274. *Derivatives of 6-Aminopenicillanic Acid. Part III.* 2,6-Dialkoxybenzoyl Derivatives.*

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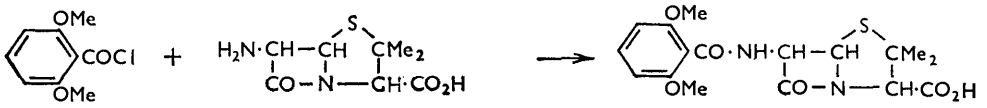
Several 2,6-dialkoxybenzoic acids have been prepared, mostly from the 2,6-dihydroxy-acid which was itself obtained in improved yield from resorcinol by a modification of the Kolbe–Schmitt reaction. Reaction of the acid chlorides with 6-aminopenicillanic acid gave a series of 2,6-dialkoxyphenylpenicillins, which are relatively resistant to inactivation by penicillinase.

In Part II * trisubstituted methylpenicillins, prepared from trisubstituted acetic acids and 6-aminopenicillanic acid, were shown to be much less readily inactivated by the

* Part II, preceding paper.

enzyme penicillinase than was any penicillin previously described. It was therefore of interest to prepare penicillins from other types of sterically hindered carboxylic acids.

Phenylpenicillin has been prepared [1] by the action of benzoyl chloride on 6-aminopenicillanic acid but its antibacterial properties were not outstanding and it was readily inactivated by penicillinase. However, if steric hindrance around the side-chain amide linkage were sufficient to produce resistance to penicillinase, then 2,6-disubstituted phenylpenicillins should be relatively stable towards the enzyme. This was confirmed when 2,6-dimethoxyphenylpenicillin (III), prepared from 2,6-dimethoxybenzoyl chloride (I) and 6-aminopenicillanic acid (II), was found to resist the action of penicillinase and in consequence to be nearly as active against penicillinase-producing resistant staphylococci as against non-resistant strains. Additional evidence for the steric origin of this effect was provided by the finding that 2,4-dimethoxyphenylpenicillin and 2,6-dimethoxybenzylpenicillin were both inactivated by penicillinase.



(I) (II) (III)

In order to prepare analogues of 2,6-dimethoxyphenylpenicillin a series of 2,6-dialkoxybenzoic acids was required. Although the dimethoxy-acid was readily prepared from 2,6-dimethoxytoluene and potassium permanganate in aqueous pyridine,[2] 2-ethoxy-6-methoxy- and 2,6-diethoxy-toluene gave only poor yields of acid by this method. 2,6-Diethoxybenzoic acid was obtained by reaction of resorcinol diethyl ether with butyllithium, followed by carbonation; [3] but the di-n-propyl ether could not be carbonated satisfactorily in this way, though 3,4-methylenedioxyanisole gave a small yield of 6-methoxy-2,3-methylenedioxybenzoic acid.

More generally applicable routes to 2,6-dialkoxybenzoic acids required 2,6-dihydroxybenzoic acid, syntheses of which have been reviewed.[4] Kolbe–Schmitt carbonation of resorcinol is the simplest, but under all conditions so far described [5] it gives poor and erratic yields of 2,6-dihydroxybenzoic acid separable only with difficulty from the 2,4-dihydroxy-isomer which constitutes the major product. A modification has now been developed in which carbon dioxide is passed into a solution of the monopotassium derivative of resorcinol in dimethylformamide at 150—155° for about 7 hr.: the yield of pure 2,6-dihydroxybenzoic acid is then 35—45%, that of the 2,4-isomer formed being too small to interfere seriously in the isolation. A mixture of resorcinol and one equivalent of anhydrous potassium carbonate could be used instead of the preformed monopotassium derivative, but the yield of 2,6-dihydroxybenzoic acid was then only 25—35%.

