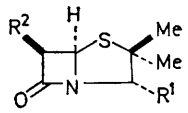
Studies Related to Penicillins. Part VII.[1] The Structure of the Epimers Derived from 6β-Substituted Penicillanic Acids

By J. R. Jackson and R. J. Stoodley,* Department of Organic Chemistry, The University, Newcastle upon Tyne NE1 7RU

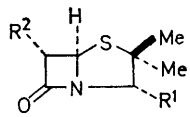
Methoxymethyl 6β-(2-hydroxy-1-naphthylmethyleneamino)penicillanate (6) is equilibrated with an epimer when treated with 1,5-diazabicyclo[4,3,0]non-5-ene in dichloromethane. Acidic hydrolysis of the epimer yields methoxymethyl 6-aminopenicillanate, which reacts with *N*-ethoxycarbonylphthalimide to give methoxymethyl 6-phthalimidopenicillanate. This last substance is identical with the epimer derived from the base-catalysed isomerisation of methoxymethyl 6β-phthalimidopenicillanate (2).

Methoxymethyl 6β-aminopenicillanate (7) and its epimer react with sodium nitrite and dilute hydrochloric acid to give methoxymethyl 6α-chloropenicillanate (17). The epimers, therefore, possess the *R*-stereochemistry at position 5 and they are 6α-substituted penicillanic acid derivatives. Methoxymethyl 6-diazopenicillanate is implicated in the deamination of both amines.

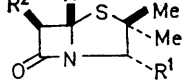
RECENTLY there has been considerable interest in the epimerisation of penicillanic acid derivatives. Wolfe and Lee [2] reported that methyl 6β-phthalimidopenicillanate (1) was converted into an epimer in the presence of a variety of bases. The β-lactam protons of the epimer showed a coupling constant of 2·0 Hz, indicating



(1) $R^1 = CO_2Me$, R^2 = Phth
(2) $R^1 = CO_2{\cdot}CH_2{\cdot}OMe$, R^2 = Phth
(3) $R^1 = CH_2{\cdot}CO_2Me$, R^2 = Phth
(4) $R^1 = CO_2H$, R^2 = Im
(5) $R^1 = CO_2{\cdot}CH_2{\cdot}OMe$, $R^2 = p\text{-}O_2N{\cdot}C_6H_4{\cdot}CH{:}N$
(6) $R^1 = CO_2{\cdot}CH_2{\cdot}OMe$, R^2 = HONp·CH:N
(7) $R^1 = CO_2{\cdot}CH_2{\cdot}OMe$, $R^2 = NH_2$
(8) $R^1 = CO_2H$, $R^2 = NH_2$



(9) $R^1 = CO_2Me$, R^2 = Phth
(10) $R^1 = CO_2{\cdot}CH_2{\cdot}OMe$, R^2 = Phth
(11) $R^1 = CH_2{\cdot}CO_2Me$, R^2 = Phth
(12) $R^1 = CO_2H$, R^2 = Im
(13) $R^1 = CO_2CH_2OMe$, $R^2 = p\text{-}O_2N{\cdot}C_6H_4{\cdot}CH{:}N$
(14) $R^1 = CO_2{\cdot}CH_2{\cdot}OMe$, R^2 = HONp·CH:N
(15) $R^1 = CO_2{\cdot}CH_2{\cdot}OMe$, $R^2 = NH_2$
(16) $R^1 = CO_2H$, $R^2 = NH_2$
(17) $R^1 = CO_2{\cdot}CH_2{\cdot}OMe$, R^2 = Cl
(18) $R^1 = CO_2H$, R^2 = Cl



(19) $R^1 = CO_2Me$, R^2 = Phth
(20) $R^1 = CO_2H$, R^2 = Im

Throughout, Phth = phthalimido, HONp = 2-hydroxy-1-naphthyl, Im = 2,2-dimethyl-5-oxo-4-phenylimidazolidin-1-yl

that they were *trans* oriented. On the basis of some chemical shift correlations, the stereochemistry at position 5 of the epimer was considered to be unchanged from that of the starting material, and, consequently, the product was assigned the 6α-structure (9).

