6. The inhibitory effect, upon Gram-positive bacteria, of diethyl stilboestrol and hexoestrol has been confirmed but was not reversed by lecithin or cholesterol. Dienoestrol is also inhibitory, but its dibenzoate and dipropionate esters are not.

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Antibacterial Substances Related to Pantothenic Acid

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It has already been shown that pantoyltaurine (Snell, 1941; McIlwain, 1939 *a, b*; McIlwain & Hawking, 1943) and other pantothenic acid derivatives (Barnett & Robinson, 1942; McIlwain, 1942) are *in vitro* antibacterial substances whose action can be antagonized by increasing the amount of pantothenic acid present in the medium. Further, it has been possible to demonstrate a therapeutic activity in rats infected with streptococci (McIlwain & Hawking, 1943) by administering large and frequent doses of pantoyltaurine. The rat was chosen because the level of circulating pantothenic acid was low enough for an effect of pantoyltaurine to be demonstrable with this animal; in mice the level was too high. The massive dosing was necessary to offset the rapid elimination of the drug from the blood stream and thus provide a concentration sufficient to antagonize the high level of pantothenic acid normally found in blood plasma. It is possible that the presence of the sulphonic acid group in pantoyltaurine was the cause of the rapid clearance rate and that a more favourable therapeutic effect might be obtainable if this group were replaced by a neutral or basic residue, retaining where possible

the β -aminoethyl configuration of the parent pantothenic acid. Recently Barnett (1944) has described certain N-pantoyl- β -aminoethylthiol, sulfoxide and sulphone compounds in which the sulphonic acid group of pantoyltaurine is replaced by other sulphur-containing groupings such as $-S-$, $-SO-$ and $-SO_2-$, but although the substances inhibited the growth of *Lactobacillus arabinosus*, *in vitro*, and this effect was reversed by pantothenic acid, it was not possible to obtain any action in rats infected with *Streptococcus pyogenes*.

The present investigation was undertaken with the object of preparing and examining further examples of pantamides in the expectation that substances of some therapeutic value might result. The substances I-XI in Table 1, collectively referred to as pantamides, were made and tested.

The reference inhibitor used was pantoyltaurine: $P-NH-CH_2-CH_2SO_3Na$ (see Table 1). Other substances, such as hydrazine, phenylhydrazine, salicylhydrazide, salicylic acid, quinine and mepacrine, were included in the biological tests in order to determine by comparison whether the antibacterial activity of some of the substances enumerated

above was due to a specific antagonism of pantothenic acid or to the presence in the molecule of other toxophore groups.

EXPERIMENTAL

Action of pantothenic acid on the bacteriostatic effect of pantamides

We are indebted to Dr F. C. Happold of Leeds University for the strain of *Lactobacillus casei* ϵ (*B. helveticus*) used in this work. The assay medium was prepared as described by Strong, Feeney & Earle (1941), but Evans's peptone (Evans Sons, Lescher and Webb Ltd.) was substituted for Bacto-peptone and the treated Difco Labco yeast was replaced by Marmite similarly treated. The strain of *L. casei* ϵ was maintained and the inoculum prepared by the method described by these authors.

Two media were prepared by taking 1 vol. of double-strength medium and adding in each case 0.5 vol. of *dl*-pantothenic acid solution of such a concentration that the final media contained 2.5 and 0.025 $\mu\text{g./ml.}$, respectively, of *dl*-pantothenic acid. After autoclaving, both media were bulk-inoculated with 0.2 ml./100 ml. of a 24 hr. old culture of *L. casei* ϵ .

In two parallel series of small tubes were placed 0.5 ml. of suitable dilutions (usually two-fold) of the compound under assay. To one series the bulk-inoculated medium (1.5 ml.) containing 2.5 $\mu\text{g./ml.}$ pantothenic acid was added and to the other the same volume of the medium containing 0.025 $\mu\text{g./ml.}$ After 18–24 hr. incubation at 37° the highest concentration of drug (in mg./ml.) just permitting growth of *L. casei* ϵ at each of the two concentrations of pantothenic acid was recorded (Table 1). The growth was determined by visual comparison.

