76. *Derivatives of 6-Aminopenicillanic Acid. Part V.*[1] *Analogues of 2,6-Dimethoxyphenylpenicillin with Enhanced Stability towards Acids.*

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Various derivatives of 2,6-dimethoxybenzoic acid containing electron-attracting substituents elsewhere in the ring have been synthesised. Analogues in which one or both of the methoxy-groups have been replaced by other substituents have also been prepared. Reaction of the acid chlorides with 6-aminopenicillanic acid gave penicillins which were less readily inactivated by acid than 2,6-dimethoxyphenylpenicillin. The stability at low pH increased with increasing strength of the side-chain acids.

Although 2,6-dimethoxyphenylpenicillin [2] (methicillin) has proved of value in the treatment of staphylococcal infections, especially those caused by penicillinase-producing strains, it suffers from the disadvantage that it must be administered by injection in large doses several times daily. Oral administration is unsatisfactory because the penicillin is very poorly absorbed from the gastrointestinal tract. This is doubtless due in part to destruction in the stomach, since the antibiotic is rapidly inactivated at low pH. It therefore seemed possible that an analogue with similar antibacterial properties but enhanced stability in acid medium might be a valuable oral antibiotic.

The introduction of electron-attracting substituents into the α-position of the side-chain of benzylpenicillin has been shown [3] to give penicillins which are relatively stable in acid. This result was attributed to the inductive effects of the substituents, which were considered to hinder the electron displacements initiating re-arrangement to the corresponding penillic acids. The same principle should apply to ring-substitution in the phenylpenicillin series, although mesomeric as well as inductive effects would then have to be considered. Nevertheless, the previously demonstrated correlation between acid-stability and the strength of the side-chain acid [3] should remain at least approximately valid. It was therefore hoped that the introduction of one or more electron-attracting substituents into 2,6-dimethoxybenzoic acid would lead to stronger acids which would in turn give penicillins with enhanced stability towards acid. Replacement of one or both

[1] Part IV, Brain, Doyle, Mehta, Miller, Nayler, and Stove, preceding paper.
[2] Doyle, Hardy, Nayler, Soulal, Stove, and Waddington, *J.*, 1962, 1453.
[3] Doyle, Nayler, Smith, and Stove, *Nature*, 1961, **191**, 1091.

of the *o*-methoxy-groups by appropriate substituents was expected to have a still more marked effect.

Treatment of 2,6-dimethoxybenzoic acid with two mol. of sulphuryl chloride appeared to give a mixture, but a pure 3,5-dichloro-derivative resulted when 2,6-dihydroxybenzoic acid was similarly treated. This product with methyl sulphate and aqueous sodium hydroxide gave chiefly 3,5-dichloro-2-hydroxy-6-methoxybenzoic acid, but complete methylation to methyl 3,5-dichloro-2,6-dimethoxybenzoate was effected in the presence of anhydrous potassium carbonate in acetone. Alkaline hydrolysis then gave 3,5-dichloro-2,6-dimethoxybenzoic acid. Treatment of 2,6-dihydroxybenzoic acid with only one mol. of sulphuryl chloride, followed by a similar reaction sequence, gave 3-chloro-2,6-dimethoxybenzoic acid.

Dibromination of 2,6-dimethoxybenzoic acid with bromine in chloroform gave a good yield of 3,5-dibromo-2-hydroxy-6-methoxybenzoic acid, the hydrogen bromide formed in the reaction presumably being responsible for the removal of one methyl group. The same product was obtained by bromination of 2-hydroxy-6-methoxybenzoic acid, there being no tendency to lose the second methyl group. Other examples of the monodemethylation of bromodimethoxybenzoyl derivatives on mild treatment with acids have been reported recently.[4] Methylation of the monohydroxy-acid with methyl sulphate and potassium carbonate in acetone gave methyl 3,5-dibromo-2,6-dimethoxybenzoate, which was readily hydrolysed to the corresponding acid. 3-Bromo-2,6-dimethoxybenzoic acid was obtained from 2,6-dihydroxybenzoic acid by treatment with one mol. of bromine, followed by methylation and hydrolysis. Alternatively, 2,6-dimethoxybenzoic acid could be monobrominated without demethylation by means of dioxan dibromide.

Treatment of 2,6-dihydroxybenzoic acid with two mol. of iodine monochloride gave the 3,5-di-iodo-derivative, which on complete methylation followed by hydrolysis yielded 3,5-di-iodo-2,6-dimethoxybenzoic acid. Attempts to obtain a monoiodo-acid in similar fashion gave no pure product, so 3-iodo-2,6-dimethoxybenzoic acid was prepared by a Sandmeyer reaction on diazotised 3-amino-2,6-dimethoxybenzoic acid.

Synthesis of 2,6-dimethoxy-3-phenylazobenzoic acid was effected from the known 2,6-dihydroxy-3-phenylazobenzoic acid [5] by complete methylation followed by saponification of the ester.

Electron-attracting substituents could not be introduced directly into the 4-position of 2,6-dimethoxybenzoic acid, but the desired compounds were obtained by applying the principle of cine-substitution.[6] Thus, treatment of 4-bromo-1,3-dimethoxybenzene with sodamide in liquid ammonia has been shown [7] to give 3,5-dimethoxyaniline. Application of the same method to 3-bromo-2,6-dimethoxytoluene has now given a fair yield of 3,5-dimethoxy-*p*-toluidine. Diazotisation of this amine, followed by a carefully controlled Sandmeyer reaction in the presence of benzene, gave a moderate yield of 4-chloro-2,6-dimethoxytoluene. Oxidation with potassium permanganate in aqueous pyridine [8] then gave 4-chloro-2,6-dimethoxybenzoic acid. 4-Bromo-2,6-dimethoxybenzoic acid was similarly prepared through 4-bromo-2,6-dimethoxytoluene. Oxidation of 2,6-dimethoxy-4-nitrotoluene, itself prepared from the corresponding diazonium fluoroborate and sodium nitrite, gave 2,6-dimethoxy-4-nitrobenzoic acid.

