THE PREPARATION OF SPARINGLY SOLUBLE PENICILLIN SALTS

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SINCE its introduction in 1948, procaine benzylpenicillin[1,2,3] has been the most widely used repository type of penicillin. Recently, however, two other amine salts of penicillin have been introduced: *N*-methyl-2-hydroxy-1 : 2-diphenylethylamine[4] and *NN'*-dibenzylethylene diamine[5]. The latter preparation especially has clinical indications because of the very prolonged, although low, blood levels[6].

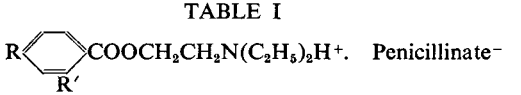
(1) *Dialkylaminoalkylbenzoates.*

We were interested to know if dialkylaminoalkyl benzoates, which, other than procaine, have practically no anæsthetic activity, would give insoluble penicillin salts.

Rhodehamel[7] has shown that the introduction of substituents in the *ortho* position with respect to the carboxyl group of procaine, has little effect on the solubility of the penicillin salts. This observation is also confirmed by the report on a sparingly soluble penicillin salt of "oxy-procaine" (diethylaminoethyl-4-aminosalicylate)[8].

We found, however, that when we replaced the *p*-amino group by other substituents, the products failed to precipitate sodium benzylpenicillin from the aqueous solution (Table I). Only the *p*-hydroxybenzoate gave an insoluble salt[9].

TABLE I



R	R'	Solubility in water at 25° C. per cent	Reference
NH_2	H	0·7–0·8	1
NH_2	Cl	0·3	7
NH_2	CH_3	0·3	7
NH_2	OH	0·6	
OH	H	0·8	

Not only the substituents of the benzene ring, but also the configuration of the ester group has a great importance. All benzoates, even the *p*-amino- or *p*-hydroxy benzoate of dimethylaminoethanol

$$(RC_6H_4COOCH_2CH_2N(CH_3)_2)$$

failed to precipitate penicillin. Anæsthetics such as larocaine or tutocaine did not give insoluble salts. Tetracaine gave an oil, which could not be crystallised.

The dimethylaminoethyl and diethylaminoethyl esters of nicotinic and *iso*nicotinic acid failed also to give insoluble salts.

(2) *Amino-acid Esters.*

Another interesting approach to the preparation of sparingly soluble penicillin salts with innocuous amines, has been described by Tosoni and Moloney[10], using esters of aromatic amino-acids. They noticed that the ethyl and *iso*amyl esters of L-tyrosine gave insoluble penicillin salts, but not the methyl or the *iso*butyl ester. These anomalous results, which we confirmed, led us to examine different phenylalanine esters. We found that all DL-phenylalanine esters, from methyl to *n*-amyl (but not the amide), gave sparingly soluble salts. No difference in solubility was noticed between the penicillin salts of D- and L-phenylalanine ethyl ester (see Table V). However, the rotation data show that DL-phenylalanine ester-penicillin, contains a larger proportion of the L-amino-acid, but the separation of one of the two optical isomers is not achieved as with *N*-methyl-2-hydroxy-1 : 2 diphenylethylamine[4].

Penicillin salts of the decarboxylation products of the amino-acids, tyramine and phenylethylamine, were also prepared, but both products were soluble in water.

From the results here described, and from experiments with other amines, as recorded in the literature and observed during our own work, we must conclude that no general rule can be drawn to relate the structure and the solubility of amine-penicillin salts.

EXPERIMENTAL

β-Dialkylaminoethyl Benzoates.

Compounds I–VI were prepared by mixing equimolecular amounts of the acyl chloride and of the β-dialkylaminoethanol dissolved in dry benzene, and heating for 2 hours on a water bath. After cooling, the solid which separated was filtered off, and recrystallised from acetone or an absolute ethanol-ether mixture. VII was prepared by catalytic reduction, in the presence of platinum oxide, of an ethanolic solution of the corresponding *p*-nitrobenzoate (VI).

The two *p*-hydroxybenzoates (IX, X) were obtained from the *p*-aminobenzoates: 0·02 mole of dimethyl- or diethyl-aminoethyl *p*-aminobenzoate (base) was dissolved in a cold mixture of 4 ml. of sulphuric acid and 6 ml. of water. The solution was cooled to 0° C., and 1·4 g. of sodium nitrite dissolved in 1 ml. of water was slowly added. The solution was subsequently heated for half an hour on a water bath, and, after cooling and adding solid sodium carbonate, extracted with ether. The ether was distilled off, and residue was dissolved in ethanol containing hydrochloric acid; the product was precipitated by cautious addition of ether when 2·75 to 3·59 g. was obtained. The product was recrystallised from an ethanol-ether mixture.

