compound was the terphenyl III resulting from the dehydrogenation of the Diels-Alder adduct X. Its infrared spectrum was identical with that of the compound obtained through the reaction of X with chloranil.

2,5-Dimethoxy-3,4,6-trimethyl-2′,3′-dicarbomethoxy-*p*-terphenyl (III).—A solution of X (0.9 g., 0.002 mole) and chloranil (0.49 g., 0.002 mole) in dry xylene (20 ml.) was refluxed for 24 hr. The red solution was cooled to room temperature and diluted with ether (50 ml.). The solution was extracted with aqueous 10% sodium hydroxide until the washings were no longer colored. The organic layer was then washed with water (100 ml.). The solvent was removed on a steam bath under a stream of nitrogen and the dark residue was triturated with petroleum ether (b.p. 60–68°). The oil solidified to a yellow solid which was recrystallized from ethanol–water. It weighed 0.7 g., 0.0016 mole, 78% yield, and had m.p. 143–144°.

Anal. Calcd. for $C_{27}H_{28}O_6$: C, 72.30; H, 6.29. Found: C, 72.30; H, 6.32.

Action of Aluminum Chloride upon III.—Aluminum chloride (1 g., 0.008 mole) was added to a solution of III (0.45 g., 0.001 mole) in *o*-dichlorobenzene (12 moles). The dark solution was refluxed for 5 min. under a nitrogen atmosphere and then cooled to room temperature. Dilute sulfuric acid was added (100 ml., 10%) and the mixture was extracted with ether (100 ml.). A yellow, insoluble material was removed by filtration. The ether solution was evaporated on a steam bath under a stream of nitrogen. After trituration with petroleum ether (b.p. 60–68°) the residue solidified and was filtered. The white solid XI was recrystallized from benzene–ethanol. It weighed 1.5 g., ethanol. It weighed 1.5 g., 0.00357 mole, 35.7% yield, and had m.p. 292–295°.

Action of Hydrobromic Acid on III.—To a solution of acetic acid (20 ml.) and aqueous hydrobromic acid (10 ml., 48%) was added III (0.55 g., 0.0012 mole). The stirred solution was refluxed; after 45 min. a precipitate formed in the red reaction mixture. Following an additional 15 min. of refluxing, the mixture was cooled to room temperature and the solid was removed by filtration and recrystallized from glacial acetic acid. It weighed 0.3 g., 0.0007 mole, 58.3% yield, and had m.p. 292–295°. The infrared spectrum of this material (XI) was identical with that of the compound produced by the action of aluminum chloride on III. The compound sublimed at 240°/0.5 mm., but this did not give a pure product, for the impurities sublimed also. No satisfactory method of purification was found for this substance.

1,4-Di(2,4,5-trimethylphenyl)but-2-yne-1,4-diol (XII).—A Grignard solution was prepared from magnesium (12 g., 0.5 g.-atom), ethyl bromide (80 g., 0.75 mole) in ether (100 cc.). Acetylene was bubbled into this solution for 12 hr., and then durylicaldehyde[11] (75 g., 0.5 mole) in ether (100 cc.) was added over the course of 1 hr. The solution was refluxed for 1.5 hr., then poured over iced sulfuric acid (500 cc., 5%), and the gray solid was removed and washed with ether. The solid was crystallized from ethanol; it then weighed 20.0 g. (0.08 mole, 32%) and melted at 214–216°.

Anal. Calcd. for $C_{22}H_{26}O_2$: C, 81.95; H, 8.13. Found: C, 81.70; H, 8.35.

1,4-Di(2,4,5-trimethylphenyl)-1,3-butadiene (XIII).—Lithium aluminum hydride (1 g., 0.044 mole) in ether (100 cc.) was added to stirred suspension of XII (2.6 g., 0.008 mole) in ether (75 cc.) at 0° and under nitrogen. The mixture was warmed slowly to room temperature; at 15° the reaction began. The mixture was then refluxed for 2 hr., cooled to 0°, and ethyl acetate (25 cc.), water (25 cc.), and sulfuric acid (20 cc., 10%) were added consecutively. More ethyl acetate (50 cc.) was added, and the organic layer was removed and dried (sodium sulfate). The solution was concentrated and cooled; the solid was removed and recrystallized from ethyl acetate. It weighed 1.0 g., 0.0034 mole, 42.5%, and melted at 199–200°,

Anal. Calcd. for $C_{22}H_{26}$: C, 91.03; H, 8.97. Found: C, 90.46; H, 9.24.

(11) R. R. Holmes, Doctoral dissertation, University of Minnesota, 1950, pp. 77, 78.

Synthesis of Specifically Iodine-131- and Carbon-14-Labeled Thyroxine[1]

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A simple synthesis of various forms of radioactive L-thyroxine, carrying the label (I^{131} or C^{14}) either in the phenolic ring or in the nonphenolic ring and in the side chain, is described. It is based on the coupling of 4-hydroxy-3,5-diiodophenylpyruvic acid and 3,5-diiodotyrosine. Labeled 4-hydroxy-3,5-diiodophenylpyruvic acid was prepared by iodination of *p*-hydroxybenzaldehyde followed by condensation with acetylglycine and hydrolysis of the azlactone formed, or by enzymic oxidative deamination of diiodotyrosine.

