Acyloxymethyl Esters of Ampicillin

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The preparation of a series of acyloxymethyl esters of D-α-aminobenzylpenicillin (ampicillin) is described. *In vitro* these esters are hydrolyzed to ampicillin by nonspecific esterases present in serum and various tissues from rat, dog, and man. Experiments performed with the hydrochloride of the pivaloyloxymethyl ester in laboratory animals and healthy volunteers show that this compound, after oral administration, is absorbed far more efficiently than ampicillin. After the absorption the compound is rapidly hydrolyzed to ampicillin with the result that high blood and tissue levels of the latter are attained.

Although D-α-aminobenzylpenicillin (ampicillin) (**1**) is relatively stable to acid it is absorbed rather inefficiently by the oral route as indicated, *e.g.*, by the fact that much higher blood levels and urinary recovery are attained after parenteral than after oral administration.[2] It is reasonable to assume that this is due to the dipolar character of the drug and that a transient masking of the carboxyl group could improve its oral absorption. Such a masking can be achieved by esterification, but since simple alkyl or aryl esters of penicillins are hydrolyzed only slowly in the human organism a special type of ester was needed.

A group of penicillin esters fulfilling the requirement of rapid hydrolysis in the organism, *viz.*, acyloxymethyl esters, has recently been reported.[3] Esters of this type have been prepared from a number of penicillins including, among others, benzylpenicillin, phenoxymethylpenicillin, 2-ethoxynaphthylpenicillin, methicillin, and cloxacillin.[3,4] The best investigated member of the group is the acetoxymethyl ester of benzylpenicillin (**2**), which is used clinically as an orally active form of benzylpenicillin giving rise to low, but prolonged blood levels of the latter. It is assumed that this compound after the absorption is hydrolyzed enzymatically by nonspecific esterases to the hydroxymethyl ester **3**, which subsequently decomposes spontaneously to benzylpenicillin (**4**) and formaldehyde.[5] With the exception of **2** esters of this type seem to be absorbed very poorly, probably due to their low solubilities.[4]

The presence of an amino group in the side chain of ampicillin will render acyloxymethyl esters of this penicillin acid soluble, a fact which may be expected to influence the oral absorption deeply. Since such esters, to our knowledge, have not been prepared previously we decided to investigate this possibility of improving the oral absorption of ampicillin, and in the following the results of our investigations are presented.

Chemistry.—Two general pathways were followed in the synthesis of acyloxymethyl esters of ampicillin. In one of these (Scheme I) the starting material was the K salt of D-α-azidobenzylpenicillin (**5**)[6] which was treated in boiling acetone with an excess of a halomethyl ester **6** to form the corresponding acyloxymethyl ester **7**. The halomethyl esters, many of which are known compounds, were prepared by treating the corresponding acid halide with paraformaldehyde.[7] Although bromomethyl esters reacted more rapidly than chloromethyl esters the latter worked quite satisfactorily when a catalytic amount of NaI was added and were generally preferred because of their better stability and ease of preparation. The D-α-azidobenzylpenicillin esters thus obtained were mostly oils which were transformed into salts of the corresponding acyloxymethyl D-α-aminobenzylpenicillinates (**8a–8h**) on catalytic hydrogenation over a Pd catalyst. The yields obtained by this method were good and the purity of the products satisfactory.

A second pathway for the preparation of such ampicillin esters is illustrated by the syntheses of the pivaloyloxymethyl ester **8f** outlined in Scheme II. Here, the first step is the preparation of pivaloyloxymethyl 6-aminopenicillanate (**10f**) which subsequently is acylated with phenylglycine. Compound **10f** could be obtained directly in 80% yield by reacting the triethylammonium salt of 6-aminopenicillanic acid (**9**) with chloromethyl pivalate in DMF and was isolated as its crystalline HCl salt or *p*-toluenesulfonate.[8] In an alternative method for the preparation of **10f**, K benzylpenicillinate (**4a**) was first transformed into pivaloyloxymethyl benzylpenicillinate (**11**). The side chain of **11** was subsequently removed by a method analogous to the elegant procedures developed for the preparation of 7-aminocephalosporanic acid[9] and 6-aminopenicillanic acid:[10] The benzylpenicillin ester (**11**) was treated with PCl_5 in $CHCl_3$ containing quinoline to form an iminochloride **12** which with *n*-PrOH afforded the imino-*n*-propyl ether **13**. This was solvolyzed with HCl to yield **10f**·HCl in an overall yield of about 90%.

Among the numerous potential methods for acylation of **10f** with phenylglycine (for a discussion of these, *cf.* Ekström, *et al.*[6]) two were found particularly convenient. In one of these D-α-phenylglycyl chloride·HCl,[11] was treated with **10f** in CH_2Cl_2 at 0°. This method gave a 85% yield of **8f**·HCl in a substantially pure form.

(1) Deceased September 1, 1969.

(2) (a) W. M. M. Kirby and C. Kind, *Ann. N. Y. Acad. Sci.*, **145**, 291 (1967); (b) Calvin M. Kunin, *ibid.*, **145**, 282 (1967).

(3) A. B. A. Jansen and T. J. Russell, *J. Chem. Soc.*, 2127 (1965).

(4) A. B. A. Jansen and T. J. Russell, U. S. Patent 3, 250,679.

(5) H. P. K. Agersborg, A. Bachelor, G. W. Cambridge, and A. W. Rule, *Brit. J. Pharmacol.*, **26**, 649 (1966).

