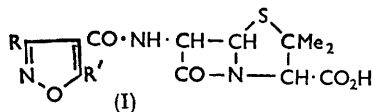


**1112. Derivatives of 6-Aminopenicillanic Acid. Part VI.<sup>1</sup> Penicillins from 3- and 5-Phenylisoxazole-4-carboxylic Acids and their Alkyl and Halogen Derivatives.**

By F. P. DOYLE, J. C. HANSON, A. A. W. LONG, J. H. C. NAYLER, and E. R. STOVE.

Condensation of benzohydroxamoyl chloride or its methyl or halogen derivatives with the sodio-derivatives of acylacetic esters gave a series of 3-arylisoxazole-4-carboxylic acids. Corresponding 5-aryl compounds were prepared from  $\alpha$ -alkanoyl- $\alpha$ -aroylacetic esters and hydroxylamine. The 3- and 5-aryl acids were conveniently differentiated by means of their ultraviolet absorption spectra. Reaction of the isoxazole acid chlorides with 6-aminopenicillanic acid gave isoxazolylpenicillins with useful antibacterial activity.

In previous Parts of this series<sup>2</sup> it has been shown that acylation of 6-aminopenicillanic acid with the chlorides of sterically hindered carboxylic acids of various types gives penicillins which resist inactivation by penicillinase. If such penicillins are to be effective when given by mouth they should preferably have a fair stability at low pH, a requirement met by various 3,5-disubstituted 4-isoxazolylpenicillins (I). Syntheses of oxacillin (I; R = Ph, R' = Me) and its isomer (I; R = Me, R' = Ph) have been described,<sup>3</sup> and the former compound has been used clinically in the oral treatment of resistant staphylococcal infections.<sup>4</sup>



<sup>1</sup> Part V, Doyle, Nayler, Waddington, Hanson, and Thomas, *J.*, 1963, 497.

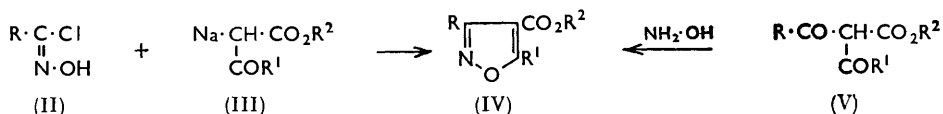
<sup>2</sup> Doyle and his co-workers, *J.*, 1962, 1445, 1453; 1963, 491.

<sup>3</sup> Doyle, Long, Nayler, and Stove, *Nature*, 1961, **192**, 1183.

<sup>4</sup> Bunn and Amberg, *New York State J. Med.*, 1961, **61**, 4158; White and Smith, *Amer. J. Med. Sci.*, 1962, **241**, 202; Rutenberg, Greenberg, Levenson, and Schweinburg, *New Engl. J. Med.*, 1962, 226, 755.

isoxazole ring has been replaced by hydrogen or a higher alkyl group, or in which the benzene ring carries methyl or halogen substituents, are described in the present paper.

Quilico and Fusco<sup>5</sup> condensed benzohydroxamoyl chloride (II; R = Ph), prepared by chlorination of benzaldoxime,<sup>6</sup> with ethyl sodioacetoacetate (III; R' = Me, R<sup>2</sup> = Et) to give ethyl 5-methyl-3-phenylisoxazole-4-carboxylate (IV; R = Ph, R<sup>1</sup> = Me, R<sup>2</sup> =



Et), which was hydrolysed by alkali to the carboxylic acid. Similar syntheses with sodio-derivatives of other acylacetic esters have now given 3-phenylisoxazole-4-carboxylic acid (IV; R = Ph, R<sup>1</sup> = R<sup>2</sup> = H) and analogues containing ethyl, propyl, isopropyl, and chloromethyl substituents in the 5-position. Other 5-alkyl-3-arylisoaxazole-4-carboxylic acids were synthesised by the same route from substituted benzohydroxamoyl chlorides (II; R = tolyl or halogenophenyl). The condensation stage was best carried out in the alcohol corresponding to the ester group of the starting material (III) when it was desired to isolate the isoxazole ester, but if this intermediate was to be hydrolysed without isolation the possibility of transesterification was no longer important and hence either methanol or ethanol was a suitable solvent. The esters were hydrolysed with alkali except in the preparation of 3-phenylisoxazole-4-carboxylic acid and its 5-chloromethyl derivative, when acid was used to avoid decomposition. Farley and his co-workers<sup>7</sup> claim to have prepared 3-*p*-chlorophenyl-5-methylisoxazole-4-carboxylic acid by this route, but the physical constants given for the intermediates and end-product are grossly different from those found by us.

Turning next to the 5-arylisoaxazole-4-carboxylic acids, we synthesised the 5-phenyl compound (IV; R = R<sup>2</sup> = H, R<sup>1</sup> = Ph) from ethyl  $\alpha$ -benzoyl- $\beta$ -ethoxyacrylate and hydroxylamine by Panizzi's method,<sup>8</sup> except that the final hydrolysis stage was more conveniently effected by heating the ester with a mixture of acetic and concentrated hydrochloric acid. The non-identity of the product with 3-phenylisoxazole-4-carboxylic acid, here prepared for the first time, confirmed the structure.

