Preparation, Hydrolysis, and Oral Absorption of Lactonyl Esters of Penicillins

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Lactonyl esters of ampicillin and other penicillins have been synthesized as prodrugs designed to improve the oral absorption of the parent penicillins. In general, the esters hydrolyzed rapidly in the presence of tissues including blood and certain of the esters were better absorbed than the parent penicillin. The phthalidyl ester of ampicillin [talampicillin (British Pharmacopoeia approved name), BRL 8988] was selected for extended studies. The compound was found to be well absorbed in various animal species and gave ampicillin serum concentrations in fasting human volunteers 2.5–3 times those obtained for ampicillin itself.

Esters of penicillins and, more recently, of cephalosporins continue to demand attention as a means of improving oral absorption of compounds that are not absorbed or only moderately absorbed by the oral route.[1,2] Since esters of penicillins are themselves devoid of antibacterial activity, it is recognized that the two essential requirements for an ester to be of clinical significance are that it should facilitate the absorption process and, once absorbed, the ester should hydrolyze readily to release the free penicillin into the blood and tissues. Simple alkyl and aryl esters of penicillins fail to meet this latter requirement in man as they are resistant to hydrolysis by human tissues.[3,4]

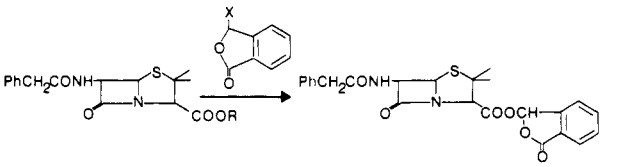
The first successful approach to the development of a penicillin ester susceptible to hydrolysis by human tissues was made by Jansen and Russell who described a series of acyloxymethyl esters of penicillin G (benzylpenicillin).[5] The acetoxymethyl ester of penicillin G, penamecillin, was investigated in some detail and was shown to be hydrolyzed by various animal tissues and by human serum.[6] Penamecillin was found to be orally absorbed to a limited extent in man resulting in serum levels of penicillin G only slightly higher than those obtained with the parent penicillin.[7]

In contrast, acyloxymethyl esters of ampicillin (D-α-aminobenzylpenicillin) were shown to be well absorbed in man, giving rise to high serum concentrations of ampicillin.[8] The ester of choice, the pivaloyloxymethyl ester, pivampicillin, was shown to produce serum concentrations of ampicillin two to three times higher than those produced by ampicillin.[9]

We now wish to describe a new class of hydrolyzable double esters of penicillins in which the second ester function is incorporated in a lactone group, preferably a phthalidyl lactone group. The preparation of these compounds, their in vitro hydrolysis rates, and oral absorption properties in man and animals are reported.

Chemistry. The penicillin lactonyl esters investigated in this study are described in Table I. It will be observed that esters 1–14 are phthalidyl or substituted phthalidyl esters, esters **15** and **16** are crotonolactonyl esters, while compounds **17** and **18** are isomeric butyrolactone esters of penicillin G. In view of the asymmetric carbon center (C-3) at the point of attachment of the thiazolidine carboxyl to the lactone ring, the esters in Table I were obtained as mixtures of epimers. In those cases examined, the proton NMR evidence suggested the epimer ratio was 1:1. The phthalidyl ester of ampicillin (**4a**) was examined in some detail. The NMR spectrum of its hydrochloride did not permit the epimer ratio to be calculated since proton signals overlapped. However, when the free base was prepared and its spectrum examined in the presence of the lanthanide shift reagent, Eu(fod)$_3$, the individual epimers could be readily distinguished in a ratio of 1:1. The thiazolidine C-3 proton appeared as two sharp singlets of equal intensity.

Scheme I[a]



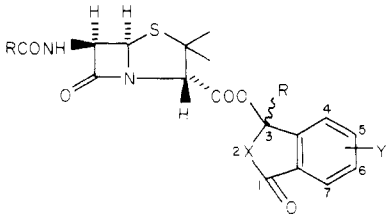
[a] Method A, R = Na; X = Br. Method B, R = Na; X = OTos. Method C, R = COOEt; X = OH.

The preparation and characterization of most of the compounds in Table I have been previously described.[10] Three general procedures were used. The preferred method—method A—was to condense an alkali or amine salt of the penicillin with the appropriate 3-halolactone. Alternatively, in place of the 3-halolactone, the corresponding arylsulfonate ester derived from the 3-hydroxylactone was used—method B. Method C consisted of preparing the penicillin mixed anhydride and reacting this with the 3-hydroxylactone. Each of these methods is illustrated in the Experimental Section and shown in Scheme I below for the phthalidyl ester of benzylpenicillin.

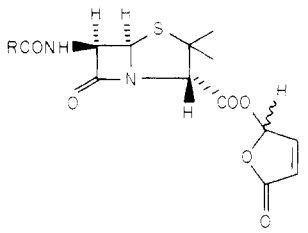
3-Bromo- or 3-chlorolactones were readily prepared by halogenation of the appropriate lactone.[10] 3-Hydroxylactones were available via the corresponding γ-keto acids with which they are in equilibrium in solution. In the preparation of the lactonyl esters of α-aminopenicillins it was desirable to protect the α-amino function prior to condensation. This was done by standard procedures, most conveniently with the enamine protecting group derived from methyl acetoacetate. This group was subsequently removed by mild acid treatment to regenerate the amine.

