[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, SCHOOL OF PHARMACY, UNIVERSITY OF CALIFORNIA AT SAN FRANCISCO]

Thyroxine Analogs. IV.[1] Synthesis of Aliphatic and Alicyclic Ethers of 3,5-Diiodo-DL-tyrosine[2]

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Several aliphatic and alicyclic ethers of 3,5-diiodo-DL-tyrosine have been prepared in order to determine the extent of aromaticity required for thyromimetic and thyroxine-antagonistic activity.

The reported success[4,5] of Woolley's 3,5-diiodo-4-*n*-butoxy-*N*-acetyl-L-phenylalanine[6] as an antagonist to thyroxine made it of interest to substitute other aliphatic groups for the phenolic ring of thyroxine in order to examine the requirement for aromatic character. If a simple aliphatic ether of 3,5-diiodotyrosine could antagonize thyroxine, it was considered possible that closely related compounds might mimic thyroxine or provide an enhancement of the antagonistic effect. To examine these possibilities, a series of aliphatic ethers of 3,5-diiodotyrosine was prepared in which enhanced binding at the biological receptor site was sought by providing the analogs with polar groups in approximately the same spatial relationship as those in the thyroid hormones. In thyroxine a chain of four carbon atoms is interposed between the ethereal and phenolic oxygens; however, due to free rotation between carbon atoms in an analogous alkyl chain, with consequent uncertainty of position of the terminal atoms (corresponding to the phenolic hydroxyl group), the synthesis of several homologous members was indicated.

Since carboxyl or amino groups could participate in ionic or hydrogen bonding, the aliphatic series studied included three acids (X–XII), an amine (XIII), and the reference butyl compound (XIV). The analogs were prepared by a potassium carbonate condensation of the appropriate halide with *N*-acetyl-3,5-diiodo-DL-tyrosine ethyl ester in 2-butanone. The condensation was successful with 3-bromobutyronitrile (I), 4-bromovaleronitrile (II), 3-dimethylaminopropyl chloride (IV), and *n*-butyl bromide (V), but failed with 5-chlorocapronitrile. Substitution of 5-iodocapronitrile, the synthesis of which is first described in this study, gave a good yield of the ethereal nitrile (III). Hydrolysis by concentrated hydrochloric acid in acetic acid gave the free amino acids (X–XIV).

To provide a closer nonaromatic analogous structure to the phenolic ring of thyroxine, a series of alicyclic ethers was synthesized comprising the cyclohexyl (VII), 3-cyclohexenyl (VIII), and 6-acetoxy-3-cyclohexenyl (IX) analogs. The allyl ether (VI) whose *N*-acetyl derivative (XV) has been prepared[7] was included in the series as a synthetic model. The lability of these ethers to acid hydrolysis did not permit obtention of the free amino acids, but a mild alkaline hydrolysis of their *N*-acetyl ethyl esters provided the *N*-acetyl free acids (XV–XVIII). The *N*-acetyl 3-cyclohexenyl ether (XVII) has been prepared[7] as an intermediate in an attempted synthesis of thyroxine.

The cyclohexyl ether, when obtained by alkaline hydrolysis (XVI, A), displayed thyromimetic activity. In view of the inactivity of all the related aliphatic and alicyclic analogs, further criteria of purity were examined for the cyclohexyl ether (XVI, A). The ultraviolet spectrum in alcoholic alkali displayed a shoulder at 310–330 mμ which could be ascribed to contamination by phenolic material. The wave length at which the absorption of the alkaline solution differed most from that of the neutral solution (λ_{max} 320 mμ) did not coincide with the maximum absorption for *N*-acetyl-3,5-diiodotyrosine (λ_{max} 315 mμ) and the presence of a second phenolic contaminant was indicated. This was confirmed when chromatography showed two contaminants, one identified as *N*-acetyl-3,5-diiodo-DL-tyrosine, the second tentatively identified as *N*-acetyl-DL-thyroxine. The latter could be formed by the base catalyzed self-condensation of *N*-acetyl-3,5-diiodo-DL-tyrosine or its ethyl ester during prolonged reaction with iodocyclohexane or during basic hydrolysis. This condensation under similar conditions has been reported by Pitt-Rivers.[8]

