MUTUAL PRO-DRUGS OF β-LACTAM ANTIBIOTICS AND β-LACTAMASE INHIBITORS

B. BALTZER, E. BINDERUP, W. VON DAEHNE, W. O. GODTFREDSEN,*
K. HANSEN, B. NIELSEN, H. SØRENSEN and S. VANGEDAL

Leo Pharmaceutical Products, DK-2750 Ballerup, Denmark

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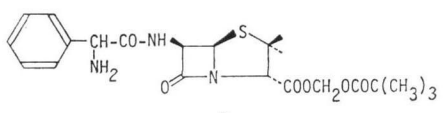
The principle of combining a β-lactam antibiotic with a β-lactamase inhibitor in a single molecule functioning as pro-drug for the two active components is illustrated by the linked esters **3** and **4** in which ampicillin and mecillinam, respectively, are combined with the β-lactamase inhibitor penicillanic acid sulfone. It is shown that in man these esters are excellently absorbed from the gastro-intestinal tract and after absorption hydrolyzed with simultaneous liberation of the active components. As a result high blood and tissue levels of antibiotic and β-lactamase inhibitor in a balanced ratio are attained. The advantages of "mutual pro-drugs" over simple combinations are discussed.

One of the ways by which it has been attempted to increase the oral absorption of β-lactam antibiotics has been to utilize the pro-drug concept, that is to synthesize derivatives of these antibiotics which are better and more reliably absorbed than their simple salts and which after absorption are converted in the organism to the parent antibiotic.

The first example of a successful application of this principle was pivampicillin (**1**) which can be regarded as a double ester of formaldehyde hydrate where one of the hydroxyl groups has been esterified with ampicillin and the other with pivalic acid. In contrast to the parent antibiotic, pivampicillin is absorbed almost completely from the gastrointestinal tract, and during or after absorption it is hydrolyzed enzymatically with liberation of ampicillin and pivalic acid[1].

In a similar way the oral absorption of other β-lactam antibiotics including various cephalosporins[2~5] and the amidinopenicillin mecillinam[6] has been drastically increased.

The same principle has also been used to improve the oral absorption of the β-lactamase inhibitor penicillanic acid sulfone (**2a**). This compound is poorly absorbed when given by mouth, but it has recently been reported that the corresponding pivaloyloxymethyl ester (**2b**) is well absorbed[7].



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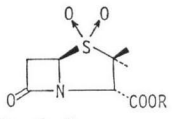


2a: R = H
2b: R = $CH_2OCOC(CH_3)_3$

When a β-lactamase inhibitor is co-administered with a β-lactam antibiotic in order to protect the latter against inactivation by the enzyme it is of course important that the antibiotic and the inhibitor are present simultaneously in appropriate balance at the site of the infection. This will usually not be the case when the two compounds are given as a combination of simple salts or pro-drugs because each drug in a combination will have its own individual profile with respect to rate of absorption, distribution,

* To whom requests should be addressed.

and duration of action.

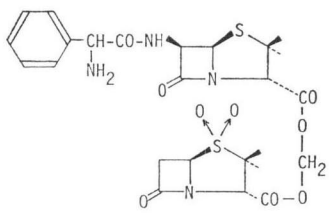
To overcome this problem we have attempted to combine β-lactam antibiotics and β-lactamase inhibitors in a single molecule which can function as a pro-drug for both of the active principles. In this way is achieved that the absorption of the two components takes place at exactly the same time and in a constant ratio.

However, in order that this principle—which may be called the mutual pro-drug principle—can work properly, a number of prerequisites have to be fulfilled:

1. The mutual pro-drug must be well absorbed.
2. After absorption the two active components must be released concomitantly and quantitatively.
3. Maximal antibacterial effect of the antibiotic-inhibitor combination must be exerted at an approximate 1 : 1 ratio.
4. The distribution and elimination of the two components must be similar.

We have chosen the antibiotic-inhibitor pairs ampicillin-penicillanic acid sulfone and mecillinam-penicillanic acid sulfone as candidates for combination in mutual pro-drug molecules because: (a) both of these combinations show optimal synergy in an approximate 1 : 1 ratio[8]*, (b) all three compounds are unsatisfactorily absorbed by the oral route[1,6,7], and (c) the two antibiotics and the inhibitor have similar volumes of distribution and similar elimination rates[6,7].

Since it is well known that esters of gem-diols are hydrolyzed relatively fast at neutral pH and their hydrolysis often is catalyzed by enzymes present in human blood and tissues, we decided to combine either of the two antibiotics with penicillanic acid sulfone by preparing double esters of formaldehyde hydrate in which one of the hydroxyl groups is esterified with ampicillin and mecillinam, respectively, and the other with penicillanic acid sulfone. It was hoped that such esters would be sufficiently stable to resist the acidic conditions in the stomach, be well absorbed, and, after absorption, be readily hydrolyzed to the parent drugs, either spontaneously or enzymatically.