In order to observe if the configuration of the ester asymmetric carbon center had any effect on hydrolysis rates and oral absorption, the individual epimers of the phthalidyl esters of benzylpenicillin and ampicillin were prepared as follows. Phthalidyl benzylpenicillinate (**3a**) was obtained in 45% yield by condensation of the potassium salt of penicillin G with 3-bromophthalide. The crystalline product, mp 158–160 °C, was shown to consist of a 1:1 mixture of epimers by the appearance in the NMR of the thiazolidine C-3 proton as two sharp singlets, δ 4.82 and 4.87 in deuteriopyridine. Repeated fractional crystallization of this product afforded the individual epimers designated **3b**, mp 179 °C, and **3c**, mp 169–171 °C, with optical rotations unchanged on further crystallization. Epimer purity was confirmed by the appearance in the NMR of single sharp singlets for the C-3 proton at δ 4.82 (**3c**) and 4.87 (**3b**). These two compounds were treated separately with phosphorus pentachloride followed by methanol to cleave the phenylacetic acid side chain. Without isolation the product, phthalidyl 6-aminopenicillinate, was reacylated with D(−)-α-aminophenylacetyl chloride hydrochloride to afford the individual

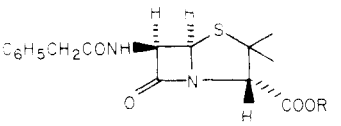
Table I. Lactonyl Esters of Penicillins[a]



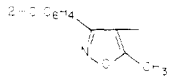
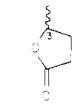
A



B



C

No.	R	R^1	X	Y	% yield	Formula	Analyses
				(A) Phthalidyl Esters			
1		H	O	H	91	$C_{27}H_{24}ClN_3O_7$	H, Cl, N; C[g]
2	$C_6H_5OCH_2$	H	O	H	60	$C_{24}H_{22}N_2O_7S$	C, H, N, S
3a	$C_6H_5CH_2$	H[b]	O	H	45	$C_{24}H_{22}N_2O_6S$	C, H, N, S
3b	$C_6H_5CH_2$	H[c]	O	H		$C_{24}H_{22}N_2O_6S$	C, H, N
3c	$C_6H_5CH_2$	H[d]	O	H		$C_{24}H_{22}N_2O_6S$	C, H, N
4a	D-$C_6H_5CH(NH_2HCl)$	H	O	H	65[j]	$C_{24}H_{24}ClN_3O_6S$	H, N; C,[g] S[h]
4b	D-$C_6H_5CH(NH_2HCl)$	H[e]	O	H	63[k]	$C_{24}H_{24}ClN_3O_6S$	H, Cl, N, S; C[g]
4c	D-$C_6H_5CH(NH_2HCl)$	H[f]	O	H	72[k]	$C_{24}H_{24}ClN_3O_6S$	H, Cl, N, S; C[g]
5	$C_6H_5CH_2$	H	O	6-OMe	34	$C_{25}H_{24}N_2O_7S$	C, H, N, S
6	D-$C_6H_5CH(NH_2HCl)$	H	O	6-OMe	51[j]	$C_{25}H_{26}ClN_3O_7S$	H, Cl, N; C,[g] S[i]
7	$C_6H_5CH_2$	H	O	5,6-$(OMe)_2$	41	$C_{26}H_{26}N_2O_8S$	H, N, S; C[g]
8	D-$C_6H_5CH(NH_2HCl)$	H	O	5,6-$(OMe)_2$	29[j]	$C_{26}H_{28}ClN_3O_8S$	H, Cl, N, S; C[g]
9	D-$C_6H_5CH(NH_2HCl)$	H	O	4,5,6-$(OMe)_3$	70[j]	$C_{27}H_{30}ClN_3O_9S$	H, Cl, N, S; C[g]
10	D-$C_6H_5CH(NH_2HCl)$	H	O	6-Cl	47[j]	$C_{24}H_{23}Cl_2N_3O_6S$	H, N, S; C[g]
11	D-$C_6H_5CH(NH_2HCl)$	H	O	6-Br	44[j]	$C_{24}H_{23}BrClN_3O_6S$	H, N, S; C[g]
12	$C_6H_5OCH_2$	CH_3	O	H	32	$C_{25}H_{24}N_2O_7S$	C, H, N
13	$C_6H_5CH_2$	C_6H_5	O	H	25	$C_{30}H_{26}N_2O_6S$	
14	$C_6H_5CH_2$	H	S	H	13	$C_{24}H_{22}N_2O_5S_2$	H, N, S; C[g]
				(B) Crotonolactonyl Esters			
15	$C_6H_5CH_2$				14	$C_{20}H_{20}N_2O_6S$	H, N; C[g]
16	D-$C_6H_5CH(NH_2)$				30	$C_{20}H_{22}ClN_3O_6S$	H, Cl, N, S; C[g]
				(C) Butyrolactonyl Esters			
17					65	$C_{20}H_{22}N_2O_6S$	
18					59	$C_{20}H_{22}N_2O_6S$	C, H, N, S

[a] All penicillins were amorphous and obtained as mixtures epimeric at C_3 in the lactone function, unless stated otherwise. [b] 1:1 epimeric mixture; $[\alpha]^{20}D$ +223° (*c* 1, MeOH); mp 158–160 °C. [c] Single epimer; $[\alpha]^{20}D$ +226° (*c* 1, MeOH); mp 179 °C. [d] Single epimer; $[\alpha]^{20}D$ +179° (*c* 0.5, MeOH); mp 169–171 °C. [e] Single epimer; $[\alpha]^{20}D$ +188° (*c* 1, MeOH). [f] Single epimer; $[\alpha]^{20}D$ +109° (*c* 1, MeOH). [g] Carbon analysis 1–2% low. [h] S: calcd, 6.19; found, 7.12. [i] S: calcd, 5.85; found, 4.51. [j] Yield is esterification and deprotection. [k] Yields of **4b** and **4c** from **3b** and **3c**, respectively.

epimers of **4a**, namely, **4b** and **4c** as their hydrochlorides.[11] The epimeric purity of **4b** and **4c** was again established as greater than 95% by NMR in deuteriopyridine where singlets for the thiazolidine C-3 proton were observed at δ 4.86 (**4b**) and 4.80 (**4c**).