Subsequently, several workers [3-7] have observed the epimerisation of 6β-substituted penicillanic acid derivatives and in all cases the 6α-structure has been assumed for the product.

Some controversy has arisen concerning the nature of the intermediate which is involved in the epimerisation. On the basis of deuterium-labelling experiments, Wolfe and Lee [2] suggested that the enethiolate (21) was an appropriate model for the transition state when potassium t-butoxide was employed as the base. In such an event the possibility that the epimer possesses the alternate structure (19) deserves serious consideration.

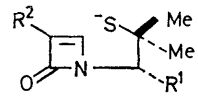
Nayler and his co-workers [4] investigated the epimerisation of hetacillin (4) in alkaline deuterium oxide and they accounted for their results in terms of the enolate (26). The product was assumed to have structure (12). However, it is conceivable that (26) may isomerise to (28) *via* the enethiolate (23) and, consequently, (20) is a possible structure for the epimer.

The reaction of compound (1) with triethylamine in dichloromethane was examined by Kovacs *et al.*[8] and the 1,4-thiazepine (29) was isolated in addition to the epimer. Under similar conditions compound (31) was obtained [9] from methoxymethyl 6β-*p*-nitrobenzylideneaminopenicillanate (5). These results provide support for the intervention of the enethiolates (21) and (24), although such species need not be involved in the rate-determining step of the epimerisation nor are they necessarily in equilibrium with the enolates (25) and (27).

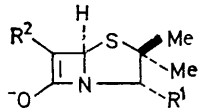
In a recent investigation [10] the reactions of methyl 6β-phthalimidohomopenicillanate (3) with tertiary organic bases was studied. In the presence of a strong base compound (3) was converted solely into an epimer

[1] Part VI, B. G. Ramsay and R. J. Stoodley, *J. Chem. Soc.* (*C*), 1971, 3864.
[2] S. Wolfe and W. S. Lee, *Chem. Comm.*, 1968, 242; S. Wolfe, W. S. Lee, and R. Misra, *ibid.*, 1970, 1067.
[3] D. A. Johnson, D. Mania, C. A. Panetta, and H. H. Silvestri, *Tetrahedron Letters*, 1968, 1903.
[4] J. P. Clayton, J. H. C. Nayler, R. Southgate, and E. R. Stove, *Chem. Comm.*, 1969, 130.
[5] R. D. G. Cooper, P. V. DeMarco, and D. O. Spry, *J. Amer. Chem. Soc.*, 1969, **91**, 1528.
[6] G. E. Gutowski, *Tetrahedron Letters*, 1970, 1779.
[7] B. G. Ramsay and R. J. Stoodley, *Chem. Comm.*, 1970, 1517.
[8] O. K. J. Kovacs, B. Ekström, and B. Sjöberg, *Tetrahedron Letters*, 1969, 1863.
[9] J. R. Jackson and R. J. Stoodley, *Chem. Comm.*, 1970, 14.
[10] B. G. Ramsay and R. J. Stoodley, *Chem. Comm.*, 1971, 450.

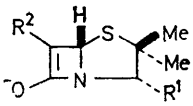
[assumed to be (11)], although as the base strength decreased the formation of (30) became increasingly important. Common anionic intermediates were implicated in the epimerisation and rearrangement processes. The precise formulation of these species was



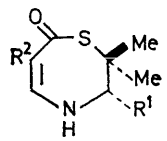
(21) $R^1 = CO_2Me$, R^2 = Phth
(22) $R^1 = CH_2{\cdot}CO_2Me$, R^2 = Phth
(23) $R^1 = CO_2^-$, R^2 = Im
(24) $R^1 = CO_2{\cdot}CH_2{\cdot}OMe$, $R^2 = p\text{-}O_2N{\cdot}C_6H_4{\cdot}CH{:}N$



