3. *Analogues of Pantothenic Acid. Part III.* Preparation of Growth-inhibiting Analogues related to* N-*Pantoyltaurine.*

By Jean Barnett.

The preparation of further sulphur-containing substances closely related to *N*-pantoyltaurine, *viz.*, *N*-pantoyl-β-aminoethylthiol, bis(pantoyl-β-aminoethyl) monosulphide, *disulphide*, sulphoxide, and -sulphone, is described. Pantoyltaurine, the sulphonic acid corresponding to pantothenic acid (I), has previously been shown to have antibacterial activity *in vitro* and *in vivo*. None of the analogues now described, when tested *in vitro* against *Lactobacillus arabinosus*, was more active than pantoyltaurine, but the disulphide and the thiol had an almost equal inhibitory action, which was reversed by pantothenic acid. *In vivo* tests against animals infected with *Streptococcus hæmolyticus* gave no indication of activity superior to that of pantoyltaurine.

In vivo experiments with *N*-pantoyltaurine (McIlwain and Hawking, *Lancet*, **1943**, **1**, 449), have shown that only in high doses does it afford protection to animals infected with *Strep. hæmolyticus*. Analogues of pantoyltaurine (II) have therefore been prepared by replacing the sulpho-group with other sulphur-containing groups, in the hope that these substances might produce a greater bacteriostatic effect. The preparation of *N*-pantoyl-β-aminoethylthiol (III), *bis*-(N-*pantoyl*-β-*aminoethyl*) *disulphide* (IV), bis-(*N*-pantoyl-β-aminoethyl) mono-

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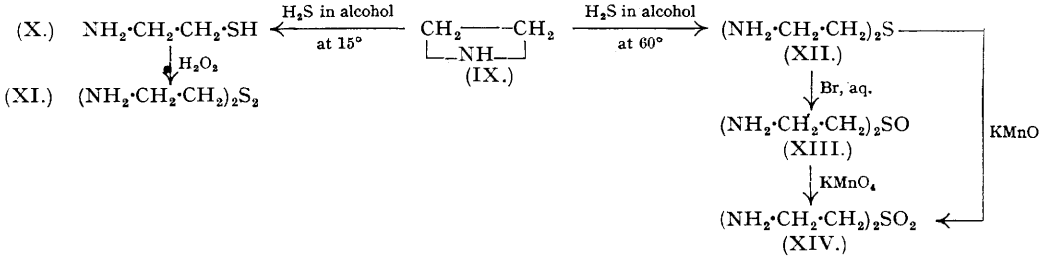
sulphide (V), bis-(*N*-pantoyl-β-aminoethyl) sulphoxide (VI), and bis-(*N*-pantoyl-β-aminoethyl)sulphone (VII) is now described.

(I.) P·NH·CH_2·CH_2·CO_2H (II.) P·NH·CH_2·CH_2·SO_3H (III.) P·NH·CH_2·CH_2·SH
(IV.) (P·NH·CH_2·CH_2)$_2S_2$ (V.) (P·NH·CH_2·CH_2)$_2S$ (VI.) (P·NH·CH_2·CH_2)$_2SO$ (VII.) (P·NH·CH_2·CH_2)$_2SO_2$
P = OH·CH_2·CMe_2·CH(OH)·CO·.

The analogues (IV) to (VII) were prepared by condensation of pantolactone (for references to methods of preparation, see Barnett and Robinson, *Biochem. J.*, 1942, **36**, 259) with the appropriate diamino-compound (XI to XIV)in boiling methyl alcohol. Pantoylaminoethylthiol (III) could not be prepared by this method, since the thiol group was completely oxidised under these conditions, and was obtained by heating the components together in a sealed tube in absence of air. In all the experiments, the extent of condensation was measured by a Van Slyke amino-nitrogen estimation.



