HCl then water and recrystallized from dimethylformamide to yield 10.8 g. (27.8%) of product, m.p. 323–325° dec.

1H-2,3,4,5-Tetrahydro-1,4-benzodiazepine (12).—3H-1,4-Benzodiazepine-2,5(1H,4H)-dione (113 g., 0.64 mole) was added to a slurry of lithium aluminum hydride (66.6 g., 1.8 moles) in 1500 ml. of tetrahydrofuran at a rate causing reflux. The mixture was refluxed with stirring for 6 hr. and left for 2 days. Excess lithium aluminum hydride was decomposed by cautious addition of 70 ml. of water, followed by 270 ml. of a saturated sodium potassium tartrate solution. Stirring was continued for 1 hr., the white slurry was filtered, the filter cake was washed well with tetrahydrofuran, and the filtrate and washings were concentrated *in vacuo* to a red oil which crystallized on slight cooling. The crude solid weighed 91.8 g. (97%), m.p. 89–94°. The dihydrochloride was prepared (alcoholic HCl) and recrystallized from methanol; m.p. 246.2–249°, lit.[3] m.p. 243–244°. The other tetrahydro-1,4-benzodiazepines were similarly prepared.

4-Methyl-1H-2,3,4,5-tetrahydro-1,4-benzodiazepine (13), free base, b.p. 70–73° (0.2 mm.), m.p. 40–43°.

4-Allyl-1H-2,3,4,5-tetrahydro-1,4-benzodiazepine (14), free base, b.p. 84–85° (0.13 mm.), $n^{26}D$ 1.5723.

7-Chloro-4-allyl-1H-2,3,4,5-tetrahydro-1,4-benzodiazepine was obtained in 100% crude yield, but we were unable to purify it either as the free base or hydrochloride. It was converted directly to the acetyl derivative.

4-Allyl-1-propionyl-1H-2,3,4,5-tetrahydro-1,4-benzodiazepine Hydrochloride (21).—Propionyl chloride (4.6 g., 0.05 mole) was added to 4-allyl-1H-2,3,4,5-tetrahydro-1,4-benzodiazepine (9.2 g., 0.05 mole) in 50 ml of $CHCl_3$. The solution became very hot and was left overnight. The $CHCl_3$ was evaporated yielding a white, crystalline mush which was treated with ether. The resulting white solid was recrystallized twice from ethanol. There was obtained 8.8 g. (62.9%) of product, m.p. 237.8–239.2° dec. The other acyl derivatives were similarly prepared.

Ethyl N-Allyl-N-(5-benzyloxy-2-nitrobenzoyl)glycinate.—Dicyclohexylcarbodiimide (45.3 g., 0.21 mole) in 300 ml. of tetrahydrofuran was added to 5-benzyloxy-2-nitrobenzoic acid (55.0 g., 0.201 mole)[15,16] and ethyl N-allylglycinate (30.0 g., 0.21 mole) in 700 ml. of tetrahydrofuran, and the mixture was left for 20 hr. The precipitate of dicyclohexylurea was collected and washed well with tetrahydrofuran. The filtrate and washings were concentrated *in vacuo* to an oil which weighed 90 g.

(15) E. Giovannini and P. Portmann, *Helv. Chim. Acta*, **31**, 1381 (1948).
(16) K. N. F. Shaw, A. Godmondson, and A. G. Morris, *Biochem. Preps.*, **9**, 12 (1962).

4-Allyl-7-benzyloxy-3H-1,4-benzodiazepine-2,5(1H,4H)-dione was prepared by iron–acetic acid reduction of the above glycinate.

4-Allyl-7-benzyloxy-2,3,4,5-tetrahydro-1H-1,4-benzodiazepine (18).—Reduction and hydrolysis were carried out as described for other tetrahydro compounds in this series.

4-Allyl-7-hydroxy-2,3,4,5-tetrahydro-1H-1,4-benzodiazepine.—Concentrated HCl (45 ml.) was added to **18** (13.1 g., 0.04 mole), and the resulting solution was immediately cooled in ice. After 1 hr., the solution was left at room temperature for 24 hr. The solution was diluted with 50 ml. of water and extracted three times with ether to remove benzyl chloride. The aqueous solution was evaporated *in vacuo* to a gum which was dissolved in 25 ml. of water and basified with 10% Na_2CO_3. Four extractions with CH_2Cl_2 and evaporation of the extracts gave 7.5 g. (82.5%) of an oil which crystallized after several days. The solid was triturated in a small amount of ethyl acetate to give a pink solid, m.p. 97–111°, which could not be recrystallized or converted to a crystalline salt.

4-Allyl-7-hydroxy-1-propionyl-2,3,4,5-tetrahydro-1H-1,4-benzodiazepine Hydrochloride (30).—A mixture of the above compound (8.5 g., 0.04 mole), 20 ml. of propionic anhydride, and 1 drop of concentrated H_2SO_4 was heated on the steam bath until a clear solution resulted. This was left overnight. Methanol (25 ml.) was added and the solution was left for 5 hr. Evaporation *in vacuo* gave the oily N,O-dipropionyl derivative. The oil was heated in an open beaker for 2 hr. on the steam bath with 5 ml. of 35% NaOH, 20 ml. of water, and enough ethanol to give a clear solution. The solution was carefully neutralized by dropwise addition of acetic acid and extracted three times with ethyl acetate. Evaporation of the solvent gave a dark oil which was dissolved in 200 ml. of ether. A small amount of amorphous brown solid was removed by filtration. Ethereal HCl was added to the filtrate, and the resulting slightly gummy solid was collected. The product was recrystallized twice from absolute methanol. There was obtained 5.4 g. (43.5%) of product, m.p. 245.0–246.0°.

