were obtained. Some of the pigment and most of the gum was removed by leaching the solid first with about 1 ml. of ice-cold acetone and then with about 1 ml. of benzene. The material, after treatment with charcoal in boiling ethanol to remove the remaining pigment and crystallization from 1·25 ml. of methanol, yielded 7·8 mg. of fine white needles, m.p. 232–237° (corr.). Mixed with authentic pregnane-3α:20α-diol (m.p. 237–238·5°, corr.) the melting point was 233–237° (corr.).

Acetylation with acetic anhydride and pyridine yielded a product which, after treatment with charcoal in boiling *n*-hexane followed by crystallization from *n*-hexane, was in the form of stout needles, m.p. 163–165° and 179–181° (corr.). Mixed with authentic pregnane-3α:20α-diol diacetate (m.p. 165–166° and 180–181°, corr.) the melting point was 162–163° and 178–181° (corr.).

SUMMARY

1. It has been shown that, using the method of Sommerville *et al.* (1948), as little as about 0·25 mg. of pregnanediol can be determined with a satisfactory degree of accuracy in one-half of a 24 hr. specimen of rabbit urine.

2. By this procedure the excretion of 'pregnanediol' in the urine of pregnant rabbits has been detected and studied quantitatively. Immediately after mating a transient excretion of 'pregnanediol' lasting for 1–3 days occurs. This is followed at about the eighth to tenth day of pregnancy by a prolonged excretion of 'pregnanediol' which is maintained until just before parturition.

3. The 'pregnanediol' excreted immediately after mating and that excreted later in pregnancy in the rabbit have been isolated and identified as pregnane-3α:20α-diol.

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Lysine Analogues as Inhibitors of Bacterial Growth

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Many investigations during recent years have demonstrated that analogues of essential metabolites may exert antibacterial action, but this approach has not so far led to the synthesis of a practical chemotherapeutic agent. It seemed to us that the ubiquity of the amino-acids, coupled with their importance in the biosynthesis of enzymes and other proteins, warranted further investigation of amino-acid analogues and peptides as potential chemotherapeutic agents. As it was impracticable to prepare analogues of every amino-acid, the choice between various alternatives was guided by existing knowledge of amino-acid metabolism.

The majority of amino-acids can undergo rapid deamination and reamination at the α-amino group (Braunstein & Kritzmann, 1937; Schoenheimer, Ratner & Rittenberg, 1939). It seemed undesirable that a potential antimetabolite should be subject to rapid metabolic transformation, and we were, accordingly, faced with the problem of designing a series of amino-acid analogues of such metabolic stability that they might retain the characteristic α-amino-carboxylic acid grouping, during protein synthesis. Experiments with ^{15}N in rats have indicated that lysine was an exception in the general

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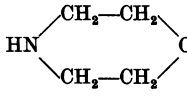
metabolic turnover, and did not receive either of its nitrogen atoms from the nitrogen of other amino-acids or from inorganic nitrogen (Schoenheimer *et al.* 1939). The failure of rats on a lysine-free diet to utilize the α-hydroxy analogue of lysine (McGinty, Lewis & Marvel, 1924) also supports the view that lysine does not participate in transamination reactions in the same way as the majority of amino-acids. This unique metabolic behaviour of lysine suggested that analogues of lysine might possess greater potentialities as antimetabolites than analogues of other amino-acids.

We accordingly synthesized and tested three types of lysine (I, $n=4$) analogue: (1) a higher straight chain homologue (I, $n=5$), 1:6-diaminohexane-1-carboxylic acid; (2) a C–N substituted analogue of lysine (II) in which the terminal amino group and the β-carbon atom of lysine were incorporated into a piperidine ring structure, β-4-piperidyl alanine; and (3) analogues formed by incorporating (II) in amide (peptide) linkage with ammonia or amino-acids.

$$NH_2.(CH_2)_n.CH(NH_2).COOH \quad (I)$$



(II)

Synthetic methods

Several interesting points arose in the synthesis of the compounds listed in Table 1, and some unexpected difficulties were encountered in attempting to extend the range of compounds under test. Synthesis of 1:6-diaminohexane-1-carboxylic acid was accomplished by an extension of the acetamidomalonic ester synthesis described by Albertson & Archer (1945). Condensation of ethyl acetamidomalonate and 1-chloro-5-benzamidopentane was slow and the isolation of a crystalline salt of homolysine was extremely tedious.

