298. *Benziminazoles related to Pteroic and Pteroylglutamic Acids.*

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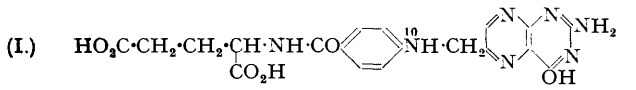
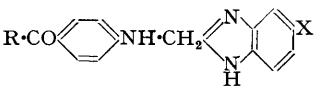
Benziminazole analogues of pteroic and pteroylglutamic acids have been prepared by condensing 2-chloromethylbenziminazole with *p*-aminobenzoic acid or its ethyl ester and with ethyl *p*-aminobenzoyl-L-glutamate. The intermediate *p*-nitrobenzoylglutamic ester was readily obtained in an optically pure condition from *p*-nitrobenzoyl chloride and ethyl L-glutamate, and the preparation of the corresponding acid from L-glutamic acid has been improved.

Several investigations on the effect on biological activity of modifications in the structure of pteroylglutamic acid (folic acid) (I) have now been reported. These mutations include the replacement in (I) of (*a*) the 4-hydroxyl by an amino-group (Seeger, Smith, and Hultquist, *J.*

(I.) 

(II.) 

Amer. Chem. Soc., 1947, **69**, 2567), (*b*) the 7-hydrogen atom by methyl (Martin, Tolman, and Moss, *Arch. Biochem.*, 1947, **12**, 318; Franklin, Stokstad, Belt, and Jukes, *J. Biol. Chem.*, 1947, **169**, 427), (*c*) the L-glutamic acid residue by L-aspartic acid (Hutchings *et al.*, *ibid.*, 1947, **170**, 323), and (*d*) of H_{10} by formyl (Gordon, Ravel, Eakin, and Shrive, *J. Amer. Chem. Soc.*, 1948, **70**, 878) and by methyl and phenacyl groups (Cosulich and Smith, *ibid.*, p. 1922). Woolley and Pringle (*J. Biol. Chem.*, 1948, **174**, 327) have described an *iso*xanthopterin-6-carboxyamidobenzoylglutamic acid, and more remote analogues of (I), *e.g.*, quinoxaline-2-carboxyamidobenzoylglutamic acid (Woolley and Pringle, *loc. cit.*) and a *p*-(4-quinazolyl)aminobenzoyl-L-glutamic acid (Martin, Moss, and Avakian, *ibid.*, 1947, **167**, 737), have also been prepared.

In general, these compounds are in varying degrees pteroylglutamic acid antagonists for several species, but the 10-formyl derivative of (I)—as would be expected from its constitution as a glutamyl derivative of the *S. fæcalis* R. factor (rhizopterin) (Wolf *et al.*, *J. Amer. Chem. Soc.*, 1947, **69**, 2753)—is a potent growth promoter for certain micro-organisms. The quinazoline is also said to have slight stimulating properties, but the observation requires confirmation in view of its exceptional nature.

The following account gives details of the synthesis of some benziminazoles structurally related to pteroylglutamic acid. While these compounds were undergoing microbiological examination, a communication appeared from Edwards, Starling, Mattocks, and Skipper (*Science*, 1948, **107**, 119) describing the results of experiments with [II; R = $NH{\cdot}CH(CO_2H){\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$, X = H], but no particulars of their method of preparation have yet been published.

The required benziminazoles were expected to be available through the imino-ether synthesis (King and Acheson, preceding paper), but the preparation of the necessary imino-ethers from cyanides of the type $R{\cdot}C_6H_4{\cdot}NH{\cdot}CH_2{\cdot}CN$ could not be accomplished on account of difficulties due to the presence of the secondary amino-group. However, a satisfactory alternative was found in the use of chloroacetimino ethyl ether hydrochloride, which very readily condensed with *o*-phenylenediamine giving 2-chloromethylbenziminazole, identical with that obtained from chloroacetic acid and the *o*-diamine by heating in hydrochloric acid (Bloom and Day, *J. Org. Chem.*, 1939, **4**, 14). The chloromethyl compound slowly reacted in boiling alcohol with ethyl *p*-aminobenzoate, forming the *hydrochloride* of *ethyl* p-(2-*benziminazolyl*)*methylaminobenzoate*, which was hydrolysed with hot 2N-alkali to the *acid* (II; R = OH, X = H), also obtained from the chloromethylbenziminazole and *p*-aminobenzoic acid.

It was not possible to synthesise the glutamic acid derivative [II; R = $NH{\cdot}CH(CO_2H){\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$, X = H] from the above acid, the insolubility of the acid chloride rendering it inactive towards aqueous alkaline solutions of glutamic acid and also of glycine. The acylation of glycine with p-(2-*benziminazolyl*)*methylaminobenzazide* under similar conditions was also unsuccessful, apparently for the same reason. The necessary azide was obtained from 1-(*p*-aminobenzoyl)-2-benzylidenehydrazine, $CHPh{:}N{\cdot}NH{\cdot}CO{\cdot}C_6H_4{\cdot}NH_2$ (Curtius, *J. pr. Chem.*, 1917, **45**, 336), and 2-chloromethylbenziminazole in boiling alcohol, followed by the action of hydrochloric acid (which removed the protecting benzylidene group) and sodium nitrite.

