decomposed to the aldehyde (m.p. 94–95°) in 50% acetic acid. A mixture of 0.7 g. of the aldehyde, 0.7 g. of fused sodium acetate, 0.27 g. of hydantoin, 5 ml. of acetic acid, and 3 drops of acetic anhydride was refluxed 2 hr. The mixture was charcoaled, cooled, treated with 2.0 ml. of water, and refrigerated. The precipitated solid was separated washed with water, and oven-dried. The straw yellow product (0.83 g., 90% yield) was recrystallized from absolute ethanol, m.p. 264–265°.

Anal. Calcd. for $C_{17}H_{13}N_3O_5$: C, 60.17; H, 3.86; N, 12.39. Found: C, 59.80; H, 4.08; N, 12.15.

Paper Chromatography.—The thyronines were chromatographed in *t*-amyl alcohol saturated with 2 *N* NH_4OH.[26] All samples except III gave one spot. R_f values observed for the substituted thyronines were: 3'-methyl-3,5-diiodo-, 0.63; 3'-*t*-butyl-3',5'-diiodo-, 0.77; 3-methyl-, 0.55; 3-methyl-3',5'-diiodo-, 0.35 (compared to 3,5-diiodothyronine, 0.58^{25}). A second minor spot in the sample of III was obtained at R_f 0.48. This may be due to the presence of a small amount of 3-methyl-3'-iodothyronine. The naphthalene derivatives were similarly chromatographed and spotted with ninhydrin or 1-nitroso-2-naphthol. R_f values obtained were: 4-(5-hydroxy-1-naphthyloxy)-3,5-diiodo-L-phenylalanine, 0.52; the acetic acid analog, 0.62; and the propionic acid analog, 0.56.

(26) G. I. Gleason, *J. Biol. Chem.*, **213**, 837 (1955).

Acknowledgment.—We are indebted to Roy G. Robinson, School of Dentistry, Department of Physiology, University of Southern California, for carrying out the tadpole assays.

Hypocholesterolemic Agents. Thyroalkanols

W. J. WECHTER, W. A. PHILLIPS, AND F. KAGAN

Research Laboratories of The Upjohn Company, Kalamazoo, Michigan

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A series of thyroalkanols was prepared and tested for hypocholesterolemic activity. The thyroalkanols, prepared by diborane reduction of the corresponding thyroalkanoic acids, showed comparable potency to the thyroalkanoic acids and less toxicity as exemplified by their effect on weight gain of treated and control rats.

The importance of thyroxine as a "metabolic regulator," and in particular the role of thyroxine in cholesterol metabolism, stimulated our interest in the chemical modification of the thyroxine side chain with the objective of effecting a split between hypocholesterolemic activity and calorigenic activity. The interesting hypocholesterolemic activity recently reported[1] for thyroalkanoic acids and the possibility that changes in the polarity of the side chain might be of importance in the absorption, distribution, metabolism, and thus the over-all activity of a thyroxine analog prompted the preparation and evaluation of a series of thyroalkanols as hypocholesterolemic agents (Table I).

The synthesis of triiodothyroethanol has been reported by Tomita and Lardy[2] who coupled an appropriately substituted phenylethanol derivative with *p*-methoxyphenol to afford a diphenyl ether bearing an ethanol side chain. Subsequent reactions yielded triiodothyroethanol. The general method of synthesis of thyroalkanols developed in this work depends upon the diborane reduction of the corresponding thyroalkanoic acids. It is of interest that diborane reduction was selective and did not adversely affect the iodinated diphenyl ether intermediates usually attacked by many other reducing agents.[2,3]

The synthesis of **8**, the thyroalkanoic acid precursor of the thyroethanol **1**, was accomplished by the Borrows[4] modification of the general method of Ullmann and Nadai.[5] Methyl 3,5-dinitro-4-hydroxyphenylacetate (**25**) was condensed with 3,5-dimethyl-4-methoxyphenol (**24**) in the presence of *p*-toluenesulfonyl chloride to afford the diphenyl ether **26** (Scheme I). Reduction, diazotization, and iodination yielded **7** which was treated with hydrogen iodide to yield **8**. The same type of synthesis in the 4'-deoxy series yielded the thyroalkanoic acid analog **10**.

Biological Methods.—Hypercholesterolemia was induced in male Sprague–Dawley rats (fasted weight about 220 g.) by using a high cholesterol diet[6] containing 10% coconut oil as fat source and 18% casein supplemented by 0.2% methionine as a protein source. Test compounds suspended in 0.25% methylcellulose at concentrations adjusted to 1 ml. of vehicle/100 g. of body weight were administered orally to groups of 10 rats. Control groups received vehicle only. The rats, weighed three times per week, were fed *ad libitum* until 17 hr. before the end of the experiment (14 days). Blood was drawn from the aorta after treatment with cyclopal.[7] Food consumption and weights were recorded at the end of each experiment.