The only commercially available radioactive thyroxine is the one in which the iodine atoms of the phenolic ring are labeled. Syntheses of other forms of radioactive thyroxine in which various other atoms carry the label, have been reported.[3–9] Most of these syntheses are either tedious or give low yields. This seems to be the only reason for the nonavailability of such other radioactive forms, which would be extremely valuable for metabolic or other studies. The major pathway

(1) A preliminary report of this work was given at the 140th National Meeting of the American Chemical Society, Chicago, Ill., September, 1961.

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(3) R. Michel, J. Roche, and J. Tata, *Bull. soc. chim. biol.*, **34**, 336, 466 (1952).

(4) S. C. Wang, J. P. Hummel, and T. Winnick, *J. Am. Chem. Soc.*, **74**, 2445 (1952).

(5) J. Nunez and C. Jacquemin, *Compt. rend.*, **249**, 138 (1959).

(6) J. R. Tata and A. D. Brownstone, *Nature*, **185**, 34 (1960).

(7) M. J. Gortatowski, L. F. Kumagai, and C. D. West, The Endocrine Society, Program of the 43rd Meeting, New York, N. Y., June 1961.

(8) R. Michel, R. Truchot, H. Tron-Loisel, and B. Poillot, *Compt. rend.*, **250**, 2632 (1960).

(9) R. Michel, R. Truchot, H. Tron-Loisel, and B. Poillot, *Bull. soc. chim. biol.*, **42**, 1207 (1960).

by which thyroxine is metabolized involves the loss of one or both iodine atoms of the phenolic ring. Since these are the atoms which are labeled in the only now easily available radioactive thyroxine, it is evident that a thyroxine in which other atoms carry the label is required for the study of the ultimate fate of thyroxine after its deiodination. Furthermore, the iodine atoms of the phenolic ring are rendered labile by the phenolic hydroxyl group in the *ortho* position and therefore exchangeable under certain conditions. Thus the use of a thyroxine in which these iodine atoms are labeled may give rise to artifacts that can be avoided with other forms of radioactive thyroxine.

We wish to report a simple synthesis of thyroxine-I^{131} in which the iodine atoms of either the phenolic or the nonphenolic ring are labeled, and also the synthesis of thyroxine-C^{14} in which either the phenolic ring or the nonphenolic ring and the aliphatic side chain are uniformly labeled.

The synthesis is based on the important finding by Meltzer and Stanaback, that 4-hydroxy-3,5-diiodophenylpyruvic acid couples with 3,5-diiodotyrosine in the presence of oxygen at a neutral or slightly alkaline *p*H to form thyroxine rapidly and in good yield.[10,11]

The formation of crude thyroxine in about 25% yield in this reaction could be confirmed in our laboratory. The same yield was obtained whether the keto acid was slowly added as a solution in 1-butanol, or as a crystalline solid. The *p*H was kept constant at 7.6 throughout the coupling reaction. As no racemization occurs[12] the pure optical isomers of labeled thyroxine can be obtained.

When either 3,5-diiodotyrosine-I^{131} or 4-hydroxy-3,5-diiodophenylpyruvic acid-I^{131} was used, the thyroxine formed was radioactive. This shows that both the amino acid and the keto acid are reaction partners and that 4-hydroxy-3,5-diiodophenylpyruvic acid does not merely serve as an oxidation catalyst which promotes the coupling of two molecules of 3,5-diiodotyrosine. No exchange of the label between 3,5-diiodotyrosine and 4-hydroxy-3,5-diiodophenylpyruvic acid takes place in the course of the reaction. This is shown by the fact that the specific activities of the labeled starting material and of the thyroxine formed (expressed in counts/minute/mmole of iodinated compound) are the same no matter whether 3,5-diiodotyrosine and an excess of 4-hydroxy-3,5-diiodophenylpyruvic acid-I^{131} or 3,5-diiodotyrosine-I^{131} and an excess of 4-hydroxy-3,5-diiodophenylpyruvic acid are permitted to react with each other.

Attempts to prepare 4-hydroxy-3,5-diiodophenylpyruvic acid-I^{131} by exchange labeling were not successful. When 4-hydroxy-3,5-diiodophenylpyruvic acid was permitted to react with iodine in the presence of sodium iodide-I^{131} under various conditions, the principal reaction products were 4-hydroxy-3,5-diiodophenylacetic acid and 4-hydroxy-3,5-diiodobenzaldehyde. The aldehyde is very easily formed from the keto acid under a variety of conditions—*e.g.*, by simple elution of the keto acid with methanol from unwashed or from prewashed Whatman paper 3MM. The keto acid and the aldehyde have the same R_f-values in a large number of solvent systems that were tried. In an acetic acid–pyridine–water mixture (1:10:89) of *p*H 6.5, the keto acid moves somewhat faster (R_f 0.77) than the aldehyde (R_f 0.64). However, chromatography in this solvent followed by elution with methanol is accompanied by a partial breakdown of the keto acid to the aldehyde. The conversion of 4-hydroxy-3,5-diiodophenylpyruvic acid to the aldehyde and to other breakdown products takes place almost instantaneously in alkaline medium. Alkaline solvent systems such as were used by various investigators[13,14] should therefore be avoided.

