763. *Some Preparative Uses of Benzylpenicillinic Ethoxyformic Anhydride.*

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The mixed anhydride (I), named in the title, which is readily prepared from a salt of benzylpenicillin and ethyl chloroformate, reacts with amines to form amides, with alcohols to form esters, and with thiols for form thiol-esters of the antibiotic. Several of the derivatives so prepared are hydrolysed in aqueous media to liberate free benzylpenicillin, a property that makes them of possible therapeutic interest.

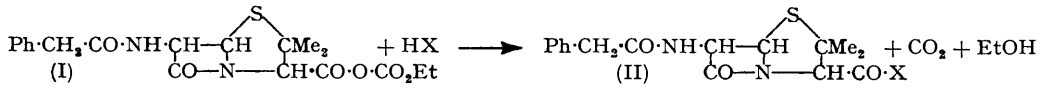
Esters of benzylpenicillin have attracted relatively little interest in the past largely because the first known members, namely, the simple alkyl and aralkyl esters, were devoid of antibacterial activity both *in vitro* and in man (though not in mice and rats). Carpenter (*J. Amer. Chem. Soc.*, 1948, **70**, 2964), however, found that the dimethylaminoethyl ester (II; X = $-O\cdot CH_2\cdot CH_2\cdot NMe_2$) exhibited full activity in the plate bioassay, owing, as he showed, to its ready hydrolysis to free penicillin and the amino-alcohol. More recently the important discovery has been made that the dialkylaminoethyl esters, particularly the hydriodide of the diethylamino-homologue (Estopen), are selectively concentrated in inflamed lung tissue : of these, however, only the last mentioned has been tested on human subjects; it gave rise to prolonged effective levels of penicillin in their sputa (Friederiksen and Nielson, XIIth Internat. Congr. Pure & Appl. Chem., New York, 1951, Abstracts, p. 284; Jensen, Dragsted, and Kjaer, *Ugeskr. Laeg.*, 1950, **112**, 1075; Heathcote and Nassau, *Lancet*, 1951, I, 1255; Ungar and Muggleton, *Brit. Med. J.*, 1952, 1211).

These findings led us to search for further esters or amides of the antibiotic that might be useful therapeutically. A necessary first objective was a preparative method of wide application, for the lability of the penicillin molecule prohibits the use of customary procedures. Before 1948 the only published method of esterification ("The Chemistry of Penicillin," Princeton, 1948, p. 92; Kirchner, McCormick, Cavallito, and Miller, *J. Org. Chem.*, 1949, **14**, 398) was the reaction of free benzylpenicillinic acid with diazoalkanes, a route severely limited by the latters' availability. Also of restricted scope are methods involving treatment of a penicillin salt with an activated halogen compound, *e.g.*, phenacyl chloride (Cooper and Binkley, *J. Amer. Chem. Soc.*, 1948, **70**, 3966; U.S.P. 2,578,570) and diethylaminoethyl chloride (B.P. 675,422). Two published methods for forming esters and amides, however, promised to be of more general application, both of them depending on the interaction of an anhydride of penicillin with the appropriate alcohol or amine.* Carpenter (*loc. cit.*) prepared benzylpenicillinic anhydride by treating a solution of triethylammonium benzylpenicillinate in pyridine with thionyl chloride, and Cooper and Binkley (*loc. cit.*) synthesised the mixed acetic benzylpenicillinic anhydride by treating acetyl chloride with sodium benzylpenicillinate in *NN*-dimethylacetamide. Both procedures have been re-examined by Holysz and Stavely (*J. Amer. Chem. Soc.*, 1950, **72**, 4760) who, though preferring the former, slightly modified, obtained from it only a 20% yield of methyl benzylpenicillinate. In our hands neither method proved particularly effective; the second, which we found the better, gave a 16% yield of the methyl ester and failed altogether with other alcohols tried.

It appeared to us that better success might be obtained with a more reactive anhydride of penicillin and that a suitable compound would be a carbonic ester anhydride, a type of intermediate that has been recently employed for peptide synthesis (Boissonas, *Helv. Chim. Acta*, 1951, **34**, 875; Vaughan, *J. Amer. Chem. Soc.*, 1951, **73**, 3547). We found that ethyl chloroformate reacted readily with a solution of triethylammonium benzylpenicillinate in chloroform, or with a suspension of the sodium salt in acetone in the presence of a trace of

* Since this paper was submitted Johnson (*J. Amer. Chem. Soc.*, 1953, **75**, 3636) has described similar reactions of benzylpenicillinic ethoxyformic anhydride.