Ferric chloride–sulfuric acid reagent was used for the determination of total sterols according to Zak, *et al.*,[8] and samples were analyzed by means of an Auto-Analyzer.[9]

Discussion

A summary of the hypocholesterolemic activity of a group of thyroalkanols and thyroalkanoic acids is

(1) G. S. Boyd and M. F. Oliver, *J. Endocrinol.*, **21**, 33 (1960); E. Curday, H. Jaffe, and D. W. Irving, *Arch. Internal Med.*, **106**, 809 (1960); W. R. Ruegamer, M. E. Alpert, and F. R. Silverman, *Endocrinology*, **66**, 160 (1960).

(2) K. Tomita and H. A. Lardy, *J. Biol. Chem.*, **235**, 3292 (1960).

(3) (a) N. G. Gaylord, "Reductions with Lithium Aluminum Hydride," Interscience Publishers, Inc., New York, N. Y., 1956, pp. 917–924. Note that no aromatic iodo compounds were stable to LAH (Table XCII); (b) T. Matsura and A. Nishinga, *J. Org. Chem.*, **29**, 3168 (1964); (c) K. Tomita, H. A. Lardy, D. Johnson, and A. Kent, *J. Biol. Chem.*, **236**, 2981 (1961); (d) J. Roche, R. Michele, and W. Wolf, *Compt. rend.*, **239**, 595 (1954); (e) R. L. Meltzer, D. M. Lustgarten, and A. Fischman, *J. Org. Chem.*, **22**, 1577 (1957).

(4) E. T. Borrows, J. C. Clayton, B. A. Heins, and A. G. Long, *J. Chem. Soc., Suppl.*, **1**, 5185 (1949).

(5) F. Ullmann and G. Nadai, *Ber.*, **41**, 1872 (1908); N. Kharasch, S. H. Kalfayan, and J. D. Arterberry, *J. Org. Chem.*, **21**, 925 (1956).

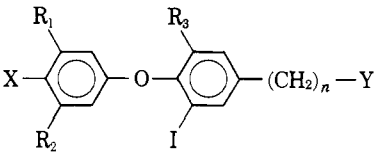
(6) W. A. Phillips and C. P. Berg, *J. Nutr.*, **53**, 481 (1954).

(7) Sodium cyclopentylallyl barbiturate at approximately 80 mg. kg. i.p.

(8) B. Zak, W. Moss, A. J. Boyle, and A. Zlatkis, *Anal. Chem.*, **26**, 776 (1954).

(9) Technicon Controls Inc., Chauncey, N. Y.