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Thyromimetics. V. The Synthesis and Biological Screening of α-Methylthyroxine

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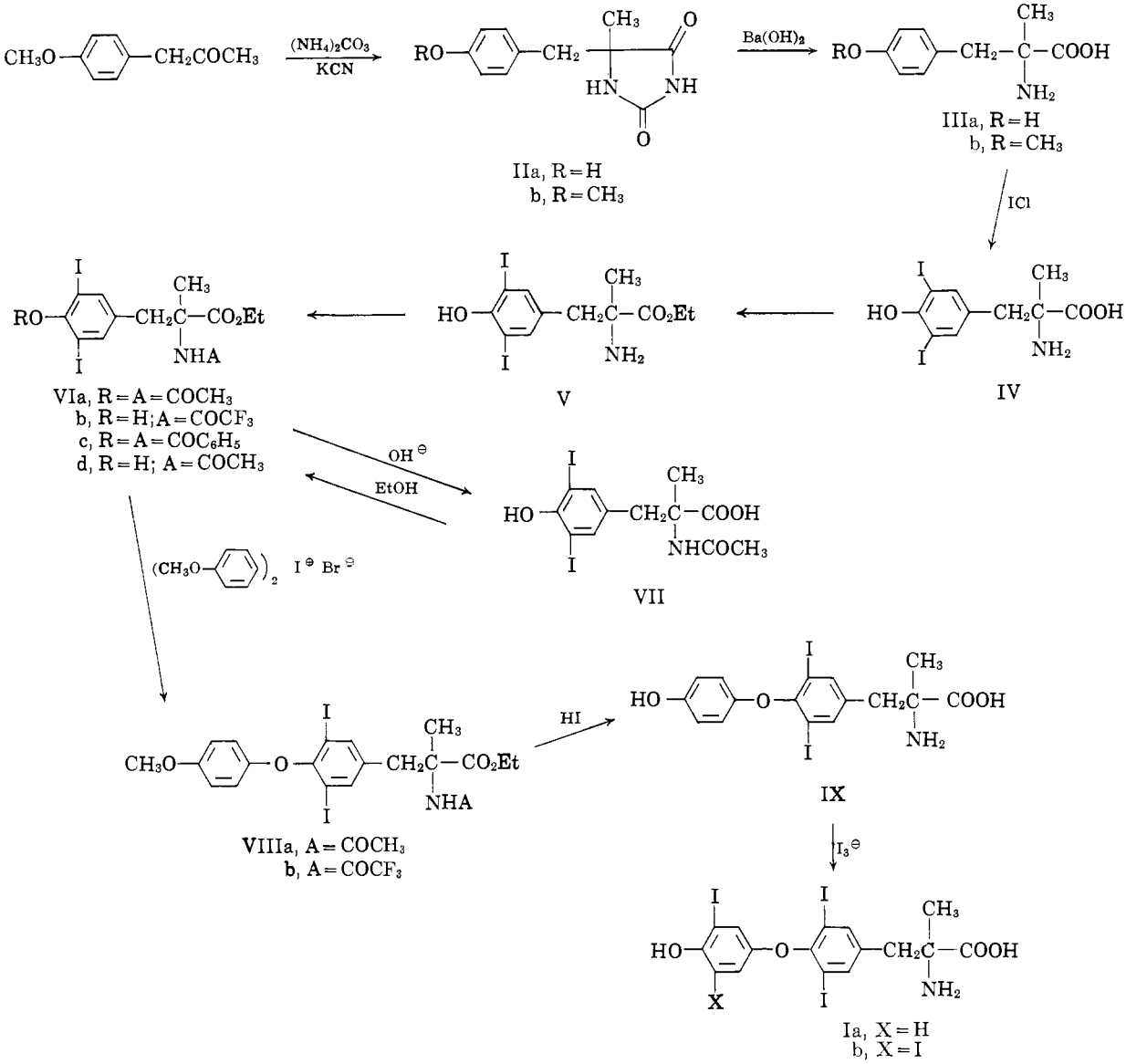
α-Methylthyroxine was prepared by a combination of established synthetic procedures and was tested for thyroxine-inhibitory and thyromimetic activities. It was found to have weak thyroxine-like activity in antigoitrogenic, cholesterol-lowering, and heart-weight assays and no activity as a thyroxine antagonist.

Despite the enormous amounts of knowledge accumulated with regard to the synthesis, metabolism, excretion, and biochemical transformations of amino acids, relatively little is known of α-methyl-α-amino acids. Sankoff and Sourkes[1] demonstrated that intraperitoneal administration of α-methyl-DL-tryptophan depressed the body weight of rats by reducing their food intake. Lin and co-workers[2] showed, *in vitro*, that α-methyl analogs of α-aminobutyric acid, methionine, and tyrosine had a reduced intestinal transport rate when compared to several nonmethylated amino acids. Christensen, *et al.*,[3] examined the tissue concentration of three α-methyl-α-amino acids and found them to be chiefly concentrated in the liver. Recent studies with α-methyl analogs of phenylalanines[4] have shown that these substances act as decarboxylase inhibitors and prevent decarboxylation of their nonmethylated counterparts. In effect, this causes these

(1) I. Sankoff and T. L. Sourkes, *Can. J. Biochem. Physiol.*, **40**, 739 (1962).
(2) T. H. Wilson, E. C. C. Lin, B. R. Landau, and C. R. Jorgensen, *Federation Proc.*, **19**, 870 (1960); E. C. C. Lin, H. Hagihira, and T. H. Wilson, *Am. J. Physiol.*, **202**, 919 (1962).
(3) H. N. Christensen, A. J. Aspen, and E. G. Rice, *J. Biol. Chem.*, **220**, 287 (1956).

CHART I



α-methyl compounds and their decarboxylation products to act as inhibitors of the products formed in the usual enzymatic decarboxylation reactions.

To further explore the actions of α-methyl-α-amino acids and to continue our studies in the thyromimetic area, we undertook the synthesis and biological study of α-methyl-3,5-diiodo-DL-thyronine, α-methyl-3,3',5-triiodo-DL-thyronine, and α-methyl-DL-thyroxine. In the light of current knowledge, we felt that these derivatives might be longer acting than the naturally occurring thyroidal hormones because of altered or decreased metabolism. Of greater interest was the possibility that these α-methyl thyrominetic analogs might act as thyroxine antagonists and be useful in hyperthyroidism.