Hydrolysis Studies in Vitro. The rates of hydrolysis of the penicillin lactonyl esters listed in Table I were determined in 90% human blood and in 0.05 M potassium phosphate buffer at pH 7.0. Esters **2** and **4a**, **6**, **8–11**, **16** of the acid-stable penicillins, penicillin V (phenoxymethylpenicillin), and ampicillin, respectively, were also examined after incubation at pH 2.0.

Results in Table II show that the rates of hydrolysis of the esters were very much greater in 90% blood than in phosphate buffer at pH 7.0. The esters of the acid-stable penicillins showed no hydrolysis (<10%) at pH 2.0 over a period of 25 min. From the point of view of oral absorption, these results suggest that the lactonyl esters possess satisfactory hydrolysis characteristics in that they might be expected to be stable in the intestinal tract but to be rapidly hydrolyzed by enzymes in the blood.

As can be seen from Table II, many of the esters were very rapidly hydrolyzed in 90% blood. It should be noted that for the ester of cloxacillin (**1**), the substrate concentration was higher than for the other compounds and this could increase the time taken for 50% hydrolysis without affecting the rate of hydrolysis. The effect of substitution of methyl (**12**) and phenyl (**13**) groups at the 3 position in the phthalidyl ring was to reduce the rate of enzymic hydrolysis compared with the unsubstituted esters. This was also the case when oxygen was replaced by sulfur in the lactone ring; an appreciably slower rate of hydrolysis in blood was observed for thioester **14** compared with its oxygen analogue **3a**.

Another structural change which might be expected to influence the rate of hydrolysis was that involving differences in configuration at the epimeric center (position 3, Table I) in the lactone ring. The results in Table III

Table II. Hydrolysis of Lactonylpenicillin Esters in Phosphate Buffer and Human Blood

Penicillin ester[a]			Time (min) taken for 50% hydrolysis	
No.	Ester group	Penicillin	In phosphate buffer, pH 7	In 90% human blood
1	Phthalidyl	Cloxacillin	>25	12[a]
2	Phthalidyl	Penicillin V	>25	<2
3a	Phthalidyl	Penicillin G	>25	<2
4a	Phthalidyl	Ampicillin	>25	<2
5	6-Methoxyphthalidyl	Penicillin G	>25	<2
6	6-Methoxyphthalidyl	Ampicillin	>25	<2
7	5,6-Dimethoxyphthalidyl	Penicillin G	>25	<2
8	5,6-Dimethoxyphthalidyl	Ampicillin	>25	5
9	4,5,6-Trimethoxyphthalidyl	Ampicillin	>25	6
10	6-Chlorophthalidyl	Ampicillin	13	2
11	6-Bromophthalidyl	Ampicillin	>25	14
12	3-Methylphthalidyl	Penicillin V	>25	>25
13	3-Phenylphthalidyl	Penicillin G	>25	10
14	2-Thiophthalidyl	Penicillin G	>25	>25
15	Crotonolactonyl	Penicillin G	22	<2
16	Crotonolactonyl	Ampicillin	>25	<2
17	3-Butyrolactonyl	Penicillin G	>25	<2
18	5-Butyrolactonyl	Penicillin G	>25	>25

[a] The concentration of all esters (not purity corrected) was such as to give 5 μg/ml of ampicillin, penicillin G, and penicillin V and 50 μg/ml of cloxacillin when completely hydrolyzed.

Table III. Hydrolysis of the Epimers of the Phthalidyl Esters of Penicillin G (3b,c) and Ampicillin (4b,c)

Hydrolyzing system	Time (min) required for 50% hydrolysis[a]			
	3b	3c	4b	4c
Phosphate buffered saline, pH 7.4	>25	>25	>25	>25
90% human blood			<2	<2
10% human blood	7	<2	3	<2
2% human blood			10	4
10% human small intestine homogenate			2	<2
0.02% human liver homogenate			25	20

[a] The concentration of ester (not purity corrected) was such as to give 5 μg/ml of parent penicillin when completely hydrolyzed.

illustrate that one epimer (**3b**) of the phthalide ester of penicillin G hydrolyzed at a slower rate than the other (**3c**) in 10% human blood. The corresponding ampicillin ester **4b**, of the same relative configuration at the ester chiral center as **3b**, was also observed to hydrolyze at a slightly slower rate than its epimer **4c** in diluted blood and in diluted human liver and intestine homogenates. In 90% blood the hydrolysis rate was too fast for differences between **4b** and **4c** to be observed.

An investigation was made of the hydrolysis rates of phthalidylampicillin (**4a**) in homogenates of tissues from the animal species used for absorption studies. The data

Table IV. Hydrolysis of the Phthalidyl Ester of Ampicillin (4a) by Tissues of Rat, Mouse, Human, and Squirrel Monkey

Species	Tissues	Time (min) required for 50% hydrolysis[a]
Rat	90% blood	<2
	2% blood	2
	10% small intestine homogenate	3
	0.02% liver homogenate	13
Mouse	90% blood	<2
Human	90% blood	<2
	10% blood	2
	2% blood	7
	10% small intestine homogenate	3
	0.02% liver homogenate	25
Squirrel monkey	90% blood	<2
	5% small intestine homogenate	<2

[a] The concentration of ester (not purity corrected) was such as to give 5 μg/ml of ampicillin when completely hydrolyzed.