An attempt was therefore made to synthesise the pteroylglutamic acid analogue from 2-chloromethylbenziminazole and *p*-aminobenzoyl-L-glutamic acid, the latter being obtained by catalytic reduction of the corresponding *p*-nitro-compound. The acylation of L-glutamic acid was carried out in sodium hydroxide solution, to avoid racemisation (cf. Carter and Stevens, *J. Biol. Chem.*, 1941, **138**, 627), and the use of only one equivalent of *p*-nitrobenzoyl chloride dissolved in dioxan was sufficient to give 70—80% of fairly pure *p*-nitrobenzoyl-L-glutamic acid. The *p*-aminobenzoylglutamic acid failed, however, to give a crystalline derivative with 2-chloromethylbenziminazole, but the synthesis of p-(2-*benziminazolyl*)*methylaminobenzoyl*-L-*glutamic acid* was finally achieved from ethyl *p*-aminobenzoylglutamate. This was easily obtained without any detectable racemisation by boiling a solution of *p*-nitrobenzoyl chloride and the hydrochloride of ethyl glutamate in benzene, the resulting *ethyl* p-*nitrobenzoyl*-L-*glutamate* being catalytically reduced. The condensation of ethyl *p*-aminobenzoyl-L-glutamate with 2-chloromethylbenziminazole proceeded smoothly in boiling ethanol, giving a *hydrochloride* from which *ethyl* p-(2-*benziminazolyl*)*methylaminobenzoyl*-L-*glutamate* was obtained and then hydrolysed to [II; R = NH·CH(CO_2H)·CH_2·CH_2·CO_2H, X = H] by cold alkali.

By very similar methods, 5-*chloro*-2-*chloromethylbenziminazole*, p-(5-*chloro*-2-*benziminazolyl*)*methylaminobenzoic acid* (II; R = OH, X = Cl), and the corresponding L-glutamic *acid* and *ethyl* ester derivatives, [II; R = NH·CH(CO_2H)·CH_2·CH_2·CO_2H, X = Cl] and [II; R = NH·CH(CO_2Et)·CH_2·CH_2·CO_2Et, X = Cl], were also prepared. Some analogous compounds were prepared from DL-methionine, the amino-acid reacting with *p*-nitrobenzoyl chloride in aqueous alkali under the usual conditions to give p-*nitrobenzoyl*-DL-*methionine*. The nitro-compound was reduced by ferrous hydroxide to p-*aminobenzoyl*-DL-*methionine*, which was condensed with 2-chloromethylbenziminazole to the *product* [II; R = NH·CH(CO_2H)·CH_2·CH_2·SMe, X = H] without the necessity for proceeding through the ester.

At one time in the course of this work some experiments were made on the synthesis of compounds of type (II) from arylaminoacetyl-*o*-phenylenediamines, Ar·NH·CH_2·CO·NH·C_6H_4·NH_2. 4-*Chloro*-2-*nitrochloroacetanilide* was prepared from chloroacetyl chloride and 4-chloro-2-nitroaniline, and the product condensed with aniline. In boiling *n*-propanol, *anilinoaceto*-4-*chloro*-2-*nitroanilide* was obtained, but with pyridine as solvent the principal product was 4-*chloro*-2-*nitroanilinoacetylpyridinium chloride*. The *o*-nitroanilide was hydrogenated over Raney nickel, and on heating the resulting *anilinoaceto*-4-*chloro*-2-*aminoanilide* under reflux with aqueous methanolic hydrogen chloride, N-(5-*chloro*-2-*benziminazolyl*)*methylaniline* was obtained, and characterised as a *picrate*. The synthesis proved much less satisfactory with the feebly reactive ethyl *p*-aminobenzoate, the condensation with 2-nitrochloroacetanilide in boiling propanol (which required pyridine as a catalyst) giving only 12% of p-*carbethoxyanilinoaceto*-2-*nitroanilide*. Catalytic reduction led to the corresponding 2-*amino*-compound, which was cyclised in boiling alcoholic acid to a hydrochloride identical with that synthesised from ethyl *p*-aminobenzoate and 2-chloromethylbenziminazole. The picrate of the benziminazole (II; R = OEt, X = H) was obtained merely on heating the *o*-diamine with alcoholic picric acid, but the poor yield obtained at the intermediate stage renders the alternative synthesis preferable, and these experiments were discontinued.

Methyl p-*nitrobenzoyl*-L-*glutamate* and 2-(2′-*chloroethyl*)*benziminazole hydrochloride* were also prepared in the course of this work.

Experimental.

2-*Chloromethylbenziminazole*.—Solutions of chloroacetiminoethyl ether hydrochloride (4·3 g., 1 mol.) and of *o*-phenylenediamine (3 g., 1 mol.) in dry ethanol (total 20 c.c.) developed heat on mixing, and ammonium chloride separated. After several hours, the mixture was diluted with water, and the precipitate (3 g., 70%) crystallised from dioxan to give colourless needles, m. p. 162°, identical with the product obtained by Bloom and Day's method (*loc. cit.*).