The earliest synthesis of 3,5-disubstituted isoxazole-4-carboxylic acids, namely, the condensation of diacylacetic esters (V) with hydroxylamine,<sup>9</sup> is also capable in theory of giving more than one isomer when the acyl groups are different. The older literature on these compounds<sup>10</sup> is further confused by reports of another synthesis leading to "unstable isomers" of 3-substituted 5-arylisoaxazole-4-carboxylic acids and their derivatives, but these have now been shown<sup>11</sup> to be highly enolised acylisoxazolones and not carboxylic acids. The only authentic 3-methyl-5-phenylisoxazole-4-carboxylic acid is that having m. p. 189°, which is conveniently prepared from  $\alpha$ -benzoylacetoacetic ester and hydroxylamine, none of the isomeric 5-methyl-3-phenylisoxazole-4-carboxylic acid being formed.<sup>12,13</sup> In extending this synthesis to thirteen other  $\alpha$ -alkanoyl- $\alpha$ -aroylacetic esters (V) we in no case obtained more than one isoxazolecarboxylic acid, although the 3-isopropyl-5-phenyl compound needed to be purified by way of the amide to remove an unidentified contaminant.

<sup>5</sup> Quilico and Fusco, *Gazzetta*, 1937, **67**, 589.

<sup>6</sup> Werner and Buss, *Ber.*, 1894, **27**, 2193; Piloty and Steinbock, *Ber.*, 1902, **35**, 3112.

<sup>7</sup> Farley, Rathmann, and Tangen, *Proc. N. Dakota Acad. Sci.*, 1959, **13**, 61.

<sup>8</sup> Panizzi, *Gazzetta*, 1943, **73**, 13.

<sup>9</sup> Claisen, *Annalen*, 1893, **277**, 174.

<sup>10</sup> Beilstein's 2nd Suppl., "Handbuch der Organischen Chemie," Springer, Vol. XXVII, pp. 382 *et seq.*

<sup>11</sup> Speroni, *Gazzetta*, 1952, **82**, 691.

<sup>12</sup> Betti and Alessandri, *Gazzetta*, 1915, **45**, 462.

<sup>13</sup> Hydorn, McGinn, Moetz, and Schwarz, *J. Org. Chem.*, 1962, **27**, 4305.

In order to confirm that the new acids were all the 5-aryl isomers it was desirable to demonstrate their non-identity with the corresponding 3-aryl compounds, here prepared unambiguously from benzohydroxamoyl chloride and its derivatives. Melting-point determinations were not always adequate for this purpose, since the values for several isomeric pairs lay close together and the depressions observed on admixture were small. Fortunately, examination of ultraviolet absorption spectra in the range 200—350  $m\mu$  proved a convenient method.

The major absorption peak of 3-phenylisoxazole-4-carboxylic acid and its various 5-alkyl derivatives is at about 230  $m\mu$ , but that of 5-phenylisoxazole-4-carboxylic acid and its 3-alkyl derivatives (which incorporate a diene chain linked head-to-tail with the benzene ring<sup>14</sup>) occurs at about 267  $m\mu$ . These results agree well with recent findings for the 3-methyl-5-phenyl and 5-methyl-3-phenyl acids.<sup>13</sup> Introduction of methyl or halogen substituents into the *para*-position of the benzene ring scarcely alters the spectra, but the effect of *ortho*-substitution is more significant. The carbocyclic and heterocyclic rings in 3-methyl-5-phenylisoxazole-4-carboxylic acid are probably almost co-planar, but when the benzene ring is *ortho*-substituted this is no longer possible and the chief absorption peak is consequently shifted by 15—30  $m\mu$  towards shorter wavelengths with broadening of the band and loss of intensity. In 5-methyl-3-phenylisoxazole-4-carboxylic acid, however, the rings are believed to be already inclined at an appreciable angle,<sup>13</sup> so it is not surprising that in this series *ortho*-substitution produces a hypsochromic shift of only 10—15  $m\mu$ .

Heating the various isoxazole-4-carboxylic acids with thionyl chloride gave the acid chlorides, of which some were purified by distillation and most were characterised as amides. Reaction of the chlorides with 6-aminopenicillanic acid occurred readily in aqueous media, and isolation of some of the resulting penicillins (I) as pure sodium salts is described in the Experimental section. The remainder were obtained sufficiently pure for initial antibacterial tests, which were carried out *in vitro* by Dr. G. N. Rolinson and his colleagues and *in vivo* by Mr. D. M. Brown and his colleagues.

All the 4-isoxazolylpenicillins showed considerable activity against most Gram-positive bacteria, but only the 3,5-disubstituted types were effective against penicillinase-producing strains of staphylococci. Evidently in 3- or 5-phenyl-4-isoxazolylpenicillin the phenyl group alone does not exert sufficient steric influence to prevent inactivation by penicillinase. The different behaviour of 2-biphenylpenicillin,<sup>15</sup> which exhibits considerable stability towards the enzyme, must be attributed to the differing geometry of the five-membered isoxazole and six-membered benzene rings.