Treatment of methyl 2,6-dihydroxybenzoate in acetone with the appropriate alkyl halides and anhydrous potassium carbonate gave methyl 2,6-di-n-propoxy-, 2,6-di-n-butoxy-, and 2,6-dibenzyloxy-benzoate. Stepwise alkylation, first with methyl sulphate and then with benzyl chloride, gave methyl 2-benzyloxy-6-methoxybenzoate. All four methyl esters were readily hydrolysed to the ether-acids by hot alkali.

Symmetrical alkyl 2,6-dialkoxybenzoates were obtained very conveniently by omitting the isolation of 2,6-dihydroxybenzoic acid. In a typical example the entire reaction mixture from the modified Kolbe–Schmitt carbonation, consisting of the mixed potassium salts of 2,6- and 2,4-dihydroxybenzoic acid in dimethylformamide, was diluted with acetone and heated under reflux with methyl sulphate and potassium carbonate. The resulting mixed esters were treated with cold alkali, which selectively hydrolysed the

[1] Doyle, Nayler, and Rolinson, B.P. Spec. 870,395/1961.
[2] Kreuchunas, *J. Org. Chem.*, 1956, **21**, 368.
[3] Cf. Gilman and Morton, *Organic Reactions*, 1954, **8**, 258.
[4] Cartwright, Jones, and Marmion, *J.*, 1952, 3499.
[5] Hale, Hawdon, Jones, and Packham, *J.*, 1952, 3503.

unhindered 2,4-dimethoxy-ester, leaving a good yield of pure methyl 2,6-dimethoxybenzoate. A similar procedure has been described before [6] but, although no yield was recorded, it must have been small because of the unfavourable conditions then used at the Kolbe–Schmitt stage. In the same way use of ethyl sulphate and allyl bromide gave ethyl 2,6-diethoxybenzoate and allyl 2,6-diallyloxybenzoate, respectively. Isopropyl 2,6-diisopropoxybenzoate was similarly prepared except that the alkylation with isopropyl bromide had to be carried out at high temperature and pressure. Vigorous hydrolysis of the various alkyl 2,6-dialkoxybenzoates with hot alkali gave the corresponding 2,6-dialkoxybenzoic acids.

The various 2,6-dialkoxybenzoic acids were converted into acid chlorides by warm thionyl chloride, alone or in an inert diluent. Some of these chlorides were thermally unstable, and undistilled products were then treated with 6-aminopenicillanic acid. These acylations could not be accomplished in aqueous media, presumably owing to preferential hydrolysis of the chlorides, but proceeded well under anhydrous conditions in the presence of triethylamine. Triethylamine was removed by dilute acid, and the 2,6-dialkoxyphenylpenicillin extracted from the organic phase into aqueous sodium hydrogen carbonate. Evaporation of the aqueous solution at low temperature and pressure then gave the sodium salt of the pencillin in a sufficiently pure form for preliminary antibacterial and pharmacological testing.

Tests carried out in these laboratories by Dr. G. N. Rolinson and Mr. D. M. Brown and their respective colleagues showed that whilst all the 2,6-dialkoxyphenylpenicillins resisted inactivation by staphylococcal penicillinase, the antibacterial activity decreased with increasing molecular weight. The first member of the series, 2,6-dimethoxyphenylpenicillin (III), was therefore examined in more detail and its complete purification as the sodium salt is described in the Experimental section. Bacteriological [7] and pharmacological [8] investigations of this penicillin have been reported, together with various clinical studies.[9]

Experimental

2-Hydroxy-6-methoxytoluene.—Methyl sulphate (50·4 g.) was slowly added to 2,6-dihydroxytoluene (49·6 g.) in 10% sodium hydroxide solution (200 ml.) at 10° and the mixture then refluxed for 2 hr., cooled, and extracted with ether. The ether layer was extracted with dilute sodium hydroxide, and these extracts were added to the original aqueous phase, which was then acidified to liberate the oily product. 2-Hydroxy-6-methoxytoluene was extracted in ether, dried, and distilled (b. p. 118—122°/10 mm.; 29 g.) (Found: C, 69·3; H, 7·4. Calc. for $C_8H_{10}O_2$: C, 69·5; H, 7·3%). Jones and Robertson [10] give b. p. 164—165°/20 mm., m. p. 47°, for the product prepared from 2-amino-6-methoxytoluene.