The method by which the bases required (X to XIV) had been prepared previously from the corresponding phthalimido-compounds (Gabriel, *Ber.*, 1889, **22**, 1137, 3098; 1891, **24**, 1111) was found tedious and difficult. A better method for the preparation of β-aminoethylthiol (X) and bis-β-aminoethyl sulphide (XII) from hydrogen sulphide and ethyleneimine (IX) (Wenker, *J. Amer. Chem. Soc.*, 1935, **57**, 2328), described by Mills and Bogert (*J. Amer. Chem. Soc.*, 1940, **62**, 1173; 1941, **63**, 2363), has been used in the present work. Bis-β-aminoethyl disulphide (XI) was obtained quantitatively from (X) by oxidation with hydrogen peroxide (cf. Gabriel and Leupold, *Ber.*, 1898, **31**, 2837; Mills and Bogert, *loc. cit.*, p. 2363). Bis-β-aminoethyl sulphoxide (XIII) was obtained in quantitative yield by oxidation of bis-β-aminoethyl sulphide with bromine water, and the corresponding sulphone (XIV) in 50% yield by oxidising the sulphide with two equivalents or the sulphoxide with one equivalent of permanganate (cf. Gabriel, *Ber.*, 1891, **24**, 3098).

Bis-(*N*-pantoyl-β-aminoethyl)sulphoxide (VI), obtained from bis-β-aminoethyl sulphoxide by refluxing the components in methyl alcohol for 1 hour, was a yellowish viscous oil. After 3 months, it crystallised and from the semi-crystalline mass, bis-(*N*-pantoyl-β-aminoethyl) disulphide, m. p. 143—144°, was isolated, identical with the material prepared from bis-β-aminoethyl disulphide and pantolactone. This fission of a sulphoxide finds a parallel in the fission of phenylsulphoxyacetic acid to thiophenol and glyoxylic acid (Pummerer, *Ber.*, 1909, **42**, 2282; 1910, **43**, 1401):

$$C_6H_5·SO·CH_2·CO_2H \longrightarrow C_6H_5·SH + CHO·CO_2H$$

If a fission such as that described above took place, the thiol so formed might be spontaneously oxidised to the disulphide. It is, however, clear from the titration of bis-β-aminoethyl sulphoxide with permanganate that no such fission had already taken place when this compound was condensed with pantolactone, otherwise more than one atom of oxygen would have been absorbed, which did not occur. Unfortunately, no initial titration with permanganate had been carried out on the product which, after 3 months' standing, yielded the disulphide. It is therefore not possible to decide whether the fission occurred during the condensation, or on standing for a long period. It is hoped to investigate this reaction further.

Biological tests on these analogues were carried out by Dr. J. Ungar. *In vitro* tests on *Lactobacillus arabinosus* showed that, though none of them was more active than pantoyltaurine, pantoylaminoethylthiol and bis(pantoylaminoethyl) disulphide were highly active as growth inhibitors, having practically the same antagonising effect as pantoyltaurine. The corresponding sulphoxide, monosulphide and sulphone had an inhibiting effect to a less degree; the effect was always reversed by addition of pantothenic acid. It may perhaps be significant that the two most highly active compounds of this series were those in which the ratio of sulphur to nitrogen is approximately the same as for pantoyltaurine, whereas compounds having only half this sulphur content were less active.

In vivo tests on rats infected with *Streptococcus hæmolyticus* gave no evidence that these analogues have an activity superior to that of pantoyltaurine; indeed, the results suggested that rats were more susceptible to the influence of the bacteria in the presence of these substances than in their absence. The pantoyl-thiol analogue was highly toxic; the others were well tolerated.

The biological results will be reported in detail elsewhere.

Experimental.