The search for substances which antagonize the action of the thyroid hormones has been long and unsuccessful.[5] Current therapy for hyperthyroidism prescribes the use of thiourea-like compounds to inhibit thyroxine synthesis and/or destruction of thyroidal tissue by radioactive iodine or surgical excision.[5b,6] The availability of a chemical agent which could inhibit the peripheral action of the thyroid hormones and/or suppress the synthesis and release of these substances from the thyroid gland would provide an attractive adjuvant to the present therapy for hyperthyroidism.

Chart I indicates the intermediates and steps employed in the synthesis of α-methylthyroxine (Ib).

p-Methoxyphenylacetone was converted to α-methyltyrosine (IIIa) *via* the Strecker hydantoin synthesis as reported by Potts.[7a] This route proved more expedient than the one employed by Stein, Bronner, and Pfister[7b] which required N-acylation of α-methylphenylalanine

(4) W. P. Burkard, K. F. Gey, and A. Pletscher, *Life Sci.*, **3**, 27 (1964); D. Horwitz and A. Sjoerdsma, *ibid.*, **3**, 41 (1964); W. Lovenberg, J. Barchas, H. Weissbach, and S. Udenfriend, *Arch. Biochem. Biophys.*, **103**, 9 (1963); C. C. Porter, J. A. Totaro, and C. M. Leiby, *J. Pharmacol. Exptl. Therap.*, **134**, 139 (1961); J. A. Oates, L. Gillespie, S. Udenfriend, and A. Sjoerdsma, *Science*, **131**, 1890 (1960); H. Dengler and G. Reichel, *Arch. Exptl. Pathol. Pharmakol.*, **234**, 275 (1958); and T. L. Sourkes, *Arch. Biochem. Biophys.*, **51**, 444 (1954).

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(6) M. W. Hamolsky, *Rhode Island Med. J.*, **47**, 336 (1964).

(7) (a) K. T. Potts, *J. Chem. Soc.*, 1632 (1955); (b) G. A. Stein, H. A. Bronner, and K. Pfister, III, *J. Am. Chem. Soc.*, **77**, 700 (1955).

with subsequent nitration, reduction, diazotization, and hydrolysis. Thus, IIIa was obtained either by demethylating the methoxyhydantoin IIb prior to ring opening or by cleaving IIb to give the methyl ether of α-methyltyrosine (IIIb) which was subsequently demethylated to IIIa. For the most part, IIIa was prepared using the latter sequence. Iodination of IIIa with iodine in sodium iodide solution as carried out by Stein, *et al.*,[7b] was less satisfactory than iodination with iodine monochloride in hydrochloric acid.

An attempt to form N-acetyl-3,5-diiodo-α-methyltyrosine (VII) directly from 3,5-diiodo-α-methyltyrosine (IV) using conditions which are successful for the preparation of N-acetyl-3,5-diiodotyrosine from 3,5-diiodotyrosine[8] led to the recovery of IV. This experiment paralleled previous results from our laboratories.[9] Consequently, acetylation of the ethyl ester of α-methyl-3,5-diiodotyrosine (V) was studied. Refluxing V for a short time with acetic anhydride gave N,O-diacetylated material (VIa) which was similar to material formed using acetic anhydride and pyridine (identical infrared spectra in Nujol with carbonyl absorptions at 5.6, 5.78, and 6.02 μ). Basic hydrolysis of VIa gave a good yield of VII, readily converted to its ethyl ester VId. Similarly, N,O-dibenzoyl-3,5-diiodo-α-methyltyrosine ethyl ester (VIc) was synthesized from V with benzoyl chloride in the presence of aqueous bicarbonate. As was the case in previous studies[8] N-acylation could be accomplished without concomitant O-acylation by using trifluoroacetic anhydride. Thus, N-trifluoroacetyl-3,5-diiodo-α-methyltyrosine ethyl ester (VIb) was prepared in one step from V, whereas VId was obtained only by the sequence V → VIa → VII → VId.

Compounds VIb and d, when allowed to react with di(*p*-anisyl)iodonium bromide, gave N-acyl-3,5-diiodo-α-methylthyronine ethyl esters (VIIIa and b). Hydrolysis of these thyronines with a mixture of acetic and hydriodic acids yielded 3,5-diiodo-α-methylthyronine (IX). Controlled iodination of IX in aqueous ethylamine afforded a mixture of tri- and tetraiodo derivatives Ia and b. Subsequent recrystallization of the mixture produced Ia containing 10–15% of Ib. This was determined by paper chromatography using an isoamyl alcohol–*t*-amyl alcohol–6 *N* ammonia system.[9,10] Attempts to obtain purer Ia *via* its hydrochloride by recrystallization from dilute hydrochloric acid[11] were unsuccessful. In contrast, α-methylthyroxine (Ib) was prepared in pure form and in good yield by the addition of excess iodine to a solution of IX in aqueous ethylamine.

Experimental Section[12]

5-(*p*-Methoxybenzyl)-5-methylhydantoin (IIb).—A solution of 164 g. (1 mole) of *p*-methoxyphenylacetone, 720 g. (7.5 moles) of $(NH_4)_2CO_3$, 156 g. (2.4 moles) of KCN, 1700 ml. of ethanol, and 1700 ml. of water was stirred at 55–60° overnight. The product was filtered after concentration of the reaction mixture to ⅓ its original volume; yield 203 g. (87%), m.p. 176–178°. Recrystallized from water, it melted at 193–194° (lit.[7a] m.p. 198°).

Anal. Calcd. for $C_{12}H_{14}N_2O_3$: C, 61.53; H, 6.02; N, 11.96. Found: C, 61.69; H, 5.70; N, 12.02.