Suitable intermediates for the synthesis of piperidyl-alanines were obtained by condensing α- and γ-picolines with chloral. Whereas 2-(3′:3′:3′-trichloro-2′-hydroxypropyl)-pyridine hydrochloride had previously been obtained in good yield (67 %; Tullock & McElvain, 1939), no satisfactory method existed for the synthesis of the corresponding 4-compound (cf. Alberts & Bachman, 1935). By keeping a mixture of γ-picoline and chloral at 37° for 8 days it was found that 4-(3′:3′:3′-trichloro-2′-hydroxypropyl)pyridine could be obtained in 68 % yield; the best yield previously reported for this compound was 16–18 % (Alberts & Bachman, 1935). Conversion of the trichlorohydroxypropylpyridines to the corresponding pyridylacrylic acids was achieved by the standard method.

Considerable difficulty seems to have been encountered by earlier investigators in attempts to reduce pyridylacrylic acids to the corresponding piperidylpropionic acids; for example, Rabe & Kindler (1919) reduced 4-pyridylacrylic acid by the use of sodium in amyl alcohol. No yields were quoted, but repetition of their method gave disappointing results. Tullock & McElvain (1939) report the failure of Raney nickel under high pressure, a failure which we can also confirm. It was found, however, that 2- and 4-pyridylacrylic acids could be quantitatively reduced at room temperature and atmospheric pressure by use of platinum oxide in the presence of a small excess of hydrochloric acid. In the absence of acid the aromatic ring remains completely unreduced, and in the presence of exactly one equivalent of acid, reduction is slow and incomplete (cf. Rubstov, 1946). The difficulty of catalytic reduction, as was pointed out by Maxted (1948), is probably associated with the existence on the pyridine nitrogen of a free electron pair which leads to the strong adsorption of such a nitrogen atom by a metal catalyst; this self-poisoning effect is eliminated in the presence of hydrochloric acid.

α-Halogenation of N-*benzoyl*-2-(4-*piperidyl*)*propionic acid* on a small scale by the method of Eck & Marvel (1934) gave no difficulty, and N-*benzoyl*-2-(4′-*piperidyl*)-1-*bromopropionic acid* was isolated in 75 % yield; on a larger scale, however, the reaction with phosphorus and bromine became violently exothermic and in the absence of a suitable solvent the reaction mixture became too viscous to allow effective stirring. The result was incomplete bromination and partial cleavage of the benzamide linkage. α-Chlorination by the method of Galat (1947) using sulphuryl chloride did not give such a good yield as the small-scale bromination with phosphorus and bromine (60 % compared with 75 %), but the reaction was mild and non-exothermic and was accordingly preferred for large-scale work.

Having successfully halogenated benzoyl-2-(4′-piperidyl)propionic acid no difficulty was anticipated in the halogenation of *benzoyl*-2-(2′-*piperidyl*)*propionic acid*; nevertheless, we were unable to find any conditions under which the 2-isomer could be α-halogenated without disruption of the *N*-benzoylpiperidyl radical. The corresponding 2-(p-*toluenesulphonyl*-2′-*piperidyl*)*propionic acid* was prepared in the hope that the toluenesulphonyl-piperidyl group would be more stable than benzoylpiperidyl, but, under the conditions which had been successful with the 4-isomer, this compound was also degraded and toluenesulphonyl chloride was recovered in almost quantitative yield from the reaction mixture. It will be remembered that von Braun (1904) prepared 1:5-dichloropentane by reaction of benzoylpiperidine with phosphorus pentachloride. It seems probable that some similar reaction may take place with benzoyl-2-piperidylpropionic acid in the presence of bromine and phosphorus.

Conversion of *benzoyl-2-(4′-piperidyl)-1-chloropropionic* acid to 2-(4′*piperidyl*)-1-*aminopropionic acid* (piperidylalanine) was effected without difficulty. Dicarbobenzyloxypiperidyl-alanine was coupled with leucine methyl ester by the azide method of Bergmann & Zervas (1932). A curious and unexplained abnormality was encountered in this coupling; a considerable quantity of *dicarbobenzyloxy-2-(4′-piperidyl)-1-aminopropionamide* was formed as a by-product in the conversion of the acid hydrazide to the peptide through the acid azide. The formation of dicarbobenzyloxylysyl amide under similar conditions and in similar yield was reported by Prelog & Wieland (1946), but repeated experiments with other carbobenzyloxy-amino-acid azides did not lead to similar amide formation.

