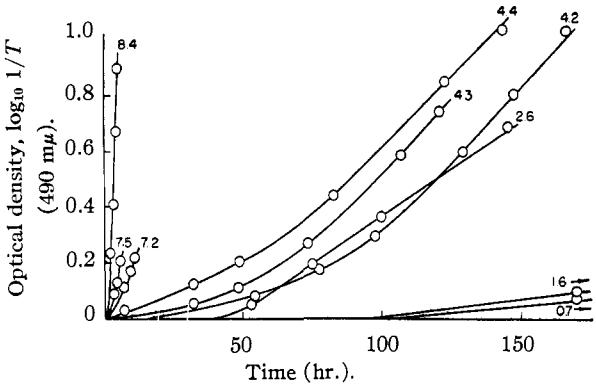

Fig. 3.—Rate of coloration of 0.25 *M* D-xylose with 0.25 *M* glycine at 65° at various *p*H (measured at the initial straight line portions of these curves) values.

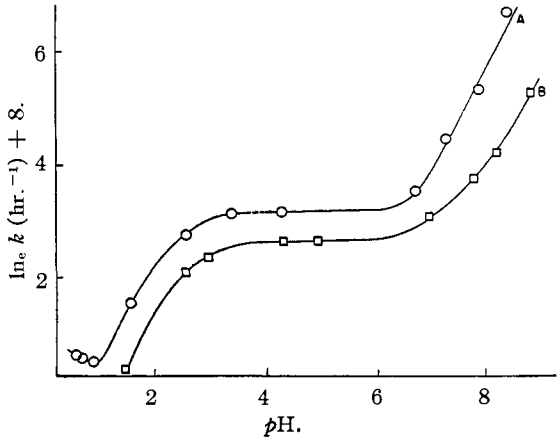

Fig. 4.—Relation between *p*H (see Fig. 3) and $\ln_e k$ for rate of coloration of 0.25 *M* (in each constituent) solutions of: A, D-xylose–glycine at 65°; B, D-xylose–DL-alanine at 65°. The lowest *p*H points on curve A were measured on brown colored solutions of an aldehydic, rather than a caramel, odor; distillation of these solutions into a solution of 2,4-dinitrophenylhydrazine yielded copious quantities of 2-furaldehyde 2,4-dinitrophenylhydrazone of m.p. 229° (dec., cor.).

before there was appreciable browning (see Table I). Curve B of Fig. 4 represents a final plot of similar data obtained for the case wherein the glycine was substituted by an equimolar amount of the closely related amino acid DL-alanine.

The curves of Fig. 2 and Fig. 4 are all of the same general type. It is probably significant that a major change in the shape of the curve occurs near *p*H 6, the isoelectric point of the amino acids. An unadjusted solution of glycine and D-xylose exhibits a *p*H near 6.5 which lowers to approximately 5 on heating.[8] Those portions of the curves between *p*H 6.5–8.5 establish strong base catalysis[9] and those between 3–5 indicate solvent or weak base catalysis. Below this a sharp change of slope may be interpreted as due to acid inhibition. In one case (curve A, Fig. 4), the reaction was followed toward still lower *p*H values and exhibited an upturn through a minimum. Here copious quantities of 2-furaldehyde were detectable, so this point represents a change in the over-all nature of the reaction and the coloration is probably due mainly to 2-furaldehyde polymerization alone. Selecting comparative values near *p*H 4 from the curves in Figs. 2 and 4 (curve A), which differ only in the temperature factor, an activation energy of 20.2 kcal. is calculable. These data then serve to show the complexity of the reaction between amino acids and reducing sugars and demonstrate that it proceeds more rapidly in the *p*H range 6.5–8.5. This is the range wherein the initial amino–carbonyl interaction is favored,[4,10] undoubtedly a significant factor.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGY, SCHOOL OF MEDICINE, UNIVERSITY OF CALIFORNIA, BERKELEY]

Synthesis of Some O-Glucuronides and O-Glucosides of Phenolic Amino Acids[1]

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Phenolic glucuronides of tyrosine and diiodotyrosine, and phenolic glucosides of tyrosine, diiodotyrosine and diiodothyronine were prepared by coupling the appropriately blocked amino acids with acetobromoglucuronic acid methyl ester or acetobromoglucose in the presence of finely divided silver oxide and quinoline. The carboxyl group of the amino acids was blocked by esterification. Trifluoroacetyl proved to be an excellent amino blocking group for the preparation of free glucuronides and glucosides because it is readily removed by treatment with dilute alkali at room temperature, a procedure which does not affect the glucoside or glucuronoside linkage.

While investigating the excretion products of I^{131}-labeled L-thyroxine in the bile of rats, we encountered an unidentified iodine-containing compound on our filter paper chromatograms.[2,3] This compound, which we named "Compound U," is the major excretion product of thyroxine in the rat. Primarily on the basis of its hydrolysis by the enzyme, β-glucuronidase, we have assumed that Compound U is a glucuronide of thyroxine.[3] Final proof of this assumption, however, must await the synthesis of thyroxine glucuronide, and the demonstration that the synthetic material is identical with Compound U.

