244. *Sulphur Derivatives of Thiazoles.*

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A number of thiazole derivatives were prepared and tested for antibacterial activity. Five thiolthiazoles and two thiazole disulphides were found to be markedly active, whereas monosulphides, sulphones, and sulphonic acids were inactive.

The antibacterial properties of sulphanilamide and pantoyl taurine are attributed to their antagonistic action towards the two growth factors, *p*-aminobenzoic acid and pantothenic acid respectively (Woods, *Brit. J. Exp. Path.*, 1940, **21**, 74; Barnett and Robinson, *Biochem. J.*, 1942, **36**, 364; McIlwain, *ibid.*, p. 417). In the course of attempts to prepare antagonists towards aneurine, it was observed that certain sulphur derivatives of thiazole inhibited the growth of bacteria, and a survey of other derivatives of thiazole was undertaken to determine the nature of the groups responsible for this activity. The antibacterial properties of the active compounds were shown not to be due to an antagonistic effect towards aneurine, for addition of aneurine to the culture media did not reverse the inhibitory action of the compounds.

Thiol derivatives of thiazole have been prepared previously by Miolati (*Gazzetta*, 1893, **23**, 575), Levi (*ibid.*, 1931, **61**, 719) and Buchman, Reims, and Sargent (*J. Org. Chem.*, 1941, **6**, 764) by the action of ammonium dithiocarbamate on halogenated ketones. Thiolbenzthiazoles have also been prepared by the action of thiourea on halogenated thiazoles (Scott and Watt, *J. Org. Chem.*, 1937, **2**, 148, and Watt, *ibid.*, 1939, **4**, 436). Sulphides can also be prepared by this reaction or by the action of potassium hydrosulphide on halogenated thiazoles. A better method of preparing sulphides, due to Ochiai and Takubo (*J. Pharm. Soc. Japan*, 1941, **61**, 1), is by the interaction of a halogenated thiazole with a thiolthiazole. Disulphides have been prepared from the corresponding thiol compounds by oxidation with persulphates, permanganates, hydrogen peroxide, hypochlorites, perchlorates, nitric acid or iodine (U.S.P. 1,880,421, 2,024,567, 2,024,575, 2,043,949 and 2,196,607; Buchman, Reims, and Sargent, *loc. cit.*). Sulphones and sulphonic acids are also prepared by oxidation of monosulphides, using stronger oxidising agents, such as acid permanganate or chromium trioxide. Sulphonic acids, however, are best prepared by sulphonation of the appropriate thiazole with fuming sulphuric acid (Ochiai, *J. Pharm. Soc. Japan*, 1938, **58**, 1040; Nagasawa, *ibid.*, 1940, **60**, 433; Ochiai and Nagasawa, *Ber.*, 1939, **72**, 1470).

Most of the compounds described in the experimental section were tested in a synthetic medium and in glucose broth against *Streptococcus hæmolyticus*, *Staphylococcus aureus*, and *B. coli*. None of the compounds had any appreciable action against *B. coli*, but 2-thiol-4-methylthiazole, *2-thiol-5-(β-hydroxyethyl)-4-methylthiazole*, *2-thiol-5-(β-acetoxyethyl)-4-methylthiazole*, and 5-(β-thiolethyl)-4-methylthiazole inhibited the growth of *Streptococcus* and *Staphylococcus* at a dilution of 1 in 100,000 in the synthetic medium and at a somewhat higher concentration in glucose broth. 2-Amino-5-thiol-4-methylthiazole hydrochloride was even more potent, as was bis-(4-methyl-2-thiazole) disulphide, but the other disulphide tested, *bis-[5-(β-acetoxyethyl)-4-methyl-2-thiazole disulphide*, was less active. The only sulphide which showed appreciable, but low, activity was *bis-(2-amino-4-methyl-5-thiazole) sulphide*. The other sulphides, the sulphones and the sulphonic acids were inactive, as were the compounds in which the thiol group of the active 2-thiol-5-(β-hydroxyethyl)-4-methylthiazole was replaced by a hydroxy, amino, methyl or chloro group. It is evident therefore that the antibacterial activity of this group of compounds resides in the thiol or the disulphide group; the latter is probably converted into the thiol group *in vivo*. The lack of activity in the sulphones, particularly in bis-(2-acetamido-4-methyl-5-thiazole) sulphone was unexpected, since the closely analogous 4 : 4′-diaminodiphenyl sulphone and its derivatives have been shown to possess valuable antibacterial propertles (Buttle, Stephenson, Smith, Dewing, and Foster, *Lancet*, 1937, **1**, 1331; Fourneau, Tréfouel, Nitti, Bovet, and Tréfouel, *Compt. rend.*, 1937, **204**, 1763). Recently, however, Bambas (*J. Amer. Chem. Soc.*, 1945, **67**, 668) prepared a number of analogues of 4 : 4′-diaminodiphenyl sulphone in which (*a*) one of the benzene nuclei was replaced by a heterocyclic nucleus and (*b*) both benzene nuclei were replaced by heterocyclic nuclei. Only compounds of the first group were active and these were less potent than 4 : 4′-diaminodiphenyl sulphone.