5-(*p*-Hydroxybenzyl)-5-methylhydantoin (IIa).—A mixture of 5 g. (0.02 mole) of IIb, 20 ml. of hydriodic acid, and 25 ml. of acetic acid was refluxed for 7.5 hr. The solution was cooled to produce a white solid which was filtered after dilution with water. After recrystallization from acetic acid (Darco) the pure product melted at 309–310° and weighed 3 g. (70%) (lit.[7a] m.p. 307° dec.).

Anal. Calcd. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 60.14; H, 5.31; N, 13.05.

***p*-Methoxy-α-methylphenylalanine (IIIb).**—A mixture of 148 g. (0.63 mole) of IIb, 835 g. of $Ba(OH)_2 \cdot 8H_2O$, and 4 l. of water was stirred under reflux for 65 hr. The mixture was cooled and adjusted carefully to pH 3 with dilute H_2SO_4. The resulting solid was filtered through Super Cel, and the filtrate was concentrated *in vacuo* to a syrup. The syrup was dissolved in hot acetone, and the solution was adjusted to pH 7–8 with diethylamine, then to pH 6 with acetic acid. The precipitated solid was cooled, filtered, triturated in acetone, and dried to give 68 g. (58%) of crude product, m.p. 279–281° dec., after recrystallization from methanol–acetone.

Anal. Calcd. for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N, 6.69. Found: C, 62.57; H, 7.11; N, 6.73.

***p*-Hydroxy-α-methylphenylalanine (α-Methyltyrosine) (IIIa).** A suspension of 61.5 g. (0.29 mole) of IIIb in 550 ml. of 47% HI was refluxed for 7 hr. The solvent was removed *in vacuo*, and the residue was evaporated twice with absolute ethanol to yield an orange syrup. The syrup was dissolved in 800 ml. of absolute ethanol and brought to pH 6.5 with diethylamine. The resulting solid was cooled, filtered, and digested with 1.5 l. of hot water. The suspension was cooled and filtered to produce 40 g. (70%) of white solid, m.p. 335° dec. (lit.[7b,13] m.p. 320° dec.). This could be recrystallized from hot water or by adding a hot 2 *N* sodium acetate solution to a solution of the amino acid in ethanol containing a few drops of concentrated HCl.

The hydroxyhydantoin IIa (1.7 g., 7 mmoles) and 9 g. of $Ba(OH)_2 \cdot 8H_2O$ in 45 ml. of water were stirred under reflux for 30 hr. The mixture was cooled and acidified to pH 3 with 2 *N* H_2SO_4. The precipitate was filtered, and the filtrate was concentrated to a white solid. The solid was azeotroped twice with fresh portions of absolute ethanol to give 0.95 g. (64%) of α-methyltyrosine, m.p. 314–315° dec. (lit.[7a] m.p. 330–332° dec.).

4-Hydroxy-3,5-diiodo-α-methylphenylalanine (3,5-Diiodo-α-methyltyrosine) (IV). Iodination of IIIa with a solution of iodine and sodium iodide[7b] produced IV in only 31% yield. To a stirred solution of 23.1 g. (0.12 mole) of IIIa in 130 ml. of water and 16 ml. of concentrated HCl at 60° was added slowly 40.3 g. (12.7 ml.) of iodine monochloride in 26 ml. of 20% HCl. The iodinating solution was added at such a rate that the temperature was maintained at 60–65°. Stirring at this temperature was continued for 2 hr. During this time the hydrochloride of IV precipitated; m.p. 245° dec. The mixture was diluted with excess aqueous $NaHSO_3$ and stirred for 30 min. The mixture was then cooled and filtered. The hydrochloride was washed with 10% $NaHSO_3$ and water, suspended in water, and converted to the free base by adjusting the pH to 5–6 with 5% Na_2CO_3 and 20% acetic acid. The resulting suspension, after cooling, was filtered and washed by suspension in water. The refiltered solid was dried overnight in a steam chest at 55–65° and then 4 hr. in a vacuum desiccator over P_2O_5 to give 44.7 g. (85%) of product, m.p. 229° dec. (lit.[7b] 215° dec.).

4-Hydroxy-3,5-diiodo-α-methylphenylalanine Ethyl Ester (V).—A suspension of 44.7 g. (0.1 mole) of IV in 1500 ml. of absolute ethanol was saturated at room temperature with HCl, whereupon the solid dissolved. The solution was cooled and resaturated with HCl. The alcohol was distilled at reduced pressure. The residue was dissolved in water, treated with Darco, and filtered, and the filtrate was brought to pH 5–6 with 5% Na_2CO_3. The precipitated product was filtered, washed with water, dried, and recrystallized from 1-butanol to give 20.1 g. of ester, m.p. 168–169° dec. The butanolic filtrate was concentrated to give a mixture of acid and ester. The mixture was suspended in dry ethanol and saturated with HCl. The resulting solution was refluxed for 3 hr., and the ester was

(8) J. H. Barnes, E. T. Borrows, J. Elks, B. A. Heins, and A. G. Long, *J. Chem. Soc.*, 2824 (1950).

(9) B. Blank, *J. Pharm. Sci.*, **53**, 1331 (1964); paper IV in this series.

(10) I. B. Eisdorfer and W. C. Ellenbogen, Abstracts of the 131st National Meeting of the American Chemical Society, Miami, Fla., April 1957, p. 42B.

(11) J. Gross and R. Pitt-Rivers, *Biochem. J.*, **53**, 645 (1953).

(12) All compounds are the racemic forms. Melting points were taken in a Thomas–Hoover capillary apparatus and are corrected.

(13) K. Pfister, III, U. S. Patent 2,868,818 (Jan. 13, 1959).

isolated and purified as described above to give an additional 8.7 g. of product, m.p. 167–168° dec., total yield 61%.

Anal. Calcd. for $C_{12}H_{15}I_2NO_3$: C, 30.34; H, 3.18; I, 53.43. Found: C, 30.13; H, 3.08; I, 53.26.