Ethyl p-(2-*Benziminazolyl*)*methylaminobenzoate*.—A solution in ethyl alcohol (100 c.c.) of 2-chloromethylbenziminazole (17·8 g., 1 mol.), ethyl *p*-aminobenzoate (17·8 g., 1 mol.), and a small quantity of sodium iodide was heated under reflux for 10 hours. The solid (25·5 g., 72%) which then slowly separated consisted of the *hydrochloride* of ethyl *p*-(2-benziminazolyl)methylaminobenzoate which, after being washed with a little alcohol, crystallised from ethanol or water in bush-like clusters of colourless prisms, m. p. 255° (decomp.) (Found: C, 61·2; H, 5·4; N, 13·0. $C_{17}H_{17}O_2N_3$,HCl requires C, 61·5; H, 5·4; N, 12·7%). Warm alcoholic solutions of the hydrochloride and picric acid deposited overnight the *picrate* in golden-yellow polyhedra, m. p. 210° (decomp.) (Found: C, 52·6; H, 3·9; N, 15·6. $C_{17}H_{17}O_2N_3$,$C_6H_3O_7N_3$ requires C, 52·7; H, 3·8; N, 16·0%). The addition of ammonia to a hot aqueous solution of the hydrocbloride liberated the *amino*-ester, crystallising from ethanol in colourless platelets, m. p. 248° (decomp.) (Found: C, 68·7; H, 5·9. $C_{17}H_{17}O_2N_3$ requires C, 69·1, H, 5·8%).

p-(2-*Benziminazolyl*)*methylaminobenzoic Acid*.—(i) A mixture of 2-chloromethylbenziminazole (8·5 g., 1 mol.) and *p*-aminobenzoic acid (7·0 g., 1 mol.) with a small amount of sodium iodide was heated in ethyl alcohol (55 c.c.) under reflux for 12 hours. After cooling, the product (8·7 g., 57%) was collected,

washed with cold alcohol, and crystallised from N-hydrochloric acid. The resulting *hydrochloride* separated in colourless long prisms, m. p. 268° (decomp.) (Found : C, 58·5; H, 4·8. $C_{15}H_{13}O_2N_3$,HCl requires C, 59·3; H, 4·6%). Its aqueous solution, treated with sodium hydrogen carbonate solution, gave p-(2-*benziminazolyl*)*methylaminobenzoic acid*, which crystallised from nitrobenzene or ethoxyethanol–water in minute elongated prisms, m. p. 281° (decomp.) (Found : C, 66·8; H, 4·7. $C_{15}H_{13}O_2N_3$ requires C, 67·4; H, 4·9%). The addition of alcoholic picric acid to a solution of the hydrochloride in warm water gave a *picrate*, slowly separating on cooling, which crystallised from alcohol in yellow prisms, m. p. 216° (decomp.) (Found : C, 50·4; H, 3·6; N, 16·6. $C_{15}H_{13}O_2N_3$,$C_6H_3O_7N_3$ requires C, 50·1; H, 3·2; N, 16·9%).

(ii) Ethyl *p*-(2-benziminazolyl)methylaminobenzoate (1·7 g.) was treated with 2N-alcoholic potassium hydroxide (20 c.c.) which was then heated to boiling for 5 minutes. After the addition of water, the cold solution was neutralised with dilute acetic acid, and the precipitated solid was collected and recrystallised from ethoxyethanol–water, giving the *p*-aminobenzoic acid derivative, m. p. 279° (decomp.).

The hydrochloride of the benziminazole (6·8 g.) was dissolved in warm phosphoryl chloride (150 c.c.) which after the addition of phosphorus pentachloride (4·7 g.) was heated under reflux for 20 minutes. Most of the phosphoryl chloride was then evaporated off under reduced pressure, and the gummy residue triturated with anhydrous ether. The product (9·7 g.), a buff powder containing phosphorus, reacted as the acid chloride in giving with ethyl alcohol the above ester, m. p. 255° (decomp.) The bulk (8·5 g.), added during 1½ hours to a solution of L-glutamic acid (3·5 g., 1 mol.) in water (100 c.c.) containing sodium hydrogen carbonate (10 g., 5 mols.) at 0°, was largely converted into a bicarbonate-insoluble product; no further precipitate was obtained on acidifying the solution with acetic acid. Similar negative results were obtained when the supposed acid chloride reacted with a solution of glycine in N-sodium hydroxide.

