expected order of activity, expressed in terms of the $-NR_1R_2$ segment, paralleling that of the respective I_{50} values (see Table II). Indeed, the significant parallel of these relationships holds true when it is considered in three additional sets of compounds listed in Table III (VI and VII, XIV and XV, and XVII and XVIII).

An additional factor which should be considered in predicting comparative anticholinesterase activities of alkyl-substituted amides is the lipophilic–lipophobic nature of the substituent groups. As the N-substituted group becomes more fat soluble, the lipophilic–lipophobic ratio for the substituent should increase in the order $H < CH_3 < C_2H_5$. This leads to the same order of activity suggested earlier.

Our study of variations in the amide structure was extended to include cyclic pyrrolidide, piperidide, and morpholide substituents. As expected, the piperidides (Table III, compounds IX, XII, and XX) were in all instances more powerful inhibitors than the corresponding pyrrolidides (VIII, XI, and XIX). Likewise, as anticipated, the morpholides (X and XIII) were weaker inhibitors than the similarly constituted pyrrolidides or piperidides. Thus, replacement of a methylene group in the 4-position of the piperidine ring by an electronegative oxygen (or introduction of oxygen into the pyrrolidine ring) results in a marked lessening of activity. This may be due to two factors: (1) the ring oxygen competes with the electron-attracting part of the amide function for the electrons of the morpholide's alkylene units, and/or (2) the decreased lipophilic character of the inhibitor molecule resulting from replacement of the piperidide with the more polar morpholide.

Isomers containing the amide function in the 4-position (Table III, footnotes *d* and *f*, compounds XXIX and XXXI) were less effective inhibitors than their 3-substituted counterparts (V and VII); this is in accordance with Wilson and Quan's conclusion,[21] relative to interatomic distances between the pertinent reactive functions.

The 3,4-unsaturated analogs of derivatives V and VII (Table III, footnotes *c* and *e*, compounds XXVIII and XXX) were less effective than the corresponding saturated compounds, confirming our earlier findings[2] concerning the introduction of 3,4-unsaturation into the piperidine moiety.

An important objective of this study was to substantiate further the data obtained in our preceding experiments.[2] We consider it an important observation that, in each instance, (1) among the decanes the monosubstituted one is the more powerful inhibitor, and (2) among the ethanes the bis-substituted derivative has the more potent inhibitory action.

A more comprehensive interpretation of this work and that currently in progress will be reported at a later date.

Acknowledgment.—We wish to acknowledge valuable discussions with Dr. Andrew Lasslo which contributed to the successful completion of this phase of our study.

(21) I. B. Wilson and C. Quan, *Arch. Biochem. Biophys.*, **73**, 131 (1958)

Thyroxine Analogs. XI.[1] Structural Isomers of 3,5,3′-Triiodo-DL-thyronine

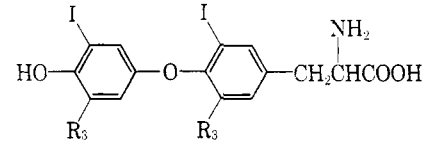
EUGENE C. JORGENSEN AND JAMES A. W. REID

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California

Received June 22, 1964

The synthesis of two structural isomers of 3,5,3′-triiodo-DL-thyronine is described, namely, 3-[2-(3-iodo-4-hydroxyphenoxy)-3,5-diiodophenyl]-DL-alanine (VIIa) and 3-[5-(3-iodo-4-hydroxyphenoxy)-2,4-diiodophenyl]-DL-alanine (VIIb). Both isomers were found to be inactive in an antigoiter assay in the rat which would have determined activity greater than 1.5% that of L-thyroxine.

The L-alanine side chains of the thyroid hormones, thyroxine (Ia) and triiodothyronine (Ib), have been replaced by a variety of ionizable groups with retention of thyroxine-like activity.[2] However, no such side-chain-substituted analog has proven more potent than the naturally occurring hormones with respect to the major physiological effects in the intact animal. Unusually high activity found for the aliphatic carboxylic acid side-chain congeners in inducing metamorphosis in tadpoles appears to be related to the absorption of the analogs from test solutions in which the tadpoles were immersed.[3] No analogs have been reported in which the side chain is moved to other positions on the same ring, although an approach to the synthesis of such isomers has been reported,[4] as have analogs with alanine side chains in both phenyl rings.[5]



Ia, $R_3 = R_3' = I$
b, $R_3 = I$; $R_3' = H$
c, $R_3 = R_3' = H$
d, $R_3 = H$; $R_3' = I$

The nature and position of substituents in the ring bearing the side chain ("inner ring") is important to

(1) (a) Paper X: E. C. Jorgensen and R. A. Wiley, *J. Med. Chem.*, **6**, 459 (1963). (b) This investigation was supported by Research Grant AM-04223 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service. (c) Presented before the Division of Medicinal Chemistry, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept., 1964.

