35. *Analogues of Pantothenic Acid. Part IV. Aryl Derivatives of Pantoyltaurine.*

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Three aryl-substituted analogues of pantoyltaurine (I) have been prepared, in an attempt to make antibacterial substances which would be growth-inhibitors by virtue of their structural relationship to pantothenic acid, but which would be less water-soluble than pantoyltaurine and so possibly have a higher *in vivo* activity. The new analogues are β-(αγ-dihydroxy-ββ-dimethylbutyramido)-α-phenylethanesulphonic acid (II), β-(αγ-dihydroxy-ββ-diphenylbutyramido)ethanesulphonic acid (III), and β-(α-tosyl-γ-hydroxy-ββ-dimethylbutyramido)-ethanesulphonic acid (IV). None of these shows bacteriostatic activity *in vitro* or *in vivo*.

The preparation of analogues of pantothenic acid possessing growth-inhibiting properties, by virtue of their structural relationship to pantothenic acid, has so far been limited to the aliphatic series (Part II, Barnett and Robinson, *Biochem. J.*, **1942**, **36**, 364; Part III, Barnett, this vol., p. 5). *N*-Pantoyltaurine (I), the sulphonic acid analogue of pantothenic acid, has been found highly active in inhibiting the growth *in vitro* of micro-organisms for which pantothenic acid is essential (McIlwain, *Biochem. J.*, 1942, **36**, 417; *Brit. J. exp. Path.*, 1942, **23**, 95; Snell, *J. Biol. Chem.*, 1941, **139**, 975; **141**, 121; Kuhn *et al.*, *Ber.*, 1941, **74**, 1605), but has an effect *in vivo* only in very high doses (McIlwain and Hawking, *Lancet*, 1943, **i**, 449). This is due, in part, to the extremely high rate of excretion of pantoyltaurine, making it difficult to maintain a bacteriostatic concentration in the blood, and, on the other hand, to the presence in the animal organism of pantothenic acid, which antagonises the effect of pantoyltaurine.

With the object of reducing the solubility of pantoyltaurine and, at the same time, preserving as far as

possible the structural relationship to pantothenic acid, two phenyl derivatives of pantoyltaurine were synthesised. It was hoped by this means to reduce the rate of excretion and yet to retain antibacterial activity. These two substances, β-(αγ-dihydroxy-ββ-dimethylbutyramido)-α-phenylethanesulphonic acid (II) and β-(αγ-dihydroxy-ββ-diphenylbutyramido)ethanesulphonic acid (III), were almost inactive *in vitro* against *Lactobacillus arabinosus*. As might have been expected, (II) was inactive *in vivo*; (III) was not tested.

A different method of reducing the rate of excretion was also tried. This involved introducing a toluenesulphonyl group on the α-hydroxy-group of pantoyltaurine to give β-(α-tosyl-γ-hydroxy-ββ-dimethylbutyramido)-ethanesulphonic acid. It was expected that this compound would have no *in vitro* activity, but would slowly hydrolyse in the body, giving pantoyltaurine; it was, however, inactive both *in vitro* and *in vivo*.

(I.) $CH_2(OH)\cdot CMe_2\cdot CH(OH)\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot SO_3H$ $CH_2(OH)\cdot CMe_2\cdot CH(OH)\cdot CO\cdot NH\cdot CH_2\cdot CHPh\cdot SO_3H$ (II.)

(III.) $CH_2(OH)\cdot CPh_2\cdot CH(OH)\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot SO_3H$ $CH_2(OH)\cdot CMe_2\cdot CH(OTs)\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot SO_3H$ (IV.)

where Ts = toluene-*p*-sulphonyl.

