272. *Derivatives of 6-Aminopenicillanic Acid. Part I. α-Aminobenzylpenicillin and Some Related Compounds.*

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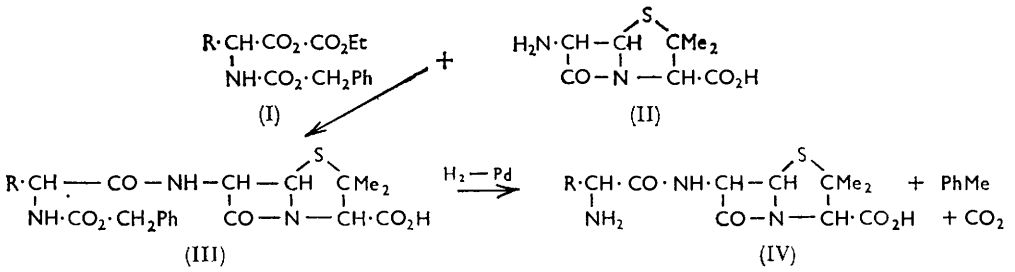
The two epimers of α-aminobenzylpenicillin, a new broad-spectrum antibiotic, have been synthesised from 6-aminopenicillanic acid. Intermediates for the preparation of some related penicillins are also described.

Despite the availability of several different penicillins [1] for the treatment of infections caused by Gram-positive bacteria, there remained a need for a broad-spectrum penicillin which would also be effective orally against Gram-negative organisms. D-4-Amino-4-carboxybutylpenicillin [2] (Cephalosporin N, Synnematin B) shows promising activity against certain Gram-negative organisms, particularly *Salmonellae*, but must be administered by injection since it is only poorly absorbed from the gut: it does not appear to have been produced on a large scale. It apparently owes its rather unusual type of antibiotic activity to the presence of the free amino-group, since when this is acylated or otherwise substituted the antibacterial spectrum reverts to the more common Gram-positive type.[3] A similar trend is observed on *N*-acylation of 4-aminobenzylpenicillin.[4] It was therefore of interest to introduce an aliphatic amino-group into the molecule of benzylpenicillin,

[1] Ing, *Proc. Chem. Soc.*, 1961, 6.
[2] Abraham, Newton, Schenck, Hargie, Olson, Schuurmans, Fisher, and Fusari, *Nature*, 1955, **176**, 551.
[3] Newton and Abraham, *Biochem. J.*, 1954, **58**, 103.
[4] Tosoni, Glass, and Goldsmith, *Biochem. J.*, 1958, **69**, 476; Doyle and Nayler, B.P. Spec. 838,974/1960.

an objective which became attainable with the isolation [5] in these laboratories of 6-aminopenicillanic acid (II).

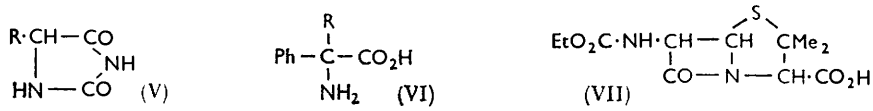
Since 6-aminopenicillanic acid is itself optically active, the desired α-aminobenzylpenicillin (IV; R = Ph) can exist in two epimeric forms. α-Aminophenylacetic acid was resolved by essentially known methods,[6,7] and each enantiomorph with benzyl chloroformate in alkali gave the *N*-benzyloxycarbonyl derivative. Each of these was then



treated with ethyl chloroformate and triethylamine, and the resulting mixed anhydride (I; R = Ph) was coupled *in situ* with 6-aminopenicillanic acid (II). Each epimeric product (III; R = Ph) was partially purified by extraction in ether as the free acid and then back into aqueous sodium hydrogen carbonate. Hydrogenolysis of the benzyloxycarbonyl group was effected at room temperature and pressure over a palladium catalyst. An unusually large proportion of palladium was used in order to overcome the tendency towards catalyst poisoning, presumably by the sulphur atom of the penicillin. Completion of hydrogenolysis was indicated by paper chromatography and the resulting epimers of α-aminobenzylpenicillin were isolated in pure crystalline form by concentration of the aqueous solutions and adjustment to pH 4.