(2) (a) H. A. Selenkow and S. P. Asper, Jr., *Physiol. Rev.*, **35**, 426 (1955); (b) T. C. Bruice, N. Kharasch, and R. J. Winzler, *Arch. Biochem. Biophys.*, **62**, 305 (1956).

(3) E. Frieden and G. Westmark, *Science*, **133**, 1487 (1961).

(4) E. L. Jackson, *J. Org. Chem.*, **25**, 2227 (1960).

(5) E. C. Jorgensen and R. Cavestri, *J. Pharm. Sci.*, **52**, 481 (1963).

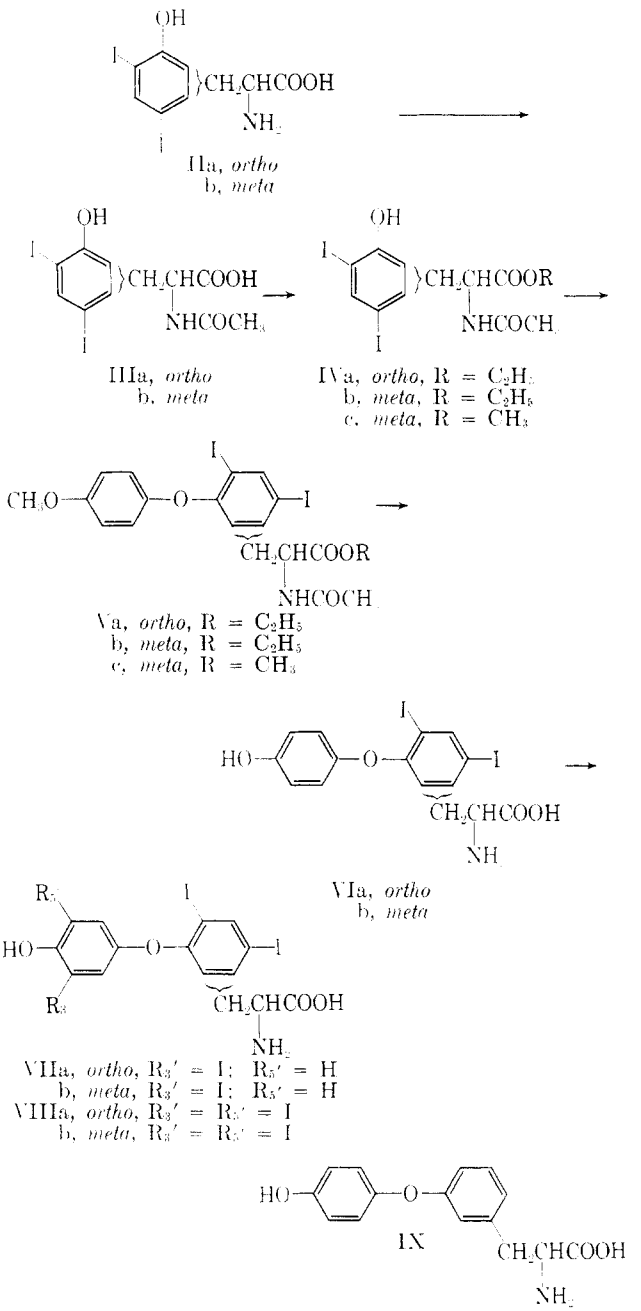
the retention of thyroxine-like biological activity. It has been observed that thyroxine analogs lacking 3,5-disubstitution, such as 3,3′-diiodothyronine (Ic) and 3,3′,5′-triiodothyronine (Id), possessed low thyroxine-like activity,[6] and also showed antagonistic effects to thyroxine.[7] Iodine has been replaced by methyl groups with retention of thyroxine-like activity,[8] while inactive analogs were produced with a variety of other substituents in the 3,5-positions.[1a] Steric effects of bulky groups in the 3,5-positions have been considered of importance in orienting the phenolic ("outer") ring.[9] In addition to this steric effect, it seemed possible that electronic contributions by the methylene group of the alanine side chain, as well as the 3,5-substituents, could act to enhance interaction of the inner ring with some biological receptor.[9] In such an event, the position of substitution of the inner-ring iodine atoms and the alanine side chain might not be important as long as these were *meta* to each other, and two of these bulky groups occupied the 3,5-positions. Such a compound is the isomer of 3,5,3′-triiodothyronine in which the positions of the alanine group and 3-iodine atom have been exchanged (VIIa). The isomer VIIb, in which the alanine side chain is *meta* to the ether linkage and which lacks a bulky 3-substituent, could be considered an analog of 3,3′-diiodothyronine (Ic), if there were no specific steric requirement for the alanine side chain. In view of these considerations, it was of interest to see if the lack of specificity regarding the nature of the side chain was also true of its position of substitution. Therefore, the synthesis and biological evaluation of the inner-ring isomers (VIIa,b and VIIIa,b) of the thyroid hormones were undertaken.