pyridine, to form the anhydride (I), which in turn underwent a vigorous reaction with *cyclo*hexylamine, as evinced by a brisk evolution of carbon dioxide, to give a practically quantitative yield of the crystalline *cyclo*hexylamide (II; X = $NH{\cdot}C_6H_{11}$):



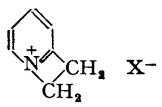
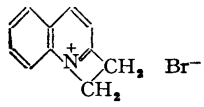
A number of other new amides, listed in Table 1, were prepared similarly. It is noteworthy that the relatively low specific rotation of the morpholide (+89°) appears to be a general property of the disubstituted amides, for it is of the same order of magnitude as the specific rotation of the piperidide (+85°) and of the diethylamide (+102°) quoted by Holysz and Stavely (*loc. cit.*). Only one of the substituted amides, namely, the morpholide, showed appreciable antibiotic activity (300 i.u./mg.) when assayed biologically by routine procedures.

The anhydride (I) also reacts with alcohols (ROH) in the presence of a tertiary base, to form the corresponding esters (II; X = OR). A complicating factor is that the ethanol liberated in the reaction may compete with the added alcohol for the remaining anhydride. Most of the alcohols in which we were interested were in fact more rapidly esterified than ethanol and the products (see Table 2) were not contaminated with ethyl benzylpenicillinate. The esterification of liberated ethanol may, moreover, sometimes be avoided by employing an excess of the alcohol to be esterified; for example, a 66% yield of methyl benzylpenicillinate was obtained in this way.

No known ester of penicillin has antibacterial activity *per se*; the aforementioned activity of the alkyl and aralkyl esters in rats and mice is due to the presence in their sera of esterases of the necessary specificity (Meyer, Hobby, and Dawson, *Proc. Soc. Exp. Biol., N.Y.*, 1943, **53**, 100; Richardson, Walker, Miller, and Hensen, *ibid.*, 1945, **60**, 272). While we do not discount the possibility of finding derivatives susceptible to the hydrolytic enzymes in man, our interest has been primarily in preparing esters hydrolysed readily by water alone (as is the diethylaminoethyl ester). To measure roughly the rates of hydrolysis of the esters prepared, we carried out standard plate assays on their aqueous-alcoholic solutions, making the assumption that the intact esters were inactive *in vitro*. The solutions were kept at room temperature for 3 hours before being plated out, as otherwise the more rapidly hydrolysed esters did not give reproducible results. It will be seen from Table 2 that the diethylaminoethyl ester hydriodide and several of its analogues, including the diester of 2:2′-dihydroxytriethylamine, which all possess the grouping $-CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}\overset{+}{N}\equiv$, were hydrolysed completely under the conditions of the assay. When the hydrocarbon chain separating the nitrogen and the oxygen atom of the diethylaminoethyl ester was increased by one or two methylene groups, *i.e.*, in the 3-hydroxypropylamine and 4-hydroxybutylamine homologues (which were somewhat impure), the extent of hydrolysis was considerably reduced, as it was also when the $-NEt_2$ group was replaced by –NHAc. The ready hydrolysis of the hydroxyethylamine esters is probably due to the formation of an ethyleneimminium-ion intermediate (see Bartlett, Ross, and Swain, *J. Amer. Chem. Soc.*, 1949, **71**, 1415).

Of related interest is the ester of 2-2′-pyridylethanol, which was obtained originally from the corresponding diazo-compound by Kirchner *et al.* (*loc. cit.*), who noted that it was active *in vitro*. We have prepared the ester by our general procedure and confirmed that hydrolysis was complete under the conditions of assay. The ease of hydrolysis does not appear to be the result of any inductive effect of the electron-attracting 2-pyridyl group, any more than the lability of the hydroxyethylamine ester salts can be ascribed to the positively charged nitrogen atom, for the 2-*p*-nitrophenylethyl and 2-4′-pyridylethyl esters are relatively stable to hydrolysis. The ester from 2-3′-pyridylethanol (the alcohol being prepared conveniently by reduction of ethyl 3-pyridylacetate with lithium aluminium hydride) was equally stable. Contrary to expectation, 2-2′-quinolylethyl benzylpenicillinate also was not readily hydrolysed. (The last three esters could not be obtained pure.) It is possible that the lability of the 2-2′-pyridylethyl ester depends on the formation of the cyclic pyridinium salt (IV; X = $C_{15}H_{17}O_2N_2S{\cdot}CO{\cdot}O{\cdot}$), for Löffler (*Ber.*, 1904, **37**, 165)

records that 2-2′-pyridylethyl bromide is converted into (IV; X = Br) when kept at room temperature, whereas the corresponding quinolylethyl bromide appears to require heating for transformation into (V) (Takahashi, Nishigaki, and Taniyana, *Chem. Abs.*, 1951, **45**, 1997).