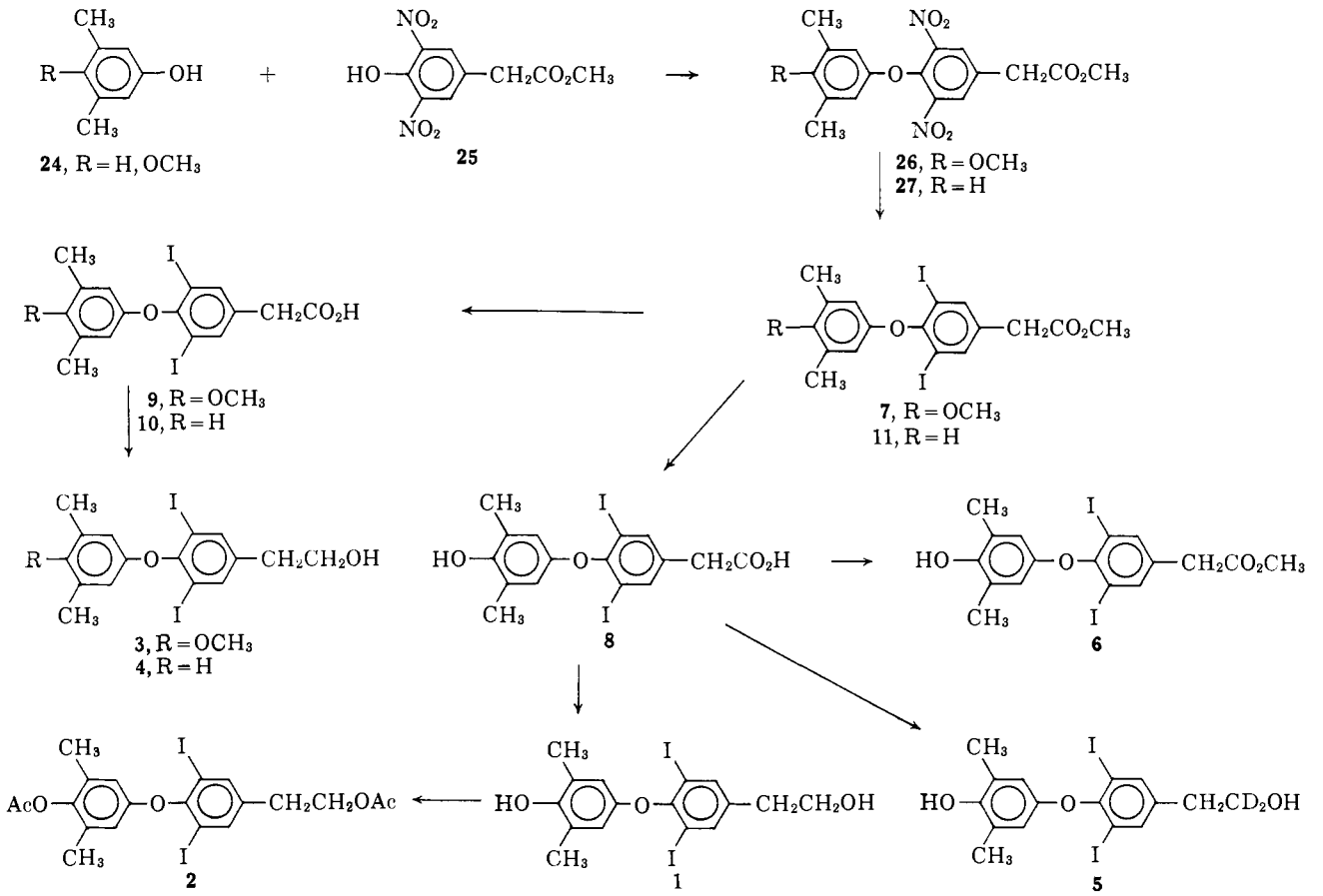
TABLE I
THYROALKANOLS AND THYROALKANOIC ACIDS



No.	X	Product R1	R2	R3	n	Y	Recrystn. solvent	M.p., °C.	% yield	Formula	Calcd., % C	H	I	Found, % C	H	I
1	HO	CH_3	CH_3	I	1	CH_2OH	Ethanol	197.5–198.5	79.5	$C_{16}H_{16}I_2O_3$	37.67	3.16	49.76	38.02	2.88	49.34
2	AcO	CH_3	CH_3	I	1	CH_2OAc	Hexane	139.5–140.5	95	$C_{20}H_{20}I_2O_5$	40.52	3.40	42.82	40.85	3.37	43.04
3	CH_3O	CH_3	CH_3	I	1	CH_2OH	Acetone–water	134.5–135.5	92	$C_{17}H_{18}I_2O_3$	38.95	3.46	48.42	38.81	3.65	48.58
4	H	CH_3	CH_3	I	1	CH_2OH	Acetone–water	143–144.5	62	$C_{16}H_{16}I_2O_2$	38.89	3.26	51.36	38.93	3.51	51.37
5	HO	CH_3	CH_3	I	1	CD_2OH	Ethanol	199–200.5	62	$C_{16}H_{14}D_2I_2O_3$	37.52	3.54[a]	49.56	37.65	3.53	
6	HO	CH_3	CH_3	I	1	CO_2CH_3	MeOH	166.5–169.5	80	$C_{17}H_{16}I_2O_4$	37.94	2.99	47.16	37.96	2.87	46.96
7	CH_3O	CH_3	CH_3	I	1	CO_2CH_3	MeOH	126.0–127.0	58.5	$C_{18}H_{18}I_2O_4$	39.15	3.28	47.97	39.54	3.59	
8	CH_3O	CH_3	CH_3	I	1	CO_2H	Ethanol–water	203.0–204.0	100	$C_{17}H_{16}I_2O_4$	37.93	3.00	47.16	38.03	2.66	46.88
9[b]	HO	CH_3	CH_3	I	1	CO_2H	Acetic acid–water	194.5–196	100							
10	H	CH_3	CH_3	I	1	CO_2H	Ethanol	219.5–222	79	$C_{16}H_{14}I_2O_3$	37.82	2.78	49.96	37.39	2.72	49.81
11	H	CH_3	CH_3	I	1	CO_2CH_3	Methanol	108–109.5	35	$C_{17}H_{16}I_2O_3$	39.10	3.09	48.61	38.83	3.08	48.37
12	HO	H	H	I	1	CH_2OH	Acetone	182.5–183.5	44	$C_{14}H_{12}I_2O_3$	34.88	2.51	52.66	34.89	2.62	52.73
13	HO	H	I	I	1	CH_2OH	Acetone	185.0–186.0	67	$C_{14}H_{11}I_3O_3$	27.65	1.82	62.62	27.65	2.08	62.49
14[c]	HO	H	H	I	0	CH_2OH	Acetone–water	173–174.5	24	$C_{13}H_{10}I_2O_3$	33.35	2.15	54.23	33.33	1.97	54.17
15	HO	H	I	I	0	CH_2OH	Methanol–water	168.5–169	64	$C_{13}H_9I_3O_3$	26.37	1.53	64.32	26.29	1.33	64.21
16	HO	I	I	I	0	CH_2OH	Acetone–water	220.5–221.5	57	$C_{13}H_8I_4O_3$	21.68	1.12	70.52	21.97	1.27	70.11
17	HO	H	H	I	2	CH_2OH	Acetone–water	192–193	80	$C_{15}H_{14}I_2O_3$	36.31	2.84	51.16	36.16	2.61	50.92
18[d]	HO	H	I	I	2	CH_2OH	Acetone	168–169	44	$C_{15}H_{13}I_3O_3$	28.96	2.10	61.21	29.34	2.27	60.78
19[e]	HO	I	I	I	0	CO_2H										
20	HO	I	I	I	2	CH_2OH	Acetone	203–204 dec.	85	$C_{15}H_{12}I_4O_3$	24.08	1.62	67.87	24.32	1.74	68.02
21	HO	I	I	I	3	CH_2OH	Ethanol–water	153.5–156.5	86	$C_{16}H_{14}I_3O_4$	25.21	1.88	66.62	25.34	1.80	66.88
22	HO	H	H	H	1	CH_2OH	Acetone–hexane	117–119	90	$C_{14}H_{13}IO_3$	47.21	3.68	35.63	47.43	4.02	35.34
23	HO	I	I	H	2	CH_2OH	CH_2Cl_2–hexane	91.5–94	47	$C_{15}H_{13}I_3O_3$	28.96	2.10	61.21	28.96	1.86	61.21