Compound (II) was prepared as follows : catalytic reduction of *iso*nitrosoacetophenone by means of palladised charcoal gave phenylethanolamine (Hartung *et al.*, *J. Amer. Chem. Soc.*, 1928, **50**, 3370; 1930, **52**, 3317; 1931, **53**, 2248, 4149) in 49% yield. This reacted with thionyl chloride at room temperature to give β-phenyl-β-chloroethylamine hydrochloride in 66% yield; the method is more convenient than that of Wolfheim (*Ber.*, 1914, **47**, 1466). The compound was converted into *α-phenyltaurine* in 40% yield by prolonged heating with sodium sulphite solution. The *sodium* salt of α-phenyltaurine was condensed with pantolactone to give (II). dl-*α-Hydroxy-ββ-diphenyl-γ-butyrolactone*, the substance required for the preparation of (III), was synthesised by a method analogous to that used for the preparation of pantolactone. Ethoxyacetic ester (*Org. Synth.*, 1933, **13**, 42) was converted, by means of a Grignard reaction, into diphenylethoxymethylcarbinol by Béhal and Sommelet's method (*Compt. rend.*, 1904, **138**, 89); this, on heating with oxalic acid (Danilov *et al.*, *Ber.*, 1926, **59**, 1032), gave diphenylacetaldehyde. This was condensed with formaldehyde by means of solid potassium carbonate. The reaction was not quantitative, however, as after a short time the mixture set to a solid mass in spite of vigorous shaking, occluding large amounts of starting material. Attempts to avoid this by carrying out a condensation in alcohol or ether were unsuccessful. The crude product was condensed with anhydrous hydrogen cyanide in ether containing a drop of piperidine. The resulting cyanohydrin was hydrolysed, giving the required butyrolactone. This was obtained in two forms, m. p. 141° and 175—176°; the one could not be converted into the other by seeding and neither form gave an amide or a phenylhydrazide.

The lack of reactivity of the lactone is consistent with the difficulty of condensing it with taurine. Various conditions for the condensation were tried, including fusion in the dry state, but in no instance did the reaction proceed to an extent greater than 50%, the best yield being obtained by refluxing in methyl-alcoholic solution for 15 hours.

The preparation of a third phenyl analogue of pantoyltaurine, namely, (α-hydroxy-β-phenyl-ββ-dimethylpropionamido)ethanesulphonic acid, had been planned, and for this purpose *α-hydroxy-β-phenylisovaleric acid* was prepared, but in view of the negative results with the above compounds, the preparation of this analogue was not completed. α-Hydroxy-β-phenyl*iso*valeric acid was prepared from phenyl*iso*butaldehyde obtained by Tiffeneau and Dorlencourt's method (*Ann. Chim. Phys.*, 1907, **10**, 247). Some difficulty was encountered, however, in preparing the cyanohydrin, since phenyl*iso*butaldehyde does not form a bisulphite compound. The condensation was therefore attempted with potassium cyanide in presence of calcium chloride (Reichstein, *Helv. Chim. Acta*, 1940, **23**, 650; Carter and Ney, *J. Amer. Chem. Soc.*, 1941, **63**, 313), but no reaction took place. Eventually the cyanohydrin was obtained by the action of anhydrous hydrogen cyanide and a drop of piperidine, but even by this method the yield of acid after hydrolysis of the cyanohydrin was only 24%.

β-(α-Tosyl-γ-hydroxy-ββ-dimethylbutyramido)ethanesulphonic acid (IV) was obtained as its *sodium* salt by the condensation of the sodium salt of taurine with *α-tosyl-ββ-dimethylbutyrolactone* (*monotosylpantolactone*) in methyl-alcoholic solution in the usual way. The monotosyl lactone was obtained in 76% yield by treatment of pantolactone in pyridine solution with tosyl chloride.

Compound (II), tested *in vitro* on *Streptococcus hæmolyticus* strain 618, showed no antibacterial activity in dilutions of from 1 : 1000 to 1 : 500,000. It was also practically inactive against *Lactobacillus arabinosus in vitro*. *In vivo* tests on rats indicated that the substance afforded no protection against *Streptococcus hæmolyticus*, the latter being recovered from their organs. Control animals, given substance (II) but not infected, remained healthy. Compound (III) also was practically inactive *in vitro* against *Lactobacillus arabinosus*, in concentrations at which pantoyltaurine inhibited growth. Compound (IV) was inactive *in vitro* against *Lactobacillus arabinosus* and against *Streptococcus hæmolyticus*, and *in vivo* it had no protective action in mice and rats infected with *Streptococcus hæmolyticus*. All the animals died during treatment and *Streptococcus hæmolyticus* was recoverable from their organs.

Experimental.

α-Phenyltaurine.—Thionyl chloride (13·5 ml.) in absolute benzene (15 ml.) was added slowly with stirring to *β*-phenyl-*β*-hydroxyethylamine hydrochloride (24 g.). When the mixture showed signs of becoming viscous, a further 10 ml. of benzene were added, and the whole was shaken thoroughly. After 12 hours, the mixture was distilled in a vacuum at 50° (bath temp.) to remove volatile products, the residue taken up in alcohol, and *β*-phenyl-*β*-chloroethylamine hydrochloride precipitated with ether (yield, 22·5 g.) and recrystallised from ethyl alcohol, giving 14 g., m. p. 157—158°.