p-(2-*Benziminazolyl*)*methylaminobenzazide*.—(*p*-Aminobenzoyl) benzylidenehydrazine (4 g.) (Curtius, *loc. cit.*), 2-chloromethylbenziminazole (2·9 g.), and a trace of sodium iodide were heated in refluxing ethanol (110 c.c.) for 15 hours. The colourless solid (4·6 g., 68%) which separated during the reaction was the *hydrochloride* of the compound (II; R = NH·N:CHPh, X = H). When collected and recrystallised from ethanol–water it formed microscopic needles, m. p. 283° (decomp.) (Found : C, 65·1; H, 4·9; N, 17·0. $C_{22}H_{19}ON_5$,HCl requires C, 65·1; H, 4·9; N, 17·2%). With picric acid in aqueous-alcoholic solution a precipitate of the *picrate* was formed, m. p. (after crystallisation from ethoxyethanol–water), 224° (decomp.) (Found, after drying at 110° in a vacuum : C, 54·8; H, 4·0; N, 18·2. $C_{22}H_{19}ON_5$,$C_6H_3O_7N_3$ requires C, 54·5; H, 4·0; N, 18·2%). A solution of the hydrochloride in 30% alcohol, neutralised with sodium hydrogen carbonate, gave the *benziminazole* (II; R = NH·N:CHPh, X = H), which crystallised from ethoxyethanol–water in colourless minute plates, m. p. 276° (Found : C, 70·7; H, 5·2. $C_{22}H_{19}ON_5$ requires C, 71·5; H, 5·2%).

The hydrochloride (4·5 g.) was dissolved in 2N-hydrochloric (250 c.c.) by heating to boiling for a few minutes. The solution was then cooled, and after removal of the benzaldehyde by ether extraction, was treated at 0° with sodium nitrite (0·9 g.). The gummy precipitate which separated slowly hardened and formed a powder. This was insoluble in the usual solvents, but the product obtained by shaking it with sodium carbonate solution, which was probably the *azide* (II; R = N_3, X = H), dissolved in warm acetone and separated on addition of light petroleum in microscopic long prisms decomposing vigorously at *ca.* 150° (Found : N, 28·0. $C_{15}H_{12}ON_6$ requires N, 28·8%). When this was added to a stirred solution of glycine in excess of sodium carbonate, apparently unchanged azide was recovered.

p-*Nitrobenzoyl*-L-*glutamic Acid*.—(i) L-Glutamic acid (19·6 g., 1 mol.) was dissolved in N-sodium hydroxide (270 c.c., 2 mols.) which was then stirred at room temperature for 1 hour during the simultaneous addition of a solution (66 c.c.) of *p*-nitrobenzoyl chloride (25 g., 1 mol.) in dioxan and of 2N-sodium hydroxide (66 c.c.). Shortly after, the solution was filtered and then acidified to Congo-red with 5N-hydrochloric acid (*ca.* 55 c.c.). When the precipitated *p*-nitrobenzoic acid had been removed, the filtrate was concentrated under reduced pressure to about 70 c.c., and the crude *p*-nitrobenzoylglutamic acid (33 g., 84%), m. p. *ca.* 110°, was collected after several hours. Crystallisation from four times its weight of water gave the pure acid, m. p. 114—116°, $[\alpha]_D^{19°}$ +16·9° in N-sodium hydroxide (Found : C, 45·4; H, 4·3; N, 8·8. Calc. for $C_{12}H_{12}O_7N_2$,H_2O : C, 45·9; H, 4·5; N, 8·9%).

(ii) Ethyl *p*-nitrobenzoylglutamate (2 g.) obtained by nitrobenzoylation of the glutamic ester (see below) was heated under reflux with 2N-hydrochloric acid (20 c.c.) until dissolved (1½ hours). On cooling, *p*-nitrobenzoic acid separated and was removed after an hour; next day the filtrate had deposited *p*-nitrobenzoylglutamic acid (0·75 g., 45%). Several crystallisations from water gave the pure product, m. p. 114—116°, $[\alpha]_D^{20°}$ +17·5° in N-sodium hydroxide.

Hydrolysis of the ester (11 g.) was also effected in acetone (160 c.c.) by shaking with 5N-sodium hydroxide (40 c.c.) at room temperature for 2 hours. The solution was acidified with hydrochloric acid, and concentrated under reduced pressure to remove acetone, whereupon nitrobenzoylglutamic acid (7·7 g., 83%) was obtained, m. p. (after recrystallisation), 113—115°, $[\alpha]_D^{19°}$ +16·9° in N-sodium hydroxide.

Ethyl p-*Nitrobenzoyl*-L-*glutamate*.—(i) *p*-Nitrobenzoyl-L-glutamic acid (3 g.) was heated under reflux in 5% ethanolic hydrogen chloride for 4 hours. On evaporation of the solvent, the ester remained as a colourless gum which slowly solidified. A solution in warm ethyl acetate, treated with light petroleum, gave a microcrystalline product (1·7 g., 50%), m. p. 89—91° (Found : C, 54·6; H, 5·6; N, 7·6. $C_{16}H_{20}O_7N_2$ requires C, 54·5; H, 5·7; N, 7·9%).