Among the disubstituted isoxazolylpenicillins the 5-alkyl-3-aryl compounds were generally more active than the corresponding 3-alkyl-5-aryl isomers. In both series the preferred alkyl substituent was methyl, the higher homologues being less active. Halogenation in the benzene ring had relatively little influence on antibacterial activity, but sometimes resulted in improved absorption into the human bloodstream after oral administration. This effect was particularly marked in cloxacillin (I;  $R = o\text{-Cl-C}_6\text{H}_4$ ,  $R^1 = \text{Me}$ ),<sup>16</sup> which has proved clinically effective in the treatment of resistant staphylococcal infections.<sup>17</sup>

#### EXPERIMENTAL

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

Ultraviolet absorption spectra of dilute methanolic solutions were measured on a Hilger

<sup>14</sup> Del Re, *J.*, 1962, 3324.

<sup>15</sup> Dolan, Bondi, Hoover, Tumilowicz, Stewart, and Ferlauto, in "Antimicrobial Agents and Chemotherapy—1961," ed. M. Finland and G. M. Savage, Amer. Soc. Microbiology, Detroit, p. 648; Gourevitch, Holdrege, Hunt, Minor, Flanagan, Cheney, and Lein, *Antibiotics and Chemotherapy*, 1962, **12**, 318.

<sup>16</sup> Nayler, Long, Brown, Acred, Rolinson, Batchelor, Stevens, and Sutherland, *Nature*, 1962, **195**, 1264; Knudsen, Brown, and Rolinson, *Lancet*, 1962, II, 632.

<sup>17</sup> A Report from Six Hospitals, *Lancet*, 1962, II, 634.

"Uvispek" instrument and we are indebted to Dr. H. D. C. Rapson and his staff for these results.

*Substituted Benzaldehydes and Benzaldoximes.*—*o*- and *p*-Bromo-, and *o*- and *p*-fluoro-benzaldehyde, were prepared as described by Coleman and Honeywell<sup>18</sup> for the *p*-bromo-compound; *o*-tolualdehyde was prepared from *o*-toluidine by Beech's method.<sup>19</sup> Oximes were made by standard methods.<sup>20</sup>

*p*-Fluorobenzoic Acid.—Solid potassium permanganate (720 g.) was added portionwise during 3 hr. to a stirred mixture of *p*-fluorotoluene (110 g.), pyridine (485 ml.), and water (1100 ml.) at 100°. Heating and stirring were continued for 1 hr. longer, then the mixture was set aside overnight. The manganese dioxide was filtered off and washed with water, and the filtrate was decolourised with sulphur dioxide and extracted with ether (1.5, 1.0 l.). Evaporation of the dried extracts left *p*-fluorobenzoic acid (120 g., 86%), m. p. 185—187° (lit.,<sup>21</sup> 186°).

*Ethyl Alkanoylacetates.*—Ethyl formylacetate was obtained as the sodio-derivative by Deuschel's method.<sup>22</sup> Ethyl propionyl-, butyryl-, and isobutyryl-acetate were prepared as described by Brändström,<sup>23</sup> and ethyl chloroacetylacetate was prepared from ethyl chloroacetate by Alexandrow's method.<sup>24</sup>

*Ethyl  $\alpha$ -Alkanoyl- $\alpha$ -aroylacetates.*—(a) Ethyl  $\alpha$ -aroylacetates were prepared as their sodio-derivatives as described by Burton and Ingold<sup>25</sup> for ethyl sodio-*p*-chlorobenzoylacetate. Treatment of the sodio-derivatives with dilute hydrochloric acid liberated the free diketo-esters which were extracted into ether. The extracts were washed with water and dried, and the solvent was evaporated, to give materials of sufficient purity for the next reaction.

(b) Ethyl  $\alpha$ -propionyl-,  $\alpha$ -butyryl-, and  $\alpha$ -isobutyrylbenzoylacetate were prepared by acylation of ethyl sodiobenzoylacetate with the appropriate acid chloride in benzene under the conditions described by Shriner, Schmidt, and Roll<sup>26</sup> for ethyl benzoylacetate.

*5-Alkyl-3-arylisoxazole-4-carboxylic Acids and their Derivatives.*—(a) *3-o-Chlorophenyl-5-methylisoxazole-4-carboxylic acid derivatives.* (i) A stirred solution of *o*-chlorobenzaldoxime (89 g.) in chloroform (500 ml.) was treated at -10° to 0° with chlorine (45 g.) in chloroform (300 ml.), and the mixture was allowed to attain room temperature overnight. Removal of the solvent *in vacuo* left crude *o*-chlorobenzohydroxamoyl chloride (107 g., 98%) as a pale yellow oil which partially solidified. A sample, crystallised from ethanol, had m. p. 54—56° (lit.,<sup>27</sup> 56°).