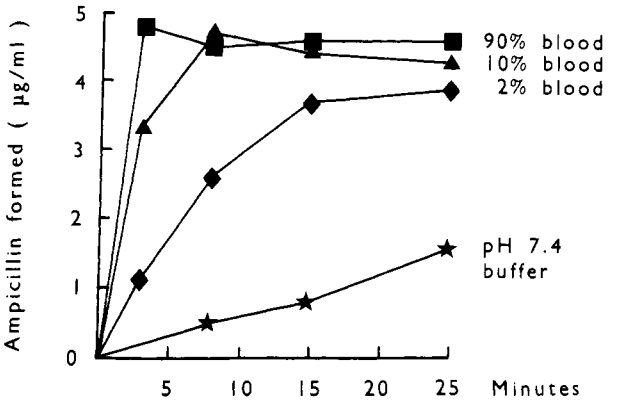


Figure 1. Rate of hydrolysis of the phthalidyl ester of ampicillin (4a) by human blood.

in Table IV show that **4a** was very rapidly hydrolyzed to ampicillin by the blood and tissue homogenates of the rat, mouse, and squirrel monkey as well as human.

Figure 1 shows the effect of different dilutions of human blood on the rate of hydrolysis of the phthalidyl ester of ampicillin **4a** to ampicillin. The rate was noticeably slower in 2% blood compared with 90% blood although in both cases hydrolysis was virtually complete after 25 min.

The mechanism of hydrolysis of lactonyl esters is not known with certainty. It was postulated by Agersborg that the acetoxymethyl ester of penicillin G hydrolyzed by initial cleavage of the terminal ester bond, followed by spontaneous breakdown of the unstable hydroxymethyl ester intermediate to give penicillin G and formaldehyde.[6] A similar mechanism was assumed for the acyloxymethyl esters of ampicillin and is represented in Scheme II, a.[1] In the case of the lactonyl esters it is suggested that initial cleavage of the lactone ring generates an unstable, substituted hydroxymethyl ester which decomposes to form the penicillin and an aldehydic acid (Scheme II, b). Phthaldehydic acid was indeed detected in further investigations in these laboratories[12] and also by independent workers as the initial product formed when phthalidylampicillin (**4a**) was incubated with various animal tissues.[13]

In support of Scheme II b was the observation that the 3-butyrolactone ester of penicillin G (**17**) was readily

Scheme II

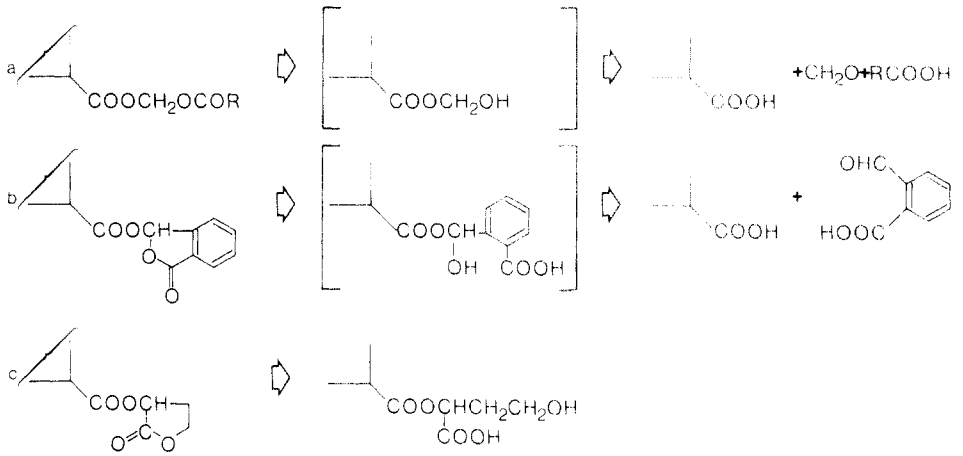


Table V. Penicillin Blood Levels in Squirrel Monkeys after Oral Administration of Lactonyl Esters[a]

Penicillin	Compd	No.	Mean blood concn of parent penicillin, μg/ml 0.5 h	1.0 h	2.0 h	4.0 h	6.0 h
Ampicillin	Phthalidyl ester	4a	7.0	8.2	7.6	1.3	0.4
	6-Methoxyphthalidyl ester	6	8.3	10.6	8.7	3.3	1.0
	5,6-Dimethoxyphthalidyl ester	8	12.7	19.5	14.8	8.0	3.0
	4,5,6-Trimethoxyphthalidyl ester	9	13.9	12.3	3.5	1.1	0.5
	6-Chlorophthalidyl ester	10	6.5	4.1	2.6	0.7	0.2
	6-Bromophthalidyl ester	11	9.9	9.0	5.5	1.5	0.2
	Crotonolactonyl ester	16	10.8	11.9	8.2	4.7	1.9
	Trihydrate		9.3	10.8	6.0	1.9	0.7
Penicillin G	Phthalidyl ester	3a	2.9	2.1	2.1	1.1	0.5
	Sodium salt		3.1	1.5	1.2	0.3	0.1
Cloxacillin	Phthalidyl ester	1	1.4	0.9	0.6	0.4	0.3
	Sodium salt		8.2	4.8	2.0	0.5	<0.5
Penicillin V	Phthalidyl ester	2	3.6	3.3	1.4	0.7	0.4
	Potassium salt		10.9	14.9	11.0	5.2	1.2