A mixture of *β*-phenyl-*β*-chloroethylamine hydrochloride (8·4 g.), sodium sulphite (12·3 g.), and water (150 ml.)

was heated under reflux on the steam-bath for 18 hours and then evaporated to dryness in an open dish. A solution of the solid in the minimum amount of boiling water was filtered hot; on cooling, *α-phenyltaurine* (3·8 g.) crystallised in white platelets, m. p. 258° (decomp.); it gave a negative test for halogen. The mother-liquor was treated with concentrated hydrochloric acid, filtered from sodium chloride, and treated with acetone; the α-phenyltaurine thereby obtained had m. p. 258° after recrystallisation from hot water (yield, 0·5 g.). Total yield, 40% (Found: C, 47·9; H, 5·25; N, 6·7; S, 16·1. $C_8H_{11}O_3NS$ requires C, 47·8; H, 5·5; N, 6·9; S, 15·9%).

The *sodium* salt was prepared by addition of sodium hydroxide (1 equiv.) to an aqueous solution of α-phenyltaurine, followed by evaporation to dryness in a vacuum and removal of traces of water by means of alcohol and absolute toluene. A white powder was obtained which was almost non-hygroscopic (Found: N, 5·6; S, 14·2; Na, 10·1. $C_8H_{10}O_3NSNa$ requires N, 6·2; S, 14·3; Na, 10·3%).

Sodium β-(αγ-Dihydroxy-ββ-dimethylbutyramido)-α-phenylethanesulphonate (as II).—The sodium salt of α-phenyltaurine (3·82 g.) was added to a solution of pantolactone (2·5 g.) in absolute methyl alcohol (30 ml.), which was then refluxed for 6 hours with exclusion of moisture. After cooling and filtration, the solution was concentrated in a vacuum at 40° to small bulk and the syrup obtained was poured into absolute ether with shaking. The granular white precipitate was collected, washed with ether (care being taken to keep it covered with ether), and dried in a vacuum over phosphoric oxide. Yield, 6·4 g. (theor., 6·3 g.) (Found: N, 4·1; S, 8·3; Na, 6·6. $C_{14}H_{20}O_6NSNa$ requires N, 4·0; S, 9·1; Na, 6·5%). It was very hygroscopic, and readily soluble in water.

β-Hydroxy-αα-diphenylpropaldehyde.—To a mixture of diphenylacetaldehyde (10 g., b. p. 170—172°/12 mm.) and 40% formalin solution (4 g.) at 0°, potassium carbonate (2·4 g.) in water (20 ml.) was added gradually with shaking, the reactants being kept cold by ice–salt. The mixture thickened after 5 minutes, and became semi-solid after ½ hour at room temperature. It was warmed at 50° for a few minutes until it was fluid and was then shaken and left at 20° overnight. The aqueous layer was decanted; the solid mass, after being shaken with a little ether, yielded *ca.* 3·8 g., m. p. *ca.* 95°, and was used for the subsequent condensation. It could be recrystallised from alcohol to give material, m. p. 137—139°.

dl-*α-Hydroxy-ββ-diphenyl-γ-butyrolactone.*—(a) *Lactone, m. p.* 141°. β-Hydroxy-αα-diphenylpropaldehyde (700 mg.) was suspended in sodium-dried ether (25 ml.), and a drop of piperidine added, followed by hydrogen cyanide (500 mg.). After 42 hours' shaking, the ether was evaporated, and the residual cyanohydrin refluxed with concentrated hydrochloric acid (10 ml) for 8 hours. The hydrolysis mixture was extracted three times with ether, the extracts washed with dilute sodium bicarbonate solution (until the washings were alkaline) and with water and dried (sodium sulphate), and the ether distilled. The brownish residue, crystallised twice from benzene after filtering through charcoal, yielded the *lactone* (120 mg.), m. p. 141° (Found: C, 75·4; H, 5·7. $C_{16}H_{14}O_3$ requires C, 75·5; H, 5·55%).

(b) *Lactone, m. p.* 174°. The reaction was carried out on about ten times the scale of the previous experiment, the only difference being that there still remained some undissolved material after 4 days' shaking; this was removed and washed with ether. The washings were added to the filtrate, and the insoluble matter was re-treated with hydrogen cyanide and piperidine, until, after a further day's shaking, it had all dissolved. On working up as before, several crops of crystalline lactone were obtained; this, after being decolorised with charcoal and recrystallised from benzene, yielded the lactone in needles (1·5 g.) m. p. 172—174°. Two more recrystallisations raised the m. p. to 175—176° (Found: C, 75·0; H, 5·7%).

