2. Evidence is presented that this substance possesses the free aldehyde structure.

3. The crystalline semicarbazone of this aldehyde has been prepared in pure form.

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THE CHEMISTRY OF DIARYL SULFIDES. III. THE SYNTHESIS OF THIOTHYRONINE[1]

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In two previous papers from this Laboratory,[2] attention has been directed to the remarkable effect of diaryl-sulfide groupings in organic compounds which have been shown to have therapeutic value as germicides or antiseptics. The outstanding derivative thus far studied is *p*-hydroxydiphenyl-sulfide which was found to possess a germicidal activity equivalent to a phenol coefficient of 115. As a result of the discovery of this unexpected increase in germicidal power, and on account of the growing interest in the biochemistry of organic sulfur groupings in general, we have now extended further our researches in this field, and have begun a study of practical methods for synthesizing sulfur-ether-α-amino acid combinations corresponding in structure to the naturally occurring protein acids, phenyl-alanine and tyrosine, and also the sulfur analog of the hormone—*thyroxine*. In this paper we describe methods of synthesizing the two new α-aminoacids—*p*-methyl-*p'*-diphenylsulfide-β-alanine and *p*-hydroxy-*p'*-diphenylsulfide-β-alanine or *thiothyronine* (desiodo-thyroxine), which are expressed by formulas I and II, respectively. The amino acid II is

$CH_3C_6H_4SC_6H_4CH_2CH(NH_2)COOH$ (I) $\quad$ $HOC_6H_4SC_6H_4CH_2CH(NH_2)COOH$ (II)

$HOC_6H_4OC_6H_4CH_2CH(NH_2)COOH$ (III) $\quad$ $HSC_6H_4CH_2CH(NH_2)COOH$ (IV)

the sulfur analog of thyronine III already described by Harington,[3] and is the first aromatic sulfur-ether derivative of the amino acid thiotyrosine IV to be described. A method for preparing *thiotyrosine* IV was first described in a paper from the Yale laboratory by Johnson and Brautlecht[4] in 1912, and so far as the authors are aware no attention has been paid to

[1] Constructed from a dissertation presented by George Hartland Law in June, 1929, to the Faculty of the Graduate School of Yale University in candidacy for the degree of Doctor of Philosophy.

[2] Hilbert and Johnson, THIS JOURNAL, **51**, 1526 (1929); Bass and Johnson, *ibid.*, **52**, 1146 (1930).

[3] Harington, *Biochem. J.*, **20**, 300 (1926); **22**, 1429 (1928); *C. A.*, **23**, 1631 (1929).

[4] Johnson and Brautlecht, *J. Biol. Chem.*, **12**, 175 (1912).

its chemistry since that date. The further study of this interesting acid and its derivatives is now in progress.

The approach to α-amino acid derivatives of diaryl sulfides has been accomplished as a result of the successful introduction of aldehyde groups into sulfide-ether constructions. Previous to our work no aldehyde derivatives of an aromatic sulfide have been described in the chemical literature. Of the various procedures previously employed for introducing the aldehyde group into the aromatic nucleus,[5] the method of Stephen,[6] involving conversion of a nitrile to an imide by reduction, and the latter to an aldehyde by hydrolysis, was the only one considered at all practical for accomplishing our purpose. This was also the method utilized by Harington and Barger[7] in their original work leading to the final synthesis of thyroxine. There is a serious objection to the application of any oxidation process for aldehyde synthesis in this series of compounds, as we are dealing with sulfur constructions which are easily altered by application of such experimental technique. The sulfides represent the lowest valency state of sulfur and all reactions must be avoided which would tend to convert the respective sulfide to a sulfoxide or a sulfone. It now seems probable from the work already done in this Laboratory that the Stephen reaction will be found of quite general application for aldehyde synthesis in the aromatic sulfide series.

The starting points for our two amino acid syntheses were *p*-methyl-*p'*-cyandiphenyl-sulfide and *p*-ethoxy-*p'*-cyandiphenyl-sulfide expressed by formulas V and VI, respectively. These were transformed into the cor-

$CH_3C_6H_4SC_6H_4CN$ V $\qquad$ $C_2H_5OC_6H_4SC_6H_4CN$ VI

responding aldehydes, VII and VIII, by application of Stephen's reaction

$CH_3C_6H_4SC_6H_4CHO$ VII $\qquad$ $C_2H_5OC_6H_4SC_6H_4CHO$ VIII

and then condensed with hydantoin according to the method of Wheeler.[8] After reducing the resulting aldehyde condensation products, and then destroying the resulting hydantoin derivatives by alkaline hydrolysis, the corresponding α-amino acids were easily obtained in good yield. Dealkylation of *p*-ethoxy-*p'*-diphenylsulfide-β-alanine by heating with hydrobromic acid gave the sulfur analog of thyronine represented by formula II. Description of the products formed in the different stages of the two syntheses