The synthesis of glucuronides has been reported by only a few investigators. Neuberg and Nei-

(1) Aided by a grant from the U. S. Public Health Service.

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mann,[4] in 1905, reported the synthesis of diacetylbromoglucuronolactone, and from it the preparation of glucuronides of phenol and euxanthone. However, Goebel and Babers,[5] in 1933, were unable to repeat this work. Goebel and Babers[6] prepared α-1-bromo-2,3,4-triacetylglucuronic acid methyl ester and from it the methyl ester of β-D-*p*-nitrobenzylglucuronide. More recently Touster and Reynolds[7] reported the synthesis of β-D-glucuronic acid-1-phosphate from acetobromoglucuronic acid methyl ester and silver phosphate. The final product was isolated as the dibrucine or dibenzylamine salt.

An improved procedure for the oxidation of glucosides to the corresponding glucuronides has been reported by Marsh.[8] Using gaseous oxygen in the presence of a platinum catalyst he prepared α- and β-menthyl glucuronides from the corresponding glucosides, and α-glucuronic acid-1-phosphate (as the tripotassium salt) from glucose-1-phosphate. This investigator was unable, however, to prepare phenyl glucuronide by this procedure.

During the preparation of the present manuscript, there appeared an article by Tsou and Seligman[9] on the synthesis of the glucuronide of 2-naphthol through oxidation of the corresponding glucoside by a method similar to that of Marsh. Triacetylglucuronolactone, which has a furanose rather than a pyranose structure, was also coupled with 2-naphthol by these workers, employing a fusion method similar to that previously used for synthesis of phenolic glucosides.[10,11]

In the present investigation, we have adopted the method which Clutton, Harington and Mead[12] employed for the synthesis of O-β-D-glucosidotyrosine. In this method, quinoline and freshly prepared silver oxide are used in the coupling of the acetohalogen derivative of the sugar with the desired aglycone. Although the method has been used for the synthesis of glucosides,[13-15] it has not, to our knowledge, been used heretofore for the synthesis of glucuronides.

In their preparation of O-β-D-glucosidotyrosine, Clutton, Harington and Mead employed carbobenzoxy substitution to block the amino group of tyrosine during the coupling procedure. For the coupling of iodinated amino acids, however, the carbobenzoxy blocking group did not seem satisfactory, since the procedure for its removal, *i.e.*, catalytic hydrogenation, could also result in deiodination. In the present investigation, therefore, we have made use of trifluoroacetyl substitution to block the amino group.[16] N-Trifluoroacetyl is easily removed by treatment with 0.2 *N* NaOH or $Ba(OH)_2$ at room temperature, a procedure which does not affect the glucoside or glucuronide linkage.

In the present report glucuronides of diiodotyrosine and tyrosine, and glucosides of tyrosine, diiodotyrosine and diiodothyronine are described. The β-configuration has been assigned to these compounds on the basis of their mode of synthesis. Unfortunately, however, the present method has not been found suitable for the preparation of thyroxine glucuronides or glucosides. A possible clue to the explanation of the failure of thyroxine derivatives to yield satisfactory glucuronides or glucosides with the present procedure is the observation that when such couplings were attempted, an intense, transitory green color appeared, suggestive of quinone formation. It is possible that the thyroxine derivative was oxidized to a quinone form by the silver oxide used as the coupling agent, thus making the phenolic hydroxyl group unavailable for reaction with the acetobromo sugar derivative. In this connection, it is of interest that Niemann[17,18] has obtained evidence that the physiological activity of thyroxine is related to its ability to be converted to a quinone form.

Experimental[19,20]

α-1-Bromo-2,3,4-triacetylglucuronic Acid Methyl Ester (I).—This was prepared as described by Goebel and Babers,[6] from glucurone supplied by Corn Products Refining Co. The compound prepared in this manner was quite unstable and had to be kept under ether in the refrigerator. Toward the end of the present investigation it was found that the compound could be recrystallized by dissolving it in a minimum of glacial acetic acid and pouring the acetic acid solution into 25 volumes of ice-water. The product prepared in this manner was a perfectly white, stable preparation which could be kept in a desiccator over calcium chloride.