(IV)  (V) 

It seemed possible that one of the contributory factors influencing the ease of hydrolysis of carboxylic esters might be the electron availability at the alcohol-oxygen atom and that a relative electron deficiency there would facilitate anionoid attack at the carboxyl-carbon atom. Convenient compounds on which to test this hypothesis are the phenols, as the electron density at their hydroxyl groups can be varied readily by the introduction of substituents into the ring. The benzylpenicillin esters of a number of phenols were prepared by our standard procedure, and it was found that their hydrolysis rates were in general accord with the known electronic effects of the substituent groups. Thus the tolyl ester had the same low activity as the phenyl ester, but the activity increased somewhat when the substituents MeO, Me_2N, NHAc, and more markedly when CO_2Et, Cl, and NO_2, were present in the aromatic nucleus. The introduction of a second substituent, as in 2 : 4-dichloro- and 2 : 4-dinitro-phenol, appeared to exert relatively little effect. The assay figures, however, indicated that less than 50% of even the most active ester had been hydrolysed under the conditions of test.

Esters derived from alcohols, in which an unsaturated or "negative" group was attached to the carbon atom bearing the hydroxyl group, were also investigated and a wide variation of activity was encountered. The preparation of several ketol esters by the interaction of sodium benzylpenicillinate and α-halogeno-ketones, such as, for example, phenacyl chloride and chloroacetone, has already been described (Cooper and Binkley, U.S.P. 2,578,570); these compounds were shown to be readily hydrolysed. We have found that the acetoin ester (prepared from bromoethyl methyl ketone) also shows a high activity on bioassay; the ester from furoin (prepared by the ethoxyformic anhydride method) is less active, and the butyroin ester (similarly prepared) is almost inactive. Other esters in this category (see Table 2) showed at best only low activity with the rather surprising exception of the furfuryl ester which gave an assay of 750 i.u./mg.

A group of compounds of particular interest were the benzylpenicillin esters of hydroxymethylene derivatives of *cyclo*hexanone, ethyl phenylacetate, and 2-phenyloxazol-5-one, which were readily prepared by the ethoxyformic anhydride route. Each of these esters, which may be regarded as the vinylogue of a mixed carboxylic acid anhydride, was extensively hydrolysed under the conditions of test.

A further application of the ethoxyformic anhydride (I) lies in its reaction with thiols

TABLE 1. *Amides.*

Amine	$[\alpha]_D^{20}$ (c = 1 in $CHCl_3$)	M. p.*	Yield (%)	Formula	Found (%) C	Found (%) H	Found (%) N	Required (%) C	Required (%) H	Required (%) N
Allylamine	+225°	154—155° (a)	54	$C_{19}H_{23}O_3N_3S$	—	—	11·0	—	—	11·25
2-Aminopyridine ...	+227	Gum	66	$C_{21}H_{22}O_3N_4S$	—	—	13·3	—	—	13·65
*cyclo*Hexylamine ...	+246	197·5—199 (b)	84	$C_{22}H_{29}O_3N_3S$	63·8	7·0	9·85	63·6	7·05	10·1
2 : 2-Dimethyl*cyclo*-hexylamine	+213	218—220 (c)	65	$C_{24}H_{33}O_3N_3S$	65·0	7·6	9·2	65·0	7·5	9·45
2-Hydroxyethyl-amine	+256	149—151 (a)	86	$C_{18}H_{23}O_4N_3S$	57·1	6·4	10·95	57·3	6·15	11·15
trans-2-Methyl*cyclo*-hexylamine	+213	218—220 (a)	50	$C_{23}H_{31}O_3N_3S$	64·2	7·4	9·85	64·3	7·3	9·8
Morpholine	+ 89	176·5—178 (a)	78	$C_{20}H_{25}O_4N_3S$	59·6	6·15	10·9	59·55	6·25	10·4
p-Toluidine	+286	192—193 (c)	86	$C_{23}H_{25}O_3N_3S$	65·3	6·3	9·95	65·25	5·95	9·9
Glycine	+200	—	30	$C_{18}H_{21}O_5N_3S$	—	—	10·2	—	—	10·75
*iso*Nicotinoylhydr-azide	+188	109—113 (d)	29	$C_{22}H_{23}O_4N_5S$	—	—	15·7	—	—	15·40

* Solvent for recrystallisation : (a) ethyl acetate–light petroleum; (b) ethyl acetate; (c) acetone; (d) aqueous ethanol.