In coupling piperidylalanine with *p*-aminobenzoic acid the acid chloride method was found to be preferable to the azide method; the low basicity of the aromatic amino group of methyl *p*-aminobenzoate prevented rapid reaction between it and the acid azide of dicarbobenzyloxypiperidylalanine.

EXPERIMENTAL

1:6-*Diaminohexane-1-carboxylic acid.* To a hot solution of Na (2·3 g.) in dry ethanol (75 ml.) was added ethylacetamidomalonate (31·2 g.) followed by 1-chloro-5-benzamidopentane (17·45 g.). The mixture was boiled for 18 hr., the ethanol removed under reduced pressure, and the oily residue mixed with water. The product was extracted into ether, dried, and the ether removed. The residual oil (24·7 g.) was mixed with aqueous KOH (20%; 10 g.) and heated at 95° for 2 hr.; a water-insoluble oil was removed and the aqueous alkaline solution acidified with excess of HCl; an oil separated which could not be crystallized. Decarboxylation of the substituted malonic acid took place rapidly when the oil was boiled with 6N-HCl and after 10 hr., hydrolysis of the benzamide and acetamido groups was also complete. Benzoic acid was removed from the acid solution which was then concentrated to a thick syrup. The syrup was extracted with ethanol, the insoluble residue discarded and the ethanolic solution of the dihydrochloride concentrated slowly. 1:6-Diaminohexane-1-carboxylic acid could not be crystallized either as the dihydrochloride or as the dipicrate. The diamine-dihydrochloride was mixed with N-HCl, and phosphotungstic acid in N-HCl was added in excess. The solid phosphotungstate was collected, suspended in water and decomposed with baryta. The filtrate and washings from the Ba phosphotungstate were concentrated and left a colourless syrup (4·2 g.). The syrup was dissolved in the minimum volume of water and HCl added to bring the pH to 5·0. 1:6-*Diaminohexane-1-carboxylic acid monohydrochloride* crystallized from the solution after cautious addition of ethanol. The product was recrystallized from aqueous ethanol as transparent needles of the monohydrate, m.p. 176° (decomp.). (Found: C, 39·1; H, 8·9; N, 13·1. $C_7H_{16}O_2N_2.HCl.H_2O$ requires C, 39·17; H, 8·86; N, 13·05%. Loss in weight at 105°/20 mm., 7·9. $1H_2O$ requires 8·4%.)

4-(3′:3′:3′-*Trichloro-2′-hydroxypropyl*)*pyridine* (I). Pure chloral (190 g.; b.p. 97°/755 mm.) and γ-picoline (106 g.) were mixed and kept at 37° for 8 days. The reaction product, a dark brown viscous oil, was diluted with ethanol (500 ml.) and heated three times with successive lots (20 g.) of charcoal. Concentration of the combined filtrates and washings left a light brown solid which crystallized from 50% aqueous methanol as needles, m.p. 160° (210 g.; 68% of theory; cf. Rabe & Kindler, 1919; Alberts & Bachman, 1935).

2-(4′-*Pyridyl*)*acrylic acid* (II). A solution of KOH (67·5 g.) in ethanol (350 ml.) was cooled in ice and an ethanolic solution of (I) (48 g.) was added slowly. The reaction mixture was stirred and the temperature allowed to rise slowly. The mixture was finally heated for 15 min. at 95°, cooled and the precipitated KCl removed. The brown residue remaining after evaporation of the ethanol was dissolved in water and neutralized with acetic acid. The sparingly soluble 2-(4′-pyridyl)acrylic acid (14·5 g.) separated slowly and was collected, m.p. 296°; the hydrochloride crystallized from ethanol as needles, m.p. 244° (cf. Rabe & Kindler, 1919).