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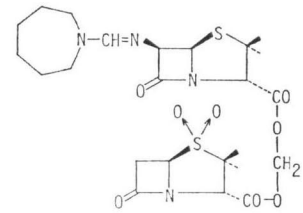

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In this report we describe the synthesis and biological properties of the linked ester **3** of ampicillin and penicillanic acid sulfone (in the following called VD 1827) and of the linked ester **4** of mecillinam and penicillanic acid sulfone (VD 1825).

Chemistry

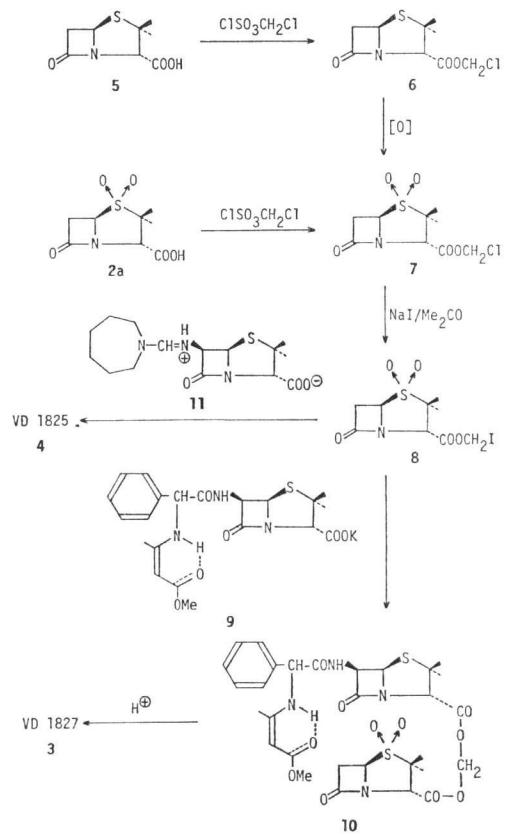
The new esters VD 1827 (**3**) and VD 1825 (**4**) were synthesized as outlined in Scheme 1.

Penicillanic acid sulfone (**2a**) was converted to the chloromethyl ester **7** by reaction of its sodium salt with chloromethyl chlorosulfate in dichloromethane-water. Alternatively, **7** could be prepared by conversion of penicillanic acid (**5**) to the chloromethyl ester **6** and subsequent oxidation of the latter with hydrogen peroxide in isopropanol in the presence of a tungstate catalyst. Treatment of **7** with

* Personal communication from N. MELCHIOR, Leo Pharmaceutical Products.

sodium iodide in acetone afforded the iodomethyl ester **8**. Reaction of **8** with the protected ampicillin derivative **9** in dimethylformamide led to the formation of the intermediate **10** which without isolation was hydrolyzed at pH 2.5 to remove the amino-protecting group. The resulting linked ester **3** (VD 1827) was finally isolated as a crystalline hydrochloride. In a similar manner the linked ester **4** (VD 1825) was obtained by reaction of **8** with mecillinam (**11**) in dimethylformamide. Also this ester was isolated as a crystalline hydrochloride.

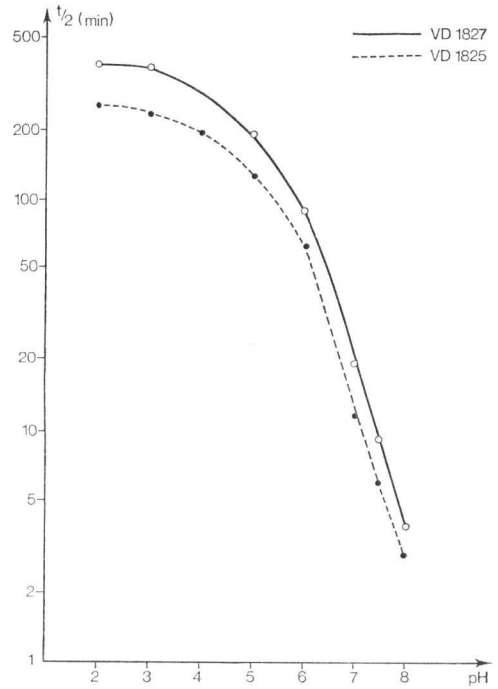
Scheme 1.