[a] Total D + H. [b] British Patent 882,401 (1962). [c] Lit.[2] m.p. 185–187° (benzene). [d] Lit.[2] m.p. 165–186° (benzene). [e] E. van Heyningen, *J. Org. Chem.*, **26**, 5005 (1961).

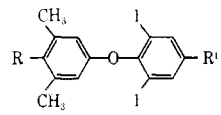
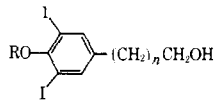
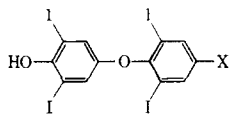
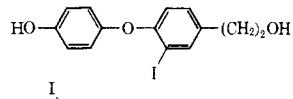
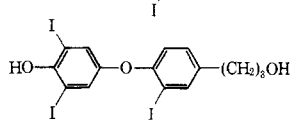
SCHEME I



presented in Table II. The following structure–activity relationships were drawn from these data. (1) Reduction of a thyroalkanoic acid (**8** or **9**) to the corresponding thyroalkanol (**3** or **1**) had no statistically significant effect on hypocholesterolemic activity but appeared to lower toxicity (as manifested by inhibition of weight gain). (2) Methylation of the 4′-hydroxyl group in both the thyroalkanoic acid series (**6** *vs.* **7** and **8** *vs.* **9**) and the thyroalkanols (**1** *vs.* **3**) had little effect on hypocholesterolemic potency and no con-

TABLE II

HYPOCHOLESTEROLEMIC ACTIVITY OF A GROUP OF THYRONOLS AND THYROALKANOIC ACIDS

Compd.	R	R', *n*, or X	Reduction in serum sterols from untreated controls,[a] % (1 run in parallel) 5 mg./kg.	0.3 mg./kg.	Inhibition of wt. gain from control, % 5 mg./kg.
					
1	OH	$(CH_2)_2OH$	76	32	N.S.
2	OAc	$(CH_2)_2OAc$	70	27	N.S.
3	OCH_3	$(CH_2)_2OH$	68	39	27
4	H	$(CH_2)_2OH$	N.S.[b]	N.S.[b]	N.S.[b]
5	OH	CH_2CD_2OH		25	N.S.
6	OH	$CH_2CO_2CH_3$	76	49	21
7	OCH_3	$CH_2CO_2CH_3$	75	38	N.S.
8	OH	CH_2CO_2H	53	51	85
9	OCH_3	CH_2CO_2H	67	59	80
10	H	CH_2CO_2H	N.S.	N.S.	N.S.
11	H	$CH_2CO_2CH_3$	N.S.	N.S.	N.S.
					