β-O-(2,3,4-Triacetyl Methyl Ester D-Glucuronosido)-N-benzoyl-L-tyrosine Ethyl Ester (II).—Two grams of I (5.0 mmoles) and 0.78 g. of N-benzoyl-L-tyrosine ethyl ester[21] (2.5 mmoles) were mixed by mortar and pestle with 1.5 cc. of redistilled quinoline; 1.27 g. of freshly prepared silver oxide (5.5 mmoles) was added with grinding, and the entire mixture was then ground for 20 minutes. The viscous reaction mixture was placed in a desiccator for 2 hours, extracted with 15–20 cc. of glacial acetic acid and centrifuged. The very dark, but clear, supernatant was poured into 75–100 cc. of crushed ice–water, and the precipitate which formed was collected by filtration and dissolved in 15 cc. of hot 9:1 alcohol–water mixture. The dark solution was treated with H_2S, and the black silver sulfide precipitate removed by filtration. Treatment of the dark filtrate with charcoal did not remove much color. The dark, clear filtrate was concentrated almost to dryness *in vacuo* at room temperature and treated with a small volume of absolute alcohol. Crystallization occurred upon cooling in an ice-bath. Filtration yielded a slightly colored product, practically all the color going into the filtrate; yield 500 mg. (32%).

The product, recrystallized from 9:1 alcohol–water, melted at 138°. *Anal.* Calcd. for $C_{31}H_{35}O_{13}N$: C, 59.13; H, 5.60; N, 2.25. Found: C, 59.01; H, 5.60; N, 2.32.

β-O-D-Glucuronosido-N-benzoyl-L-tyrosine.—0.76 g. of II (1.2 mmoles) was suspended in a solution containing

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(18) C. Niemann and J. F. Mead, *ibid.*, **63**, 2685 (1941).
(19) Carbon, hydrogen and iodine analyses were carried out by the Microanalytical Laboratories, Chemistry Department, University of California, Berkeley. Nitrogen analyses were performed in our own laboratory by the Kjeldahl method.
(20) All melting points were performed on a Fisher Melting Point Block and are uncorrected.
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2.1 g. of $Ba(OH)_2 \cdot 8H_2O$ (6.7 mmoles) in 50 cc. of water and the mixture was stirred mechanically for 4 hours; 4–5 cc. of ether was added and the stirring continued for another 20 hours. The reaction mixture was diluted with about 50 cc. of water, warmed to 50° and saturated with CO_2. The $BaCO_3$ was removed by filtration and the filtrate was concentrated *in vacuo* (bath temp. 60–70°) to approximately 1/2 volume. An additional precipitate was filtered off and the filtrate was concentrated to dryness *in vacuo*. The residue was completely dissolved in a small quantity of hot water and the barium salt of the glucuronide was precipitated by the addition of 2 volumes of ethanol. Precipitation was completed by cooling in an ice-bath, and the product was collected by filtration and washed with cold absolute alcohol. The barium salt was redissolved in a small amount of water and the free acid was precipitated by the addition of an equivalent amount of 1 *N* HCl. After standing in the refrigerator overnight the precipitate was collected by filtration and washed with a small volume of 0.1 *N* HCl, in which it was appreciably soluble; yield 0.35 g. (58%), m.p. 168–169°. *Anal.* Calcd. for $C_{22}H_{23}O_{10}N \cdot 2H_2O$: C, 53.12; H, 5.45; H_2O, 7.2. Found: C, 53.28; H, 5.55; H_2O (Karl Fischer), 8.3.

β-O-(2,3,4,6-Tetracetyl-D-glucosido)-N-benzoyltyrosine Ethyl Ester (III).—1.35 g. of 1-bromo-2,3,4,6-tetraacetylglucose[22] (IV) (3.3 mmoles), 0.51 g. of N-benzoyltyrosine ethyl ester (1.6 mmoles), 2 cc. of redistilled quinoline and 0.81 g. of freshly prepared silver oxide (3.5 mmoles) were mixed as described for II. The reaction mixture, after standing in a desiccator for 2 hours, was extracted with 15–20 cc. of glacial acetic acid. The extract was centrifuged and the clear, orange colored supernatant was poured into 75–100 cc. of crushed ice–water. After about 10 minutes, the light colored precipitate which had formed was collected by filtration and dissolved in 50 cc. of 9:1 alcohol–water. The solution was treated with H_2S and charcoal and filtered. The orange colored filtrate, upon being concentrated *in vacuo*, deposited a heavy precipitate of fine needles. The product was collected after cooling in an ice-bath. It was almost perfectly white and weighed 700 mg. An additional yield of 130 mg. was obtained by concentrating the mother liquor and cooling; total yield 79%.

Upon recrystallization from absolute ethanol, the product melted at 147–148° and gave the following analysis: Calcd. for $C_{32}H_{37}O_{13}N$: C, 59.72; H, 5.80; N, 2.18. Found: C, 59.82; H, 5.84; N, 2.18.