The penicillin (IV; R = Ph) derived from D(−)-α-aminophenylacetic acid proved to be more soluble in water than its epimer and was also the more active against most bacteria, and its bacteriological and preliminary pharmacological investigation has been reported.[8] Its satisfactory absorption after oral administration is doubtless due in part to its unusual stability towards acid.[9]

Numerous other penicillins containing amino-substituted side-chains were prepared from 6-aminopenicillanic acid essentially by the same method. The presence of the α-amino-substituent always led to penicillins with marked stability to acids, but this was lost when the substituent was remote from the amide linkage, as in 5-aminopentylpenicillin. In these experiments optically inactive or racemic amino-acids were used and complete purification of the penicillins was not attempted. For this reason, only the preparation of the intermediate amino-acids and their *N*-benzyloxycarbonyl derivatives is reported in the Experimental section: details of the penicillins obtained therefrom are in a patent.[10]



In this work several new DL-α-aminoarylacetic acids were prepared through the corresponding 5-arylhydantoins (V) essentially as described by Harvill and Herbst,[11] but the

[5] Batchelor, Doyle, Nayler, and Rolinson, *Nature*, 1959, **183**, 257; Doyle, Nayler, and Rolinson, B.P. Spec. 870,396/1961.
[6] Betti and Mayer, *Ber.*, 1908, **41**, 2073.
[7] Ingersoll and Adams, *J. Amer. Chem. Soc.*, 1925, **47**, 1168.
[8] Rolinson and Stevens, *Brit. Med. J.*, 1961, **2**, 191; Brown and Acred, *ibid.*, p. 197.
[9] Doyle, Nayler, Smith, and Stove, *Nature*, 1961, **191**, 1091.
[10] Doyle, Nayler, and Smith, B.P. Spec. 873,049/1961.
[11] Harvill and Herbst, *J. Org. Chem.*, 1944, **9**, 21.

isolation procedure could often be simplified and the yields improved by using sodium hydroxide instead of barium hydroxide for the final hydrolysis. Treatment of the amino-acids in dilute aqueous sodium hydroxide with benzyl chloroformate generally gave good yields of the *N*-benzyloxycarbonyl derivatives, but this procedure failed with α-amino-α-phenylpropionic acid (VI; R = Me) and α-aminodiphenylacetic acid (VI; R = Ph) for which special methods were devised. The *N*-benzyloxycarbonyl derivatives of the last two acids were also exceptional in that attempted coupling with 6-aminopenicillanic acid by way of the mixed ethoxyformic anhydrides merely gave ethoxypenicillin (VII), identified by paper chromatography. Similar abnormal acylations have been noted with the ethoxyformic anhydrides of other trisubstituted acetic acids and can probably be ascribed to steric factors.[12]

The completeness of the hydrogenolysis of the various benzyloxycarbonylamino-substituted penicillins was established by paper chromatography. This technique also revealed that cleavage of the benzyloxycarbonyl group from the chloro-compounds (III; R = *o*-, *m*-, or *p*-Cl·C_6H_4) was accompanied by extensive dehalogenation, much α-amino-benzylpenicillin (IV; R = Ph) being produced.

Experimental

Chromatography of penicillins on paper strips was carried out by Mr. F. R. Batchelor and his assistants. The solvent system was usually butanol–ethanol–water (4 : 1 : 5, top layer) and the chromatograms were developed on agar plates seeded with *B. subtilis*.

5-*Arylhydantoins*.—(*a*) *m*-Chlorobenzaldehyde (50 g.) in ethanol (350 ml.) was added with stirring to a solution of sodium cyanide (26·2 g.) and ammonium carbonate (120 g.) in water (350 ml.). The mixture was stirred at room temperature for 7 days, then concentrated under reduced pressure to one-third of its volume and acidified with concentrated hydrochloric acid. After 1 hr. the yellow solid was collected, washed, and dried (61 g.; m. p. 210—213°). This material was suitable for hydrolysis to the amino-acid, but a portion was recrystallised to constant m. p. from water to give pure 5-m-*chlorophenylhydantion*, m. p. 177° (Found: C, 51·3; H, 3·5; N, 13·4; Cl, 16·6. $C_9H_7ClN_2O_2$ requires C, 51·3; H, 3·4; N, 13·3; Cl, 16·8%).

(*b*) In the same way *p*-tolualdehyde gave 5-p-*tolylhydantoin* (85%), m. p. 155° (from aqueous alcohol) (Found: C, 63·4; H, 5·5; N, 14·8. $C_{10}H_{10}N_2O_2$ requires C, 63·1; H, 5·3; N, 14·7%).

(*c*) Similarly 1-naphthaldehyde gave 5-1′-*naphthylhydantoin* (65%), m. p. 225—227° (from alcohol) (Found: C, 69·3; H, 4·6; N, 12·4. $C_{13}H_{10}N_2O_2$ requires C, 69·0; H, 4·4; N, 12·4%).