(ii) A suspension of L-glutamic acid (20 g.) in ethanol (150 c.c.) was saturated with dry hydrogen chloride. More anhydrous alcohol (300 c.c.) was then added, and the mixture gently heated under reflux for 3 hours and finally evaporated under reduced pressure. To remove the last traces of alcohol, pure benzene (50 c.c.) was added and distilled. Finally, a solution of *p*-nitrobenzoyl chloride (27 g., 1·05 mols.) in dry benzene was introduced, and the mixture heated under reflux for several hours until evolution of hydrogen chloride had ceased. The benzene solution, cooled and washed with 5% aqueous bicarbonate, was then dried ($CaCl_2$) and evaporated, leaving the *p*-nitrobenzoyl ester as a colourless solid (33 g., 70%) identical when crystallised from 50% alcohol or ether–light petroleum with the product, m. p. 94—95°, obtained by esterification of the acid.

Ethyl p-*Aminobenzoyl*-L-*glutamate* (cf. Waller *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 20).—The nitro-ester

was hydrogenated in alcoholic solution over palladised charcoal at room temperature and 2—3 atm. The *p*-aminobenzoylglutamate was isolated by evaporating the filtered solution and treatment with water. Crystallisation from a little alcohol gave small prisms, m. p. 140—141° (Found: C, 59·3; H, 6·8; N, 8·5. Calc. for $C_{16}H_{22}O_5N_2$: C, 59·6; H, 6·9; N, 8·9%).

p-*Aminobenzoyl*-L-*glutamic Acid*.—A solution of the nitro-acid (10·8 g.) in ethanol (100 c.c.) was hydrogenated over palladised charcoal at room temperature and 2—3 atm. Evaporation of the filtered solution left a gum, which was dissolved in a little hot water. The aminobenzoylglutamic acid (8·8 g., 90%) crystallising on cooling had m. p. 169—170° and $[\alpha]_D^{22°}$ +29·2° in N-sodium hydroxide.

Ethyl p-(2-*Benziminazolyl*)*methylaminobenzoyl*-L-*glutamate*.—2-Chloromethylbenziminazole (3·5 g.), ethyl *p*-aminobenzoylglutamate (7 g.), and a little sodium iodide in ethanol (25 c.c.) were heated under reflux for 10 hours. The solvent was evaporated (charcol) to half bulk, and the solution left until next day. The product which had then separated was a *hydrochloride* (3·8 g., 36%), which crystallised from alcohol, containing hydrogen chloride, in minute elongated prisms, m. p. 232° (decomp.) (Found: C, 58·7; H, 5·9; N, 11·5; Cl, 7·4. $C_{24}H_{28}O_5N_4$,HCl requires C, 58·9; H, 5·9; N, 11·5; Cl, 7·3%). The *ethyl* p-(2-*benziminazolyl*)*methylaminobenzoylglutamate*, colourless minute needles from 50% alcohol, had m. p. 125° (Found: N, 12·6. $C_{24}H_{28}O_5N_4$ requires N, 12·4%). The *picrate* separated from alcohol in yellow prisms, m. p. 179° (Found: C, 52·6; H, 4·5; N, 14·5. $C_{24}H_{28}O_5N_4$,$C_6H_3O_7N_3$ requires C, 52·4; H, 4·5; N, 14·4%).

p-(2-*Benziminazolyl*)*methylaminobenzoyl*-L-*glutamic Acid*.—The ethyl ester (1 g.) was dissolved in ethanol (10 c.c.) to which a solution of sodium hydroxide (0·5 g.) in water (3 c.c.) was added. After 2 hours the solution was partly evaporated under reduced pressure and treated with a little water containing concentrated hydrochloric acid (1·6 c.c.). The oily precipitate (0·8 g., 90%) shortly solidified, and when crystallised from 2N-hydrochloric acid gave the *hydrochloride* of the substituted glutamic acid in the form of minute prisms, m. p. 201° (Found: C, 52·9; H, 5·0; Cl, 7·55. $C_{20}H_{29}O_5N_4$,HCl,H_2O requires C, 53·3; H, 5·1; Cl, 7·9%. Found, after drying at 100° in a vacuum: C, 55·6; H, 4·7; N, 12·6; Cl, 8·1. $C_{20}H_{29}O_5N_4$,HCl requires C, 55·5; H, 4·9; N, 12·9; Cl, 8·2%).

5-*Chloro*-2-*chloromethylbenziminazole*.—4-Chloro-*o*-phenylenediamine dihydrochloride (5·4 g., 1 mol.) and chloroacetic acid (3·6 g., 1½ mols.) were dissolved in 3N-hydrochloric acid (37 c.c.) which was then heated under reflux for ¾ hour. After being left overnight and treatment with charcoal, the solution was diluted with water (50 c.c.) and basified with 6N-ammonia. The buff precipitate (5·3 g.) was collected, dried, and extracted with cold acetone, which left a residue (3—3·5 g.) probably a condensate of 2 molecules of the benziminazole. The product obtained by evaporating the acetone solution was dissolved in alcoholic hydrogen chloride (charcoal), from which 5-*chloro*-2-*chloromethylbenziminazole hydrochloride* was precipitated by ether as light pink microscopic prisms (0·8 g.), m. p. 213—214° (decomp.) (Found: C, 37·9; H, 3·4. $C_8H_6N_2Cl_2$,HCl,H_2O requires C, 37·6; H, 3·5%). The *base* was precipitated from an aqueous solution of the salt with ammonia as a colourless solid difficult to recrystallise, m. p. after drying over phosphoric anhydride, 140° (Found: C, 47·8; H, 3·1; N, 13·3; Cl, 34·6. $C_8H_6N_2Cl_2$ requires C, 47·8; H, 3·0; N, 13·9; Cl, 35·3%). The *picrate* was prepared from the hydrochloride and picric acid in ethanol and crystallised in yellow elongated prisms, m. p. 195—196° (Found: N, 16·1; Cl, 16·4. $C_8H_6N_2Cl_2$,$C_6H_3O_7N_3$ requires N, 16·3; Cl, 16·5%).

