40. Analogues of Pantothenic Acid

2. Preparation of Growth Inhibitors

By **Jean W. Barnett** and **F. Arnold Robinson**, *Chemical Research Department, Glaxo Laboratories Ltd., Greenford, Middlesex*

(*Received 25 March 1942*)

Of the various theories advanced to explain the bacteriostatic effect of sulphanilamide and related compounds, that which has received most widespread acceptance is the 'enzyme-interference' or the 'anti-metabolite' theory.

This theory is based on evidence published by Woods [1940] and Selbie [1940]. The former found that *p*-aminobenzoic acid antagonized the bacteriostatic effect of sulphanilamide *in vitro* and the latter obtained the same result *in vivo*. Fildes [1940] suggested that the bacteriostatic action of sulphanilamide was due to competition for an enzyme containing *p*-aminobenzoic acid, which he regarded as an essential metabolite for such organisms as are inhibited by sulphanilamide. The mutually antagonistic action of *p*-aminobenzoic acid and sulphanilamide was confirmed by Rubbo & Gillespie [1940], Spink & Jermsta [1941] and Strauss *et al.* [1941], whilst the general theory received support from McIlwain's observation that nicotinic acid and pyridine-β-sulphonic acid also antagonize one another [McIlwain, 1940; 1941]. (See Table 2 for formulae.)

Arising out of the work on pantothenic acid described in Part 1, it occurred to us that since pantothenic acid is known to be an essential metabolite for a number of organisms, it should be possible to prepare analogues of similar structure which would antagonize the effect of pantothenic acid, just as sulphanilamide neutralizes the growth-effect of *p*-aminobenzoic acid.

Table 1

No.	Amino-acid	Lactone	Analogue	Inhibitory effect
1	Taurine	α-Hydroxy-ββ-dimethyl-γ-butyrolactone	$CH_2OH—C(CH_3)_2—CHOH—CO—NH—CH_2—CH_2—SO_3H$ Pantoyltaurine	Highly inhibitory to *Strep. haemolyticus* and some strains of *Corynebact. diphtheriae*. Effect reversed by pantothenic acid
2	Taurine	β-Hydroxy-γγ-dimethyl-δ-valerolactone	$CH_2OH—C(CH_3)_2—CHOH—CH_2—CO—NH—CH_2—CH_2—SO_3H$ Homopantoyltaurine	Inhibitory to *Strep. haemolyticus*, but less active than 1. Effect reversed by pantothenic acid
3	Taurine amide	As 1	$CH_2OH—C(CH_3)_2—CHOH—CO—NH—CH_2—CH_2—SO_2NH_2$ Pantoyltauramide	As 2
4	β-Alanine	ββ-Dimethyl-γ-butyrolactone	$CH_2OH—C(CH_3)_2—CH_2—CO—NH—CH_2—CH_2—COOH$ Deoxypantothenic acid	Showed some inhibition to *B. coli*. Effect not reversed by pantothenic acid
5	β-Alanine	γ-Butyrolactone	$CH_2OH—CH_2—CH_2—CO—NH—CH_2—CH_2—COOH$ *bisnor*Deoxypantothenic acid	Inhibitory in high dilution towards *S. haemolyticus*, *C. diphtheriae*, *B. coli*, *Pr. vulgaris*, and *S. aureus*. With all organisms except *Pr. vulgaris*, effect not reversed by pantothenic acid
6	β-Alanine	γ-Valerolactone	$CH_3—CHOH—CH_2—CH_2—CO—NH—CH_2—CH_2—COOH$ *isonor*Deoxypantothenic acid	Similar to 5 but less marked

The preparation of pantoyltaurine (Table 1, no. 1) (for an explanation of the nomenclature adopted, see Part 1) was suggested to us by Dr H. McIlwain, and we accordingly prepared this compound and related substances which are listed in Table 1. While this work was in progress, two papers were published by Snell [1941, 1, 2] in which the preparation of pantoyltaurine (but not of any other analogues) was described.* Snell found that pantoyltaurine inhibited the growth of *Lactobacillus arabinosus*, *Streptococcus lactis* and *Propionibacterium pentosaceum*, but he did not apparently carry out any tests with *Streptococcus haemolyticus* or *Corynebacterium diphtheriae*, with which the most significant results in the present investigation were obtained. The present work was carried out independently of Snell, although we cannot claim priority in the preparation of pantoyltaurine.

The methods of testing these analogues for inhibitory effect on the growth of microorganisms and the results obtained will be discussed in detail in a subsequent communication by Dr H. McIlwain who performed the bacteriological tests. The compounds were tested on *Bact. coli*, *Proteus morgani*, *Pr. vulgaris*, *Staphylococcus aureus*, *Streptococcus haemolyticus* and *Corynebacterium diphtheriae*; the results are summarized in Table 1.