[5] Reimer–Tiemann method, *Ber.*, **9**, 824 (1876); Gattermann, *Ann.*, **347**, 347 (1906); *ibid.*, **357**, 313 (1907); Guyot, *Compt. rend.*, **149**, 788 (1909); Bouveault, *ibid.*, **122**, 1543 (1896); Gattermann and Maffezzoli, *Ber.*, **36**, 4152 (1903); Bodroux, *Compt. rend.*, **138**, 92, 700 (1904); Bouveault, *Bull. soc. chim.*, [III] **31**, 1322 (1904); Étard, *Ann. chim.*, **22**, 218 (1881); Rosenmund, *Ber.*, **51**, 585 (1918).

[6] Stephen, *J. Chem. Soc.*, **127**, 1874 (1925).

[7] Harington and Barger, *Biochem. J.*, **21**, 169 (1927).

[8] Wheeler and Hoffman, *Am. Chem. J.*, **45**, 368 (1911).

are given in the experimental part of this paper. Further work dealing with the preparation and study of halogen derivatives of aromatic sulfide amino acids is now in progress.

Experimental Part

***p*-Ethoxythiophenol,** $C_2H_5OC_6H_4SH$.—This thiophenol has previously been prepared by Lagai[9] and also Gattermann.[10] By application of the technique of Leuckart[11] we succeeded in preparing the same compound in good yield (77%) from *p*-phenetidine. Ninety-six grams of the amine is productive of 83–85 g. of the thiophenol distilling between 238–241°.

***p*-Ethoxy-*p'*-nitrodiphenyl-sulfide,** $C_2H_5OC_6H_4SC_6H_4NO_2$.—This is formed by digesting in alcoholic solution the sodium salt of *p*-ethoxythiophenol with *p*-nitrochlorobenzene. The sulfide is easily obtained in a yield of 93% of the theoretical and crystallizes from alcohol in needles melting at 96°. It is insoluble in water but soluble in all common organic solvents except petroleum ether.

Anal. Calcd. for $C_{14}H_{13}NSO_3$: N, 5.09; S, 11.63 Found: N, 4.90; S, 11.49.

***p*-Methyl-*p'*-nitrodiphenyl-sulfide,** $CH_3C_6H_4SC_6H_4NO_2$, is obtained from *p*-thiocresol and *p*-nitrochlorobenzene in a yield of 95% of the theoretical. It crystallizes from alcohol in yellow needles melting at 81.5°.

Anal. Calcd. for $C_{13}H_{11}O_2NS$: N, 5.71; S, 12.06. Found: N, 5.63; S, 13.12.

Reduction of these two nitro compounds with stannous chloride in dilute alcohol solution acidified with hydrochloric acid leads to the formation of the corresponding amino derivatives.

***p*-Ethoxy-*p'*-aminodiphenyl-sulfide,** melting at 53° from alcohol. The hydrochloride melts at 184–185°.

Anal. Calcd. for $C_{14}H_{15}ONS$: N, 5.71; S, 13.06 Found: N, 5.47; S, 13.01.

***p*-Methyl-*p'*-aminodiphenyl-sulfide,** melting at 73.5° from alcohol. The hydrochloride melts at 183–184° and is obtained in a yield of 84% of the theoretical.

Anal. Calcd. for $C_{13}H_{13}NS$: N, 6.51; S, 14.88. Found: N, 6.39; S, 15.01.

***p*-Ethoxy-*p'*-cyandiphenyl-sulfide. VI.**—From the corresponding amine by application of Sandmeyer's reaction. This was purified by distillation under diminished pressure and finally by crystallization from dilute acetic acid. It separated in colorless prisms melting at 95–96°. The nitrile is insoluble in water, but soluble in all the common organic solvents.

Anal. Calcd. for $C_{15}H_{13}ONS$: N, 5.49; S, 12.55. Found: N, 5.45; S, 12.39.

***p*-Methyl-*p'*-cyandiphenyl-sulfide. V.**—This was prepared from *p*-aminobenzonitrile[12] and *p*-thiocresol according to Ziegler's technique,[13] and also from *p*-methyl-*p'*-aminodiphenylsulfide by application of Sandmeyer's reaction. The yield by either method is about 45% of the theoretical. This nitrile is easily purified by crystallization from dilute acetic acid and crystallizes in plates melting at 102–103°.

