664. N-*Alkyl Derivatives of Penicillin V*

By T. Leigh

6-Alkylamino- and 6-dialkylamino-penicillanic acids have been prepared from 6-aminopenicillanic acid by reductive condensation of the last-named with aldehydes and ketones. Acylation of 6-alkylaminopenicillanic acids with phenoxyacetyl chloride gave the corresponding 6-(*N*-alkyl-*N*-phenoxyacetylamino)penicillanic acids or *N*-alkyl derivatives of penicillin V. Quaternary ammonium salts were obtained from 6-dimethylaminopenicillanic acid and alkyl halides.

Phenoxymethylpenicillin or penicillin V, together with wholly natural penicillins suffers from the disadvantage that they are hydrolysed by the enzyme penicillinase to the corresponding penicilloic acids. Semi-synthetic penicillins derived from 6-aminopenicillanic acid and hindered carboxylic acids, for example, 2,6-dimethoxyphenylpenicillin, are relatively resistant to penicillinase.[1] It was considered that enzymatic hydrolysis might be arrested in an alternative manner by the introduction of an alkyl group at the amidic nitrogen atom of the penicillin molecule. Sheehan *et al.*[2] have prepared 6-tritylamino- and 6-(2,4-dinitrophenylamino)-penicillanic acids by the alkylation of 6-aminopenicillanic acid with the corresponding halides but, owing to steric hindrance, it is not possible to acylate these substituted penicillanic acids. It was thought that if the simpler n-alkylaminopenicillanic acids could be obtained then acylation of these should be possible.

The reductive condensation of amines with aldehydes or ketones in the presence of

[1] F. P. Doyle, K. Hardy, J. H. C. Nayler, M. J. Sonlal, E. R. Stove, and H. R. J. Waddington, *J.*, 1962, 1453.

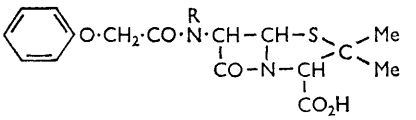
[2] J. C. Sheehan and K. R. Henery-Logan, *J. Amer. Chem. Soc.*, 1959, **81**, 5838; W. A. Bolhofer, J. C. Sheehan, and E. L. A. Abrams, *ibid.*, 1960, **82**, 3437.

hydrogen and a catalyst [3] appeared to be a method suitable for the alkylation of 6-aminopenicillanic acid. This procedure has been applied by Bowman and Stroud [4] for the preparation of alkyl and dialkyl derivatives of amino-acids. These authors found that, with a straight-chain aldehyde, monoalkylation only occurs if the amino-acid has a branched chain in the β- or γ-position to the carboxyl group and that with aldehydes branched in the α-position all the amino-acids examined yielded monoalkyl derivatives. An exception was formaldehyde; with this aldehyde the only product obtained from highly hindered amino-acids was the dimethyl derivative.

6-Aminopenicillanic acid was alkylated by reductive condensation with both aldehydes and ketones. Catalytic hydrogenation of aliphatic aldehydes in the presence of platinum oxide and one equivalent of 6-aminopenicillanic acid gave mixtures of 6-alkylamino-6-dialkylamino-, and unchanged 6-aminopenicillanic acids. In contrast to the findings of Bowman and Stroud in the simpler amino-acid series it was possible to prepare the monomethyl derivative. The alkylaminopenicillanic acids, in particular 6-methylaminopenicillanic acid, proved to be too unstable to permit isolation and, accordingly, the crude mixtures from the alkylation reaction were treated with phenoxyacetyl chloride and the resulting mixtures of 6-dialkylamino-, 6-(*N*-alkyl-*N*-phenoxyacetylamino)-, and 6-phenoxyacetylamino-penicillanic acids were then separated. Both the *N*-butyl and *N*-isobutyl derivatives of penicillin V were readily separated from the accompanying unalkylated penicillin by partition chromatography but resolution of the mixture of penicillin V and *N*-methylpenicillin V was not possible by this method. Fortunately, the solubilities in ethanol of the sodium salts of the last two compounds proved to be widely different. Striking characteristics of these *N*-alkyl derivatives of penicillin V are much increased optical activity, resistance to the action of penicillinase, the relatively high stability of the two butyl derivatives towards alkali, the low stability of *N*-methylpenicillin V in the presence of acid, and poor antibacterial activity. This last property provided an unwelcome but reliable measure of freedom from contamination with unsubstituted penicillin V.

Reductive condensation of 6-aminopenicillanic acid with an excess of formaldehyde afforded an excellent yield of the 6-dimethylamino-derivative. With ketones, only monosubstitution occurred and 6-isopropylaminopenicillanic acid was prepared both by the catalytic method and by the addition of sodium borohydride to a mixture of the amino-acid, water, and acetone. Other monoalkylated penicillanic acids were prepared from p-chlorobenzyl methyl ketone, salicylaldehyde, and 3,5-dichlorosalicylaldehyde.