2-Ethoxy-6-methoxytoluene.—A stirred mixture of 2-hydroxy-6-methoxytoluene (25 g.), ethyl iodide (38 g.), anhydrous potassium carbonate (45 g.), and acetone (125 ml.) was refluxed for 4 hr., then concentrated to remove acetone. The residue was dissolved in water and extracted with ether. The extracts were washed with dilute sodium hydroxide solution, then with water, dried, and distilled, giving *2-ethoxy-6-methoxytoluene* (18 g.), b. p. 108—109°/10 mm. (Found: C, 72·1; H, 8·5. $C_{10}H_{14}O_2$ requires C, 72·3; H, 8·5%).

2,6-Diethoxytoluene.—2,6-Dihydroxytoluene was alkylated as described by Kreuchunas [2] for the dimethoxy-analogue, but with ethyl instead of methyl sulphate, giving *2,6-diethoxytoluene* (58%), b. p. 127—128°/16 mm. (Found: C, 73·1; H, 9·1. $C_{11}H_{16}O_2$ requires C, 73·3; H, 9·0%).

2-Ethoxy-6-methoxybenzoic Acid.—Oxidation of 2-ethoxy-6-methoxytoluene by Kreuchunas's

[6] Clewer, Green, and Tutin, *J.*, 1915, **107**, 835.
[7] Rolinson, Stevens, Batchelor, Cameron-Wood, and Chain, *Lancet*, 1960, 564.
[8] Brown and Acred, *Lancet*, 1960, 568; Acred, Brown, Turner, and Wright, *Brit. J. Pharm.*, 1961, **17**, 70.
[9] Editorial, *Brit. Med. J.*, 1960, 720, and references cited therein.
[10] Jones and Robertson, *J.*, 1930, 1699.

method [2] for the dimethoxy-compound gave this acid in 26% yield. After crystallisation from aqueous alcohol the compound melted at 127°, re-solidified, and melted again at 142—143° (lit.,[11] 128°) (Found: C, 61·2; H, 6·4. Calc. for $C_{10}H_{12}O_4$: C, 61·2; H, 6·2%).

2,6-*Diethoxybenzoic Acid.*—(*a*) Application of Kreuchunas's oxidation procedure [2] to 2,6-diethoxytoluene gave only 10% of 2,6-*diethoxybenzoic acid* which, crystallised from ethyl acetate–light petroleum, had m. p. 132—134° (Found: C, 63·0; H, 6·8. $C_{11}H_{14}O_4$ requires C, 62·8; H, 6·7%). (*b*) The same acid was obtained in 68% yield by treating resorcinol diethyl ether with butyl-lithium as described for the dimethyl ether [12] and pouring the resulting lithium derivative on solid carbon dioxide. (*c*) Ethyl 2,6-diethoxybenzoate (see below) was refluxed with a mixture of 50% aqueous potassium hydroxide and butanol to give, after concentration and acidification, the same acid, m. p. and mixed m. p. 132—134°.