The same general synthetic route was used for both isomers (see Chart I). *o*- and *m*-Tyrosine were iodinated to yield the diiodo derivatives IIa and IIb. Jackson[10] has established the positions of the iodine atoms in diiodo-*m*-tyrosine (IIb). The positions of the iodine atoms in IIa have not been rigorously established; however, under the conditions of the reaction, *ortho* and *para* substitution relative to the phenolate ion of *o*-tyrosine would be expected to take place, yielding the diiodo compound IIa.

The amino and carboxyl groups of the side chain were then protected by acetylation and esterification. The yields of the esterification reaction to form the ethyl esters (IIIa,b → IVa,b) were low, and the products were difficult to purify. By using diazomethane to prepare the methyl ester IVc, some improvement in yield and purity was obtained.

The condensations of 4,4′-dimethoxydiphenyliodonium bromide with IVa and IVb (and IVc) in the presence of triethylamine and copper gave the diphenyl ethers Va and Vb (also Vc), respectively, according to the method of Bevilacqua, *et al.*[11] Hydrolysis of Va

CHART I[a]



[a] "*ortho*" and "*meta*" refer to the position of the alanine side chain relative to the OH group in compounds II–IV and to the diphenyl ether oxygen in the thyronine analogs (V–VIII).

with a hydriodic–acetic acid mixture proceeded normally to give the expected amino acid VIa. The hydrolysis of Vc by this method gave, as the only isolable product, the deiodinated derivative IX (see Chart I).[12] It appears that the treatment of derivatives of iodophenyl phenyl ethers (X) with hydriodic acid results readily in the loss of iodine when the 5-position is vacant.[13] During removal of an O-methyl protective group, Meltzer, *et al.*[14] reported that treatment of β-

(6) (a) C. L. Gemmill, *Am. J. Physiol.* **186**, 1 (1956); (b) C. J. Shellabarger, *Endocrinology*, **65**, 503 (1959); (c) E. G. Tomich, E. A. Wollett, and M. A. Pratt, *J. Endocrinol.*, **20**, 65 (1960).

(7) S. B. Barker, C. S. Pittman, J. A. Pitman, Jr., and S. R. Hill, Jr., *Ann. N. Y. Acad. Sci.*, **86**, 545 (1960).

(8) E. C. Jorgensen and R. A. Wiley, *J. Med. Pharm. Chem.*, **5**, 1307 (1962).

(9) E. C. Jorgensen, P. A. Lehman, C. Greenberg, and N. Zenker, *J. Biol. Chem.*, **237**, 3832 (1962).

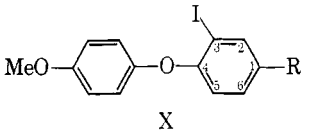
(10) E. L. Jackson, *J. Am. Chem. Soc.*, **77**, 486 (1955).

(11) P. F. Bevilacqua, J. T. Plati, and W. Wenner, U. S. Patent 2,895,927 (July 21, 1959).

(12) We are indebted to Dr. Hans J. Cahnmann for informing us of the facile deiodination of Vb, which greatly aided our work. See T. Shiba, A. Höfer, and H. J. Cahnmann, *J. Org. Chem.*, **29**, 3171 (1964).

(13) A study of the relative ease of deiodination by hydriodic acid of some iodinated aryl ethers has been carried out, and a hypothesis has been advanced regarding the reason for differences in deiodination rates: E. C. Jorgensen and J. A. W. Reid, *ibid.*, **29**, 3396 (1964).

(14) R. I. Meltzer, S. Farber, E. Merrill, and A. Caro, *ibid.*, **26**, 1413 (1961).



X

[3-iodo-4-(4-methoxyphenoxy)phenyl]propionic acid (X, R = CH_2CH_2COOH) with a mixture of hydriodic and acetic acids gave β-[4-(4-hydroxyphenoxy)-phenyl]propionic acid and not the expected 3-iodo derivative. However, treatment of X (R = CH_2-CH_2COOH) with modified conditions developed by Block and Powell[15] gave the required 3-iodo derivative in low yield. Gemmill, *et al.*,[16] successfully converted α-benzamido-3-iodo-4-(4-methoxyphenoxy)cinnamic acid to DL-3-iodothyronine with a mixture of hydriodic, hydrobromic, and acetic acids containing red phosphorus. In this case it appears that the hydriodic acid was used to reduce the double bond of the cinnamic acid derivative, leading to reaction conditions for cleavage of the methyl ether which would not result in loss of the labile 3-iodine. We have found that a mixture of hydrobromic and acetic acids containing red phosphorus gave VIb in 77% yield from Vb (and Vc).