Pure 4-hydroxy-3,5-diiodophenylpyruvic acid-I^{131} was prepared by total synthesis from *p*-hydroxybenzaldehyde. Treatment of this aldehyde with iodine monochloride in the presence of sodium iodide-I^{131} gave 4-hydroxy-3,5-diiodobenzaldehyde-I^{131}. Condensation with acetylglycine yielded 4-(4-acetoxy-3,5-diiodobenzal)-2-methyl-5-oxazolone-I^{131}. Acid hydrolysis of this azlactone gave 4-hydroxy-3,5-diiodophenylpyruvic acid-I^{131}. The absence of 4-hydroxy-3,5-diiodobenzaldehyde is easily revealed by ultraviolet spectroscopy (see below).

This synthesis is not easily applicable to the preparation of microgram quantities of labeled keto acid. Such small amounts were prepared by oxidative deamination of either iodine- or carbon-labeled 3,5-diiodotyrosine in the presence of rattlesnake venom, oxygen, and catalase.[15] The keto acid formed should not be purified by paper chromatography nor should a 3,5-diiodotyrosine eluted from filter paper be used as a starting material as this will cause a partial breakdown of the keto acid to 4-hydroxy-3,5-diiodobenzaldehyde. No aldehyde was formed when the keto acid was extracted with ether from the reaction mixture. Evaporation of the ether extract leaves

(10) R. I. Meltzer, Conference on Derivatives and Isomers of the Thyroid Hormones, University of Pennsylvania, Pa., February, 1960, Summaries of papers, p. 1.

(11) R. I. Meltzer and R. J. Stanaback, *J. Org. Chem.*, **26**, 1977 (1961). The authors wish to thank Drs. Meltzer and Stanaback for their kind permission to read their manuscript before its publication, as well as for helpful discussions.

(12) This statement is based on optical rotation measurements. The usually much more sensitive hog kidney D-amino acid oxidase test cannot be applied since D-thyroxine is not a substrate for that enzyme (S. M. Birnbaum, personal communication).

(13) W. Tong, A. Taurog, and I. L. Chaikoff, *J. Biol. Chem.*, **207**, 59 (1954).

(14) M. Nakano, T. S. Danowski, and A. Utsumi, *Endocrinology*, **65**, 242 (1959).

(15) The authors wish to thank Dr. B. N. LaDu of the Natl. Inst. of Arthritis and Metabolic Diseases, Natl. Inst. of Health, Bethesda, Md., for his valuable advice concerning this reaction.

a residue which is a mixture of the desired keto acid and 4-hydroxy-3,5-diiodophenylacetic acid. Figure 1 shows the ultraviolet absorption spectra of the residue from the ether extract, of authentic 4-hydroxy-3,5-diiodophenylpyruvic acid, of authentic 4-hydroxy-3,5-diiodophenylacetic acid, of an equimolar mixture of these two compounds, and of 4-hydroxy-3,5-diiodobenzaldehyde. Table I shows the wave lengths of maximal absorption and the corresponding molar extinction coefficients of the same compounds. It can be seen from Fig. 1 that the spectra of the residue from the ether extract and of an equimolar mixture of 4-hydroxy-3,5-diiodophenylpyruvic acid and 4-hydroxy-3,5-diiodophenylacetic acid are almost superimposable. It can further be seen from both Fig. 1 and Table I

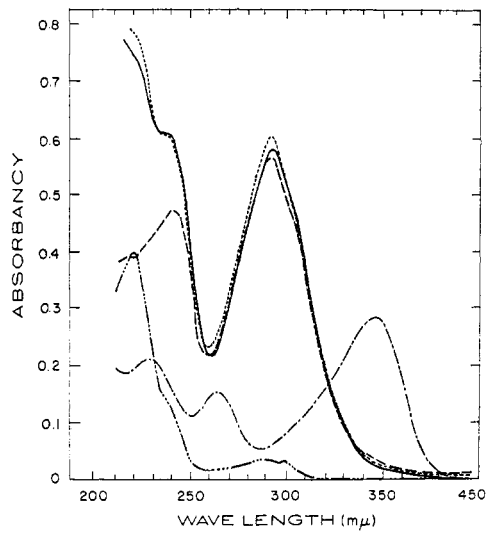

Fig. 1.—Ultraviolet absorption spectra (in methanol). Product of the reaction of L-3,5-diiodotyrosine with rattlesnake venom, residue of the ether extract (arbitrary concentration) ———; 4-hydroxy-3,5-diiodophenylpyruvic acid (10 μmoles/l.) – – – –; 4-hydroxy-3,5-diiodophenylacetic acid (10 μmoles/l.) — · · · —; equimolar mixture of 4-hydroxy-3,5-diiodophenylpyruvic acid and 4-hydroxy-3,5-diiodophenylacetic acid (10 μmoles + 10 μmoles/l.) .....; 4-hydroxy-3,5-diiodobenzaldehyde (10 μmoles/l.) — · —

TABLE I

WAVE LENGTHS OF MAXIMAL ABSORPTION IN THE ULTRAVIOLET AND MOLAR EXTINCTION COEFFICIENTS

	λ_{max}	ϵ_{max}	
4-Hydroxy-3,5-diiodophenylpyruvic acid	291	57,000	
	241	47,600	
	220	39,000	(shoulder)
4-Hydroxy-3,5-diiodophenylacetic acid	297	3,400	
	288	3,700	
	240	12,800	(shoulder)
	220	40,000	
4-Hydroxy-3,5-diiodobenzaldehyde	345	28,700	
	263	15,400	
	212	21,200	

that 4-hydroxy-3,5-diiodobenzaldehyde, if it were formed in the course of the oxidative deamination of 3,5-diiodotyrosine could easily be detected spectrophotometrically. The spectrophotometrically determined yields of 4-hydroxy-3,5-diiodophenylpyruvic acid from 3,5-diiodotyrosine are quite erratic. They varied from about 8 to 30%. The ratio of 4-hydroxy-3,5-diiodophenylpyruvic acid to 4-hydroxy-3,5-diiodophenylacetic acid in the ether extract varied from 1:4 to 1:1. Attempts to improve the yield of the keto acid at the expense of the acetic acid analog of 3,5-diiodotyrosine, which presumably is formed from the keto acid by oxidative decarboxylation, have not as yet been successful.