6-*Methoxy*-2,3-*methylenedioxybenzoic Acid.*—Sodium hydroxide (16 g.) in water (60 ml.) was added dropwise during 20 min. to a stirred mixture of 3,4-methylenedioxyphenol [13] (13·7 g.), methyl sulphate (11·4 ml.), and water (100 ml.). The mixture was refluxed for 2 hr., cooled, and extracted with ether (3 × 50 ml.). Distillation of the dried extracts gave the semi-solid methyl ether (4·98 g.), b. p. 100—106°/1 mm., which was then treated in dry ether (50 ml.) with butyl-lithium (from 0·5 g. of lithium [3]) in the same solvent. The mixture was refluxed for 1 hr., poured on solid carbon dioxide, left to attain room temperature, and treated with 5N-sulphuric acid (75 ml.). The ether phase was washed with water (2 × 30 ml.), then extracted with sodium hydrogen carbonate solution. Acidification with hydrochloric acid gave needles of 6-*methoxy*-2,3-*methylenedioxybenzoic acid* (1·5 g.), m. p. 142° (Found: C, 55·5; H, 4·2. $C_9H_8O_5$ requires C, 55·1; H, 4·1%).

2,6-*Dihydroxybenzoic Acid.*—A stirred mixture of resorcinol (330 g.), potassium hydroxide (168 g.), and xylene (750 ml.) was refluxed under a Dean and Stark head until water ceased to collect. Xylene was decanted from the cooled mixture and the residual monopotassium derivative of resorcinol was treated with dimethylformamide (750 ml.) and heated to 150°. Carbon dioxide was passed continuously through the stirred mixture, at 150—155°, for 7 hr. The cooled mixture was diluted with acetone (3 l.), filtered, and evaporated *in vacuo*, to remove acetone and as much dimethylformamide as possible. The residue was dissolved in water (300 ml.), filtered, acidified with concentrated hydrochloric acid (275 ml.), and again filtered. The small quantity of solid removed at this stage was washed with water (2 × 50 ml.). The combined filtrate and washings were diluted with water (300 ml.), stirred, seeded with 2,6-dihydroxybenzoic acid, and kept at 15° whilst the product crystallised. Filtration gave a first crop of colourless 2,6-dihydroxybenzoic acid (167·6 g.), m. p. 160—161° (decomp.); cooling the mother-liquors in the refrigerator overnight gave a light brown second crop (30·7 g.), m. p. 159—160° (decomp.). Recrystallisation of the main crop from water (1676 ml.; $\nearrow$75°) gave pure 2,6-dihydroxybenzoic acid monohydrate (151·2 g.), m. p. 166—168° (decomp.). Prolonged drying over phosphorus pentoxide *in vacuo* gave the anhydrous acid which with boiling methanolic hydrogen chloride (24 hr.) gave methyl 2,6-dihydroxybenzoate (36%), m. p. 69—71° (from methanol) (lit.,[14] m. p. 67—68°).

Dialkylation of Methyl 2,6-*Dihydroxybenzoate.*—(*a*) A stirred mixture of methyl 2,6-dihydroxybenzoate (16·8 g., 0·1 mole), anhydrous potassium carbonate (36 g., 0·26 mole), n-propyl iodide (34 g., 0·2 mole), and dry acetone (100 ml.) was refluxed for 4 hr. The solvent was evaporated and the residue dissolved in water and extracted with ether. The extracts were washed with dilute sodium hydroxide, then with water, dried, and distilled to give methyl 2,6-dipropoxybenzoate (55%), b. p. 172—176°/10 mm. Without further purification, this ester was dissolved in ethanol (25 ml.), treated with 40% aqueous potassium hydroxide (100 ml.), and refluxed for 3 hr. The cooled mixture was diluted with water, clarified by ether-extraction, and acidified to give 2,6-*dipropoxybenzoic acid* (74%) which, crystallised from cyclohexane, had m. p. 54—56° (Found: C, 65·6; H, 7·8. $C_{13}H_{18}O_4$ requires C, 65·5; H, 7·6%).

(*b*) A similar alkylation with butyl bromide gave methyl 2,6-dibutoxybenzoate (48%), b. p. 190—194°/12 mm., which on hydrolysis yielded 2,6-*dibutoxybenzoic acid* (66%), m. p. 82—84° (from light petroleum) (Found: C, 67·1; H, 7·9. $C_{15}H_{22}O_4$ requires C, 67·4; H, 8·3%).