Analogues of 2,6-dimethoxybenzoic acid in which one methoxy-group was replaced by a different type of substituent were next considered. The previously reported oxidation of 2-methoxy-6-nitrotoluene to 2-methoxy-6-nitrobenzoic acid [9] was improved by using alkaline permanganate instead of the neutral reagent. Alkaline permanganate also proved suitable for the oxidation of 2-chloro-6-methoxytoluene. Carbonation of the

[4] Allen, Promislow, and Moir, *J. Org. Chem.*, 1961, **26**, 2906.
[5] Gore, Panse, and Venkataraman, *Proc. Indian Acad. Sci.*, 1949, **29**, *A*, 289.
[6] Bunnett and Zahler, *Chem. Rev.*, 1951, **49**, 382.
[7] Benkeser, Hickner, Hoke, and Thomas, *J. Amer. Chem. Soc.*, 1958, **80**, 5289.
[8] Kreuchunas, *J. Org. Chem.*, 1956, **21**, 368.
[9] Roberts, Wiles, and Kent, *J.*, 1932, 1792.

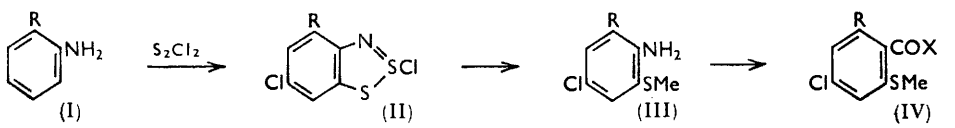
lithium derivative prepared from 3,5-diethylanisole gave 2,4-diethyl-6-methoxybenzoic acid. Complete methylation of 3-chloro-6-hydroxy-2,4-dimethylbenzoic acid [10] followed by saponification gave 3-chloro-6-methoxy-2,4-dimethylbenzoic acid.

Analogues of 2,6-dimethoxybenzoic acid in which both methoxy-groups were replaced by other substituents were mostly prepared by published methods, but side-chain bromination of 2-bromo-6-chlorotoluene, followed by permanganate oxidation, proved a more convenient route to 2-bromo-6-chlorobenzoic acid than the previously reported use of dilute nitric acid in sealed tubes.[11]

A novel synthesis of three 2-substituted 4-chloro-6-methylthiobenzoic acids (IV; X = OH, R = Me, MeO, and MeS) has been developed from appropriately substituted 1,3,2-benzothiazathiolium chlorides (II) ("Herz compounds"), which are readily prepared [12] from an *ortho*-substituted aniline (I) and sulphur chloride. Hydrolysis and reduction of the Herz compounds to *o*-aminothiols has been described,[13] but for the present purpose it was convenient to methylate them *in situ* and isolate the amino-sulphides (III). The free bases (III) proved to be unstable in air, so they were normally stored as the hydrochlorides.

The amines (III) were diazotised and converted into the corresponding nitriles by the Sandmeyer reaction. Hydrolysis of these hindered nitriles, even under vigorous conditions, gave chiefly the amides (IV; X = NH_2). Conversion into the carboxylic acids (IV; X = OH) was best effected by treating the amides with exactly one equivalent of nitrous acid. If nitrous acid was used in excess the methylthio-group suffered oxidation; in one such experiment the only product isolated from 4-chloro-2-methyl-6-methylthiobenzamide (IV; R = Me, X = NH_2) was 4-chloro-2-methyl-6-methylsulphinylbenzoic acid.

The various substituted benzoic acids were treated with thionyl chloride to give the acid chlorides, which were not usually purified. Reaction with 6-aminopenicillanic acid was effected in anhydrous solvents in the presence of triethylamine, essentially as in the preparation of methicillin.[2] In most cases the resulting penicillins were only partially purified, but the complete purification of 3-bromo-2,6-dimethoxyphenylpenicillin is described in the Experimental section.



(I) (II) (III) (IV)

Comparison of the stability in acid solution of a representative group of the new penicillins was made by a previously described method; [3] pK_a values were determined

Side-chain acid	pK_a in 50% aq. EtOH	Half-life (min.) of derived penicillin *
Two methoxy-groups.		
2,6-Dimethoxybenzoic	5·1	2—3
4-Chloro-2,6-dimethoxybenzoic	4·9	8
4-Bromo-2,6-dimethoxybenzoic	5·0	8
3-Chloro-2,6-dimethoxybenzoic	4·5	35
3-Bromo-2,6-dimethoxybenzoic	4·4	42
2,6-Dimethoxy-4-nitrobenzoic	4·3	46
2,6-Dimethoxy-3-nitrobenzoic	3·7	105
One methoxy-group.		
2-Methoxy-1-naphthoic	5·1	7
2-Chloro-6-methoxybenzoic	4·4	73
2-Methoxy-6-nitrobenzoic	3·8	177
No methoxy-group.		
2-Methyl-1-naphthoic	4·8	63
2,6-Dimethylbenzoic	4·9	65
2-Chloro-6-methylbenzoic	4·2	212
2,6-Dichlorobenzoic	3·5	330

* In 50% aqueous ethanol at pH 1·3 and 35°.

[10] Stollé, *Ber.*, 1921, **54**, 1219.
[11] Cohen and Raper, *J.*, 1904, **85**, 1268.
[12] Cassella and Co., G.P. 360,690/1922.
[13] Cassella and Co., G.P. 367,346/1923.

for the corresponding side-chain acids. As expected, the stability of the penicillins in acid tended to increase with increasing strength of the side-chain acids. The effect is seen most clearly when the compounds are considered in three groups, as shown in the Table. Structures containing two methoxy-groups form a fairly regular series, as do those containing only one methoxy-group and those containing no such group. That the effect is not confined to the simple benzene series is shown by the marked increase in acid-stability brought about by replacing the methoxy-group of 2-methoxy-1-naphthylpenicillin [1] by a methyl group.

Antibacterial testing of the new penicillins was carried out *in vitro* by Dr. G. N. Rolinson and his colleagues and *in vivo* by Mr. D. M. Brown and his colleagues. As expected, all the compounds proved to be essentially stable towards staphylococcal penicillinase. Unfortunately, the antibacterial activity of the more acid-stable penicillins never exceeded, and was usually less than, that of methicillin.