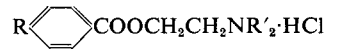
The derivatives of the *p*-alkoxybenzoic acids are described elsewhere[15].

The threshold anæsthetic concentration, as determined on the cornea of the rabbit[15], for these compounds is 15 per cent. or higher, except for VII (8 per cent.) and IV (5 per cent).

The LD50 for mice (mg./kg.) has been determined for some compounds

VII: 202, VIII: 250, IX: 360. Some characteristics of the benzoates are given in Table II.

TABLE II



	R	R′	m.pt. °C.	Nitrogen: Calculated per cent.	Nitrogen: Found per cent.	Reference
I	H	C_2H_5	124 to 125	—	—	11
II	H	CH_3	140 to 141	—	—	11
III	Cl	C_2H_5	136 to 137	—	—	12
IV	Cl	CH_3	196 to 197	5·30	5·40, 5·35	—
V	NO_2	C_2H_5	177 to 178	—	—	13
VI	NO_2	CH_3	171 to 173	—	—	13
VII	NH_2	C_2H_5	153 to 156	—	—	Procaine
VIII	NH_2	CH_3	181 to 183	—	—	13
IX	HO	C_2H_5	185 to 186	5·11	5·12, 5·07	14
X	HO	CH_3	140 to 142	5·69	5·65, 5·60	—

Derivatives of Nicotinic and iso*Nicotinic Acid.*

Nicotinyl chloride-hydrochloride was prepared by refluxing for 2 hours 15 g. of nicotinic acid in 100 ml. of colourless thionyl chloride. A large proportion of the excess of reagent was distilled off, and the residue was taken up in chloroform. The crystals were filtered off, washed with a small amount of chloroform and dried *in vacuo*. Yield 90 per cent., m.pt. 150 to 155° C.[16,17]. *iso*Nicotinyl chloride hydrochloride was prepared in the same manner; m.pt. 164° to 165° C.

Dialkylaminoethyl nicotinates: equimolecular amounts (0·1 mol.) of the acyl chloride-hydrochloride and of β-dimethyl- or diethyl-aminoethanol were dissolved in 50 ml. of pyridine, and heated for 1 hour on the water bath. The mixture was cooled, and after concentrating *in vacuo*, the crystals were filtered off, and recrystallised from a mixture of absolute ethanol and ether. Good yields (70 to 80 per cent.) were obtained with nicotinyl chloride, but this method was not well suited for condensations with *iso*nicotinyl chloride (yield about 15 per cent.). Their characteristics are given in Table III.

TABLE III

$C_5H_4N{\cdot}COOCH_2CH_2NR_2{\cdot}HCl$

	R	m.pt. °C.	Nitrogen: Calculated per cent.	Nitrogen: Found per cent.	Reference
Nicotinyl ..	CH_3	163 to 164	12·14	12·10, 12·28	—
	C_2H_5	129 to 130	—	—	18, 19
*iso*Nicotinyl ..	C_2H_5	114·6	—	—	19

Hydrochlorides of Phenylalanine Esters.

A stream of hydrogen chloride gas is passed through a suspension of 3 g. of DL-phenylalanine in 100 ml. of the alcohol, and after saturation, the solution is heated for 2 hours on a water bath. After concentration *in vacuo*, and cooling, the crystals are filtered off and recrystallised from the corresponding alcohol. Their characteristics are given in Table IV.

TABLE IV

$C_6H_5 \cdot CH_2 \cdot CH(NH_2) \cdot COOR \cdot HCl$

	R	m.pt. °C.	Nitrogen		Reference
			Calculated per cent.	Found per cent.	
DL	CH_3	159 to 160	—	—	20
"	C_2H_5	124 to 125	—	—	20 21
"	*n*-C_3H_7	122 to 123	5·76	5·78, 5·78	—
"	*iso* C_3H_7	192 to 195	5·76	5·76, 5·74	—
"	*n*-C_4H_9	125 to 126	5·43	5·43, 5·42	—
"	*iso*C_4H_9	129·5 to 130·5	5·43	5·46, 5·43	—
"	*n*-C_5H_{11}	118 to 130	5·11	5·16, 5·14	—
D	C_2H_5	154 to 156	$[\alpha]_D^{20°\,C.}$ +7·5 (c=1·0 water) −33·7 (c=1·0 ethanol abs).		
L	C_2H_5	154 to 156	$[\alpha]_D^{22°\,C.}$ −8·1 (c=1·0 water) +33·5 (c=1·0 ethanol abs).		