Table 1. *Antibacterial activity of pantamides on L. casei* ϵ and *Strep. pyogenes*

Compound no. Reference inhibitor	<i>dl</i> -Na pantothenate added to medium ($\mu\text{g./ml.}$):	<i>L. casei</i> ϵ		<i>Strep. pyogenes</i>
		2.5	0.025	Nil
		Highest concentration of drug (mg./ml.) which will just permit growth		
	*P—NH—CH ₂ —CH ₂ SO ₃ Na (pantoyltaurine)	10	0.6	0.04
I	P—NH ₂	10	1.25	—
II	P—NH—NH ₂	5	0.006	1
III	P—NH—(CH ₂) ₂ N(C ₂ H ₅) ₂	5	2.5	0.3
IV	$\begin{array}{c} \text{CH}_3 \\ \\ \text{P—NH—CH} \\ \\ (\text{CH}_2)_2\text{N(C}_2\text{H}_5)_2 \end{array}$	1.2	1.2	1
V	$\begin{array}{c} \text{COOH} \\ \\ \text{P—NH—CH} \\ \\ \text{CH}_2-\text{C}_6\text{H}_5 \end{array}$	—	—	0.3
VI	$\begin{array}{c} \text{CH}_2-\text{CH}_2 \\ \qquad \\ \text{P—NH—CH} \quad \text{CH}_2 \\ \qquad \\ \text{CH—CH}_2 \\ \\ \text{COONa} \end{array}$	10	10	1
VII	$\begin{array}{c} \text{CH}_2-\text{CH}_2 \\ \qquad \\ \text{P—N} \quad \text{CH}_2 \\ \qquad \\ \text{CH}_2-\text{CH} \\ \\ \text{COONa} \end{array}$	10	10	—
VIII	$\text{P—NH(CH}_2)_2\text{SO}_2-\text{C}_6\text{H}_4-\text{CH}_3$	2.5	0.16	0.1

Table 1 (cont.).

Compound no.	<i>dl</i> -Na pantothenate added to medium (μg./ml.):	<i>L. casei</i> ε		<i>Strep. pyogenes</i>
		2.5	0.025	Nil
		Highest concentration of drug (mg./ml.) which will just permit growth		
IX		5	0.16	0.01
X		5	0.04	0.01
XI		—	—	0.01
XII		0.16	0.16	—
XIII		0.16	0.16	—
XIV		0.16	0.16	—
XV		1.25	1.25	—
XVI	Quinine	0.6	0.6	—
XVII	Mepacrine (Atebrine)	0.04	0.04	—

* P = $dl\text{-HO-CH}_2\text{-C(CH}_3\text{)}_2\text{-CHOH-CO-}$ = pantoyl residue.

Table 2. Antagonism of pant-hydrazide and pantothenic acid with *L. casei* ε as test organism

Concentration of pant-hydrazide (II) (mg./ml.)	Concentration of <i>dl</i> -sodium pantothenate (μg./ml.)							
	1	0.33	0.11	0.04	0.01	0.004	0.001	Nil
12.5	tr.	tr.	—	—	—	—	—	—
4.1	tr.	tr.	—	—	—	—	—	—
1.4	±	±	—	—	—	—	—	—
0.45	±	±	tr.	—	—	—	—	—
0.15	±	±	tr.	—	—	—	—	—
0.05	+	+	±	—	—	—	—	—
0.017	++	+	+	tr.	—	—	—	—
0.006	++	++	+	tr.	tr.	tr.	tr.	tr.
0.002	++	++	+	±	±	tr.	tr.	tr.
Nil	++	++	++	+	±	±	±	±

— = no growth. tr. = trace growth. ++ = full growth.

In order to demonstrate the specific nature of the antagonism, quinine, mepacrine, salicylic acid, hydrazine, salicylhydrazide and phenylhydrazine were also included. The hydrazides were included on account of the outstanding properties of pant-hydrazide (II) as a pantothenic acid antagonist. In view of this remarkably high activity a more detailed experiment was carried out with this substance in which a series of concentrations of pantothenic acid was used (Table 2).