Carbon-labeled 3,5-diiodotyrosine was prepared by treating L-tyrosine-U-C^{14} with iodine monochloride. The reaction product was not purified by paper chromatography but was used directly either for coupling with 4-hydroxy-3,5-diiodophenylpyruvic acid or for the enzymic synthesis of 4-hydroxy-3,5-diiodophenylpyruvic acid-U-C^{14}. In cases where a high specific activity was not required, it was mixed with unlabeled L-3,5-diiodotyrosine and the mixture was then recrystallized from 8 *M* acetic acid.

For the synthesis of thyroxine-I^{131} orthyroxine-C^{14} of relatively low specific activity the coupling reaction was carried out with about 1 to 2 grams of 3,5-diiodotyrosine and of 4-hydroxy-3,5 diiodophenylpyruvic acid. In this case the procedure used was essentially the one described by Meltzer and Stanaback[11] for the synthesis of unlabeled thyroxine. The synthesis of thyroxine of high specific activity required certain modifications of the procedure because the reaction had to be carried out with microgram quantities of labeled starting material. A large excess of the nonlabeled reaction partner was used in this case and the reaction mixture was much more diluted.

In the large scale experiments thyroxine was purified by recrystallization from a 0.5% sodium carbonate solution. A good criterion for the purity of the sodium salt of thyroxine thus obtained is, besides elemental analysis after drying to constant weight at 100°, the determination of the ratio of the absorbancies at 325 (λ_{max}) and 310 mμ (1.35). In small scale experiments thyroxine was purified by repeated paper chromatography on thoroughly washed paper. For the determination of the ratio of absorbancies, a blank eluate from a similar prewashed paper must be used in the reference cell of the spectrophotometer.

Yields of thyroxine in the coupling reaction were about 20% in large scale experiments and varied from 12 to 20% (based on the labeled starting material, either 3,5-diiodotyrosine or 4-hydroxy-3,5-diiodophenylpyruvic acid) when labeled thyroxine was prepared on a microgram scale.

Thyroxine-I^{131} in which the iodine atoms of either the phenolic or of the nonphenolic ring are labeled and thyroxine-C^{14} in which the carbon atoms of either the phenolic ring or of the nonphenolic ring and the aliphatic side chain are

labeled are the only forms of labeled thyroxine prepared so far in this laboratory. It is evident that a number of other labeled forms—*e.g.*, uniformly iodine- or carbon-labeled thyroxine, tritium-labeled thyroxine, doubly labeled thyroxine (with I^{131} and C^{14}) or thyroxine labeled exclusively in the side chain—can be prepared equally well by carrying out the same coupling reaction but using the appropriately labeled starting materials. This opens the way for the use of a variety of tailor-made tracers for the study of the metabolism of thyroxine.

Experimental[16]

4-Hydroxy-3,5-diiodobenzaldehyde-I^{131}.—This compound was prepared from 9.8 g. (0.08 mole) of *p*-hydroxybenzaldehyde according to the procedure described previously for the synthesis of the unlabeled aldehyde,[17] except that one drop (about 5 μc.) of a carrier-free solution of sodium iodide I^{131} [18] was added to the solution of iodine monochloride. The crude reaction product was recrystallized from aqueous ethanol; m.p. 199.5–200.5°; yield 20.5 g. (69%). The yield before recrystallization was not determined in this case but was found to be quantitative in similar preparations of the unlabeled 4-hydroxy-3,5-diiodobenzaldehyde.

4-(4-Acetoxy-3,5-diiodobenzal)-2-methyl-5-oxazolone-I^{131}.—A mixture of 11.2 g. (0.03 mole) of 4-hydroxy-3,5-diiodobenzaldehyde-I^{131}, 3.5 g. (0.03 mole) of acetylglycine, 2.5 g. (0.03 mole) of freshly fused sodium acetate, and 10.7 g. (0.105 mole) of acetic anhydride was heated on a steam bath for 2 hr. After cooling, water containing some cracked ice was added to the reaction mixture which was then broken up by means of a glass rod. The crystalline mass was filtered and washed with water, dried *in vacuo* over potassium hydroxide, then recrystallized from benzene–petroleum ether. The first crop of crystals (1.5 g.) was mixed with 7.5 g. of unlabeled 4-(4-acetoxy-3,5-diiodobenzal)-2-methyl-5-oxazolone,[19] prepared in the same manner from unlabeled 3,5-diiodobenzaldehyde. The mixture was recrystallized from benzene. The first crop of crystals obtained (3.9 g., m.p. 221–222°) was used for the next step.[20]