Properties of some *N*-alkyl derivatives of penicillin V



R	Molecular rotation of Na salt in water	Half life in minutes in N-NaOH	Half life in minutes in N-HCl	Minimum inhibitory concentration *vs. S. aureus* in μg./ml.
Me	+1380°	Few	60	20
Bu^n	1373	30	180	20
Bu^i	1507	60	180	20
H	915	Few	180	0·02

In contrast with 6-methylaminopenicillanic acid, the dimethyl derivative is stable and unlike the parent amino-acid it forms a stable hydrochloride. Quaternary ammonium salts were quickly formed when methyl iodide or benzyl bromide was added to a solution of 6-dimethylaminopenicillanic acid in acetonitrile. Attempts to acylate 6-isopropylaminopenicillanic acid were unsuccessful; this secondary amine did, however, form a

[3] A. Skita and F. Keil, *Ber.*, 1928, **61***B*, 1452, 1682.
[4] R. E. Bowman and H. H. Stroud, *J.*, 1950, 1342, 1346.

nitroso-derivative. By the addition of sodium nitrite to a mixture of an aqueous solution of 6-isopropylaminopenicillanic acid hydrochloride and benzene, 6-(*N*-nitroso-*N*-isopropylamino)penicillanic acid was obtained. Complete transfer of optical activity from the aqueous to the benzene phase suggested that this reaction proceeded in excellent yield but evaporation of the benzene solution left a pale yellow oil which decomposed when exposed to the atmosphere. Infrared absorption confirmed the presence of the *N*-nitroso-group but attempts to reduce the compound to the hydrazine failed.

Antibacterial tests on these derivatives of 6-aminopenicillanic acid were carried out by Dr. A. R. Martin and Dr. D. W. F. Wheater. None of the compounds showed useful activity.

Experimental

N-*Methylpenicillin V*.—A mixture of sodium 6-aminopenicillanate (53 g.), 40% aqueous formaldehyde (30 c.c.), water (600 c.c.), and platinum oxide (5 g.) was stirred in the presence of hydrogen at 100 atm. and 25° for 2 hr. After removal of the platinum, acetone (500 c.c.) was added followed by phenoxyacetyl chloride (36 g.). The last-named was added with stirring during 30 min. and during this time *N*-methylmorpholine (30 g.) was added to maintain the pH at 6·0—7·0. Phosphoric acid was added until the pH was 2·5 and the mixture was extracted with ethyl acetate (800 c.c.). Sufficient N–sodium hydroxide was added to the extract until the pH was 7·0. The mixture was separated and n-butanol (1000 c.c.) was added to the aqueous phase. The resulting mixture was distilled *in vacuo* until the volume was reduced to one-half of the original, when a large part of the sodium salt of penicillin V was precipitated. The latter was filtered off and the filtrate was distilled *in vacuo* at a temperature not exceeding 30° until a viscous oily residue remained. The residue was triturated with ether (200 c.c.) when it solidified. The mixture was filtered and the solid was washed with ether whereby a crude mixture of the sodium salts of *N*-methylpenicillin V and penicillin V was obtained. The mixture was dissolved in water, the solution was acidified, and the mixed penicillins were extracted with ethyl acetate (100 c.c.). The ethyl acetate extract was poured on to a column prepared from a mixture of silica gel (300 g.), water (120 c.c.), *N*-ethylhexamethyleneimine (17 g.), and ethyl acetate. The mixture of penicillins was eluted from the column with wet ethyl acetate. Surprisingly, even when the loading was greatly reduced, neither this column nor a phosphate buffer–silica column resolved this mixture of penicillins. The column did, however, remove small amounts of decomposition products which otherwise interfered with the subsequent separation by crystallisation. The eluate was shaken with phosphoric acid, the mixture was separated, and sufficient N-sodium hydroxide was added to the ethyl acetate to raise the pH to 7·0. The aqueous phase was separated and dried *in vacuo* from the frozen state. The residue was stirred with ethanol (50 c.c.), the mixture was filtered to remove the undissolved sodium salt of penicillin V, and the filtrate was evaporated to dryness *in vacuo*. Crystallisation of the residue from anhydrous acetone gave pure *sodium* 6-(N-*methyl*-N-*phenoxyacetylamino*)*penicillanate monohydrate*, $[\alpha]_D^{25}$ +330° (*c* 1% in water) (Found: C, 50·7; H, 5·3; N, 6·3. $C_{17}H_{19}N_2NaO_5S,H_2O$ requires C, 50·5; H, 5·2; N, 6·9%). The yield from 6-aminopenicillanic acid was 5%.