Experimental.

(Analyses are by Drs. Weiler and Strauss, Oxford; all m. p.'s are uncorrected.)

2-Thiol-4-methylthiazole, m. p. 88°, was prepared from ammonium dithiocarbamate and monochloroacetone by Levi's modification (*loc. cit.*) of Miolati's method (*loc. cit.*).

2-Thiol-5-(β-acetoxyethyl)-4-methylthiazole.—Ammonium dithiocarbamate (28 g.) was suspended in dry ether (200 c.c.) and γ-aceto-γ-bromopropyl acetate (25 g.) added. The reaction mixture was cooled and left for 50 hours at room temperature. The ethereal extract was decanted and the residue extracted several times with ether, once with ethyl acetate, and finally with water. The residue was crystallised twice from ethyl alcohol to give *2-thiol-5-(β-acetoxyethyl)-4-methylthiazole* in the form of glistening white platelets, m. p. 102° (Found: C, 44·5; H, 4·64; N, 6·65; S, 30·1. $C_8H_{11}O_2NS_2$ requires C, 44·2; H, 5·07; N, 6·45; S, 29·5%). This compound was prepared, but not described, by Gravin (*J. App. Chem. U.S.S.R.*, 1943, **16**, 105) using a different method.

2-Thiol-5-(β-hydroxyethyl)-4-methylthiazole.—Ammonium dithiocarbamate (60 g.) was added to a solution of γ-aceto-γ-bromopropyl alcohol (50 g.) in dry ether. After five days the ether was decanted, and the residue extracted twice with ether and once with ethyl acetate. The residue was stirred with water (80 c.c.), and the aqueous solution extracted with ether and then with ethyl acetate. The combined extracts on evaporation yielded *2-thiol-5-(β-hydroxyethyl)-4-methylthiazole* which was crystallised three times from alcohol, forming small white prisms, m. p. 157° (Found: C, 41·1; H, 5·16; N, 8·33; S, 37·4. $C_6H_9ONS_2$ requires C, 41·1; H, 5·14; N, 8·0; S, 36·6%).

Bis-(4-methyl-2-thiazole) sulphide was prepared from 2-chloro-4-methylthiazole and 2-thiol-4-methylthiazole by refluxing in CS_2 in presence of NH_4Cl, a method used by Ochiai and Takubo (*loc. cit.*), who obtained the compound as an oil, b. p. 134—135°/1 mm. The product, now obtained, solidified and was recrystallised from aqueous ethanol, forming white needles, m. p. 190°. It yielded a picrate, m. p. 136°, identical with that obtained by Ochiai and Takubo. The sulphide was also prepared by the following method. 2-Bromo-4-methylthiazole (3 g.) was added to an alcoholic solution (100 c.c.) of KOH (2 g.) saturated with H_2S. The mixture was left for 1½ hours with H_2S passing through it and then allowed to stand for 2 days and heated at 55° for 2 hours. The mixture was neutralised with a little 2N-H_2SO_4 and evaporated to dryness. The residue was stirred with water, collected and recrystallised from aqueus ethanol. It yielded white needles, m. p. 190°.