N-Trifluoroacetyl-L-tyrosine Ethyl Ester (V).—Two grams of l-tyrosine ethyl ester (10 mmoles, Eastman Kodak Co.) was suspended in 80 cc. of 1:1 ethyl acetate–chloroform in a separatory funnel. Four cc. of trifluoroacetic anhydride (Minnesota Mining and Manufacturing Co., redistilled at 40°), contained in several cc. of ethyl acetate, was added in portions, shaking after each addition. All the solid material dissolved. The reaction mixture was shaken with 30 cc. of saturated $KHCO_3$, and then washed twice with 30-cc. portions of water. The ethyl acetate–chloroform solution was dried with $CaCl_2$, filtered and concentrated *in vacuo* to about 25 cc. The product was precipitated by the addition of 75 cc. of petroleum ether (b.p. 30–60°), and recrystallized from ethyl acetate by addition of petroleum ether; yield 1.78 g., m.p. 174–176°. A second crop of product, obtained from the concentrated mother liquor by addition of petroleum ether, weighed 0.50 g., m.p. 175–176°, total yield 75%. *Anal.* Calcd. for $C_{13}H_{14}O_4NF_3$: N, 4.59. Found: N, 4.59.

β-O-(2,3,4,6-Tetraacetyl-D-glucosido)-N-trifluoroacetyl-L-tyrosine Ethyl Ester (VI).—0.76 g. of V (2.5 mmoles), 2.0 g. of IV (5.0 mmoles), 3 cc. of redistilled quinoline and 1.20 g. of freshly prepared silver oxide (5.2 mmoles) were mixed and worked up as described for II. The orange-brown filtrate, after H_2S and charcoal treatment, deposited a mass of fine crystals upon standing in the refrigerator overnight. The precipitate, which was collected by filtration and washed with a minimum of cold absolute ethanol, was almost perfectly white and weighed 860 mg., m.p. 157–158°. An additional yield of 100 mg. was obtained from the concentrated mother liquor on standing in the refrigerator for several days; total yield 61%.

Anal. Calcd. for $C_{27}H_{32}O_{13}NF_3$: C, 51.02; H, 5.08; N, 2.20. Found: C, 51.58; H, 5.26; N, 2.17.

β-O-D-Glucosido-L-tyrosine (VII).—0.40 g. of VI (0.63 mmoles) was suspended in a solution containing 25 cc. of water and 1.05 g. of $Ba(OH)_2 \cdot 8H_2O$ (3.3 mmoles). The mixture was stirred mechanically and the solid material slowly went into solution. After 18 hours, the slightly yellow solution was treated with CO_2, and the $BaCO_3$ was removed by filtration. The filtrate was concentrated *in vacuo* (bath temp. 60–70°) to about 5 cc., treated with charcoal and filtered. The filtrate was acidified with 1 cc. of 2 *N* acetic acid and treated with about 5 volumes of alcohol. Crystallization soon occurred, and was completed by standing in the refrigerator for several hours. The product was collected and dried *in vacuo* over P_2O_5 at 100°; 0.19 g. (88%), m.p. 250–257° dec. (lit.[11] 282° dec.). *Anal.* Calcd. for $C_{15}H_{21}O_8N$: C, 52.47; H, 6.17; N, 4.08. Found: C, 51.82; H, 6.20; N, 3.94.

The completeness of removal of the trifluoroacetyl blocking group was determined by analyzing the final product for fluorine. Thirty mg. of the sample was fused with 200 mg. of Na_2CO_3 in a platinum crucible. The white residue was dissolved in water to give a clear solution, neutralized with HCl, and analyzed for fluoride by a slight modification of the method of Bumsted and Wells.[23] The quantity of fluoride present was below the limit of detection of the method (approximately 30 μg.). Therefore, not more than 1% of the trifluoroacetyl groups could have failed to be removed by the hydrolysis procedure.

N-Acetyl-L-tyrosine Ethyl Ester (VIII).—N-Acetyl-L-tyrosine was prepared by the method of du Vigneaud and Meyer.[24] We were unable, however, to obtain a crystalline product with this procedure. The product, in the form of a yellow oil, obtained from 7.5 g. of starting L-tyrosine, was dried by repeated evaporation with absolute alcohol. The oily residue was dissolved in 70 cc. of absolute ethanol, and esterification was carried out by treatment with HCl gas. The acid ethanol solution was concentrated to low volume *in vacuo*, and treated with excess 1 *M* Na_2CO_3. When the solution was further evaporated on the steam-bath and then cooled in an ice-bath, it deposited slightly yellow crystals (4.6 g., 44% based on starting tyrosine). Recrystallization from boiling water and charcoal yielded 4.2 g. of white crystals, m.p. 78–79° (lit.[25] 79–80°).

N-Acetyl-L-tyrosine ethyl ester was also obtained in the following manner[26]: 3.3 g. of tyrosine ethyl ester (16 mmoles, Eastman Kodak Co.) was dissolved in 15 cc. of chloroform; 1.5 cc. of acetyl chloride (21 mmoles) was added and the solution refluxed for 30 hours. The yellow chloroform solution was decanted from a small amount of gummy solid material which adhered to the sides of the boiling flask, and concentrated on the steam-bath. The oily residue was dissolved in methanol. On the addition of water an oily precipitate settled out which partially crystallized on cooling in the refrigerator for several days. The mixture was clarified by boiling with charcoal and filtering. The filtrate deposited long, needle crystals on standing in the refrigerator for 2 weeks; yield 2.1 g. (53%), m.p. 78–80°.