An alternate synthetic route to the cyclohexyl ether involving hydrogenation of the 3-cyclohexenyl ether (XVII) was successful. The product isolated showed a small amount of contamination by *N*-

(1) Paper III, E. C. Jorgensen, N. Zenker, and C. Greenberg, *J. Biol. Chem.*, **235**, 1732 (1960).

(2) Reported in part before the Division of Medicinal Chemistry at the 136th National Meeting of the American Chemical Society, Atlantic City, N. J., September 1959.

(3) In partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of California, June 1960. Present address: Laboratorios "Hormona," S.A., Laguna de Mayrán 411, Mexico D.F., Mexico.

(4) R. H. Williams, R. F. Tagnon, H. Jaffe, B. T. Towery, and W. F. Rogers, Jr., *J. Clin. Endocrinol.*, **8**, 597 (1948).

(5) C. E. Searle and A. Lawson, *J. Endocrinol.*, **7**, xxxvii (1950–51).

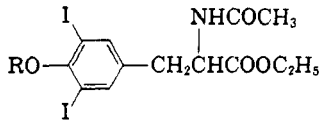
(6) D. W. Woolley, *J. Biol. Chem.*, **164**, 11 (1946).

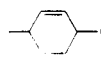
(7) J. H. Barnes, E. T. Borrows, J. Elks, B. A. Hems, and A. G. Long, *J. Chem. Soc.*, 2824 (1950).

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

TABLE I

ALIPHATIC AND ALICYCLIC ETHERS OF 3,5-DIIODO-DL-TYROSINE-*N*-ACETYL ETHYL ESTER



No.	R	Reaction Conditions[a] Halide	Mole, halide	Reflux, hr.	Yield, %	M.P.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
I	$(CH_2)_3CN$	Br[b]	0.028	7.25	90	126–128	$C_{17}H_{20}O_4N_2I_2$	35.81	36.10	3.54	3.77
II	$(CH_2)_4CN$	Br[b]	0.028	5.75	76	98–100	$C_{18}H_{22}O_4N_2I_2$	37.01	37.36	3.80	3.84
III	$(CH_2)_5CN$	I[c]	0.020	5.00	83	101–104	$C_{19}H_{24}O_4N_2I_2$	38.15	38.05	4.04	3.94
IV	$(CH_2)_3N(CH_3)_2$	Cl[d]	0.040	10.00	65	112–117	$C_{18}H_{26}O_4N_2I_2$	36.75	37.10	4.46	4.51
V	$(CH_2)_3CH_3$	Br[b]	0.028	6.00	80	93–95	$C_{17}H_{23}O_4NI_2$	36.51	36.36	4.15	3.98
VI	$CH_2CH{=}CH_2$	Br[b]	0.030	2.50	84	121–126	$C_{16}H_{19}O_4NI_2$	35.38	35.40	3.53	3.60
VII		I[e]	0.060	29.00	43	Oil	$C_{19}H_{25}O_4NI_2$[f]	38.99	39.10	4.31	4.59
VIII		Br[g]	0.030	10.00	63	66–68	$C_{19}H_{23}O_4NI_2$	39.12	37.67	3.97	4.40
IX		Br[h]	0.032	11.00	40	115–145[i]	$C_{21}H_{25}O_6NI_2$	39.33	39.35	3.93	3.97