Toxicity tests indicate that pantoyltaurine is non-toxic to mice in a dose of 5 g. per kg. *In vivo* animal tests are in progress, and these results will be published in a later communication.

Chemical method

The general method of condensation was that described in Part 1, where the preparation of compounds 4, 5 and 6 has already been given, and the preparation of the taurine analogues will be described here. With compounds 1 and 2, the method of refluxing the components together in methyl alcohol was found more convenient than the fusion method [Williams, 1940; Snell, 1941, 1, 2], the latter yielding a solid glass which was difficult to handle. Both products, however, showed a similar degree of inhibition. With pantoyltauramide, which was soluble in ether, the refluxing method had no advantage over the fusion method.

Taurine sodium salt

Taurine, M.P. 299° with decomposition, was prepared from ox-bile. The sodium salt was prepared by adding an exact equivalent of 10 % NaOH, boiling for 1 min., filtering from a trace of flocculent precipitate which always appeared, and evaporating to dryness *in vacuo*. Addition of absolute alcohol and toluene, followed by evaporation to dryness was carried out three times, to ensure that the product was quite dry. It was finally ground up with ethyl alcohol, filtered, and washed with absolute alcohol and absolute ether. (Found: N, 9·3 %. Calc.: N, 9·53 %.)

Condensation

Pantoyltaurine (*no.* 1). The condensation of pantolactone with taurine was effected both by the fusion method and by the refluxing method.

(*a*) *Fusion method.*. The sodium salt of taurine (581 mg.) and pure dry pantolactone (502 mg.) were heated together in an oil-bath at 120° for 3 hr. Yield 600 mg. (Found: N, 4·81 %. Calc.: N, 5·06 %.)

(*b*) *Refluxing method.* The same amounts of the reactants, as above, were refluxed in 5 ml. of absolute methyl alcohol for 3½ hr. (Refluxing for 1 hr. only, gave incomplete condensation.) Yield 750 mg. (Found: N, 5·10 %. Calc.: N, 5·06 %.)

Homopantoyltaurine (*no.* 2). The sodium salt of taurine (581 mg.) was fused with 550 mg. of homopantoyllactone (β-hydroxy-γγ-dimethyl-δ-valerolactone) at 120° for 3 hr. in the usual way. Yield 700 mg. (Found: N, 4·38 %. Calc.: N, 4·81 %.)

* There has recently come to our notice a paper by Kuhn *et al.* [1941] in which the preparation of the *d*- and *l*-pantoyltaurines is described, and their inhibitory effects on the growth of *Streptobacterium plantarum* noted.

Pantoyltauramide (no. 3). Taurine amide hydrochloride was prepared by the method of Miller [1940], M.P. 132–133°. The free amide was obtained from the hydrochloride by dissolving the latter in water, adding an exact equivalent of 20% aqueous NaOH and evaporating the water *in vacuo* at 50°. Absolute ethyl alcohol and toluene were added and distilled off *in vacuo* at 50°, this procedure being repeated twice more to remove all traces of water. Finally, five parts of absolute ethyl alcohol were added, the precipitated sodium chloride was filtered off and washed well with alcohol, and the filtrate was evaporated to dryness *in vacuo* at 40°. The residue crystallized at once in flat platelets.

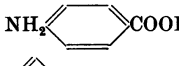
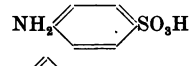
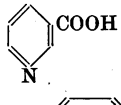
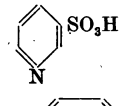
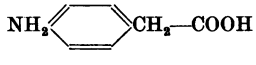
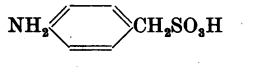
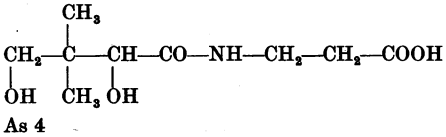
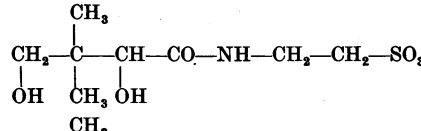
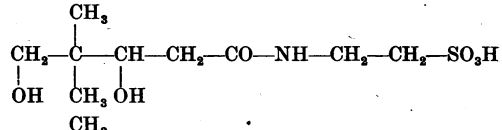
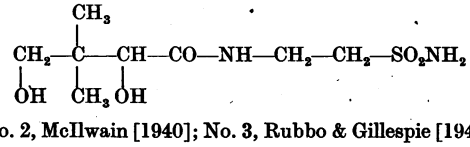
Condensation. Taurine amide (1·11 g.) and pantolactone (1·17 g.) were heated together for 3½ hr. at 120°. The mass melted to a viscous liquid, but did not give a precipitate when a trial portion was dissolved in methyl alcohol and added to ether, and it was therefore tested in the form of a yellowish glass-like solid.

