1120. *Derivatives of 6-Aminopenicillanic Acid. Part VIII.*[1] *Further Analogues of 3-o-Chlorophenyl-5-methyl-4-isoxazolylpenicillin*

By J. C. Hanson, A. A. W. Long, J. H. C. Nayler, and E. R. Stove

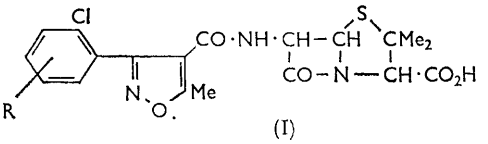
Various analogues of 3-*o*-chlorophenyl-5-methylisoxazole-4-carboxylic acid containing additional substituents in the benzene ring have been synthesised. Chlorides of these acids, together with certain other dihalogen derivatives of 5-methyl-3-phenyl- and 3-methyl-5-phenyl-isoxazole-4-carbonyl chlorides, were condensed with 6-aminopenicillanic acid to give new penicillins with useful antibacterial properties.

In Part VI of this Series [2] we described the synthesis of 3-*o*-chlorophenyl-5-methyl-4-isoxazolylpenicillin (cloxacillin) (I; R = H), which proved effective in the parenteral and oral treatment of infections caused by Gram-positive bacteria, including penicillinase-producing strains of Staphylococci.[3] This prompted the preparation of further halogenated phenylisoxazolylpenicillins, most of which could be formally derived from cloxacillin by the introduction of a second substituent into the benzene ring (R in I).

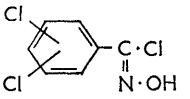
Each of the six possible dichlorobenzohydroxamoyl chlorides (II) was condensed with methyl sodioacetoacetate by the general procedure of Quilico and Fusco,[4] and the resulting

[1] Part VII, F. P. Doyle, J. C. Hanson, A. A. W. Long, and J. H. C. Nayler, *J.*, 1963, 5845.
[2] F. P. Doyle, J. C. Hanson, A. A. W. Long, J. H. C. Nayler, and E. R. Stove, *J.*, 1963, 5838.
[3] A Report from Six Hospitals, *Lancet*, 1962, II, 634.
[4] A. Quilico and R. Fusco, *Gazzetta*, 1937, **67**, 589.

ester hydrolysed to give the appropriate 3-(dichlorophenyl)-5-methylisoxazole-4-carboxylic acid. Other 3-aryl-5-methylisoxazole-4-carboxylic acids were similarly synthesised from 2,3,6- and 2,4,6-trichloro-, 2,6-difluoro-, 2-chloro-6-fluoro-, 2-bromo-6-chloro-, 2-bromo-6-fluoro-, 2-chloro-3-methoxy-, 2-chloro-4-nitro-, and 2-chloro-5-nitro-benzohydroxamoyl chlorides. Only in the last of these reactions was the yield unsatisfactory, and then a



(I)



(II)

convenient alternative route to the desired 3-(2-chloro-5-nitrophenyl)-5-methylisoxazole-4-carboxylic acid was available in the nitration of 3-*o*-chlorophenyl-5-methylisoxazole-4-carboxylic acid.

Heating 3-(2-chloro-3-methoxyphenyl)-5-methylisoxazole-4-carboxylic acid with aluminium chloride gave the 3-hydroxy-acid, whilst the 4- and 5-hydroxy-isomers were obtained by reducing the corresponding nitro-compounds with stannous chloride and diazotising the resulting amines. The diazonium sulphates were converted into the phenols by heating at 120—135° in moderately strong sulphuric acid containing sodium sulphate, since an attempt to prepare the 5-hydroxy-compound in weaker acid at 60—65° led to the recovery of the crystalline diazonium salt.

Five of the six possible 5-(dichlorophenyl)-3-methylisoxazole-4-carboxylic acids were obtained by condensing the appropriate α-dichlorobenzoyl derivative of acetoacetic ester with hydroxylamine, as previously described for the monochloro-analogues,[2] and hydrolysing the resulting esters.

The various isoxazole-4-carboxylic acids were heated with thionyl chloride and, after removing the excess of the reagent under reduced pressure, the residual acid chlorides were usually pure enough for reaction with 6-aminopenicillanic acid. However, the crude oily chlorides so obtained from the three 3-(chlorohydroxyphenyl)-5-methylisoxazole-4-carboxylic acids retained thionyl chloride with exceptional tenacity, possibly in chemical combination with the phenolic group, and gave erratic results on reaction with 6-aminopenicillanic acid. These oily products were therefore exposed to moist air with trituration, until liberation of sulphur dioxide ceased. After this treatment two of the chlorides crystallised, and all three were readily converted into hydroxy-penicillins (I; R = OH). We considered the possibility that a biologically active metabolite[5] which could be detected chromatographically in the blood and urine of animals or humans after administration of cloxacillin (I; R = H) might be one of these three hydroxy-derivatives, or a glucuronide thereof, but biochemical studies by Mr. K. R. L. Mansford and Mr. F. R. Batchelor did not support the suggestion.