N,O-Diacetyl-4-hydroxy-3,5-diiodo-α-methylphenylalanine Ethyl Ester (VIa).—A suspension of 4.8 g. (0.01 mole) of V in 25 ml. of pyridine was stirred at room temperature while 5 ml. of acetic anhydride was added slowly. The resulting clear solution was stirred an additional 3 hr. and left overnight at room temperature. The solution was poured into several volumes of ice–water, and this mixture was stirred several hours. The formed crystals were filtered, washed with water, and dried; m.p. 140°. After recrystallization from aqueous ethanol the crystals weighed 4.5 g. (87%), m.p. 143–145°.

Anal. Calcd. for $C_{16}H_{19}I_2NO_5$: C, 34.37; H, 3.43; I, 45.40. Found: C, 34.83; H, 3.42; I, 45.30.

N,O-Dibenzoyl-4-hydroxy-3,5-diiodo-α-methylphenylalanine Ethyl Ester (VIc).—To a stirred mixture of 10.8 g. (0.023 mole) of V, 8 g. of $NaHCO_3$, 100 ml. of 5% aqueous $NaHCO_3$, and 350 ml. of tetrahydrofuran at 0–5° was added 10 ml. of benzoyl chloride. The mixture was stirred 15 min. with cooling and 2 hr. at room temperature, diluted with 3 vol. of ice–water, and extracted with $CHCl_3$. The $CHCl_3$ solution was washed twice with 5% $NaHCO_3$ and twice with water, dried (Na_2SO_4), and evaporated. The solid residue was suspended in ether and filtered to give 13.7 g. of crude product, m.p. 180°. After one recrystallization from aqueous ethanol, it weighed 13.1 g. (98%) and melted at 184–188°. For analysis a sample was recrystallized twice from acetonitrile; m.p. 187–189°.

Anal. Calcd. for $C_{26}H_{23}I_2NO_5$: C, 45.70; H, 3.39; I, 37.15. Found: C, 45.85; H, 3.20; I, 37.21.

N-Trifluoroacetyl-4-hydroxy-3,5-diiodo-α-methylphenylalanine Ethyl Ester (VIb).—In a separatory funnel was placed a suspension of 8.3 g. (0.02 mole) of V in 80 ml. of ethyl acetate and 80 ml. of $CHCl_3$. To this was added in four portions, 8 ml. of trifluoroacetic anhydride in 50 ml. of ethyl acetate. The funnel was shaken thoroughly with caution after each addition. At the time of the last addition the solid was completely dissolved. The solution was washed with water, aqueous $NaHCO_3$, and again with water and dried (Na_2SO_4). The solvents were removed, and the residue was crystallized and recrystallized from methanol–water; yield 9.1 g. (90%), m.p. 94–96°.

Anal. Calcd. for $C_{14}H_{14}F_3I_2NO_4 \cdot 0.5H_2O$: C, 28.98; H, 2.61; I, 43.76. Found: C, 29.37; H, 2.99; I, 43.49.

N-Acetyl-4-hydroxy-3,5-diiodo-α-methylphenylalanine (VII).—Material from the two different preparations of VIa (6.2 g., 0.01 mole) was dissolved in 65 ml. of ethanol, 10 ml. of 40% NaOH was added, and the solution was stirred 1.5 hr. at room temperature. After diluting with water, cooling, and acidifying with dilute HCl, the solid was filtered, washed with water, and dried to yield 5.4 g. of VII, m.p. 208° dec. Recrystallized from aqueous ethanol, the crystals weighed 4.5 g. (84%) and melted at 211–213° dec.

Anal. Calcd. for $C_{12}H_{13}I_2NO_4$: C, 29.47; H, 2.68; I, 51.90. Found: C, 29.65; H, 2.46; I, 51.70.

N-Acetyl-4-hydroxy-3,5-diiodo-α-methylphenylalanine Ethyl Ester (VId).—A mixture of 4.5 g. (9 mmoles) of VII, 10 ml. of dry ethanol, 0.1 g. of *p*-toluenesulfonic acid monohydrate, and 100 ml. of $CHCl_3$ was stirred under reflux overnight with azeotropic distillation of water. An additional 5 ml. of ethanol was added after heating for 3 hr. and again after 4 hr. The clear, pale yellow solution was cooled and extracted several times with 5% Na_2CO_3 and three times with water. The combined aqueous extracts were acidified to pH 1–2 with 3 *N* HCl. The mixture of gum and solid was cooled, scratched, filtered, washed with water, and dried to give 3.9 g. (85%) of VId, m.p. 140° with preliminary softening and sintering. Recrystallized from ethanol–water, it had m.p. 141–143°.

Anal. Calcd. for $C_{14}H_{17}I_2NO_4$: C, 32.52; H, 3.32; I, 49.08. Found: C, 32.32; H, 3.26; I, 48.53.

N-Trifluoroacetyl-3-[3,5-diiodo-4-(4-methoxyphenoxy)phenyl]-2-methylalanine Ethyl Ester (VIIIb).—A mixture of 12.6 g. (0.03 mole) of di(*p*-anisyl)iodonium bromide,[14] 8.7 g. (0.015 mole) of VIb, 2 g. of copper powder, and 2 ml. of triethylamine in 200 ml. of methanol was stirred 24 hr. at room temperature. The solution was filtered, and the filtrate was evaporated. The residue was dissolved in benzene, and the benzene solution was washed twice, successively, with dilute HCl, water, 5% $NaHCO_3$, and water. The dried (Na_2SO_4) benzene solution was evaporated, and the residue was triturated with petroleum ether (b.p. 40–60°) to precipitate an oil. The solvent was decanted, and the oil was dissolved in methanol. This solution was diluted with water until the oil began to separate, then was heated to give a clear solution. Cooling and scratching produced crystals. Evaporation of the decanted petroleum ether and treatment of this residue with methanol and water as described gave more solid. The combined solids, after a recrystallization from aqueous methanol, weighed 7.1 g. and melted at 105–115° with sintering. A second crop weighed 1.1 g.; total yield 8.2 g. (81%). Further recrystallization from aqueous methanol produced an analytical sample melting at 125–126°.