Experimental

3,5-*Dichloro*-2,6-*dihydroxybenzoic Acid*.—A solution of 2,6-dihydroxybenzoic acid (30·8 g.) in dry ether (300 ml.) was refluxed whilst sulphuryl chloride (32·5 ml.) was added dropwise during 3 hr. and then for 2 hr. more. The cooled mixture was washed with water (8 × 100 ml.). The ether layer was then extracted with 3% aqueous sodium hydrogen carbonate (7 × 100 ml.), each extract being diluted with sufficient water to dissolve the suspended solid. Acidification of the combined diluted extracts (4 l.) with concentrated hydrochloric acid (100 ml.) gave a clear solution which slowly deposited colourless needles of 3,5-*dichloro*-2,6-*dihydroxybenzoic acid* (37·6 g.), m. p. 216—218° (decomp.), unchanged by recrystallisation from benzene (Found: C, 37·7; H, 2·4; Cl, 32·1. $C_7H_4Cl_2O_4$ requires C, 37·7; H, 1·8; Cl, 31·8%).

3,5-*Dichloro*-2-*hydroxy*-6-*methoxybenzoic Acid*.—Methyl sulphate (10·2 ml.) was added to a stirred solution of 3,5-dichloro-2,6-dihydroxybenzoic acid (6·7 g.) in 10% aqueous sodium hydroxide (96 ml.) and the mixture was heated on the steam-bath for 2½ hr., cooled, and washed with ether (3 × 50 ml.). Acidification of the aqueous layer gave the crude product as a buff solid. Recrystallisation from water gave pure 3,5-*dichloro*-2-*hydroxy*-6-*methoxybenzoic acid* (3·5 g.) as colourless plates, m. p. 190—192° (Found: C, 41·0; H, 3·2; Cl, 30·1. $C_8H_6Cl_2O_4$ requires C, 40·5; H, 2·5; Cl, 30·0%).

Methyl 3,5-*Dichloro*-2,6-*dimethoxybenzoate*.—Methyl sulphate (39·2 ml.) was added dropwise to a stirred mixture of 3,5-dichloro-2,6-dihydroxybenzoic acid (32·2 g.), anhydrous potassium carbonate (79·6 g.), and dry acetone (120 ml.). The mixture was refluxed for 3 hr., then evaporated under reduced pressure. The residue was dissolved in water (1·5 l.) and extracted with ether (4 × 200 ml.). The combined extracts were washed with 2·5N-sodium hydroxide, then with water, dried, and distilled, to give the pale yellow *ester* (31 g.), b. p. 168°/16 mm., n_D^{21} 1·5275 (Found: Cl, 26·8. $C_{10}H_{10}Cl_2O_4$ requires Cl, 26·8%).

3,5-*Dichloro*-2,6-*dimethoxybenzoic Acid*.—The methyl ester (31 g.) and a solution of sodium hydroxide (9·6 g.) in 50% aqueous methanol (30 ml.) were refluxed together for 3 hr., then the solution was cooled, diluted with water, and washed with ether (2 × 100 ml.). Acidification of the aqueous solution gave a precipitate which was collected and further purified by dissolution in sodium hydrogen carbonate solution and re-precipitation with acid. Recrystallisation from light petroleum gave needles of 3,5-*dichloro*-2,6-*dimethoxybenzoic acid* (20 g.), m. p. 104—106° (Found: C, 42·6; H, 3·8; Cl, 28·4. $C_9H_8Cl_2O_4$ requires C, 43·0; H, 3·2; Cl, 28·3%).

3-*Chloro*-2,6-*dihydroxybenzoic Acid*.—Sulphuryl chloride (8·1 ml.) was added dropwise during 15 min. to a stirred solution of 2,6-dihydroxybenzoic acid (15·4 g.) in dry ether (150 ml.). The mixture was refluxed for 4 hr., cooled, washed with water (4 × 100 ml.), and then extracted with 3% sodium hydrogen carbonate solution (400 ml.). Acidification of the extracts precipitated 3-chloro-2,6-dihydroxybenzoic acid as a white granular solid (12·2 g.), m. p. 189°. Cartwright *et al.*,[14] who prepared it from the dimethoxy-acid and aluminium chloride, give m. p. 193°.

3-*Chloro*-2,6-*dimethoxybenzoic Acid*.—Methyl sulphate (20·5 ml.) was added during 15 min. to a stirred mixture of 3-chloro-2,6-dihydroxybenzoic acid (12·2 g.), anhydrous potassium carbonate (35·6 g.), and dry acetone (50 ml.). The mixture was refluxed for 3 hr., then

[14] Cartwright, Jones, and Marmion, *J.*, 1952, 3499.

evaporated under reduced pressure. The residue was treated with water (500 ml.) and extracted with ether (4 × 100 ml.). The combined ether extracts were washed with 10% sodium hydroxide solution (2 × 10 ml.), then with water (2 × 50 ml.), dried, and distilled, to give methyl 3-chloro-2,6-dimethoxybenzoate (12·2 g.), b. p. 174—176°/15 mm., m. p. 84°. This ester and potassium hydroxide (5·9 g.) in 50% aqueous methanol (10 ml.) were refluxed together for 2 hr., cooled, and diluted with water (250 ml.). After extraction with ether (3 × 100 ml.), the aqueous phase was acidified, to give 3-chloro-2,6-dimethoxybenzoic acid as a white microcrystalline powder (10·7 g.), m. p. 133°. Cartwright *et al.*[14] obtained a small quantity of the acid, m. p. 132°, as a by-product in the hypochlorite oxidation of 2,6-dimethoxyacetophenone.

3,5-*Dibromo*-2-*hydroxy*-6-*methoxybenzoic Acid*.—Bromine (32 g.) was added dropwise during 3 hr. to a stirred mixture of 2,6-dimethoxybenzoic acid (18·2 g.) and chloroform (150 ml.) at 50°. The mixture was then refluxed for 1 hr., cooled, and filtered. Recrystallisation of the solid from benzene gave colourless needles of 3,5-*dibromo*-2-*hydroxy*-6-*methoxybenzoic acid* (22 g.), m. p. 200—202° (Found: C, 29·6; H, 2·1; Br, 48·9. $C_8H_6Br_2O_4$ requires C, 29·5; H, 1·8; Br, 49·1%).