β-O-(2,3,4,6-Tetraacetyl-D-glucosido)-N-acetyltyrosine Ethyl Ester.—0.63 g. of VIII (2.5 mmoles), 2.05 g. of IV (5.0 mmoles), 3 cc. of redistilled quinoline and 1.27 g. of freshly prepared silver oxide (5.5 mmoles) were mixed and worked up as described for III; yield 0.66 g. (45%), m.p. 173–174°. *Anal.* Calcd. for $C_{27}H_{35}O_{13}N$: C, 55.75; H, 6.07. Found: C, 55.04; H, 6.24.

β-O-(2,3,4,6-Tetraacetyl-D-glucosido)-N-benzoyl-3,5-diiodo-L-tyrosine Ethyl Ester.—1.58 g. of N-benzoyl-L-diiodotyrosine ethyl ester[27] (2.8 mmoles), 2.31 g. of IV (5.6 mmoles), 3.0 cc. of redistilled quinoline and 1.38 g. of freshly prepared silver oxide were mixed and extracted with glacial acetic acid as described in III. After H_2S and charcoal treatment, the solution was concentrated to low

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volume and cooled in the refrigerator. An oily precipitate settled out which could not be crystallized. The material was redissolved in ethanol and precipitated by pouring into ice-water. The light-colored, semi-crystalline material was repeatedly extracted with ether. Addition of petroleum ether to the yellow ether extract precipitated a light-colored crystalline substance, 1.78 g. (71%), m.p. 85–95°. The product was recrystallized from ether–petroleum ether. It still melted over a range of several degrees (87–95°), but analysis showed it to be the desired product. Calcd. for $C_{32}H_{35}O_{13}NI_2$: C, 42.92; H, 3.94; N, 1.56. Found: C, 43.31; H, 4.10; N, 1.55.

β-O-(2,3,4-Triacetyl Methyl Ester D-Glucuronosido) N-Benzoyl-3,5-diiodo-L-tyrosine Ethyl Ester.—2.82 g. of N-benzoyl-3,5-diiodo-L-tyrosine ethyl ester (5.0 mmoles), 4.0 g. of I (10 mmoles), 5.0 cc. of redistilled quinoline, and 2.5 g. of freshly prepared silver oxide were mixed and extracted with glacial acetic acid (50 cc.) as described for II. After H_2S and charcoal treatment, the filtrate was concentrated *in vacuo* at room temperature and treated with ether. The crystals which separated on cooling were filtered off and washed with cold absolute ethanol; yield 800 mg. An additional 370 mg. of product was obtained from the mother liquor by concentrating to low volume and cooling; total yield 25%.

Recrystallized from absolute ethanol, the product melted at 102–103°, resolidified and melted again at 177–179°. *Anal.* Calcd. for $C_{31}H_{33}O_{13}NI_2$: C, 42.24; H, 3.77; N, 1.59; I, 28.80. Found: C, 42.04; H, 4.00; N, 1.60; I, 28.68.

β-O-(2,3,4-Triacetyl Methyl Ester Glucuronosido)-N-acetyl-3,5-diiodo-L-tyrosine Methyl Ester.—N-Acetyl-3,5-diiodo-L-tyrosine was prepared by the method of Pitt-Rivers.[28] The product, although it did not have a sharp melting point (120–126°, Pitt-Rivers reports 125°), was esterified by solution in absolute methanol and treatment with HCl gas. The solution was neutralized with Na_2CO_3 and poured into water. The resulting product even after recrystallization from methanol–water melted over the range 140–153° (lit.[29] 152–153.5°), but was sufficiently pure for the preparation of the glucuronide. Calcd. for $C_{12}H_{13}O_4NI_2$: N, 2.86. Found: N, 2.86.

1.22 g. of the above N-acetyldiiodotyrosine methyl ester (2.5 mmoles), 2.0 g. of I (5.0 mmoles), 3 cc. of redistilled quinoline and 1.27 g. of silver oxide (5.2 mmoles) were mixed and worked up as described for III; yield 1.59 g. (80%), m.p. 199–201°. *Anal.* Calcd. for $C_{25}H_{29}O_{13}NI_2$: C, 37.28; H, 3.63; N, 1.74. Found: C, 37.42; H, 3.60; N, 1.75.

N-Trifluoroacetyl-3,5-diiodo-L-tyrosine Ethyl Ester (IX).—This was prepared as described above for V, using the following quantities: 4.60 g. of diiodo-L-tyrosine ethyl ester,[27] 300 cc. of 1:1 ethyl acetate–chloroform and 4.0 cc. of trifluoroacetic anhydride; yield 5.40 g. (97%), m.p. 172–173°. Calcd. for $C_{13}H_{12}O_4NF_3I_2$: N, 2.50. Found: N, 2.50.