(25) $R^1 = CO_2Me$, R^2 = Phth
(26) $R^1 = CO_2^-$, R^2 = Im
(27) $R^1 = CO_2{\cdot}CH_2{\cdot}OMe$, $R^2 = p\text{-}O_2N{\cdot}C_6H_4{\cdot}CH{:}N$



(28) $R^1 = CO_2^-$, R^2 = Im



(29) $R^1 = CO_2Me$, R^2 = Phth
(30) $R^1 = CH_2CO_2Me$, R^2 = Phth
(31) $R^1 = CO_2CH_2OMe$, $R^2 = p\text{-}O_2N{\cdot}C_6H_4{\cdot}CH{:}N$

not considered to be critical providing that they retained at least partial 1,5-bonding: thus, they may possess enolate- or enethiolate-like character or the charge may be delocalised between the oxygen and sulphur atoms. The conversion of these anionic intermediates into the enethiolate (22) was postulated to be the slow step in thiazepine formation. An essential feature of this proposal is that the enethiolate plays no role in the epimerisation reaction and, therefore, the epimers are 6α-substituted penicillanic acid derivatives.

A short while ago the behaviour of some 6α-arylmethyleneaminopenicillanic acid esters with 1,5-diazabicyclo[4,3,0]non-5-ene (DBN) was examined.[11] In contrast to the foregoing examples, the Schiff bases were in equilibrium with their epimers. Thus, in the case of methoxymethyl 6β-(2-hydroxy-1-naphthylmethyleneamino)penicillanate (6) in dichloromethane solution, the epimer comprised *ca.* 60% of the equilibrium mixture. The optical rotation of the epimer {$[\alpha]_D$ +337° ($CHCl_3$)} was much larger than that of (6) {$[\alpha]_D$ +27° ($CHCl_3$)}. This result appears to be exceptional, since a comparison of the rotations of other epimers with those of the corresponding 6β-substituted penicillanic acid derivatives (Table) indicates that the epimers possess the lower values. It, therefore, seemed necessary to correlate the Schiff base epimers with those derived from 6β-phthalimidopenicillanic acid and to place the absolute configurations at positions 5 and 6 of such derivatives on a firmer basis.

Optical rotations of 6β-substituted penicillanic acid derivatives and the corresponding epimers

	$[\alpha]_D$	$[\alpha]_D$ of epimer
Methyl 6β-phthalimidopenicillanate	+279° ($CHCl_3$) [a]	+228° ($CHCl_3$) [a]
Methyl 6β-phthalimidohomopenicillanate	+251° ($CHCl_3$) [b]	+169° ($CHCl_3$) [c]
Hetacillin	+343° (pyridine) [d]	+232° (pyridine) [d]
Potassium benzylpenicillinate	+310° (H_2O) [e]	+196° (H_2O) [f]
Methyl benzylpenicillinate	+286° ($CHCl_3$) [g]	+119° ($CHCl_3$) [f]
6β-Aminopenicillanic acid	+273° (0·1N-HCl) [h]	+262° (H_2O) [f]
6β-Trimethylammoniopenicillanate-hemihydroiodide	+212° (H_2O) [i]	+151° (H_2O) [i] †

† Determined for the hydroiodide.

[a] Ref. 2. [b] B. G. Ramsay and R. J. Stoodley, *J. Chem. Soc.* (*C*), 1969, 1319. [c] Ref. 10. [d] Ref. 3. [e] 'The Chemistry of Penicillin,' ed. H. T. Clarke, J. R. Johnson, and R. Robinson, Princeton University Press, New Jersey, 1949, p. 89. [f] D. A. Johnson and D. Mania, *Tetrahedron Letters*, 1969, 267. [g] Ref. *e*, p. 94. [h] J. C. Sheehan and K. R. Henery-Logan, *J. Amer. Chem. Soc.*, 1959, **81**, 5838. [i] Ref. 4.