4-Hydroxy-3,5-diiodophenylpyruvic Acid-I^{131}. A. By Hydrolysis of 4-(4-Acetoxy-3,5-diiodobenzal)-2-methyl-5-oxazolone-I^{131}.—A suspension of 3.9 g. (7.85 mmoles) of 4-(4-acetoxy-3,5-diiodobenzal)-2-methyl-5-oxazolone-I^{131} in a mixture of 150 ml. of glacial acetic acid and 37 ml. of 6 *N* hydrochloric acid was heated on a steam bath for 4 hr. The reaction mixture was kept overnight at 4° and then filtered. The crystalline precipitate was washed with aqueous ethanol (1:1), then dried *in vacuo* over potassium hydroxide to remove traces of acetic acid. After one recrystallization from aqueous ethanol there was no further significant change in specific activity; yield 2.7 g. (63%); m.p. 241–243° dec.[21]

B. By Oxidative Deamination of 3,5-Diiodo-L-tyrosine-I^{131}.—To a solution of 2.0 mg. (4.3 μmoles) of L-3,5-diiodotyrosine dihydrate[22] in 5 ml. of 0.2 *M* phosphate buffer (*p*H 6.5) an appropriate amount of a solution of L-3,5-diiodotyrosine-I^{131},[23] 0.5 ml. of a 1% solution of rattlesnake venom[24] and 0.1 ml. of a solution of catalase[25] were added. The mixture was kept at room temperature for 2 hr. (in some runs 2.5 hr.) with occasional shaking. The enzymic reaction was stopped by the addition of a few drops of 4 *N* hydrochloric acid and the reaction mixture extracted four times with 10 ml. of peroxide-free ether. If the extraction is done carefully, the formation of emulsions can usually be avoided. Should nevertheless an emulsion form, it can be broken by the addition of water or a small amount of ethanol, or by centrifugation. The combined ether extracts were washed once with water, dried over sodium sulfate, and evaporated under reduced pressure. The residue can be used without further purification for the coupling with 3,5-diiodotyrosine.

3,5-Diiodo-L-tyrosine-U-C^{14} Dihydrate.—A solution of 2.0 mg. (12 μmoles) of iodine monochloride in 9 μl. of 20% hydrochloric acid was added to a solution of 0.9 mg. (5 μ moles) of L-tyrosine-U-C^{14} [26] in 0.1 ml. of 1 *N* hydrochloric acid. The mixture was kept at room temperature for 2 hr. with occasional shaking. Then the solution was decolorized by the addition of about two drops of a freshly prepared saturated aqueous solution of sulfur dioxide and evaporated under reduced pressure. The residue was used without further purification for the coupling with 4-hydroxy-3,5-diiodophenylpyruvic acid or for the preparation of 4-hydroxy-3,5-diiodophenylpyruvic acid-U-C^{14}. Chromatography of an aliquot showed that the conversion of tyrosine to 3,5-diiodotyrosine was virtually complete.

4-Hydroxy-3,5-diiodophenylpyruvic Acid-U-C^{14}.—The above mentioned residue of 3,5-diiodotyrosine-U-C^{14} (from 0.9 mg. of tyrosine-U-C^{14}) was dissolved in 5 ml. of 0.2 *M* phosphate buffer, *p*H 6.5. A 1% solution of rattlesnake venom[24] (0.5 ml.) and a solution of catalase[25] (0.1 ml.) were added, and the mixture was incubated and worked up as described above for the preparation of 4-hydroxy-3,5-diiodophenylpyruvic acid-I^{131}.

Sodium Salt of L-3-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodo-I^{131}-phenyl]alanine. (Sodium Salt of L-Thyroxine-3,5-I^{131}).—Pure L-3,5-diiodotyrosine-I^{131} dihydrate was prepared by repeated recrystallization of a mixture of L-3,5-diiodotyrosine and an appropriate amount of a solution of L-3,5-diiodotyrosine-I^{131} [23] from 8 *M* acetic acid until a constant specific activity (16,500 counts/min./mmole) of

(16) Elemental analyses were carried out by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y., and by Mr. McCann and his associates of the Analytical Service Laboratory of this Institute. Melting points were determined in capillary tubes and are not corrected. The radioactivity of I^{131}-containing samples was determined in a scintillation well counter, the radioactivity of C^{14}-containing samples in an automatic liquid scintillation counter. A solution of the sample—*e.g.*, an aliquot of an eluate of a paper chromatogram—was mixed with 2 ml. of "Hydroxide of Hyamine," Packard Instrument Co., La Grange, Ill., and with 13 ml. of toluene containing 0.457% of 2,5-diphenyloxazole, Pilot Chemicals, Watertown, Mass., and 0.01% of "POPOP," Packard Instrument Co. Filter Paper Whatman 3 MM was used for chromatography. This was thoroughly washed, first with 2 *N* acetic acid in 95% ethyl alcohol, then with the chromatographic solvent to be used, and finally with the eluent to be used. Strip counting of radioactive chromatograms was done with a Geiger-Mueller end window tube connected to a rate counter and pen recorder.

(17) T. Matsuura and H. J. Cahnmann, *J. Am. Chem. Soc.*, **81**, 871 (1959).

(18) Obtained from the Oak Ridge National Laboratory, Oak Ridge, Tenn.

(19) This material is now commercially available from Chemed, Inc., Odenton, Md.

(20) If the keto acid to be prepared from the azlactone is to be used not for specific activity measurements but only for preparative purposes, subsequent crops of crystals may also be used provided that the melting point is above 210°.