*Bacteriostatic action of pantamides on
Streptococcus pyogenes*

(a) *In vitro experiments.* We are indebted to Dr H. McIlwain of Sheffield University for the culture of *Strep. pyogenes*, 'Richards' strain, used by us for this examination. The medium employed for the *in vitro* examination was a slight modification of that used by McIlwain (1939 *a, b*). The following components of his 'mixture A'—pimelic acid, uracil, cytosine, thymine, guanine and riboflavin—were omitted and to the completed medium 0.2% peptone was added. Without the latter, growth of the organism almost always failed. The drug dilutions to be examined were placed in sterile tubes in 1 ml. volumes. To each tube were added 9 ml. of a previously prepared mixture of basal solution, supplements and inoculum (in the correct proportions). In this way 10 ml. of medium, sown with the test organism and containing a known amount of drug, were obtained. After incubation at 37° for 24 hr. the tubes were examined for growth; the results are given in Table 1.

(b) *In vivo experiments.* The *in vivo* action of compounds II, VIII, IX and XI was examined in rats infected intraperitoneally with 0.2 ml. of a 1 : 10,000 dilution of an 18 hr. blood-broth culture of *Strep. pyogenes*, 'Richards'. The drugs were administered subcutaneously.

With compound IX given in doses of 75 mg./100 g. rat at 0, 3, 6, 9, 24, 27, 30, 39 hr. after infection, a definite but slight therapeutic effect, as measured by the survival time, was obtained, although much less than that produced by pantoyletaurine in the same experiment. Compounds VIII and XI failed to show any therapeutic effect when administered in the highest tolerated doses (25 mg./100 g. rat, administered subcutaneously at 0, 3½, 5½ and 12 hr. after infection); compound II was likewise ineffective in doses of 100 mg./100 g. rat.

Methods of preparation of compounds used

The general method of synthesis adopted for the compounds described was to fuse the pantoyl-lactone with an equivalent amount of the appropriate amine or of the sodium salt of the amino-acid. This, however, was found to be applicable only in

the case of aliphatic or alicyclic amines. Aromatic amines did not appear to be sufficiently reactive, and to acylate them with the pantoyl residue it was necessary to proceed *via* reaction with diacetyl pantoyl chloride and subsequent hydrolysis of the resulting diacetyl derivative.

Some of the compounds were hygroscopic viscous oils or glassy solids, which could not easily be obtained in a form suitable for analysis. Routine analysis would not be an indication of condensation. In such cases physical and chemical properties were used to confirm that condensation had occurred. All melting points are uncorrected.

dl-Pantamide (I). *dl*-Pantolactone (2 g.) was added to dry methanol (60 ml.) saturated with ammonia at 0°. After standing 5 hr. at this temperature and 18 hr. at room temperature the methanol was removed under reduced pressure at room temperature. The crystalline residue was recrystallized from acetone. The amide separated in small colourless prisms (1.4 g.), m.p. 127–128°. (Found: C, 48.9; H, 8.45; N, 10.0. Calc. for $C_6H_{13}O_5N$: C, 49.0; H, 8.8; N, 9.5%.)

dl-Pant-hydrazide (II). *dl*-Pantolactone (13.0 g.) was added to hydrazine hydrate (15 ml. 50% solution). Heat was evolved. After standing at room temperature for 24 hr. the excess hydrazine hydrate was removed under reduced pressure at 50°. The remaining viscous syrup crystallized slowly when stored over phosphorus pentoxide in a vacuum desiccator for 2–3 days. It crystallized from absolute ethanol (40 ml.) in small prisms (11.0 g.), m.p. 104–105°. (Found: C, 44.65; H, 8.75; N, 17.6. $C_6H_{14}O_5N_2$ requires C, 44.4; H, 8.6; N, 17.3%.)

dl-N-(β-diethylaminoethyl)-pantamide (III). *dl*-Pantolactone (1.3 g.) and β-diethylaminoethylamine (1.2 g.) were heated together, with occasional stirring and exclusion of moisture, in an oil-bath at 100–110° for 2½ hr. The product, a viscous syrup, was cooled and triturated with light petroleum (10 ml., b.p. 60–80°), the solvent decanted and the process repeated. The syrup was finally warmed to 50° with light petroleum (5 ml.) and, after the bulk of it was decanted, the remaining solvent was removed *in vacuo* at 50°. The residual viscous syrup did not form a picrate under identical conditions where β-diethylaminoethylamine, either alone or as a mixture with an equivalent of lactone, gave an almost quantitative precipitate of picrate. The amide is readily soluble in water, ethanol and ether.

dl-N-(5-diethylamino-2-pentyl)-pantamide (IV). *dl*-Pantolactone (1.3 g.) and 5-diethylaminopentyl-2-amine (1.6 g.) were heated together in an oil-bath at 105–110° for 2½ hr. with exclusion of moisture. The original fluid mixture became very viscous. The product, a viscous syrup, was treated as in the preceding preparation.