All the new 3-aryl-5-methyl-4-isoxazolylpenicillins described here are essentially stable towards staphylococcal penicillinase and, in tests carried out by Dr. G. N. Rolinson and his colleagues *in vitro*, and by Mr. D. M. Brown and his colleagues *in vivo*, exhibited considerable activity against Gram-positive bacteria. The 3-(2,6-dihalogenophenyl)-5-methyl-4-isoxazolypenicillins[6] (I; R = 6-F, 6-Cl, or 6-Br) constituted a particularly promising group, since when given by mouth to human volunteers they were exceptionally well absorbed into the bloodstream. One of them, 3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolylpenicillin (dicloxacillin), has proved to be of clinical value in the oral treatment of staphylococcal infection.[7]

[5] J. H. C. Nayler, A. A. W. Long, D. M. Brown, P. Acred, G. N. Rolinson, F. R. Batchelor, S. Stevens, and R. Sutherland, *Nature*, 1962, **195**, 1264.

[6] J. H. C. Nayler, B.P. 978,299.

[7] P. Naumann and B. Kempf, *Arzneim-Forsch.*, 1965, **15**, 139; G. Mössner, H. Maurer, and C. Meisel, *ibid.*, p. 344.

In view of the special interest in the above structure (I; R = 6-Cl), we also condensed 2,6-dichlorobenzohydroxamoyl chloride with the sodio-derivative of formylacetic ester and, from the resulting 3-(2,6-dichlorophenyl)isoxazole-4-carboxylic acid, prepared an analogous penicillin lacking the methyl substituent on the isoxazole ring. In agreement with previous experience in the isoxazole series [2] it was found that resistance to penicillinase was lost when this substituent was omitted, although the penicillin retained high activity against streptococci and strains of staphylococci which did not produce the enzyme.

Experimental

Representative experiments are described; other *compounds*, further details, and analyses appear in the Tables.

Ultraviolet absorption spectra of dilute solutions in methanol were measured on a Hilger " Uvispek " instrument by Mr. K. Austin and his assistants.

Substituted Benzaldehydes and Benzaldoximes.—2,3-Dichlorotoluene was brominated at 120—230° under ultraviolet irradiation, and the resulting crude 2,3-dichlorobenzal bromide was treated with concentrated sulphuric acid at 90—95° to give 2,3-dichlorobenzaldehyde (68%), m. p. 61—61·5° (lit.,[8] 64°).

2,5-Dichloro-, m. p. 57—59° (lit.,[9] 56—58°), 3,5-dichloro-, m. p. 64—65° (lit.,[10] 65°), 2-chloro-6-fluoro-,[11] 2-bromo-6-fluoro-, 2-bromo-6-chloro-, m. p. 85—86° (lit.,[12] 79·5—81°), and 2-chloro-4-nitro-benzaldehyde, m. p. 71—73° (lit.,[13] 74°) were prepared similarly.

Chlorination of 2,6-dichlorobenzal chloride at 60—70° in the presence of antimony trichloride gave 2,3,6-trichlorobenzal chloride, m. p. 79—80° (lit.,[14] 78—80°), treatment of which with 98% sulphuric acid at 90—95° gave 2,3,6-trichlorobenzaldehyde (83%), m. p. 87—88° (lit.,[14] 87—88°).

2,6-Difluoro-,[11] 2,6-dichloro-,[15] and 2,4,6-trichlorobenzaldehyde [16] were obtained similarly from the corresponding benzal chlorides.

2,5-Dichloro-[9] and 2,4,6-trichloro-benzaldehyde [16] were obtained also from 2,5-dichloro- and 2,4,6-trichloro-aniline by Beech's method.[17]

Nitration of 2-chlorobenzaldehyde as described by Hodgson and Beard [18] gave 2-chloro-5-nitrobenzaldehyde, m. p. 80—81° (lit., 79—80°).

Oximes were prepared by standard methods.[19] 2,3-Dichlorobenzaldoxime had m. p. 131—132° (lit.,[8] 121°) (Found: C, 44·0; H, 2·85; Cl, 37·35; N, 7·3. Calc. for $C_7H_5Cl_2NO$: C, 44·2; H, 2·65; Cl, 37·35; N, 7·35%).

Hydroxamoyl Chlorides.—A solution of 2-chloro-3-methoxybenzaldoxime in ether was treated with nitrosyl chloride (freshly prepared from butyl nitrite and acetyl chloride [20]) to give 2-chloro-3-methoxybenzohydroxamoyl chloride.

The remaining hydroxamoyl chlorides were obtained by treating the appropriate benzaldoxime, dissolved in chloroform, with a solution of chlorine in the same solvent.