Note. All melting points are uncorrected. Micro-analyses were carried out by Dr G. Weiler of Oxford. Our thanks are due to Misses E. Paxton, K. N. Speyer, P. Turner-Jones and B. Wilson for assistance in the preparation of some of the intermediate compounds, and to Mr A. L. Bacharach for the toxicity determination.

Discussion

The bacteriological results with analogues 1, 2 and 3 provide further confirmation of the general anti-metabolite theory, of which three examples have already been reported in the literature. These are given, together with the substances now described, in Table 2;

Table 2. *Table showing pairs of substances so far found to be mutually antagonistic to one another*

No.	Growth stimulator	Corresponding inhibitor
1		
2		
3		
4		
5	As 4	
6	As 4	

References: No. 1, Fildes [1940], Woods [1940]; No. 2, McIlwain [1940]; No. 3, Rubbo & Gillespie [1942]; No. 4, Snell [1941, 1, 2]; Kuhn *et al.* [1941].

the components of each pair of compounds are mutually antagonistic; that is, the effect of one may be nullified by addition of a sufficient quantity of the other. In each instance, the two compounds are closely related chemically. The degree of inhibition by pantoyltaurine depends on the amount of pantothenic acid present in the medium and is reversed in a regular manner by addition of pantothenic acid, just as in the case of *p*-aminobenzoic

acid and sulphanilamide. It might be worth noting here that the chemical structure of the inhibitor does not seem to be necessarily so specific as that of the growth-promoter, since homopantoyltaurine (Table 2, no. 5) inhibits growth and is reversed by pantothenic acid, whereas its analogue homopantothenic acid (Part 1) does not act as a growth-stimulator.

The inhibition shown by compounds 4, 5 and 6 (Table 1), all of which lack the α-hydroxy group of pantothenic acid, appears to be of a different type from that produced by the sulphonic acids, for the inhibition is not reversed by addition of pantothenic acid. These three analogues (nos. 4, 6 and 5, Table 1) show an increasing degree of inhibition in that order and this may be correlated with an increasing divergence in structure from that of pantothenic acid. The inhibition produced by the sulphonic acids is of the competitive type, whereas the inhibition produced by the deoxypantothenic acids is of the non-competitive type, since it is not reversed on addition of pantothenic acid.

The preparation of further analogues is in progress.

Summary

1. Analogues of pantothenic acid obtained by condensing α-hydroxy-ββ-dimethylbutyrolactone with taurine and taurine amide and by condensing β-hydroxy-γγ-dimethylvalerolactone with taurine have been prepared and have been found to be highly inhibitory to *Streptococcus haemolyticus* and to some strains of *Corynebacterium diphtheriae*. The inhibitory effect is reversed by pantothenic acid.

2. Other analogues have been prepared which show an inhibitory activity which is not reversed by pantothenic acid. In all these compounds, the α-hydroxy group of pantothenic acid is absent.

REFERENCES

Fildes, P. [1940]. *Lancet*, **1**, 956.
Kuhn, R., Wieland, T. & Müller, E. F. [1941]. *Ber. dtsch. chem. Ges.* **74**, 1605.
McIlwain, H. [1940]. *Brit. J. exp. Path.* **21**, 136.
—— [1941]. *Brit. J. exp. Path.* **22**, 148.
Miller, E. [1940]. *J. Amer. chem. Soc.* **62**, 2099.
Rubbo, S. D. & Gillespie, J. M. [1940]. *Nature, Lond.*, **146**, 838.
—— —— [1942]. *Lancet*, **1**, 36.
Selbie, F. R. [1940]. *Brit. J. exp. Path.* **21**, 90.
Snell, E. E. [1941, 1]. *J. biol. Chem.* **139**, 975.
—— [1941, 2]. *J. biol. Chem.* **141**, 121.
Spink, W. W. & Jermsta, J. [1941]. *Proc. Soc. exp. Biol., N.Y.*, **47**, 395.
Strauss. E., Dingle, J. H. & Finland, M. [1941]. *Proc. Soc. exp. Biol., N.Y.*, **46**, 131, 133.
Williams, R. J. [1940]. *J. Amer. chem. Soc.* **62**, 1784.
Woods, D. D. [1940]. *Brit. J. exp. Path.* **21**, 74.