3,5-*Dibromo*-2,6-*dimethoxybenzoic Acid*.—A stirred mixture of 3,5-dibromo-2-hydroxy-6-methoxybenzoic acid (8·15 g.), anhydrous potassium carbonate (13·8 g.), dry acetone (50 ml.), and methyl sulphate (6·8 ml.) was refluxed for 3 hr., then evaporated under reduced pressure. The residue was dissolved in water (200 ml.) and extracted with ether (3 × 75 ml.). The combined extracts were washed with 10% sodium hydroxide solution, then with water, dried, and distilled, to give pale yellow methyl 3,5-dibromo-2,6-dimethoxybenzoate (6·2 g.), b. p. 190°/19 mm. This ester was hydrolysed with potassium hydroxide (2 g.) in a mixture of methanol (15 ml.) and water (45 ml.) (2 hours' refluxing). The mixture was cooled and extracted with ether (3 × 30 ml.), then the aqueous phase was acidified and the product collected. Recrystallisation from light petroleum gave needles of 3,5-*dibromo*-2,6-*dimethoxybenzoic acid* (4·7 g.), m. p. 109—111° (Found: C, 32·0; H, 2·3; Br, 46·8. $C_9H_8Br_2O_4$ requires C, 31·8; H, 2·3; Br, 47·0%).

3-*Bromo*-2,6-*dihydroxybenzoic Acid*.—Bromine (16 g.) was added dropwise during 1 hr. to a stirred solution of 2,6-dihydroxybenzoic acid (15·4 g.) in acetic acid (150 ml.) at room temperature. The mixture was heated on the steam-bath for 1 hr., then cooled and filtered. Recrystallisation of the solid from water gave needles of 3-*bromo*-2,6-*dihydroxybenzoic acid* (14 g.), m. p. 198° (decomp.) (Found: Br, 34·2. $C_7H_5BrO_4$ requires Br, 34·3%).

Methyl 3-*Bromo*-2,6-*dimethoxybenzoate*.—The above dihydroxy-acid was completely methylated with methyl sulphate and potassium carbonate in acetone, as described for the 3-chloro-analogue, giving the *ester* (88%), b. p. 190°/19 mm., m. p. 81° (Found: Br, 29·1. $C_{10}H_{11}BrO_4$ requires Br, 29·1%).

3-*Bromo*-2,6-*dimethoxybenzoic Acid*.—(*a*) The last preceding ester was hydrolysed as described for the 3-chloro-analogue, to give the *acid* (64%), plates (from benzene–light petroleum), m. p. 146° (Found: Br, 30·9. $C_9H_9BrO_4$ requires Br, 30·7%).

(*b*) (With K. Hardy) Dioxan dibromide (200 g.) was dissolved in dry ether (1600 ml.) and treated with 2,6-dimethoxybenzoic acid (146 g.) in the same solvent (800 ml.). The mixture was set aside for 30 min., with occasional stirring, then filtered to remove a little insoluble matter. The filtrate was thoroughly washed with water, then exhaustively extracted with saturated sodium hydrogen carbonate solution. Acidification of the extracts precipitated 3-bromo-2,6-dimethoxybenzoic acid (174 g.), having m. p. 146—148° after recrystallisation from aqueous alcohol.

3,5-*Di-iodo*-2,6-*dimethoxybenzoic Acid*.—Iodine monochloride (31 g.) in acetic acid (80 ml.) was added during 5 min. to a stirred solution of 2,6-dihydroxybenzoic acid (13·8 g.) in acetic acid (120 ml.). Water (350 ml.) was added dropwise and the temperature was slowly raised to 80° and held thus for 20 min., then the mixture was cooled in ice and the crude purple-brown 2,6-dihydroxy-3,5-di-iodobenzoic acid (26 g.; m. p. 138—140°) was collected, washed with dilute acetic acid, and dried *in vacuo*. Complete methylation with methyl sulphate and potassium carbonate in acetone in the usual way gave methyl 3,5-di-iodo-2,6-dimethoxybenzoate, b. p. 160°/0·5 mm. This ester was refluxed for 5 hr. with potassium hydroxide (3 g.) in 50% aqueous methanol to give, after the usual isolation procedure, 3,5-*di-iodo*-2,6-*dimethoxybenzoic acid* (7·3 g.), which crystallised from benzene–light petroleum in buff-coloured needles, m. p. 136—138° (Found: C, 25·2; H, 2·0; I, 58·3. $C_9H_8I_2O_4$ requires C, 24·9; H, 1·8; I, 58·5%).

3-*Iodo*-2,6-*dimethoxybenzoic Acid*.—A solution of 3-amino-2,6-dimethoxybenzoic acid [1]

(5 g.) in 5N-sulphuric acid (50 ml.) was diazotised with 10% sodium nitrite solution (20 ml.), stirred at 0° for 15 min., then added to a solution of potassium iodide (6·5 g.) in 5N-sulphuric acid (25 ml.) and water (30 ml.). The mixture was refluxed for 15 min., then cooled, and the crude product was collected. Recrystallisation from water (charcoal) gave colourless needles of 3-*iodo*-2,6-*dimethoxybenzoic acid* (3·4 g.), m. p. 162° (Found: C, 35·1; H, 3·2; I, 41·2. $C_9H_9IO_4$ requires C, 35·1; H, 2·9; I, 41·2%).

Methyl 2,6-*Dimethoxy*-3-*phenylazobenzoate*.—A mixture of 2,6-dihydroxy-3-phenylazobenzoic acid [5] (11 g.), anhydrous potassium carbonate (24 g.), dry acetone (400 ml.), and methyl sulphate (13 ml.) was refluxed with stirring for 4 hr., cooled, and filtered. Evaporation of the combined filtrate and acetone washings left a red oil, which solidified on trituration with light petroleum. Recrystallisation from alcohol gave orange-red plates of the *ester* (7·7 g.), m. p. 110° (Found: C, 64·1; H, 5·6; N, 9·4. $C_{16}H_{16}N_2O_4$ requires C, 64·0; H, 5·4; N, 9·3%).