dl-N-pantoyl-phenylalanine (sodium salt) (V). *dl*-Pantolactone (1.3 g.) was melted in an oil-bath at 100–110° with exclusion of moisture, and dry powdered sodium salt of phenylalanine (1.87 g. = 1 g. mol.) stirred in. After 4 hr. at 100–110° with occasional stirring the viscous product was allowed to cool. It set to a hard glass which was powdered and stored over phosphorus pentoxide.

dl-N-pantoylhexahydroanthranilic acid (sodium salt) (VI). Hexahydroanthranilic acid, 1.43 g. (Einhorn & Meyerberg, 1894), was converted to the sodium salt, dried, finely

powdered and added with stirring to melted pantolactone (1.4 g.) in an oil-bath at 110°. The mixture was kept at 110–115° for 6 hr. with exclusion of moisture and occasional stirring. It slowly became viscous and set to a brittle glassy solid. It was powdered and stored over phosphorus pentoxide.

dl-N-pantoyl-nipecotinic acid (sodium salt) (VII). Finely powdered, dry, sodium nipecotinate (1.5 g.: Ladenburg (1892)) was added, with stirring, to pantolactone (1.3 g.) melted in an oil-bath at 100°. The mixture was heated for 5 hr. at 100–110° with occasional stirring and exclusion of moisture. On cooling, a highly hygroscopic brittle glass was obtained, which was powdered and stored over phosphorus pentoxide *in vacuo*.

dl-β-(N-pantoylaminoethyl)-p-tolyl sulphone (VIII). Sodium (2.5 g.) was dissolved in absolute ethanol (80 ml.), and *p*-tolyl mercaptan (12.4 g.) added, followed by *β*-bromoethylphthalimide (28 g. (Gilman, 1932)) and the mixture heated under reflux on the steam-bath for 1 hr. The separated sodium bromide (8.7 g.) was filtered and washed with a little ethanol. On cooling, the filtrate deposited *p*-tolyl-*β*-phthalimidoethyl sulphide (22 g.; 73% of theoretical yield) in colourless prisms, m.p. 81°. The sulphide (9.0 g.) was dissolved in glacial acetic acid (25 ml.) and perhydrol (12 ml.) added to the hot solution. After heating on the steam-bath for 1½ hr. and cooling, the crystalline solid was separated by filtration, washed with a little 50% acetic acid, and dried at 100°. Yield 9.0 g.; m.p. 130–132°. Recrystallization from ethanol gave *p*-tolyl-*β*-phthalimidoethyl sulphone in colourless needles, m.p. 140–141°. (Found: C, 61.75; H, 4.5; N, 4.45. $C_{17}H_{15}O_4NS$ requires C, 62.0; H, 4.6; N, 4.3%.)

The sulphone (8.5 g.) was heated under reflux for 1½ hr. with ethanol (50 ml.) and hydrazine hydrate (2.2 g.; 50% solution). Solid soon separated from the originally clear solution. The ethanol was removed under reduced pressure and the solid residue heated on the steam-bath for ½ hr. with hydrochloric acid (20 ml., *d.* 1.18 diluted with 20 ml. water) and filtered whilst hot. The solid on the filter was washed with a little dilute hydrochloric acid and finally with water. The clear filtrate was made strongly alkaline with NaOH. An ether-insoluble oil separated. This was extracted at 5° with ethyl acetate (5 × 30 ml.). The combined extract was dried over anhydrous sodium sulphate and the solvent removed, the last portions under reduced pressure. *β*-(*p*-Tolylsulphonyl)ethylamine was left as an oil (3.8 g.) which slowly crystallized. Recrystallized from benzene/light petroleum (b.p. 60–80°) mixture (4:1), it was obtained in colourless plates, m.p. 66–67°. (Found: C, 54.3; H, 7.0; N, 7.3. $C_9H_{13}O_2NS$ requires C, 54.3; H, 6.5; N, 7.0%.)