3-Arylisoxazole-4-carboxylic Acids and their Derivatives.—(*a*) *3-(2,6-Dichlorophenyl)-5-methylisoxazole-4-carboxylic acid derivatives.* (i) A solution of methyl sodioacetoacetate [from sodium (9·2 g.), methanol (300 ml.), and methyl acetoacetate (47 g.)] was added slowly to a stirred solution of 2,6-dichlorobenzohydroxamoyl chloride [from 2,6-dichlorobenzaldoxime (75·5 g.) and chlorine (30 g.)] in methanol (300 ml.) at 0—10°. The mixture was allowed to attain room

[8] G. Lock, *Monatsh.*, 1959, **90**, 683.
[9] H. S. Sharadamma, S. N. Kulkarni, P. B. Sattur, and K. S. Nargund, *J. Karnatak Univ.*, 1956, **1**, 61.
[10] F. Asinger and G. Lock, *Monatsh.*, 1933, **62**, 344.
[11] G. Lock, E. Stoits, and H. Glassner, *Ber.*, 1936, **69**, 2253.
[12] J. F. Bunnett, J. H. Miles, and K. V. Nahabedian, *J. Amer. Chem. Soc.*, 1961, **83**, 2512.
[13] L. Chardonnens and P. Heinrich, *Helv. Chim. Acta*, 1940, **23**, 292.
[14] H. C. Brimelow, R. L. Jones, and T. P. Metcalfe, *J.*, 1951, 1208.
[15] G. Stork and W. N. White, *J. Amer. Chem. Soc.*, 1956, **78**, 4609.
[16] V. M. Berenfel'd, L. N. Yakhontov, N. A. Yanbukhtin, D. M. Krasnokutskaya, S. V. Yatsenko, and M. V. Rubtsov, *Zhur. obshchei Khim.*, 1962, **32**, 2169.
[17] W. F. Beech, *J.*, 1954, 1297.
[18] H. H. Hodgson and H. G. Beard, *J.*, 1926, 147.
[19] R. P. Linstead and B. C. L. Weedon, " A Guide to Qualitative Organic Chemical Analysis," Butterworths, London, 1956, p. 27.
[20] H. Franzen and F. Zimmermann, *Ber.*, 1907, **40**, 2009.

temperature overnight. The precipitated solid was collected, then extracted with ether (200 ml.) and water (200 ml.), and the aqueous phase was extracted with more ether (4 × 200 ml.). Evaporation of the combined ether extracts yielded methyl 3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxylate (57·5 g.). Evaporation of the filtrate from the crude product, followed by a similar extraction and washing procedure, yielded a second crop of the ester (29·1 g.).

(ii) The ester (84·4 g.), dissolved in ethanol (370 ml.) was treated with potassium hydroxide (25 g.) in water (125 ml.) and heated under reflux for 6 hr. After distillation of most of the ethanol, water (75 ml.) was added, the solution was washed with ether, and acidified to precipitate crude 3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxylic acid (96%).

(iii) The acid (66·4 g.) and thionyl chloride (70 ml.) were boiled together under reflux for 7 hr. and the excess of thionyl chloride was removed *in vacuo* to give crystalline 3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbonyl chloride (99%), m. p. 88—91°.

TABLE 1

3-Arylisoxazole-4-carboxylic acid derivatives

	Subst. at position						
No.	3	5	4	M. p.	Cryst. from *	Yield (%)	$\lambda_{max.}$ (mμ) (ε in parentheses)
1	2,6-Cl_2·C_6H_3	H	CO_2H	179°	A	17	215sh (11,350) 220br (11,760) 277br (390)
2	2,6-F_2·C_6H_3	Me	CO_2H	173—174	A	27	211sh (8340) 222br (9490) 265br, sh (1290)
3	2-F, 6-Cl·C_6H_3	Me	CO_2Me	55—58	D; H	65	
4			CO_2H	206—208	C; E; B–E	55	220 (12,770) 270 (1530)
5			$CONH_2$	141—142	B–E	—	
6	2-F, 6-Br·C_6H_3	Me	CO_2H	212—214	E; B–C	43	220br (11,820) 272br (1260)
7	2,3-Cl_2·C_6H_3	Me	CO_2Me	101·5—102·5	I; E	86	
8			CO_2H	204—205	E; A	85	217br (17,140)
9			$CONH_2$	153—154	E; G	—	
10	2,4-Cl_2·C_6H_3	Me	CO_2Me	75	F	60	
11			CO_2H	207—208	B–E	98	215 (16,950) 223sh (15,610)
12			$CONH_2$	170—171	E	—	
13	2,5-Cl_2·C_6H_3	Me	CO_2Me	88·5—89	E; D	80	
14			CO_2H	237—238	E; I; B–E	82	215br (16,750) 225br, sh (16,480)
15			$CONH_2$	176—177	E	—	
16	2,6-Cl_2·C_6H_3	Me	CO_2Me	116—117	H; F	76	
17			CO_2H	220—221	B–E; C	96	218 (16,510)
18			CONHPh	147—148	E; B–E; A; E	93	
19	3,4-Cl_2·C_6H_3	Me	CO_2H	239—240	B–E	75	216 (18,470) 236br, sh (13,790) 280br, sh (410)
20			$CONH_2$	223—224	E; C	—	
21	3,5-Cl_2·C_6H_3	Me	CO_2Me	106—107	B–E	70	
22			CO_2H	179—181	B–E; A	96	218 (19,040) 234sh (9360) 286br (190)
23			$CONH_2$	226—228	E	—	
24	2-Cl, 6-Br·C_6H_3	Me	CO_2H	231—232	C; B–E	40	217 (17,880)
25	2,3,6-Cl_3·C_6H_2	Me	CO_2Me	159	F; H	82	
26			CO_2H	246	E	78	217 (20,750) 291br (460)
27	2,4,6-Cl_3·C_6H_2	Me	CO_2Me	118—119	E	54	
28			CO_2H	228—229	E	69	217br (21,090) 226sh (18,450)
29	2-Cl, 3-MeO·C_6H_3	Me	CO_2Me	102—104	E	70	217 (18,440) 286br (2700)
30			CO_2H	238—240	E	97	216 (17,360) 286br (2590)
31	2-Cl, 4-O_2N·C_6H_3	Me	CO_2Me	154—155	F	51	
32			CO_2H	218—220	E; C	91	219 (18,720) 263br (11,240)