2-(4′-*Piperidyl*)*propionic acid* (III). The hydrochloride of (II) (46 g.) in water (300 ml.) containing one equivalent of HCl was hydrogenated at room temperature and atmospheric pressure using Adams's platinum oxide catalyst. Hydrogenation was complete after 60 hr. The catalyst was removed and the solution concentrated. The product was crystallized from hot ethanol. During this procedure some esterification of the piperidylpropionic acid took place and only the first crop (30 g.) of crystalline material which separated on addition of acetone to the ethanolic solution was the hydrochloride of (III), needles, m.p. 244°. (Found: C, 49·8; H, 8·4; N, 7·3. $C_8H_{15}O_2N.HCl$ requires C, 49·6; H, 8·3; N, 7·2%.) Addition of ether to the mother liquor of the acid hydrochloride gave a second crop of crystalline material (11 g.), m.p. 129–130°, which was identified as the hydrochloride of *ethyl-2-(4′-piperidyl)propionate.* (Found: C, 54·4; H, 9·3; N, 6·2. $C_{10}H_{19}O_2N.HCl$ requires C, 54·2; H, 9·0; N, 6·3%.)

Benzoyl 2-(4′-*piperidyl*)*propionic acid* (IV). Benzoylation was carried out at 0° by dropwise addition of benzoyl chloride (15 g.) to an alkaline solution (50 ml. 2N-NaOH) of the hydrochloride of (III) (19 g.). A further equivalent of alkali (25 ml., 2N) was added during the period of benzoylation. The product separated on acidification of the reaction mixture and was crystallized from ethanol as white prisms, m.p. 148° (21·8 g.). (Found: C, 69·2; H, 7·4; N, 5·4. $C_{15}H_{19}O_3$ requires C, 69·0; H, 7·3; N, 5·4%.)

Benzoyl 2-(4′-*piperpidyl*)-1-*bromopropionic acid* (V). The method of bromination was essentially that of Eck & Marvel (1934). Benzoyl-2-(4′-piperidyl)propionic acid (4·9 g.) was mixed with red P (0·83 g.) and allowed to react at 0° with Br_2 (12 g.). The reaction was completed by heating and the α-bromo acid (5·0 g., 78% theory) was isolated by the method recommended by Eck & Marvel. After repeated crystallization from ethanol the product (3·4 g.) was obtained in the form of white plates melting at 183–184°. (Found: C, 53·3; H, 5·5; Br, 23·2. $C_{15}H_{18}O_3NBr$ requires C, 53·0; H, 5·3; Br, 23·5%.)

Benzoyl 2-(4′-*piperidyl*)-1-*chloropropionic acid* (VI). Chlorination was effected by the method of Galat (1947). Benzoyl 2-(4′-piperidyl) propionic acid (30 g.) was intimately mixed with powdered I_2 (0·5 g.) and the mixture added to SO_2Cl_2 (70 ml.). The reaction mixture was heated at 60–65° for 2 hr. and then gradually brought to the boil to complete the reaction. Sulphuryl chloride was removed under reduced pressure and the viscous residue poured into a mixture of ice and water. The material which separated gradually solidified.

The solid (33·5 g.) was ground to a fine powder, washed thoroughly, dried and dissolved in hot ethanol. The product crystallized from ethanol as pale yellow prisms (16 g.), m.p. 170°. The mother liquors were reworked by evaporation to dryness, trituration with benzene and crystallization of the benzene-insoluble residue from 80% ethanol. The combined crops of crystalline material (24 g.) were recrystallized from ethanol, yield 20 g. (60% theory), m.p. 174°. (Found: C, 60·6; H, 6·1; Cl, 12·4. $C_{15}H_{18}O_3NCl$ requires C, 60·9; H, 6·1; Cl, 12·0%.)

2-(*Benzoyl-4'-piperidyl*)-1-*aminopropionic acid* (VII). The chloroacid (VI) (11 g.) was mixed with NH_3 (175 ml., *d* 0·88) and heated under pressure for 12 hr. at 100°. Excess of ammonia was removed under reduced pressure and Cl^- eliminated by addition of Ag_2SO_4. Excess Ag was removed as the sulphide and SO_4 as $BaSO_4$. The product was a crystalline solid (7·5 g.), m.p. 216–218°, which was used for the next stage without further purification.