(6) B. Ekström, A. Gomez-Revilla, R. Mollberg, H. Thelin, and B. Sjöberg, *Acta Chem. Scand.*, **19**, 281 (1965).

(7) L. H. Ulich and R. Adams, *J. Amer. Chem. Soc.*, **43**, 662 (1921).

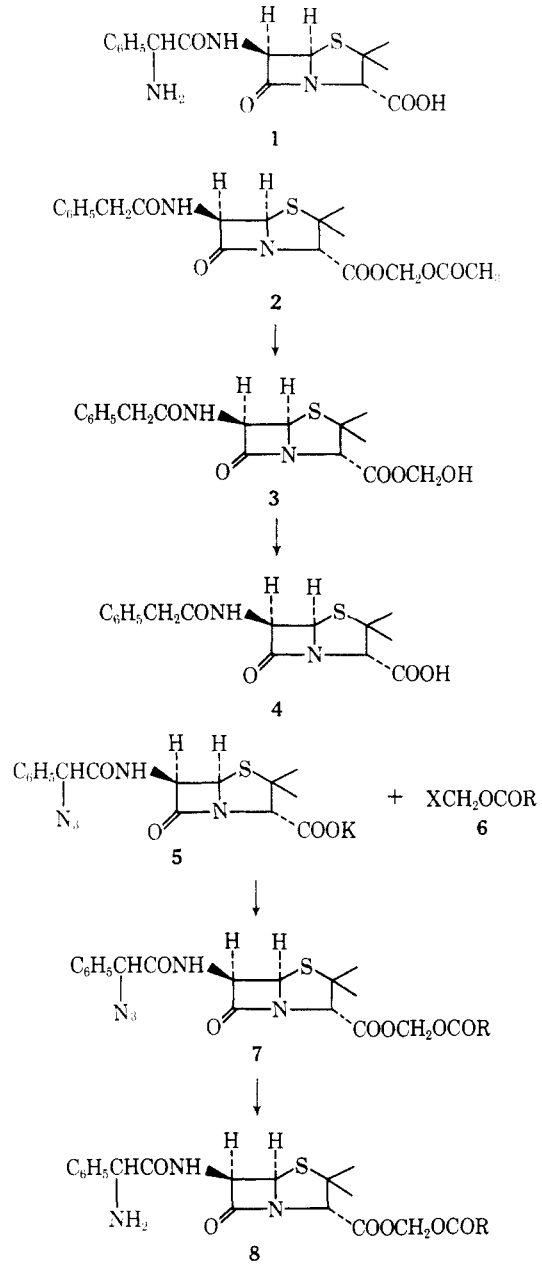
(8) A number of other 6-APA acyloxymethyl esters were prepared analogously.

(9) B. Fechtig, H. Peter, H. Bickel, and E. Vischer, *Helv. Chim. Acta*, **51**, 1108 (1968).

(10) H. W. O. Weissenburger and M. G. van der Hoeven, U. S. Patent 3,499,909.

(11) G. A. Hardcastle, Jr., D. A. Johnson, and C. A. Panetta, *J. Org. Chem.*, **31**, 897 (1966).

Scheme I



a, R = CH_3; **b**, R = CH_2CH_3; **c**, R = $(CH_2)_2CH_3$; **d**, R = $CH(CH_3)_2$; **e**, R = $(CH_2)_3CH_3$; **f**, R = $C(CH_3)_3$; **g**, R = $CH(CH_2CH_3)_2$; **h**, R = C_6H_5

Racemization, which could have been expected to be a problem, was negligible.

β-Dicarbonyl compounds have been used to protect amino functions during peptide synthesis and their application to ampicillin has been reported.[12] In an analogous procedure the mixed anhydride **14** was treated with **10f** with formation of the protected intermediate **15**. This was not isolated but hydrolyzed directly to **8f**·HCl. The yield of this process, during which some racemization took place, was about 65%.

Biological Properties.—Hydrolysis *in vitro*.—We have been able to show that the acyloxymethyl esters of ampicillin described in this paper are hydrolyzed with liberation of ampicillin under the influence of enzymes present in serum and tissue homogenates from rat, dog, and man. Although the exact mechanism of this reaction has not been elucidated, it is reasonable to assume that it proceeds analogously to the conversion of **2** into **4**.

The rate of hydrolysis of the ampicillin esters **8a**–**8h** has been studied at pH 7.4 and 37° in the presence of 10% human serum. The method is based on the fact that intact ester can be removed from the reaction mixture by extraction with EtOAc whereas ampicillin remains in the aq phase and can be determined microbiologically. To give a rough impression of the relative rates of hydrolysis the degree of hydrolysis after incubation for 30 min is indicated in Table I, from which it will be seen that unbranched esters such as **8a**, **8b**, and **8c** are hydrolyzed more rapidly than the branched esters **8f** and **8g**.

Table I

Hydrolysis of Acyloxymethyl Esters of Ampicillin at pH 7.4 and 37° in the Presence of 10% Human Serum[a]

Ester	% hydrolyzed[b] after 30 min
Acetoxymethyl (**8a**)	89
Propionyloxymethyl (**8b**)	80
n-Butyryloxymethyl (**8c**)	85
Isobutyryloxymethyl (**8d**)	89
Pivaloyloxymethyl (**8f**)	22
α-Ethyl-*n*-butyryloxymethyl (**8g**)	23
Benzoyloxymethyl (**8h**)	67

[a] The starting concentration of the esters was 1/35 mmol ~10 μg/ml of free ampicillin. [b] The figures do not indicate the exact degree of hydrolysis since—under the applied conditions—ampicillin as well as its esters undergo transformations (probably polymerization) resulting in loss at antibacterial activity.