Bis-[5-(β-acetoxyethyl)-4-methyl-2-thiazole] Sulphide.—2-Hydroxy-5-(β-acetoxyethyl)-4-methylthiazole was prepared by the method of Andersag and Westphal (*Ber.*, 1937, **70**, 2035) by the action of $Ba(SCN)_2$ on γ-chloro-γ-acetylpropyl acetate; it had m. p. 85—86° (Found: C, 47·9; H, 5·40; N, 7·14; S, 16·1. Calc. for $C_8H_{11}O_2NS$: C, 47·8; H, 5·47; N, 6·97; S, 15·9%). It was converted into 2-chloro-5-(β-acetoxyethyl)-4-methylthiazole by refluxing with $POCl_3$, the method used by Andersag and Westphal (Found: Cl, 16·4. Calc. for $C_8H_{10}O_2NSCl$: Cl, 16·2%). 2-Chloro-5-(β-acetoxyethyl)-4-methylthiazole was also prepared from γ-thiocyano-γ-acetylpropyl acetate, obtained by the action of NaCNS on γ-chloro-γ-acetylpropylacetate. This compound (246 g.) was saturated at 0° with dry HCl (54 g.) for a period of 5 hours, and then left overnight. The product was neutralised and steam-distilled. The distillate was saturated with salt and extracted with ether, and the extract distilled, the fraction (55 g.), b. p. 101—112°/1 mm., being collected (Found: Cl, 17·8%).

A mixture of 2-chloro-5-(β-acetoxyethyl)-4-methylthiazole (25 g.), 2-thiol-5-(β-acetoxyethyl)-4-methylthiazole (25 g.), NH_4Cl (25 g.) and CS_2 (250 c.c.) was treated according to the method of Ochiai and Takubo (*loc. cit.*). The resulting *bis-[5-(β-acetoxyethyl)-4-methyl-2-thiazole] sulphide*, a yellow oil, had b. p. 230—240°/0·5 mm., but underwent slight decomposition on distillation (Found: C, 47·2; H, 5·6; N, 6·8; S, 24·3. $C_{16}H_{20}O_4N_2S_3$ requires C, 48·0; H, 5·0; N, 7·0; S, 24·0%).

2-Hydroxy-5-(β-hydroxyethyl)-4-methylthiazole.—2-Hydroxy-5-(β-acetoxyethyl)-4-methylthiazole (2 g.) was heated for 1 hour in a boiling water-bath with 2N-H_2SO_4 (20 c.c.), and the solution neutralised with NaOH and extracted with ether. The residue, left on evaporation of the ethereal extract, was crystallised from ether, forming pale buff prisms, m. p. 135—136° (Found: C, 45·4; H, 6·15; N, 8·55; S, 19·4. $C_6H_9O_2NS$ requires C, 45·3; H, 5·65; N, 8·80; S, 20·1%).

2-Amino-5-(β-hydroxyethyl)-4-methylthiazole hydrochloride and picrate. These were prepared by the method of Basu and Das Gupta (*J. Ind. Chem. Soc.*, 1938, **15**, 160) from γ-chloro-γ-acetylpropyl alcohol and thiourea. The hydrochloride had m. p. 154° and the picrate m. p. 210°. Todd *et al.* (*J.*, 1936, 1601) did not purify the hydrochloride, whilst Basu and Das Gupta record m. p. 138° for the hydrochloride, and Jensen and Thorsteinsson (*Dansk. Tidsskr. Farm.*, 1941, **15**, 41) give m. p. 150° (Found for the hydrochloride: C, 37·3; H, 5·92; N, 14·1; Cl, 18·6; S, 16·6. Calc. for $C_6H_{11}ON_2ClS$: C, 37·0; H, 5·66; N, 14·4; Cl, 18·3; S, 16·5%).

5-(β-Hydroxyethyl)-2 : 4-dimethylthiazole was prepared from thioacetamide and γ-bromo-γ-acetylpropyl alcohol according to the method of Pesina (*J. Gen. Chem. U.S.S.R.*, 1939, **9**, 804). It was an oil, b. p. 130—135°/6 mm.

Bis-(4-methyl-2-thiazole) disulphide. 2-Thiol-4-methylthiazole (1·3 g.) was dissolved in NaOH solution (15%, 3·3 c.c.) and iodine (2·4 g.) added with shaking over a period of 30 minutes. The resulting oil was taken up in ether, and the extract washed with dilute alkali and water. The solid, left on evaporation of the ether, was recrystallised from alcohol, forming yellow prisms, m. p. 65° (Found: C, 37·0; H, 3·56; N, 10·3; S, 49·2. Calc. for $C_8H_8N_2S_4$: C, 36·9; H, 3·08; N, 10·7; S, 49·2%). Buchman, Reims, and Sargent (*loc. cit.*) prepared this compound by oxidation with H_2O_2; they record m. p. 61—61·5°.