TABLE 2. *Esters.*

Alcohol	$[\alpha]_D^{20}$ *	M. p.†	Time (hr.)	Temp.	Yield (%)	Formula	Found (%) N		Required (%) N		Bio-assay (i.u./mg.)
Methanol	+172°	97—98° (a)	See text		66		—	—	—	—	102
Diethylaminoethanol, hydriodide	+158 (β)	177—178	,,		84·5	—	—	—	—	—	1040
2-Morpholinoethanol, hydriodide	+148 (β)	175—177 (c)	,,		70	$C_{22}H_{29}O_5N_3S,HI$	7·3	—	7·3	—	1060
2-(2 : 6-Dimethylmorpholino)ethanol, hydriodide	+145 (β)	164—165 (d)	,,		14·3	$C_{24}H_{33}O_5N_3S,HI$	6·9	I, 20·9	6·95	I, 21·05	930
1-Morpholinopropan-2-ol, hydriodide	+ 87	—	,,		51	$C_{23}H_{31}O_5N_3S,HI$	6·5	I, 21·3	7·15	I, 21·55	800
2 : 2′-Dihydroxytriethylamine, hydrochloride	+144	—	,,		25	$C_{38}H_{47}O_8N_5S_2,HCl$	—	Cl, 4·3	—	Cl, 4·40	1200
2 : 2′-Dihydroxytriethylamine, hydriodide	+140	—	,,		29	$C_{38}H_{47}O_8N_5S_2,HI$	—	I, 14·5	—	I, 14·2	1270
3-Diethylaminopropanol, hydriodide	+137 (α)	—	,,		44	$C_{23}H_{33}O_4N_3S,HI$	7·3	I, 20·6	7·3	I, 22·05	190
4-Diethylaminobutanol, hydriodide	+ 99 (α)	—	,,		44	$C_{24}H_{35}O_4N_3S,HI$	6·6	I, 20·4	7·15	I, 21·55	130
2-Acetamidoethanol	+151	154—156 (b)	0·5	18°	44	$C_{20}H_{27}O_5N_3S$	10·1	—	9·95	—	200
2-2′-Pyridylethanol	+140	—	12	18	60	$C_{23}H_{25}O_4N_3S$	9·5	—	9·55	—	1540
2-4′-Pyridylethanol	+114	—	4	18	42	$C_{23}H_{25}O_4N_3S$	8·6	—	9·55	—	60
2-*p*-Nitrophenylethanol	+149	—	0·5	60	64	$C_{24}H_{25}O_6N_3S$	8·5	—	8·7	—	40
2-3′-Pyridylethanol	+144	—	12	18	52	$C_{23}H_{25}O_4N_3S$	8·9	—	9·55	—	200
2-2′-Quinolylethanol	+120	—	12	18	64	$C_{27}H_{27}O_4N_3S$	8·1	—	8·6	—	205
Phenol	+151	154—155 (e)	12	0	27	$C_{22}H_{22}O_4N_2S$	7·05	—	6·85	—	125
p-Cresol	+152	154·5—156·5 (f)	1	18	68	$C_{23}H_{24}O_4N_2S$	6·5	—	6·6	—	130
p-Methoxyphenol	+147	113·5—114·5 (g)	2	18	75	$C_{23}H_{24}O_5N_2S$	6·5	—	6·35	—	250
p-Dimethylaminophenol	+149	109—111 (g)	1	0	42	$C_{24}H_{27}O_4N_3S$	9·65	—	9·25	—	310
p-Acetamidophenol	+136	—	1	60	31	$C_{24}H_{25}O_5N_3S$	9·4	—	9·0	—	350
Ethyl *p*-hydroxybenzoate	+103·5	—	0·5	60	50	$C_{25}H_{26}O_6N_2S$	5·6	—	5·8	—	400
p-Chlorophenol	+130	—	0·1	60	97	$C_{22}H_{21}O_4N_2SCl$	5·9	—	6·3	—	600
p-Nitrophenol	+131	—	1	18	75	$C_{22}H_{21}O_6N_3S$	9·2	—	9·25	—	560
2 : 4-Dichlorophenol	+112	—	0·5	60	88	$C_{22}H_{20}O_4N_2SCl_2$	5·4	—	5·85	—	470
2 : 4-Dinitrophenol	+132	—	0·5	60	60	$C_{22}H_{20}O_8N_4S$	10·8	—	11·2	—	670
Acetoin	+137	—	See text		—	$C_{20}H_{24}O_5N_2S$	6·45	—	6·95	—	1590
Butyroin	+117	—	24	18	80	$C_{24}H_{32}O_5N_2S$	5·8	—	6·1	—	26
Furoin	+131	—	0·5	60	78	$C_{26}H_{24}O_7N_2S$	5·8	—	5·5	—	405
Allyl alcohol	+155	—	1·5	18	59	$C_{19}H_{22}O_4N_2S$	7·4	—	7·5	—	50
Diphenylmethanol	+ 97·5	—	1	60	78	$C_{29}H_{28}O_4N_2S$	5·3	—	5·6	—	0
2-Pyridylmethanol	+150	94—96 (g)	0·25	60	16·5	$C_{22}H_{23}O_4N_3S$	10·2	—	9·9	—	trace
3-Pyridylmethanol	+ 99·5	—	1	60	50	$C_{22}H_{23}O_4N_3S$	9·45	—	9·9	—	60
2-Quinolylmethanol	+134	—	60	0	90	$C_{26}H_{25}O_4N_3S$	8·65	—	8·85	—	450
4-Quinolylmethanol	+125	—	12	18	72	$C_{26}H_{25}O_4N_3S$	8·5	—	8·85	—	290
p-Nitrobenzyl alcohol	+121	—	1	18	54	$C_{23}H_{23}O_6N_3S$	8·5	—	8·95	—	235
Furfuryl alcohol	+173	136—138 (a)	1·5	18	63	$C_{21}H_{22}O_5N_2S$	6·8	—	6·75	—	750
2-Hydroxymethylene*cyclo*hexanone	+145	—	24	0	90 ‡	$C_{23}H_{26}O_5N_2S$	6·1	—	6·35	—	1500
Ethyl hydroxymethylenephenylacetate	+138	—	26	0	90 ‡	$C_{27}H_{28}O_6N_2S$	5·4	—	5·5	—	940
4-Hydroxymethylene-2-phenyloxazol-5-one	+137	—	See text		35	$C_{26}H_{23}O_6N_3S$	7·9	—	8·3	—	840
Thiophenol	+294	140—142 (a)	1	18	60 ‡	$C_{22}H_{22}O_3N_2S$	6·7	S, 14·7	6·55	S, 15·0	150
2-Diethylaminoethanethiol	—	85—86 (g)	See text		72	$C_{22}H_{31}O_3N_3S_2$	9·35	S, 14·4	9·35	S, 14·25	700
2-Diethylaminoethanethiol, hydrochloride	+304	183—184 (i)	,,		92	$C_{22}H_{31}O_3N_3S_2,HCl$	9·0	Cl, 6·9; S, 13·5	8·65	Cl, 7·25; S, 13·2	600
2-Diethylaminoethanethiol, hydriodide	+249	174—175	,,		92	$C_{22}H_{31}O_3N_3S_2,HI$	—	I, 21·5; S, 10·7	—	I, 22·0; S, 11·1	550