TABLE 1 (*Continued*)

	(Found (%))					Required (%)			
No.	C	H	Hal	N	Formula	C	H	Hal	N
1	46·85	2·05	27·25	5·25	$C_{10}H_5Cl_2NO_3$	46·5	1·95	27·5	5·4
2	54·95	2·9	16·1	5·65	$C_{11}H_7F_2NO_3$	55·2	2·95	15·9	5·85
3	53·1	3·5	Cl: 13·35 F: 7·6	5·2	$C_{12}H_9ClFNO_3$	53·4	3·35	Cl: 13·15 F: 7·05	5·2
4	51·5	3·0	Cl: 13·7 F: 7·65	5·45	$C_{11}H_7ClFNO_3$	51·6	2·75	Cl: 13·9 F: 7·45	5·5
5	51·5	3·35	Cl: 13·9 F: 7·55	11·1	$C_{11}H_8ClFN_2O_2$	51·85	3·15	Cl: 13·95 F: 7·45	11·0
6	44·2	2·5	Br: 26·25 F: 6·45	4·7	$C_{11}H_7BrFNO_3$	44·0	2·35	Br: 26·65 F: 6·35	4·65
7	50·4	3·35	25·05	4·7	$C_{12}H_9Cl_2NO_3$	50·35	3·15	24·8	4·9
8	48·55	2·2	25·9	5·0	$C_{11}H_7Cl_2NO_3$	48·55	2·55	26·1	5·15
9	48·85	2·6	25·95	10·1	$C_{11}H_8Cl_2N_2O_2$	48·7	2·95	26·2	10·35
10	50·1	3·4	24·9	4·7	$C_{12}H_9Cl_2NO_3$	50·35	3·15	24·8	4·9
11	48·4	2·85	25·9	5·15	$C_{11}H_7Cl_2NO_3$	48·55	2·55	26·1	5·15
12	48·6	2·7	25·5	10·15	$C_{11}H_8Cl_2N_2O_2$	48·7	2·95	26·2	10·35
13	50·6	3·5	24·7	4·85	$C_{12}H_9Cl_2NO_3$	50·35	3·15	24·8	4·9
14	48·45	2·85	25·9	4·95	$C_{11}H_7Cl_2NO_3$	48·55	2·55	26·1	5·15
15	48·7	2·75	26·5	10·75	$C_{11}H_8Cl_2N_2O_2$	48·7	2·95	26·2	10·35
16	50·6	3·4	24·75	5·1	$C_{12}H_9Cl_2NO_3$	50·35	3·15	24·8	4·9
17	48·6	2·8	26·05	4·95	$C_{11}H_7Cl_2NO_3$	48·55	2·55	26·1	5·15
18	58·85	3·6	20·1	8·15	$C_{17}H_{12}Cl_2N_2O_2$	58·8	3·45	20·5	8·05
19	48·6	3·0	25·7	5·05	$C_{11}H_7Cl_2NO_3$	48·55	2·55	26·1	5·15
20	48·45	2·85	26·5	10·15	$C_{11}H_8Cl_2N_2O_2$	48·7	2·95	26·2	10·35
21	50·5	3·3	24·7	4·6	$C_{12}H_9Cl_2NO_3$	50·35	3·15	24·8	4·9
22	48·5	2·4	26·2	5·45	$C_{11}H_7Cl_2NO_3$	48·55	2·55	26·1	5·15
23	48·9	3·0	25·95	10·1	$C_{11}H_8Cl_2N_2O_2$	48·7	2·95	26·2	10·35
24	41·4	2·6	37·1 †	4·7	$C_{11}H_7BrClNO_3$	41·7	2·2	36·5 †	4·4
25	44·8	2·45	33·15	4·6	$C_{12}H_8Cl_3NO_3$	44·9	2·5	33·2	4·35
26	43·3	2·25	34·5	4·6	$C_{11}H_6Cl_3NO_3$	43·1	1·95	34·75	4·55
27	45·2	2·6	33·1	4·5	$C_{12}H_8Cl_3NO_3$	44·9	2·5	33·2	4·35
28	43·0	2·2	34·75	4·8	$C_{11}H_6Cl_3NO_3$	43·1	1·95	34·75	4·55
29	55·75	4·35	12·95	5·25	$C_{13}H_{12}ClNO_4$	55·4	4·25	12·6	5·0
30	53·7	3·9	13·05	4·95	$C_{12}H_{10}ClNO_4$	53·85	3·75	13·3	5·25
31	48·8	3·2	12·4	9·45	$C_{12}H_9ClN_2O_5$	48·55	3·05	12·0	9·45
32	46·9	2·8	12·9	9·8	$C_{11}H_7ClN_2O_5$	46·75	2·5	12·55	9·9

* A = Benzene; B = water; C = acetic acid; D = light petroleum; E = ethanol; F = methanol; G = toluene; H = cyclohexane; I = acetonitrile. † Total halogen.