Using a similar technique it has been shown that the half-life of the pivaloyloxymethyl ester **8f** in heparinized human whole blood at 37° is approx 5 min (average of 8 persons) and in whole blood from the dog 2.5–4 min. It has also been demonstrated that homogenates of gastric mucosa, intestinal mucosa, and liver from the dog hydrolyze **8f** rapidly.

Absorption and Distribution in Rats and Dogs.—Serum and tissue concentrations of ampicillin after oral administration of equivalent amounts of ampicillin and the hydrochloride of its pivaloyloxymethyl ester **8f**, respectively, to rats and dogs are shown in Tables II and III. The fact that the serum and tissue levels obtained after administration of the ester are much higher than those obtained after administration of ampicillin indicates a superior absorption of the former in these species. A detailed account of these experiments will be published elsewhere.[13]

Absorption and Excretion in Man.—In Tables IV and V and in Figures 1 and 2 are shown the serum levels of ampicillin attained after oral administration of ampicillin and **8f**·HCl, respectively, to healthy volunteers in two cross-over studies. The higher and earlier peak serum levels and the overall greater area under the serum level–time curves attained after administration of the ester clearly show that this compound is more efficiently absorbed from the gastrointestinal tract than ampicillin. As will appear from Tables IV and V the superior absorption is also re-

(12) E. Dane and T. Dockner, *Chem. Ber.*, **98**, 789 (1965).

(13) (a) K. Roholt and B. N. Nielsen, in preparation; (b) K. Roholt, S. B. Wolfson, and B. Skov, in preparation.

SCHEME II

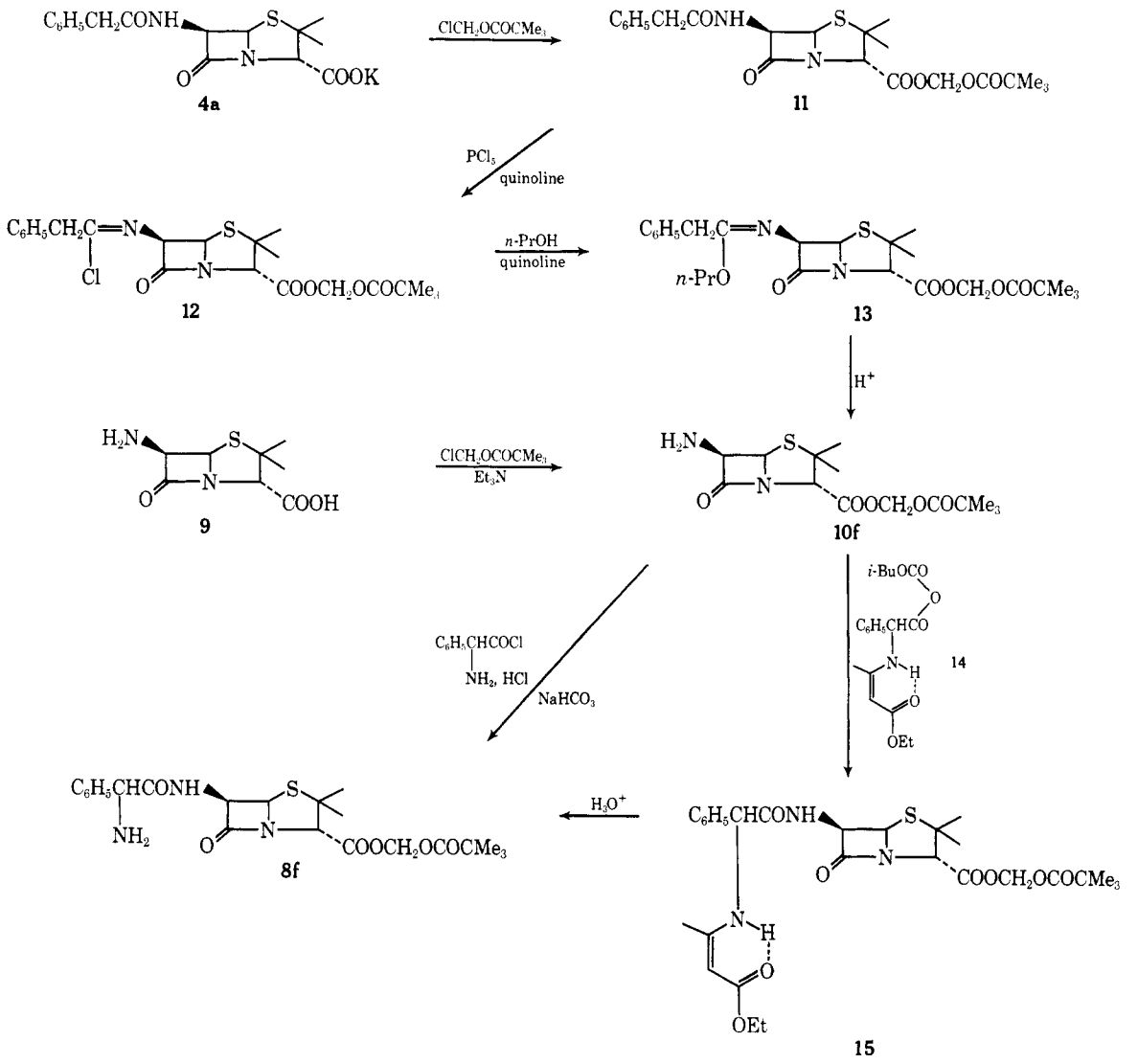


TABLE II

MEAN AMPICILLIN CONCENTRATIONS IN PLASMA AND TISSUES 2 HR FOLLOWING ORAL ADMINISTRATION OF A AND B TO GROUPS OF 5 RATS[a]