* $c = 1$. Solvent: α signifies acetone; β 80% aqueous acetone. In all other cases chloroform was employed.

† Solvent for crystallisation: (a) CCl_4; (b) EtOAc–$COMe_2$; (c) $COMe_2$; (d) $COMe_2$–EtOH; (e) EtOAc–Et_2O; (f) EtOAc–light petroleum; (g) Pr^iOH; (h) $CHCl_3$–light petroleum; (i) MeOH–Et_2O.

‡ Product not chromatographed.

(RSH), in the presence of a base, to form the corresponding benzylpenicillin thio-esters (II; X = SR) (cf. Wieland, Schafer, and Bokelmann, *Annalen*, 1951, **573**, 99). In this way we prepared the ester of thiophenol, which was slightly more active than the phenyl ester, and the thio-analogue (X = $-S\cdot CH_2\cdot CH_2\cdot NEt_2$) of the diethylaminoethyl ester, which had only half the activity of the oxygen compound.

Experimental

Benzylpenicillinic Ethoxyformic Anhydride (I).—Ethyl chloroformate (1·1 g., 0·01 mole) was added to an ice-cooled solution of triethylammonium benzylpenicillinate (4·35 g., 0·01 mole) in dry chloroform (10 c.c.). After 10 min. the solution was washed twice with ice-cold water to remove the triethylamine hydrochloride, dried ($MgSO_4$), and evaporated at 0°/0·1 mm. The *anhydride* obtained thereby was an almost colourless gum (1·95 g.), $[\alpha]_D$ +159° (c = 1 in $CHCl_3$) (Found: C, 55·9; H, 5·65; N, 6·95. $C_{19}H_{22}O_6N_2S$ requires C, 56·15; H, 5·45; N, 6·9%).