(iv) The chloride (3·3 g.) was dissolved in acetone (20 ml.), and aniline (1·5 ml.) was added. The solution was diluted with water (70 ml.) to precipitate the crude anilide.

(*b*) The other 3-arylisoxazole-4-carboxylic acids and their derivatives listed in Table 1 were obtained similarly except that ethyl 3-(2,6-dichlorophenyl)isoxazole-4-carboxylate and methyl 3-(2-chloro-4-nitrophenyl)-5-methylisoxazole-4-carboxylate were both hydrolysed with a mixture of equal volumes of acetic and concentrated hydrochloric acids, rather than with alkali.

3-(2-*Chloro*-3-*hydroxyphenyl*)-5-*methylisoxazole*-4-*carboxylic Acid*.—Anhydrous aluminium chloride (190 g.) was added to nitro-benzene (700 ml.), the mixture was heated to 80°, 3-(2-chloro-3-methoxyphenyl)-5-methylisoxazole-4-carboxylic acid (30 g.) was added, and the clear solution was stirred at 95—100° for 4 hr. The mixture was set aside at room temperature overnight, and then poured on to ice (1·8 kg.). The mixture was adjusted to pH 0·5 by the addition of concentrated hydrochloric acid, and the crude product was collected and dissolved in N-sodium hydrogen carbonate (200 ml.). The solution was warmed to 55°, ether (100 ml.) was added, and the mixture was digested, then filtered to remove impurity. The aqueous phase was separated, washed with more ether (50 ml.), treated with charcoal, and filtered after 1 hr. The filtrate was adjusted to pH 1—1·5 by the addition of concentrated hydrochloric acid (19 ml.), and the product (21·2 g., 75%) was collected, washed with water, and dried at 60°. Pure 3-(2-*chloro*-3-*hydroxyphenyl*)-5-*methylisoxazole*-4-*carboxylic acid*, crystallised from aqueous ethanol, had m. p. 268—270° (Found: C, 52·15; H, 3·25; Cl, 14·2; N, 5·55. $C_{11}H_8ClNO_4$ requires C, 52·1; H, 3·2; Cl, 14·0; N, 5·5%). $\lambda_{max.}$ 216 and 287 mμ (ε 16,220 and 2920).

3-(4-*Amino*-2-*chlorophenyl*)-5-*methylisoxazole*-4-*carboxylic Acid*.—3-(2-Chloro-4-nitrophenyl)-5-methylisoxazole-4-carboxylic acid (34·1 g.) was added to a mixture of stannous chloride

TABLE 2

5-Dichlorophenyl-3-methylisoxazole-4-carboxylic acid derivatives

No.	Subst. at position 5	Subst. at position 4	M. p.	Cryst. from *	Yield (%)	$\lambda_{max.}$ (mμ) (ε in parentheses)
1	2,3-Cl_2·C_6H_3	CO_2H	207°	I; B–C	67	218sh (17,140), 221 (17,540), 240br, sh (7340)
2		$CONH_2$	199—200	E	95	
3	2,4-Cl_2·C_6H_3	CO_2H	198	C	72	217 (16,180), 249br (9380)
4		$CONH_2$	187—189	B–E; A	—	
5	2,5-Cl_2·C_6H_3	CO_2H	196—197	B–E; B–C	69	225 (18,300), 240br, sh (6750), 277br, sh (1960)
6		$CONH_2$	195—196	B–E; A	—	
7	3,4-Cl_2·C_6H_3	CO_2Et	100—101	E	78	
8		CO_2H	220—221	C; E	90	212sh (14,040), 218 (14,990), 275br (14,310)
9		$CONH_2$	186—187	E	87	
10		CONH·Ph	163—164	E	89	
11	3,5-Cl_2·C_6H_3	CO_2Et	93·5	E	73	
12		CO_2H	181—183	B–E; A	89	216 (17,600), 222 (18,660), 268br (12,350)
13		$CONH_2$	201—202	E; G; I	—	