The amine (2.0 g.) and pantolactone (1.3 g.) were melted together in an oil-bath at 90–100° and heated at this temperature for 4 hr. with occasional stirring and exclusion of moisture. The resulting viscous syrup crystallized very slowly on standing and was recrystallized from benzene (about 30 ml.). *dl-β-(N-pantoylaminoethyl)-p-tolyl sulphone* separated in colourless prisms (2.4 g.), m.p. 96–97°. Soluble in ethanol and water, sparingly soluble in benzene. (Found: C, 54.7; H, 6.7; N, 4.4. $C_{15}H_{23}O_5NS$ requires C, 54.7; H, 7.0; N, 4.3%.)

dl-β-(N-pantoylaminoethyl)-p-aminophenyl sulphone (IX). *β*-Bromoethylphthalimide (26 g.) dissolved in ethanol (300 ml.) was heated under reflux for 4 hr. with sodium *p*-nitrothiophenate (17.7 g.). Water (500 ml.) was added

and the solution cooled. The solid which separated was removed by filtration and washed with 50% ethanol. Yield, 35 g. Dried at 100°. Not purified further for next stage. (Recrystallization of a small specimen from acetic acid gave *p*-nitrophenyl-*β*-phthalimidoethyl sulphide in small yellow plates, m.p. 175–176°.) Oxidation with perhydrol in acetic acid gave *p*-nitrophenyl-*β*-phthalimidoethyl sulphone in faintly yellow prisms (from acetic acid), m.p. 211–212°. (Found: C, 53.2; H, 3.7; N, 8.5. $C_{16}H_{14}O_6N_2S$ requires C, 53.3; H, 3.3; N, 7.8%.)

The sulphone (31 g.) was powdered and heated under reflux with stirring for 5 hr. with ethanol (900 ml.), water (100 ml.), conc. hydrochloric acid (1 ml.) and iron pin dust (90 g.). The mixture was made faintly alkaline to brilliant yellow with ammonia and filtered hot. On cooling, the filtrate deposited *p*-aminophenyl-*β*-phthalimidoethyl sulphone in long pale orange-yellow needles (19.7 g.), m.p. 199–200°. A further amount (3.5 g.) was recovered by concentration of the mother liquors. A specimen recrystallized from ethanol separated in long pale yellow needles, m.p. 200°. (Found: C, 57.75; H, 4.2; N, 8.45. $C_{16}H_{14}O_4N_2S$ requires C, 58.2; H, 4.2; N, 8.5%.)

The phthalimido-sulphone (16.5 g.) was decomposed with hydrazine hydrate in the manner already described. *β*-Aminoethyl-*p*-aminophenyl sulphone was obtained in colourless plates (from benzene), m.p. 116–117°. Soluble in water, ethanol, acetone, ethyl acetate. (Found: C, 48.4; H, 5.8; N, 13.75. $C_8H_{12}O_2N_2S$ requires C, 48.0; H, 6.0; N, 14.0%.)

The amino-sulphone (2.0 g.) and pantolactone (1.3 g.) were heated together in an oil-bath at 110° for 4 hr., with occasional stirring and exclusion of moisture. *dl-β-(N-pantoylaminoethyl)-p-aminophenyl sulphone* was obtained as a clear glass which could not be induced to crystallize. It contained a diazotizable amino group, was soluble in water but did not form a picrate. Sparingly soluble in benzene, soluble in acetone and ethyl acetate.

dl-β-(N-pantoylaminoethyl)-p-methoxyphenyl sulphone (X). Sodium (2.3 g.) was dissolved in absolute ethanol (200 ml.), and *p*-methoxyphenyl mercaptan (14.0 g.) added, followed by *β*-bromoethylphthalimide (25 g.). The mixture was heated under reflux for 10 hr. Most of the ethanol was removed on the steam-bath and ice-water added to the residue. On standing, with occasional stirring, a solid crystallized out which was separated. Recrystallization from ethanol gave *p*-methoxyphenyl-(*β*-phthalimidoethyl) sulphide in small plates (23 g.), m.p. 96°.

By oxidation with perhydrol in acetic acid *p*-methoxy-(*β*-phthalimidoethyl) sulphone (22 g.; m.p. 136°) was obtained and hydrolysis of the phthalimido compound (22.0 g.) was effected as previously described, by means of hydrazine hydrate and hydrochloric acid. The crude product (12.0 g.), crystallized on standing. Recrystallization from ethyl acetate/light petroleum (b.p. 60–80°) mixture (3:1) gave *β*-aminoethyl-*p*-methoxyphenyl sulphone in colourless prisms (5.2 g.), m.p. 56–67°. A further 3.0 g. was recovered from the mother liquors on dilution with light petroleum. (Found: C, 50.15; H, 6.15; N, 6.6. $C_9H_{13}O_3NS$ requires C, 50.2; H, 6.05; N, 6.5%.)