When treated with DBN compound (6) readily equilibrated with an epimer which was isolated (35%) by silica gel chromatography. The imino-linkage of the epimer was hydrolysed on brief contact with hydrochloric acid in acetone to give an epimer of (7). The latter substance reacted with *N*-ethoxycarbonylphthalimide to afford a material identical with the product obtained from methoxymethyl 6β-phthalimidopenicillanate (2) and DBN. Acidic hydrolysis of the latter derivative yielded an acid which was converted into a methyl ester with diazomethane. The ester was indistinguishable from the epimer isolated by Wolfe and Lee [2] from the reaction of compound (1) with potassium t-butoxide. Thus the epimers derived from the Schiff bases and from 6β-phthalimidopenicillanic acid possess the same absolute stereochemistry at positions 5 and 6.

On the basis of n.m.r. spectroscopy the β-lactam protons of the epimers are *trans*-oriented. Their total absolute stereochemistry may, therefore, be deduced if the configuration at either position 5 or 6 is defined. There is little doubt [12] that the reaction of 6β-aminopenicillanic acid (8) with sodium nitrite and hydrochloric acid leads to 6α-chloropenicillanic acid (18). A single deuterium atom is incorporated into position 6 of the product when the reaction is conducted in deuteriochloric acid. This experiment implicates the intermediacy of 6-diazopenicillanic acid (33; R = H) in the reaction and provides convincing evidence for the preservation of the stereochemistry at position 5.

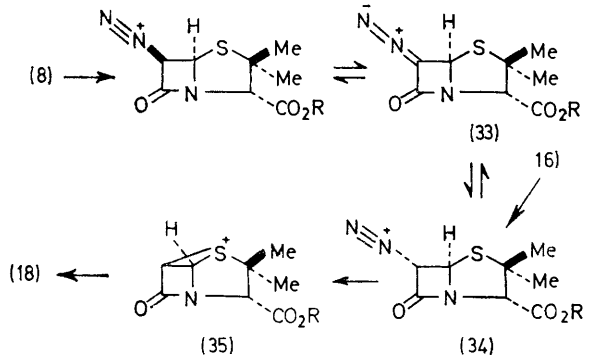
When treated with sodium nitrite and hydrochloric

[11] J. R. Jackson and R. J. Stoodley, *Chem. Comm.*, 1971, 648.
[12] I. McMillan and R. J. Stoodley, *Tetrahedron Letters*, 1966, 1295; *J. Chem. Soc.* (*C*), 1968. 2533.

acid both compound (7) and its epimer gave methoxymethyl 6α-chloropenicillanate (17). Consequently, the epimers differ from their 6β-substituted penicillanic acid precursors only in the configuration at position 6.

We have previously[12] commented on the stereochemical course of the reaction of compound (8) with sodium nitrite and hydrochloric acid. The failure to detect 6β-chloropenicillanic acid in the product implies that the addition of hydrochloric acid to 6-diazopenicillanic acid (33; R = H) is highly stereoselective. A possible explanation of this result is that the episulphonium ion (35; R = H) intervenes. As suggested in the Scheme, the diazonium cation (34; R = H) derived from 6α-aminopenicillanic acid (16) may afford species (35; R = H) without necessarily equilibrating with compound (33; R = H). Consequently, the amine (16) may be converted into the chloro-derivative (18) without complete exchange of the hydrogen atom at position 6.

In an attempt to provide some evidence for sulphur participation, the reactions of compounds (7) and (15) with sodium nitrite and deuteriochloric acid were investigated. In each case substantial deuterium exchange at position 6 resulted. Since a control experiment indicated that compound (17) did not undergo



SCHEME

deuterium exchange under the reaction conditions, methoxymethyl 6-diazopenicillanate (33; R = MeO·CH$_2$) is implicated as a common intermediate in the deamination of both compounds (7) and (15). Consequently, if species (35; R = MeO·CH$_2$) is a precursor of (17), the rate of its formation from the diazonium cation (34; R = MeO·CH$_2$) must be slow compared to the rate with which the latter intermediate undergoes deuterium exchange at position 6.