(ii) A solution of methyl sodioacetoacetate [from sodium (13.4 g.), methanol (400 ml.), and methyl acetoacetate (64 ml.)] was added slowly to a stirred solution of *o*-chlorobenzohydroxamoyl chloride (107 g.) in methanol (400 ml.) at 0°, the mixture was allowed to warm to room temperature overnight, and the solvent was evaporated *in vacuo*. The residue was shaken with water (200 ml.) and ether (200 ml.), the aqueous phase was extracted with more ether (2  $\times$  200 ml.), the combined ether extracts were washed with 5% sodium hydroxide solution, then with water, dried, and distilled to give *methyl 3-o-chlorophenyl-5-methylisoxazole-4-carboxylate* (88.3 g.), b. p. 120—134°/0.05 mm., m. p. 57—58°.

(iii) The ester (85 g.), dissolved in ethanol (500 ml.), was treated with potassium hydroxide (32 g.) in water (70 ml.) and refluxed for 2 hr. After distillation of most of the ethanol, water (400 ml.) was added, and the solution was clarified by ether-extraction, and acidified to precipitate crude pale yellow *3-o-chlorophenyl-5-methylisoxazole-4-carboxylic acid* (75.2 g.), m. p. 194—196°.

(iv) When the acid (75.2 g.) and thionyl chloride (40 ml.) had been boiled together for 1 hr. and the excess of thionyl chloride removed *in vacuo*, distillation of the residue yielded *3-o-chlorophenyl-5-methylisoxazole-4-carbonyl chloride* (73.5 g., 91%), b. p. 130°/0.5 mm., which solidified.

<sup>18</sup> Coleman and Honeywell, *Org. Synth.*, Coll. Vol. II, p. 89.

<sup>19</sup> Beech, *J.*, 1954, 1297.

<sup>20</sup> Linstead and Weedon, "A Guide to Qualitative Organic Chemical Analysis," Butterworths Scientific Pubns., London, 1956, p. 27.

<sup>21</sup> Schiemann and Winkelmüller, *Org. Synth.*, Coll. Vol. II, p. 299.

<sup>22</sup> Deuschel, *Helv. Chim. Acta*, 1952, **35**, 1587.

<sup>23</sup> Brändström, *Acta. Chem. Scand.*, 1951, **5**, 820.

<sup>24</sup> Alexandrow, *Ber.*, 1913, **46**, 1022.

<sup>25</sup> Burton and Ingold, *J.*, 1928, 919.

<sup>26</sup> Shriner, Schmidt, and Roll, *Org. Synth.*, Coll. Vol. II, p. 266.

<sup>27</sup> Rheinboldt, Dewald, Jansen, and Schmitz-Dumont, *Annalen*, 1926, **451**, 161.

TABLE I.  
3-Aryloxazole-4-carboxylic acids and their derivatives.