β-Aminoethylthiol (X) —(The successful preparation of this substance from ethyleneimine depends very much on conditions; unless the optimum conditions described below are rigidly adhered to, none is obtained.) An alcoholic

solution of ethyleneimine (dried finally over sodium and redistilled over potassium hydroxide) was run during ½ hour into absolute ethyl alcohol saturated with hydrogen sulphide, kept between 10° and 20°, and shaken continuously while hydrogen sulphide was passing through the liquid. The mixture was left overnight at 10°, and the solvent evaporated in a vacuum. β-Aminoethylthiol was obtained as a hygroscopic white solid, which was sublimed at 70—80°/15 mm. just before use. The purity, as determined by titration with 0·1N-iodine (·SH ⟶ ·S·S·), was 97%.

Dinitrobenzoyl thioether. Resublimed β-aminoethylthiol (150 mg.) was dissolved in absolute alcohol (4 ml.), N-potassium hydroxide (1·8 ml.) added, and the mixture added to 1-chloro-2 : 4-dinitrobenzene (400 mg.) in absolute alcohol (2 ml.); an immediate precipitate formed. The solution was refluxed for 3 minutes and filtered hot. On cooling, orange needles (75 mg.) were deposited, m. p. 93·5—94·5° after recrystallisation from absolute alcohol (Found: S, 13·1. $C_8H_9O_4N_3S$ requires S, 13·2%).

N-*Pantoyl-β-aminoethylthiol* (III).—Pure resublimed β-aminoethylthiol (480 mg.) and redistilled pantolactone (820 mg.) were heated in a vacuum in a sealed tube at 100° for 1 hour. The resulting yellow oil was heated at 70° in a vacuum to remove any unchanged thiol. Titration with iodine indicated that the substance was 86% pure, and a Van Slyke estimation showed the presence of 1·7% of free amino-nitrogen, corresponding to 78% condensation.

Bis-β-aminoethyl Disulphide (XI).—This was prepared by oxidation of the pure resublimed thiol (10 g.) with hydrogen peroxide (Mills and Bogert, *loc. cit.*). The crude dihydrochloride (yield, 80%), recrystallised from 95% alcohol, gave bis-β-aminoethyl disulphide dihydrochloride in needles, m. p. 200—203°. Gabriel and Leupold (*loc. cit.*) record m. p. 203°, and Mills and Bogert (*loc. cit.*) m. p. 217°. The base was obtained by addition of exactly 1 equiv. of 10% aqueous sodium hydroxide to an aqueous solution of the dihydrochloride and evaporation to dryness in a vacuum at 50°, followed by repeated extraction with hot absolute alcohol.

Bis-(N-*pantoyl-β-aminoethyl*) *Disulphide* (IV).—Bis-β-aminoethyl disulphide (6·5 g.) was dissolved in absolute methyl alcohol (13 ml.), treated with redistilled pantolactone (2 equivs; 11·1 g.), and refluxed for 1 hour with exclusion of moisture. After cooling, the almost clear solution was filtered, evaporated in a vacuum to constant weight, and sealed up in ampoules. A Van Slyke estimation indicated the presence of 0·8% of amino-nitrogen, corresponding to 90% condensation. The syrupy disulphide (1 g.) partly crystallised after 3 months. The semi-solid mass was digested with acetone and filtered quickly, without exposure to the air. After washing with dry acetone and finally with absolute ether, a white crystalline mass was obtained (400 mg.), m. p. 128—132°. It was dissolved in a large volume of boiling acetone (redistilled over potassium permanganate), and the filtered solution evaporated to small bulk and seeded. After 2 days' standing at 0°, the crystalline *bis-*(N-*pantoyl-β-aminoethyl*) *disulphide* was collected and washed with acetone and ether; it was no longer hygroscopic. Yield 160 mg., m. p. 140—142°. For analysis it was recrystallised again from acetone; m. p. 141—144° (Found: C, 46·1; H, 7·9; N, 6·2. $C_{16}H_{32}O_6N_2S_2$ requires C, 46·6; H, 7·8; N, 6·8%). For permanganate titration, see under bis-(*N*-pantoyl-β-aminoethyl) sulphoxide.