No.	Found (%) C	H	Cl	N	Formula	Required (%) C	H	Cl	N
1	48·8	2·8	25·85	4·85	$C_{11}H_7Cl_2NO_3$	48·55	2·55	26·1	5·15
2	48·9	2·5	26·25	10·0	$C_{11}H_8Cl_2N_2O_2$	48·7	2·95	26·2	10·35
3	48·2	2·75	26·3	4·9	$C_{11}H_7Cl_2NO_3$	48·55	2·55	26·1	5·15
4	48·5	2·65	26·25	10·6	$C_{11}H_8Cl_2N_2O_2$	48·7	2·95	26·2	10·35
5	48·8	2·85	26·05	5·45	$C_{11}H_7Cl_2NO_3$	48·55	2·55	26·1	5·15
6	48·85	2·75	26·5	10·05	$C_{11}H_8Cl_2N_2O_2$	48·7	2·95	26·2	10·35
7	52·2	3·45	23·95	4·3	$C_{18}H_{11}Cl_2NO_3$	52·0	3·65	23·65	4·65
8	48·9	2·9	26·1	4·85	$C_{11}H_7Cl_2NO_3$	48·55	2·55	26·1	5·15
9	48·6	3·15	26·6	10·55	$C_{11}H_8Cl_2N_2O_2$	48·7	2·95	26·2	10·35
10	59·2	3·85	20·55	7·5	$C_{17}H_{12}Cl_2N_2O_2$	58·8	3·45	20·5	8·05
11	51·95	3·6	23·4	4·6	$C_{18}H_{11}Cl_2NO_3$	52·0	3·65	23·65	4·65
12	48·5	2·6	26·35	5·2	$C_{11}H_7Cl_2NO_3$	48·55	2·55	26·1	5·15
13	48·6	2·9	26·1	10·3	$C_{11}H_8Cl_2N_2O_2$	48·7	2·95	26·2	10·35

* See footnote to Table 1.

dihydrate (82·5 g.) and concentrated hydrochloric acid (111 ml.), and the mixture was heated to boiling. Water (290 ml.) was added, then the mixture was boiled for 3 hr., and refrigerated overnight. The solution was adjusted to pH 8, filtered to remove tin compounds, and the filtrate was adjusted to pH 3·6 to precipitate the crude product (27·0 g.). Crystallisation from ethanol (charcoal) gave pure 3-(4-*amino*-2-*chlorophenyl*)-5-*methylisoxazole*-4-*carboxylic acid*, m. p. 197—198° (decomp.) (Found: C, 52·6; H, 3·9; Cl, 14·1; N, 10·75. $C_{11}H_9ClN_2O_3$ requires C, 52·25; H, 3·55; Cl, 14·05; N, 11·1%), $\lambda_{max.}$ 218, 259br, 296sh mμ (ε 16,290, 9290, 3370).

3-(2-*Chloro*-4-*hydroxyphenyl*)-5-*methylisoxazole*-4-*carboxylic Acid*.—3-(4-Amino-2-chlorophenyl)-5-methylisoxazole-4-carboxylic acid (14·3 g.) in a mixture of concentrated sulphuric acid (4·6 ml.) and water (114 ml.) was diazotised at 0° with sodium nitrite (3·96 g.) in water (11·5 ml.). The mixture was stirred at 0° for 3 hr., urea was added to destroy any remaining nitrous acid, and the solution was added dropwise during *ca.* 4 hr. to a mixture of sodium sulphate (51·6 g.), concentrated sulphuric acid (36 ml.), and water (36 ml.), held at 120°. The mixture was diluted with water, then refrigerated overnight, and the product (13·9 g., 97%) was collected. Crystallisation several times from aqueous ethanol (charcoal) yielded pure 3-(2-*chloro*-4-*hydroxyphenyl*)-5-*methylisoxazole*-4-*carboxylic acid* as needles, m. p. 252° (decomp.) (Found: C, 52·35; H, 3·35; Cl, 13·95; N, 5·4. $C_{11}H_8ClNO_4$ requires C, 52·1; H, 3·2; Cl, 14·0; N, 5·5%), $\lambda_{max.}$ 216, 278 br,sh, 286br,sh mμ (ε 16,530, 1620, 630).

Nitration of 3-o-*Chlorophenyl*-5-*methylisoxazole*-4-*carboxylic Acid*.—A solution of 3-*o*-chlorophenyl-5-methylisoxazole-4-carboxylic acid (47·6 g.) in concentrated sulphuric acid (70 ml.) was stirred and treated dropwise at 5—10° with fuming nitric acid (50 ml.). When the

addition was complete, the temperature of the mixture was allowed to rise to 15° during 2 hr., the solution was poured on to a mixture of ice (500 g.) and water (500 ml.), and the product was collected and dissolved in warm N-sodium hydrogen carbonate solution (500 ml.). The solution was extracted with ether (250 ml.), treated with charcoal, filtered, and the filtrate adjusted to pH 1·5 by the addition of N-hydrochloric acid (300 ml.). The product was collected, washed with water until the washings were free from chloride ions, and dried at 60° to give 3-(*2-chloro-5-nitrophenyl*)-5-*methylisoxazole*-4-*carboxylic acid* (52·3 g., 93%), m. p. 204°. A sample, crystallised as needles from propan-2-ol, had m. p. 204—204·5° (Found: C, 47·0; H, 2·8; Cl, 12·45; N, 10·1. $C_{11}H_7ClN_2O_5$ requires C, 46·7; H, 2·5; Cl, 12·55; N, 9·9%), $\lambda_{max.}$ 220, 273br mμ (ε 17,210, 10,040).

An identical product was obtained in 3% yield from 2-chloro-5-nitrobenzohydroxamoyl chloride in the normal manner.