2-(*4'-piperidyl*)-1-*aminopropionic acid* (VIII). The product of the previous experiment (6·5 g.) was debenzoylated by boiling for 10 hr. with 6 N-HCl (120 ml.). The acid solution was freed from benzoic acid and concentrated to dryness. The residue was crystallized by the careful addition of ether to an ethanolic solution of the dihydrochloride containing a few drops of water. The pure dihydrochloride monohydrate (4·2 g.) separated from aqueous acetone or from a mixture of ethanol and ether as plates, m.p. 160°. (Found: C, 36·5; H, 7·6; N, 10·4. $C_8H_{16}O_2N_2.2HCl.H_2O$ requires C, 36·5; H, 7·6; N, 10·6%. Loss of weight at 120°, 6·6%. $1H_2O$ requires 6·8%. Anhydrous dihydrochloride: N, 11·6. $C_8H_{16}O_2N_2.2HCl$ requires N, 11·4%.)

Dicarbobenzyloxy-2-(*4'-piperidyl*)-1-*aminopropionic acid* (IX). The dicarbobenzyloxy derivative was prepared according to the method of Bergmann, Zervas & Ross (1935). From 4 g. of 2-(4'-piperidyl)-1-aminopropionic acid dihydrochloride we obtained 6·2 g. of the dicarbobenzyloxy derivative as a colourless syrup which could not be crystallized.

Dicarbobenzyloxy - 2 - (*4'-piperidyl*) - 1 - *aminopropionamide* (X). The product (IX) from the above experiment was esterified by treatment with excess diazomethane. A portion of the ester (1·8 g.) was mixed with excess ammoniacal methanol (saturated at 0°) and heated under pressure at 37° for 48 hr. The solvent was removed and the product crystallized from ethanol, prisms, m.p. 134° (1·4 g.). (Found: C, 65·4; H, 6·5; N, 9·8. $C_{24}H_{29}O_5N_3$ requires C, 65·6; H, 6·6; N, 9·6%.)

2 - (*4' - Piperidyl*) - 1 - *aminopropionamide dihydrochloride* (XI). Compound (X) (1·1 g.) in methanol (30 ml.) containing 2 equiv. acid (0·18 ml. of 10 N-HCl) was hydrogenated in the presence of palladium black (300 mg.). When evolution of CO_2 was complete (3 hr.) the catalyst was removed, the solvent evaporated, and the residual amide dihydrochloride (0·5 g.) crystallized as plates from a mixture of methanol and acetone, m.p. 292° (decomp.). (Found: C, 39·1; H, 7·9; N, 17·5. $C_8H_{17}ON_3.2HCl$ requires C, 39·3; H, 7·8; N, 17·2%.)

Dicarbobenzyloxy - 2 - (*4' - piperidyl*) - 1 - *aminopropionic acid hydrazide* (XII). Excess of hydrazine (1 ml., 90%) was added to a solution of dicarbobenzyloxy 2-(4'-piperidyl)-1-aminopropionic acid methyl ester (2 g.) in methanol (20 ml.). After 24 hr. at room temperature the solvent was removed from the mixture and the residual oil crystallized from a mixture of ethyl acetate and ligroin. The pure hydrazide (1·8 g.) separated as plates, m.p. 115°. (Found: C, 63·7; H, 6·6; N, 12·3. $C_{24}H_{30}O_5N_4$ requires C, 63·4; H, 6·6; N, 12·3%.)

Dicarbobenzyloxy - 2 - (*4' - piperidyl*) - 1 - *aminopropionyl*-DL-*leucine methyl ester* (XIII). A. *Azide method.* The hydrazide (XII) (1·6 g.) was dissolved in glacial acetic acid (5 ml.) and diluted with N-HCl (15 ml.). The mixture was cooled to 0° and a cooled solution of $NaNO_2$ (1·1 g.) in water (10 ml.) was added dropwise to the stirred solution. The precipitated acid azide was extracted into ethyl acetate (50 ml.) and the ethyl acetate washed successively at 0° with pre-cooled water, with cooled saturated $NaHCO_3$ solution and again with water. The neutral solution of azide in ethyl acetate was quickly dried over Na_2SO_4 and added during 10 min. to a cooled solution of leucine methyl ester (0·5 g.) in ether (25 ml.). The mixture was left for 24 hr. and then extracted with N-HCl. Excess leucine ester was recovered from the aqueous acid solution. The organic solvent mixture was then washed with $NaHCO_3$, with water, and dried over Na_2SO_4. Removal of solvent *in vacuo* left a pale yellow gum (0·6 g.) which crystallized after trituration with ethyl acetate. The crystalline product (0·2 g.) was not the dipeptide but dicarbobenzyloxy-2-(4'-piperidyl)-1-aminopropionamide; plates, m.p. 132–133°. (Found: C, 65·9; H 6·6; N, 9·7. $C_{24}H_{29}O_5N_3$ requires C, 65·6; H, 6·6; N, 9·6%.) The required dipeptide, m.p. 104° (0·25 g.), was isolated from the ethyl acetate mother liquors of the amide by addition of light petroleum. (Found: C, 65·8; H, 7·4; N, 7·6. $C_{31}H_{41}O_7N_3$ requires C, 65·6; H, 7·2; N, 7·4%.)