2,6-*Dimethoxy*-3-*phenylazobenzoic Acid*.—The above ester (6 g.) in ethanol (70 ml.) was refluxed for 3 hr. with potassium hydroxide (15 g.) in water (30 ml.). The red solution was evaporated *in vacuo* and the residue was re-dissolved in warm water and acidified, to give a bulky orange precipitate, which was collected and dried *in vacuo*. Recrystallisation from alcohol (100 ml.) gave orange plates of 2,6-*dimethoxy*-3-*phenylazobenzoic acid* (5·4 g.), m. p. 214—215° (Fnund: C, 62·9; H, 5·2; N, 9·9. $C_{15}H_{14}N_2O_4$ requires C, 62·9; H, 4·9; N, 9·8%).

3-*Bromo*-2,6-*dimethoxytoluene*.—Dioxan dibromide (248 g.) in ether (500 ml.) was added during 20 min. to a stirred solution of 2,6-dimethoxytoluene (152 g.) in ether (1 l.). The solution was stirred for 30 min. more, then washed successively with water, aqueous sodium hydrogen carbonate, then water again, and distilled, to give 3-bromo-2,6-dimethoxytoluene (191 g.), b. p. 106°/3 mm. (lit.,[15] b. p. 106—107°/5·5 mm.).

3,5-*Dimethoxy*-p-*toluidine*.—3-Bromo-2,6-dimethoxytoluene (185 g.) was added dropwise during 30 min. to a freshly prepared solution of sodamide (from 36·8 g. of sodium) in liquid ammonia (1·6 l.). The mixture was stirred for 4 hr., then ammonium chloride (84 g.) was added cautiously during 20 min. Benzene (450 ml.) was added and the ammonia allowed to evaporate. The benzene solution was washed with water (3 × 100 ml.) and then shaken with 6N-hydrochloric acid (250 ml.), whereupon the solid amine hydrochloride separated. The latter was collected, washed with ether, and converted into the solid base by stirring with 10N-sodium hydroxide (200 ml.) for 1 hr. The buff solid (40 g.) was collected, washed with water, and dried. Recrystallisation of a portion from benzene–light petroleum gave buff needles of 3,5-*dimethoxy*-p-*toluidine*, m. p. 124—126° (Found: C, 65·1; H, 7·7; N, 8·0. $C_9H_{13}NO_2$ requires C, 64·7; H, 7·8; N, 8·4%).

4-*Chloro*-2,6-*dimethoxytoluene*.—A solution of 3,5-dimethoxy-*p*-toluidine (16·7 g.) in 4N-hydrochloric acid (100 ml.) was diazotised at 0—5° by means of sodium nitrite (7 g.), then added during 20 min. to a vigorously stirred mixture of benzene (250 ml.) and a solution of cuprous chloride (10·2 g.) in concentrated hydrochloric acid (40 ml.). The mixture was stirred at room temperature for a further 30 min., then kept at 35° for 1 hr. The layers were separated and the aqueous phase was extracted with benzene (3 × 50 ml.). The combined benzene solutions were dried and distilled, to give a greenish liquid, b. p. 65—75°/0·3 mm., which slowly crystallised. Recrystallisation from a little methanol gave 4-*chloro*-2,6-*dimethoxytoluene* (6 g.) as yellow prisms, m. p. 60—61° (Found: C, 58·2; H, 6·2; Cl, 18·9. $C_9H_{11}ClO_2$ requires C, 57·9; H, 5·9; Cl, 19·0%).

4-*Bromo*-2,6-*dimethoxytoluene*.—A solution of 3,5-dimethoxy-*p*-toluidine (16·7 g.) in a mixture of 60% hydrobromic acid (32 ml.) and water (70 ml.) was diazotised at 0—5° by means of sodium nitrite (7 g.), then added during 20 min. to a vigorously stirred mixture of benzene (200 ml.) and a solution of cuprous bromide (14·5 g.) in 60% hydrobromic acid (30 ml.). The mixture was stirred at room temperature for a further 30 min., then kept at 35° for 1 hr., and finally worked up as described for the 4-chloro-analogue, to give 4-*bromo*-2,6-*dimethoxytoluene* (2·1 g.), b. p. 70—80°/0·6 mm., m. p. 53—54° (yellow needles from methanol) (Found: Br, 34·7. $C_9H_{11}BrO_2$ requires Br, 34·6%.)

2,6-*Dimethoxy*-4-*nitrotoluene*.—A solution of 3,5-dimethoxy-*p*-toluidine (15 g.) in 40% fluoroboric acid (40 ml.) was diazotised at 0—5° with sodium nitrite (6·3 g.) in water (10 ml.). The diazonium solution was then added during 20 min. to a stirred mixture of benzene (200 ml.), copper bronze (14 g.), and a solution of sodium nitrite (72 g.) in water (140 ml.) at room temperature. The mixture was stirred for 30 min. more, then filtered and the benzene

[15] Sibita, *J. Pharm. Soc. Japan*, 1939, **59**, 323.

layer was separated. The aqueous phase was extracted with benzene (3 × 50 ml.), and the combined benzene solutions were dried and distilled, to give an oil, b. p. 120—130°/0·5 mm., which rapidly crystallised. Recrystallisation from methanol gave bright yellow needles of 2,6-*dimethoxy*-4-*nitrotoluene* (3·8 g.), m. p. 123—124° (Found: C, 55·2; H, 5·8; N, 7·1. $C_9H_{11}NO_4$ requires C, 54·8; H, 5·6; N, 7·1%).