[a] The general procedure is given in the Experimental section. The limiting reagent, 3,5-diiodo-DL-tyrosine-*N*-acetyl ethyl ester, was held constant at 0.020 mole. [b] Aldrich Chemical Co., Milwaukee, Wis. [c] Synthesis described in the Experimental section. [d] Michigan Chemical Corp. [e] H. Stone and H. Shechter, *J. Org. Chem.*, **15**, 491 (1950). [f] Analysis on an intermediate fraction chromatographed on alumina with chloroform as the eluant. [g] K. Ziegler, A. Späth, E. Schaff, W. Schumann, and E. Winkelman, *Ann. der Chem.*, **551**, 80 (1942). [h] M. A. Berlande, *Bull. Soc. Chem. Fr.*, **9**, 644 (1942). [i] The broad melting range indicates possible admixture of the allylic isomer, *N*-acetyl-3,5-diiodo-4-(4-acetoxy-3-cyclohexenyloxy)-DL-phenylalanine ethyl ester.

acetyl-3,5-diiodo-DL-tyrosine when examined by paper chromatography and ultraviolet spectroscopy. The absence of this compound in the starting material indicated that it was formed by hydrogenolysis of the allylic ether. The cyclohexyl ether (XVI, B) obtained by hydrogenation was inactive in the rat antigoiter assay both as a mimetic and an antagonist.

Whereas Woolley[6] found that the *n*-butyl ether (XIV) as the acetamido acid antagonized the lethal action of thyroxine on tadpoles, no antagonistic properties could be detected in the rat antigoiter assay[1] for the related acetylated and nonacetylated aliphatic and alicyclic-DL-ethers (X–XVIII) at a molar dose 200 times that of L-thyroxine. Those (XI, XIII–XVIII) tested in the rat antigoiter assay for thyroxine-like properties at a molar dose 200 times the effective dose of L-thyroxine were found to be without activity.

EXPERIMENTAL

All melting points were taken on a Fisher-Johns melting point apparatus and are given uncorrected.

Microanalyses were carried out by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley. All compounds were dried for at least 24 hr. over phosphorus pentoxide at 3 mm. and 100°.

5-Iodocapronitrile. The method of Newman and Closson[9] was employed. To a solution of sodium iodide (46.5 g., 0.31 mole) in dry acetone (250 ml.) was added 5-chlorocapronitrile (39.3 g., 0.30 mole; Aldrich Chemical Co.). The mixture was allowed to stand for 5 hr. After filtering off the sodium chloride precipitate, the filtrate was refluxed 8.5 hr. in a dry system with intermittent removal of the precipitate as it formed. The volume was reduced by distillation to 50 ml. and the sodium chloride removed. An equal volume of benzene was added, and the mixture washed with 5% sodium bisulfite and water. The benzene was removed by distillation at atmospheric pressure, and the residue distilled *in vacuo*. There was collected 26.9 g. (36%) of material, b.p. 138–147° at 12 mm. The analysis of a middle cut, b.p. 141–144° at 12 mm., showed that the product was contaminated, probably with some starting material. This degree of purity was adequate for its use in the next step.

Anal. Calcd. for $C_6H_{10}NI$: C, 32.31; H, 4.52. Found: C, 35.54; H, 4.83.

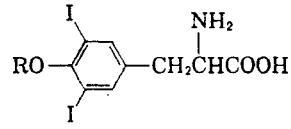
Aliphatic and alicyclic ethers of 3,5-diiodo-DL-tyrosine-N-acetyl ethyl ester, Table I. 3,5-Diiodo-DL-tyrosine-*N*-acetyl ethyl ester[7] (10.6 g., 0.02 mole) in 100 ml. of 2-butanone was heated under reflux with anhydrous potassium carbonate (8.3 g., 0.06 mole). The halide (0.02 to 0.06 mole) was added slowly and the mixture was refluxed for a total of 3 to 29 hr., depending on the reactivity of the halide. After cooling, the supernatant was decanted from the residue which was washed by refluxing several times with small volumes of fresh 2-butanone. The combined supernatant and washings were filtered and allowed to evaporate to dryness in a current of air. With the more unreactive halides, it was sometimes necessary to wash an ether solution of the residue with 2*N* potassium carbonate in order to remove any unchanged phenol. The brown glassy residue was crystallized with Norit decolorization from aqueous ethanol.