12	$4\text{-}OHC_6H_4$	1	62	N.S.	N.S.
13	$3\text{-I-}4\text{-}OHC_6H_3$	1	72	58	98
14	$4\text{-}OHC_6H_4$	0	N.S.	N.S.	N.S.
15	$3\text{-I-}4\text{-}OHC_6H_3$	0	50	N.S.	N.S.
16	$3,5\text{-}I_2\text{-}4\text{-}OHC_6H_2$	0	30	N.S.	N.S.
17	$4\text{-}OHC_6H_4$	2	N.S.	N.S.	N.S.
18	$3\text{-I-}4\text{-}OHC_6H_3$	2	73	61	105
					
19		COOH	41	N.S.	N.S.
20		$(CH_2)_3OH$	N.S.	N.S.	N.S.
21		$(CH_2)_4OH$	29	N.S.	N.S.
22			N.S.	N.S.	N.S.
23			N.S.	N.S.	N.S.

[a] All values shown are statistically significant ($P \leq 0.05$). [b] N.S. = no significant change.

sistent effect on toxicity.[10] (3) Acetylation of both the phenolic and alcoholic hydroxyl groups of **1** had no effect on either hypocholesterolemic activity or toxicity (*cf.* **1** *vs.* **2**). (4) Removal of the 4′-hydroxyl group and substitution of hydrogen in **8** and **1** destroyed activity[11] (*cf.* **8** *vs.* **10** and **1** *vs.* **4**). (5) Removal of the 3′,5′-substituents in the thyroalkanols reduced hypocholesterolemic potency (*cf.* 0.3-mg./kg. dose in **12**). Introduction of a single iodine returned hypocholesterolemic potency to that comparable to **1**; however, the toxicity was increased markedly in some cases as exemplified by its effect on weight gain (*cf.* **13** *vs.* **12** and **18** *vs.* **17**). In compounds showing relatively low activity (**15** *vs.* **14**), the effect on weight gain was not discernible. (6) Shortening the side chain of the thyroalkanols from two carbons to one lowered activity (*cf.* **12** *vs.* **14** and **13** *vs.* **15**).

Since these biological results were not definitive in the determination of whether the thyroalkanols really possessed a favorable split between their hypercholesterolemic activity and calorigenicity, further studies were carried out by Phillips and Nelson[12] utilizing a gas chromatographic technique to determine calorigenicity. Their findings showed that **1** possessed a greater dissociation between hypocholesterolemic and calorigenic activities than either L- or D-thyroxine in the cholesterol-induced hypercholesterolemic rat.

In summary, a series of thyroalkanols was prepared and tested for hypocholesterolemic activity. The

(10) This is in agreement with the findings of B. Blank, C. M. Greenberg, and J. F. Kerwin, *J. Med. Chem.*, **7**, 53 (1964), on the effect on potency of 4-methylation in a series of iodinated thyronines and thyroalkanoic acids; however, they found an improved therapeutic ratio in their series.

(11) Of the 4′-unsubstituted thyroxine analogs which have been tested for thyromimetic activity, only the 2′,3′-dimethylphenoxy analog has shown activity: *cf.* E. C. Jorgensen, N. Zenker, and C. Greenberg, *J. Biol. Chem.*, **235**, 1732 (1960).

(12) W. A. Phillips and N. A. Nelson, to be published. A paper on this new methodology has been submitted to *Proc. Soc. Exptl. Biol. Med.*

thyroalkanols showed comparable potency to the thyroalkanoic acids and less toxicity as exemplified by effect on weight gain of treated and control rats.

Experimental[13]

Diborane Reduction of Acids to Alcohols. General Method.—A solution of thyroalkanoic acid (2.0 g.) in 35 ml. of tetrahydrofuran (THF) was chilled to 0° and treated with 10 ml. of THF saturated at 0° with diborane (1.9 *M*).[14] The stirred solution was allowed to come to room temperature during 1 hr., after which it was again chilled and ice chips were added cautiously to the reaction mixture to destroy the excess diborane. The solution was then diluted with water to 200 ml. and the majority of the THF distilled under reduced pressure. The solid product which separated (usually crystalline) at this point was collected, washed thoroughly with water, and dried (*in vacuo* 60°). The alcohols were then recrystallized from suitable solvents and analyzed. In Table I are summarized the recrystallizing solvents, melting points, yields, and analytical data of all thyroalkanols.