Ph	Subst. at position		M. p. or b. p./mm.	Cryst. from *	Yield (%)	Found (%)				Formula	Required (%)				$\lambda_{\text{max}}$ (m $\mu$ ) ( $\epsilon$ in parentheses)							
	3	4				C	H	Hal	N		C	H	Hal	N								
	H	CO <sub>2</sub> Et	110-111 <sup>o</sup> /0.1 <sup>†</sup>		12	66.5	5.05			6.15					66-35	5.1				6-45		
	CO <sub>2</sub> H		165-166	A	79	63.15	4.0			7.1					63-5	3.7				7-4	230.5 (8960)	
	Et	CO <sub>2</sub> H	154-155	B-C; A-D; B-E	36	66.1	5.4			6.1					66-35	5.1					227 (11,180)	
	CO <sub>2</sub> NH <sub>2</sub>		150-150.5	B-E	—	66.8	6.0			12.85					66-65	5.55					12.95	
	CO <sub>2</sub> H		156-157	A; B-C	23	67.8	5.8			5.8					67.5	5.65					6.05	226 (12,010)
	CO <sub>2</sub> H		177-178	B-C; A; B-E	43	67.4	5.7			5.9					67.5	5.65					6.05	227 (11,440)
	CO <sub>2</sub> NH <sub>2</sub>		123-123	B-E; A-D; B-E	—	67.5	6.4			11.9					67.8	6.1					12.2	
	CH <sub>2</sub> Cl	CO <sub>2</sub> H	143-144	A-D	14	55.75	3.55	15.0		5.7				14.95	3.4						5.9	226 (13,730)
	Me	CO <sub>2</sub> H	160-160.5	A; B-E	24	66.6	5.2			5.95				66.35	5.1						6.45	216.5 (12,260)
	Me	CO <sub>2</sub> Me	66-67	F	69	67.75	6.0			5.85				67.5	5.65						6.05	
	CO <sub>2</sub> H		206-207	B-E	95	66.15	5.3			5.95				66.35	5.1						6.45	226 (11,090)
	CO <sub>2</sub> NH <sub>2</sub>		194-195	E	—	66.4	5.6			12.95				66.65	5.55						12.95	
	Me	CO <sub>2</sub> Me	120-122/0.5		59																	
	CO <sub>2</sub> H		191-192	G; C; B-E	93	60.0	3.9			6.0				59.75	3.6						6.3	217.5 (12,240)
	CO <sub>2</sub> H		203-204	B-E; G; B-C	27	60.45	3.8			6.3				59.75	3.6						6.3	226 (10,340)
	CO <sub>2</sub> NHPh		173-173.5	E	—					9.2				68.95	4.4						9.45	
	Me	CO <sub>2</sub> Me	59-60	H	62	57.5	4.2	14.1		5.4				57.2	4.0						5.6	
	CO <sub>2</sub> H		195-196	B-E	94	55.9	3.55	14.4		5.7				55.6	3.4						5.6	219 (12,780)
	CO <sub>2</sub> NH <sub>2</sub>		145-146	G; B-E	—	55.25	3.65	15.25		11.5				55.85	3.8						11.85	
	Et	CO <sub>2</sub> Et	124-128/0.1		59																	
	CO <sub>2</sub> H		165-166	G-D; B-C; A-D	78	57.1	3.95	13.8		5.5				57.2	4.0						5.6	220 (13,430)
	CO <sub>2</sub> NH <sub>2</sub>		109-109.5	A-D; B-E	64	57.1	4.45	14.35		11.05				57.5	4.4						11.2	
	CO <sub>2</sub> Et		136-142/0.02		—																	
	CO <sub>2</sub> H		136-137	H; A; I	53	58.7	4.8	13.5		5.35				58.85	4.5						5.25	220 (13,800)
	CO <sub>2</sub> NH <sub>2</sub>		108-109	B-C; A-D	—	58.7	5.4	13.7		10.5				59.0	4.9						10.6	
	CO <sub>2</sub> H		132-134	D	49	58.4	4.6	13.2		5.0				58.85	4.5						5.25	218.5 (15,270)
	CO <sub>2</sub> NH <sub>2</sub>		140-142	B-E; A-D	—	59.1	4.85	13.65		10.4				59.0	4.9						10.6	
	CO <sub>2</sub> H		174-175	B-C; G; F	63	55.6	3.8	14.8		5.8				55.6	3.4						5.9	216 (13,130); 228sh (10,330)
	CO <sub>2</sub> NH <sub>2</sub>		200-201	E	—	56.0	3.85			11.55				55.85	3.8						11.85	
	CO <sub>2</sub> Me		63-64	D	90 <sup>†</sup>	57.5	4.3			5.6				57.2	4.0						5.6	
	CO <sub>2</sub> Et		59-60	D	93 <sup>†</sup>	59.1	4.9			5.0				58.85	4.5						5.25	
	CO <sub>2</sub> H		213-214	A; B-E	65	55.5	3.6	15.3		5.6				55.6	3.4						5.9	232 (14,600)
	COCl		146/0.25		95	51.85	3.15	28.2		5.2				51.55	2.75						5.45	
	CO <sub>2</sub> NH <sub>2</sub>		207-208	E	—	55.65	3.95	14.95		12.1				55.85	3.8						11.85	
	CO <sub>2</sub> H		187-188	C; G; E	30	57.3	4.1	14.45		5.35				57.2	4.0						5.6	232.5 (15,220)
	CO <sub>2</sub> NH <sub>2</sub>		179-180	E; G	—	57.5	4.4	14.3		10.95				57.5	4.4						11.2	
	CO <sub>2</sub> Me		66-67	D; H	58	48.5	3.65	27.25		4.5				48.65	3.4						4.75	
	CO <sub>2</sub> H		196.5-197.5	G; B-E	93	46.85	2.95	28.45		4.95				46.8	2.85						4.95	217.5 (13,200)
	CO <sub>2</sub> NH <sub>2</sub>		133-134	E	—	47.45	3.2	28.2		9.95				46.8	3.2						9.95	
	CO <sub>2</sub> H		219.5-220	A; G; B-C	25	47.2	2.9	28.25		4.8				46.8	2.85						4.95	237 (14,180)
	CO <sub>2</sub> NHPh		185-185.5	E	—	57.0	3.6	22.55		7.5				57.15	3.65						7.85	

\* A = Benzene; B = water; C = acetic acid; D = light petroleum; E = ethanol; F = methanol; G = toluene; H = cyclohexane; I = acetonitrile.  
<sup>†</sup>  $n_D^{20}$  1.5445. <sup>‡</sup> Prepared from the chloride and the appropriate alcohol. § Some of these acids also show a weak absorption ( $\epsilon < 1500$ ) at 265-275 m $\mu$ .

TABLE 2.  
3-Alkyl-5-arylisoxazole-4-carboxylic acids and their derivatives.