Iodination of VIa and VIb using 70–90% of the calculated amount of iodine for monoiodination resulted in mixtures containing at least two components as shown by paper chromatography. Purification by means of repeated isoelectric precipitations gave the required iodinated derivatives VIIa and VIIb.

An attempt was made to prepare the tetraiodo analog VIIIa from VIa, but the addition of more than 75% of the required amount of iodine for diiodination caused the reaction mixture to turn dark brown in color. Elemental analysis on the isolated product was low in iodine.[17] No attempt was made to make VIIIb because of insufficient quantities of VIb and because of the failure to make VIIIa. A similar phenomenon has been observed in the iodination of 3,5-dimethylthyroformic acid,[18] where the 3′-iodo but not the 3′,5′-diiodo derivatives could be prepared.

Biological Results and Discussion

The compounds VIIa and VIIb were tested for thyroxine-like activity by the rat antigoiter procedure as described previously.[19] The results and estimates of potency relative to L-thyroxine are shown in Table I. The isomer VIIa was tested at molar ratios of 50:1, 10:1, and 2:1 with respect to a standard effective dose of 3 γ of sodium L-thyroxine pentahydrate for each 100 g. of body weight. On the same molar scale, VIIb was tested at ratios of 50:1 and 10:1. Both compounds at all dose levels produced thyroid weights which were the same as the thiouracil control and were significantly larger than those thyroid weights produced by the standard doses of L-thyroxine. The isomers were therefore inactive at these levels. Comparisons with the effects produced by graded doses of L-thyroxine (Table I) showed that compounds

(15) P. Block and G. Powell, *J. Am. Chem. Soc.*, **64**, 1070 (1942).
(16) C. L. Gemmill, J. J. Anderson, and A. Burger, *ibid.*, **78**, 2434 (1956).
(17) *Anal.* for VIIIa. Calcd. for $C_{15}H_{11}I_4NO_4$: C, 23.18; H, 1.43; I, 65.35. Found: C, 23.33; H, 1.80; I, 59.76.
(18) E. Van Heyningen, *J. Org. Chem.*, **26**, 3850 (1961).
(19) E. C. Jorgensen and P. Slade, *J. Med. Pharm. Chem.*, **5**, 729 (1962).

TABLE I

RAT ANTIGOITER ASSAY OF THYROXINE ANALOGS[a]

Food	Compd. injected	Daily dose, γ/100 g.	Molar ratio	Thyroid wt. mg./100 g. ± S.D.	Approx. activity
Untreated	...	...	...	9.0 ± 1.10	...
Tu[b]	...	...	...	27.0 ± 6.1	...
Tu	Thyroxine[c]	2.0	0.67	13.7 ± 1.9	100
Tu	Thyroxine	3.0	1.0	12.9 ± 4.5	100
Tu	Thyroxine	4.5	1.5	6.6 ± 1.6	100
Tu	VIIa	4.5	2	23.0 ± 4.6	...
Tu	VIIa	22.5	10	27.7 ± 3.9	...
Tu	VIIa	112.5	50	31.1 ± 4.8	<1.5
Tu	VIIb	22.5	10	26.4 ± 5.2	...
Tu	VIIb	112.5	50	30.1 ± 7.2	<1.5

[a] Six rats were used at each control and dose level. [b] Thiouracil, 0.3%. [c] Sodium L-thyroxine pentahydrate.

VIIa and VIIb are either inactive or have less than 1.5% the activity of L-thyroxine.

As the isomers VIIa and VIIb have all the "prime-ring" substituents necessary for maximum thyromimetic activity (*cf.* 3,5,3′-triiodothyronine, Ia), it appears that this activity is almost completely lost when the alanine side chain is moved from its position *para* to the diphenyl ether linkage. Since the biological evaluation was carried out in intact animals, certain limitations are imposed on interpretation of the results. The loss of activity observed could be due to the effect of chemical alteration on the normal pattern of distribution and metabolism. For example, decreased ability to bind with transport proteins would be expected to alter activity. If, however, normal transport to the biological receptor proposed earlier[9] occurs, it is apparent that a high degree of specificity is associated with the position of substitution of the alanine side chain with respect to binding and action at the site. The inactivity shown by the analogs VIIa and VIIb could be related to an inability to bind at the receptor due to the change in the normal 1,4-relationship of the ether oxygen and the side chain. Alternatively, the requirements for binding by the inner ring may be preserved, but the critical position in space for the phenolic outer ring would be altered, and interaction with a "functional" portion of the receptor could not occur. Further studies on the protein-binding affinities of the analogs, and their abilities to act as thyroxine antagonists may help to clarify these points.

In summary, the loss in activity shown by isomers of triiodothyronine suggests that the position of the ionizing side chain *para* to the diphenyl ether linkage is essential in conferring significant thyroxine-like activity.

