Notes

Thyroxine Analogs. XVII.[1] 3,5-Di(ethylthio)- and 3,5-Di(phenylthio)-DL-thyronines and Their 3′-Isopropyl Analogs

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A wide variety of groups have replaced the 3′-iodine atom of 3,5,3′-triiodo-L-thyronine with retention of thyroid hormonal activity.[2] However, requirements for the 3,5-iodine atoms have been more restrictive, only bromine[1,3] or methyl[4] substitution leading to active analogs among the limited series studied.[5] The synthesis of analogs containing 3,5-di(ethylthio) and 3,5-di(phenylthio) substituents on the thyronine nucleus was undertaken to provide additional data on substituent requirements for the alanine-bearing ring, and in an attempt to prepare a halogen-free thyromimetic compound.

A positive correlation has been noted between thyroxine-like effects in rodents, and lipophilic and electronic character of the 3′- and 5′-halogen substituents of thyroxine analogs.[6] A similar relationship has been reported for the alkyl, aryl, and halogen substituents in the 3′ position of 3,5-diiodothyronines.[7] Activity rises to a maximum with increasing lipophilic character, as measured by the Hansch substituent constant, π.[8] Activity is further enhanced by electron-donating groups, as measured by the Hammett σ_p value.[9] Thus, 3,5-diiodo-3′-isopropyl-L-thyronine ($\pi_{i\text{-Pr}} = 1.30$; $\sigma_{i\text{-Pr}} = -0.15$) is 700–1200% as active as L-thyroxine, the isopropyl group currently being the most effective 3′ substituent.[2c] The activities of compounds containing substituents other than iodine in the 3,5 positions, such as 3,5-dibromo-3′-isopropyl-L-thyronine[10] (170% of L-thyroxine; $\pi_{Br} = 0.94$; $\sigma_{Br} = 0.23$) and 3,5-dimethyl-3′-iodo-DL-thyronine[4] (3% of L-thyroxine; $\pi_{Me} = 0.51$, $\sigma_{Me} = -0.17$), indicated that such a correlation might be extended to substituents in the 3,5 positions. The ethylthio group (C_2H_5S) has a lipophilic character ($\pi_{C_2H_5S} = 1.13$)[11] close to that of iodine ($\pi_I = 1.15$),[6] and an electronic character ($\sigma_{C_2H_5S} = 0.03$) between that of groups which confer activity in the 3,5 positions, iodine ($\sigma_I = 0.28$) and methyl ($\sigma_{CH_3} = -0.17$). The phenylthio group was selected as representative of a highly lipophilic ($\pi_{C_6H_5S} = 2.0$)[12] substituent. In both the ethylthio and phenylthio series, analogs containing the biologically activating 3′-isopropyl substituent were included.

N-Acetyl-3,5-diiodo-L-tyrosine ethyl ester (**1**) was converted into its 3,5-di(ethylthio) derivative (**2**) by reaction with cuprous ethyl mercaptide (see Scheme I). However, **2** did not react with di(*p*-anisyl)iodonium bromide (**3a**) in the presence of Cu powder and NEt_3[13,14] or KO-*t*-Bu in *t*-BuOH.[4] Therefore, the intermediate diiododiphenyl ethers (**4a**, **4b**) were formed by reaction of **1** with the diaryliodonium salts (**3a**, **3b**). The intermediate **4b** was identical with a sample prepared by a longer route,[15] which established the position of the 3′-isopropyl group. Reaction of **4a** and **4b** with cuprous ethyl mercaptide yielded the ethylthio ethers (**5a**, **5b**), and with cuprous phenyl mercaptide, the phenylthio ethers (**8a**, **8b**) by methods developed by Adams, *et al.*[16] Reactions carried out under N_2 resulted in purer products in higher yield than those reactions carried out in the absence of nitrogen. Relative to the nmr spectra of the 3,5-diiododiphenyl ethers[17] (**4a**, **4b**), 3,5-di(phenylthio) substitution (**8a**, **8b**) produced an upfield shift of about 0.22 ppm for the 2,6-protons and for protons in the N-acetylalanine ethyl ester side chain. Ethylthio substitution (**5a**, **5b**) did not produce this effect, which must have been due to the positioning of the 2,6- and side-chain protons above or below the planes of the phenylthio groups.[17]

Hydrolysis of **8a** and **8b** with HBr yielded the phenylthio-substituted amino acids (**9a**, **9b**). However, treatment of **5b** under these conditions caused partial loss of the ethylthio group,[18] under conditions which

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(11) Estimated[6,8] by $\pi_{SCH_3}(0.62) + \pi_{CH_3}(0.51)$.