Subst. at position		M. p. or b. p./mm.	Cryst. from*	Yield (%)	Found (%)			Required (%)			$\lambda_{\text{max.}}$ ( $m\mu$ ) ( $\epsilon$ in parenthesis)			
5	3				4	C	H	Hal	N	C		H	Hal	N
Ph	Et	CO <sub>2</sub> Et	130°/0.5	57	66.5	5.45	5.9	66.35	5.1			6.45	267 (13,130)	
		CO <sub>2</sub> H	179-180	A; I	83	67.45	5.95	13.15	66.65	5.55			12.95	
		CO-NH <sub>2</sub>	181-182	E	87	67.25	5.65	5.7	67.5	5.65			6.05	267.5 (13,380)
<i>o</i> -Me-C <sub>6</sub> H <sub>4</sub>	Me	CO <sub>2</sub> Et	150-158/1.0	87	68.15	6.35	12.15	67.8	6.1			12.2		
		CO <sub>2</sub> H	115-117	B-C; I; H	72	66.4	5.3	6.2	66.35	5.1			6.45	247.5 (8030)
		CO-NH <sub>2</sub>	162-163	E	73	66.85	5.9	13.35	66.65	5.55			12.95	
<i>p</i> -Me-C <sub>6</sub> H <sub>4</sub>	Me	CO <sub>2</sub> Et	150/1.5 †	82	66.0	5.1	6.3	66.35	5.1			6.45	272.5 (15,650)	
		CO <sub>2</sub> H	149-150	B-E	83	66.9	5.75	13.0	66.65	5.55			12.95	
		CO-NH <sub>2</sub>	91-92	B-E	80	59.6	3.8	6.2	59.75	3.6			8.6	253 (9860)
<i>o</i> -F-C <sub>6</sub> H <sub>4</sub>	Me	CO <sub>2</sub> Et	213-214	80	59.65	3.2	6.1	59.75	3.6			8.6	267 (12,640)	
		CO <sub>2</sub> H	124-126/0.4	B-E; G	91	59.7	4.15	12.6	60.0	4.1			8.65	12.75
		CO-NH <sub>2</sub>	183.5-184	E; I	96	60.2	3.8	6.0	59.75	3.6			8.6	267 (12,660)
<i>m</i> -FC <sub>6</sub> H <sub>4</sub>	Me	CO <sub>2</sub> Et	118-120/0.2	76	59.4	4.1	12.8	60.0	4.1			8.65	12.75	
		CO <sub>2</sub> H	182-183	E; C	77	55.5	3.6	14.9	55.6	3.4			14.95	5.9
		CO-NH <sub>2</sub>	190-191	A	77	55.8	4.05	11.6	55.85	3.8			15.0	11.85
<i>p</i> -F-C <sub>6</sub> H <sub>4</sub>	Me	CO <sub>2</sub> Et	117-121/0.1	76	55.5	3.6	14.9	55.6	3.4			14.95	5.9	
		CO <sub>2</sub> H	191.5-192	A; B-E	98	55.8	4.05	11.6	55.85	3.8			15.0	11.85
		CO-NH <sub>2</sub>	203-204	E; C	77	55.55	3.65	15.0	55.6	3.4			14.95	5.9
<i>o</i> -Cl-C <sub>6</sub> H <sub>4</sub>	Me	CO <sub>2</sub> Et	159-160	77	55.65	4.05	15.2	55.85	3.8			15.0	11.85	
		CO <sub>2</sub> H	140-141	E	77	55.4	3.1	14.6	55.6	3.4			14.95	5.9
		CO-NH <sub>2</sub>	140-147/0.01	F; I	77	55.65	3.8	11.4	55.85	3.8			15.0	11.85
<i>m</i> -Cl-C <sub>6</sub> H <sub>4</sub>	Me	CO <sub>2</sub> Et	176-178 †	86	55.4	3.1	14.6	55.6	3.4			14.95	5.9	
		CO <sub>2</sub> H	190-191.5	E	86	55.65	4.05	15.2	55.85	3.8			15.0	11.85
		CO-NH <sub>2</sub>	61-62	A	78	55.4	3.1	14.6	55.6	3.4			14.95	5.9
<i>p</i> -Cl-C <sub>6</sub> H <sub>4</sub>	Me	CO <sub>2</sub> Et	204-205	98	55.65	3.8	11.4	55.85	3.8			15.0	11.85	
		CO <sub>2</sub> H	227-228	B-C; C	54	50.7	4.0	25.85	50.3	3.85			25.8	4.5
		CO-NH <sub>2</sub>	126-134/0.4	A; B-E	98	46.95	2.95	28.4	46.8	2.85			28.35	4.95
<i>o</i> -Br-C <sub>6</sub> H <sub>4</sub>	Me	CO <sub>2</sub> Et	175-176	98	46.7	3.4	28.1	47.0	3.2			28.45	9.95	
		CO <sub>2</sub> H	159-161	B-E	85	47.0	3.0	28.4	46.8	2.85			28.35	4.95
		CO-NH <sub>2</sub>	195	G	85	46.9	3.0	28.55	47.0	3.2			28.45	9.95
<i>p</i> -Br-C <sub>6</sub> H <sub>4</sub>	Me	CO <sub>2</sub> Et	225-226	E; I	45	40.3	2.65	38.7	40.15	2.45			28.35	4.95
		CO <sub>2</sub> H	171-172	A	45	40.1	2.95	38.8	40.25	2.75			38.6	4.25
		CO-NH <sub>2</sub>	205-206.5	E; C	—	40.1	2.95	38.8	40.25	2.75			38.7	8.55

\* See footnotes to Table 1. † Needles, m. p. 47-48°. ‡ Khromov and Porai-Koshits [J. Gen. Chem. (U.S.S.R.), 1947, 17, 1816] give m. p. 176°. § These acids also show absorption in the region 210-225  $m\mu$  ( $\epsilon$  4000-11,000).