3-(5-*Amino*-2-*chlorophenyl*)-5-*methylisoxazole*-4-*carboxylic Acid*.—3-(2-Chloro-5-nitrophenyl)-5-methylisoxazole-4-carboxylic acid (18·6 g.) was washed with water (70 ml.) into concentrated hydrochloric acid (60 ml.) containing stannous chloride dihydrate (45 g.), and the mixture was boiled under reflux for 30 min., during which time more water (45 ml.) was added, to give a clear solution. This was cooled to room temperature, the crystalline precipitate was collected, suspended in water (70 ml.) and treated with 5N-sodium hydroxide (80 ml.). The solution was adjusted to pH 8 by the addition of 5N-hydrochloric acid, and the tin salts were filtered off. The filtrate was treated with 5N-hydrochloric acid (11 ml.) to give pH 3·8, and the 3-(5-*amino*-2-*chlorophenyl*)-5-*methylisoxazole*-4-*carboxylic acid* was collected after 1 hr. (14·4 g., 87%). It had m. p. 166—168° (Found: C, 52·2; H, 3·75; Cl, 13·9; N, 11·1. $C_{11}H_9ClN_2O_3$ requires C, 52·3; H, 3·6; Cl, 14·1; N, 11·1%), $\lambda_{max.}$ 218br, 245br,sh, 311br mμ (ε 14,710, 8550, 1710).

3-(2-*Chloro*-5-*hydroxyphenyl*)-5-*methylisoxazole*-4-*carboxylic Acid*.—(*a*) 3-(5-Amino-2-chlorophenyl)-5-methylisoxazole-4-carboxylic acid (5·0 g.), in a mixture of water (30 ml.) and concentrated sulphuric acid (3·0 ml.), was diazotised at 0° with sodium nitrite (1·38 g.) in water (6·0 ml.). The mixture was held at 0—10° for 1 hr., then the temperature was raised gradually to 60—65° and held at that level for 2½ hr. to give a clear solution which gave only a weakly positive test for nitrous acid. The solution was set aside at room temperature for 2 days, and the orange crystals, m. p. 135° (decomp.), presumably of the *diazonium hydrogen sulphate* of the starting acid, were collected (3·0 g., 42%) (Found: C, 36·2; H, 2·45; Cl, 9·8; N, 11·35. $C_{11}H_8ClN_3O_7S$ requires C, 36·5; H, 2·2; Cl, 9·85; N, 11·6%).

(*b*) 3-(5-Amino-2-chlorophenyl)-5-methylisoxazole-4-carboxylic acid (10·0 g.) in a mixture of water (60 ml.) and concentrated sulphuric acid (6·0 ml.) was diazotised at 0° with sodium nitrite (2·76 g.) in water (12 ml.). The mixture was allowed to attain room temperature during 1 hr., then heated at 65—70° for 30 min.; the temperature was then raised to 90°, and the solution filtered hot. The filtrate was treated with anhydrous sodium sulphate (100 g.) and concentrated sulphuric acid (60 ml.), heated at 130—135° for 30 min., and cooled to room temperature. Water (300 ml.) was added, and the aqueous solution was decanted from the crude resinous product. The resin was triturated with ether (100 ml., 200 ml.), and the aqueous phase was extracted with the same solvent (200 ml.). Evaporation of the combined ether extracts left an oil which solidified (7·29 g.). This was extracted with boiling water (1500 ml.), and the extract, decanted from the undissolved tar, was boiled with charcoal, and filtered. Extraction of the filtrate with ether (3 × 250 ml.) and evaporation of the ether extracts gave a solid (4·61 g.), m. p. 199—206°. Two further recrystallisations from hot water (charcoal) followed by extraction into sodium hydrogen carbonate and reprecipitation with dilute hydrochloric acid did not raise the melting point. Crystallisation from di-n-butyl ether gave 3-(2-*chloro*-5-*hydroxyphenyl*)-5-*methylisoxazole*-4-*carboxylic acid*, m. p. 213—214·5° (Found: C, 51·8; H, 3·35; Cl, 13·9; N, 5·55. $C_{11}H_8ClNO_4$ requires C, 52·1; H, 3·2; Cl, 14·0; N, 5·5%), $\lambda_{max.}$ 216, 293br mμ (ε 16,100, 1040).

3-(*Chlorohydroxyphenyl*)-5-*methylisoxazole*-4-*carbonyl Chlorides and Derivatives*.—(*a*) Finely powdered 3-(2-chloro-3-hydroxyphenyl)-5-methylisoxazole-4-carboxylic acid (5·07 g.) and thionyl chloride (15 ml.) were heated together under reflux for 11 hr. to give a clear solution. The excess of thionyl chloride was distilled off *in vacuo*, and the residual oil was dried azeotropically with benzene. Trituration of the product in moist air gave crystals of 3-(2-*chloro*-3-*hydroxyphenyl*)-5-*methylisoxazole*-4-*carbonyl chloride* (5·38 g., 99%), m. p. 91—93° (Found: C, 48·35; H, 2·3; Cl, 25·95; N, 5·05. $C_{11}H_7Cl_2NO_3$ requires C, 48·55; H, 2·55; Cl, 26·1; N, 5·15%).

(*b*) 3-(2-*Chloro*-4-*hydroxyphenyl*)-5-*methylisoxazole*-4-*carbonyl chloride* (94%), m. p. 151—152°, was obtained similarly (Found: C, 48·25; H, 2·6; Cl, 25·75; N, 4·5. $C_{11}H_7Cl_2NO_3$ requires C, 48·55; H, 2·55; Cl, 26·1; N, 5·15%).