Bis-β-aminoethyl Sulphide (XII).—This was prepared, according to Mills and Bogert (*loc. cit.*), in 65% yield, b. p. 118—120°/17 mm. Permanganate titration (·S· ⟶ ·SO_2·) showed the purity to be 99%. The benzoyl derivative had m. p. 106—107° (Gabriel, *loc. cit.*, gives 106°).

Bis-(N-*pantoyl-β-aminoethyl*) *Sulphide* (V).—Bis-β-aminoethyl sulphide (2 g.) and redistilled pantolactone (4·3 g.) were dissolved in absolute methyl alcohol (4 ml.) and, after 12 hours, refluxed for 1 hour with exclusion of moisture. The solvent was evaporated in a vacuum at 40°, leaving a viscous oil. It was extracted twice with absolute ether to remove traces of unchanged aminoethyl sulphide, and a portion was then titrated in 50% acetic acid with permanganate; the result indicated a purity of 100%. A Van Slyke estimation showed the presence of 0·6% of amino-nitrogen, equivalent to 14% of unchanged bis-β-aminoethyl sulphide, *i.e.*, 86% condensation.

Bis-β-aminoethyl Sulphoxide (XIII).—A solution of bis-β-aminoethyl sulphide (1·2 g.) in water (10 ml.) was cooled to 0°, and bromine water added gradually until a slight permanent yellow colour remained. The solution was at once distilled in a vacuum, being kept cold until all bromine had been removed. The concentrated residue, while still warm, was treated at once with boiling absolute alcohol (10 ml.). The *sulphoxide dihydrobromide* crystallised in fine needles (2·82 g.; 97%). For recrystallisation it was dissolved in the minimum amount of warm water, and hot ethyl alcohol added until cloudiness just appeared; m. p. 201—202° (Found: N, 9·1; S, 10·5; Br, 54·4. $C_4H_{12}ON_2S,2HBr$ requires N, 9·4; S, 10·7; Br, 53·7%). Permanganate titration (·SO· ⟶ ·SO_2·) showed the purity to be *ca.* 100%.

The dihydrobromide (2·98 g.) was dissolved in water (5 ml.), and sodium ethoxide (2 equivs.; 0·46 g. of sodium in 20 ml. of absolute alcohol) added. The solvent was evaporated in a vacuum at 40°. Repeated addition of absolute alcohol and toluene (*ca.* 10 ml.), followed by evaporation in a vacuum, removed the last traces of water. Alcohol was then added, and sodium bromide (1·1 g.) removed. The filtrate was treated gradually with absolute ether (2 vols.) and kept until the precipitate had coagulated. Sodium bromide was again removed (total, 1·85 g.; theo., 2·06 g.). The filtrate, evaporated to dryness in a vacuum at 40°, left a syrupy residue (1·55 g.; theo., 1·36 g.).

This oil (117 mg.) was dissolved in ethyl alcohol (2 ml.) and treated with alcoholic hydrogen chloride; 160 mg. of aminoethyl sulphoxide dihydrochloride (theo., 178 mg.), m. p. 220°, were obtained. Permanganate titration of the free base in aqueous acetic acid indicated that the product was 97% pure.

Bis-(N-*pantoyl-β-aminoethyl*) *Sulphoxide* (VI).—Bis-β-aminoethyl sulphoxide (1·5 g.) and redistilled pantolactone (2·6 g.; 2 mols.) were dissolved in absolute methyl alcohol (4 ml.) and kept at 20° for 3 days. The slightly cloudy solution was filtered, and the solvent removed in a vacuum at 40°. The syrup obtained (4·1 g.) was sealed up in absence of air. Permanganate titration in aqueous acetic acid indicated the addition of 1·2 atoms of oxygen. On the assumption that this increased titre corresponds to a partial fission of the sulphoxide, as already discussed, the amount of sulphoxide in the mixture is *ca.* 92%. A Van Slyke determination showed 0·6% of amino-nitrogen, indicating 93% conversion.