(12) Estimated[6,8] by $\pi_{C_6H_5}(1.89) + \pi_{SCH_3}(0.62) - \pi_{CH_3}(0.51)$.

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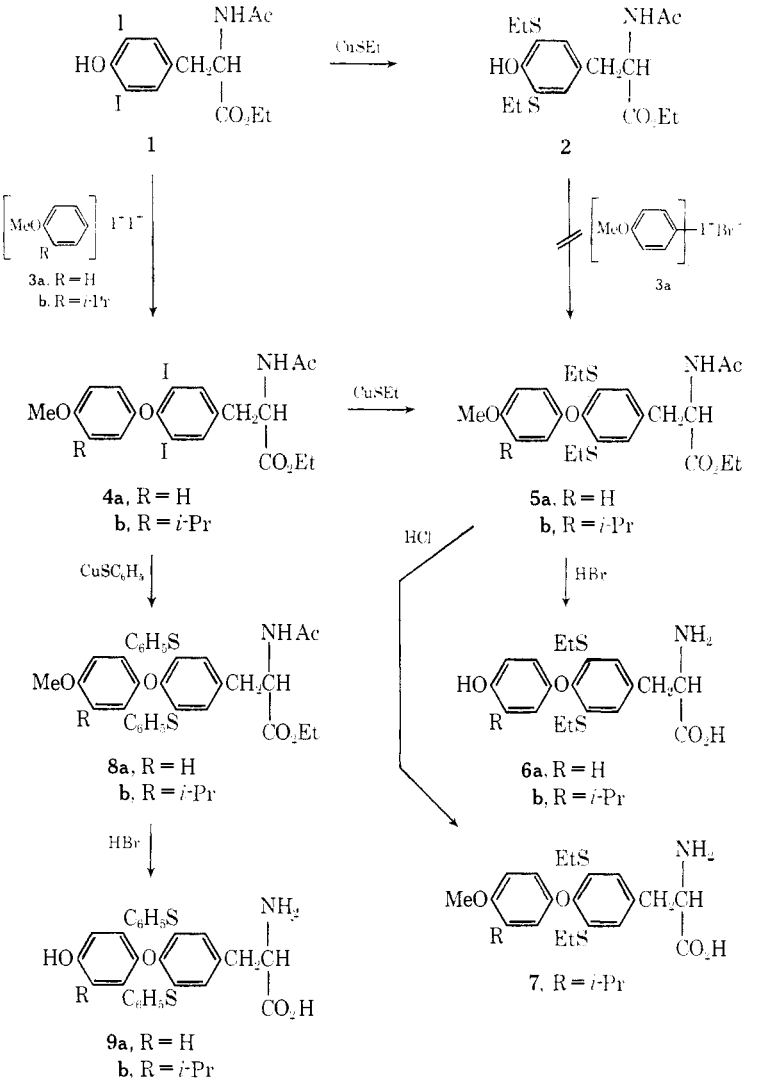
SCHEME I



were necessary for complete hydrolysis of the 4′-methoxyl group. 3,5-Di(ethylthio)-3′-isopropyl-L-thyronine was obtained as a mixture of two amino acids which was submitted as such to biological evaluation. Since the 4′-methoxy derivatives of active thyroxine analogs are themselves highly active, **5b** was hydrolyzed with HCl to yield the 3,5-di(ethylthio)-3′-isopropyl-4′-methoxy-L-thyronine (**7**). The less sterically hindered 3,5-di(ethylthio)-L-thyronine (**6a**) was obtained by HBr hydrolysis of **5a**.

Pharmacology.[19]—Compounds **6a**, **6b**, **7**, **9a**, and **9b** were inactive when tested for thyroxine-like activity by the rat antigoiter method[19] at dosage levels on a molar basis 100 times that of an effective dose of L-thyroxine (3 μg of sodium L-thyroxine pentahydrate/100 g of body weight). Compounds **6a**, **9a**, and **9b** were inactive as thyroxine antagonists[2b] when administered in 100-fold excess together with L-thyroxine (3 μg) to thiouracil-fed rats.