Methyl 3,5-Dinitro-4-(3,5-xylyloxy)phenylacetate (27).—Methyl 3,5-dinitro-4-hydroxyphenylacetate (25.6 g., 0.1 mole) and *p*-toluenesulfonyl chloride (20.0 g., 0.105 mole) were dissolved in 50 ml. of pyridine and heated (protected from water) on a steam bath for 10 min. 3,5-Dimethylphenol (20.0 g., 0.164 mole) was added to the reaction mixture which was in turn heated under reflux for 1 hr. The majority of the pyridine was removed under reduced pressure with last traces being removed by codistillation with toluene. The residue was taken up in 200 ml. of acetone and treated with Darco G-60 and filtered. The acetone solution was then taken to dryness *in vacuo* and redissolved in 350 ml. of benzene. The benzene solution was washed twice with water, filtered through Celite, then washed consecutively with 1 *N* KOH, 1 *N* HCl, and saturated NaCl solution, and dried (Na_2SO_4). The benzene was then removed under reduced pressure, and the residue was recrystallized from absolute ethanol; m.p. 115.5–117.0°, yield 13.5 g. A sample was recrystallized once for analysis; m.p. 116.5–117.5°; ν_{max} 3080, 1735, and 1150 cm.$^{-1}$; λ_{max}^{EtOH} 234.5 mμ (ε 14,400).

Anal. Calcd. for $C_{17}H_{16}N_2O_7$: C, 56.67; H, 4.47; N, 7.78. Found: C, 56.48; H, 4.93; N, 7.70.

Methyl 3,5-Dinitro-4-(3,5-dimethyl-4-methoxyphenoxy)phenylacetate (26).—Methyl 3,5-dinitrophenylacetate (25.6 g., 0.1 mole) was condensed with 4-methoxy-3,5-xylenol as above (*cf.* **27**). The crude product (after removal of pyridine) was taken up in 200 ml. of acetone and percolated through a column of neutral activity I alumina (made up with acetone, 2.8 × 30 cm.). The column was eluted with an additional 1.5 l. of acetone and the total eluate was taken to dryness under reduced pressure. The residue was dissolved in 400 ml. of benzene and washed with water, then 1 *N* KOH. After filtering it was washed further with 1 *N* HCl and dried (Na_2SO_4). The benzene solution was then adsorbed onto a column of neutral activity I alumina (made up with benzene, dimensions 2.8 × 40 cm.) and the product was eluted with 3 l. of benzene. The benzene eluate was taken to dryness under reduced pressure and the residual light yellow oil crystallized from ethanol. After refrigeration (4°) the product was collected and dried *in vacuo* (at 60°) giving 17.4 g. of product, m.p. 95–96°. A sample was recrystallized from ethanol for analysis, m.p. 96.0–97.0°.

Anal. Calcd. for $C_{18}H_{18}N_2O_8$: C, 55.38; H, 4.65; N, 7.18. Found: C, 55.64; H, 4.70; N, 7.68.

Methyl 3,5-Diiodo-4-(3,5-dimethyl-4-methoxyphenoxy)phenylacetate (7).—To the dinitro ester **26** (17.4 g., 0.0444 mole) dissolved in 300 ml. of glacial acetic acid was added 10% palladium-on-carbon catalyst and hydrogenation was carried out on a Parr low-pressure apparatus until the theoretical amount of hydrogen had been absorbed (0.266 mole). The acetic acid solution was filtered directly into a stirred mixture of 50 ml. of concentrated H_2SO_4, 50 ml. of acetic acid, and 8.0 g. of $NaNO_2$ chilled in an ice–salt bath. The rate of addition was controlled so that the temperature of the reaction mixture remained between −5 and 2°. After the addition of the diamine was complete (*ca.* 1 hr.) the ice–salt bath was replaced with an ice bath and the reaction mixture was stirred for 1 hr. at 0°. Ice water (25 ml.) was then added to the suspension. The resulting solution was then poured into a vigorously stirred mixture of water (500 ml.) and chloroform (200 ml.) containing 32 g. of NaI, 6.4 g. of urea, and 16 g. of iodine. Stirring was continued for 2 hr., the chloroform layer was separated, and the aqueous layer was washed with 200 ml. of $CHCl_3$. The combined chloroform extracts were washed consecutively with water, 4% $NaHSO_3$ solution, and water, dried (Na_2SO_4), and taken to dryness under reduced pressure. The dark residue was taken up in 300 ml. of boiling methanol and treated with Darco, and the carbon-free solution was concentrated to 75 ml. of a steam bath. Upon refrigeration, the product crystallized, yield 14.4 g., m.p. 116.5°; it resolidified and remelted at 123°.

Methyl 3,5-Diiodo-4-(3,5-xylyloxy)phenylacetate (11).—The dinitro ester **27** (11.5 g.) dissolved in 200 ml. of glacial acetic acid was reduced on a Parr hydrogenator employing 3 g. of 10% Pd–C. The procedure employed for the synthesis of **7** (⅔ scale) was followed. After treatment of the methanol solution with Darco G-60 it was taken to dryness on a rotary evaporator. The residue was dissolved in benzene and adsorbed onto a column of activity I alumina made up with benzene. The product was eluted with ten 150-ml. portions of benzene. Fractions 1–6 were combined and recrystallized from methanol to give 4.9 g. of colorless prisms, m.p. 108.0–109.5°. A sample was recrystallized once for analysis[12]; m.p. 108–109.5°; ν_{max} 3050, 1730, and 1715 cm.$^{-1}$; λ_{max}^{EtOH} 226 mμ (ε 34,700) and 280 (1950).

