in an ice-bath, and then filtered free of the small amount of the double anhydride already formed. On adding several volumes of cold water to the filtrate, a yellow amorphous mass separated immediately. This was soluble in alcohol and ethyl acetate, and, after drying, yielded the acetyl anhydride when treated again with excess acetic anhydride.

The amorphous substance was dissolved in as small an amount of hot chloroform as possible, and petroleum ether added carefully, to precipitate the yellow material. On filtering out the latter, and adding more petroleum ether, a white, flocculent precipitate was obtained. This was dissolved in chloroform. On allowing the solvent to evaporate spontaneously, the product crystallized out in short rods which sintered at 75° and melted at 135 to 138°. The compound gave a strongly positive color reaction with nitrous acid, and on titrating it in alcohol with sodium hydroxide, using phenolphthalein as the indicator, the alkali was neutralized rapidly, at first, to give a temporary end-point, and then more slowly until a permanent end-point was reached after several hours when two equivalents of sodium hydroxide had been added. Thus, the compound titrated typically like an azlactone. It reacted with ammonia to form the amide of N-acetyldiiodo-*d,l*-tyrosine and was slowly hydrolyzed to the monoacetyl.

Anal. Calcd. for $C_{11}H_9O_3NI_2$: C, 28.66; H, 1.97; I, 55.58; neut. equiv. (150 mg in alcohol), 6.3 cc. of 0.1 *N* NaOH (phenolphthalein). Found: C, 28.58; H, 1.93; I, 55.43; neut. equiv., 6.23 cc. of 0.1 *N* NaOH.

Azlactone of O-Acetyl-N-acetyldiiodo-*d,l*-tyrosine.—This was obtained by heating diacetyldiiodo-*l*-tyrosine for one hour at 100° with 15 cc. of acetic anhydride. The excess acetic anhydride was removed by evaporation under diminished pressure, the last part being removed by repeated evaporation in a vacuum with xylene. The residual sirup was dissolved in a small amount of chloroform, and the yellow impurities precipitated by careful addition of petroleum ether. Further addition of petroleum ether precipitated the azlactone, which was finally obtained by allowing its chloroform solution to evaporate spontaneously, as stout prisms which sintered at 60°, melted at 87 to 89° and gave no color reaction with nitrous acid. The product titrated with alkali like a typical azlactone, formed the amide of acetyldiiodotyrosine when treated with concentrated ammonium hydroxide at room temperature, and was slowly hydrolyzed, on standing with water for two or three days, to diacetyldiiodotyrosine. Like the azlactone of acetylphenylalanine,[7] it is comparatively stable to alcohol.

Anal. Calcd. for $C_{13}H_{11}O_4NI_2$: C, 31.20; H, 2.20; I, 50.90. Found: C, 31.31; H, 2.22; I, 50.79.

O-Methyl-N-acetyl-*d,l*-thyroxine.—To a solution of 400 mg. of acetyl-*d,l*-thyroxine in 25 cc. of alcohol containing 1.0 cc. of 5 *N* sodium hydroxide, were added 0.5-cc. portions of dimethyl sulfate, alternately, with 1.0-cc. portions of 5 *N* sodium hydroxide while shaking. The solution no longer gave a color reaction with nitrous acid, after 5 cc. of 5 *N* sodium hydroxide and 2.5 cc. of dimethyl sulfate had been used. The alcoholic

solution was acidified with 3 cc. of 20% hydrochloric acid. If this neutralization is carried out in aqueous solution or in the absence of sufficient alcohol, a decomposition of the methylated product occurs with the liberation of iodine. The acidified solution was slowly poured into 175 cc. of water at 90°. After standing in the refrigerator for several hours, the separated product was redissolved in 40 cc. of alcohol and treated with charcoal. The filtrate from the charcoal was poured into 200 to 250 cc. of hot water containing 2 cc. of concentrated hydrochloric acid, and the alcohol removed on the steam-bath. On cooling, the acetyl of the methyl ether of thyroxine separated in tufts of short needles which softened at 205° and melted, without decomposition, at 214 to 217°. The nitrous acid color reaction was negative; yield, 355 mg.

Anal. Calcd. for $C_{18}H_{15}O_5NI_4$: C, 25.93; H, 1.80; I, 60.98. Found: C, 25.82; H, 1.82; I, 60.32.

O-Methyl-*d,l*-thyroxine.—In a large Pyrex tube, 200 mg. of O-methyl-N-acetyl-*d,l*-thyroxine was dissolved in 15 cc. of alcohol containing 0.5 cc. of 5 *N* sodium hydroxide. The alcohol was replaced with water on the steam-bath and the total volume made up to 20 cc. with water containing 3.5 cc. of 5 *N* sodium hydroxide to make a solution of 4% sodium hydroxide. The solution was heated at 100° for twenty-four hours, the test-tube being fitted with a one-holed rubber stopper carrying a large pipet to act as a reflux condenser. After cooling, 3 cc. of concentrated sodium hydroxide was added, and the solution allowed to stand in the refrigerator for several hours. The sodium salt, which separated, was centrifuged out, dissolved in 15 to 20 cc. of alcohol, the solution acidified with 20% hydrochloric acid, and then poured into 200 cc. of hot water to precipitate the product. For purification, the methyl ether was dissolved in hot alcohol, treated with a small amount of charcoal, and obtained in the form of long, meshed needles after the addition of several volumes of hot water to the alcoholic filtrate. These showed no color reaction with nitrous acid and decomposed with frothing at 210 to 213°. The methyl ether is insoluble in water, ether and chloroform, but moderately soluble in alcohol when freshly precipitated from solution and still moist.

Anal. Calcd. for $C_{16}H_{13}O_4NI_4$: C, 24.27; H, 1.64; I, 64.22. Found: C, 24.20; H, 1.69; I, 64.09.

Summary

The syntheses of the following are described: O-methyl-, O-methyl-N-acetyl, N-acetyl- and its methyl ester and O-acetyl-N-acetyl-, diiodo-*l*-tyrosine; N-acetyldiiodo-*d,l*-tyrosine and its amide, azlactone and methyl, ethyl and phenyl esters; O-acetyl-N-acetyl-diiodo-*d,l*-tyrosine and its azlactone; O-methyl- and O-methyl-N-acetyl-, *d,l*-thyroxine.

The compound described in the literature as acetyldiiodotyrosine is shown to be a condensation product resulting from the action of excess acetic anhydride on diiodotyrosine and involving two molecules of acetyldiiodotyrosine. Some chemical and physical properties of this product are described, and its structure is considered.

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