These results indicate that the balance of lipophilic and electronic properties correlated with activity of a substituent in the 3′ position of the thyronine nucleus may not be extended to the corresponding 3,5 positions. However, the effects produced by the thio ether substituents of the present study have not been investigated in the 3′ position, and it is possible that these groups are too labile under biological conditions to survive long enough to exert their effects. The susceptibility of alkylthio and arylthio substituents to metabolic oxidation to the more polar sulfoxides and sulfones is well known.[20]

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Experimental Section[21]

N-Acetyl-3,5-di(ethylthio)-L-tyrosine Ethyl Ester (2).—A mixture of N-acetyl-3,5-diiodo-L-tyrosine ethyl ester[22] (**1**, 5.0 g,

(20) R. T. Williams, "Detoxication Mechanisms," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1959.

(21) Melting points were determined with a Thomas–Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by the Microanalytical Laboratory, University of California, Berkeley, Calif. Nmr spectra were obtained in $CDCl_3$ on a Varian A-60 (Me_4Si). Tlc (BAW) is BuOH–HOAc–H_2O 10:3:1 solvent system. Ir spectra were obtained with a Beckman IR-8 instrument. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. The absence of I_2 in compounds was determined by the sensitive test of heating a sample with concentrated H_2SO_4 over an open flame, and noting the absence of purple vapors. Nmr and ir spectra were consistent for all structures assigned. Compounds named as thyronine derivatives in the text, are named as in *Chemical Abstracts* in the Experimental Section.

(22) J. H. Barnes, E. T. Borrows, J. Elks, B. A. Hems, and A. G. Long, *J. Chem. Soc.*, 2824 (1949).

10 mmoles), cuprous ethyl mercaptide[16b] (3.8 g, 30 mmoles), quinoline (10 ml), and pyridine (0.5 ml) was stirred and heated under N_2 at a bath temperature of 175–178° for 3 hr. The black reaction mixture was poured onto a mixture of concentrated HCl (40 ml) and ice (200 g) and allowed to stand overnight. The solid was removed by filtration and extracted with three 100-ml portions of Et_2O and four 100-ml portions of EtOAc. The combined Et_2O and EtOAc extracts were washed with 5% HCl and with H_2O, dried (Na_2SO_4), and evaporated to yield 1.45 g of a brown solid. Crystallization from EtOH gave 0.89 g (22%) of **2** as yellow-white crystals, mp 103–103.5°, iodine absent. *Anal.* ($C_{17}H_{25}NO_4S_2$) C, H, S.

No formation of **5a** was detected by the attempted condensation of **2** with di(*p*-anisyl)iodonium bromide (**3a**) in the presence of Cu powder and Et_3N,[13,14] or KO-*t*-Bu in *t*-BuOH.[4]

N-Acetyl-3,5-diiodo-4-(3′-isopropyl-4′-methoxyphenoxy)phenyl-L-alanine Ethyl Ester (4b).—N-Acetyl-3,5-diiodo-L-tyrosine ethyl ester[22] (2.6 g, 5.2 mmoles), di(3-isopropyl-4-methoxyphenyl)iodonium iodide[23] (5.15 g, 9.3 mmoles), powdered Cu (45 mg), MeOH (62 ml), and Et_3N (0.77 ml) were stirred at room temperature for 24 hr. Additional MeOH (50 ml), Et_3N (0.8 ml), and Cu (45 mg) were added and stirring was continued for 24 hr. After filtration, the solvent was evaporated, and the residue was taken up in C_6H_6 (70 ml) and 3% HCl (50 ml) and shaken for 5 min. The precipitated $Et_3N \cdot HCl$ was removed by filtration, the C_6H_6 solution was washed (H_2O, 1 *N* NaOH, H_2O) and dried (Na_2SO_4), and the C_6H_6 was removed *in vacuo*. The addition of petroleum ether (bp 30–60°) (density, 0.67–0.69) gave a white solid, mp 129–131°. Crystallization from H_2O–EtOH yielded 1.2 g (36%); mp 129–131°, lit.[15] mp 129–131°; tlc (EtOAc), one spot, R_f 0.75; nmr and ir spectra were identical with a sample prepared by the method of Blank.[15] *Anal.* ($C_{23}H_{27}I_2NO_5$) C, H, I.