Aliphatic ethers of 3,5-diiodo-DL-tyrosine, Table II. The aliphatic ethers of 3,5-diiodo-DL-tyrosine-*N*-acetyl ethyl ester (I–V, 6.0 g., *ca.* 0.01 mole) were heated under reflux for 6 hr. in 30 ml. of concd. hydrochloric acid and 30 ml. of glacial acetic acid, then refrigerated until crystallization of the hydrochloride had ceased. After filtration and washing with cold glacial acetic acid, the hydrochloride was sus-

(9) M. S. Newman and R. D. Closson, *J. Am. Chem. Soc.*, **66**, 1553 (1944).

TABLE II

ALIPHATIC ETHERS OF 3,5-DIIODO-DL-TYROSINE

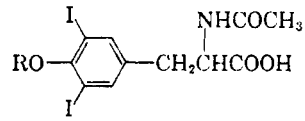


No.	R	Yield, %	M.P., Dec.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Iodine, % Calcd.	Iodine, % Found
X	$(CH_2)_3COOH$	77	213–215	$C_{13}H_{15}O_5NI_2$[a]	30.08	29.91	2.91	3.28		
XI	$(CH_2)_4COOH$	44	217–219	$C_{14}H_{17}O_5NI_2$[b]	31.54	31.41	3.22	3.33		
XII	$(CH_2)_5COOH$	82	202–204	$C_{15}H_{19}O_5NI_2$[c]	32.93	32.67	3.50	3.61		
XIII	$(CH_2)_3N(CH_3)_2$	10	217–219	$C_{14}H_{20}O_3N_2I_2$[d]	28.47	28.32	4.27	3.85	42.97	42.26
XIV	$(CH_2)_3CH_3$	81	205–223	$C_{13}H_{17}O_3NI_2$	31.92	31.87	3.50	3.47	51.90	51.72

[a] Calcd.: N, 2.70. Found: N, 2.62. Hydrochloride, m.p. 158–160° dec. [b] Calcd.: N, 2.63. Found: N, 2.54. Hydrochloride, m.p. 184–190° dec. [c] Calcd.: N, 2.56. Found: N, 2.69. Hydrochloride, m.p. 138–146° dec. [d] Hydrochloride dihydrate.

TABLE III

ALLYLIC AND ALICYCLIC ETHERS OF *N*-ACETYL-3,5-DIIODO-DL-TYROSINE



No.	Method	R	Yield, %	M.P.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Iodine, % Calcd.	Iodine, % Found
XV	A	$CH_2CH=CH_2$	90	178–182[a]	$C_{14}H_{15}O_4NI_2$						
XVI	A		10	165–178	$C_{17}H_{21}O_4NI_2$	36.65	36.43	3.80	3.80	45.56	45.45
	B		61	167–172			36.36		3.91		45.35
XVII	A		55	160–162[b]	$C_{17}H_{19}O_4NI_2$	36.78	37.00	3.45	3.50	45.72	45.46
XVIII	A		65	159–165[c]	$C_{17}H_{19}O_5NI_2$	35.75	35.79	3.35	3.46	44.44	44.28

[a] Lit.[7] m.p. 176.5°. [b] Lit.[7] m.p. 147° dec. [c] Possible admixture of the allylic isomer, *N*-acetyl-3,5-diiodo-4-(4-hydroxy-3-cyclohexenyloxy)-DL-phenylalanine.

pended in *ca.* 70 ml. of water and the free amino acid was obtained by adjusting the *p*H to the isoelectric point. Further purification was achieved by dissolving the compound in aqueous sodium hydroxide and adjusting the *p*H with aqueous hydrochloric acid to 3.0 for monoaminodicarboxylic acids (X–XII), to 5.0 for the monoaminomonocarboxylic acid (XIV) and to 10.0 for the diaminomonocarboxylic acid (XIII).