β-(αγ-Dihydroxy-ββ-diphenylbutyramido)ethanesulphonic Acid (III).—*dl-α*-Hydroxy-ββ-diphenyl-γ-butyrolactone (173 mg., m. p. 174—176°) (10% excess) and the sodium salt (90 mg.) of taurine were refluxed in absolute methyl-alcoholic solution (3 ml.) for 15 hours. Absolute ether was added to the cooled concentrated solution, and the precipitate collected rapidly (yield of powder dried over phosphoric oxide, 120 mg.). From the ethereal filtrate unchanged lactone was recovered, m. p. 175—176° after recrystallisation from benzene–petroleum (yield 75 mg.). The mother-liquors yielded a further 12 mg., m. p. 171—173°; the total weight recovered (87 mg.) indicates 55% condensation. A Van Slyke determination gave 2·5% of free amino-nitrogen, which represents 51% condensation.

α-Hydroxy-β-phenylisovaleric Acid.—(*a*) Phenyl*iso*butaldehyde (5 g., b. p. 98—101°/12 mm.) was treated at 0° with anhydrous hydrogen cyanide (1·1 g.; 20% excess) and a drop of piperidine. The mixture was allowed slowly to attain room temperature, concentrated hydrochloric acid (20 ml.) added after 4 days, and the liquid refluxed for 9 hours. Two layers persisted throughout. After being made alkaline, the mixture was extracted twice with ether to remove unchanged phenyl*iso*butaldehyde. It was then acidified with hydrochloric acid and extracted three times with ether; the extracts were washed with a little water and dried, and the ether removed. The residue formed a crystalline cake, which, after two recrystallisations from benzene–light petroleum, yielded α-hydroxy-β-phenyl*iso*valeric acid (670 mg.), m. p. 91—92°.

(*b*) The reactants were mixed exactly as under (*a*) and left at room temperature for 3 weeks; the product was then hydrolysed and worked up as above. The yield of *α-hydroxy-β-phenylisovaleric acid* (1·55 g.), m. p. 94—95° after recrystallisation from benzene–light petroleum, was 24% (Found: C, 68·2; H, 7·3. $C_{11}H_{14}O_3$ requires C, 68·0; H, 7·3%).

Monotosyl Ester of Pantolactone.—A solution of dry redistilled pantolactone (3 g.) in absolute pyridine (10 ml.) was cooled in ice, and an ice-cold solution of toluene-*p*-sulphonyl chloride (5·7 g.) in pyridine (20 ml.) added slowly, with exclusion of moisture. After being left at room temperature for 16 hours, the mixture was heated at 100° for 1 hour, cooled, and poured into ice-cold dilute hydrochloric acid. An oil separated and solidified on stirring. It was collected, washed with water, and dissolved in chloroform. The chloroform solution was washed successively with dilute hydrochloric acid, dilute sodium bicarbonate solution, and water, the washings being extracted twice more with small amounts of chloroform. The extracts, after drying over anhydrous sodium sulphate, were evaporated in a vacuum to dryness. On addition of methyl alcohol to the residue, crystals (4·4 g.) were at once obtained, m. p. 113—114°. A second crop (0·75 g.) from the mother-liquor, after charcoal treatment, had m. p. 113—115°. Recrystallisation from methyl alcohol gave pure *monotosylpantolactone* in white needles, m. p. 114—115° (Found: C, 55·1; H, 6·0; S, 10·6. $C_{13}H_{16}O_5S$ requires C, 54·9; H, 5·7; S, 11·3%).

Sodium β-(α-Tosyl-γ-hydroxy-ββ-dimethylbutyramido)ethanesulphonate (*as* IV).—Monotosylpantolactone (1·42 g.) and the sodium salt (0·73 g.) of taurine were refluxed in absolute methyl alcohol (12 ml.) for 5 hours with exclusion of moisture. The practically clear solution was filtered and worked up as described for (II). Yield, 1·5 g. (71%) of a white hygroscopic powder, from ether (Found: N, 3·7; S, 13·9; Na, 6·5. $C_{15}H_{22}O_8NS_2Na$ requires N, 3·3; S, 14·8; Na, 5·4%). Van Slyke determination of free amino-nitrogen showed 0·86%, indicating 77% condensation.

Melting points are all uncorrected.

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