[a] Single oral dose of ester equivalent to 100 mg/kg of free acid.

hydrolyzed to penicillin G by human blood (see Table II), whereas no penicillin G was liberated when the isomeric ester **18**, in which the carbonyl and oxygen functions are reversed, was incubated in blood. This is illustrated in Scheme II c where it can be seen that the initial product from **18** is an ester in which the thiazolidine carboxyl group is linked to the 2-hydroxyl of 2,4-dihydroxybutyric acid. Such an intermediate would not be expected to hydrolyze further. Additional evidence relating to the initial cleavage of **18** came from the observation that when **18** was incubated at 1 mg/ml in 10% human blood the pH of the solution fell as it did with the control compounds **4a** and **17**. The fall in pH suggests an initial cleavage of the lactone ring to form a stable hydroxy acid ester as depicted in the Scheme II c.

Absorption Studies. Ten of the lactonyl esters described in Table I and their parent penicillins were each administered as a single oral dose to squirrel monkeys and the blood samples assayed for antibacterial activity in terms of the parent penicillins (Table V). As hydrolysis studies had shown that all of the esters were converted within 30 min to their parent penicillins in squirrel monkey blood and in view of the likely hydrolysis in the gut wall and liver, the antibacterial material in the blood was assumed to be all in the form of the parent penicillin.

The phthalidyl (**4a**), 6-methoxyphthalidyl (**6**), and 6-bromophthalidyl (**11**) esters of ampicillin gave rise to ampicillin blood levels of the same order as those produced by ampicillin trihydrate, and there was some enhancement of ampicillin blood levels after dosing the trimethoxy-substituted phthalidyl ester **9** and the crotonolactonyl ester **16**. The 5,6-dimethoxyphthalidyl ester **9** produced the highest levels of ampicillin, and these were about twice as high as those found after administration of the unsubstituted phthalidyl ester **4a** or ampicillin itself.

The extent to which the phthalidyl ester group influenced the absorption of cloxacillin, penicillin V, and penicillin G was variable and there was no correlation between the oral absorption of the parent penicillins and the penicillin blood levels produced by their esters. For example, the phthalidyl esters of two moderately well-absorbed penicillins, penicillin V and cloxacillin, produced blood levels notably lower than those obtained with the parent penicillins. Penicillin G was poorly absorbed by the oral route and no improvement in levels was found after dosing its phthalidyl ester to squirrel monkeys.

Esterification of the thiazolidine carboxyl group of a penicillin increases its lipid solubility and the ester might be expected to demonstrate improved oral absorption properties. However, the poor oral absorption of the phthalidyl esters of penicillin G, penicillin V, and cloxacillin is presumably a consequence of their inadequate aqueous solubility in fluids within the gastrointestinal tract. On the other hand, although the phthalidyl ester of ampicillin is more lipophilic than ampicillin it retains some degree of aqueous solubility by virtue of the hydrophilic amino group.

The absorption of the phthalidyl ester of ampicillin (**4a**) was further compared with that of ampicillin in the mouse, rat, and fasting human volunteers in Table VI. Ester **4a** was well absorbed and gave ampicillin blood levels two to three times higher than produced by ampicillin.

The serum and urinary concentrations of ampicillin produced in fasting human subjects by oral administration

Table VI. Ampicillin Blood Levels in Mouse, Rat, and Man after Oral Administration of the Phthalidyl Ester of Ampicillin (4a)[a]

Species	Dose[b]	Compd	Mean concn of ampicillin, μg/ml							
			10 min	20 min	30 min	40 min	60 min	120 min	240 min	360 min
Mouse	50 mg/kg	Ester 4a	7.6	5.0	3.2		1.2	0.4	0.1	
		Ampicillin	2.2	1.4	1.5		1.3	0.4	0.1	
Rat	100 mg/kg	Ester 4a			1.5		2.7	1.5	0.8	0.5
		Ampicillin			1.2		1.4	0.7	0.2	<0.1
Man	250 mg	Ester 4a		1.8		5.8	6.5	3.6	0.8	0.3
		Ampicillin		0.3		1.7	2.4	2.2	0.9	0.2

[a] Blood concentrations in man were measured in serum. [b] Dose is expressed in terms of ampicillin free acid.

Table VII. Absorption of Ampicillin Phthalidyl Esters in Human Volunteers

Ester	Dose, mg[a]	No. of subjects	Mean ampicillin serum concn, μg/ml							Urinary excretion of ampicillin, 0-6 h	
			20 min	40 min	60 min	90 min	120 min	240 min	360 min	Concn, μg/ml	% of dose
Phthalidyl (4a)	371	10	1.8	5.8	6.5	4.7	3.6	0.8	0.3	440	73
Phthalidyl single epimer (4b)	371	9	1.5	5.8	5.6	5.4	3.1	0.6	0.2	517	72
Phthalidyl single epimer (4c)	371	9	1.5	5.5	5.3	4.1	2.5	0.5	0.1	465	68
5,6-Dimethoxyphthalidyl (8)	413	10	1.6	6.3	7.6	4.8	4.0	1.0	0.4	494	81
Ampicillin	250	10	0.3	1.7	2.4	2.3	2.2	0.9	0.2	295	43

[a] Weight of ester hydrochloride equivalent to an oral dose of 250 mg of ampicillin free acid.

of ester **4a**, a 1:1 mixture of epimers, were compared with those obtained by administration of the individual epimers **4b** and **4c** and with ampicillin itself (Table VII). All three esters produced very similar ampicillin concentrations in serum which were two to three times higher than those obtained with ampicillin. Likewise, the amount of ampicillin excreted in the urine in the 0–6-h period after dosing was significantly higher with the esters (68–73%) compared with ampicillin (43%). Results are also shown in Table VII for the 5,6-dimethoxyphthalidyl ester of ampicillin (**8**), and it can be seen that levels of ampicillin were slightly higher than those found after administration of the unsubstituted phthalidyl ester.