EXPERIMENTAL

For general experimental details see Part I.[12]

Methoxymethyl 6β-(2-Hydroxy-1-naphthylmethyleneamino)-penicillanate (6).—Triethylamine (1·12 ml, 0·008 mol) and 6β-(2-hydroxy-1-naphthylmethyleneamino)penicillanic acid[13] (2·96 g, 0·008 mol) were added to dichloromethane (20 ml) and the resulting solution was cooled in acetone–solid carbon dioxide. Chloromethyl methyl ether (1·20 g, 0·016 mol) in dichloromethane (6 ml) was added dropwise during 10 min to the stirred, cooled solution, which was then allowed to warm to room temperature. The solution was washed (twice) with water, dried ($MgSO_4$), and evaporated to give an orange syrup which deposited yellow crystals of the *methoxymethyl ester* (6) (2·90 g, 87%) on addition of ethanol; m.p. 105—106° (from ethanol), $[\alpha]_D$ +27° (0·5% in $CHCl_3$), $\nu_{max.}$ (KBr) 1775 (azetidinone), 1750 (ester C=O), and 1630 (C=N) cm^{-1}, τ ($CDCl_3$) 8·40 and 8·27 (each 3H, s, *gem*-Me_2), 6·43 (3H, s, OMe), 5·40 (1H, s, 3-H), 4·60 (3H, superimposed signals, 6-H and O·CH_2), 4·22 (1H, d, *J* 5 Hz, 5-H), 3·0—1·7 (6H, m, aromatic protons), and 0·55 (1H, s, CH=N) [Found: C, 60·8; H, 5·65; N, 6·6%; *M* (mass spectrum), 414. $C_{21}H_{22}N_2O_5S$ requires C, 60·9; H, 5·3; N, 6·75%; *M*, 414].

Reaction of the Imine (6) *with DBN*.—A solution of the imine (6) (0·497 g, 0·0012 mol) in dichloromethane (8 ml) was treated dropwise with DBN (0·025 ml). After *ca.* 10 min the solution, which had changed in colour from yellow to wine red, was diluted with dichloromethane, washed with dilute hydrochloric acid followed by water, and dried ($MgSO_4$). Evaporation left a syrup (0·437 g, 88%) which, on the basis of n.m.r. spectroscopy, contained starting material and epimer in the ratio of 2 : 3.

The mixture was fractionated on a silica gel (Mallinckrodt) column [chloroform–ether (2 : 1) as eluant] Elution was monitored by n.m.r. spectroscopy, and methoxymethyl 6α-(2-hydroxy-1-naphthylmethyleneamino)penicillanate (14) (0·152 g, 35%) was obtained as an orange syrup from the early fractions; $[\alpha]_D$ +337° (0·67% in $CHCl_3$), $\nu_{max.}$ (film) 1775 (azetidinone), 1750 (ester C=O), and 1630 (C=N) cm^{-1}, τ ($CDCl_3$) 8·39 and 8·25 (each 3H, s, *gem*-Me_2), 6·39 (3H, s, OMe), 5·28 (1H, s, 3-H), 4·90 (1H, d, *J* 2 Hz, 6-H), 4·54 (2H, s, O·CH_2), 4·35 (1H, d, *J* 2Hz, 5-H), 2·9—1·4 (6H, m, aromatic), and 0·53 (1H, s, CH=N).

The middle fractions afforded a mixture of compounds (6) and (14) as an orange syrup (0·06 g, 12%). By combining the late fractions some starting material (0·117 g, 24%) was recovered; this crystallised (0·084 g, 19%) on addition of methanol; m.p. 105—106°.