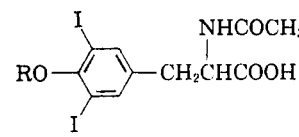
The dimethylaminopropyl ether (XIII) could not be purified sufficiently in this way, but an acceptable analysis was obtained on its amino acid hydrochloride dihydrate. The hydrochlorides (X–XIII) were obtained by dissolving the amino acid in glacial acetic acid, adding a few drops of concentrated hydrochloric acid, and refrigerating overnight. The resulting solid was washed with a small amount of 1:1 concentrated hydrochloric acid: glacial acetic acid and dried thoroughly.

*Allylic and alicyclic ethers of N-acetyl-3,5-diiodo-*DL*-tyrosine*, Table III, Method A. The allylic and alicyclic ethers of 3,5-diiodo-DL-tyrosine-*N*-acetyl ethyl ester (VI–IX, 3.0 g., *ca.* 0.005 mole) in 50 ml. of absolute ethanol and 9 ml. of 40% aqueous sodium hydroxide (0.09 mole) were stirred at room temperature for 1–1.5 hr. After addition of an equal volume of water, the *p*H was adjusted to 3.0 with 6*N* hydrochloric acid. The resulting oil or amorphous powder was crystallized from aqueous ethanol or acetone.

In the case of the cyclohexyl analog (VII), hydrolysis resulted in a 10% yield of material which could be crystal-

TABLE IV

CHROMATOGRAPHY[a] OF PRODUCTS FROM ALTERNATE SYNTHESES OF 3,5-DIIODO-4-CYCLOHEXYLOXY-*N*-ACETYL-DL-PHENYLALANINE



R	R_f Values XVIA	XVIB[b]	XVII[b]
H[c]	0.22	0.29	—
3-Cyclohexenyl	—	—	0.71
Cyclohexyl[d]	0.66	0.75	—
3,5-Diiodo-4-hydroxyl-phenoxy[e]	0.92	—	—

[a] Whatman paper 3 MM. Ascending solvent: butanol-dioxane-2*N*-ammonia (4:1:5). Compounds detected by quartz envelope ultraviolet light and by the ceric sulfate-sodium arsenite reagent of Bowden, Maclagan, and Wilkinson.[10] [b] Run on the same paper. [c] Authentic sample run as a control. [d] The major spot in XVI A and XVI B. [e] No authentic sample available; tentative identification.

lized from acetic acid. Although this product (XVI A) gave a satisfactory analysis, ultraviolet spectra with strong absorption at 320 mμ in alkali indicated the presence of a phenolic impurity. This was confirmed by chromatography (Table IV) which gave three spots with R_f values of 0.22, 0.66 and 0.92. Comparison with an authentic sample showed the minor spot with R_f 0.22 to be *N*-acetyl-3,5-diiodo-DL-tyrosine. No authentic sample was available for comparison, but the other minor spot with R_f 0.92 was assumed to be *N*-acetyl-DL-thyroxine on the basis of the transformation of *N*-acetyl-3,5-diiodotyrosine to *N*-acetylthyroxine under similar conditions.[8] Both the R_f 0.92 spot and thyroxine-like activity were lost when the alternate synthetic Method B was applied. The spot varying between R_f 0.65–0.75 made up the major chromatographic component by both methods of synthesis, and on the basis of analytical and spectral data was assumed to be the desired cyclohexyl ether.

*N-Acetyl-3,5-diiodo-4-cyclohexyloxy-*DL*-phenylalanine*, (XVI B), Method B. *N*-Acetyl-3,5-diiodo-4-(3-cyclohexenyloxy)-DL-phenylalanine (XVII, 220 mg., 0.5 mmole) was dissolved in absolute ethanol (10 ml.), and shaken with hydrogen at atmospheric pressure and room temperature in the presence of platinum oxide (10 mg.) until the theoretical amount of hydrogen had been absorbed. The catalyst was filtered off, water was added to the hot solution which was then chilled until crystallization ceased. There was obtained 135 mg. (61%) of colorless prisms, m.p. 167–172°, which did not decolorize bromine water. Analytical and bioassay samples were prepared by recrystallization from aqueous acetone.