The phthalidyl ester of ampicillin (talampicillin, BRL 8988) was selected for further studies in animals and in man, and results obtained with this ester have been reported from these laboratories and, independently, by a separate group of workers.[13,14]

Experimental Section

Chemistry. Melting points were determined using a Buchi melting point apparatus and are recorded uncorrected. The structures of all compounds were confirmed by their ir and NMR spectra, the latter of which were determined as solutions in either $CDCl_3$ or Me_2SO-d_6. The ir spectra were obtained with a Perkin-Elmer 457 spectrophotometer and the NMR spectra with a Varian Associates spectrometer, Model A-60A. Optical rotations were measured in a Perkin-Elmer polarimeter. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical values.

The three methods used for preparation of the esters in Table I are illustrated below.

Method A. (i) Phthalidyl Benzylpenicillinate (3a–c). Potassium benzylpenicillinate (2.0 g, 0.0054 mol) was dissolved in dry DMF (5 ml) and cooled to 0 °C. 3-Bromophthalide (1.15 g, 0.0054 mol) in dry DMF (2 ml) was added in one portion to the stirred penicillin solution. The reaction mixture was allowed to warm to room temperature and then stirred for a further 2 h. The mixture was then poured into ice-cold water (60 ml) and stirred vigorously. The white solid which separated was collected and washed well with H_2O and dried (1.1 g, 45%). The amorphous solid was rapidly crystallized from ethanol to give epimeric **3a**: mp 158–160 °C; $[\alpha]^{20}D$ +223° (*c* 1, MeOH). Repeated fractional crystallization from 2-propanol of **3a** afforded the individual epimers **3b** [mp 179 °C; $[\alpha]^{20}D$ +266° (*c* 1, MeOH)] and **3c** [mp 169–171 °C; $[\alpha]^{20}D$ +179° (*c* 0.5, MeOH)].

(ii) Phthalidyl D-α-Aminobenzylpenicillinate Hydrochloride (4a). A fine suspension of potassium *N*-(1-methoxycarbonylpropen-2-yl)-D-α-aminobenzylpenicillinate (2.52 g, 0.005 mol) and 3-bromophthalide (1.06 g, 0.005 mol) was stirred at ambient temperature in a 1:2 mixture of Me_2CO–EtOAc (150 ml) for 24 h. After filtration the organic layer was washed twice with 25-ml portions of aqueous $NaHCO_3$, followed by H_2O, and dried. The solution was concentrated in vacuo. Addition of a small volume of Et_2O to the solution gave phthalidyl (1-methoxycarbonylpropen-2-yl)-D-α-aminobenzylpenicillinate as a white amorphous solid (2.3 g, 81%).

The enamine-protecting group was removed from this product by dissolving it (1 g) in aqueous Me_2CO (1:1, 25 ml) and vigorously stirring this solution at pH 2.5 for 1 h. Acetone was removed in vacuo and deprotected **4a** was salted out of the aqueous phase as a sticky yellow gum. The material was dissolved in EtOAc (20 ml), washed with H_2O, and dried. Careful addition of dry ether (ca. 5 ml) to the EtOAc solution of the penicillin ester yielded **4a** as a fine white amorphous solid (0.7 g, 80%).

Method B. 3-Methylphthalidyl Phenoxymethylpenicillinate (12). *o*-Acetylbenzoic acid (4.92 g, 0.03 mol) was dispersed in dry EtOAc (100 ml) and cooled to 0 °C. Dry pyridine (2.37 g, 0.03 mol) was then added to the stirred suspension, followed by the dropwise addition of benzenesulfonyl chloride (5.31 g, 0.03 mol). The mixture was left overnight at 4 °C. Potassium phenoxymethylpenicillinate (11.64 g, 0.03 mol) was added with stirring to the benzenesulfonate ester solution and was allowed to react for a further 18 h at ambient temperature.

The reaction mixture was filtered and the filtrate washed with aqueous $NaHCO_3$, followed by H_2O, and dried. Removal of the solvent in vacuo yielded a yellow oil which was purified by chromatography over silica gel using EtOAc–ligroine [bp 60–80 °C (4:1 ratio)] as eluent. 3-Methylphthalidyl phenoxymethylpenicillinate (**12**) was obtained as a white amorphous solid (2.5 g, 17%), with identical ir and NMR spectral properties as the sample obtained by method C.