Anal. Calcd. for $C_{21}H_{20}F_3I_2NO_5$: C, 37.24; H, 2.98; I, 37.48. Found: C, 37.29; H, 2.95; I, 37.68.

N-Acetyl-3-[3,5-diiodo-4(4-methoxphenoxy)phenyl]-2-methylalanine Ethyl Ester (VIIIa).—A mixture of 3.7 g. (7.2 mmoles) of VId, 6 g. (0.0143 mole) of di(*p*-anisyl)iodonium bromide,[14] 1 g. of copper powder, and 1 ml. of triethylamine in 100 ml. of methanol was stirred for 24 hr. The solution was filtered, and the filtrate was evaporated to give 9 g. of a brown oil. The oil was triturated with petroleum ether, and the solid formed was filtered. Concentration of the filtrate gave a second crop of solid. The combined solids were recrystallized from aqueous ethanol (Darco) and twice from aqueous methanol. The product melted from 60–100°. After drying in a steam chest, the first-crop material melted at 119–121° and weighed 1.9 g. The recrystallization filtrates were concentrated and cooled to give an additional 1.5 g. of solid; total yield 3.4 g. (76%). After drying at 40° *in vacuo* over P_2O_5, the first-crop material melted at 128–130°.

Anal. Calcd. for $C_{21}H_{23}I_2NO_5$: C, 40.47; H, 3.72; I, 40.73. Found: C, 40.88; H, 3.84; I, 40.47.

3-[4-(4-Hydroxyphenoxy)-3,5-diiodophenyl]-2-methylalanine (3,5-Diiodo-α-methylthyronine) (IX).—A solution of 3.4 g. (5.5 mmoles) of crude VIIIa in 35 ml. of HI and 40 ml. of acetic acid was refluxed for 4 hr. The solution was cooled, diluted with ice–water, and neutralized to pH 4–5 with 10% NaOH. The precipitate was cooled, filtered, washed with water, and purified by precipitation from a hot ethanolic solution containing a few drops of concentrated HCl by the addition of hot 2 *N* sodium acetate to pH 4–6; yield 1.3 g. (43%), m.p. 282° dec.

In a similar fashion 8.2 g. (0.01 mole) of VIIIb was converted to 3.8 g. (59%) of IX, m.p. 286° dec., after drying *in vacuo* at 100°.

Anal. Calcd. for $C_{16}H_{15}I_2NO_4 \cdot 0.5H_2O$: C, 35.06; H, 2.94; I, 46.31. Found: C, 35.27; H, 3.11; I, 45.92.

3-[4-(4-Hydroxy-3-iodophenoxy)-3,5-diiodophenyl]-2-methylalanine (3,3',5-Triiodo-α-methylthyronine) (Ia).—A solution of 0.6 g. (2.35 mmoles) of iodine in 10 ml. of 10% KI was added slowly to a stirred solution of 1.3 g. (2.4 mmoles) of IX in 15 ml. of 33% aqueous ethylamine. The solution was stirred 1.5 hr., then 25 ml. of saturated NaCl was added along with 1 g. of solid NaCl. The mixture was stirred 1 hr., and the solids were allowed to settle overnight.[15] The solids were filtered and the filtrate was acidified with 3 *N* HCl to give additional solid. The combined solids were purified by two isoelectric precipitations to give 1 g. (63%) of solid, m.p. 252–254° dec. Two further purifications, the first by precipitation from ethanol–10% NaOH with acetic acid and the second from ethanol–HCl as described above gave material melting at 264–266° dec. Examination of this material by paper chromatography showed it to be a mixture containing a trace of IX and 10–15% of the thyroxine analog. The R_f values in an isoamyl alcohol–*t*-amyl alcohol–6 *N* ammonia system (1:1:2) after spraying with ninhydrin and Emerson's reagent[9,10] were 0.26 for the tetraiodo component, 0.35 for the triiodo component, and 0.47 for the diiodo component.

3-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]-2-methylalanine (α-Methylthyroxine) (Ib).—To a solution of 1.2 g. (2 mmoles) of IX in 25 ml. of 33% aqueous ethylamine was added slowly with stirring over 1 hr., 1.1 g. (4.2 mmoles) of iodine in 25 ml. of 10% aqueous KI. After the addition, the solution was cooled and stirred, with cooling, for 2 hr. It was poured into ice–water and acidified to pH 1–2 with concentrated HCl. The solid was filtered after the addition of a little $NaHSO_3$, washed with water, and purified by two isoelectric precipitations from acidic ethanol. This yielded 950 mg. (60%) of product,

(14) J. T. Plati, U. S. Patent 2,839,583 (June 17, 1958).

(15) Method of H. Nahm and W. Siedel, *Chem. Ber.*, **96**, 1 (1963).

m.p. 270° dec. Paper chromatography of this material in the system described above showed two components, one with R_f 0.26 (major) and a second with R_f 0.36 (trace, small amount). Consequently the product was reiodinated with excess iodine and purified as described above to give a product which produced a single spot on paper chromatograms, R_f 0.27. This product melted at 257–259° dec. when dried *in vacuo* at 100°.

Anal. Calcd. for $C_{16}H_{13}I_4NO_4$: C, 24.30; H, 1.66; I, 64.18. Found: C, 24.63; H, 1.78; I, 64.11.

Biological Screening.—Antigoitrogenic[16,17] and heart-weight[18] assays were determined in rats as described previously with the test compound (Ib) administered subcutaneously at doses of 69, 138, 276, and 552 μg. These results are shown in Table I.