General Procedure for the Preparation of Amides.—The following method was employed for synthesising most of the amides listed in Table 1. When novel features were introduced the preparations are described separately below.

A solution of the anhydride (I) (0·01 mole) in chloroform (10 c.c.) was prepared as above, save that water washing was omitted, and the appropriate amine (0·01 mole) was added, causing usually a vigorous evolution of carbon dioxide. The reaction was complete after 30 min. at 0° and the solution was washed successively with water, 0·1M-citric acid, 0·2M-disodium hydrogen phosphate, and water. The product was isolated by evaporation of the solvent under reduced pressure and recrystallisation of the residue. Data relating to the individual amides and the solvents employed for their crystallisation are given in Table 1.

Alternatively the anhydride could be prepared by treating sodium penicillinate (3·56 g., 0·01 mole) in acetone (25 c.c.) containing pyridine (3 drops) with ethyl chloroformate (1·1 g.). A rapid reaction ensued in which the sodium penicillinate dissolved and sodium chloride was precipitated. After filtration the resulting solution could be used for the preparation of amides as described above, the obvious modifications being introduced during working up to take account of the change of solvent.

Benzylpenicillinoylglycine.—The anhydride (I) (prepared as above from 8·7 g. of triethylammonium benzylpenicillinate) in chloroform (40 c.c.) was added to a solution of glycine (2·25 g.) in water (25 c.c.) to which sufficient N-sodium hydroxide (2—3 c.c.) had been added to bring the pH to 7·5—8. The mixture was stirred vigorously, more sodium hydroxide solution being added at intervals to maintain the pH at the initial value. Stirring was continued for 1 hr. after the mixture had ceased to require the addition of further alkali, then the aqueous layer was separated and acidified. The gummy solid was collected in chloroform and washed with water and dilute acid, and the solvent evaporated. The residue was purified by trituration with dry ether to give a white amorphous solid. The acid and its triethylamine and 1-ethylpiperidine salt failed to crystallise.

N-*Benzylpenicillinoyl*-N′-iso*nicotinoylhydrazine.*—The preparation was carried out by the general procedure, save that triethylamine (4 drops) was added to the mixture to promote reaction between the ethoxyformic anhydride and *iso*nicotinoylhydrazine and stirring was continued at room temperature until all the solid had dissolved (45 min.).

General Procedure for the Preparation of Esters.—The anhydride (I) was prepared in chloroform as before and the alcohol (0·01 mole) and triethylamine (5 drops) were added. The reaction was allowed to proceed under conditions appropriate to the particular alcohol (see Table 2), and the chloroform solution was then washed successively with water, 0·1M-citric acid (omitted with basic esters), 0·2M-disodium hydrogen phosphate solution, and water, Most of the solvent was evaporated under reduced pressure and the residue was washed with chloroform or ethyl acetate through a column of alumina (100 g.; Peter Spence, Type H, weakened by treatment with 10 c.c. of 5% aqueous acetic acid) which removed any unchanged anhydride and other impurities. Evaporation of the solvent, finally in a high vacuum, left the desired ester, usually substantially pure. The physical constants, analyses, and activities, by bioassay, of the esters, together with the solvents employed for crystallisation where this proved possible, are given in Table 2.

The preparation of a few of the esters differed from the standard procedure in certain details, which are described in the following notes.

Methyl Benzylpenicillinate.—A solution of the anhydride (I) in acetone (25 c.c.) containing pyridine (5 drops) was prepared from sodium penicillin (3·56 g.) and ethyl chloroformate (1·1 g.)

as described above and methanol (10 c.c.) was added. After 1 hr. at 0° the mixture was poured into a large volume of water, and the product collected in chloroform. Evaporation of the solution, after washing in the usual way, and recrystallisation of the residue from carbon tetrachloride–light petroleum yielded the pure ester (2·12 g., 66%), m. p. 96·5—97·5°, not depressed on mixture with an authentic specimen.

Diethylaminoethyl Benzylpenicillinate Hydriodide.—Ethyl chloroformate (54·25 g.) in ether (50 c.c.) was added gradually to an ice-cooled stirred suspension of benzylpenicillin 1-ethylpiperidine salt (223·5 g.) in acetone (300 c.c.) and ether (300 c.c.) with pyridine (5 drops). After 45 min. the mixture was filtered and the filtrate added to an ice-cold solution of diethylaminoethanol (64·35 g.) in acetone (50 c.c.). Most of the solvent was removed *in vacuo* and the residual yellow oil (266 g.) was dissolved in a cold solution of acetic acid (40 c.c.) in water (670 c.c.). After treatment with charcoal (40 g.) the filtered solution was stirred in an ice-bath while sodium iodide (100 g.) in water (500 c.c.) was added dropwise during 2 hr., seed crystals of the product being added when the mixture became cloudy. The colourless crystalline hydriodide (236 g.) that separated was collected, washed with water, and dried *in vacuo*.