Organ	Concentration in μg/ml or μg/g of wet weight A	B
Plasma	0.84	4.9
Liver	9.0	39
Kidneys	7.0	34
Spleen	0.34	1.4
Lungs	0.57	3.1

[a] A is 100 mg/kg of ampicillin; B is 143 mg/kg of **8f**·HCl (~100 mg/kg of ampicillin).

TABLE III

MEAN AMPICILLIN CONCENTRATIONS IN BODY FLUIDS AND TISSUES 2 HR FOLLOWING ORAL ADMINISTRATION OF A AND B TO GROUPS OF 5 DOGS[a]

Organ	Concentration in μg/ml or μg/g of wet weight A	B
Blood	2.0	5.7
Liver	5.5	13
Bile	28	147
Kidneys	18	69
Urine	847	2932
Spleen	1.1	2.3
Lungs	2.1	4.1

[a] A is 30 mg/kg of ampicillin; B is 43 mg/kg of **8f**·HCl (~30 mg/kg of ampicillin).

flected in the urinary recovery. Thus, the amount of ampicillin excreted in the urine in 0–6 hr averages 70% of the administered dose in the case of the ester, but only 27% after administration of ampicillin. It is interesting to note that the degree of absorption of **8f** is practically the same whether the compound is given on an empty stomach or in connection with a meal. The fact that both the peak serum levels and the urinary excretion of ampicillin obtained after *oral* administration of the ester are very similar to the values reported after *intramuscular* injection of an equivalent amount of ampicillin[14] suggest an almost quantitative absorption of the ester.

To study the hydrolysis of **8f** *in vivo*, 10 healthy volunteers were given 715 mg of its HCl salt (~500 mg of ampicillin) orally. Blood samples were drawn 15, 60, and 120 min after administration, and the concentrations of ampicillin and intact **8f** determined microbiologically. This investigation, details of which will

(14) J. O. Klein and M. Finland, *Amer. J. Med. Sci.*, **245**, 544 (1963).

TABLE IV

SERUM CONCENTRATIONS AND URINARY EXCRETION OF AMPICILLIN AFTER ORAL ADMINISTRATION OF A AND B TO NORMAL VOLUNTEERS ON AN EMPTY STOMACH.[a] BOTH COMPOUNDS WERE GIVEN IN CAPSULES

	μg of ampicillin per ml of serum—Hr after administration																Excretion, % 0-6 hr	
	0.25		0.5		1		1.5		2		3		4		6			
Subject	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
GK	0.03	0.50	0.17	5.2	1.3	8.9	2.3	6.0	2.2	3.3	0.96	1.4	0.43	0.62	0.09	0.17	27	95
DR	0.20	0.70	0.67	3.0	1.3	4.7	0.96	5.0	1.0	4.1	0.96	2.7	0.65	1.1	0.35	0.38	22	63
KE	<0.03	<0.03	0.43	1.8	1.3	4.4	1.3	8.4	0.81	5.4	0.46	2.0	0.37	1.0	0.08	0.33	17	70
FK	<0.03	1.6	0.24	2.9	1.7	4.6	2.3	5.8	2.1	3.9	0.83	1.5	0.40	0.66	0.11	0.22	25	77
LT	<0.03	6.4	0.39	9.8	1.4	5.0	1.3	3.3	1.1	2.2	1.0	1.1	0.30	0.49	0.07	0.16	18	57
PC	<0.03	0.61	0.74	6.7	1.8	8.9	1.7	4.4	1.5	2.3	1.2	0.96	0.62	0.50	0.10	0.16	29	79
TJ	<0.03	0.03	0.17	2.3	1.4	6.1	2.2	6.6	1.7		1.8	1.7	0.87	0.72	0.16	0.23	53	83
BN	0.07	0.46	0.74	2.7	1.9	4.0	1.7	5.0	2.8	3.2	1.3	1.5	0.77	0.64	0.17	0.19	40	57
Mean	0.056	1.29	0.44	4.30	1.51	5.83	1.72	5.56	1.65	3.49	1.06	1.61	0.55	0.72	0.14	0.23	29	73

[a] A is 250 mg of ampicillin; B is 358 mg of **8f**·HCl (~250 mg of ampicillin).