4-*Chloro*-2,6-*dimethoxybenzoic Acid*.—Potassium permanganate (26 g.) was added portionwise during 1 hr. to a stirred mixture of pyridine (21 ml.), water (67 ml.), and 4-chloro-2,6-dimethoxytoluene (10·25 g.) at 45—50°. The mixture was stirred at 50° for 2 hr. more, and then filtered, the manganese dioxide being washed separately with ether (50 ml.). The aqueous filtrate was concentrated *in vacuo* to half its volume, then cooled and washed with ether (2 × 20 ml.). From the combined ether washings unchanged 4-chloro-2,6-dimethoxytoluene (3 g.) was recovered. Acidification of the aqueous solution gave 4-*chloro*-2,6-*dimethoxybenzoic acid* (3·2 g.) which crystallised from water in colourless needles, m. p. 189—190° (Found: C, 50·1; H, 4·0. $C_9H_9ClO_4$ requires C, 49·9; H, 4·2%).

4-*Bromo*-2,6-*dimethoxybenzoic Acid*.—Permanganate oxidation of 4-bromo-2,6-dimethoxytoluene as described for the chloro-analogue gave the *acid* (61%), colourless needles (from water), m. p. 201—202° (Found: C, 42·0; H, 3·5; Br, 30·1. $C_9H_9BrO_4$ requires C, 41·4; H, 3·5; Br, 30·6%).

2,6-*Dimethoxy*-4-*nitrobenzoic Acid*.—2,6-Dimethoxy-4-nitrotoluene was oxidised as before to give the *acid* in 90% yield (after allowance for recovered toluene). This crystallised from methanol in colourless needles, m. p. 225—226° (Found: N, 5·9. $C_9H_9NO_6$ requires N, 6·2%).

2-*Methoxy*-6-*nitrobenzoic Acid*.—Potassium permanganate (15 g.) in water (500 ml.) was treated with 2-methoxy-6-nitrotoluene [9] (5 g.) and 5% sodium hydroxide solution (50 ml.). The mixture was refluxed for 3 hr., then filtered hot, and the filtrate evaporated to low bulk. Acidification gave colourless crystals of 2-methoxy-6-nitrobenzoic acid (3·5 g.), m. p. 178° (lit.,[9] m. p. 180°).

2-*Chloro*-6-*methoxybenzoic Acid*.—2-Chloro-6-methoxytoluene (22·2 g.) was mixed with water (2 l.), potassium permanganate (66 g.), and 5% sodium hydroxide solution (230 ml.). The stirred mixture was heated on the steam-bath for 4 hr., then filtered hot, and the solid was washed with hot water. The combined filtrate and washings were cooled, clarified by ether-extraction, and concentrated to 600 ml. Acidification gave 2-*chloro*-6-*methoxybenzoic acid* (13 g.), m. p. 141—144° unchanged by recrystallisation from aqueous alcohol (Found: C, 51·7; H, 4·2; Cl, 19·3. $C_8H_7ClO_3$ requires C, 51·5; H, 3·8; Cl, 19·0%).

2,4-*Diethyl*-6-*methoxybenzoic Acid* [with E. G. Brain].—A 1·25N-solution of butyl-lithium in ether (230 ml.) was treated with 3,5-diethylanisole [16] (23·5 g.) in ether (50 ml.), and the stirred mixture was refluxed under nitrogen for 13 hr., then kept at room temperature for 30 hr. The yellow mixture was cooled to −40° and treated with an excess of solid carbon dioxide powder, becoming deep red and then colourless. A white precipitate separated but redissolved at room temperature, and the resulting solution was washed with dilute hydrochloride acid, then with water, and finally extracted with 2N-sodium hydroxide. The alkaline extracts were acidified, to give 2,4-*diethyl*-6-*methoxybenzoic acid* (11·9 g.), m. p. 112·5—113·5° (Found: C, 69·5; H, 7·8 $C_{12}H_{16}O_3$ requires C, 69·2; H, 7·7%).

3-*Chloro*-6-*methoxy*-2,4-*dimethylbenzoic Acid*.—A mixture of 3-chloro-6-hydroxy-2,4-dimethylbenzoic acid [10] (5 g.), potassium carbonate (9 g.), dry acetone (40 ml.), and methyl sulphate (6·6 g.) was refluxed for 3 hr., then evaporated *in vacuo*. The residue was treated with water (50 ml.), and the product extracted into ether (2 × 50 ml.), washed, and dried. Removal of ether left crude methyl 3-chloro-6-methoxy-2,4-dimethylbenzoate (5·36 g.), m. p. 64—66°. A portion (2·1 g.) of this ester in butanol (6 ml.) was treated with potassium hydroxide (0·8 g.) in water (1 ml.) and refluxed for 3 hr. The mixture was evaporated *in vacuo* and the residue was taken up in water, clarified by ether-extraction, and acidified to precipitate colourless 3-*chloro*-6-*methoxy*-2,4-*dimethylbenzoic acid* (1·74 g.) which, crystallised from light petroleum, had m. p. 162—164° (Found: C, 56·5; H, 5·5; Cl, 16·3. $C_{10}H_{11}ClO_3$ requires C, 55·9; H, 5·2; Cl, 16·5%).

2-*Bromo*-6-*chlorotoluene*.—3-Chloro-*o*-toluidine (18 g.) was dissolved in a hot mixture of 48% hydrobromic acid (73 ml.) and water (200 ml.). The stirred solution was cooled rapidly to 0° and diazotised during 45 min. with sodium nitrite (8·8 g.) in water (50 ml.). It was then stirred

[16] Kruber and Lauenstein, *Chem. Ber.*, 1948, **81**, 221.

at 0·5° for 1½ hr. whilst a solution of cuprous bromide (from 68 g. of cupric sulphate) in 48% hydrobromic acid (56 ml.) was freshly prepared. The diazonium salt solution was added to the cuprous bromide reagent during 1 hr. at room temperature, then the mixture was heated at 65° for 2 hr., cooled, diluted with an equal volume of water, and extracted with ether (4 × 100 ml.). The combined extracts were washed with water, then dilute sodium hydroxide, then water again, and dried. Distillation gave pale yellow 2-bromo-6-chlorotoluene (19·6 g.), b. p. 92—94°/10 mm. (lit.,[11] b. p. 118—120°/40 mm.).