N-Acetyl-3,5-di(ethylthio)-4-(3′-isopropyl-4′-methoxyphenoxy)-phenyl-L-alanine Ethyl Ester (5b).—N_2 was passed through a stirred mixture of **4b** (3.2 g, 4.9 mmoles), cuprous ethyl mercaptide[16b] (3.25 g, 26.1 mmoles), quinoline (8.2 ml), and pyridine (0.41 ml), heated in a bath at 160–190° for 3.5 hr. The reaction mixture was poured onto HCl (40 ml) and ice (300 g) and allowed to stand overnight. The precipitated solid was removed by filtration and extracted with Et_2O (1 l.). The Et_2O solution was washed with 5% HCl (750 ml) and H_2O (800 ml), dried (Na_2SO_4), and evaporated to give 2.4 g of oily solid which was dissolved in C_6H_6 and chromatographed on acid-washed alumina (30 g). Elution with C_6H_6 and 20% $CHCl_3$–C_6H_6 and evaporation of the combined eluate gave a solid which was crystallized from EtOAc–hexane; mp 70–85°. Repeated washes with cold Et_2O yielded 100 mg (4%) of a white solid, mp 108–110°. An analytical sample was recrystallized from EtOAc–hexane and dried over P_2O_5 *in vacuo;* mp 114–116°, tlc, one spot (EtOAc), R_f 0.58. *Anal.* ($C_{27}H_{37}NO_5S_2$) C, H, S.

N-Acetyl-3,5-di(ethylthio)-4-(4′-methoxyphenoxy)phenyl-L-alanine Ethyl Ester (5a).—A mixture of N-acetyl-3,5-diiodo-4-(4′-methoxyphenoxy)phenyl-L-alanine ethyl ester[14] (**4a**, 3.8 g, 6 mmoles), cuprous ethyl mercaptide[16b] (4.0 g, 32 mmoles), quinoline (10 ml), and pyridine (0.5 ml) was stirred and heated for 3.5 hr at an oil bath temperature of 175–178°, then poured into concentrated HCl (40 ml) and ice (300 g). After standing for 8 hr, the precipitated solid was removed by filtration, then extracted with $CHCl_3$ (800 ml). The $CHCl_3$ extract was washed with 5% HCl (800 ml) and H_2O (500 ml), dried (Na_2SO_4), and evaporated *in vacuo*. The residual black oil was chromatographed on acid-washed alumina (70 g). Four 250-ml fractions were collected: C_6H_6, 20% $CHCl_3$ in C_6H_6, 50% $CHCl_3$ in C_6H_6, and $CHCl_3$. The residue from evaporation of the 50% $CHCl_3$ in C_6H_6 was chromatographed on silicic acid (35 g). After 200 ml of C_6H_6, 50% $CHCl_3$ in C_6H_6 was the eluting solvent. Evaporation yielded a brown solid which was recrystallized from EtOAc–hexane (Norit) to give 60 mg (2%) of **5a**: mp 107–108°; iodine absent; tlc, one spot, R_f 0.71 (EtOAc). *Anal.* ($C_{24}H_{31}NO_5S_2$) C, H, S.

N-Acetyl-3,5-di(phenylthio)-4-(3′-isopropyl-4′-methoxyphenoxy)phenyl-L-alanine Ethyl Ester (8b).—A stirred mixture of **4b** (3.2 g, 4.9 mmoles), cuprous phenyl mercaptide[16a] (4.5 g, 26 mmoles), quinoline (8.2 ml), and pyridine (0.4 ml), through which N_2 was passed, was heated at a bath temperature of 168–170° for 3.5 hr. The reaction mixture was poured onto a mixture of ice (300 g) and HCl (40 ml) and allowed to stand overnight. The precipitated solid was removed by filtration, extracted with Et_2O (1 l.), H_2O (1.5 l.), 10% $NaHSO_3$ (100 ml), and H_2O (200 ml), and dried (Na_2SO_4). The ether was removed by distillation, leaving a dark oil (3.4 g) which solidified on standing; colorless crystals from Et_2O, 1.21 g (40%), mp 94–99°. An analytical sample was recrystallized from Et_2O; mp 97–99°, iodine absent. *Anal.* ($C_{35}H_{37}NO_5S_2$) C, H; S: calcd, 10.41; found, 9.89.

The same reaction carried out in the absence of N_2 resulted in a significantly lower yield.

N-Acetyl-3,5-di(phenylthio)-4-(4′-methoxyphenoxy)phenyl-L-alanine Ethyl Ester (8a).—A mixture of **4a** (3.8 g, 6 mmoles), cuprous phenyl mercaptide[16a] (5.3 g, 32 mmoles), quinoline (10 ml), and pyridine (0.5 ml) was heated at a bath temperature of 175–183° for 3.5 hr without N_2 cover. The reaction mixture was treated as was **8b**, to yield 4.6 g of a dark oil which solidified on standing. Crystallization (EtOAc–heptane) followed by washes with cold Et_2O gave 130 mg (4%) as colorless crystals, mp 115–117°, iodine absent. *Anal.* ($C_{32}H_{31}NO_5S_2 \cdot 0.5H_2O$) C, H, S.