(*c*) Benzyl bromide similarly afforded methyl 2,6-dibenzyloxybenzoate (76%), b. p. 242—

[11] Libermann and Moyeux, *Bull. Soc. chim. France*, 1956, 166.
[12] Gilman, Willis, Cook, Webb, and Meals, *J. Amer. Chem. Soc.*, 1940, **62**, 667.
[13] Boeseken, *Rec. Trav. chim.*, 1936, **55**, 815.
[14] Mauthner, *J. prakt. Chem.*, 1930, **124**, 319.

246°/0·5 mm., and 2,6-*dibenzyloxybenzoic acid* (91%), m. p. 124—126° (from ethyl acetate–light petroleum) (Found: C, 75·1; H, 5·4. $C_{21}H_{18}O_4$ requires C, 75·4; H, 5·4%).

Methyl 2-Hydroxy-6-methoxybenzoate.—A stirred mixture of methyl 2,6-dihydroxybenzoate (50 g., 0·298 mole), anhydrous potassium carbonate (60 g.), methyl sulphate (37·8 g., 0·3 mole), and acetone (300 ml.) was refluxed for 2 hr. Acetone was removed *in vacuo* and the residue was treated with water and extracted with ether. The ether solution was extracted with 2N-sodium hydroxide, and the alkaline solution acidified. The product was extracted in ether, dried, and distilled, giving *methyl 2-hydroxy-6-methoxybenzoate* (14 g.), b. p. 134—138°/9 mm. (Found: C, 59·4; H, 5·7. $C_9H_{10}O_4$ requires C, 59·3; H, 5·7%).

Alkaline hydrolysis gave 2-hydroxy-6-methoxybenzoic acid, m. p. 136—138° (from alcohol) (Found: C, 57·3; H, 4·8. Calc. for $C_8H_8O_4$: C, 57·1; H, 4·8%). This acid had been obtained previously from natural sources [6,15] and by two other syntheses.[16]

Methyl 2-Benzyloxy-6-methoxybenzoate.—A stirred mixture of methyl 2-hydroxy-6-methoxybenzoate (19·5 g.), benzyl chloride (28 g.), anhydrous potassium carbonate (43 g.), and acetone (100 ml.) was refluxed for 4 hr. Acetone was removed *in vacuo* and the residue was treated with water and extracted with ether. The extracts were washed with dilute sodium hydroxide, then with water, dried, and distilled, to give *methyl 2-benzyloxy-6-methoxybenzoate* (10 g.), b. p. 172—178°/0·5 mm. (Found: C, 70·8; H, 5·9. $C_{16}H_{16}O_4$ requires C, 70·6; H, 5·9%).

2-Benzyloxy-6-methoxybenzoic Acid.—A mixture of methyl 2-benzyloxy-6-methoxybenzoate (8·2 g.), potassium hydroxide (8·4 g.), butanol (140 ml.), and water (8 ml.) was refluxed for 2 hr., then evaporated to small volume. The residue was treated with water and extracted with ether. Acidification of the aqueous layer gave *2-benzyloxy-6-methoxybenzoic acid* (5·7 g.), which, crystallised from benzene–light petroleum, had m. p. 104—106° (Found: C, 70·1; H, 5·5. $C_{15}H_{14}O_4$ requires C, 69·8; H, 5·4%).