TABLE V

SERUM CONCENTRATIONS AND URINARY EXCRETION OF AMPICILLIN AFTER ORAL ADMINISTRATION OF A AND B TO NORMAL VOLUNTEERS IMMEDIATELY AFTER BREAKFAST.[a] BOTH COMPOUNDS WERE GIVEN IN CAPSULES

	μg of ampicillin per ml of serum—Hr after administration																Excretion, % 0-6 hr	
	0.25		0.5		1		1.5		2		3		4		6			
Subject	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
GK	<0.03	0.96	0.24	1.8	1.0	6.4	1.4	5.2	1.2	4.7	1.1	1.9	0.54	0.71	0.15	0.21	35	55
EN	<0.03	<0.03	<0.03	0.21	0.77	7.0	1.7	6.7	1.4	3.4	0.76	1.5	0.39	0.57	0.14	0.19	26	60
AH	<0.03	<0.03	0.10	0.16	0.66	2.3	1.0	5.3	1.1	6.9	1.0	3.0	0.54	1.5	0.17	0.36	31	52
DR	<0.03	<0.03	<0.03	0.28	0.74	6.7	1.9	8.0	2.0	6.2	1.3	3.0	0.96	1.3	0.28	0.33	21	63
KE	<0.03	0.08	<0.03	1.7	0.08	4.9	0.39	4.4	1.0	4.7	1.7	2.1	1.1	1.5	0.39	0.29	21	68
AF	<0.03	<0.03	<0.03	<0.03	0.67	2.3	1.3	6.1	1.6	6.9	1.0	2.7	0.71	1.1	0.37	0.30	21	74
LT	<0.03	2.3	<0.03	8.6	0.14	8.5	0.52	5.2	1.2	3.8	1.1	1.5	0.57	0.70	0.17	0.25	18	84
TJ	<0.03	0.04	0.16	1.3	0.71	3.9	1.2	4.1	1.7	3.8	1.5	2.9	0.50	0.96	0.17	0.30	27	80
BN	<0.03	<0.03	<0.03	1.7	0.68	5.0	1.2	4.2	1.7	3.8	1.2	2.0	0.70	0.72	0.23	0.17	26	71
Mean	<0.03	0.39	0.076	1.75	0.61	5.22	1.19	5.47	1.43	4.91	1.18	2.29	0.67	1.01	0.23	0.27	25	67

[a] A is 250 mg of ampicillin; B is 358 mg of **8f**·HCl (~250 mg of ampicillin).

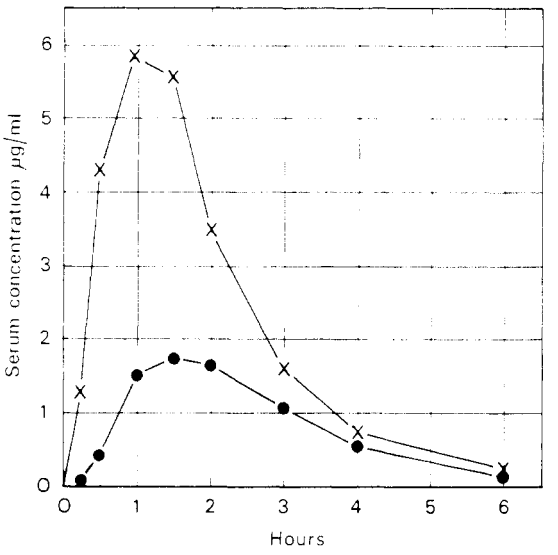


Figure 1.—Mean serum levels of ampicillin in normal volunteers following oral administration of 250 mg of ampicillin (●-●-●) and 358 mg of **8f**·HCl (≈250 mg of ampicillin) (×-×-×) on an empty stomach.

be published elsewhere,[13] showed that the concentration of **8f** in no case exceeded 2% of the corresponding ampicillin concentration and generally was considerably lower. These results as well as the fact that attempts to demonstrate **8f** in the urine have failed indicate that the hydrolysis is practically complete and takes place rapidly after the absorption—in good agreement with the high enzymatic activity demonstrated *in vitro* in blood and various tissues.

Clinical trials with **8f** are in progress.

Experimental Section

All melting points are corrected. Optical rotations were determined at 20° with a Perkin-Elmer 141 polarimeter. The ir spectra were obtained with a Perkin-Elmer 21 spectrophotometer with a NaCl prism and the nmr spectra with a Varian Associates spectrometer, Model A-60A. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

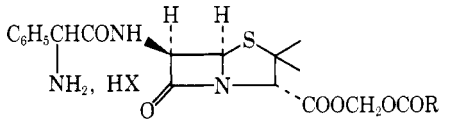
Acetoxymethyl D-α-Azidobenzylpenicillinate (7a).—K D-α-azidobenzylpenicillinate (**5**)[6] (8.26 g, 0.02 mol), $KHCO_3$ (1.0 g, 0.01 mol), and bromomethyl acetate[7] (4.0 ml, 0.04 mol) were refluxed for 1 hr in a mixture of Me_2CO (50 ml) and H_2O (1 ml). After cooling, the suspension was filtered, the filtrate evaporated *in vacuo*, and the residue washed repeatedly by decantation with petroleum ether to remove excess of bromomethyl acetate. The oily residue thus obtained was taken up in EtOAc (50 ml); the resulting solution was washed with aq $NaHCO_3$ followed by H_2O and dried. Removal of the solvent *in vacuo* gave 10.0 g of **7a** as a gum which did not crystallize. Ir and nmr spectra were as expected.

Pivaloyloxymethyl D-α-Azidobenzylpenicillinate (7f).—To a suspension of K D-α-azidobenzylpenicillinate (**5**)[6] (206.8 g, 0.5 mol) in Me_2CO (2 l.) and 10% aq NaI (40 ml) was added chloromethyl pivalate[15] (148 ml, 1.0 mol) and the mixture was refluxed for 3.5 hr. After cooling, the suspension was filtered and the

(15) M. Rasmussen and N. J. Leonard, *J. Amer. Chem. Soc.*, **89**, 5442 (1967).