2-*Morpholinoethyl Benzylpenicillinate Hydriodide.*—Ethyl chloroformate (10·85 g., 0·1 mole) in dry ether (40 c.c.) was added with stirring to an ice-cooled suspension of finely divided sodium penicillinate (35·6 g., 0·1 mole) in acetone (125 c.c.) containing pyridine (5 drops). After 30 min. the solution was filtered through a kieselguhr pad into a solution of morpholinoethanol (13·1 g., 0·1 mole) in acetone (50 c.c.) cooled in ice. The mixture was stirred for 10 min. and then cooled more strongly while a solution (50 c.c.; 16%) of acetic acid in acetone was added at such a rate that the temperature did not exceed 0°. The solvent was removed at 0° under reduced pressure until the residue weighed *ca.* 90 g. (*i.e.*, a 55—60% solution of the base acetate). Ice-water (400 c.c.) was added with vigorous stirring to give a clear solution, which was seeded and then treated dropwise with sodium iodide (20 g.) in water (100 c.c.). Further water (200 c.c.) was added and stirring continued until the precipitate, which was initially gummy, had solidified (1 hr.). The solid *hydriodide* was collected, washed with a little water, dried, and purified by refluxing for a short time with acetone (160 c.c.) to give colourless prisms (40·5 g.), m. p. 173—175°. A sample for analysis, recrystallised from 80% aqueous acetone (recovery only 57%), had m. p. 175·5—178·5°.

2-(2′ : 6′-*Dimethylmorpholino*)*ethyl Benzylpenicillinate Hydriodide.*—The ester base was prepared by the general method (on five times the scale described), the reaction being allowed to proceed for 1 hr. at room temperature. The resulting gum, isolated without chromatography, was taken up in *iso*propanol (100 c.c.), and constant-boiling hydriodic acid (*ca.* 7 c.c.) was added until a drop of the solution on moist Universal Indicator paper showed a pH of <3. When crystals began to form, more *iso*propanol (150 c.c.) was added gradually with shaking (too rapid addition precipitates a gum) and the solid (8·65 g.), m. p. 140—147°, was collected. The crude *product* could be recrystallised from ethanol or from methanol–ether, but purification was effected most economically by trituration with a cold mixture of acetone (12 c.c.) and ethanol (12 c.c.), which gave colourless prisms (4·3 g.), m. p. 164—165°.

1-*Morpholinoprop-2-yl Benzylpenicillinate Hydriodide.*—Ethyl chloroformate (2·2 g.) was added to a stirred suspension at 0° of sodium benzylpenicillinate (7·1 g.) in acetone (50 c.c.) containing pyridine (2 drops); after 10 min. the precipitated sodium chloride was removed by filtration through a pad of kieselguhr. 1-Morpholinopropan-2-ol (3·9 g.) was added to the filtrate, and the solution was concentrated *in vacuo* to a small volume. The addition of light petroleum precipitated the desired ester, which was freed from the supernatant liquid and was then taken up in ether (100 c.c.) and filtered from any solid material. Treatment of this solution dropwise at 0° with an ethereal solution of hydriodic acid (0·2N; 80 c.c.) precipitated the *hydriodide* as a pale yellow amorphous powder (6 g.), which decomposed when heated.

2 : 2′-*Dihydroxytriethylamine Diester Hydrochloride of Benzylpenicillin.*—The ester base, prepared by the general procedure (on twice the scale described) with a reaction time of 3 days at 0°, was obtained as a yellow gum (7·1 g.). This was dissolved in *iso*propanol (100 c.c.) and treated with *iso*propanolic hydrogen chloride (N; 10 c.c.) and then with a large volume of ether. Repeated trituration of the gum that separated with fresh ether gave a pale buff amorphous *hydrochloride* (4·1 g.) of indefinite m. p. The *hydriodide* was prepared similarly from the ester base (2·5 g.), by means of ethereal hydrogen iodide, as a yellow amorphous powder.

3-*Diethylaminopropyl Benzylpenicillinate Hydriodide.*—The ester base was prepared by the general procedure save that triethylamine was omitted, with reaction for 30 min. at 0°. The oily product (8·3 g.) in *iso*propanol (100 c.c.) was treated with constant-boiling hydriodic acid (1·6 c.c.) and the mixture warmed to effect dissolution. After dilution of the solution with dry

ether (100 c.c.) and cooling, the solvent was decanted from the precipitated gum, which was triturated repeatedly with fresh portions of ether until a pale yellow amorphous solid (5·6 g.) was obtained. The *salt* was of indefinite m. p. and did not crystallise.