Method C. 3-Methylphthalidyl Phenoxymethylpenicillinate (12). Potassium phenoxymethylpenicillinate (11.64 g, 0.03 mol) was dispersed in dry CH_2Cl_2 (200 ml) at −5 °C. To the stirred

suspension, EtOCOCl (3.24 g, 0.03 mol) was added dropwise, together with a catalytic quantity of pyridine, and the mixture stirred at −5 °C for 0.5 h. A solution of *o*-acetylbenzoic acid (4.92 g, 0.03 mol) was added to the mixed anhydride of the penicillin and the reaction continued at ambient temperature for 18 h. Removal of the solvent in vacuo gave a yellow oil. The oily residue was taken up in EtOAc (250 ml); the resulting solution was washed with aqueous $NaHCO_3$, followed by H_2O, and dried. The product obtained after removal of EtOAc in vacuo was purified by column chromatography over silica gel using EtOAc–ligroine [bp 60–80 °C (4:1 ratio)] as eluent. 3-Methylphthalidyl phenoxymethylpenicillinate (**12**) was obtained as a white amorphous solid (4.7 g, 32%).

Tissue Hydrolysis Studies. Animals were killed by mechanical means and the liver and entire small intestine (pyloric junction to caecal junction) removed immediately. The small intestines were flushed with water to remove gut contents and all tissues kept frozen (−15 °C) until needed. Human liver and jejunum were removed at postmortem examination after death from bronchitis and emphysema. The tissues were immediately frozen and kept frozen until needed. Liver samples were cut into small pieces, washed with cold buffer, and blotted dry to remove excess blood before homogenizing. The entire small intestine from the rat was homogenized. The small intestine of the squirrel monkey was cut into four sections of equal length and approximately 3 cm removed from the proximal end of each length and homogenized. Tissues were homogenized at approximately 10% (w/v) in cold (about 5 °C) 0.05 M potassium phosphate buffer, pH 7.4, containing 0.15 M sodium chloride, using an Ultra Turrax homogenizer and diluted to the required concentration with the same buffered saline.

Blood samples were taken from live animals and healthy human volunteers, heparinized and used immediately, neat, or diluted with 0.05 M potassium phosphate buffer, pH 7.4, containing 0.15 M sodium chloride.

The ampicillin esters, being hydrochlorides, were dissolved in distilled water at a concentration equivalent to 1 mg/ml of sodium ampicillin. Esters of other penicillins were dissolved in acetone at a concentration equivalent to 5 mg/ml of the sodium salt of the parent penicillin. No correction was made for the purity of the esters which was generally estimated at 90% or more. The above solutions were immediately diluted with water to a concentration of 50 μg/ml. A one in ten dilution of this solution was then rapidly made into the blood sample, tissue homogenate, or 0.05 M potassium phosphate buffer so that the final concentration of substrate ester was equivalent to 5 μg/ml of the sodium salt of the parent penicillin. However, because of the lower antibacterial activity of cloxacillin the final concentration of its ester was equivalent to 50 μg/ml of sodium cloxacillin.

Reaction mixtures were incubated at 37 °C and sampled at 3, 8, 15, and 25 min after mixing tissue with substrate. The 5-μl samples were assayed immediately for liberated penicillin by the bioelectrophoretic method.

Bioelectrophoresis Assay. Electrophoretic separation of ester from liberated penicillin was carried out on starch–agar gel plates at pH 5.5, a pH at which further enzymatic hydrolysis is markedly reduced. Each plate was prepared by autoclaving a mixture of 1% soluble starch (Analar) + 1% agar (Oxoid Ionagar) in pH 5.5 buffer (0.87 g/l. of KH_2PO_4 + 0.047 g/l. of $Na_2HPO_4 \cdot 2H_2O$) at 121 °C for 15 min to form a molten gel. Approximately 120 ml of this was poured into a 20 × 30 cm frame on a glass sheet. When the gel was set the frame was removed and two rows of nine 1-mm holes were punched along the length of the plate using a Pasteur pipet. Four pairs of holes were then loaded with 5-μl aliquots of reaction mixtures or control solutions, the remaining five pairs of holes being filled with 5 μl of standard solutions of parent penicillin dissolved in gut homogenate, liver homogenate, blood, or buffer as appropriate. When loaded, a voltage of 15 V/cm was applied across the plate for 30 min. Under these conditions ampicillin esters migrate to the cathode at a faster rate than ampicillin which stays near the origin. Esters of the other penicillins being neutral remain at the origin, whereas their free acids migrate to the anode.

The plates were next covered with blood agar base (Oxoid) inoculated with *Sarcina lutea* NCTC 8340. This was achieved by inoculating molten blood agar base at approximately 45 °C with *S. lutea* and pouring into the frame as for the electrophoresis plates. When set, but still warm, this gel layer was slid from the glass sheet onto the electrophoresis gel, any trapped air being removed by gently running a clean glass rod over the surface. The plates were then incubated at 30 °C overnight (18 h), after which the diameters of the zones of inhibition due to liberated penicillin were measured and used to calculate the concentration in the reaction mixtures and controls.

Absorption in Experimental Animals. The esters were administered orally as aqueous suspensions by intubation to (a) squirrel monkeys (*Saimiri sciureus*) weighing 500–1000 g, (b) male albino mice, CSI strain, weighing 18–22 g, and (c) male albino rats, Sprague–Dawley OLAC strain, weighing 350–500 g. The compounds were administered at a dose of ester equivalent to 100 mg of penicillin free acid/kg to groups of six squirrel monkeys or groups of five rats. Venous blood samples were taken at 0.5, 1, 2, 4, and 6 h after dosing. Mice were dosed with ester at a dose equivalent to 50 mg of penicillin free acid/kg. Groups of five were killed at 10, 20, 60, 120, and 240 min after dosing, and blood was sampled from the cut axilla region. Blood specimens were heparinized, stored at 4 °C, and assayed on the day of sampling.