Bis-[5-(β-acetoxyethyl)-4-methyl-2-thiazole] Disulphide.—2-Thiol-5-(β-acetoxyethyl)-4-methylthiazole (4·1 g.) was dissolved in warm dilute NaOH solution (30 c.c.) and a warm aqueous solution (25 c.c.) of potassium persulphate (2·7 g.) added with shaking. After 1 hour, the mixture was extracted with ether, the extract washed with alkali and water and evaporated. A yellow syrup, which did not crystallise, remained (Found: S, 30·2. $C_{16}H_{20}O_4N_2S_4$ requires S, 29·7%).

Bis-(4-methyl-2-thiazole) sulphone. Bis-(4-methyl-2-thiazole) sulphide (5·7 g.) was dissolved in glacial acetic acid (50 c.c.), and $KMnO_4$ solution (3%, 160 c.c.) added slowly with cooling. Water (100 c.c.) was added, and SO_2 passed in until the solution became colourless. The deposited solid was separated and crystallised from methanol, forming white prisms (2·7 g.), m. p. 124—125°. Ochiai and Takubo (*loc. cit.*) prepared the compound by oxidation of the sulphide with H_2O_2.

Bis-[5-(β-acetoxyethyl)-4-methyl-2-thiazole] Sulphone.—Bis-[5-(β-acetoxyethyl)-4-methyl-2-thiazole] sulphide (5·8 g.) was dissolved in glacial acetic acid (30 c.c.), and 3% $KMnO_4$ solution (90 c.c.) added slowly with cooling. The solution was decolorised with SO_2 and worked up as described above. The solid product was recrystallised from alcohol, forming white leaflets, m. p. 104—105° (Found: C, 44·4; H, 5·7; N, 6·7; S, 22·4. $C_{16}H_{20}O_6N_2S_3$ requires: C, 44·4; H, 4·6; N, 6·5; S, 22·3%).

5-(β-Acetoxyethyl)-4-methylthiazole-2-sulphonic Acid.—Crude 5-(β-acetoxyethyl)-4-methylthiazole (10 g.), prepared by the method of Buchman (*J. Amer. Chem. Soc.*, 1936, **58**, 1803), was heated for 9 hours on a boiling water bath with acetic anhydride (30 c.c.) and conc. H_2SO_4 (10 g.). The mixture was cooled, ether (100 c.c.) added, and the ethereal solution decanted from the resulting oily layer. The latter was stirred with water (50 c.c.) and barium carbonate (20

g.) and filtered after standing overnight. The filtrate was diluted to 200 c.c., and dilute sulphuric acid, equivalent to the barium content, added and the mixture filtered. The filtrate was evaporated, left to crystallise and the product recrystallised from water. It formed glistening white platelets, m. p. 242° (Found: C, 35·7; H, 4·15; N, 5·15; S, 24·0. $C_8H_{11}O_5NS_3$ requires C, 36·2; H, 4·15; N, 5·28; S, 24·1%).

2-Amino-5-thiol-4-methylthiazole Hydrochloride and Bis-(2-amino-4-methyl-5-thiazole) Sulphide.—(i) 2-Amino-4-methylthiazole, prepared from monochloroacetone and thiourea, was brominated as described by Ochiai and Nagasawa (*loc. cit.*). The resulting crude 5-bromo-2-amino-4-methylthiazole (57 g.) was dissolved in a solution of KSH, made by saturating a solution of KOH (34 g.) in methanol (640 c.c.) with H_2S. The reaction mixture was left for 1½ hours with H_2S passing through it and was then allowed to stand overnight. It was boiled for 1 hour under reflux and the methanol removed by distillation. The residue was stirred with water and filtered from crude bis-(2-amino-4-methyl-5-thiazole) sulphide (23 g.), which was recrystallised from aqueous alcohol, forming pale-yellow prisms, m. p. 190° (see below). The filtrate was neutralised and extracted with ether, and the ether extract distilled. The residue was acidified to Congo red with dilute HCl and cooled to 0°, and the crude *2-amino-5-thiol-4-methylthiazole hydrochloride* (7 g.) filtered off and recrystallised from dilute HCl. It formed pale yellow needles, m. p. 200°, and was highly sternutatory (Found: C, 26·9; H, 3·65; N, 15·2; S, 34·5; Cl, 19·2. $C_4H_7N_2S_2Cl$ requires C, 26·3; H, 3·84; N, 15·34; S, 35·1; Cl, 19·4%).