4-Diethylaminobutyl Benzylpenicillinate Hydriodide.—This *compound* was prepared in the same way as its lower homologue and was obtained as an almost white amorphous solid.

2-3′-Pyridylethanol.—A standard ethereal solution (0·8M; 22 c.c.) of lithium aluminium hydride was added dropwise to a vigorously stirred solution of methyl 3-pyridylacetate (3·4 g.) (Schwenk and Papa, *J. Org. Chem.*, 1946, **11**, 798; Malan and Dean, *J. Amer. Chem. Soc.*, 1947, **69**, 1797) in dry ether (50 c.c.) at such a rate that the mixture refluxed gently. Stirring was continued for 45 min. after the addition was complete and the solid complex was then decomposed with a saturated solution of sodium potassium tartrate (5 c.c.). The supernatant liquid was decanted and the residue washed several times with chloroform. Evaporation of the combined solutions and distillation of the product yielded a colourless oil (2·1 g.), b. p. 148°/15 mm. [phenylurethane, m. p. 101—102° (from ethanol) (lit., m. p. 100—102°)].

1-Methylacetonyl Benzylpenicillinate.—A mixture of triethylammonium benzylpenicillinate (2·17 g.), 1-bromoethyl methyl ketone (1·5 g.), pyridine (3 drops), and acetone (10 c.c.) was refluxed for 30 min. The resulting solution was filtered from the triethylamine hydrobromide and evaporated under reduced pressure. The residue was taken up in chloroform and washed and chromatographed, as in the general procedure, to yield a gummy *ester* (1·9 g., 94%) that failed to crystallise.

2-Pyridylmethanol.—Ethyl picolinate (7·55 g.) was reduced with lithium aluminium hydride (38 c.c. of M-solution) as described above for methyl 3-pyridylacetate. The product was obtained as a colourless oil (2·4 g.), b. p. 110°/12 mm. (Found: N, 12·4. Calc. for C_6H_7ON: N, 12·8%) [picrate (from ethanol), m. p. 157—159° (lit., m. p. 159°)].

3-Pyridylmethanol (cf. B.P. 631,078).—This compound was prepared similarly, but on twice the scale, from ethyl nicotinate (15·1 g.), being obtained as a colourless oil (8·3 g.), b. p. 141—142°/14 mm. [picrate (from ethanol), m. p. 159—160° (lit., m. p. 128°, 158°)] (Found: N, 16·9. Calc. for $C_{12}H_{10}O_8N_4$: N, 16·5%).

4-Benzylpenicillinoyloxymethylene-2-phenyloxazol-5-one.—4-Hydroxymethylene-2-phenyloxazolone (0·9 g.) was added to a chloroform solution of the anhydride (I) containing triethylamine, as in the general procedure, and the reaction mixture was kept at room temperature for 5 min. only. The solution was extracted in the normal way, but the treatment with weakened alumina was omitted, as the product was too strongly adsorbed. The solvent was evaporated *in vacuo* and the residue was thoroughly triturated with dry ether, which was discarded. The remaining gum was dissolved in a little methanol and ether was added. The resulting solution was decanted from the tar that separated and was evaporated under reduced pressure, leaving a brittle gummy *ester* (0·8 g.).

2-Diethylaminoethyl Benzyl(thiolpenicillinate).—Reaction of 2-diethylaminoethanethiol with the anhydride (I) under the standard conditions (triethylamine being omitted) was rapid and required only 10 min. at 0°. The crude basic *ester* was unstable in solution, but was isolated as a pale pink crystalline solid, m. p. 82—84°, by pouring the concentrated chloroform solution into a large volume of light petroleum and cooling in a refrigerator for several hours. A sample for analysis was recrystallised from warm *iso*propanol with rapid cooling in ice and gave colourless laths, m. p. 85—86°.

The crude basic ester (1·1 g.) in *iso*propanol (20 c.c.) was treated with *iso*propanolic hydrogen chloride solution (N; 2·9 c.c.), whereupon the *hydrochloride* (1·1 g.), m. p. 180—181°, crystallised on cooling. A sample, recrystallised from methanol–ether, gave colourless needles, m. p. 183—184°. *The hydriodide*, m. p. 162—165°, prepared similarly, formed prisms, m. p. 171—174°, from methanol containing a trace of ether.

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