β-O-(2,3,4-Triacetyl Methyl Ester D-Glucuronosido)-N-trifluoroacetyl-3,5-diiodo-L-tyrosine Ethyl Ester (X).—0.95 g. of I (2.4 mmoles), 0.67 g. of IX (1.2 mmoles), 2.0 cc. of redistilled quinoline and 0.58 g. of freshly prepared silver oxide (2.5 mmoles) were mixed and worked up as described for III; yield 0.85 g. (81%), m.p. 186–187°. *Anal.* Calcd. for $C_{26}H_{28}O_{13}NI_2F_3$: C, 35.76; H, 3.23; N, 1.60; I, 29.07. Found: C, 35.96; H, 3.32; N, 1.61; I, 29.1.

β-O-Glucuronosido-3,5-diiodo-L-tyrosine Barium Salt (XI).—0.42 g. of X (0.48 mmoles) was dissolved in a mixture of 5 cc. of acetone and 15 cc. of alcohol. Two cc. of carbonate-free 2 *N* NaOH was added and the mixture stirred with a magnetic stirrer. A precipitate started to appear in about 5 minutes, and gradually became heavier. After 3 hours the precipitate was collected by centrifugation, washed with acetone, and redissolved in 10 cc. of water. One cc. of carbonate-free 2 *N* NaOH was added and the mixture allowed to stand for 1 hour. Addition of 1 cc. of 1 *M* $BaCl_2$ precipitated the insoluble barium salt of diiodotyrosine glucuronide. This was isolated by centrifugation after standing in the refrigerator for 2 hours, and washed well with water. The free acid was soluble in water and in alcohol–water mixtures and was not isolated. The barium salt was dried *in vacuo* over P_2O_5 at 100° and was extremely hygroscopic; yield 0.27 g. (76%). *Anal.* of barium salt. Calcd. for $C_{15}H_{15}O_9NI_2Ba$: C, 24.20; H, 2.03; N, 1.88; I, 34.10. Found: C, 24.16 (cor. for $BaCO_3$ residue); H, 2.50; N, 1.83; I, 32.6.

The barium salt was analyzed for fluorine as described above for VII. The quantity of fluoride present was again below the limit of detection of the method and it was concluded that not more than 1% of the trifluoroacetyl groups could have failed to be removed by the hydrolytic procedure.

N-Benzoyl-3,5-diiodo-L-thyronine Methyl Ester (XII).—Five grams of 3,5-diiodo-L-thyronine[30] was converted to the methyl ester according to the method of Ashley and Harington.[31] The melting point of the ester was 174–175° (Ashley and Harington report 174–175° for the m.p. of the DL ester). 4.2 g. of the ester (8.0 mmoles) was suspended in 68 cc. of anisole in a separatory funnel; 0.94 cc. (8 mmoles) of benzoyl chloride was added in portions, shaking after each addition. Thirty cc. of 0.7 *N* Na_2CO_3 was added and the mixture shaken vigorously. 0.94 cc. more of benzoyl chloride was added in portions, shaking after each portion. The cloudy anisole layer was separated and concentrated *in vacuo* (bath temp. 95°). The residue was dissolved in 10 cc. of chloroform and treated with petroleum ether. A gummy precipitate formed which was taken up in hot benzene. The hot benzene solution was cooled and agitated with a glass rod, whereupon a mass of crystals formed. Recrystallization from benzene yielded 3.48 g. of white product, m.p. 105–106°. A second crop of 0.40 g. was obtained from the concentrated mother liquor; total yield 78%.

Anal. Calcd. for $C_{23}H_{19}O_5NI_2$: C, 42.94; H, 2.98; N, 2.18. Found: C, 42.86; H, 2.87; N (Dumas), 2.32.

β-O-(2,3,4,6-Tetraacetyl-D-glucosido)-N-benzoyl-3,5-diiodo-L-thyronine Methyl Ester.—0.85 g. of XII (1.3 mmoles), 2.0 g. of IV (4.9 mmoles), 2.0 cc. of redistilled quinoline, 1.27 g. of freshly prepared silver oxide (5.5 mmole) were mixed and worked up as described for III. The filtrate, after H_2S and charcoal treatment, was concentrated almost to dryness *in vacuo* at room temperature and the residue taken up in ether. The insoluble material was filtered off and the ether solution was cooled in the refrigerator. The precipitate which settled out was recrystallized from alcohol–ether; yield 0.25 g. (26%), m.p. 200–202°. After a second recrystallization from absolute alcohol the product gave the following. *Anal.* Calcd. for $C_{37}H_{37}O_{14}NI_2$: C, 45.65; H, 3.83. Found: C, 45.80; H, 4.21.