Alkylation of Mixed 2,6- and 2,4-Dihydroxybenzoic Acid.—(*a*) Resorcinol (55 g.) was converted into the monopotassium derivative and treated with carbon dioxide in dimethylformamide at 150—155°, as in the preparation of 2,6-dihydroxybenzoic acid. The resulting solution of potassium 2,6-dihydroxybenzoate with some of the 2,4-dihydroxy-isomer in dimethylformamide was cooled and diluted with acetone (500 ml.). Potassium carbonate (228 g.) was added and the mixture stirred whilst methyl sulphate (156 ml.) was added dropwise. The mixture was then refluxed with stirring for 3 hr., cooled, and filtered. The solid was dissolved in water (900 ml.) and extracted with ether (2 × 100 ml.), the extracts being added to the filtrate. The solvents were removed *in vacuo* and the residue distilled to give a semi-solid mixture of methyl 2,6- and 2,4-dimethoxybenzoate (58 g.), b. p. 160—179°/16 mm. The mixed esters were dissolved in methanol (580 ml.), treated with 5N-sodium hydroxide (116 ml.), and set aside at room temperature for 24 hr., then concentrated *in vacuo*. The residue was made into a slurry with water, and methyl 2,6-dimethoxybenzoate was collected as a white solid (46 g.), m. p. 88—90°. Crystallisation from dibutyl ether gave large needles of unchanged m. p. (lit.,[6] m. p. 88°).

(*b*) Resorcinol (55 g.) was carbonated as before but the mixture was alkylated with ethyl sulphate (216 ml.). The resulting mixture of ethyl 2,6- and 2,4-diethoxybenzoate (70·9 g.), b. p. 122—132°/0·4 mm., was treated in methanol (600 ml.) with 5N-sodium hydroxide (120 ml.) and set aside at room temperature for 24 hr., during which some *ethyl 2,6-diethoxybenzoate* crystallised. A further crop of the hindered ester was obtained by removal of methanol *in vacuo* and addition of water (total yield 60·4 g.). It crystallised from light petroleum in needles, m. p. 57—59° (Found: C, 65·8; H, 8·0. $C_{13}H_{18}O_4$ requires C, 65·5; H, 7·6%). Acidification of the aqueous filtrate gave 2,4-diethoxybenzoic acid (7·8 g.), m. p. 100—103° (from aqueous alcohol) (lit.,[17] m. p. 99°).

(*c*) The experiment was repeated with allyl bromide (143 ml.) as the alkylating agent. The resulting mixture (105 g.) of allyl 2,6- and 2,4-diallyloxybenzoate was not distilled but was treated in methanol (1·5 l.) with 5N-sodium hydroxide (200 ml.) and kept at room temperature for 41 hr. Methanol was removed *in vacuo* and the residue was diluted with water and extracted with ether. Evaporation of the extracts left the hindered ester as a crude oil (83 g.) which was refluxed with potassium hydroxide (26 g.) in butanol (180 ml.) and water (26 ml.)

[15] Beer, Karapetyan, Kolesnikov, and Snezirev, *Doklady Akad. Nauk S.S.S.R.*, 1949, **67**, 883; Santavy, Hoscalkova, Podivinsky, and Potesilova, *Coll. Czech. Chem. Comm.*, 1954, **19**, 1289.

[16] Limaye and Kelkar, *Rasayanam*, 1936, **1**, 24; Santucci and Gilman, *J. Amer. Chem. Soc.*, 1958, **80**, 4537.

for 9 hr., then evaporated *in vacuo*. The residue was dissolved in water and acidified, giving *2,6-diallyloxybenzoic acid* (33 g.), m. p. 71—72° (from carbon tetrachloride) (Found: C, 66·3; H, 6·1. $C_{13}H_{14}O_4$ requires C, 66·6; H, 6·0%).

Treatment with thionyl chloride below 40°, followed by evaporation *in vacuo*, gave the crude oily acid chloride. A portion with ammonia in chloroform gave *2,6-diallyloxybenzamide*, m. p. 130—131° (from benzene–cyclohexane) (Found: C, 66·8; H, 6·6; N, 5·8. $C_{13}H_{15}NO_3$ requires C, 67·0; H, 6·4; N, 6·0%); similarly it gave the *anilide*, m. p. 90—91° (from cyclohexane) (Found: C, 73·9; H, 6·5; N, 4·2. $C_{19}H_{19}NO_3$ requires C, 73·8; H, 6·2; N, 4·5%).