Hydrolysis of 5-Arylhydantoins.—(*a*) A mixture of 5-*m*-chlorophenylhydantoin (69·6 g.) and 10% sodium hydroxide solution (320 ml.) was refluxed with stirring for 24 hr., then the yellow solution was treated with charcoal and filtered. The filtrate was brought to pH 7 with concentrated hydrochloric acid and kept at 5° for 10 hr., whereupon the product crystallised. A second crop, obtained by concentrating the mother-liquor *in vacuo*, was combined with the main crop, suspended in water, and treated with sufficient concentrated hydrochloric acid to give a clear solution. On neutralisation with 2N-sodium hydroxide pure *α-amino-α-m-chlorophenylacetic acid* (46 g., 75%), having m. p. 250—254° (decomp.) was obtained (Found: C, 52·0; H, 4·6; Cl, 18·9; N, 7·3. $C_8H_8ClNO_2$ requires C, 51·8; H, 4·4; Cl, 19·1; N, 7·5%).

(*b*) By this simplified hydrolysis procedure the following α-aminoacetic acids were obtained in the improved yields indicated: α-*p*-tolyl- (58%), m. p. 260—265° (with sublimation) (Darapsky *et al.*[13] report sublimation at 228°); α-*p*-methoxyphenyl- (76%), m. p. 264—266° (with sublimation) [Harvill and Herbst [11] report sublimation at 230°, m. p. 284—285° (decomp.)]; α-*o*-chlorophenyl- (95%), m. p. 218—220° (decomp.) (lit.,[11] m. p. 219·5°); α-*p*-chlorophenyl- (86%), m. p. 269—271° (decomp.) [lit.,[11] m. p. 261—262° (decomp.)].

N-*Benzyloxycarbonyl Derivatives of Amino-Acids*.—(*a*) Benzyl chloroformate (25 g.) and N-sodium hydroxide were simultaneously added dropwise during 45 min. to a stirred solution of D(−)-α-amino-α-phenylacetic acid [6] {22 g., $[\alpha]_D^{20}$ −158° (*c* 7% in H_2O containing 1·36 equiv. of HCl)} in N-sodium hydroxide (146 ml.) at 0°, the rates of addition being such as to keep the mixture at pH 8—9 throughout. The solution (pH 8) was stirred for 30 min. at 0°, then for

[12] Brain, Doyle, Hardy, Long, Mehta, Miller, Nayler, Soulal, Stove, and Thomas, following paper.
[13] Darapsky, Germscheid, Kreuter, Engelmann, Engels, and Trinius, *J. prakt. Chem.*, 1936, **146**, 219.

a further 30 min. whilst it attained room temperature, and washed with ether (2 × 150 ml.). It was then cooled to 0° and added slowly with stirring to ice-cold 5N-hydrochloric acid (15 ml.). The oil which separated soon solidified and was collected, washed with water, and dried *in vacuo* (yield 40·3 g.). Recrystallisation from ethanol–water gave needles of D(−)-*α-benzyloxycarbonylamino-α-phenylacetic acid*, m. p. 130—130·5°, $[\alpha]_D^{21}$ −119° (*c* 4% in EtOH) (Found: C, 67·3; H, 5·3; N, 4·8. $C_{16}H_{15}NO_4$ requires C, 67·4; H, 5·3; N, 4·9%).

(*b*) L(+)α-Amino-α-phenylacetic acid [7] {$[\alpha]_D^{20}$ +158° (*c* 7% in H_2O containing 1·36 equiv. of HCl)} similarly gave L(+)-*α-benzyloxycarbonylamino-α-phenylacetic acid*, needles (from ethanol–water), m. p. 130—130·5°, $[\alpha]_D^{21}$ +117° (*c* 4% in EtOH), not increased by further crystallisation from ethanol, benzene, or chloroform (Found: C, 67·3; H, 5·7; N, 4·9%).

(*c*) In the same way DL-α-amino-α-phenylacetic acid gave DL-*α-benzyloxycarbonylamino-α-phenylacetic acid*, m. p. 134—135° (Found: C, 67·3; H, 5·5; N, 4·9%).

(*d*) The DL-α-amino-acids listed in the Table were similarly converted into N-*benzyloxycarbonyl derivatives* in yields of 50—90%.

N-Benzyloxycarbonyl derivatives of DL-α-amino-acids.