2-Bromo-6-chlorobenzoic Acid.—2-Bromo-6-chlorotoluene (13·4 g.) was heated at 175° whilst bromine (10·7 g.) was added dropwise with stirring during 1½ hr. The crude brominated product was added to a stirred solution of sodium hydroxide (3·25 g.) in water (250 ml.), and the mixture was heated on the steam-bath whilst potassium permanganate (15 g.) was added portionwise until the purple colour persisted. This colour was discharged by the addition of a little ethanol, the hot mixture was filtered, and the solid washed with hot water (100 ml.). The combined filtrate and washings were cooled, clarified by ether-extraction, and acidified. Some product crystallised and the rest was isolated by ether-extraction. The combined crops (9·7 g.) crystallised from benzene–light petroleum in needles of 2-bromo-6-chlorobenzoic acid, m. p. 144—146° (lit.,[11] m. p. 143—144°).

2-Substituted 4-Chloro-6-methylthioanilines (III).—(*a*) A suspension of 6-chloro-4-methyl-1,3,2-benzothiazathiolium chloride [12] (164 g.) in water (1·5 l.) was stirred at room temperature for 1 hr. and then filtered. The almost colourless solid was added cautiously with vigorous stirring to 40% sodium hydroxide solution (250 ml.). After 15 min., the solution was cooled and sodium dithionite (165 g.) was added, followed by methyl sulphate (84·5 ml.). The mixture was stirred at 80° for 1 hr., cooled, and extracted with ether (4 × 100 ml.). The combined extracts were washed, dried, and distilled to give yellow *4-chloro-2-methyl-6-methylthioaniline* (87 g.), b. p. 136—140°/5 mm. (Found: C, 51·6; H, 5·1; N, 7·7; S, 17·3. $C_8H_{10}ClNS$ requires C, 51·2; H, 5·3; N, 7·5; S, 17·1%). The bulk of the amine (86 g.) was dissolved in alcohol (100 ml.), treated with concentrated hydrochloric acid (70 ml.), and evaporated under reduced pressure to leave the stable hydrochloride (90 g.), which was dried over potassium hydroxide *in vacuo*.

(*b*) 6-Chloro-4-methoxy-1,3,2-benzothiazathiolium chloride [12] (213 g.) was made into a slurry with methanol (800 ml.) and added to a stirred solution of sodium hydroxide (200 g.) in water (1300 ml.) and methanol (800 ml.). Sodium dithionite (80 g.) was added and the mixture was heated at 60° for 1 hr., then concentrated to remove most of the methanol, cooled to 40°, and treated with methyl sulphate (94 ml.). The mixture was heated on the steam-bath for 1 hr., cooled, and extracted with ether (4 × 200 ml.). Distillation of the washed and dried extracts gave colourless 4-chloro-2-methoxy-6-methylthioaniline (75·5 g.), b. p. 146°/5 mm. This was dissolved in dry ether (500 ml.) and saturated with hydrogen chloride to give the *hydrochloride* as a white powder (87 g.), m. p. 184° (decomp.). Crystallisation of a portion from alcohol gave colourless needles, m. p. 189° (decomp.) (Found: Cl, 29·7; S, 13·3. $C_8H_{11}Cl_2NOS$ requires Cl, 29·6; S, 13·3%).

(*c*) 6-Chloro-4-methylthio-1,3,2-benzothiazathiolium chloride was prepared as a purple powder (84%) from *o*-methylthioaniline hydrochloride and sulphur chloride as described for the 4-methoxy-analogue.[12] This substance (240 g.) was successively hydrolysed, reduced, and methylated as in (*b*), to give yellow *4-chloro-2,6-di(methylthio)aniline* (137 g.), b. p. 152—160°/3 mm. (Found: C, 44·1; H, 4·6; Cl, 16·0; N, 6·3. $C_8H_{10}ClNS_2$ requires C, 43·7; H, 4·6; Cl, 16·2; N, 6·4%). This base was stabilised by conversion into the colourless hydrochloride as in (*a*).

2-Substituted 4-Chloro-6-methylthiobenzonitriles.—(*a*) A mixture of 4-chloro-2-methyl-6-methylthioaniline hydrochloride (90 g.), concentrated hydrochloric acid (56 ml.), and water (150 ml.) was diazotised at 0° with sodium nitrite (30·4 g.) in water (50 ml.). After 30 min. the diazonium salt solution was added during 20 min. to a stirred solution of sodium cyanide (47 g.), and cuprous cyanide monohydrate (47 g.) in water (225 ml.) at room temperature. The mixture was stirred on the steam-bath for 3 hr., then cooled and filtered. The dried solid was extracted with hot acetone (2 × 500 ml.), and the extract concentrated to give *4-chloro-2-methyl-6-methylthiobenzonitrile* (44 g.), which on recrystallisation formed orange needles, m. p. 122° (Found: Cl, 17·8; N, 6·9; S, 16·1. C_9H_8ClNS requires Cl, 18·0; N, 7·1; S, 16·4%).

(*b*) In the same way 4-chloro-2-methoxy-6-methylthioaniline hydrochloride yielded *4-chloro-2-methoxy-6-methylthiobenzonitrile* (26%), yellow needles (from alcohol), m. p. 136—138°

(Found: C, 51·0; H, 3·7; Cl, 16·6; N, 6·7; S, 15·1. C_9H_8ClNOS requires C, 50·6; H, 3·8; Cl, 16·7; N, 6·6; S, 15·0%).

(*c*) Similarly 4-chloro-2,6-dimethylthioaniline hydrochloride gave *4-chloro-2,6-di(methylthio)benzonitrile* (39%), bronze needles (from ethyl acetate), m. p. 186—188° (Found: C, 46·8; H, 3·7; Cl, 15·6; N, 6·1; S, 28·1. $C_9H_8ClNS_2$ requires C, 47·0; H, 3·5; Cl, 15·5; N, 6·1; S, 27·9%).