B. *Acid chloride method.* As an alternative to the above method the same peptide was prepared by the conversion of the acid (IX) (1·0 g.) to the acid chloride by treatment with PCl_5 (0·5 g.) in ether (20 ml.) at 0°. When the PCl_5 had entirely dissolved, the solution was filtered and diluted with light petroleum. The solvent was decanted from the precipitated acid chloride which was immediately dissolved in ether, washed quickly in ice-cold water (10 ml.) and dried over Na_2SO_4. DL-Leucine methyl ester hydrochloride (1·5 g.) was dissolved in water (10 ml.) and the aqueous solution covered with ether (20 ml.). The mixture was cooled to −10° and the amino-acid ester forced into the ether layer by gradual addition of a large excess of anhydrous K_2CO_3. The ether layer was decanted from the K_2CO_3 sludge, quickly dried over Na_2SO_4 and filtered. The acid chloride solution already prepared was added dropwise, with shaking, to 2 equiv. of the ester solution, and after 1 hr. the leucine methyl ester hydrochloride which had separated was removed by filtration. The ethereal filtrate was washed successively with water, N-HCl, $KHCO_3$ (7%) and water, dried and concentrated *in vacuo*. The product crystallized from a mixture of ethyl acetate and light petroleum as platelets, m.p. 104–106° (0·25 g.) (Found: C, 65·9; H, 7·4; N, 7·8. $C_{31}H_{41}O_7N_3$ requires C, 65·6; H, 7·2; N, 7·4%.)

2-(*4'-Piperidyl*)-1-*aminopropionyl*-DL-*leucine methyl ester* (XIV). The carbobenzyloxy groups were removed from (XIII) (0·15 g.) by catalytic hydrogenation by the same method as was used in (XI). The dihydrochloride was hygroscopic and could not be crystallized. As removal of carbobenzyloxy groups by this method is essentially quantitative, the product was deemed pure enough for test without crystallization.

Dicarbobenzyloxy - 2 - (*4' - piperidyl*) - 1 - *aminopropionyl* - p-*aminobenzoic acid methyl ester* (XV). An ethereal solution of the acid chloride of (IX) prepared as already described from (IX) (1·0 g.) was added dropwise, with shaking, to a solution of 2 equiv. (0·7 g.) of methyl-*p*-aminobenzoate in dry $CHCl_3$ (20 ml.). After leaving for 18 hr. at 37°, crystalline methyl-*p*-aminobenzoate hydrochloride (0·38 g.) was re-

moved and the $CHCl_3$-ether solution washed successively with dilute acid, bicarbonate and water. Removal of the solvent left a syrup (0·8 g.) which was redissolved in ethyl acetate and boiled with activated charcoal. The product, a colourless viscous oil (0·5 g.), could not be crystallized and was used for the next stage without further purification.

2 - (4' - *Piperidyl*) - 1 - *aminopropionyl* - p - *aminobenzoic acid* (XVI). The acylated peptide ester (XV) (0·5 g.) was hydrolysed in a mixture of dioxane (5 ml.) and N-NaOH (5 ml.). After 30 min. at room temperature the mixture was acidified and diluted with water; the oil which separated was extracted into $CHCl_3$. The $CHCl_3$ was removed *in vacuo* and the residue hydrogenated in the presence of palladium black (100 mg.) and 2 equiv. of HCl. The dihydrochloride dihydrate of (XVI) crystallized as needles (0·1 g.), m.p. 190–192° (decomp.), when ether was added slowly to a methanolic solution of the product of hydrogenation. (Found: C, 45·0; H, 7·0; N, 10·7. $C_{15}H_{21}O_3N_3.2HCl.2H_2O$ requires C, 45·0; H, 6·8; N, 10·5%. Loss in weight at 105°, 8·4%. $2H_2O$ requires 9·0%.)