p-(5-*Chloro*-2-*benziminazolyl*)*methylaminobenzoic Acid*.—The unpurified 5-chloro-2-chloromethylbenziminazole (2 g.), *p*-aminobenzoic acid (1·4 g.), and sodium iodide (0·1 g.) were treated with hot alcohol (25 c.c.), and after filtration the solution was heated under reflux. Colourless solid began to separate in 4 hours, and after 18-hours' heating the product (1·1 g., 37%) was collected, washed with cold ethanol, and dissolved in boiling water (60—70 c.c.) containing a little hydrochloric acid. When kept, the *hydrochloride* crystallised in prisms, m. p. 276° (decomp.) (Found: C, 52·9; H, 4·0; N, 12·3; Cl, 21·4. $C_{15}H_{12}O_2N_3Cl$,HCl requires C, 53·3; H, 3·85; N, 12·4; Cl, 21·0%). Addition of sodium acetate to its solution in water gave the *amino*-acid, crystallising as an alcoholate from aqueous ethanol in tiny plates, sintering at *ca.* 150°, m. p. 237° (decomp.) (Found: C, 58·8; H, 5·1; Cl, 10·4. $C_{15}H_{12}O_2N_3Cl$,C_2H_6O requires C, 58·7; H, 5·2; Cl, 10·2%). The *picrate*, yellow needles from alcohol, had m. p. *ca.* 235° (decomp.) (Found: C, 47·4; H, 2·6; Cl, 6·9. $C_{15}H_{12}O_2N_3Cl$,$C_6H_3O_7N_3$ requires C, 47·5; H, 2·8; Cl, 6·7%).

Ethyl p-(5-*Chloro*-2-*benziminazolyl*)*methylaminobenzoylglutamate*.—The *p*-aminobenzoylglutamic ester (1·85 g.) and 5-chloro-2-chloromethylbenziminazole (1·15 g.) gave the required product after boiling in alcohol (10 c.c.) with sodium iodide (0·1 g.) for 15 hours. It was isolated by concentrating the solution (charcoal) and leaving it in the cold, the *hydrochloride* (0·85 g., 30%) separating from ethanol as colourless minute prisms, m. p. 219—220° (Found: C, 54·9; H, 5·2; N, 11·2; Cl, 14·0. $C_{24}H_{27}O_5N_4Cl$,HCl requires C, 55·1; H, 5·35; N, 10·7; Cl, 13·6%). The *amino*-ester crystallised from 50% aqueous alcohol in clusters of colourless needles, m. p. 123° (Found: Cl, 7·4. $C_{24}H_{27}O_5N_4Cl$ requires Cl, 7·2%); it formed a *picrate*, sparingly soluble in alcohol, m. p. 195° (Found: C, 50·6; H, 4·2; N, 13·5; Cl, 5·2. $C_{24}H_{27}O_5N_4Cl$,$C_6H_3O_7N_3$ requires C, 50·3; H, 4·2; N, 13·7; Cl, 5·0%).

p-(5-*Chloro*-2-*benziminazolyl*)*methylaminobenzoylglutamic Acid*.—The ester (0·3 g.) was dissolved in ethanol (3 c.c.), and a solution of sodium hydroxide (0·15 g.) in water (1 c.c.) added. After 2 hours the mixture was concentrated in a vacuum at room temperature and acidified with 12% hydrochloric acid (1·5 c.c.). The resultant gummy precipitate hardened on rubbing, and the amino-acid *hydrochloride* (0·17 g., 62%) obtained on crystallisation from dilute hydrochloric acid had m. p. 199—202° (Found: C, 49·7; H, 4·6; N, 11·5; Cl, 14·3. $C_{20}H_{19}O_5N_4Cl$,HCl,H_2O requires C, 49·5; H, 4·5; N, 11·5; Cl, 14·6%. Found, after drying at 140°: C, 51·7; H, 4·5; loss, 3·6. $C_{20}H_{19}O_5N_4Cl$,HCl requires C, 51·4; H, 4·3; loss 3·7%).