No.	Derivative of α-amino-acid	Cryst. from	M. p.
1	α-Amino-octanoic	Aq. EtOH	93—94°
2	α-Amino-cyclohexyl	Aq. EtOH	136—137
3	α-Amino-*p*-tolyl	CCl_4	114
4	α-Amino-*p*-methoxyphenyl	Aq. EtOH, then CCl_4	105
5	α-Amino-*o*-chlorophenyl	CCl_4	109
6	α-Amino-*m*-chlorophenyl	CCl_4	110
7	α-Amino-*p*-chlorophenyl	CCl_4, then C_6H_6	135
8	α-Amino-*m*-nitrophenyl	Aq. EtOH, then CCl_4	130
9	α-Amino-1-naphthyl *	Aq. EtOH, then C_6H_6	144—145
10	α-Amino-2-furyl	,, ,,	121—122
11	*threo*-β-phenylserine	Aq. EtOH, then CCl_4	96
12	α-*N*-Methylamino-α-phenylacetic	Light petroleum	93—94

	Found (%)					Required (%)			
No.	C	H	N	Cl	Formula	C	H	N	Cl
1	65·6	7·9	4·9	—	$C_{16}H_{23}NO_4$	65·5	7·9	4·8	—
2	66·2	7·5	4·6	—	$C_{16}H_{21}NO_4$	66·0	7·3	4·8	—
3	68·0	6·0	4·7	—	$C_{17}H_{17}NO_4$	68·2	5·7	4·7	—
4	65·0	5·5	4·1	—	$C_{17}H_{17}NO_5$	64·8	5·4	4·4	—
5	60·0	4·3	4·5	10·9	$C_{16}H_{14}ClNO_4$	60·1	4·4	4·4	11·1
6	60·2	4·8	4·5	11·1	$C_{16}H_{14}ClNO_4$	60·1	4·4	4·4	11·1
7	60·0	4·3	4·4	11·0	$C_{16}H_{14}ClNO_4$	60·1	4·4	4·4	11·1
8	58·3	4·6	8·2	—	$C_{16}H_{14}N_2O_6$	58·2	4·3	8·5	—
9	71·5	5·4	4·4	—	$C_{20}H_{17}NO_4$	71·6	5·1	4·2	—
10	61·3	4·7	4·9	—	$C_{14}H_{13}NO_5$	61·1	4·8	5·1	—
11	63·3	5·4	4·3	—	$C_{17}H_{17}NO_5,0·5H_2O$	63·0	5·4	4·3	—
12	68·3	6·0	4·7	—	$C_{17}H_{17}NO_4$	68·2	5·7	4·7	—

* The crude product from the hydrolysis of 5-1′-naphthylhydantoin was used without purification.

(*e*) DL-α-Amino-α-phenylpropionic acid [14] (10 g.) was dissolved in a mixture of water (500 ml.), acetone (500 ml.), and pyridine (34 ml.) by refluxing for 7 hr., then cooled to 0°. Benzyl chloroformate (50 g.) was added and the mixture was stirred at 0° for 3 hr., then concentrated under reduced pressure to half its volume and acidified. The oil which separated was extracted in ether (3 × 80 ml.), and the extracts were concentrated to 100 ml., then extracted with N-sodium hydroxide (200 ml. in 3 portions). The aqueous solution was acidified and set aside at 0°, whereupon the oily product solidified. DL-*α-Benzyloxycarbonylamino-α-phenylpropionic acid* (16·7 g.) was collected, washed with water, dried *in vacuo*, and crystallised from carbon tetrachloride; it had m. p. 107—109° (Found: C, 68·0; H, 5·9; N, 4·4. $C_{17}H_{17}NO_4$ requires C, 68·2; H, 5·7; N, 4·7%).

(*f*) α-Amino-αα-diphenylacetic acid [15] was stirred in water with triethylamine (2 equiv.) until a clear solution resulted, which was then evaporated *in vacuo*. The resulting triethylamine salt (11·2 g., dried over P_2O_5 *in vacuo*) was suspended in dimethylformamide (100 ml.), cooled to −5°, and treated with stirring with triethylamine (19 ml.) and benzyl chloroformate

[14] Steiger, *Org. Synth.*, Coll. Vol. III, p. 88.
[15] Newman and Edwards, *J. Amer. Chem. Soc.*, 1954, **76**, 1842.

(18 g.). The mixture was stirred for 13 hr. at 0—5°, then diluted with ice–water (100 ml.). After 1 hr. at room temperature the resulting clear solution was evaporated *in vacuo* and the residual moist solid suspended in water (100 ml.) and brought to pH 10 by addition of 2N-sodium hydroxide. The solution was clarified by ether extraction, then slowly added to an excess of dilute hydrochloric acid with stirring at 0°; this precipitated an oil which gradually solidified and was collected, dried *in vacuo*, and crystallised from carbon tetrachloride and then from light petroleum, to give *α-benzyloxycarbonylamino-αα-diphenylacetic acid* (3 g.), m. p. 130—130·5° (Found: C, 73·1; H, 5·4; N, 3·9. $C_{22}H_{19}NO_4$ requires C, 73·1; H, 5·3; N, 3·9%).