Experimental

All melting points were taken with a Thomas–Hoover capillary melting point apparatus and are corrected. Microanalyses were carried out by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, Calif. Paper chromatography was carried out on Whatman No. 1 paper which had been washed with 2 *N* ammonium hydroxide. Compounds were detected using 0.5% ninhydrin in butanol.

DL-*o*-Tyrosine.—The method of Sealock, Speeter, and Schweet[20] for preparing DL-*m*-tyrosine was used. Salicylalde-

(20) R. R. Sealock, M. E. Speeter, and R. S. Schweet, *J. Am. Chem. Soc.* **73**, 5386 (1951).

hyde[21] was converted in 26% yield to DL-*o*-tyrosine, m.p. 245° dec. Crystallization of a sample from a water–methanol mixture (equal volumes) raised the m.p. to 250–251° dec. (lit.[22] m.p. 249–250° dec.).

3-(2-Hydroxy-3,5-diiodophenyl)-DL-alanine (IIa).—A modification of Dickinson and Marshall's[23] method was used. DL-*o*-Tyrosine (34.0 g., 0.20 mole) was dissolved in 500 ml. of concentrated ammonium hydroxide (*d* 0.90), and the solution was cooled in ice. A 32% iodine solution (in KI) was added slowly with occasional shaking until the color of the solution became dark brown. The pH was adjusted to 5 with concentrated HCl to give the crude diiodo compound (68.2 g., 92%), m.p. 212° dec. (lit.[23] m.p. 211° dec.). Purification by means of two isoelectric precipitations gave the amino acid (IIa), m.p. 222° dec.

N-Acetyl-3-(2-hydroxy-3,5-diiodophenyl)-DL-alanine (IIIa).—The method of Barnes, *et al.*,[24] was used. A solution of IIa (30.0 g., 0.071 mole) in 2 *N* sodium hydroxide (300 ml.) was stirred at 5–10° while acetic anhydride (32 ml.) was added during 1 hr. After standing overnight, the solution was treated with sodium hydroxide (15.0 g.) in water (75 ml.) and left at room temperature for 3 hr. Ethanol (50 ml.) was added and the solution was adjusted to pH 2 with concentrated HCl to give the N-acetyl derivative (IIIa) as a semicrystalline sirup (~28.0 g.). This was used in the preparation of IVa without further purification.

N-Acetyl-3-(2-hydroxy-3,5-diiodophenyl)-DL-alanine Ethyl Ester (IVa).—The method of Barnes, *et al.*,[24] was used. The N-acetyl derivative (IIIa) (~28.0 g.), *p*-toluenesulfonic acid (3.0 g.), ethanol (30 ml.), and chloroform (500 ml.) were heated under reflux for 8 hr. with a Soxhlet extraction system containing anhydrous sodium sulfate. On cooling, the solution was washed with aqueous sodium bicarbonate. Evaporation of the solvent gave a semisolid residue (19.4 g.) which was treated twice with Norit A in aqueous ethanol to give the ethyl ester (IVa) (10.0 g., 28% based on IIa), m.p. 147–148°.

Anal. Calcd. for $C_{13}H_{15}I_2NO_4$: C, 31.04; H, 3.00; I, 50.47. Found: C, 30.99; H, 2.82; I, 50.18.

N-Acetyl-3-[2-(4-methoxyphenoxy)-3,5-diiodophenyl]-DL-alanine Ethyl Ester (Va).—The method of Bevilacqua, *et al.*,[11] was used. A mixture of IVa (5.7 g., 0.011 mole), di(*p*-anisyl)-iodonium bromide[25] (9.6 g., 0.023 mole), methanol (150 ml.), triethylamine (2.0 ml.), and powdered copper (0.06 g.) was stirred at room temperature for 24 hr. Evaporation of the methanol gave a residue which was shaken vigorously for 5 min. with benzene (100 ml.) and 3% aqueous HCl (70 ml.). The benzene layer was washed with water (50 ml.), extracted twice with *N* sodium hydroxide (30 ml.), washed three times with water (40 ml.), and dried with sodium sulfate. After evaporation of the benzene, petroleum ether (60 ml.) (b.p. 30–60°) was added to the residue and, on standing overnight at 0°, the diphenyl ether (Va) (3.90 g., 55%), m.p. 89–93°, was removed by filtration. Treatment with Norit A in aqueous ethanol gave an analytical sample of the ether Va, m.p. 113–114°.

Anal. Calcd. for $C_{20}H_{21}I_2NO_5$: C, 39.44; H, 3.48; I, 41.68. Found: C, 39.22; H, 3.54; I, 41.78.