Cholesterol-lowering activity was determined in mice[16] fed a diet containing 1% ethyl linoleate for 4 days prior to subcutaneous treatment with the test compounds which was continued for 11 days. These results are presented in Table II.

thyromimetic activities. Since certain of these previous modifications of T_3 have been rather drastic and yet have yielded compounds with interesting activities, the activity of Ib was rather disappointing.

The current studies show Ib to be weakly active as an antigoitrogenic or cardiac-stimulatory agent, being 0.02 as active as DL-T_3 (or about 0.1 as active as DL-T_4) in either assay. As a thyroxine antagonist Ib was inactive at a molar ratio of 40:1 and caused no change in the weights of a variety of organs after chronic administration. The results from the cholesterol-lowering assay, unfortunately, do not permit any definite conclusions (no dose response, see Table II). All that can be said is that Ib appears to be a weak hypocho-

TABLE I

ANTIGOITROGENIC AND HEART-WEIGHT RESULTS[a]

Treatment	Dose, μg. 2 ml./kg.	Mean thyroid wt. (mg. 100 g.) ± S.D.	Mean heart wt. (mg. 100 g.) ± S.D.	Mean body wt. change (g. rat) ± S.D.
None (0.9% alkaline saline, untreated controls)	...	5.9 ± 0.7	305 ± 22	103.4 ± 19.3
Thiouracil (0.1% + 0.9% alkaline saline)	...	12.8 ± 1.4	287 ± 21	98.6 ± 10.7
Thiouracil (0.1%) + DL-T_3	2.5	11.4 ± 2.2	312 ± 20[c]	99.7 ± 13.4
Thiouracil (0.1%) + DL-T_3	3.5	11.0 ± 1.5[b]	308 ± 27	87.3 ± 12.3[c]
Thiouracil (0.1%) + DL-T_3	4.9	9.4 ± 2.0[b]	310 ± 19[c]	101 ± 12.4
Thiouracil (0.1%) + DL-T_3	6.9	6.6 ± 1.2[b]	313 ± 23[c]	96.6 ± 18.1
Thiouracil (0.1%) + Ib	69.0	10.7 ± 2.1[c]	297 ± 16	106.2 ± 23.3
Thiouracil (0.1%) + Ib	138.0	9.8 ± 1.6[b]	308 ± 21[c]	105 ± 14.1
Thiouracil (0.1%) + Ib	276.0	9.9 ± 2.2[b]	311 ± 18[c]	94.3 ± 15.4
Thiouracil (0.1%) + Ib	552.0	8.6 ± 2.1[b]	324 ± 21[b]	91.1 ± 20.0

[a] Results are for groups of 10 rats. [b] When compared with the thiouracil-treated animals, significant at $P = 0.05$. [c] When compared with the thiouracil-treated animals, significant at $P = 0.01$.

TABLE II

PLASMA CHOLESTEROL CHANGES AND BODY WEIGHT GAIN

Treatment	Dose, μg./kg. b.i.d.	Plasma total cholesterol, mg. %	Body wt. gain, g.
Control (0.9% saline)		168.3	+1.3
Ib	8.3	130.8	+2.3
Ib	16.6	153.9	+1.7
Ib	33.2	135.0	+2.9
Ib	66.4	143.7	+1.4

The activity of Ib as a thyroxine antagonist was studied in rats by determining the effect of Ib on the reversal of thiouracil-induced goiters by thyroxine (DL-T_4).[5c,d] The compounds were administered subcutaneously simultaneously at different sites, Ib at 4000 μg. and DL-T_4 at 98 μg. (molar ratio of 40:1). Ib had no effect in this experiment.

The effect of chronic administration of Ib was examined by giving subcutaneous doses of Ib (4 mg. and 8 mg.) once daily for 20 days. The animals were then sacrificed and organs were removed and weighed. No significant changes in heart, thyroid, pituitary, adrenal, or body weights were noted.

Discussion

Chemical manipulation of the structures of triiodothyronine (T_3) and thyroxine in our laboratories[18,19] has led to compounds with enhanced thyromimetic activity as well as to compounds with a separation of lesteremic agent. Thus our hope of preparing a thyroid antagonist or a thyromimetic substance with an enhanced duration of activity was not realized. However, it can be noted that by simply placing a methyl group in the α-position of the alanine side chain of T_4, a 10-fold decrease in its thyromimetic activity has been effected.

In our earlier studies it was shown that (1) replacement of the 3′-iodine of T_3 with alkyl groups of comparable size produced compounds with activity similar to or greater than that of T_3,[18,19a] (2) replacement of the alanine side chain of T_3 with diethylaminoethyl esters of acetic and propionic acids yielded compounds which had a separation of activities,[19b] and (3) the formation of 4′-methyl ethers together with a combination of the above modifications caused a significant enhancement in the separation of cholesterol lowering from calorigenic and cardiac activities.[19c]

It is also known that triiodothyroacetic (T_3A) and -propionic (T_3P) acids have less thyromimetic activity than T_3 in various assay procedures. For example in antigoitrogenic assays T_3A has about 0.1[5a,18] times the activity and T_3P about 0.05[17] times the activity of T_3; in their effects on heart-weight increase, these analogs were, respectively, about 0.1 and 0.02 times as active as T_3.[19c] Such results lead to the conclusion that the alanine side chain of the thyroid hormones plays a role in eliciting certain of the responses of the thyroid hormones. The importance of the position of the side

(16) C. M. Greenberg, C. A. Bocher, J. F. Kerwin, S. M. Greenberg, and T. H. Lin, *Am. J. Physiol.*, **201**, 732 (1961).

(17) C. M. Greenberg, L. F. Mansor, C. A. Bocher, H. L. Saunders, and J. F. Kerwin, *Endocrinology*, **70**, 365 (1962).

(18) C. M. Greenberg, B. Blank, F. R. Pfeiffer, and J. F. Pauls, *Am. J. Physiol.*, **205**, 821 (1963).