(*d*) A solution of potassium 2,6-dihydroxybenzoate with some of the 2,4-dihydroxy-isomer in dimethylformamide was prepared as before from resorcinol (55 g.), diluted with acetone (500 ml.), and filtered. The filtrate was transferred to an autoclave and stirred with isopropyl bromide (320 ml.) and anhydrous potassium carbonate (228 g.) at 110—160° for 4½ hr. After removal of the inorganic salts, the mixture was distilled, giving a mixture of isopropyl 2,6- and 2,4-di-isopropoxybenzoate, b. p. 104—126°/0·05 mm. Selective alkaline hydrolysis as previously described left *isopropyl 2,6-di-isopropoxybenzoate* (40 g.), b. p. 99—101°/0·1 mm., n_D^{20} 1·4892 (Found: C, 68·4; H, 8·7. $C_{16}H_{24}O_4$ requires C, 68·5; H, 8·6%). More vigorous hydrolysis of this hindered ester with a mixture of 50% aqueous potassium hydroxide and butanol (10 hours' refluxing) gave *2,6-di-isopropoxybenzoic acid*, m. p. 107—108° (from carbon tetrachloride) (Found: C, 65·2; H, 7·7. $C_{13}H_{18}O_4$ requires C, 65·5; H, 7·6%).

Treatment with thionyl chloride below 40°, followed by evaporation *in vacuo*, gave the crude oily acid chloride that, as above, afforded *2,6-di-isopropoxybenz-amide*, m. p. 106—107° (from cyclohexane) (Found: N, 5·6. $C_{13}H_{19}NO_3$ requires N, 5·9%), and *-anilide*, m. p. 153—154° (from cyclohexane) (Found: C, 73·1; H, 7·6. $C_{19}H_{23}NO_3$ requires C, 72·8; H, 7·4%).

2,6-Dimethoxyphenylpenicillin.—A solution of 2,6-dimethoxybenzoyl chloride (500 g.) in dry alcohol-free chloroform (3·75 l.) was added with stirring during 20 min. to an ice-cold suspension of 6-aminopenicillanic acid (540 g.) in chloroform (3·75 l.) and triethylamine (697 ml.). The mixture was stirred at room temperature for 1 hr. and then treated, with continued stirring, with sufficient 0·87N-hydrochloric acid (3 l.) to give an aqueous phase of pH 1. The chloroform layer was rapidly separated, washed with water, and stirred with sufficient N-sodium hydrogen carbonate (3·2 l.) to bring the aqueous phase to pH 7. The resulting solution of the sodium salt of the penicillin was separated, washed with ether (1 l.), evaporated at low temperature and pressure until the concentrate weighed 1415 g., thoroughly mixed with dry acetone (22 l.), and filtered. After addition of more acetone (4 l.) to the filtrate, crystallisation commenced and was allowed to proceed for 16 hr. at 0—3°. The product (563 g.) was collected and a second crop obtained by diluting the filtrate with dry ether (7·5 l.). The combined crops (766 g., 73%) of *sodium 2,6-dimethoxyphenylpenicillin monohydrate* had $[\alpha]_D^{25}$ +219° (*c* 5 in H_2O). Recrystallisation of a portion by dissolution in acetone containing 10—20% of water, followed by dilution with dry acetone, gave the pure colourless hydrated salt, m. p. 181—182° (decomp.), $[\alpha]_D^{20}$ +230° (*c* 5 in H_2O) (Found: C, 48·9; H, 5·2; N, 7·1; S, 8·0; Na, 5·5; H_2O, 4·3. $C_{17}H_{19}N_2O_6SNa,H_2O$ requires C, 48·6; H, 5·0; N, 6·7; S, 7·6; Na, 5·5; H_2O, 4·3%). The water of crystallisation was removed *in vacuo* at 115°, but the anhydrous salt was very hygroscopic.

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