p-*Nitrobenzoyl*-DL-*methionine*.—*p*-Nitrobenzoyl chloride (10 g.) in dioxan (30 c.c.) and aqueous sodium hydroxide (2·2 g. in 50 c.c.) were added simultaneously to a stirred solution of DL-methionine (8 g.) in a further equal quantity of alkali at room temperature in the course of 25—30 minutes. Stirring was continued until the red-purple solution was almost colourless, and concentrated hydrochloric acid (7—8 c.c.) was then added. The precipitated p-*nitrobenzoyl*-DL-*methionine* had m. p. 145—160° after crystallisation from aqueous alcohol, being contaminated with *p*-nitrobenzoic acid. This was removed

by trituration with ether, in which it is less soluble; crystallisation from hot water containing ethanol then gave the methionine derivative in colourless long thin prisms, m. p. 169—170° (Found: C, 48·4; H, 4·8; N, 9·35; S, 10·6. $C_{12}H_{14}O_5N_2S$ requires C, 48·3; H, 4·7; N, 9·40; S, 10·7%).

p-*Aminobenzoyl*-DL-*methionine*.—*p*-Nitrobenzoylmethionine (3 g.) was dissolved in water (25 c.c.) containing a little ammonia, and a solution of ferrous sulphate heptahydrate (20 g.) in boiling water (40 c.c.) was added. Further additions of aqueous ammonia (total, 40—45 c.c.; *d* 0·88) were made to the boiling liquid until alkaline, and after further brief heating it was filtered and the filtrate and washings concentrated. Neutralisation with acetic acid precipitated the p-*aminobenzoyl*-DL-*methionine* in long colourless needles, m. p. 169—171°, rising when recrystallised to 172° (Found: C, 54·1; H, 6·15. $C_{12}H_{16}O_3N_2S$ requires C, 53·7; H, 6·0%).

p-(2-*Benziminazolyl*)*methylaminobenzoyl*-DL-*methionine*.—A solution of *p*-aminobenzoyl-DL-methionine (1·2 g.), 2-chloromethylbenziminazole (0·8 g.), and sodium iodide (0·1 g.) in ethanol (13 c.c.) was heated under reflux for 5 hours. Ether was added, precipitating a gum which was purified by dissolving it in very dilute hydrochloric acid and reprecipitating with sodium hydrogen carbonate. Several crystallisations from alcohol gave the *benziminazolylmethylaminobenzoylmethionine* in buff globules, m. p. *ca.* 195° (Found: C, 59·3; H, 5·7; N, 14·5. $C_{20}H_{22}O_3N_4S$ requires C, 60·3; H, 5·5; N, 14·1%).

4-*Chloro*-2-*nitrochloroacetanilide*.—Chloroacetyl chloride (22·8 g., 1·1 mols.) was carefully mixed with 4-chloro-2-nitroaniline (28 g., 1 mol.), and after the initial reaction, the mixture was heated until molten. The product was then cooled, ground with water, and dissolved in boiling alcohol. On cooling, the *anilide* (38 g., 79%), m. p. 139°, separated, and two further crystallisations gave long thin pale yellow prisms, m. p. 141° (Found: C, 38·5; H, 2·5; N, 11·7. $C_8H_6O_3N_2Cl_2$ requires C, 38·6; H, 2·4; N, 11·2%).

Anilinoaceto-4-*chloro*-2-*nitroanilide*.—The chloroacetanilide (2·3 g., 1 mol.) and aniline (1·8 g., 2·1 mols.) were heated in propanol (14 c.c.) under reflux for 3 hours, and the product precipitated by water. The *anilinoacetanilide* (2 g., 71%), m. p. 132—133°, after recrystallisation from water formed orange-yellow plates, m. p. 134—135° (Found: C, 55·2; H, 4·0; Cl, 11·8. $C_{14}H_{12}O_3N_3Cl$ requires C, 55·0; H, 3·9; Cl, 11·6%).

When the chloroacetanilide (1·2 g.) and aniline (0·45 g., 1 mol.) were heated in pyridine (3 c.c.), a solid began to separate, and this was redissolved by the addition of more pyridine, which was then refluxed for ½ hour. Next day, the anilinoaceto-4-chloro-2-nitroanilide (0·12 g.) was obtained by filtering the pyridine solution from a crystalline solid and adding water. The crystalline residue was water-soluble and consisted of 4-*chloro*-2-*nitroanilinoacetylpyridinium chloride* (1 g., 61%), which separated from ethanol in pale yellow needles, m. p. 232° (decomp.) (Found: C, 47·4; H, 3·3; N, 13·1. $C_{13}H_{11}O_3N_3Cl_2$ requires C, 47·6; H, 3·4; N, 12·8%).

N-(5-*Chloro*-2-*benziminazolyl*)*methylaniline*.—Anilinoaceto-4-chloro-2-nitroanilide, dissolved in methanol, was reduced over Raney nickel at room temperature under 2 atm. hydrogen pressure. The corresponding 2-*amino*-*anilide* separated from aqueous alcohol in colourless plates, m. p. 163° (Found: Cl, 12·5. $C_{14}H_{14}ON_3Cl$ requires C, 12·9%). The amine was warmed with aqueous methanolic hydrogen chloride, and the resulting benziminazole isolated as an unstable *dihydrochloride* in colourless prisms, m. p. 250—251° (Found: C, 51·4; H, 4·8. $C_{14}H_{12}N_3Cl,2HCl$ requires C, 50·8; H, 4·2%). The *picrate*, yellow prisms from aqueous alcohol, had m. p. 214—215° (decomp.) (Found: C, 49·4; H, 3·2; N, 17·6. $C_{14}H_{12}N_3Cl,C_6H_3O_7N_3$ requires C, 49·3; H, 3·1; N, 17·3%).