3-[2-(4-Hydroxyphenoxy)-3,5-diiodophenyl]-DL-alanine (VIa).—A mixture of 3.0 g. of Va, 47% aqueous hydriodic acid (30 ml.), and glacial acetic acid (30 ml.) was heated under reflux for 4 hr. The excess hydriodic and acetic acids were removed by distillation, and the residue was dissolved in water (50 ml.) containing ethanol (10 ml.). The pH was adjusted to 5 to give the amino acid (VIa) (1.6 g., 94%), m.p. 257–258° dec. A second isoelectric precipitation raised the m.p. to 264° dec., R_f 0.72 (solvent system: isoamyl alcohol–2 *N* ammonium hydroxide).

Anal. Calcd. for $C_{15}H_{13}I_2NO_4$: C, 34.31; H, 2.50; I, 48.38. Found: C, 34.55; H, 2.76; I, 48.05.

3-[2-(3-Iodo-4-hydroxyphenoxy)-3,5-diiodophenyl]-DL-alanine (VIIa).—The method used is that described by Chalmers, *et al.*,[26] for preparing thyroxine from 3,5-diiodothyronine. Iodine (0.45 g., 1.7 mmoles) in concentrated potassium iodide solution (5.0 ml.) was added dropwise to a stirred solution of VIa (1.0 g., 1.9 mmoles) in 33% aqueous ethylamine (10 ml.) at 10°. Stirring was continued for 5 min., the pH was adjusted to 4.5 with 16% aqueous HCl, while keeping the temperature of the solution below 20°, and the yellow precipitate was removed by filtration. This was dissolved in aqueous 2 *N* NaOH, filtered, and the pH of the filtrate was adjusted to 5 with concentrated HCl to give a pale yellow solid, m.p. 200–205° dec. Two more isoelectric precipitations gave a product which was dissolved in aqueous 2 *N* HCl–ethanol (1:1). The pH was adjusted to 5 to give VIIa as the monohydrate (95 mg., 8.0%), m.p. 207–208° dec., R_f 0.72 (solvent system: isoamyl alcohol–2 *N* ammonium hydroxide).

Anal. Calcd. for $C_{15}H_{12}I_3NO_4 \cdot H_2O$: C, 26.93; H, 2.11; I, 56.92. Found: C, 27.09; H, 2.20; I, 57.18.

Paper chromatography showed that the initial crude product contained traces of a slower moving impurity (R_f 0.51). This was probably the tetraiodo derivative (VIIIa) which was removed during the final precipitation.

DL-*m*-Tyrosine.—*m*-Hydroxybenzaldehyde[27] (122.0 g., 1 mole) was converted in 52% yield by the method of Sealock, Speeter, and Schweet[20] to DL-*m*-tyrosine, m.p. 274–275° dec. (lit.[20] m.p. 283° dec.).

3-(5-Hydroxy-2,4-diiodophenyl)-DL-alanine (IIb).—DL-*m*-Tyrosine (37.0 g., 0.2 mole) was iodinated as described in the preparation of IIa. Two isoelectric precipitations on the crude product gave 38 g. (43%) of IIb, m.p. 228° dec. (lit.[23] m.p. 230° dec.).

N-Acetyl-3-(5-hydroxy-2,4-diiodophenyl)-DL-alanine (IIIb).—IIb (38.0 g., 0.087 mole) was acetylated as described in the preparation of IIIa. The crude product IIIb (33.0 g., 80%) solidified on standing at 0° for 24 hr., m.p. 195–199° (lit.[10] m.p. 200°). IIIb was used in the next step without purification.

Esterification of N-Acetyl-3-(5-hydroxy-2,4-diiodophenyl)-DL-alanine. A. Ethyl Ester (IVb).—IIIb (33.0 g., 0.07 mole) was esterified as described in the preparation of IVa, yielding 18.0 g. (51%) of IVb as a glassy brown solid. This was purified by dissolving in ethyl acetate and filtering through acid-washed alumina, followed by three treatments with Norit A in aqueous ethanol to give IVb (2.0 g.), m.p. 153–154°.

Anal. Calcd. for $C_{13}H_{15}I_2NO_4$: C, 31.04; H, 3.00; I, 50.47. Found: C, 30.90; H, 3.07; I, 50.22.

B. Methyl Ester (IVc).—IIIb (40.0 g., 0.084 mole) in ethanol (200 ml.) was treated with diazomethane (0.09 mole) in ether (500 ml.) at 5°. Evaporation of the ether and ethanol gave the crude methyl ester (IVc) (25.0 g., 60%) which solidified on treatment with petroleum ether at 0°, m.p. 90–145°. Two further recrystallizations from methanol gave white crystals which melted slowly over the range 95–160°.

Anal. Calcd. for $C_{12}H_{13}I_2NO_4$: C, 29.47; H, 2.67. Found: C, 29.49; H, 2.68.

N-Acetyl-3-[5-(4-methoxyphenoxy)-2,4-diiodophenyl]-DL-alanine Ethyl Ester (Vb).—The crude ethyl ester (IVb, 18.0 g., 0.03 mole) was condensed with di(*p*-anisyl)iodonium bromide as described in the preparation of Va. This gave 4.0 g. (20%) of Vb, m.p. 110–115°. Treatment with Norit A in ethanol gave white crystals (1.7 g.), m.p. 173–174°.