Reaction of the 6α-Epimer (14) *with Hydrochloric Acid*.—N-Hydrochloric acid (7·5 ml) was added to a solution of compound (14) (0·263 g, 0·00066 mol) in acetone (15 ml). After 5 min the solution was diluted with water and washed with chloroform. The aqueous layer was neutralised with sodium hydrogen carbonate solution and extracted with chloroform. The organic layer was dried ($MgSO_4$) and concentrated to leave methoxymethyl 6α-aminopenicillanate (7) (0·112 g, 68%) as a pale yellow syrup, $\nu_{max.}$ (film) 3400 (NH), 1770 (azetidinone), and 1750 (ester C=O) cm^{-1}, τ ($CDCl_3$) 8·46 and 8·38 (each 3H, s, *gem*-Me_2), 7·90br (2H, s, exchanged with D_2O, NH_2), 6·45 (3H, s, OMe), 5·76br (1H, s, 6-H), 5·48 (1H, s, 3-H), 4·84 (1H, d, *J* 2 Hz,5-H), and 4·64 (2H, s, O·CH_2).

Reaction of the 6α-Amine (7) *with N-Ethoxycarbonylphthalimide*.—A solution of compound (7) (0·112 g, 0·00043 mol) and *N*-ethoxycarbonylphthalimide (0·10 g, 0·00046 mol) in chloroform (5 ml) was left at room temperature for 3 h. The solvent was evaporated off and the resulting syrup was dissolved in acetone (4 ml) and treated with sodium hydrogen carbonate (0·04 g) in water (4 ml). The solution was diluted with water and extracted with chloroform.

[13] S. Wolfe, J. C. Godfrey, C. T. Holdrege, and Y. G. Perron, *J. Amer. Chem. Soc.*, 1963, **85**, 463; *Canad. J. Chem.*, 1968, **46**, 2549.

The extract was dried ($MgSO_4$) and evaporated to a pale yellow syrup, which deposited crystals of methoxymethyl 6α-phthalimidopencillanate (10) (0·049 g, 29%), m.p. 174—175°, $[\alpha]_D$ +197° (0·4% in $CHCl_3$), on addition of ethyl acetate. The i.r. and n.m.r. spectra of the sample were identical with those of the epimer derived from the reaction of compound (2) with DBN.

Methoxymethyl 6β-Phthalimidopenicillanate (2).—6β-Phthalimidopenicillanic acid [14] (2·08 g, 0·006 mol) was converted into compound (2) by the procedure used to prepare compound (6). The product afforded crystals of the *methoxymethyl ester* (2) (2·04 g, 87%) on addition of petroleum (60—80°); m.p. 124—125°, $[\alpha]_D$ +267° (0·5% in $CHCl_3$), $\nu_{max.}$ (KBr) 1775 and 1725 (phthalimido C=O), 1775 (azetidinone), and 1740 (ester C=O) cm^{-1}, τ ($CDCl_3$) 8·43 and 8·16 (each 3H, s, *gem*-Me_2), 6·64 (3H, s, OMe), 5·28 (1H, s, 3-H), 4·63 (2H, s, O·CH_2), 4·32 (2H, ABq, *J* 5 Hz, β-lactam protons), and 2·13 (4H, s, aromatic) [Found: C, 55·3; H, 4·5; N, 7·15%; *M* (mass spectrum), 390. $C_{18}H_{18}N_2O_6S$ requires C, 55·4; H, 4·65; N, 7·2%; *M*, 390].

Reaction of Compound (2) *with DBN*.—DBN was added dropwise to a solution of compound (2) (0·78 g, 0·002 mol) in dichloromethane (4 ml) and the reaction was followed by n.m.r. spectroscopy. When the reaction was complete the solution was diluted with chloroform and washed with dilute hydrochloric acid and water. The dried ($MgSO_4$) organic layer was concentrated to a syrup which afforded crystals of the *methoxymethyl ester* (10) (0·488 g, 63%) on addition of ethyl acetate; m.p. 174—176° (from acetate), $[\alpha]_D$ +185° (0·4% in $CHCl_3$), $\nu_{max.}$ (KBr) 1770 and 1725 (phthalimide C=O), 1770 (azetidinone), and 1745 (ester C=O) cm^{-1}, τ ($CDCl_3$) 8·45 and 8·32 (each 3H, s, *gem*-Me_2), 6·45 (3H, s, OMe), 5·31 (1H, s, 3-H), 4·63 (2H, s, O·CH_2), 4·57 and 4·36 (each 1H, d, *J* 2 Hz, β-lactam protons), and 2·12br (4H, s, aromatic) [Found: C, 55·3; H, 4·65; N, 7·1%; *M* (mass spectrum), 390. $C_{18}H_{18}N_2O_6S$ requires C, 55·4; H, 4·65; N, 7·2%; *M*, 390].