Phenylalanineamide $C_6H_5 \cdot CH_2 \cdot CH(NH_2) \cdot CONH_2$.

5 g. of DL-phenylalanine methyl ester hydrochloride was suspended in 75 ml. of concentrated ammonium hydroxide solution, and kept during one night at room temperature. The crystalline platelets, which separated, were filtered off. Yield 2·2 g. m.pt. 138° to 139° C.[22].

With the ethyl ester, the reaction was slower (3 to 4 days), and the product obtained was less pure.

The phenylalanine amide was converted into its hydrochloride by dissolving in ethanol containing the theoretical amount hydrochloric acid, and precipitated with ether. After recrystallisation from an absolute ethanol-ether mixture, m.pt. 243° to 244° C. (decomp.); nitrogen, calculated 14·00; found 13·98 to 14·03 per cent.

Penicillin Salts.

Equimolecular amounts of the hydrochloride of β-dialkylaminoethyl benzoate or nicotinate, or of the phenylalanine ester and of sodium or potassium benzylpenicillin were dissolved in a minimum amount of water, and the two solutions mixed with stirring. The crystals which

TABLE V

$C_6H_5 \cdot CH_2 \cdot CH(NH_2)COOR \cdot C_{16}H_{18}N_2O_4S$

	R	m.pt. °C. (decomp.)	Nitrogen		Solubility in water (25° C.) per cent.	Ref.
			Calculated per cent.	Found per cent.		
DL	CH_3	112 to 113	8·35	8·33, 8·33	2·1	—
"	C_2H_5	115 to 117	$[\alpha]_D^{20°}$ +196 (c=1·0 ethanol abs.)		2·3	10
"	*n*-C_3H_7	85 to 86	7·76	7·58, 7·60	2·1	—
"	*iso* C_3H_7*	75 to 78	7·76	7·76, 7·79	1·6	—
"	*n*-C_4H_9	60 to 63	7·56	7·52, 7·55	2·1	—
"	*iso* C_4H_9	90 to 92	7·56	7·52, 7·56	1·7	—
"	*n*-C_5H_{11}	81 to 83	7·38	7·38, 7·36	1·4	—
D	C_2H_5	96 to 97	$[\alpha]_D^{20°}$ +179 (c=1·0 ethanol abs.)		2·3	—
L	C_2H_5	139 to 140	$[\alpha]_D^{20°}$ +205 (c=1·0 ethanol abs.)		2·3	—
$HOC_6H_4COO(CH_2)_2N(C_2H_5)_2 \cdot C_{16}H_{18}N_2O_4S$		109·5–10 †	7·35	7·21, 7·22	0·8	—

* This penicillin salt is relatively unstable.

† Abildgaard-Elling gives 89 to 90° C. for the salt containing 1 mol. of water of crystallisation.

separate, were filtered off, washed and dried *in vacuo* over phosphorus pentoxide at room temperature.

All salts were assayed biologically, and had the theoretical benzylpenicillin content, within the limits of error. The solubility was also determined by biological assay. These, together with some additional characteristics, are shown in Table V.

Phenylethylamine penicillin. To a solution of penicillin, obtained by acidification of an aqueous solution of the salt and extraction with ether, an equimolecular amount of phenylethylamine dissolved in a small volume of *n*-butanol was added. The crystalline precipitate was filtered off and dried; m.pt. 92° to 93° C. Nitrogen, calculated 9·30, found 9·24, 9·29 per cent.

Tyramine penicillin was obtained by the same method; m.pt. 120° to 190° C. These salts had a good solubility in water, ethanol and acetone, but were insoluble in ether and benzene.

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SUMMARY

1. The preparation of sparingly penicillin salts with dialkylaminoalkyl benzoates has been investigated. Only the diethylaminoethyl ester of *p*-hydroxybenzoic acid has the same properties as diethylaminoethyl *p*-aminobenzoate (procaine).

2. Phenylalanine esters (methyl to *n*-amyl) give sparingly soluble penicillin salts. The anomalies, described with different L-tyrosine-esters, were not observed in this series.

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