Ultraviolet absorption spectra in acidic and basic ethanolic solutions showed the presence of a small amount of phenolic contamination (λ_{max} 315 mμ in alcoholic alkali) and its absence in the starting material. This was confirmed by chromatography (Table IV) which showed one spot (R_f 0.71) for the starting material, and two for the hydrogenated product (R_f 0.29 and 0.75). The slower moving component was identified as *N*-acetyl-3,5-diiodo-DL-tyrosine, and its concentration in the preparation was estimated from the absorbance at 6%.

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(10) C. H. Bowden, N. F. Maclagan, and J. H. Wilkinson, *Biochem. J.*, 59, 93 (1955).

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, SCHOOL OF PHARMACY, UNIVERSITY OF CALIFORNIA AT SAN FRANCISCO]

Thyroxine Analogs. V.[1] Synthesis of Some 1- and 2-Naphthyl Ethers of 3,5-Diiodo-DL-Tyrosine[2]

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In an investigation of the importance of aromatic character and steric requirements for the phenolic ring of thyroxine, a series of analogs was prepared replacing the benzene with the naphthalene ring.

The finding that a diverse series of aliphatic and alicyclic ethers substituted in the 4-position of 3,5-diiodotyrosine was devoid of either thyromimetic or thyroxine antagonistic properties,[1] together with the observation that such properties were displayed by a related series of alkyl-substituted phenyl ethers[4] led to the conclusion that aromaticity of the "prime" ring was a requisite for activity. In order to determine the effect of aromatic systems other than the benzenoid, synthesis of a series of naphthyl ethers was carried out.

The unsubstituted 1-naphthyl and 2-naphthyl ethers of 3,5-diiodo-DL-tyrosine were selected since they could be considered as sterically related to the 2′,3′-dimethylphenoxy[5] and the 3′,4′-dimethylphenoxy[6] analogs which have shown mimetic[4] and antagonistic[7] properties, respectively. The naphthalene ring of 3,5-diiodo-4-(1-naphthyl) ethers possesses a relatively fixed stereochemical orientation, being forced to occupy a position in space perpendicular to the plane of and distal to the alanine-bearing ring in order to provide a minimal interaction with the 3,5-diiodophenyl structure.[8] The 4-hydroxy-1-naphthoxy analog was of interest in providing comparison with its nonhydroxylated analog, and as a precursor to a halogenated derivative whose halogen substituent would occupy a

(1) E. C. Jorgensen and P. A. Lehman, Paper IV, *J. Org. Chem.*, 26, 894 (1961).

(2) Reported in part before the Division of Medicinal Chemistry at the 138th National Meeting of the American Chemical Society, New York, N. Y., September 1960.

(3) In partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of California, June 1960. Present address: Laboratorios "Hormona," S.A., Laguna de Mayrañ 411, Mexico D.F., Mexico.

(4) E. C. Jorgensen, N. Zenker, and C. Greenberg, *J. Biol. Chem.*, 235, 1732 (1960).

(5) E. C. Jorgensen and P. N. Kaul, *J. Am. Pharm. Assoc., Sci. Ed.*, 48, 653 (1959).

(6) J. H. Barnes, J. Elks, F. F. Stephens, and G. J. Waller, *J. Chem. Soc.*, 764 (1953).

(7) To the calorigenic effect of thyroxine: Footnote 6. To the antigoitrogenic effect of thyroxine: E. C. Jorgensen, unpublished observation.

(8) N. Zenker and E. C. Jorgensen, *J. Am. Chem. Soc.*, 81, 4643 (1959).