Anal. Calcd. for $C_{20}H_{21}I_2NO_5$: C, 39.44; H, 3.48; I, 41.68. Found: C, 39.03; H, 3.38; I, 41.56.

N-Acetyl-3-[5-(4-methoxyphenoxy)-2,4-diiodophenyl]-DL-alanine Methyl Ester (Vc).—The methyl ester (IVc) was converted in 27% yield to Vc as described in the preparation of Va. The white crystals (from methanol) had m.p. 146°.

Anal. Calcd. for $C_{19}H_{19}I_2NO_5$: C, 38.35; H, 3.22. Found: C, 38.61; H, 3.04.

3-[5-(4-Hydroxyphenoxy)-2,4-diiodophenyl]-DL-alanine (VIb).—A mixture of Vb (1.65 2.70 g., mmoles), glacial acetic acid (48 ml.), red phosphorus (2.4 g.), and aqueous 48% HBr (6.0 ml.) was heated under reflux for 3.25 hr. The volume of the reaction mixture was reduced one-half by distillation and, after filtration, water (30 ml.) was added. The pH was adjusted to 5 with sodium acetate to give the free amino acid (VIb) as a white solid (0.96 g., 77%), m.p. 257° dec. The amino acid was dissolved

(21) Salicylaldehyde (from bisulfite compound), Eastman Organic Chemicals.

(22) W. P. Dickinson and P. G. Marshall, *J. Chem. Soc.*, 1495 (1929).

(23) W. P. Dickinson and P. G. Marshall, *ibid.*, 2289 (1930).

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

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

(26) J. R. Chalmers, G. T. Dickson, J. Elks, and B. A. Hems, *J. Chem. Soc.*, 3421 (1949).

(27) Eastman practical grade *m*-hydroxybenzaldehyde was crystallized from water to constant m.p. 103–104° (lit.[28] m.p. 101–102°). A comparison of the infrared spectra of *o*-, *m*-, and *p*-hydroxybenzaldehyde showed no contamination of the purified *meta* isomer.

(28) R. B. Woodward and W. E. Doering, *J. Am. Chem. Soc.*, **67**, 860 (1945).

in aqueous 2 *N* NaOH and the pH was adjusted to 5 with acid to give VIb (0.7 g.), m.p. 237° dec. as the monohydrate, R_f 0.67 (solvent system: isoamyl alcohol–6 *N* ammonium hydroxide).

Anal. Calcd. for $C_{15}H_{13}I_2NO_4 \cdot H_2O$: C, 33.20; H, 2.75; I, 46.74. Found: C, 33.32; H, 2.77; I, 46.94.

The methyl ester Vc was hydrolyzed in comparable yield as described above to give the amino acid VIb as a white solid, m.p. 235° dec. There was no depression of melting point on admixture with an analytically correct sample prepared from the ethyl ester; the infrared spectra were identical.

Treatment of Vc with Hydriodic Acid.—Vc (200 mg.) was heated under reflux for 4 hr. with 47% aqueous hydriodic acid (3.0 ml.) and glacial acetic acid (3.0 ml.). The reaction mixture was poured into water (20 ml.) and the pH was adjusted to 4.5 with sodium acetate. The precipitate was treated with Norit A at 80° for 1 min. in 2 *N* aqueous HCl. Adjustment of the pH to 4.5 gave a white precipitate of 3-[3-(4-hydroxyphenoxy)-phenyl]-DL-alanine (15 mg., 17%), m.p. 239–240° dec. There was depression of melting point on admixture with a sample of VIb.

Anal. Calcd. for $C_{15}H_{15}NO_4$: C, 65.93; H, 5.54. Found: C, 65.76; H, 5.19.

3-[5-(3-Iodo-4-hydroxyphenoxy)-2,4-diiodophenyl]-DL-alanine (VIIb).—VIb (0.6 g., 1.1 mmoles) was iodinated as described in the preparation of VIIa. The crude product was dissolved in a mixture of 2 *N* NaOH (10 ml.) and ethanol (20 ml.). Adjustment of the pH to 5 gave a yellow precipitate which was removed by filtration. The volume of the filtrate was reduced one-half by evaporation. Addition of water (5 ml.) precipitated the amino acid (VIIb). This procedure was repeated four times to give VIIb (72 mg., 9%), m.p. 197–198° dec. as the hemihydrate, R_f 0.58 (solvent system: isoamyl alcohol–6 *N* ammonium hydroxide).

Anal. Calcd. for $C_{15}H_{12}I_3NO_4 \cdot 0.5H_2O$: C, 27.30; H, 1.99; I, 57.69. Found: C, 27.73; H, 2.28; I, 57.42.