Reaction of the Ester (10) *with Hydrochloric Acid*.—Concentrated hydrochloric acid (6·4 ml) was added to a solution of compound (10) (2·60 g, 0·0067 mol) in acetone (160 ml). After 45 min the solution was diluted with chloroform, washed (twice) with water, dried ($MgSO_4$), and evaporated to a syrup. Addition of ether afforded *6α-phthalimidopenicillanic acid* (1·79 g, 77%), m.p. 195—197° (decomp.) (from chloroform–ether), $[\alpha]_D$ +208° (0·4% in $CHCl_3$), $\nu_{max.}$ (KBr) 1780 and 1725 (phthalimide C=O), 1760 (azetidinone), and 1725 (CO_2H) cm^{-1}, τ [$(CD_3)_2SO$] 8·50 and 8·39 (each 3H, s, *gem*-Me_2), 5·36 (1H, s, 3-H), 4·66 and 4·32 (each 1H, d, *J* 2 Hz, β-lactam protons), and 1·91 (4H, s, aromatic) (Found: C, 55·3; H, 4·3; N, 7·9. $C_{16}H_{14}N_2O_5S$ requires C, 55·5; H, 4·05; N, 8·1%).

The acid was treated with ethereal diazomethane to give methyl 6α-phthalimidopenicillanate (9), m.p. 176—177° (from chloroform–ether), $[\alpha]_D$ +206° (0·5% in $CHCl_3$), identical with a sample prepared by the method of Wolfe and Lee.[2]

Methoxymethyl 6β-Aminopenicillanate (7).—Triethylamine (3 ml, 0·02 mol) and the acid (8) (2·16 g, 0·01 mol) were stirred in dichloromethane (25 ml) until a solution was obtained. The solution was then cooled in acetone–solid carbon dioxide and chloromethyl methyl ether (1·61 g, 0·02 mol) in dichloromethane (5 ml) was added dropwise during 10 min. The solution was warmed to room temperature, washed (twice) with water, dried ($MgSO_4$), and evaporated to leave a pale yellow syrup. The syrup was dissolved in acetone–ether (1 : 1; 30 ml) and treated with a solution of toluene-*p*-sulphonic acid monohydrate (1·9 g, 0·01 mol) in acetone–ether (1 : 1; 30 ml) to yield the crystalline *salt* (2·7 g, 62%). The salt was recrystallised from methanol–ether but showed no distinct m.p.; $[\alpha]_D$ +142° (0·2% in EtOH), $\nu_{max.}$ (KBr) 1770 (azetidinone) and 1725 (ester C=O) cm^{-1} (Found: C, 47·1; H, 5·65; N, 6·4. $C_{17}H_{24}N_2O_7S_2$ requires C, 47·2; H, 5·55; N, 6·5%).

The salt (0·623 g, 0·015 mol) was suspended in chloroform and the mixture was shaken with sodium hydrogen carbonate solution. The organic layer was dried ($MgSO_4$) and evaporated to give the ester (7) (0·294 g, 75%) as a syrup, $\nu_{max.}$ (film) 3390 and 3340 (NH), 1775 (azetidinone), and 1750 (ester C=O) cm^{-1}, τ ($CDCl_3$) 8·46 and 8·39 (each 3H, s, *gem*Me_2), 6·7br (2H, s, exchanged with D_2O, NH_2), 6·53 (3H, s, OMe), 5·57 (1H, s, 3-H), 5·42 (1H, d, *J* 5 Hz, 6-H), 4·67 (2H, s, O·CH_2), and 4·48 (1H, d, *J* 5 Hz, 5-H).