(21) The melting point varies considerably with the rate of heating. The highest melting point obtained in preparations of unlabeled 4-hydroxy-3,5-diiodophenylpyruvic acid was 243–245° dec. Considerably lower melting batches gave equally good elemental analyses and identical infrared spectra.

(22) Prepared by recrystallization of commercial L-3,5-diiodotyrosine from 8 *M* acetic acid.

(23) Obtained from Abbott Laboratories, Oak Ridge, Tenn.

(24) Snake venom from *Crotalus adamenteus* was obtained from the Ross Allen Reptile Institute, Silver Springs, Fla. Its freshly prepared solution was clarified by centrifugation.

(25) A suspension of crystalline beef liver catalase (minimum activity 150,000 units/ml.) was obtained from the Worthington Biochemical Corp., Freehold, N. J. The suspension was diluted with 4 vol. of water.

(26) The specific activity varied from run to run. The highest specific activity used was 10.1 Mc./mmole.

diiodotyrosine was reached. To a solution of 2.17 g. (4.63 mmoles) of this L-3,5-diiodotyrosine-I^{131} dihydrate in a mixture 50 of ml. of 0.2 *M* borate buffer (*p*H 7.6), 17.5 ml. of 1 *N* sodium hydroxide, and 17.5 ml. of a saturated aqueous solution of sodium sulfate enough 4 *N* hydrochloric acid was added to adjust the *p*H to 7.6. After the addition of 10 ml. of a 1% solution of *t*-butyl hydroperoxide[27] in 1-butanol, a solution of 2.59 g. (6.0 mmoles) of 4-hydroxy-3,5-diiodophenylpyruvic acid[19] in 50 ml. of 1-butanol was added dropwise to the vigorously stirred reaction mixture over a period of 1 hr. and 40 min. During the addition oxygen was bubbled through the reaction mixture and the *p*H kept constant at 7.6 by adding 2 *N* sodium hydroxide slowly to the reaction mixture by means of an immersed thin polyethylene tubing. The rate of addition was automatically controlled with a *p*H-stat.[28] Stirring and bubbling of oxygen were continued for another hour. After addition of 30 ml. of 10 *N* sodium hydroxide and 50 ml. of 1-butanol the reaction mixture was mixed by shaking and the butanol layer separated. The aqueous layer was then extracted twice with 100 ml. of 1-butanol. The combined butanol layers were washed with 150 ml. of 1 *N* sodium hydroxide, then with 100 ml. of water, and evaporated under reduced pressure in a bath of 30°. The residue was recrystallized from about 20 ml. of an 0.5% aqueous sodium carbonate solution. The crystals of the pentahydrate of the sodium salt of L-thyroxine thus obtained were washed with a small amount of ice-cold water and dried to constant weight *in vacuo* at 100°; yield 240 mg. of sodium salt of L-thyroxine-3,5-I^{131} (6.5%)[29]; $[\alpha]^{26}D$ −9.9° ± 2.3° (solvent: 1 vol. of 1 *N* sodium hydroxide + 2 vol. of ethanol, *c* 0.323); lit. values for unlabeled thyroxine in various alkaline solvents vary between −3.2° and −5.7°).

Anal. Calcd. for $C_{15}H_{10}I_4NNaO_4$: C, 22.55; H, 1.26; I, 63.54; N, 1.75. Found: C, 22.20; H, 1.62; I, 63.22; N, 1.93.

Ultraviolet Spectrum. Solvent: 0.1 *N* sodium hydroxide. Ratio of absorbancies at 325 (λ_{max}) and 310 mμ: 1.35 (ratio for pure thyroxine obtained by repeated recrystallization of a commercial sample of the sodium salt of thyroxine from a 0.5% aqueous sodium carbonate solution: 1.35; ratio of the commercial sample before recrystallization: 1.31).

Specific Activity.—16,600 counts/min./mmole of thyroxine; relative specific activity (specific activity of the 3,5-diiodotyrosine used as starting material = 100.0): 100.2 ± 1.9. Samples of about 30 mg. were used. 3,5-Diiodotyrosine was dissolved in 2 ml. of 2 *N* sodium hydroxide, the sodium salt of thyroxine in a mixture of 1 ml. of 2 *N* sodium hydroxide and 1 ml. of ethanol.

Microgram quantities of L-thyroxine-3,5-I^{131} were prepared according to the procedure described below for the synthesis of L-thyroxine-C^{14} (labeled in the nonphenolic ring and in the side chain).

Sodium Salt of L-3-[4-(4-Hydroxy-3,5-diiodo-I^{131}-phenoxy)-3,5-diiodophenyl]alanine. (Sodium Salt of L-Thyroxine-3′,5′-I^{131}.)—The procedure was essentially the same as the one described above for the large scale synthesis of the sodium salt of L-thyroxine-3,5-I^{131}, except that the keto acid (1.30 g., 3.0 mmoles) was iodine-labeled (specific activity: 34,910 counts/min./mmole) and the 3,5-diiodotyrosine dihydrate (1.17 g., 2.5 mmoles) unlabeled. The keto acid was added over a period of 70 min. and stirring and bubbling of oxygen were continued for another 90 min.; yield 160 mg. (8.0%);[29] $[\alpha]^{25}D$ −7.7° ± 0.2° (solvent: 1 vol. of 1 *N* sodium hydroxide +2 vol. of ethanol; *c* 1.85).