N-*n-Butylpenicillin V and* N-*Isobutylpenicillin* V.—These compounds were prepared in 25% yield by a procedure similar to that described above. Unlike *N*-methylpenicillin V they were very easily separated from the accompanying penicillin V by partition chromatography. They quickly travelled down the silica–water–tertiary base column leaving behind the penicillin V. *Sodium* 6-(N-*n-butyl*-N-*phenoxyacetylamino*)*penicillanate* crystallised (acetone) as the *hemihydrate*, $[\alpha]_D^{25}$ +314° (*c* 0·5% in water) (Found: C, 55·4; H, 6·2; N, 5·7. $C_{20}H_{25}N_2NaO_5,\frac{1}{2}H_2O$ requires C, 54·9; H, 6·0; N, 6·4). *Sodium* 6-(N-*isobutyl*-N-*phenoxyacetylamino*)*penicillanate* crystallised from acetone as the *monohydrate monoacetonate*, $[\alpha]_D^{25}$ +299° (*c* 0·5% in water) (Found: C, 54·8; H, 6·5; N, 5·7. $C_{20}H_{25}N_2NaO_5S,C_3H_6O,H_2O$ requires C, 54·8; H, 6·5; N, 5·6%).

6-*Dimethylaminopenicillanic Acid Hydrochloride*.—A mixture of 6-aminopenicillanic acid (8·8 g.), water (50 c.c.), *N*-methylmorpholine (4·2 g.), platinum oxide (1 g.), and 40% aqueous formaldehyde solution (18 c.c.) was stirred in the presence of hydrogen at 100 atm. and 25° for 20 hr. Sufficient concentrated hydrochloric acid was added to reduce the pH to 4·0 and the mixture was filtered. More hydrochloric acid was added to the filtrate until the pH was 1·4 and the solution was concentrated *in vacuo*, at below 30°, to a syrup. Ethanol (20 c.c.) was

added and a crystalline solid was obtained (5·7 g.). Recrystallisation from ethanol and water gave pure 6-*dimethylaminopenicillanic acid hydrochloride*, m. p. 175°, $[\alpha]_D^{25}$ +272° (*c* 0·5% in water) (Found: C, 42·9; H, 6·0; Cl, 12·6; N, 9·8. $C_{10}H_{16}N_2O_3S$,HCl requires C, 42·9; H, 6·1; Cl, 12·7; N, 10·0%).

6-*Dimethylaminopenicillanic Acid Methiodide*.—The addition of methyl iodide (1·5 c.c.) to a solution of 6-dimethylaminopenicillanic acid (2·4 g.) in acetonitrile (10 c.c.) caused the precipitation of a crystalline *solid* (2·1 g.) which analysed as a 1 : 1 complex of the methiodide and the corresponding betaine, m. p. 175°, $[\alpha]_D^{23}$ +219° (*c* 0·3% in water) (Found: C, 40·5; H, 6·0; I, 19·7; N, 8·7. $2(C_{11}H_{18}N_2O_3S)$,HI requires C, 41·0; H, 5·8; I, 19·7; N, 8·7%).

6-*Isopropylaminopenicillanic Acid*.—A mixture of sodium 6-aminopenicillanate (8·8 g.), water (50 c.c.), acetone (12 c.c.), and platinum oxide (0·5 g.) was stirred with hydrogen at 100 atm. for 20 hr. Concentrated hydrochloric acid was added until the pH was reduced to 3·3. The mixture was filtered, enough sodium hydroxide was added to the filtrate to raise the pH to 4·2, and the solution was evaporated to dryness *in vacuo* at below 30°. The residue was extracted with ethanol (500 c.c.) and the extract was concentrated *in vacuo* to approximately 30 c.c. when 6-isopropylaminopenicillanic acid quickly crystallised (3·6 g.), m. p. 158° $[\alpha]_D^{25}$ +288° (*c* 0·5% in ethanol). The *hydrochloride*, m. p. 165°, was prepared for analysis (Found: C, 44·5; H, 6·8; Cl, 12·1; N, 9·2. $C_{11}H_{18}N_2O_3S$,HCl requires C, 44·9; H, 6·3; Cl, 12·1; N, 9·5%).

Reductive alkylation of 6-aminopenicillanic acid with *p*-chlorobenzyl methyl ketone gave 6-(1-p-*chlorophenyl*-2-*propylamino*)*penicillanic acid*, m. p. 152°, $[\alpha]_D^{23}$ +213° (*c* 0·3% in ethanol) (Found: C, 55·7; H, 5·8; Cl, 9·2; N, 7·5. $C_{17}H_{19}ClN_2O_3S$ requires C, 55·7; H, 5·2; Cl, 9·6; N, 7·6%). From salicylaldehyde there was obtained 6-(2-*hydroxybenzylamino*)*penicillanic acid*, m. p. 164°, $[\alpha]_D^{25}$ +261° (*c* 0·5% sodium salt in water) (Found: C, 56·0; H, 5·6; N, 8·5. $C_{15}H_{18}N_2O_4S$ requires C, 55·9; H, 5·6; N, 8·7%). Reductive alkylation with 3,5-dichlorosalicylaldehyde gave 6-(3,5-*dichloro*-2-*hydroxybenzylamino*)*penicillanic acid*, m. p. 168, $[\alpha]_D^{25}$ +172° (*c* 0·5% in acetone).

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