Paper chromatography of the crude product showed traces of a slower moving impurity (R_f 0.30) which probably corresponds to the tetraiodinated derivative (VIIIb). This was removed during the purification procedure, and paper chromatography also showed the absence of the starting material VIb (R_f 0.67).

Lower 2′-Alkylthio Analogs and Derivatives of Griseofulvin *via* a Mercaptanolysis Reaction[1]

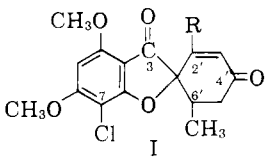
B. K. Koe and W. D. Celmer

Medical Research Laboratories, Chas. Pfizer and Company, Inc., Groton, Connecticut

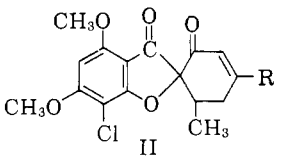
Received May 27, 1963

The synthesis of several homologous 2′,4′,4′-tris(alkylthio)-7-chloro-4,6-dimethoxy-6′-methyl-2′-grisen-3-ones by the acid-catalyzed reaction of griseofulvin with lower alkanethiols is described. A convenient route to the corresponding 2′-alkylthio analogs of griseofulvin is provided by the facile removal of the 4′-mercaptole group from the novel tris(alkylthio) derivatives. In a disk–plate assay against *Phoma*, the following biological activities *in vitro* are observed: 2′-methylthio and 2′-ethylthio analogs, as active as griseofulvin; 2′-ethoxy analog, more active than griseofulvin; 2′-*n*-propylthio, 2′-isopropylthio, and 2′-*n*-butylthio analogs, less active than griseofulvin; isogriseofulvin, 4′-alkylthio analogs, and tris(alkylthio) derivatives, inactive.

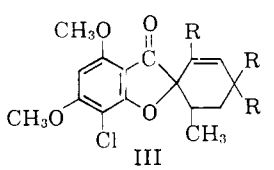
The therapeutic usefulness of griseofulvin (If) as an antifungal agent has stimulated considerable research into the relationship of structure and activity and towards discovering more effective analogs.[2] Recently, Stephenson, *et al.*,[3] prepared a number of 2′-alkylthio analogs of griseofulvin and 4′-alkylthio analogs of isogriseofulvin (IIf) by nucleophilic displacement of the chloro substituent with alkanethiols in 2′,7-dichloro-4,6-dimethoxy-6′-methyl-2′-grisene-3,4′-dione (Ig) and 4′,7-dichloro-4,6-dimethoxy-6′-methyl-3′-grisene-2′,3-dione (IIg), respectively. For the 2′-alkylthio analogs their synthesis starting from griseofulvin involved four steps: If → IIf → Ig → I (R = alkylthio). Our investigation of the direct acid-catalyzed reaction of griseofulvin with the lower alkanethiols (mercaptanolysis) as a synthetic route to the corresponding 2′-alkylthio analogs is reported below.



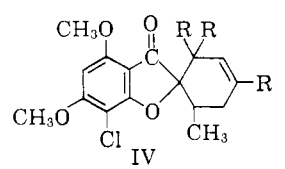
I



II



III



IV

a, R = CH_3S
b, R = C_2H_5S
c, R = *n*-C_3H_7S
d, R = *i*-C_3H_7S
e, R = *n*-C_4H_9S
f, R = CH_3O
g, R = Cl
h, R = C_2H_5O

When griseofulvin is shaken with an excess of methanethiol in the presence of small amounts of *p*-toluenesulfonic acid, a novel tris(methylthio) derivative crystallizes analytically pure in 70% yield from the reaction mixture shortly after the dissolution of If. Refluxing an acetone solution of the tris(methylthio) compound with the same catalyst results in the formation of pure 7-chloro-4,6-dimethoxy-6′-methyl-2′-methylthio-2′-grisene-3,4′-dione (Ia). Ethanethiol, 1-propanethiol, and 1-butanethiol also react with griseofulvin to give the corresponding tris(alkylthio) compounds, which can be converted in the same manner to the respective 2′-alkylthio analogs (Ib, Ic, and Ie). Two structures are possible for the tris(alkylthio) compounds (III or IV, R = alkylthio), in which two of the alkylthio groups are present as a mercaptole group. The assignment of structure III (R = alkylthio) to these compounds is discussed in a subsequent section.

In contrast to the alcoholysis of griseofulvin which usually forms substantial amounts of the 4′-alkoxy

(1) Presented in part at the 144th National Meeting of the American Chemical Society, Division of Medicinal Chemistry, Los Angeles, Calif., April, 1963.

(2) J. F. Grove, *Quart. Rev.* (London), **17**, 1 (1963).

(3) L. Stephenson, T. Walker, W. K. Warburton, and G. B. Webb, *J. Chem. Soc.*, 1282 (1962).