2 - (3':3':3':- *Trichloro* - 2' - *hydroxypropyl*) *pyridine hydrochloride* (XVII). The method used was based on earlier methods described by Einhorn & Liebrecht (1887), Löffler & Kaim (1909) and Tullock & McElvain (1939). Anhydrous chloral (110 ml.) was mixed with α-picoline (130 ml.) and dry amyl acetate (200 ml.). The mixture was heated for 14 hr. at 130° and then steam distilled to remove solvent and excess α-picoline. The non-volatile residue was decolorized with charcoal and concentrated *in vacuo*. After drying thoroughly over H_2SO_4, the product was crystallized from ethanol as needles, m.p. 200–201° (120 g.) (cf. Tullock & McElvain, 1939).

2-(2'-*Piperidyl*)*propionic acid hydrochloride* (XVIII). 2-(2'-Pyridyl)acrylic acid was prepared by the method of Löffler & Kaim (1909); the acrylic acid was reduced by the method used for the isomeric acid (III) and the product (XVIII) crystallized from aqueous acetone as the hydrochloride, m.p. 191–192°. The yield in the reduction was almost quantitative and gave none of the difficulties reported for other methods (Tullock & McElvain, 1939; Löffler & Kaim, 1909). (Found: C, 49·5; H, 8·3; N, 7·1. $C_8H_{15}O_2N.HCl$ requires C, 49·6; H, 8·3; N, 7·2%.)

Benzoyl-2-(2'-*piperidyl*)*propionic acid* (XIX). The method used for the isomeric compound (IV) was applied successfully to (XVIII) and the product crystallized from ethanol as needles, m.p. 158°. (Found: C, 69·0; H, 7·3; N, 5·7. $C_{15}H_{19}O_3N$ requires C, 69·0; H, 7·3; N, 5·4%.)

p-*Toluenesulphonyl*-2-(2'-*piperidyl*)*propionic acid* (XX). Tosylation was effected by the method of Thomas & Goerne (1919). A solution of (XVIII) (2 g.) in N-NaOH (25 ml.) was shaken vigorously with p-toluenesulphonyl chloride (2·5 g.) for 3 hr. Unchanged acid chloride was removed by filtration and the filtrate acidified. The precipitate was crystallized from 80% ethanol, prisms, m.p. 108–109° (1·0 g.) (Found: C, 57·6; H, 7·0; N, 4·7. $C_{15}H_{21}O_4NS$ requires C, 57·9; H, 6·8; N, 4·5%.)

MICROBIOLOGICAL RESULTS

When tested *in vitro* against *Streptococcus haemolyticus* (in Hartley's digest broth), *Staphylococcus aureus* (in medium of Strauss, Dingle & Finland, 1941) and *Escherichia coli* (in medium of MacLeod, 1940, supplemented with casein), analogues of lysine were found to be slightly antibacterial. Furthermore, the antibacterial activity of a simple analogue (β-4-piperidylalanine) was enhanced by its incorporation in peptide linkage with other amino-acids as indicated in Table 1.

Table 1. *Minimum inhibitory concentrations* (M) *of lysine analogues as inhibitors of bacterial growth*

Compound	Haemolytic streptococci		*Staph. aureus*		*Esch. coli*	
	Blood	Broth	Broth	Synthetic	Broth	Synthetic
1:6-Diaminohexane-1-carboxylic acid mono HCl	—	10^{-3}	$>10^{-2}$	—	$>10^{-2}$	—
2(4'-Piperidyl)-1-aminopropionic acid dihydrochloride	2×10^{-2}	8×10^{-3}	$>4\times10^{-2}$	2×10^{-2}	$>4\times10^{-2}$	$>4\times10^{-2}$
2(4'-Piperidyl)-1-aminopropionamide dihydrochloride	—	4×10^{-3}	$>4\times10^{-3}$	—	$>4\times10^{-3}$	—
2(4'-Piperidyl)-1-aminopropionyl-*p*-aminobenzoic acid dihydrochloride	—	$1{\cdot}5\times10^{-3}$	$>6\times10^{-3}$	6×10^{-3}	$>6\times10^{-3}$	$>6\times10^{-3}$
2(4'-Piperidyl)-1-aminopropionyl-DL-leucine methyl ester dihydrochloride	—	$1{\cdot}5\times10^{-3}$	$>1{\cdot}5\times10^{-3}$	—	$>1{\cdot}5\times10^{-3}$	—