Anal. Calcd. for $C_{15}H_{10}I_4NNaO_4$: C, 22.55; H, 1.26; N, 1.75. Found: C, 22.83; H, 1.45; N, 1.58.

Ultraviolet Spectrum.—Solvent: 0.1 *N* sodium hydroxide. Ratio of absorbancies at 325 and 310 mμ: 1.35.

Specific Activity.—35,380 counts/min./mmole of thyroxine; relative specific activity (specific activity of the 4-hydroxy-3,5-diiodophenylpyruvic acid used as starting material = 100.0): 101.3 ± 0.9. Samples of about 20–40 mg. were used. Both the keto acid and the sodium salt of thyroxine were dissolved in 2 ml. of methanol.

Microgram quantities of L-thyroxine-3′,5′-I^{131} were prepared according to the procedure described below for the synthesis of L-thyroxine-C^{14} (labeled in the phenolic ring).

L-3[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl-1,2,3,4,5,6-C^{14}]alanine-1,2,3-C^{14}. [L-Thyroxine-C^{14} (All Carbon Atoms of the Nonphenolic Ring and of the Side Chain Uniformly Labeled).]—In a 10-ml. beaker about 1.5 mg. (3.2 μmoles) of 3,5-diiodotyrosine-U-C^{14} dihydrate (10.0 μc/μmole) was dissolved in a mixture of 0.5 ml. of 0.2 *M* borate buffer (*p*H 7.6), 0.18 ml. of 1 *N* sodium hydroxide, and 0.18 ml. of a saturated aqueous solution of sodium sulfate. The *p*H was adjusted to 7.6 with 4 *N* hydrochloric acid and two small drops of *t*-butyl hydroperoxide[27] were added. A solution of 8.2 mg. (19.0 μmoles) of 4-hydroxy-3,5-diiodophenylpyruvic acid[19] in 500 μl. of 1-butanol was then added over a period of 45 min. to the vigorously stirred reaction mixture by means of a very thin immersed polyethylene tubing. The slow addition was controlled by a motor-driven syringe.[30] Oxygen was bubbled through the reaction mixture throughout the addition of the keto acid. An electrode was immersed in the reaction mixture to permit *p*H-measurements, but no *p*H-stat was used. The *p*H was kept sufficiently constant by the occasional addition of a trace of 1 *N* sodium hydroxide. Stirring and bubbling of oxygen were continued for another 1.5 hr. After addition of 0.9 ml. of 4 *N* sodium hydroxide the reaction mixture was extracted four times with 200 μl. of 1-butanol. The combined extracts were placed on a washed filter paper strip and chromatographed in 1-butanol–dioxane–2 *N* ammonia (4:1:5). The thyroxine spot, revealed by visual observation in short wave ultraviolet light and by strip counting, was eluted with 12 ml. of 8 *N* ammonium hydroxide. Chromatography and elution were then repeated. The ultraviolet spectrum of the second eluate showed that it contained practically pure thyroxine. The yield based on the radioactivities of 3,5-diiodotyrosine and thyroxine was 12%.

L-3-[4-(4-Hydroxy-3,5-diiodophenoxy-1,2,3,4,5,6-C^{14})-3,5-diiodophenyl]alanine. [L-Thyroxine-C^{14} (All Carbon Atoms of the Phenolic Ring Uniformly Labeled).]—The ether extract of the reaction product obtained in the enzymic preparation of 4-hydroxy-3,5-diiodophenylpyruvic acid-U-C^{14} from about 5 μmoles of 3,5-diiodotyrosine-U-C^{14} (10.1 μc./μmole) was evaporated in a small beaker. To the residue was added at once a solution of 108.5 mg. (231 μmoles) of 3,5-diiodotyrosine dihydrate in a mixture of 2.5 ml. of 0.2 *M* borate buffer (*p*H 7.6), 0.9 ml. of 1 *N* sodium hydroxide, and 0.9 ml. of a saturated aqueous solution of sodium sulfate, adjusted to *p*H 7.6 with 4 *N* hydrochloric acid.[31] Two small drops of *t*-butyl hydroperoxide[27] were added and oxygen was bubbled vigorously through the stirred reaction mixture for 2 hr. The *p*H was kept constant in the same manner as described in the preceding experiment. After addition of 1.2 ml. of 4 *N* sodium hydroxide the reaction mixture was extracted four times with 200 μl. of

(27) Lucidol Division, Wallace and Tiernan, Buffalo, N. Y.

(28) Radiometer, Copenhagen, Denmark.

(29) This yield is not typical. A large second crop can be obtained from the mother liquor. The purpose of the experiment was to obtain not a good yield but a very pure sodium salt of thyroxine for specific activity measurements. In other runs (with unlabeled starting materials) practically pure thyroxine was obtained in about 20% yield.

(30) A synchronous motor turning at a speed of 2/3 r.p.m. was used to drive a syringe equipped with a homemade polyethylene adapter. The motor was made by the Cramer Controls Corp., Centerbrook, Conn. (Type 117). The syringe, an "Agla" micrometer syringe, and the syringe holder were obtained from Burroughs Wellcome and Co., London, England.

(31) In other runs the residue from the ether extract was first dissolved in 200 μl. of methanol.

1-butanol. The extracts were worked up as described in the preceding experiment. The yield of thyroxine based on the amount of 4-hydroxy-3,5-diiodophenylpyruvic acid-U-C^{14} present in the ether extract used as a starting material was 17%. The yields in a number of experiments varied from 12 to 20%.