3,5-Di(phenylthio)-4-(4′-hydroxyphenoxy)phenyl-L-alanine (9a).—N_2 was passed through a solution of **8a** (267 mg, 0.45 mmole) in 4.3 ml of HOAc for 30 min. HBr (48%, 1.1 ml) was added and the solution was heated under N_2 and refluxed for 3.5 hr. A white precipitate formed on addition of H_2O. The solvents were removed *in vacuo*, the residue was dissolved in 5% NaOH (70 ml) and filtered, and the pH was adjusted to 5.0 with 4 *N* HCl giving 172 mg (77%) of a white solid, mp 225–245° dec. Two more isoelectric precipitations gave 132 mg (60%) of a white solid: mp 232–236° dec; tlc (BAW), one spot, ninhydrin positive, Pauly positive. *Anal.* ($C_{27}H_{23}NO_4S_2$) C, H; S: calcd, 13.10; found, 12.49.

3,5-Di(phenylthio)-4-(3′-isopropyl-4′-hydroxyphenoxy)phenyl-L-alanine (9b).—The N-acetyl ester (**8b**) (130 mg, 0.21 mmole) was heated under reflux (N_2) in HOAc (2.2 ml) and 48% HBr (0.6 ml) for 3.5 hr. Work-up as described for **9a** gave 68 mg (61%); mp 154–159° dec; tlc (BAW), one spot, ninhydrin positive, Pauly positive. *Anal.* ($C_{30}H_{29}NO_4S_2 \cdot 0.5H_2O$) C, H, S.

Attempted Formation of 3,5-Di(ethylthio)-4-(3′-isopropyl-4′-hydroxyphenoxy)phenyl-L-alanine (6b).—The N-acetyl ethyl ester (**5b**) (100 mg) in 2 ml of HOAc and 0.5 ml of 40% HBr was heated under reflux for 4 hr (N_2). Additional HOAc (1 ml) and HBr (0.3 ml) were added at 1, 2, and 3 hr. Work-up as described for **9a** gave 64 mg, mp 132–220°. Nmr and ir showed absence of OCH_3, amide, and ester groups, and a partial loss of SC_2H_5; tlc (BAW), two spots, ninhydrin positive, Pauly positive. The mixture was used in biological testing. *Anal.* Calcd for $C_{22}H_{29}NO_4S_2$: C, 60.66; H, 6.71; N, 3.21; S, 14.72. Found: C, 57.09; H, 5.95; N, 3.20; S, 10.33.

3,5-Di(ethylthio)-4-(4′-hydroxyphenoxy)phenyl-L-alanine (6a).—The N-acetyl ethyl ester **5a** (200 mg) in 4 ml of HOAc and 1 ml of 48% HBr was heated under reflux for 3 hr. The solvents were removed *in vacuo*, and the residue was dissolved in 5% NaOH and precipitated at pH 5.0 by addition of dilute HCl. The precipitate was filtered, dissolved in dilute HCl, and precipitated at pH 5.0 with 5% NaOH, giving 125 mg (72%); mp 205° dec; tlc (BAW), one spot, ninhydrin positive, Pauly positive; λ_{max} (0.05 *N* NaOH) 301 mμ (ϵ 4120), λ_{max} (dilute HCl) 284 mμ (ϵ 3940). *Anal.* ($C_{19}H_{25}NO_5S_2$) H, S; C: calcd, 55.45; found, 56.04.

3,5-Di(ethylthio)-4-(3′-isopropyl-4′-methoxyphenoxy)phenyl-L-alanine (7).—The N-acetyl ethyl ester (**5b**) (100 mg) in 3.6 ml of HOAc and 2.4 ml of HCl was heated under reflux for 3.5 hr (N_2). Additional 1-ml portions of HCl and HOAc were added at 1 hr and 2.5 hr. Addition of 2 *N* NaOH to pH 5.0 gave a precipitate which was collected, dissolved in HOAc–HCl, and reprecipitated at pH 5.0 with 2 *N* NaOH to give 32 mg (36%) of a white solid: mp 175–180° dec; tlc (BAW), one spot, ninhydrin positive, Pauly negative. *Anal.* ($C_{23}H_{31}NO_4S_2 \cdot H_2O$) C; H: calcd, 7.11; found, 6.46; S: calcd, 13.71; found, 12.96.

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