TABLE VI

ACYLOXYMETHYL D-α-AMINOBENZYLPENICILLINATES



No.	R	X	Mp, °C dec	[α]D, °	Purity, %[a]	Formula	Analysis
8a	CH_3	Cl	Amorphous	+194[e]	88	$C_{19}H_{24}ClN_3O_6S$	
		p-$CH_3C_6H_4SO_2$	163–164[b]	+153[f]		$C_{26}H_{31}N_3O_8S_2 \cdot 0.5\ H_2O$	C, H, N, S
8b	CH_2CH_3	Cl	Amorphous	+191[e]	90	$C_{20}H_{26}ClN_3O_6S$	
		p-$CH_3C_6H_4SO_2$	159–160[b]	+154[f]		$C_{27}H_{33}N_3O_8S_2 \cdot 0.5\ H_2O$	C, H, N, S
8c	$(CH_2)_2CH_3$	Cl	Amorphous	+190[e]	96	$C_{21}H_{28}ClN_3O_6S$	
8d	$CH(CH_3)_2$	Cl	Amorphous	+189[e]	96	$C_{21}H_{28}ClN_3O_6S$	
8e	$(CH_2)_3CH_3$	Cl	Amorphous	+186[e]	99	$C_{22}H_{30}ClN_3O_6S$	
8f	$C(CH_3)_3$	Cl	155–156[c]	+200[e]		$C_{22}H_{30}ClN_3O_6S \cdot 0.5\ H_2O$	C, H, N, S, H_2O
		p-$CH_3C_6H_4SO_2$	151–152[b]	+151[f]		$C_{29}H_{37}N_3O_8S_2$	C, H, N, S
		p-$CH_3C_6H_4SO_3$	144–145[d]	+150[f]		$C_{29}H_{37}N_3O_9S_2$	C, H, N, S
8g	$CH(CH_2CH_3)_2$	Cl	Amorphous	+176[e]	86	$C_{23}H_{32}ClN_3O_6S$	
		p-$CH_3C_6H_4SO_2$	149–150[b]	+146[f]		$C_{30}H_{39}N_3O_8S_2$	C, H, N, S
8h	C_6H_5	Cl	Amorphous	+182[e]	96	$C_{24}H_{26}ClN_3O_6S$	

[a] Determined iodometrically using ampicillin trihydrate as standard. [b] Recrystallized from MeOH–H_2O. [c] Recrystallized from EtOH–*i*-Pr_2O. [d] Recrystallized from EtOAc. [e] H_2O (*c* 1). [f] MeOH (*c* 1).

filtrate was evaporated *in vacuo*. The oily residue thus obtained was dissolved in EtOAc (800 ml); the resulting solution was washed with aq $NaHCO_3$ followed by H_2O, dried, and evaporated *in vacuo* to leave a yellowish gum, which crystallized from Et_2O to afford 186 g (76%) of **7f** as colorless needles, mp 113–114°. An analytical sample was prepared by recrystallization from EtOAc: mp 114–115°; [α]D +42° (*c* 1, $CHCl_3$); ir and nmr spectra were as expected. *Anal.* ($C_{22}H_{27}N_3O_6S$) C, H, N, S.

In a similar way the acyloxymethyl D-α-azidobenzylpenicillinates **7a–7e** and **7g–7h** were prepared from **5** and the corresponding chloromethyl esters.[16] Apart from **7h** [mp 127–128°; [α]D +46° (*c* 1, $CHCl_3$)] the compounds were obtained as gums, which did not crystallize. Their ir and nmr spectra were as expected and the compounds were hydrogenated to the corresponding ampicillin esters without further purification.

Acetoxymethyl D-α-Aminobenzylpenicillinate (8a).—To a solution of crude acetoxymethyl D-α-azidobenzylpenicillinate (**7a**) (10 g, *ca.* 0.02 mol) in EtOAc (150 ml), placed in a 4-necked 500 ml-flask equipped with an efficient stirrer, gas inlet and outlet tubes, a glass–calomel combination electrode, and a buret controlled by an automatic titrator, were added H_2O (100 ml) and 10% Pd–C (5 g). The system was flushed with N_2, and thereafter H_2 was bubbled through the suspension with stirring, a pH value of 3.0 being maintained in the aq phase by addition of 1 *N* HCl *via* the titrator. When the consumption of acid stopped, the flask was flushed with N_2 and the catalyst filtered off. After separation of the phases the organic layer was extracted with H_2O. The combined aqueous phases were washed with Et_2O and freeze-dried to afford 6.5 g of **8a**·HCl as a colorless amorphous powder, [α]D +194° (*c* 1, H_2O). The product had a purity of 88%. Ir and nmr spectra were as expected. A crystalline ***p*-toluenesulfinate** was obtained by adding 0.5 *M* aq sodium *p*-toluenesulfinate to a 20% aq solution of the amorphous hydrochloride. The analytical sample was prepared by recrystallization from MeOH–H_2O: mp 163–164° dec; [α]D +153° (*c* 1, MeOH). *Anal.* ($C_{26}H_{31}N_3O_8S_2 \cdot 0.5\ H_2O$) C, H, N, S.

Analogously to the preparation of **8a** the ampicillin esters listed in Table VI were obtained by hydrogenation of the corresponding acyloxymethyl D-α-azidobenzylpenicillinates. The yields were in the range of 60–80% and all ir and nmr spectra were as expected.