p-*Carbethoxyanilinoaceto*-2-*nitroanilide*.—2-Nitrochloroacetanilide (4·3 g., 1 mol.), ethyl *p*-aminobenzoate (3·3 g., 1 mol.), and pyridine (1·6 c.c.) were heated in refluxing *n*-propanol (10 c.c.) for 30 hours. Pouring into water gave p-*carbethoxyanilinoaceto*-2-*nitroanilide* which, after crystallisation from ethanol and then *n*-propanol, formed irregular yellow plates (0·82 g., 12%), m. p. 186° (Found: C, 59·4; H, 5·0; N, 12·0. $C_{17}H_{17}O_5N_3$ requires C, 59·5; H, 5·0; N, 12·2%). The omission of pyridine or heating for a shorter time resulted in a still smaller yield.

Hydrogenation of the 2-nitroanilide in methanol at room temperature and 2 atm. gave the corresponding 2-*amino*-compound, which crystallised from alcohol in clusters of colourless needles, m. p. 169° (Found: C, 64·9; H, 6·4; N, 13·0. $C_{17}H_{19}O_3N_3$ requires C, 65·2; H, 6·1; N, 13·4%). Boiling the base in 95% alcoholic hydrogen chloride and evaporation gave the hydrochloride of ethyl *p*-(2-benziminazolyl)methylaminobenzoate, which crystallised from alcohol in prisms, m. p. 255° (decomp.) alone or mixed with the product prepared from 2-chloromethylbenziminazole. The benziminazole picrate, m. p. and mixed m. p. 210°, was obtained by boiling *p*-carbethoxyanilinoacet-2-aminoanilide in saturated ethanolic picric acid (2 mols.) for 4 hours (Found: C, 53·1; H, 3·9; N, 16·1%).

Methyl p-*Nitrobenzoyl*-L-*glutamate*.—Methyl L-glutamate was prepared by heating the acid (3 g.) under reflux with 3% methyl-alcoholic hydrogen chloride. The glassy residue of ester hydrochloride obtained after evaporating the alcohol and triturating with benzene was heated under reflux in benzene (25 c.c.) with *p*-nitrobenzoyl chloride (4 g.) for 7 hours. When cold, the solution was twice washed with aqueous bicarbonate, dried, and evaporated. The residue of *methyl* p-*nitrobenzoyl*-L-*glutamate* (3·1 g., 47%) crystallised from aqueous methanol in hair-fine needles, m. p. 96—97° (Found: C, 51·8; H, 4·9; N, 8·4. $C_{14}H_{16}O_7N_2$ requires C, 51·8; H, 4·9; N, 8·6%).

2-(2′-*Chloroethyl*)*benziminazole*.—A solution of *o*-phenylenediamine (5·4 g., 1 mol.) and β-chloropropionic acid (8·1 g., 1·5 mols.) in 4N-hydrochloric acid (50 c.c.) was boiled for ¾ hour. The solution was then filtered, diluted with water (100 c.c.), cooled to 0°, and neutralised with 6N-ammonia. The nearly colourless precipitate of the benziminazole, rapidly dried over phosphoric anhydride in a vacuum, had m. p. *ca.* 85° (2·5 g., 28%), but it darkened and resinified in a day or two. When the freshly prepared material was dissolved in ethanolic hydrogen chloride (20 c.c.) and the solution treated with ether, a precipitate of 2-(2′-*chloroethyl*)*benziminazole hydrochloride* separated, which, if dissolved in cold acetone–ethanol, could be obtained by the addition of ether as colourless needles, m. p. 180° (Found: C, 49·9; H, 4·7; N, 12·9; Cl, 32·6. $C_9H_9N_2Cl,HCl$ requires C, 49·8; H, 4·6; N, 12·9; Cl, 32·7%). The benziminazole was also formed on adding 2-chloropropionimino ethyl ether hydrochloride to a solution of *o*-phenylenediamine in dry ethanol, heat being evolved and ammonium chloride precipitated; the product was isolated by adding water and ammonia, and identified with the compound obtained by the previous method.

Biological data on the compound [II; R = NH·CH(CO_2H)·CH_2·CH_2·CO_2H, X = H] have already been published (King, Spensley, and Nimmo-Smith, *Nature*, 1948, **162**, 153) and show that it has a low order of inhibitory activity for *S. fæcalis* R. and *L. casei*. The inhibitory power of the 5-chloro-analogue [II, R = NH·CH(CO_2H)·CH_2·CH_2·CO_2H, X = Cl] for *L. casei* is somewhat more marked; its bacterial index is 10^6. The simpler acid (II; R = OH, X = Cl) is inactive for *L. casei*, as is the methionine derivative [II, R = NH·CH(CO_2H)·CH_2·CH_2·SMe, X = H] for *S. fæcalis* R.

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