α-Aminobenzylpenicillin.—(*a*) Ethyl chloroformate (4·8 ml.) was added to an ice-cold solution of D(−)-α-benzyloxycarbonylamino-α-phenylacetic acid (14·3 g.) and triethylamine (8·3 ml.) in dry acetone (420 ml.). The mixture was stirred at 0° for 5 min., during which triethylamine hydrochloride was precipitated and the mixed anhydride formed in solution. The suspension was cooled to −50° and stirred vigorously during addition as rapidly as possible of an ice-cold solution of 6-aminopenicillanic acid (13 g.) in 3% sodium hydrogen carbonate solution (420 ml.), the temperature of the mixture being kept at <0°. The resulting clear solution was stirred for 30 min. at 0°, then for a further 30 min. whilst it attained room temperature, and was finally extracted with ether (3 × 400 ml.), only the aqueous phase being retained. This aqueous solution was brought to pH 2 by the addition of hydrochloric acid and the 6-[D(−)-α-(benzyloxycarbonylamino)-α-phenylacetamido]penicillanic acid (III; R = Ph) so liberated was extracted into ether (150 ml. in 3 portions). This intermediate was partially purified by re-extracting it into aqueous sodium hydrogen carbonate as the sodium salt and then, after re-adjustment to pH 2, back into ether as the free acid. Finally, it was re-converted into the sodium salt by shaking the ether solution with sufficient 3% sodium hydrogen carbonate to give a neutral aqueous phase, separating the latter, and evaporating it at low temperature and pressure. Drying over phosphorus pentoxide *in vacuo* gave the moderately pure sodium salt (13 g.).

A solution of this intermediate (20·4 g.) in water (250 ml.) was added to an aqueous suspension (125 ml.) of 30% palladium–barium carbonate (38 g.) previously shaken under hydrogen for 1 hr. The mixture was hydrogenated at room temperature and pressure for 1 hr., then filtered. The combined filtrate and aqueous washings were treated with N-hydrochloric acid to pH 2, then washed with ether (3 × 100 ml.). The aqueous phase was adjusted to pH 4·65 by means of aqueous 3% sodium hydrogen carbonate and then concentrated at low temperature and pressure to a volume of about 50 ml., whereupon fine needles separated. After 30 min., these were collected, washed with a little cold water, and dried *in vacuo* (P_2O_5) to give pure *6-[D(−)-α-amino-α-phenylacetamido]penicillanic acid monohydrate* (5·5 g.), $[\alpha]_D^{21}$ +281° (*c* 1 in H_2O), decomp. *ca.* 202°. Recrystallisation from water did not change the rotation (Found: C, 52·5; H, 5·7; N, 11·9; S, 8·9. $C_{16}H_{19}N_3O_4S,H_2O$ requires C, 52·3; H, 5·8; N, 11·4; S, 8·7%). A further 9 g. of less pure product was obtained by concentrating the aqueous filtrate. Like the first crop, it gave only a single zone of antibiotic activity on a paper chromatogram, the R_F value being less than that of the benzyloxycarbonyl intermediate.

(*b*) L(+)-α-Benzyloxycarbonylamino-α-phenylacetic acid (14·3 g.) was converted into the mixed anhydride with ethyl chloroformate as in (*a*) and coupled with 6-aminopenicillanic acid (13 g.) to give 17·6 g. of moderately pure sodium 6-[L(+)-α-(benzyloxycarbonylamino)-α-phenylacetamido]penicillanate. Hydrogenation of this intermediate on the scale and by the method described in (*a*) gave a first crop (6·2 g.) of pure anhydrous *6-[L(+)-α-amino-α-phenylacetamido]penicillanic acid*, $[\alpha]_D^{20}$ +209° (*c* 0·2 in H_2O), decomp. *ca.* 205°. Recrystallisation from water did not change the rotation (Found: C, 54·9; H, 5·6; N, 11·8; S, 9·2. $C_{16}H_{19}N_3O_4S$ requires C, 55·0; H, 5·5; N, 12·0; S, 9·2%). A further 6 g. of less pure product was obtained by concentrating the aqueous filtrate. Like the first crop, it gave only a single zone of antibiotic activity on a paper chromatogram, the R_F value being less than that of the benzyloxycarbonyl intermediate.

The authors thank Mr. R. G. Marsh for experimental assistance.

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BROCKHAM PARK, BETCHWORTH, SURREY. [*Received, October 27th*, 1961.]