(19) (a) B. Blank, F. R. Pfeiffer, C. M. Greenberg, and J. F. Kerwin, *J. Med. Chem.*, **6**, 554 (1963); (b) B. Blank, F. R. Pfeiffer, C. M. Greenberg and J. F. Kerwin, *ibid.*, **6**, 560 (1963); (c) B. Blank, C. M. Greenberg, and J. F. Kerwin, *ibid.*, **7**, 53 (1964).

chain relative to the diphenyl ether bond has been examined recently by Jorgensen and Reid[20a] and Shiba, Höfer, and Cahnmann.[20b] Jorgensen and Reid found that compounds in which the side chain of T_3 was placed *ortho* or *meta* to the diphenyl ether bond were inactive in the prevention of thiouracil-induced goiters.

The reduction in thyromimetic activities caused by the introduction of an α-methyl group into the side chain of T_4 is further evidence for the importance of the unmodified alanine moiety for maximum biological response. The reduction in activity seen with Ib may be a consequence of impaired binding to transport proteins or to sites of biological action which in turn may lead to more rapid metabolism (degradation) and/or excretion. Another less attractive possibility, since currently there are few data to substantiate such an event,[21] is that α-methyl-T_4 is incapable of being converted to an "active form" of the hormone.

Nonetheless, whatever the specific reason(s), it seems apparent from this and previous studies that the position and nature of the side chain in thyroxine-like substances help to determine the character and potency of the biological response evoked.[22]

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(20) (a) E. C. Jorgensen and J. A. W. Reid, *J. Med. Chem.*, **7**, 701 (1964); (b) T. Shiba, A. Höfer, and H. J. Cahnmann, *J. Org. Chem.*, **29**, 3171 (1964).

(21) E. C. Wolff and J. Wolff in "The Thyroid Gland," Vol. I, R. Pitt-Rivers and W. R. Trotter, Ed., Butterworth and Co. Ltd., London, 1964, p. 239.

(22) After this manuscript had been prepared it was noted that compounds Ia, Ib, and IX had been disclosed in the Eire Patent 362/65 (May 5, 1965).

Fusidic Acid Derivatives. I. Relationship between Structure and Antibacterial Activity

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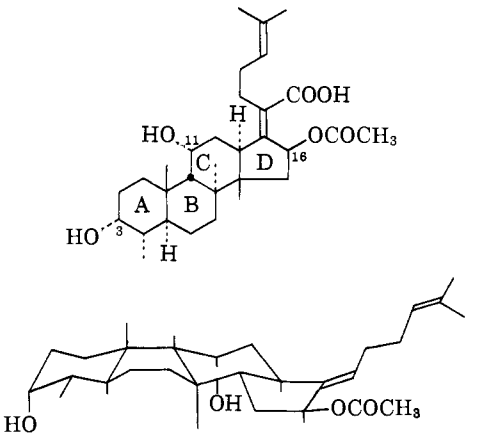
A series of derivatives of the antibiotic fusidic acid has been prepared and their antibacterial activities have been determined. The relationship between structure and antibacterial activity of this group of compounds is discussed.

It has recently been shown[1-3] that fusidic acid (**1a**), an antibiotic formed by *Fusidium coccineum*,[4] has the structure and stereochemistry depicted below. One of the most remarkable features of this structure is the unusual stereochemistry of the cyclopentanoperhydrophenanthrene ring system which differs fundamentally from that of other tetracyclic triterpenes and sterols. In contrast to the usual *trans,anti,trans* arrangement of rings A, B, and C, we find in fusidic acid a *trans,syn,-trans* arrangement of these rings which forces ring B into the boat conformation as illustrated in the perspective formula.

The elucidation of this unusual structure stimulated our inherent interest in studying the influence of structural modifications on the antibacterial activity of this antibiotic which has found a well-established use in the treatment of staphylococcal infections in man.[5,6] In this paper the effect of a number of variations concerning both the two carbon–carbon double bonds and the functional groups at C-3, C-11, C-16, and C-21 on the inhibitory activity against a number of bacteria will be discussed. Some of the compounds studied have previously been described in connection with the structural work, whereas others are new.



1a

Chemistry.—The two carbon–carbon double bonds in fusidic acid (**1a**) can be hydrogenated stepwise.[1a,4] Hydrogenation over a palladium catalyst yields 24,25-dihydrofusidic acid (**2a**) while reduction of the latter over a platinum catalyst in acetic acid (Chart I) affords a tetrahydrofusidic acid (**3a**) in which the side chain is α-orientated. A tetrahydrofusidic acid with a β-orientated side chain has not been obtained so far.

When a solution of fusidic acid (**1a**) in ethanol or dioxane was irradiated with ultraviolet light from a medium-pressure mercury lamp (Hanovia, Type 509),

(1) (a) W. O. Godtfredsen and S. Vangedal, *Tetrahedron*, **18**, 1029 (1962); (b) D. Arigoni, W. von Daehne, W. O. Godtfredsen, A. Marquet, and A. Melera, *Experientia*, **19**, 521 (1963); (c) D. Arigoni, W. von Daehne, W. O. Godtfredsen, A. Melera, and S. Vangedal, *ibid.*, **20**, 344 (1964); (d) W. O. Godtfredsen, W. von Daehne, S. Vangedal, A. Marquet, D. Arigoni, and A. Melera, *Tetrahedron*, in press.

(2) A. Cooper, *ibid.*, in press.

(3) R. Bucourt, M. Legrand, M. Vignau, J. Tessier, and V. Delaroff, *Compt. rend.*, **257**, 2679 (1963).

(4) W. O. Godtfredsen, S. Jahnsen, H. Lorck, K. Roholt, and L. Tybring, *Nature*, **193**, 987 (1962).

(5) M. Barber and L. P. Garrod, "Antibiotic and Chemotherapy," E. and S. Livingstone Ltd., Edinburgh, 1963, pp. 85–87.

(6) G. T. Stewart, *Pharmacotherapia*, **2**, 136 (1964).