Methoxymethyl 6α-Chloropenicillanate (17).—The chloro-acid (18) [12] (7·05 g, 0·03 mol) was converted into the ester (17) by the procedure used to prepare compound (6). The *methoxymethyl ester* was obtained as a pale yellow syrup (7·42 g, 88%), $\nu_{max.}$ (film) 1785 (azetidinone) and 1745 (ester C=O) cm^{-1}, τ ($CDCl_3$) 8·46 and 8·37 (each 3H, s, *gem*-Me_2), 6·47 (3H, s, OMe), 5·41 (1H, s, 3-H), 5·21 (1H, d, *J* 1·5 Hz, 6-H), and 4·66 (3H, superimposed signals, O·CH_2 and 5-H) [Found: *M* (mass spectrum), 279. $C_{10}H_{14}ClNO_4S$ requires *M*, 279].

Reaction of the Amine (7) *with Sodium Nitrite and Hydrochloric Acid*.—(*a*) Sodium nitrite (0·061 g, 0·00088 mol) in water (0·5 ml) was added dropwise during 10 min to a solution of the amine (7) (0·208 g, 0·0008 mol) in N-hydrochloric acid (2·7 ml) at 0°. The mixture was stirred for for 30 min, neutralised with sodium hydrogen carbonate solution, and extracted (twice) with chloroform. The extracts were washed with water, dried ($MgSO_4$), and concentrated to a syrup which was fractionated by silica gel chromatography. The derived pale yellow syrup was identical (i.r., n.m.r., and mass spectroscopy) with the chloro-ester (17).

(*b*) A solution of the amino-ester (7) (0·127 g, 0·00049 mol) in methan[^{2}H]ol (1·5 ml) and 2N-deuteriochloric acid (1·0 ml) at 0° was treated with a solution of sodium nitrite (0·69 g, 0·001 mol) in deuterium oxide (0·5 ml) during 10 min. The solution was stirred for 30 min, neutralised with sodium hydrogen carbonate solution, and extracted with chloroform. The organic layer was washed with water, dried ($MgSO_4$), and evaporated to leave a syrup which afforded a pure sample of the chloro-ester (17) (0·03 g, 22%) after alumina chromatography. The sample possessed an n.m.r. spectrum ($CDCl_3$) identical with that of unlabelled (17) except that the signal at τ 5·21 was absent; ; its mass spectrum showed a molecular ion at *m/e* 280. Low voltage studies indicated that the material was >90 mol % monodeuteriated.

Reaction of the Amino-ester (15) *with Sodium Nitrite and Hydrochloric Acid*.—(*a*) Compound (15) (0·148 g, 0·0057 mol) was treated with sodium nitrite and hydrochloric acid in a manner similar to that described for compound (7) [procedure (*a*)]. Work-up gave a syrup which was purified by alumina chromatography to afford the chloro-ester (17) (0·018 g, 11%) identical (i.r., n.m.r., and mass spectro-

[14] J. C. Sheehan and K. R. Henery-Logan, *J. Amer. Chem. Soc.*, 1962, **84**, 2983.

scopy) with the material obtained from the deamination of compound (7).

(*b*) Compound (15) (0·127 g, 0·00049 mol) was treated with sodium nitrite in deuteriochloric acid and methan[^{2}H]ol as described for compound (7) [procedure (*b*)]. The purified product (11%) was indistinguishable by n.m.r. and mass spectroscopy from the material which was isolated from compound (7). Low voltage studies indicated that the substance was >95 mol % monodeuteriated.

Reaction of the Chloro-ester (17) *with Sodium Nitrite and Hydrochloric Acid.*—A sample of the chloro-ester (17) (0·14 g, 0·005 mole) was treated with sodium nitrite in deuteriochloric acid and methan[^{2}H]ol as described for compound (7) [procedure (*b*)]. The n.m.r. and mass spectra of the product (0·10 g, 71% were identical with those of the starting material.

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