Pivaloyloxymethyl 6-Aminopenicillanate (10f). A. From 6-APA.—To a suspension of 6-APA (54 g, 0.25 mol) in DMF (250 ml) was added Et_3N (49 ml, 0.35 mol) and, after stirring for 0.5 hr, chloromethyl pivalate[15] (74 ml, 0.5 mol). After stirring at 26–28° for 4 hr, the mixture was diluted with EtOAc (750 ml); the precipitated Et_3N·HCl was filtered off, and the filtrate was washed with H_2O (4 × 250 ml) to remove the greater part of DMF and unreacted 6-APA. The organic layer was dried and concentrated to about half the volume under reduced pressure (bath temp 30–35°). Treatment of the stirred solution of the crude ester with 0.5 *M* TsOH in EtOAc (450 ml) at 25° precipitated the crystalline ***p*-toluenesulfonate** which was filtered off, washed with EtOAc followed by Et_2O, and dried to afford 101.2 g (80.6%) of colorless needles, mp 148–149° dec. Recrystallization from MeOH–EtOAc furnished the analytical sample: mp 150–151° dec; [α]D +133° (*c* 1, MeOH); ir and nmr spectra were as expected. *Anal.* ($C_{21}H_{30}N_2O_8S_2$) C, H, N, S.

A crystalline **hydrochloride** [mp 156–160° dec, [α]D +183° (*c* 1, 0.1 *N* HCl)] was obtained by adding 1 *N* HCl in *i*-PrOH to a stirred solution of crude 6-APA pivaloyloxymethyl ester in EtOAc. *Anal.* ($C_{14}H_{23}ClN_2O_5S$) C, H, Cl, N, S.

B. From 11.—To a stirred solution of PCl_5 (33.6 g, 0.16 mol) in dry, EtOH-free $CHCl_3$ (320 ml) were added quinoline (36.2 ml, 0.31 mol) and, after cooling to −10°, pivaloyloxymethyl benzylpenicillinate (**11**) (64 g, 0.14 mol). After stirring for 15 min at −10°, *n*-PrOH (105 ml, 1.4 mol) was added over a period of 5 min. The mixture was kept at −10° for a further 15 min, then a solution of NaCl (50 g) in H_2O (220 ml) was added with vigorous stirring. During this process the tempera-

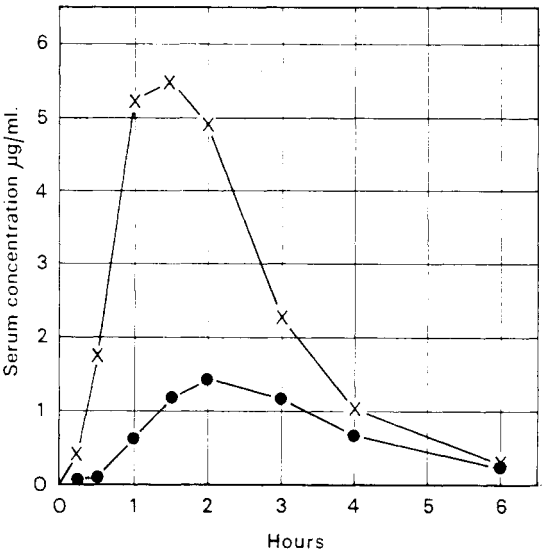
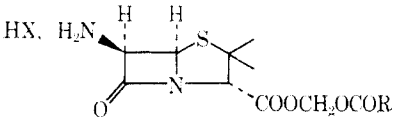


Figure 2.—Mean serum levels of ampicillin in normal volunteers following oral administration of 250 mg of ampicillin (●-●-●) and 358 mg of **8f**·HCl (≈250 mg of ampicillin) (×-×-×) immediately after breakfast.

(16) The chloromethyl esters of propionic, *n*-butyric, valeric, and benzoic acid have previously been described.[7] Chloromethyl isobutyrate (bp[15] 44–46°) and chloromethyl α-ethyl-*n*-butyrate (bp[15] 66–72°) were prepared analogously.

TABLE VII

ACYLOXYMETHYL 6-AMINOPENICILLANATES



No.	R	X	Mp, °C dec	$[\alpha]_D$, °	Formula	Analysis
10a	CH_3	p-$CH_3C_6H_4SO_3$	147–148[a]	+133[c]	$C_{18}H_{24}N_2O_8S_2$	C, H, N, S
10b	CH_2CH_3	p-$CH_3C_6H_4SO_3$	135–136[a]	+128[c]	$C_{19}H_{26}N_2O_8S_2$	C, H, N, S
10d	$CH(CH_3)_2$	p-$CH_3C_6H_4SO_3$	132–133[a]	+133[c]	$C_{20}H_{28}N_2O_8S_2$	C, H, N, S
10e	$(CH_2)_3CH_3$	p-$CH_3C_6H_4SO_3$	131–132[a]	+126[c]	$C_{21}H_{30}N_2O_8S_2$	C, H, N, S
10f	$C(CH_3)_3$	p-$CH_3C_6H_4SO_3$	150–151[a]	+133[c]	$C_{21}H_{30}N_2O_8S_2$	C, H, N, S
		Cl	156–160[b]	+183[d]	$C_{14}H_{23}ClN_2O_5S$	C, H, Cl, N, S
10g	$CH(CH_2CH_3)_2$	p-$CH_3C_6H_4SO_3$	139–140[a]	+123[c]	$C_{22}H_{32}N_2O_8S_2$	C, H, N, S
10h	C_6H_5	p-$CH_3C_6H_4SO_3$	143–144[a]	+121[c]	$C_{23}H_{26}N_2O_8S_2$	C, H, N, S