Isolation of Bis(pantoylaminoethyl) Disulphide (IV) *from partly Crystalline Bis(pantoylaminoethyl) Sulphoxide Syrup.*—The partly crystalline syrup (7 g.) (prepared by refluxing the components together for 1 hour, evaporating the solvent in a vacuum, and keeping the residual syrup in a sealed tube for 3 months) was digested with acetone (dried, and redistilled over potassium permanganate). After filtration and washing with acetone and absolute ether, the crystals (2·6 g.) had m. p. 129—133° (to an opaque liquid). After recrystallisation from acetone, as described under the disulphide, they had m. p. 141—143°, raised after one more recrystallisation to 143—144° (sharp, to a clear liquid), not depressed by bis(pantoylaminoethyl) disulphide (Found: C, 47·0; H, 7·9; N, 6·8; S, 14·8. Calc. for $C_{16}H_{32}O_6N_2S_2$: C, 46·6; H, 7·8; N, 6·8; S, 15·5%). Permanganate titration of this crystalline material in glacial acetic acid represented an uptake of about 4·1 atoms of oxygen, the disulphide requiring 4 atoms of oxygen.

Bis-β-aminoethylsulphone Dihydrochloride.—(a) *Preparation from the sulphoxide.* 3% Potassium permanganate solution (1 equiv.) was added gradually to an ice-cold solution of the sulphoxide dihydrochloride (500 mg.) in 50% acetic acid (1 ml.). After decoloration with sulphur dioxide the solvent was evaporated in a vacuum. The solid residue was digested with absolute alcohol, excess of 10% alcoholic ammonia added, the inorganic salts removed and re-extracted three times with boiling alcohol, and the united extracts and filtrate evaporated to dryness in a vacuum; the residue was freed from traces of inorganic salts by repeated treatment with alcohol. The filtrate was treated with excess of alcoholic hydrogen chloride, and the precipitate collected and washed with alcohol and ether; needles (200 mg.) were

obtained, m. p. 226—228° after recrystallisation from 90% alcohol (Found: C, 21·5; H, 6·4; N, 12·6; S, 14·1; Cl, 31·1. $C_4H_{12}O_2N_2S,2HCl$ requires C, 21·3; H, 6·3; N, 12·4; S, 14·2; Cl, 31·5%).

(b) *Preparation from the sulphide.* Bis-β-aminoethyl sulphide (18 g.), dissolved in 180 ml. of 50% acetic acid, was cooled to 0°, and potassium permanganate (4 equivs.; 20·7 g. as a 3% solution in 50% acetic acid) added dropwise. The mixture was worked up as described under (a). The crude aminoethylsulphone dihydrochloride (29·7 g.), recrystallised from aqueous alcohol, gave needles (8·92 g.), m. p. 225—226°, not depressed by the *dihydrochloride* obtained under (a). Addition of alcohol to the filtrate gave a second crop (4·84 g.), m. p. 215°. A mixture of rosettes and needles (4 g.), m. p. 203°, obtained by concentrating the mother-liquors and adding more alcohol, was clearly a mixture; the nature of the impurity was not investigated. Only the high-melting material was used for condensations. The total yield was therefore only *ca.* 50%.

Bis-(N-pantoyl-β-aminoethyl)sulphone.—Bis-β-aminoethylsulphone (3·86 g.) (prepared from the dihydrochloride) and pantolactone (2 equivs.; 6·62 g.) were dissolved in absolute methyl alcohol (8 ml.), refluxed for 1 hour with exclusion of moisture, and left overnight at 20°. Evaporation of the solvent in a vacuum left a syrup (10·4 g.) which has not yet crystallised. Van Slyke determination showed 0·19% of free amino-nitrogen, which represents 97% condensation.

For *in vivo* tests, about 30 g. of each of the above analogues were prepared.

Microanalyses were carried out by Drs. Weiler and Strauss of Oxford.

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