3,5-Diiodo-4-(3,5-dimethyl-4-methoxyphenoxy)phenylacetic Acid (9).—Methyl 3,5-diiodo-4-(3,4-dimethyl-4-methoxyphenoxy)phenylacetate (**7**) (20.0 g.) was suspended in 350 ml. of 1 *N* NaOH, heated to reflux for 1 hr., and diluted with an additional 100 ml. of water, and the boiling suspension was filtered free of insoluble material. Upon cooling, the sodium salt crystallized in large white plates. The salt was filtered, washed with water, then suspended in 250 ml. of water, and acidified with 3 *N* HCl, and the suspension was stirred for 1 hr. The acid was collected, washed with water, and dried (*in vacuo*, 60°). Recrystallization from ethanol–water afforded 15 g. of fine needles, m.p. 203–204°. A sample was recrystallized for analysis[12]; m.p. 203.0–204.0°; ν_{max} 3090, 3040, 1705, and 1595 cm.$^{-1}$; λ_{max}^{EtOH} 224 mμ (ε 37,800), 272 (2800), and 286 sh (2200).

3,5-Diiodo-4-(3,5-xylyloxy)phenylacetic Acid (10).—The ester **11** (19.5 g.) was suspended in 100 ml. of 1 *N* NaOH and the suspension was heated to reflux with stirring for 2 hr. The clear hot solution was acidified with concentrated HCl. After cooling, the crystalline product was isolated, washed thoroughly with water, and dried at 60° *in vacuo* giving 4.4 g. of crude **10**. The solid was dissolved in ethanol, the solution was filtered, and the product was allowed to crystallize as colorless plates, 3.6 g., m.p. 219.5–222.0°. A sample was recrystallized once for analysis; m.p. 219–221°; ν_{max} 2620, 2540, 1710, 1620, and 1095 cm.$^{-1}$; λ_{max}^{EtOH} 225 mμ (ε 38,400), 271 (2350), and 279 (2100).

3,5-Diiodo-4-(3,5-dimethyl-4-hydroxyphenoxy)phenylacetic Acid (8).—The ester **7** (3.0 g.) was dissolved in a 1:1 mixture of acetic acid and 47% HI and the resulting solution was heated at reflux for 2 hr. Upon cooling the product crystallized. It was washed with 10 ml. of 50% aqueous acetic acid and dried *in vacuo* at 60° giving 2.9 g. (100%) of product, m.p. 194.5–196.0°. This material proved to be identical with an authentic sample by infrared and mixture melting point.[15]

Methyl 3,5-Diiodo-4-(3,4-dimethyl-4-hydroxyphenoxy)phenylacetate (6).—A 2.5-g. sample of the acid **8** dissolved in 25 ml. of methanol was treated with 1 ml. of boron trifluoride etherate and allowed to stand at room temperature overnight. Upon refrigeration (4°) a white crystalline solid separated and was isolated, 2.0 g., m.p. 166.5–169.5°. A sample was recrystallized from methanol for analysis[12]; m.p. 166.5–169.5°; ν_{max} 3440, 3055, 1712, 1602, 1576, and 1535 cm.$^{-1}$; λ_{max}^{EtOH} 226 mμ (ε 34,500) and 281 (3700).

3,5-Diiodo-4-(3,5-dimethyl-4-hydroxyphenoxy)phenethyl-α,α-d_2 Alcohol (5).—A 2.0-g. (3.63-mmole) sample of the acid **8** dissolved in 30 ml. of freshly purified THF was treated at 0°

(13) Melting points were taken in capillary tubes and are corrected. Infrared spectra were taken in Nujol mulls and ultraviolet spectra in 95% ethanol. Yields, analytical data, etc. are summarized in Table I. The acids serving as starting materials for compounds **13**, **15–17**, and **19–23** were obtained from Cyclo Chemical Corp., Los Angeles, Calif. Diborane gas was obtained from Callery Chemical Co., Philadelphia, Pa.

(14) H. C. Brown, "Hydroboration," W. A. Benjamin, Inc., New York, N. Y., 1962, Chapter 5.