[a] Recrystallized from MeOH–EtOAc. [b] Recrystallized from MeOH–*i*-PrOH. [c] MeOH (*c* 1). [d] 0.1 *N* HCl (*c* 1).

ture in the mixture rose to 0°. Petroleum ether (450 ml) was added, and the mixture was seeded with crystals from an earlier preparation of **10f**·HCl. After stirring for 10 min at 0°, another portion of petroleum ether (500 ml) was added over a period of 10 min. Stirring at the low temperature was continued for 15 min, then the aq phase was separated and the precipitate in the organic phase collected. The filter cake was slurried in a mixture of saturated aq NaCl (200 ml) and H_2O (10 ml). The slurry was filtered and the solid was washed with saturated aq. NaCl followed by Et_2O to yield 52.4 g of a crystalline product: $[\alpha]_D$ +161° (*c* 1, 0.1 *N* HCl); corresponding to a 89% yield of pure **10f**. Apart from a content of NaCl the product was pure and could be used directly for the acylation step.

Analogously to the preparation of **10f** (method A) the acyloxymethyl 6-aminopenicillanates listed in Table VII were prepared from 6-APA and the corresponding chloromethyl esters.

Pivaloyloxymethyl Benzylpenicillinate (11).—To a suspension of K benzylpenicillinate (95 g, 0.25 mol) in Me_2CO (1 l.) was added chloromethyl pivalate[15] (41.5 ml, 0.28 mol) followed by 25% aq NaI (25 ml). The mixture was refluxed for 5 hr with stirring. After cooling, water (1 l.) was added to precipitate 103 g (92%) of pure **11**: mp 114–115°; $[\alpha]_D$ +144° (*c* 1, $CHCl_3$). *Anal.* ($C_{22}H_{28}N_2O_6S$) C, H, N.

Pivaloyloxymethyl D-α-Aminobenzylpenicillinate (8f) by Acylation of 10f. A. From D-α-Phenylglycyl Chloride·HCl.—To a stirred suspension of D-α-phenylglycyl chloride·HCl[11] (49.6 g, 0.25 mol) in CH_2Cl_2 (1 l.) was added at 0° $NaHCO_3$ (42 g, 0.50 mol) followed by **10f**·HCl (73.4 g, 0.2 mol). After vigorous stirring at 0° for 1.5 hr, the mixture was filtered through Celite; *i*-PrOH (300 ml) was added to the filtrate which then was concentrated *in vacuo*. After removal of the greater part of CH_2Cl_2 crystallization of a colorless product began. To the mixture was added *i*-PrOH (300 ml) and Et_2O (700 ml), and, after cooling, the precipitate was filtered off to yield 85 g (85%) of **8f**·HCl, mp 155–156° dec, identical in every respect with that obtained by the azidomethod described above.

B. *Via* β-Dicarbonyl Protecting Group.—To a solution of K *N*-[1-methyl-2-carbethoxyvinyl]-D-α-amino-α-phenylacetate hemihydrate[12] (155.2 g, 0.5 mol) in EtOAc (2 l.) was added *N*-methylmorpholine (2.5 ml) and isobutyl chloroformate (70 ml) at −15° with stirring. KCl separated immediately, and the mixture was kept at −15° for 6 min. To the solution of the mixed anhydride **14** thus obtained was added with stirring an ice-cold solution of **10f** (liberated from 251.3 g (0.5 mol) of the *p*-toluenesulfonate of this compound) in EtOAc (1 l.). During the addition and then for a further 10 min, the temperature was kept between −14° and −12°. Thereafter, the cooling bath was removed, and stirring continued until the temperature had risen to 10° (about 40 min). The mixture was washed with H_2O (500 ml), 0.5 *M* aq $NaHCO_3$ (500 ml), and H_2O (2 × 250 ml), dried, and evaporated *in vacuo*. The residual yellow oil was taken up in THF (1 l.); H_2O (900 ml) was added, and the apparent pH value of the mixture was adjusted to 2.5 by addition of 4 *N* HCl with stirring. During the hydrolysis this pH was maintained by addition of further HCl *via* an automatic titrator. The consumption of acid ceased when a total of 105 ml of 4 *N* HCl (84% of the theoretical amount) had been added. THF was removed from the mixture at reduced pressure (bath 30–35°), and the remaining aqueous phase was extracted with EtOAc (4 × 150 ml). To the combined EtOAc extracts were added petroleum ether (600 ml) and H_2O (200 ml); the pH value of the aqueous phase was adjusted to 2.5 by addition of dil HCl with stirring, and the aq extract was separated. To the combined aq phases (*ca.* 1.2 l.) thus obtained, was added NaCl (240 g); the mixture was shaken vigorously, and the resulting yellowish organic layer (*ca.* 400 ml) was separated. After extraction of the aq phase with EtOAc (200 ml) the combined organic phases were dried and *i*-PrOH (800 ml) was added. The mixture was concentrated to about half the volume at reduced pressure, and another portion of *i*-PrOH (800 ml) was added. Repeated concentration of the mixture to about 600–800 ml *in vacuo* furnished precipitation of a colorless crystalline product, which was kept at 4° overnight. The precipitate was filtered off, washed with ice-cold *i*-PrOH (2 × 100 ml) followed by Et_2O (2 × 100 ml), and dried to yield 149.2 g (58.6%) of **8f**·HCl, mp 155–156° dec. On concentration of the mother liquor *in vacuo* another 17.0 g (6.6%) of the desired product, mp 155–156° dec, was obtained.

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