N-Trifluoroacetyl-3,5-diiodo-L-thyronine Methyl Ester (XIII).—This was prepared as described for V, using the following quantities: 1.51 g. of diiodothyronine methyl ester (2.8 mmoles), 80 cc. of 1:1 ethyl acetate–chloroform, and 1.1 cc. of trifluoroacetic anhydride; yield 1.78 g. (100%). This product had a double melting point, melting at 136°, resolidifying and melting again at 166–169°. *Anal.* Calcd. for $C_{18}H_{14}O_5F_3NI_2$: N, 2.20. Found: N, 2.10.

β-O-(2,3,4,6-Tetraacetyl-D-glucosido)-N-trifluoroacetyl-3,5-diiodo-L-thyronine Methyl Ester (XIV).—1.27 g. of XIII (2.0 mmoles), 1.71 g. of IV (4.1 mmoles), 2.2 cc. of distilled quinoline and 0.97 g. of freshly prepared silver oxide (4.2 mmoles) were mixed and worked up as described for III; yield 0.90 g. (47%), m.p. 134–135°. *Anal.* Calcd. for $C_{32}H_{32}O_{14}NF_3I_2$: C, 39.81; H, 3.34; N, 1.45. Found: C, 39.57; H, 3.37; N, 1.43.

Several attempts to prepare the corresponding glucuronoside of XIII by the above procedure were not successful.

β-O-D-Glucosido-3,5-diiodo-L-thyronine.—Four hundred mg. of XIV (0.41 mmole) was dissolved in a mixture of 5 cc. of acetone and 15 cc. of absolute ethanol. Two cc. of 2 *N* NaOH was added and the reaction mixture stirred with a magnetic stirrer. The sodium salt started to separate out after about 30 minutes and the precipitate became gradually heavier. After 5 hours, the precipitate was collected by centrifugation and washed with acetone. This sodium salt was quite insoluble in cold water, but readily soluble in hot water. It was recrystallized twice from water, and converted to the free acid by dissolving it in hot water and adding an excess of acetic acid. The free acid crystallized readily from the aqueous solution. The free acid was washed

(28) R. Pitt-Rivers, *Biochem. J.*, **43**, 223 (1948).

(29) C. S. Myers, THIS JOURNAL, **54**, 3718 (1932).

(30) We are indebted to Dr. B. A. Hems of Glaxo Laboratories for a generous supply of 3,5-diiodo-L-thyronine and -L-thyroxine.

(31) J. N. Ashley and C. R. Harington, *Biochem. J.*, **22**, 1436 (1928).

well with water, and dried *in vacuo* over P_2O_5 at 100°; yield 0.26 g. (91%), m.p. 255–260° (dec.). Calcd. for $C_{21}H_{23}O_3NI_2$: C, 36.70; H, 3.40; N, 2.04; I, 36.94. Found: C, 36.41; H, 3.60; N, 2.05; I, 36.27.

Fluorine analysis on the free glucoside was carried out as described for VII and XI. Again, the results indicated that not more than 1% of the trifluoroacetyl groups could have resisted the hydrolytic treatment.

N-Trifluoroacetyl-DL-thyroxine Methyl Ester.—DL-Thyroxine (Hoffmann–La Roche)[32] was esterified according to Ashley and Harington.[31] 0.73 g. of thyroxine methyl ester (m.p. 148–151° dec.) was suspended in 30 cc. of 1:1 ethyl acetate–chloroform, treated with 0.4 cc. of trifluoroacetic anhydride, and worked up as described for V; yield 0.76 g. (93%), m.p. 203–206°. *Anal.* Calcd. for $C_{18}H_{12}O_5NF_3I_4$: C, 24.37; H, 1.35; N, 1.58; I, 57.24. Found: C, 24.46; H, 1.54; N, 1.55; I, 57.8.

This derivative of thyroxine failed to couple with either acetobromoglucose or acetobromoglucuronic acid methyl ester by the methods described here.

N-Benzoyl-L-thyroxine Methyl Ester.—L-Thyroxine[30] was esterified in the same manner as the DL-isomer (treatment of a methanol suspension of the amino acid with dry HCl gas and removal of HCl from the resulting hydrochloride with an equivalent amount of NaOH). The ester prepared by this procedure was not entirely satisfactory. It started to melt at approximately 100°, but melted over a range of 10–15° to give a sticky, non-flowing melt. Several different preparations all behaved in this manner, differing somewhat in the temperature at which melting first started. The preparation used for the N-benzoyl derivative gave the following analysis. Calcd. for $C_{16}H_{13}O_4NI_4$: C, 24.28; H, 1.66. Found: C, 24.40; H, 1.70.

1.32 g. of L-thyroxine methyl ester (1.67 mmoles) was dissolved in 50 cc. of chloroform in a separatory funnel. Six cc. of 2 *N* Na_2CO_3 was added, and then in portions, 0.25 cc. of benzoyl chloride (2.1 mmoles) contained in several cc. of chloroform, was added with shaking. The chloroform layer was drawn off, washed with 10 cc. of 2 *N* Na_2CO_3, twice with water, and dried with $CaCl_2$. The chloroform solution was concentrated *in vacuo* at room temperature, and the residue was redissolved in a small volume of warm benzene. After charcoal treatment and concentration *in vacuo* the benzene solution was treated with several volumes of petroleum ether, whereupon the product started to crystallize. Precipitation was completed by standing in the refrigerator overnight; yield 1.22 g. (82%), m.p. 200–206°. *Anal.* Calcd. for $C_{23}H_{17}O_5NI_4$: C, 30.82; H, 1.91. Found: C, 31.44; H, 2.13.