2-Substituted 4-Chloro-6-(methylthio)benzamides.—(*a*) A solution of 4-chloro-2-methyl-6-methylthiobenzonitrile (30 g.) and potassium hydroxide (40 g.) in alcohol (400 ml.) was refluxed for 4 hr., then poured into water (3 l.). The precipitate was collected, dried, and recrystallised from ethyl acetate, to give colourless needles of *4-chloro-2-methyl-6-(methylthio)benzamide* (17·2 g.), m. p. 200—202° (Found: C, 50·5; H, 4·9; Cl, 16·7; N, 6·4; S, 14·9. $C_9H_{10}ClNOS$ requires C, 50·1; H, 4·6; Cl, 16·5; N, 6·5; S, 14·9%).

(*b*) A mixture of 4-chloro-2-methoxy-6-methylthiobenzonitrile (5·6 g.), potassium hydroxide (10 g.), water (400 ml.), and alcohol (120 ml.) was refluxed for 72 hr., then poured into water (2 l.). The precipitate was collected, dried, and crystallised first from ethyl acetate and then from alcohol, to give colourless needles of *4-chloro-2-methoxy-6-(methylthio)benzamide* (3·6 g.), m. p. 226—227° (Found: C, 47·0; H, 4·5; Cl, 15·5; N, 5·8; S, 14·3. $C_9H_{10}ClNO_2S$ requires C, 46·7; H, 4·3; Cl, 15·3; N, 6·0; S, 13·8%).

(*c*) 4-Chloro-2,6-di(methylthio)benzonitrile (46 g.) was hydrolysed as in (*b*), to give *4-chloro-2,6-di(methylthio)benzamide* (16 g.) as colourless needles (from ethyl acetate), m. p. 218—220° (Found: Cl, 14·5; N, 5·6; S, 25·9. $C_9H_{10}ClNOS_2$ requires Cl, 14·3; N, 5·7; S, 25·9%).

2-Substituted 4-Chloro-6-(methylthio)benzoic Acids.—(*a*) A solution of 4-chloro-2-methyl-6-(methylthio)benzamide (11 g.) in water (45 ml.) and concentrated sulphuric acid (84 ml.) was stirred at 5° whilst sodium nitrite (3·5 g.) in water (10 ml.) was introduced beneath the surface during 15 min. The mixture was stirred at 5° for 1 hr., then heated on the steam-bath for 30 min., and finally poured into ice–water (200 ml.). The crude product was collected, partially purified by dissolution in sodium hydrogen carbonate solution and reprecipitation with acid, and then recrystallised from light petroleum, to give colourless needles of *4-chloro-2-methyl-6-(methylthio)benzoic acid* (2·45 g.), m. p. 132—134° (Found: C, 49·5; H, 45; Cl, 16·6; S, 15·0. $C_9H_9ClO_2S$ requires C, 49·8; H, 4·2; Cl, 16·4; S, 14·8%).

In one experiment in which sodium nitrite was used in excess, the only isolable product proved to be *4-chloro-2-methyl-6-methylsulphinylbenzoic acid*, which crystallised from alcohol in colourless needles, m. p. 160° (decomp.) (Found: C, 46·7; H, 4·1; S, 13·8. $C_9H_9ClO_3S$ requires C, 46·5; H, 3·9; S, 13·8%). The infrared absorption spectrum of this compound, kindly measured by Mr. K. Austin, showed a very strong band at 9·96 μ and a weaker band at 9·73 μ. These bands, assigned to the sulphoxide structure, were absent from the spectrum of the corresponding sulphide.

(*b*) Treatment of 4-chloro-2-methoxy-6-(methylthio)benzamide with one equivalent of nitrous acid as in (*a*) gave *4-chloro-2-methoxy-6-(methylthio)benzoic acid* (74%) as colourless needles (from aqueous alcohol), m. p. 176—178° (Found: C, 46·5; H, 4·2; Cl, 15·5; S, 13·9. $C_9H_9ClO_3S$ requires C, 46·5; H, 3·9; Cl, 15·2; S, 13·8%).

(*c*) In the same way 4-chloro-2,6-di(methylthio)benzamide gave *4-chloro-2,6-di(methylthio)benzoic acid* (35%) which was crystallised first from methanol and then from benzene, to give pale yellow needles, m. p. 202—205° (Found: C, 43·6; H, 3·8; Cl, 14·2; S, 25·6. $C_9H_9ClO_2S_2$ requires C, 43·5; H, 3·6; Cl, 14·3; S, 25·8%).

3-Bromo-2,6-dimethoxyphenylpenicillin.—3-Bromo-2,6-dimethoxybenzoic acid (5·2 g.) and thionyl chloride (5 ml.) were refluxed for 1 hr., then volatile material was removed *in vacuo*. The residual crude acid chloride was dissolved in chloroform (20 ml.) and added dropwise during 15 min. to a stirred mixture of 6-aminopenicillanic acid (4·3 g.), triethylamine (5·6 ml.), and chloroform (60 ml.). The mixture was stirred at room temperature for 1 hr., then washed with water (200 ml.), to which was added sufficient N-hydrochloric acid to give an aqueous phase of pH 2. The chloroform phase was washed with water and then extracted with sufficient dilute sodium hydrogen carbonate to give an aqueous phase of pH 7. Evaporation of the neutral extracts at low temperature and pressure left the crude sodium salt of the pencillin as a white powder (7·9 g.). A portion (5 g.) of this material was dissolved in a mixture of water (8 ml.) and acetone (16 ml.), more acetone (176 ml.) was added, and the solution was clarified by filtration and then set aside overnight, whereupon the pure *sodium salt* separated in plates (3·5 g.), m. p. 190—200°, $[\alpha]_D^{21}$ +200° (*c* 1 in H_2O) (Found: C, 40·9; H, 4·4; Br, 16·2;

N, 5·8; S, 6·4; Na, 4·4. $C_{17}H_{18}BrN_2O_6SNa,H_2O$ requires C, 40·9; H, 4·0; Br, 16·0; N, 5·6; S, 6·4; Na, 4·6%).

We acknowledge the skilled experimental assistance of Messrs. B. W. Hatt, R. D. Hill, M. Mardle, and D. Neal. We also join with the authors of the preceding paper in thanking Sir Robert Robinson, F.R.S., Dr. A. H. Cook, F.R.S., Professor E. B. Chain, F.R.S., and Dr. J. Farquharson for their interest.

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