The amino-sulphone, m.p. 56–57° (2.15 g.) and pantolactone (1.3 g.) were melted together at 110–115° and held at this temperature, with occasional stirring for 3 hr. The resulting *dl-β-(N-pantoylaminoethyl)-p-methoxyphenyl sulphone* was a resin which could not be crystallized.

dl-*N*-pantoyl-*p*-anisidine (XI). α -Diacetoxy- β -dimethylbutyric acid was prepared both by acetylation of sodium α -dihydroxy- β -dimethylbutyrate (Harris, Boyack & Folkers, 1941) and by acetylation of dl-pantamide followed by treatment with amyl nitrite in acetic acid. As reported by Harris *et al.* (1941) the former method yields chiefly the monoacetyl lactone.

In both methods the neutral fraction was removed by extraction of an alkaline solution with ethyl acetate and the diacetoxy acid then liberated with hydrochloric acid and extracted with ethyl acetate. The ethyl acetate extract was dried over anhydrous sodium sulphate and the solvent removed, last portion under reduced pressure. The residual α -diacetoxy- β -dimethylbutyric acid was used without further purification. The crude acid (4.8 g.) was heated on the steam-bath for $\frac{1}{2}$ hr. with thionyl chloride (3 ml., freshly distilled) and the excess then removed under reduced pressure. *p*-Anisidine (2.4 g.) dissolved in dry pyridine (5 ml.) was added all at once and the mixture heated on the steam-bath for $\frac{1}{2}$ hr. Pyridine was removed under reduced pressure and the residual viscous syrup dissolved in chloroform (35 ml.), washed twice with *N*-hydrochloric acid (2 $\times$ 25 ml.), once with water, then with cold saturated sodium bicarbonate solution and finally with water. After drying over calcium chloride the chloroform was removed on the steam-bath, the last portion under reduced pressure. A very viscous brown syrup remained (6.5 g.), which was insoluble in water and hydrochloric acid, and gave no diazo reaction; it was very soluble in acetone, soluble in benzene, and insoluble in light petroleum (60–80°).

Crystallized from acetone/petrol or benzene/petrol. *N*-diacetoxy-pantoyl-*p*-anisidine separated in colourless felted needles from acetone/light petroleum (prisms from benzene/light petroleum) (3.2 g.), m.p. 86–87°. (Found: C, 60.9; H, 7.2; N, 4.6. $C_{17}H_{23}O_6N$ requires C, 60.5; H, 6.8; N, 4.2%.)

Hydrolysis was effected by adding a solution of the diacetyl compound (1.13 g.) in ethanol (5 ml.) to cold *N*-potassium hydroxide (10 ml.) and allowing to stand for 18 hr. at room temperature. As much solvent as possible was then removed under reduced pressure without heating and the residual oil extracted with benzene. On concentration and cooling, the benzene deposited a crystalline solid which was recrystallized from benzene (charcoal treatment). *N*-pantoyl-*p*-anisidine separated in colourless plates (0.5 g.), m.p. 116°. Soluble in water. (Found: N, 5.8. $C_{13}H_{19}O_4N$ requires N, 5.5%.)

DISCUSSION

From the results recorded above and those obtained by the other workers already referred to, it is apparent that many compounds derived from pantothenic acid have the property of antagonizing this substance with respect to the growth of bacteria. Moreover, these substances may have nothing more in common with pantothenic acid than the presence within the molecule of the pantamide residue. In the above experiments the activities of the compounds examined against *L. casei* ϵ and *Strep.*

pyogenes did not in every case run parallel. With the latter organism some difficulty was experienced in obtaining consistently reproducible results. This disadvantage did not attend the use of *L. casei* ϵ as the indicator organism, so that the results obtained with it are more suitable for the discussion of comparative activity.