Anal. Calcd. for $C_{14}H_{11}NS$: N, 6.22; S, 14.22. Found: N, 6.14; S, 14.08.

***p*-Ethoxy-diphenyl-sulfide-*p'*-carboxylic acid,** melts at 201–202° (calcd.: S, 11.68. Found: S, 11.75).

[9] Lagai, *Ber.*, **25**, 1838 (1892).

[10] Gattermann, *ibid.*, **32**, 1149 (1899).

[11] Leuckart, *J. prakt. Chem.*, **41**, 179 (1890).

[12] Bogert and Wise, THIS JOURNAL, **32**, 1494 (1910).

[13] Ziegler, *Ber.*, **23**, 2471 (1890).

p-**Methyl-diphenyl-sulfide-*p'*-carboxylic acid,** melts at 197–198° (calcd.: S, 13.12. Found: S, 13.21).

p-**Ethoxy-*p'*-aldehyde-diphenyl-sulfide. VIII.**—Thirty grams of anhydrous stannous chloride is added to 900 cc. of dry ether and the solvent saturated at low temperature with hydrochloric acid gas. Eight grams of *p*-ethoxy-*p'*-cyandiphenyl-sulfide dissolved in 30 cc. of chloroform is then added and after thorough mixing the solution is allowed to stand in an ice chest for several hours. Saturation with hydrochloric acid gas is then repeated and the mixture again allowed to stand for eight to ten hours. The imide hydrochloride is then separated by filtration and immediately converted to the aldehyde by warming with very dilute hydrochloric acid solution. The aldehyde crystallizes from dilute acetic acid in plates which melt at 83°. It is insoluble in water, but soluble in all the common organic solvents.

Anal. Calcd. for $C_{15}H_{14}O_2S$: S, 12.41. Found: S, 12.42.

Its phenylhydrazone, $C_{21}H_{20}ON_2S$, crystallizes from alcohol in yellow needles melting at 126–127° (calcd.: N, 8.04. Found: N, 7.82).

p-**Methyl-*p'*-aldehyde-diphenyl-sulfide. VII.**—This is obtained from *p*-methyl-*p'*-cyandiphenyl-sulfide by application of Stephen's reaction in a yield of 81% of the theoretical. The aldehyde is purified by crystallization from dilute acetic acid and separates in plates melting at 69°.

Anal. Calcd. for $C_{14}H_{12}OS$: S, 14.04. Found: S, 14.17.

Its phenylhydrazone, $C_{20}H_{18}N_2S$, crystallizes from alcohol in yellow plates melting at 149–150° (calcd.: N, 8.80. Found: N, 8.61).

p-**Methyl-diphenylsulfide-*p'*-aldal-hydantoin,** $CH_3C_6H_4SC_6H_4CH{=}\overline{CCONHCONH}$.—This is formed in a yield of 71% of the theoretical by interaction of *p*-methyl-*p'*-aldehydodiphenyl-sulfide (15.5 g.) with hydantoin (6.9 g.) and sodium acetate (26 g.) in a mixture of glacial acetic and acetic anhydride heated at its boiling point for eight hours. On pouring into cold water the hydantoin separates and is purified by crystallization from dilute alcohol. It melts at 239–240° and is insoluble in water and ether and only sparingly soluble in alcohol and acetic acid.

Anal. Calcd. for $C_{17}H_{14}O_2N_2S$: N, 9.03; S, 10.32. Found: N, 8.83; S, 10.19.

p-**Ethoxy-diphenyl-sulfide-*p'*-aldal-hydantoin,** $C_2H_5OC_6H_4SC_6H_4CH{=}\overline{CCONHCONH}$.—This hydantoin crystallizes from dilute alcohol in needles which melt at 254° with decomposition. The hydantoin is insoluble in water and ether and only sparingly soluble in alcohol and acetic acid.

Anal. Calcd. for $C_{18}H_{16}O_3N_2S$: N, 8.24; S, 9.41. Found: N, 8.16; S, 9.22.