245 br. sh. (6350)

275 (18,360)

287.5 (14,410),  
255 v br. sh.

(v) Treatment of the acid chloride in acetone solution with an excess of aqueous ammonia ( $d$  0.88) afforded 3-*o*-chlorophenyl-5-methylisoxazole-4-carboxamide.

(b) The other 3-arylisoxazole-4-carboxylic acids and their derivatives listed in Table 1, were obtained similarly, except that ethyl 3-phenylisoxazole-4-carboxylate and its 5-chloromethyl derivative were both hydrolysed with a mixture of equal volumes of acetic and concentrated hydrochloric acids (5 hours' refluxing) rather than with alkali.

**3-Alkyl-5-arylisoxazole-4-carboxylic Acids and their Derivatives.**—(a) 5-*o*-Bromophenyl-3-methylisoxazole-4-carboxylic acid derivatives. (i) Crude ethyl  $\alpha$ -*o*-bromobenzoylacetate (46.5 g.), in ethanol (150 ml.) was treated with hydroxylamine hydrochloride (21.5 g.) in water (24 ml.), and the mixture was boiled for 10 min., cooled to room temperature overnight, diluted with water (300 ml.), and extracted with ether ( $2 \times 250$  ml.). The ether solution was washed with dilute sodium hydroxide solution until the alkaline extracts gave no precipitate upon acidification, then with water, dried, and distilled, to give ethyl 5-*o*-bromophenyl-3-methylisoxazole-4-carboxylate (25 g.).

(ii) The ester (24.5 g.) in ethanol (150 ml.) containing potassium hydroxide (7 g.) and water (45 ml.) was refluxed for 2 hr., the bulk of the solvent was removed, and the residual solution was diluted with water, extracted with ether, and acidified, to precipitate crude 5-*o*-bromophenyl-3-methylisoxazole-4-carboxylic acid (22 g.).

(iii) The acid (16 g.) and thionyl chloride (12 ml.) were refluxed together for 2 hr., the excess of thionyl chloride removed *in vacuo*, and the residual oil dried azeotropically with benzene and then distilled, gave 5-*o*-bromophenyl-3-methylisoxazole-4-carbonyl chloride (15.1 g., 89%), b. p. 118—120°/0.75 mm.

(iv) The chloride, when treated in acetone with aqueous ammonia ( $d$  0.88), afforded the amide.

(b) 3-Isopropyl-5-phenylisoxazole-4-carboxylic acid derivatives. (i) Crude ethyl  $\alpha$ -isobutyrylbenzoylacetate (106 g.), ethanol (350 ml.), hydroxylamine hydrochloride (52 g.), and water (70 ml.), when boiled for 10 min., cooled to room temperature overnight and worked up as in (i) above, yielded an ester (42 g.), b. p. 142—152°/1.3 mm.

(ii) Hydrolysis of the ester (42 g.) with potassium hydroxide (13.5 g.), ethanol (250 ml.), and water (65 ml.) under reflux for 2½ hr., removal of most of the solvent, dilution with water, clarification by ether-extraction, and acidification precipitated the crude acid (26.2 g., 67%), m. p. 140—150°. Crystallisation from light petroleum (b. p. 100—120°), aqueous acetic acid, and benzene-light petroleum failed to improve the m. p. of the product, which, however, gave the expected elemental analysis (Found: C, 67.5; H, 5.85; N, 5.65.  $C_{13}H_{13}NO_3$  requires C, 67.5; H, 5.65; N, 6.05%) and had  $\lambda_{max}$  217, 235, and 267 m $\mu$  ( $\epsilon$  7800, 7500, and 8920).

(iii) The impure acid (17.8 g.) and thionyl chloride (20 ml.) were refluxed together for 4 hr., the excess of thionyl chloride was removed *in vacuo*, and the residue was distilled, giving 3-isopropyl-5-phenylisoxazole-4-carbonyl chloride (17.5 g., 91%), b. p. 115°/0.3 mm.

(iv) The chloride (17.5 g.), gave, as above, in acetone, the amide (15.8 g., 97%), m. p. 155—160°. Crystallisation from aqueous ethanol, benzene-light petroleum (b. p. 80—100°), ethanol, and isobutyl methyl ketone raised the m. p. to 168—169°. The amide had  $\lambda_{max}$  216 and 262 m $\mu$  ( $\epsilon$  7060 and 14,810) (Found: C, 68.05; H, 6.4; N, 12.2.  $C_{13}H_{14}N_2O_2$  requires C, 67.8; H, 6.1; N, 12.2%).

(v) The amide (8.5 g.) and potassium hydroxide (20 g.) in 50% aqueous ethanol (100 ml.) were refluxed for 7 days, most of the solvent was distilled off, and the aqueous residue was diluted with water (100 ml.), extracted with ether ( $2 \times 100$  ml.), adjusted to pH 6.3 with 5*N*-hydrochloric acid, filtered, and acidified strongly to precipitate the crude product (6.9 g.), m. p. 148—155°. Crystallisation from benzene, ethanol, and then aqueous ethanol gave pure 3-isopropyl-5-phenylisoxazole-4-carboxylic acid (4.7 g.), m. p. 159—160°,  $\lambda_{max}$  213 and 265 m $\mu$  ( $\epsilon$  8660 and 13,700) (Found: C, 67.8; H, 5.8; N, 6.05.  $C_{13}H_{13}NO_3$  requires C, 67.5; H, 5.65; N, 6.05%).