The Synthesis of Dihydrotriacanthine[1]

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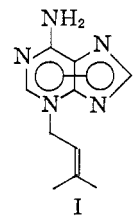
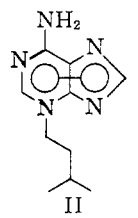
3-Isopentyladenine has been synthesized and shown to be identical with dihydrotriacanthine (II).

In a companion article[2] we have provided physical and chemical evidence that the structure of triacanthine, naturally occurring in the new leaves of *Gleditsia triacanthos* L., is 6-amino-3-(γ,γ-dimethylallyl)purine (I). Moreover, we have reported a synthesis, novel but equivocal, of I, along with syntheses of the isomeric 6-amino-7-(γ,γ-dimethylallyl)- and 6-amino-9-(γ,γ-dimethylallyl)purines,[3] all by the alkylation of adenine. An unequivocal synthesis of triacanthine or one of its derivatives was desirable for final structure proof, and we elected to synthesize dihydrotriacanthine (II) following a method which Elion[4] had established as unambiguous for 3-methyladenine.[5]

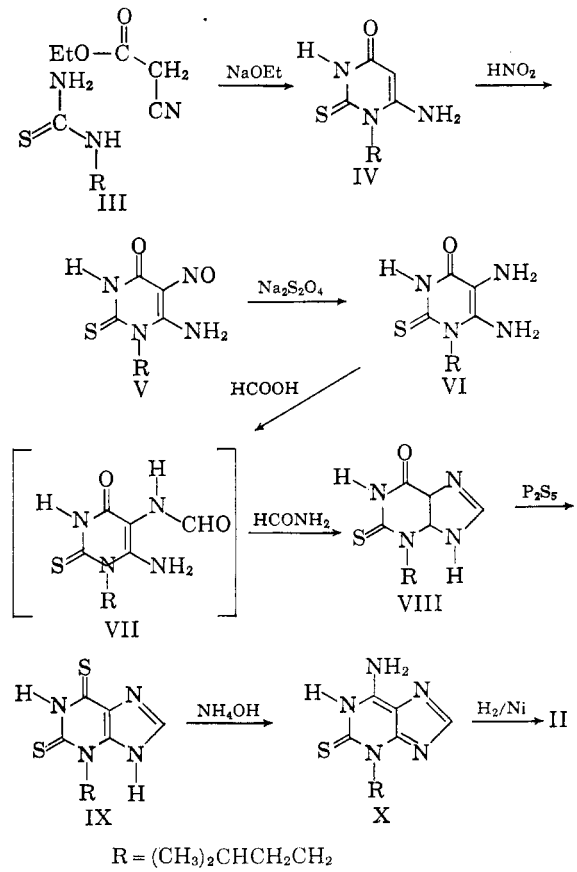


I



II

6-Amino-1-isopentyl-2-thiouracil (IV) was prepared according to the general method of Traube and Winter,[6] by the almost unidirectional condensation of isopentylthiourea (III) with ethyl cyanoacetate, followed by nitrosation to give 6-amino-1-isopentyl-5-nitroso-2-thiouracil (V, a possible equilibrium of tautomeric forms is recognized). Reduction of V with sodium hydrosulfite[7-9] yielded 5,6-diamino-1-isopentyl-2-thiouracil (VI). The imidazole ring was closed by formic acid and/or formamide, and



R = $(CH_3)_2CHCH_2CH_2$

the product, 3-isopentyl-2-thioxanthine (VIII), was converted to 3-isopentyl-2,6-dithioxanthine (IX) using phosphorus pentasulfide.[4] Replacement of the sulfur at C-6 by an amino group to give 3-isopentyl-2-mercaptoadenine (X) and hydrogenolysis of the C-2 sulfur linkage by means of Raney nickel completed the synthesis of 3-isopentyladenine (6-amino-3-isopentylpurine) (II). Yields at each stage averaged 80%. The only

(1) This investigation was supported in part by a research grant (USPHS-RG5829) from the National Institutes of Health, U. S. Public Health Service.

(2) N. J. Leonard and J. A. Deyrup, *J. Am. Chem. Soc.*, in press.

(3) 6-(γ,γ-Dimethylallyl)aminopurine has also been made in this laboratory (Dr. T. Fujii, research in progress).

(4) (a) G. B. Elion, "Ciba Foundation Symposium on the Chemistry and Biology of Purines," G. E. W. Wolstenholme and C. M. O'Connor, eds., Little, Brown and Co., Boston, 1957, p. 39; (b) G. B. Elion, private communication; article now in press, *J. Org. Chem.*

(5) Another method of synthesis of 3-methyladenine and of triacanthine (I) will be reported independently by R. Denayer, A. Cavé, and R. Goutarel, *Compt. rend.*, in press. The authors are grateful to Dr. Goutarel for providing a copy of this note prior to publication.

(6) W. Traube and F. Winter, *Arch. Pharm.*, **244**, 11 (1906).

(7) M. T. Bogert and D. Davidson, *J. Am. Chem. Soc.*, **55**, 1667 (1933).

(8) G. B. Elion, E. Burgi, and G. H. Hitchings, *ibid.*, **74**, 411 (1952).

(9) G. Levin, A. Kalmus, and F. Bergmann, *J. Org. Chem.*, **25**, 1752 (1960).