Absorption in Human Volunteers. Ampicillin esters **4a–c** and **8** were administered as their unformulated hydrochlorides in plain gelatin capsules to groups of ten fasting human volunteers as single oral doses equivalent to 250 mg of ampicillin pure free acid. The dose of the phthalidyl ester was 371 mg and of the 5,6-dimethoxyphthalidyl ester, 413 mg. Ampicillin was administered as its trihydrate. Venous blood was taken at 20 min, 40 min, 1 h, 1.5 h, 2 h, 4 h, and 6 h after administration and urine was collected over the 6-h period after dosing.

Microbiological Assay. Concentrations of penicillins in specimens of blood or urine after administration of the esters were measured by large-plate microbiological assay with *Sarcina lutea* NCTC 8340 as assay organism. Specimens of animal blood or human serum were assayed against standard solutions of the appropriate penicillin prepared in animal blood or in pooled human serum. Urine specimens of ampicillin were assayed against ampicillin standard solutions prepared in 0.05 M phosphate buffer, pH 7.0. The assay plates were incubated overnight at 30 °C, inhibition zone diameters were measured, and the penicillin concentrations of the test specimens were derived from standard lines constructed from the standard solutions.

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Trichothecene Analogues. 1. 1,5-Dioxaspiro[2.5]octanes

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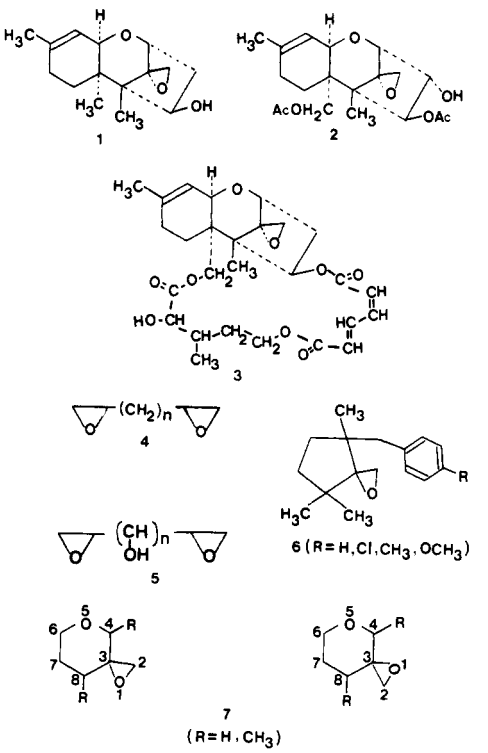
Seven 1,5-dioxaspiro[2.5]octanes were synthesized and tested in the mouse P388 lymphocytic leukemia screen and the mouse Ehrlich ascites screen. These compounds possess the "epoxypyran" structure which has been believed to be the active portion of the trichothecene class of sesquiterpene tumor inhibitors. Three of the compounds were found to have marginal to moderate activity in the Ehrlich ascites screen (inhibition 74.1–86.3%) and low activity in the P388 screen (T/C = 126–131). A carbocyclic analogue, 1-oxaspiro[2.5]octane (**9**), was moderately active in both screens (inhibition 78.8%, T/C = 140). In the Ehrlich ascites screen, T-2 toxin (**2**) was about 25 times more potent than **9**. None of the spirooctanes studied caused any skin irritation in 10-mg doses on the skin of rabbits, whereas **2** caused extensive necrosis at 0.1-mg doses.

The trichothecenes, illustrated by triochodermol (**1**), T-2 toxin (**2**), and the related macrocyclic esters, the verrucarins and roridins, e.g., verrucarin A (**3**), are a large group of fungal metabolites which have a wide variety of biological activities (see Chart I).[1-5] For example, at 1 ng/ml verrucarin A (**3**) and other trichothecenes cause a 50% inhibition in mouse tumor cell (p815) growth, making them among the most active cytostatic agents known.[1,2,6] Some trichothecenes, e.g., T-2 toxin, are potent skin irritants and can cause extensive dermal necrosis.[1,2,7] Many are potent mycotoxins of significant agricultural–economic importance, being implicated in moldy corn toxicosis, fescue foot diseases, and alimentary toxic aleukia in farm animals.[1,2,8] Several trichothecenes have been found to have antifungal activity in vitro, and a few have been used as agricultural fungicides.[2,6] Insecticidal and larvicidal activities have even been reported.[9,10]

The chemistry and biological activities of the trichothecenes have recently been reviewed by Bamburg,[1,2] Ciegler,[3] Tamm,[4] and Wogan.[5] Two syntheses of trichothecenes have been reported[11,12] and other papers have reported the syntheses of possible synthetic intermediates.[13-16] However, little is known about the structure–activity relationships of the trichothecenes. Although not all compounds of this structure have been tested, and only selected activities have been evaluated, some preliminary generalizations have been made.[2,17] The epoxypyran system appears to be the "active center" of the trichothecenes, although other structural features may contribute to maximal activity. Acid-catalyzed rearrangement to the apotrichothecene structure (Scheme I) also abolishes activity.[17,18] Furthermore, alcohol derivatives of the trichothecene esters are not as active as the esters themselves. Hydrogenation of the double bond reduces activity.[17,18] It should also be noted that bisepoxides, for example, **4** and **5**, have been found to have moderate to good antitumor activity, at least when "*n*" is small (0–2).[19-21]

The significant cytostatic antitumor activity of the trichothecenes, combined with their other varied activities, made us interested in studying spirane structure–activity relationship in greater detail. This interest has been shared by McChesney and Corbin at the University of Kansas who have recently synthesized the spirane epoxides of general

Chart I



Scheme I