(c) The other 3-alkyl-5-arylisoxazole-4-carboxylic acids and their derivatives listed in Table 2 were prepared as described for the *o*-bromo-compound in (a) above.

**3-Aryl-5-methyl-4-isoxazolylpenicillins.**—(a) A stirred suspension of 6-aminopenicillanic acid (216 g.) in water (2 l.) was adjusted to pH 7.1 with *N*-sodium hydroxide, and the resulting solution was treated with 3-*o*-chlorophenyl-5-methylisoxazole-4-carbonyl chloride (256 g.) in isobutyl methyl ketone (3 l.). The mixture was stirred vigorously for 90 min., then the organic layer was separated, washed with saturated brine (1 l.), and treated with 2*N*-sodium

2-ethylhexanoate in propan-2-ol (500 ml.), whereupon the sodium salt of the penicillin (414 g., 87%) crystallised. A portion was dissolved in propan-2-ol containing 20% of water, diluted with propan-2-ol to a water content of 5%, and chilled, and the salt was collected and crystallised again in similar fashion, made into a slurry with dry acetone, finally collected, and dried at 40° in air, to give colourless *sodium 3-o-chlorophenyl-5-methyl-4-isoxazolylpenicillin monohydrate*, m. p. 170° (decomp.),  $[\alpha]_D^{20} +163^\circ$  (*c* 1 in H<sub>2</sub>O) (Found: C, 47.75; H, 4.05; Cl, 7.7; N, 8.65; S, 6.75; Na, 5.0; H<sub>2</sub>O, 3.9. C<sub>19</sub>H<sub>17</sub>ClN<sub>3</sub>NaO<sub>5</sub>S.H<sub>2</sub>O requires C, 47.95; H, 4.0; Cl, 7.45; N, 8.85; S, 6.75; Na, 4.85; H<sub>2</sub>O, 3.8%).

In similar fashion were prepared *sodium 3-o-fluoro-* (90%),  $[\alpha]_D^{20} +187^\circ$  (*c* 1 in H<sub>2</sub>O) (Found: C, 50.3; H, 4.4; N, 9.15; S, 7.0; Na, 4.8. C<sub>19</sub>H<sub>17</sub>FN<sub>3</sub>NaO<sub>5</sub>S.H<sub>2</sub>O requires C, 49.7; H, 4.15; N, 9.15; S, 6.95; Na, 5.0%), and *3-m-chloro-phenyl-5-methyl-4-isoxazolylpenicillin monohydrate* (86%), m. p. 170° (decomp.),  $[\alpha]_D^{21} +179^\circ$  (*c* 1 in H<sub>2</sub>O) (Found: C, 48.15; H, 4.5; Cl, 7.35; N, 8.45; S, 6.4; Na, 4.5. C<sub>19</sub>H<sub>17</sub>ClN<sub>3</sub>NaO<sub>5</sub>S.H<sub>2</sub>O requires C, 47.95; H, 4.0; Cl, 7.45; N, 8.85; S, 6.75; Na, 4.85%).

(b) A stirred suspension of 6-aminopenicillanic acid (64.8 g.) in water (600 ml.) was adjusted to pH 7.2 with *N*-sodium hydroxide. The resulting solution was diluted with acetone (300 ml.) and then 3-*p*-chlorophenyl-5-methylisoxazole-4-carbonyl chloride (76.8 g.) in acetone (600 ml.) was added in one lot, crystallisation of the product beginning almost immediately. The mixture was stirred for 1 hr. at room temperature and then 2 hr. at 0°, and the hydrated penicillin acid was collected, washed with 50% aqueous acetone and then with water; a solution of this product was dissolved in isobutyl methyl ketone (1.5 l.), washed with saturated brine (750 ml.), filtered, and treated with 2*N*-sodium 2-ethylhexanoate in propan-2-ol (150 ml.), and the resulting colourless platelets of the penicillin sodium salt were collected and dried in air at room temperature and then at 65°. A solution of this product (111.4 g.) in acetone (110 ml.) and water (55 ml.) was diluted with acetone (1 l.), filtered rapidly, and treated with more acetone (1 l.), to give pure *sodium 3-p-chlorophenyl-5-methyl-4-isoxazolylpenicillin monohydrate* (93.6 g.), m. p. 192° (decomp.),  $[\alpha]_D^{20} +187^\circ$  (*c* 1 in H<sub>2</sub>O) (Found: C, 48.2; H, 4.25; Cl, 7.45; N, 9.0; S, 6.6; Na, 4.8; H<sub>2</sub>O, 4.0. C<sub>19</sub>H<sub>17</sub>ClN<sub>3</sub>NaO<sub>5</sub>S.H<sub>2</sub>O requires C, 47.95; H, 4.0; Cl, 7.45; N, 8.85; S, 6.75; Na, 4.85; H<sub>2</sub>O, 3.8%).

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