DISCUSSION

At the time (1946) when the present investigation was planned some analogues of amino-acids were already known to exhibit limited antibacterial action: thus Fildes (1941) showed that indole-acrylic acid, an analogue of tryptophan, inhibited growth of *Esch. coli* at a concentration of 8×10^{-3}M; the effect was reversed by tryptophan. McIlwain (1941) had shown that α-aminosulphonic acids had some growth inhibitory action; Harris & Kohn (1941) had reported inhibition of growth of *Esch. coli* by ethionine; Roblin, Lampen, English, Cole & Vaughan (1945) had found that methoxinine, the oxygen analogue of methionine, inhibited *Esch. coli* and *Staph. aureus* in synthetic media; du Vigneaud, McKennis, Simmonds, Dittmer & Brown (1945) had shown that β-2-thienyl-alanine acted as a phenyl-

alanine antagonist, and Waelsh, Owades, Miller & Borek (1946) had studied methionine sulphoxide, methionine sulphone and benzylhomocysteine sulphoxide as antimetabolites of glutamic acid.

While the present investigation was under way the published knowledge of amino-acid analogues as growth inhibitors was augmented by a paper by Mitchell & Nieman (1947) on halogenated phenylalanines and tyrosines, of which 3-fluoro-DL-phenylalanine and 3-fluoro-L-tyrosine were the most active; by a communication from Elks, Hems & Ryman (1948) on α-amino acids with longer than normal aliphatic chains which were ineffective as growth inhibitors, and by a paper by Elliott, Fuller & Harington (1948) on alanines substituted in the β-position with pyridine, quinoline and basic derivatives of benzene. Significant inhibitions were observed only with β-ω-amino-*p*-tolylalanine, β-6-methoxyquinolyl-4-alanine and β-pyridyl-4-alanine; these compounds inhibited growth of *Strep. pyogenes* in broth at concentrations of 2×10^{-3}, $1{\cdot}5 \times 10^{-3}$ and 8×10^{-3} M respectively. Other substituted alanines showing significant action as inhibitors of bacterial growth were β-2-furylalanine and β-2-pyrrolealanine (Clark & Dittmer, 1948; Herz, Dittmer, and Cristol, 1948).

Without comparative tests, using the same strain of organism and the same batch of culture medium, it is impossible to make any comparison in absolute terms between the antibacterial efficacy of amino-acid analogues prepared in different laboratories. If any conclusion can be drawn from the experimental evidence available, it is that, as possible chemotherapeutic agents, essential amino-acid analogues do not offer great promise. The results obtained in the present investigation (Table 1) indicate, however, that in our series antibacterial activity was increased when an analogue of lysine (β-4-piperidyl alanine) was combined in peptide linkage with other amino-acids. This led to the abandonment of the present work, and to a study of synthetic peptides as possible antibacterial compounds; the results obtained will be reported in future papers.

SUMMARY

1. The reasons for synthesizing analogues of lysine as potential chemotherapeutic agents are discussed.

2. The following analogues have been synthesized and tested as inhibitors of bacterial growth against *Streptococcus haemolyticus*, *Staphylococcus aureus* and *Escherichia coli*: (1) 1:6-diaminohexane-1-carboxylic acid (homolysine); (2) 2-(4′-piperidyl)-1-aminopropionic acid (β-4-piperidyl alanine); (3) 2-(4′-piperidyl)-1-aminopropionyl-*p*-aminobenzoic acid; (4) 2-(4′-piperidyl)-1-aminopropionyl-DL-leucine methyl ester.

3. No striking growth inhibitory properties were encountered but increased inhibitory action was apparent when (2) was incorporated in peptide linkage with *p*-aminobenzoic acid and DL-leucine.

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