(ii) 5-Bromo-2-amino-4-methylthiazole (18 g.) was added to a solution of thiourea (7·2 g.) in ethanol (65 c.c.) and left for 3 days at room temperature with occasional shaking. The crude *bis-(2-amino-4-methyl-5-thiazole) sulphide* (8 g.) was separated and recrystallised from aqueous alcohol, m. p. 190° (Found: C, 37·5; H, 4·4; N, 21·3; S, 37·0. $C_8H_{10}N_4S_3$ requires C, 37·2; H, 3·9; N, 21·7; S, 37·2%). It yielded a crystalline diacetyl compound, m. p. 360°. The filtrate, on being worked up as in (i), yielded 2-amino-5-thiol-4-methylthiazole hydrochloride (5 g.) (see above). A small quantity of a crystalline picrate, m. p. 225° decomp., was also obtained from the mother liquors. This appeared to be the *2-amino-5-hydroxy-4-methylthiazole picrate* (Found: C, 36·0; H, 3·07; N, 18·6; S, 9·95. $C_{10}H_9O_8N_5S$ requires: C, 33·4; H, 2·50; N, 19·4; S, 8·92%).

Bis-(2-acetamido-4-methyl-5-thiazole) Sulphone.—Bis-(2-amino-4-methyl-5-thiazole) sulphide (14·5 g.) was refluxed for 30 minutes with 75 c.c. of acetic anhydride. The product was poured into water, and the *bis-(2-acetamido-4-methyl-5-thiazole) sulphide* separated. It was crystallised from hot acetic acid, forming pale yellow needles, m. p. 360° (decomp.) (Found: C, 42·2; H, 4·36; N, 16·25; S, 27·6. $C_{12}H_{14}O_2N_4S_3$ requires: C, 42·1; H, 4·1; N, 16·4; S, 28·1%). To a suspension of this compound (9 g.) in glacial acetic acid (150 c.c.) was added powdered chromium trioxide (9 g.) over a period of 30 minutes, the temperature being kept below 20°. After 1 hour, the solution was heated to boiling point and then left overnight. Unchanged sulphide was separated and the filtrate diluted with 3 to 4 volumes of water. On cooling to 0°, small colourless prismatic crystals of *bis-(2-acetamdio-4-methyl-5-thiazole) sulphone* separated, m. p. 325° (decomp.) (Found: C, 39·2; H, 4·0; N, 15·6; S, 25·7. $C_{12}H_{14}O_4N_4S_3$ requires: C, 38·5; H, 3·7; N, 15·0; S, 25·7%). All attempts to convert this compound into the amino compound were unsuccessful. Oxidation of bis-(2-acetamido-4-methyl-5-thiazole) sulphide (1 g.) with nitric acid (48%, 2·5 c.c.) yielded *5-nitro-2-acetamido-4-methylthiazole*, which was recrystallised from alcohol, forming bright yellow needles, m. p. 227° (decomp.) (Found: C, 36·5; H, 3·73; N, 20·2; S, 16·2. $C_6H_7O_3N_3S$ requires C, 35·8; H, 3·48; N, 20·9; S, 15·9%).

2-Amino-4-methylthiazole-5-sulphonic acid, m. p. 350°, was prepared by the action of fuming H_2SO_4 on 2-amino-4-methylthiazole; Ochiai and Nagasawa (*loc. cit.*) record m. p. 340° (decomp.). Attempts to convert it to the acid chloride and amide were unsuccessful. The preparation of 2-amino-4-methylthiazole-5-sulphonamide was recently described by Backer and de Jonge (*Rec. trav. chim.*, 1943, **62**, 163). It was without antibacterial activity.

5-(β-Thiolethyl)-4-methylthiazole.—5-(β-Bromoethyl)-4-methylthiazole (4 g.), obtained by the action of thioformamide on 1 : 3-dibromo-2-pentanone (Delaby, *Compt. rend.*, 1923, **176**, 1153), was added to an alcoholic solution (20 c.c.) of KOH (2 g.), saturated with H_2S, and H_2S passed into the mixture for 1 hour. The reaction mixture was heated at 50° for 2 hours, and the alcohol removed by distillation. Water was added to the residue, neutralised with dilute acid and extracted with ether. The oil, remaining after removal of the ether, was fractionally distilled, the fraction boiling at 75°/0·5 mm. being collected. This pale yellow oil did not crystallise; it possessed an unpleasant odour (Found: S, 40·8. $C_6H_9NS_2$ requires S, 40·2%).

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