(*c*) Similar treatment of 3-(2-chloro-5-hydroxyphenyl)-5-methylisoxazole-4-carboxylic acid (0·4 g.) with thionyl chloride (2·0 ml.) gave the chloride as an oil which did not crystallise. The chloride, dissolved in acetone (4·0 ml.) was added to a mixture of aniline (0·2 ml.), acetone (4·0 ml.), and N-sodium hydrogen carbonate (8·0 ml.). Most of the acetone was evaporated off, the solid was collected, dissolved in isobutyl methyl ketone (20 ml.), and the solution was washed with 0·2N-hydrochloric acid (2 × 10 ml.), then with water (2 × 10 ml.), treated with charcoal, filtered, and evaporated. Crystallisation of the residue from toluene (15 ml.) gave 3-(2-*chloro*-5-*hydroxyphenyl*)-5-*methylisoxazole*-4-*carboxyanilide* (0·39 g.), colourless needles, m. p. 183—184° (Found: C, 62·3; H, 4·05; Cl, 10·85; N, 8·1. $C_{17}H_{13}ClN_2O_3$ requires C, 62·1; H, 4·0; Cl, 10·8; N, 8·5%).

5-*Dichlorophenyl*-3-*methylisoxazole*-4-*carboxylic Acids and their Derivatives.*—(*a*) 5-(3,5-*Dichlorophenyl*)-3-*methylisoxazole*-4-*carboxylic acid derivatives.* (i) Crude ethyl α-(3,5-dichlorobenzoyl)acetoacetate (80 g.) in ethanol (330 ml.) was treated with a solution of hydroxylamine hydrochloride (36 g.) in water (47 ml.), the mixture was boiled for 10 min., cooled to room temperature overnight, diluted with water (300 ml.), and extracted with ether (3 × 500 ml.). The combined ether extracts were washed with 5% sodium hydroxide solution (2 × 100 ml.), then with water, dried (Na_2SO_4), and the ether was distilled off to give *ethyl* 5-(3,5-*dichlorophenyl*)-3-*methylisoxazole*-4-*carboxylate* (58 g., 73%).

(ii) The ester (57 g.) in ethanol (250 ml.) containing potassium hydroxide (16 g.) and water (100 ml.) was boiled under reflux for 4 hr., the bulk of the ethanol was distilled off, water (400 ml.) was added, and the solution acidified to precipitate crude 5-(3,5-*dichlorophenyl*)-3-*methylisoxazole*-4-*carboxylic acid* (52 g.).

(iii) The acid (20 g.) and the thionyl chloride (20 ml.) were heated together under reflux for 2½ hr., the excess of thionyl chloride was removed *in vacuo*, final traces being removed azeotropically with benzene, to give crude 5-(3,5-dichlorophenyl)-3-methylisoxazole-4-carbonyl chloride (21·4 g.).

(iv) Treatment of the chloride, in acetone, with aqueous ammonia (*d* 0·88) afforded the *amide*.

(*b*) The other 5-*dichlorophenyl*-3-*methylisoxazole*-4-*carboxylic acids* and their *derivatives* listed in Table 2 were prepared similarly.

Acylation of 6-*Aminopenicillanic Acid.*—(*a*) A stirred suspension of 6-aminopenicillanic acid (52·5 g.) in water (484 ml.) was adjusted to pH 7·2 with N-sodium hydroxide, 3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbonyl chloride (70·5 g.) in isobutyl methyl ketone (726 ml.) was added, and the mixture was stirred vigorously for 90 min. The organic layer was separated, washed with saturated brine, and treated with 2N-sodium 2-ethylhexanoate in propan-2-ol (125 ml.), whereupon the sodium salt of the penicillin crystallised (64 g.). A portion (9·8 g.) was dissolved in a mixture of propan-2-ol (25 ml.) and water (5 ml.) at 50°. Diethyl ether (30 ml.) was added, and the mixture was stood for 1 hr. at room temperature, then overnight at 0°, and pure *sodium* 3-(2,6-*dichlorophenyl*)-5-*methyl*-4-*isoxazolylpenicillin monohydrate* (8·3 g.) was collected, $[\alpha]_D^{20}$ +134·4° (*c* 1 in H_2O), m. p. 214° (decomp.) (Found: C, 44·2; H, 3·8; N, 8·3; Na, 3·9; S, 6·6; H_2O, 3·4. $C_{19}H_{16}Cl_2N_3NaO_5S,H_2O$ requires C, 44·7; H, 3·5; N, 8·2; Na, 4·5; S, 6·3; H_2O, 3·5%).

On occasions, the product, although analytically pure, was obtained in a slightly hygroscopic, amorphous form, and had a modified infrared (i.r.) spectrum in the solid state. Such material, when stirred with acetone at ordinary temperature, first dissolved and then rapidly separated in the non-hygroscopic crystalline form. Various other amorphous specimens of penicillin salts, particularly in the isoxazole series, could likewise be induced to crystallise by exposure to ketonic solvents.

(*b*) The other penicillins were prepared similarly, but crystallisation to analytical purity was omitted.

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