The most potent antibacterial effect was obtained with pant-hydrazide (II) although only in the presence of the lower concentration of added pantothenic acid. Hydrazine, phenyl-hydrazine and salicylhydrazide were also antibacterial but the action was independent of the concentration of supplement. Antagonism was also apparent with the simple amide (I) and with the β -diethylaminoethylamide (III) but not with the β -diethylaminoamylamide (IV). The antibacterial activity of mepacrine and quinine, which also carry basic alkyl side chains, was not reversed by pantothenic acid. Compounds VI and VII are closely related to the growth factor in that they are pantamidocarboxylic acids in which the amide nitrogen occupies a β position with respect to the carboxylic acid group. Neither inhibited the growth of the bacterium at the maximum concentration examined. Compounds VIII, IX and X can be regarded as derived from pantoyltaurine by replacing the hydroxyl of the sulphonic acid group by a substituted phenyl nucleus. Compound IX is also related to sulphanilamide and the *p*-aminophenyl sulphones, and the antibacterial activity might therefore be attributed to this relationship. Separate experiment, which has not been recorded here in detail, has shown, however, that the growth-inhibiting effect of this substance against *Strep. pyogenes* is not antagonized by a concentration of 1/1000 of *p*-aminobenzoic acid. It can be regarded as functioning, therefore, primarily as a pantothenic acid antagonist. This observation is of importance since this substance was the only member of the series examined *in vivo* (II, VIII, IX and XI) which exhibited any activity.

SUMMARY

1. Eleven amides derived from pantoic acid have been prepared and examined as antagonists of pantothenic acid with respect to bacterial growth.

2. Pant-hydrazide showed marked antagonism *in vitro* with respect to *Lactobacillus casei* ϵ but exhibited no therapeutic action in rats infected with *Streptococcus pyogenes*.

3. The amide derived from 4-aminophenyl- β -amino ethylsulphone showed slight therapeutic activity under similar conditions.

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Antibacterial Activity of Substances Related to *p*-Aminobenzoic Acid

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The current view of the mode of action of the sulphanilamide drugs is that these substances interfere with the growth of bacteria by virtue of a competitive inhibition of *p*-aminobenzoic acid, or some substance related thereto, which serves as an essential metabolite for the organisms. Further, this competitive inhibition is probably connected with a similarity in the spatial arrangement of the substituent groupings within the molecule of the growth-factor and growth-inhibitor, which provides them with an affinity for the same hypothetical enzyme system. It was, on this view, likely that substances which antagonize *p*-aminobenzoic acid might be devised from the parent structure, leaving the existing substituent groups intact, by introducing further substituents into the benzene nucleus. This has proved to be the case in certain instances.

While this paper was being prepared the results were published of similar investigations by Wyss, Rubin & Strandskov (1943) and by Johnson, Green & Pauli (1944), who have examined many of the compounds listed below. Nevertheless the present work has included compounds not used by the American workers and in addition more attention has been paid to *in vivo* experiments.

Derivatives of *p*-aminobenzoic acid employed

The substances shown in Tables 1 and 2, many of which are known compounds, were employed in the experiments. Table 1 contains the nuclear substituted derivatives and Table 2 a number of miscellaneous derivatives which were included in the investigation. The preparation of the new compounds will be described elsewhere.

Table 1. Nuclear substituted 4-aminobenzoic acids

Compound	Substituent(s)
I*	3-Chloro
II* †	2-Chloro
III*	3-Iodo
IV* †	3-Methyl
V*	2-Methyl
VI*	3-Hydroxy
VII* †	3-Methoxy
VIII*	2-Methoxy
IX †	3-Ethoxy
X*	3-Amino
XI*	3-Carboxy
XII †	3-Methylmercapto
XIII †	3-Ethylmercapto
XIV †	3-Methylsulphonyl
XV †	3-Ethylsulphonyl
XVI	3:5-Dichloro
XVII	3:6-Dichloro
XVIII	3-Chloro-6-amino
XIX	3-Chloro-6-acetylamino
XX	3:5-Dibromo
XXI †	3:6-Dimethyl
XXII †	3:6-Dimethoxy
XXIII †	2-Methoxy-5-methyl
XXIV †	3-Methoxy-6-methyl

* Compounds used by Wyss, Rubin & Strandskov (1943).

† Compounds used by Johnson, Green & Pauli (1944).

‡ New compounds.

Behaviour towards streptococci in vitro

The general plan of all the experiments was the same. The organism used was *Streptococcus pyogenes*, 'Kruger' strain (Lancefield Group A), maintained by weekly subculture on agar. All tests were carried