Reduction of the Unsaturated Hydantoins.—This was accomplished by dissolving the respective hydantoin in warm alcohol and then reducing it at the double bond by slow addition of 2.5% sodium amalgam to the solution. When the reduction was complete the solution was acidified with hydrochloric acid and the reduced hydantoin precipitated by addition of water. The yield of crude hydantoin is about 85–90% of the theoretical.

p-**Methyl-diphenyl-sulfide-*p'*-aldyl-hydantoin,** $CH_3C_6H_4SC_6H_4CH_2\overline{CHCONHCONH}$, crystallizes from dilute alcohol in plates which melt at 187–189°. It is insoluble in water and ether, and soluble in acetic acids.

Anal. Calcd. for $C_{17}H_{16}O_2N_2S$: N, 8.97; S, 10.25. Found: N, 8.82; S, 10.09.

p-**Ethoxy-diphenyl-sulfide-*p'*-aldyl-hydantoin,** $C_2H_5OC_6H_4SC_6H_4CH_2\overline{CHCONHCONH}$, crystallizes from dilute alcohol in needles which melt at 184–185°. It is insoluble in water and ether, but soluble in the common organic solvents.

Anal. Calcd. for $C_{18}H_{18}O_3N_2S$: N, 8.18; S, 9.36. Found: N, 8.09; S, 9.45.

***p*-Methyl-*p'*-diphenyl-sulfide-β-alanine. I.**—This α-amino acid is formed by heating its hydantoin (above) in strong aqueous barium hydroxide at the boiling point of the solution for several hours (forty-eight). On acidifying the alkaline solution with hydrochloric acid and cooling, the hydrochloride of the amino acid separated as clusters of colorless prismatic crystals. The yield was 81% of the theoretical and the salt melted at 205–207° with decomposition. The free amino acid was precipitated from an aqueous solution of its hydrochloride by addition of sodium acetate. This proved to be a very insoluble substance, and was purified by repeatedly dissolving in alkaline solution and precipitating with acetic acid. The sodium salt was found to crystallize from dilute sodium hydroxide solution as needles. The acid gives the normal ninhydrin test characteristic of α-amino acids. The acid melts at 198–200° with great decomposition.

Anal. Calcd. for $C_{16}H_{17}O_2NS$: N, 4.88; S, 11.15. Found: N, 4.80; S, 11.74.

***p*-Ethoxy-*p'*-diphenyl-sulfide-β-alanine,** $C_2H_5OC_6H_4SC_6H_4CH_2CH(NH_2)COOH$.—This is formed by hydrolysis of its corresponding hydantoin (above) with barium hydroxide (heating at 100° for forty-eight hours). The yield of hydrochloride was 82% of the theoretical, and it melted at 218–220° with decomposition. The free amino acid melts at 208–210° with intense decomposition and is slightly soluble in dilute solutions of mineral acids and alkali and insoluble in water. It responds to the ninhydrin test for α-amino acids.

Anal. Calcd. for $C_{17}H_{19}O_3NS$: N, 4.41; S, 10.09. Found: N, 4.38; S, 10.21.

***p*-Hydroxy-*p'*-diphenyl-sulfide-β-alanine or Thiothyronine. II.**—This amino acid is formed by heating the corresponding ethyl ether with a mixture of 48% hydrobromic acid and acetic anhydride at 90° for several hours (forty-eight). The reaction mixture was then diluted with water and the hydrobromic acid neutralized by addition of sodium acetate using alizarine as an indicator. The amino acid separated at once and was further purified by dissolving in sodium hydroxide solution and precipitating with acetic acid. Further purification was effected by dissolving the amino acid in hydrochloric acid and reprecipitating by adding to the solution sodium acetate. This amino acid crystallizes in the form of colorless spherical crystals which darken when heated at 230° and melt with decomposition at 240–242°. It is insoluble in cold water, very slightly soluble in hot water, and also sparingly soluble in dilute acid and alkaline solutions. It gives a positive ninhydrin test but does not give as distinct and decisive a Millon's test as tyrosine. This later color change is undoubtedly influenced by the presence of sulfur in the sulfide molecule.

Anal. Calcd. for $C_{15}H_{15}O_3NS$: C, 62.2; H, 5.19; N, 4.84. Found: C, 62.0; H, 5.26; N, 5.00.

Summary

1. The Stephen reaction for preparing aldehydes from cyanides has been applied with success in the diaryl-sulfide series.

2. The two aldehydes $CH_3C_6H_4SC_6H_4CHO$ and $C_2H_5OC_6H_4SC_6H_4CHO$ condense smoothly with hydantoin, giving condensation products which can be transformed into characteristic α-amino acids.

3. Three new α-amino acids have been described, including thiothyronine, the sulfur analog of thyronine or desiodo-thyroxine.

4. The study of these amino acids and their halogen substitution products is being continued.

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