(15) Our original supplies of this material were furnished by Farbwerke Hoechst, A. G., Frankfurt, Germany: W. Siedel, H. Nahm, and J. Konig, German Patent 683,174 (1964).

under nitrogen with diborane-d_6 gas generated from 1 g. (55 mmoles) of lithium aluminum deuteride in 50 ml. of ether and 4.8 g. of freshly distilled boron trifluoride etherate in 10 ml. of dry ether. After the generation of the diborane-d_6 was complete, the THF solution was stirred at room temperature under nitrogen for 1 hr. The excess diborane-d_6 was destroyed by the addition of ice chips to the reaction mixture after which it was diluted to 100 ml. with water. The THF was removed under reduced pressure whereupon the product crystallized. The isolated product was washed thoroughly with water, air dried, and recrystallized from ethanol giving 1.2 g. of material, m.p. 196.5–199.8°. N.m.r.[16] confirmed the structure and the absence of hydrogen at the α-ethyl position: 131 (s) (CH_3), 168 (singlet) (β-CH_2), 382 (s) (Me_2ArH_2), and 470 c.p.s. (s) (I_2ArH_2).

(16) N.m.r. spectrum was determined on a 5–10% solution in CDCl at 60 Mc. with a Varian A-60 spectrometer, employing tetramethylsilane as an internal reference. Frequencies are reported in cycles per second relative to tetramethylsilane as 0 c.p.s.

3,5-Diiodo-4-(3,5-dimethyl-4-hydroxyphenoxy)phenethyl Alcohol Diacetate (2).—A 1.0-g. sample of the thyroethanol **1** was dissolved in 10 ml. of pyridine and 1 ml. of acetic anhydride and allowed to stand at room temperature overnight. The reaction mixture was poured into 100 ml. of water and allowed to stand until an amorphous solid formed. The product (1.1 g.) was filtered, washed with water, and dried *in vacuo*, 60°. Recrystallization from Skellysolve B gave 930 mg. of a substance, m.p. 137.5–139.0°. A sample was recrystallized for analysis[17]: m.p. 139.5–140.5°; ν_{max} 1753, 1740, 1595, and 1537 cm.$^{-1}$; λ_{max}^{EtOH} 223 mμ (ε 35,850), 272 (2650), and 279 sh (2350).

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The Synthesis of Tenuazonic and Congeneric Tetramic Acids

STANTON A. HARRIS,[1] LINDA V. FISHER, AND KARL FOLKERS

Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey

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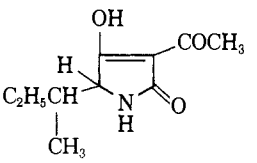
The structure of tenuazonic acid as 3-acetyl-5-*sec*-butyltetramic acid has been verified by total synthesis from L-isoleucine and diketene. A new series of crystalline-N-acetoacetylamino acids is described. For the purpose of correlating structure *vs.* biological activity, a series of tetramic acids having various substituents at the 1-, 3-, and 5-positions has been synthesized. An enhancement of the *in vitro* antibacterial activity of N-substituted tetramates has been confirmed.

Tenuazonic acid and several related tetramates have been synthesized from amino acids and diketene for study in human tumor and other biological systems.

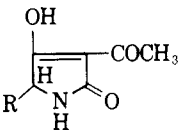
Hadacidin[2] was recently discovered as a new growth-inhibitory substance in human tumor systems, and further research led to the discovery of another crystalline human antitumor substance which was identified[3] as the known tenuazonic acid (I).[4,5] Recently Miller, *et al.*,[6] reported that synthetic tenuazonic acid showed antiviral activity at rather high dose levels but that it was inactive against bacteria and yeast. We were interested in varying the substituents on the tetramic acid skeleton of tenuazonic acid in order to make possible a study of the effect of these changes on their biological activities.

Our synthesis of tenuazonic acid differs slightly from that of Lacey.[7] In this process, we were able to isolate and characterize as crystalline compounds a new series of N-acetoacetylamino acids which are given in Table I. This was the basis for the synthesis of the substituted tetramic acids which are described in Table II, in which variations have been made in the alkyl group at position 5 and substitutions have been made at position 1 (N). Several 3-acetyltetramic acids having the following groups in position 5, benzyl, isopropyl, methylthioethyl, ethyl, phenyl, dimethyl, *n*-butyl, methyl, hydrogen and isobutyl, have already been described.[8] There was no N-substitution on these compounds.

3-Acetyl and 3-acetyl-5-methyltetramic acids have been synthesized by Lacey[7] who allowed the methyl ester of glycine and DL-alanine to react with diketene, and then carried out the cyclization to give II and III.



I



II, R = H
III, R = CH_3

Since Lacey did not start with optically active amino acids, his products could not reveal the stereochemistry of C-5. The product which we synthesized by these reactions and L-isoleucine was identical in all respects with tenuazonic acid (I).

Table III lists a few tetramic acids in which the acetyl group at position 3 has been replaced by other carbonyl functions.

The tumor-inhibiting properties of tenuazonic acid against a human tumor growing on chick embryos are described by Gitterman, *et al.*[9] The activities of the substituted tetramates in this system are described[10]

(1) To whom inquiries should be addressed.

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