An attempt to couple the above thyroxine derivative with acetobromoglucuronic acid methyl ester was not successful.

BERKELEY, CALIF.

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Synthesis of 11-Keto Steroids

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A general synthesis of 11-keto steroids is described in which the prerequisite $\Delta^{7,9(11)}$-*allo*-steroid is converted through successive stages to a Δ^7-9α,11α-epoxide, a Δ^8-7ξ,11α-diol, a Δ^8-7,11-dione, and finally to a 7,11-dione. The latter can be reduced preferentially at the 7-position to an 11-keto steroid. By means of this procedure ergosterol, stigmasterol and diosgenin can be converted to *allo*-pregnan-3β-ol-11,20-dione acetate (XV) which can be used for the synthesis of cortical steroids.

In an earlier communication, we described briefly the synthesis of *allo*pregnan-3β-ol-11,20-dione acetate (XV) from ergosterol, stigmasterol and diosgenin.[1] Since that time other investigators have also reported the preparation of 11-oxygenated steroids from $\Delta^{5,6}$-steroids devoid of functional groups in ring C.[2] Of these publications only two have described the synthesis of the diketo *allo*pregnane (XV).[1,2b] We now wish to describe the results presented in our first communication and additional pertinent data.

In the course of research on the introduction of 11-oxygen into the above-mentioned steroids, the oxidation of $\Delta^{7,9(11)}$-steroid dienes by peracids was studied. In contradistinction to earlier literature on the action of perbenzoic acid on conjugated dienes[3] we observed that $\Delta^{7,9(11)}$-dienes react in an orderly stepwise manner with perbenzoic acid. Thus, from the reaction between $\Delta^{7,9(11),22}$-ergostatrien-3β-ol acetate (I) (ergosterol-D acetate)[4] and perbenzoic acid in benzene at 10° a mono-epoxide, a di-epoxide, and a tri-epoxide can be isolated by the use of one, two or three moles of the peracid, respectively. Of these various derivatives, the mono-epoxide proved most useful for the synthesis of 11-keto steroids.

Since the mono-epoxide obtained from ergosterol-D acetate exhibits end absorption only in the ultraviolet above 220 mμ, it is evident that the 7,9(11)-diene system is attacked in preference to the side-chain function. In fact the dienic function is attacked with such rapidity that the mono-epoxide is obtained almost exclusively from the reaction of I with one mole of perbenzoic acid. Similarly $\Delta^{7,9(11)}$-5α,22a-spirostadien-3β-ol acetate (XVI) can be selectively oxidized to a mono-epoxide and to a di-epoxide.

After our initial communication,[1] Jeger and his co-workers[2e] reported the conversion of I into the mono-epoxide and ascribed to the latter a Δ^7-9α,11α-epoxide structure (II). Our experience likewise indicates that these mono-epoxides from $\Delta^{7,9(11)}$-*allo*-steroids are best described as Δ^7-9α,11α-mono-epoxides.[5]

(1) E. M. Chamberlin, W. V. Ruyle, A. E. Erickson, J. M. Chemerda, L. M. Aliminosa, R. L. Erickson, G. E. Sita and M. Tishler, THIS JOURNAL, **73**, 2396 (1951).

(2) (a) L. F. Fieser, J. E. Herz and Wei-Yuan Huang, *ibid.*, **73**, 2397 (1951); (b) G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 3546 (1951); (c) L. F. Fieser, J. C. Babcock, J. Herz, Wei-Yuan Huang and W. P. Schneider, *ibid.*, **73**, 4053 (1951); (d) C. Djerassi, O. Mancera, G. Stork and G. Rosenkranz, *ibid.*, **73**, 4496 (1951); (e) H. Heusser, K. Eichenberger, P. Kurath, H. R. Dällenbach and O. Jeger, *Helv. Chim. Acta*, **34**, 2106 (1951); (f) R. C. Anderson, R. Budziarek, G. T. Newbold, R. Stevenson and F. S. Spring, *Chemistry and Industry*, 1035 (1950); *J. Chem. Soc.*, 2892 (1952).

(3) A. Windaus and A. Lüttringhaus, *Ann.*, **481**, 119 (1930); A. Windaus, O. Linsert and H. J. Eckhardt, *ibid.*, **534**, 22 (1938).

(4) An improved method of synthesis of this substance and other $\Delta^{7,9(11)}$-steroids will be presented later in THIS JOURNAL.

(5) A report covering additional reactions of the mono-epoxide will be presented later.