Hydrolysis *In Vitro*

The hydrolysis of VD 1827 and VD 1825 was studied at 37°C over the pH-range of 2~8. It was followed by HPLC determination of residual intact esters and was found to observe pseudo first order kinetics. By plotting the half-lives against pH the pH-rate profiles shown in Fig. 1 were obtained. As evident from these data, both esters are relatively stable in acid solution but are rapidly hydrolyzed in neutral solution. At pH 7.4, half-lives of 9.2 and 6.0 minutes were found for VD 1827 and VD 1825, respectively.

Fig. 1. pH-Rate profiles for VD 1825 and VD 1827.



The rates of hydrolysis of VD 1827 and VD 1825 in the presence of serum and tissue homogenates from various species were investigated at

Fig. 2. Formation of ampicillin and penicillanic acid sulfone during hydrolysis of VD 1827 at 37°C and pH 7.4 in the presence of 10% human serum.

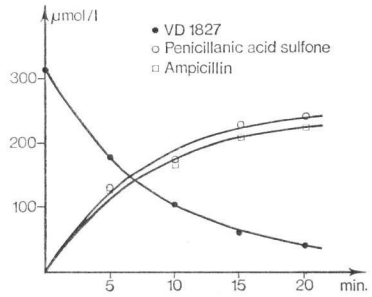
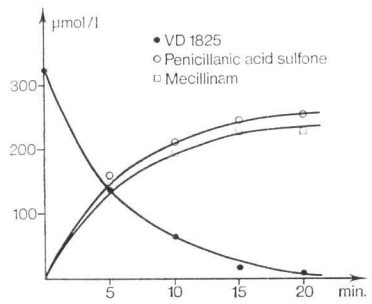


Table 1. Hydrolysis of VD 1827 and VD 1825 at 37°C and pH 7.4 in the presence of various biological fluids.

Enzyme source	Half-life* (min.)	
	VD 1827	VD 1825
None	9.2	6.0
Mouse serum, 1 %	2.6	<1.0
Dog serum, 10 %	4.0	4.4
Liver homogenate from the dog, 10 %	1.1	1.7
Homogenate of gastric mucosa from the dog, 10 %	5.1	4.7
Homogenate of intestinal mucosa from the dog, 10 %	3.5	4.8
Human serum, 10 %	5.9	4.5
Human serum, 30 %	2.7	3.1

* In all experiments, the starting concentration of VD 1827, HCl and VD 1825, HCl, respectively, was 200 μg/ml.

Fig. 3. Formation of mecillinam and penicillanic acid sulfone during hydrolysis of VD 1825 at 37°C and pH 7.4 in the presence of 10% human serum.



37°C and pH 7.4, remaining intact esters being determined by HPLC as described before. The results, summarized in Table 1, indicate that the hydrolysis of both esters is considerably faster in the presence of biological fluids, probably due to catalysis by esterases.

To ensure that the active components are formed simultaneously during hydrolysis of the linked esters, additional studies were performed where the formation of the hydrolysis products was determined concurrently with the disappearance of the esters.

The results of these studies, which were performed at 37°C and pH 7.4 in the presence of 10% human serum, are presented in Figs. 2 and 3. It will be seen that antibiotic and inhibitor indeed are liberated concomitantly in an equimolar ratio.

Absorption and Elimination in Humans

The absorption and elimination of VD 1827 (**3**) in man was studied by dosing a group of six healthy, fasting volunteers with gelatine capsules containing 475 mg of the hydrochloride salt - an amount equimolar to 250 mg of anhydrous ampicillin.

Blood samples were drawn at 1/2, 1, 2, 4, and 6 hours after administration and serum analyzed for ampicillin and penicillanic acid sulfone. Urine was collected from 0~6 and 6~24 hours and analyzed in the same way.

In Table 2 are presented individual values for peak concentrations (C_{max}), time for onset of peak (T_{max}), and areas under the serum level *versus* time curves (AUC), calculated according to a method developed by ENGBERG-PEDERSEN[9], as well as the urinary recovery of both components expressed in per cent of the theoretically possible amounts. Computer generated mean serum level *versus* time curves for ampicillin and penicillanic acid sulfone are shown in Fig. 4.

Table 2. Individual values of maximum serum concentrations (C_{max}) of ampicillin (A) and penicillanic acid sulfone (B), the time for their occurrence (T_{max}), the area under the serum level/time curves (AUC), and urinary excretions after oral administration of 475 mg of VD 1827, HCl to six healthy, fasting volunteers.

Subject	C_{max} μg/ml		T_{max} hours		AUC μg/ml×hours		Urinary excretion %			
							0~6 hours		0~24 hours	
	A	B	A	B	A	B	A	B	A	B
GK	7.94	11.77	0.96	0.80	12.68	19.63	68.3	79.3	70.2	84.8
KM	11.39	7.07	0.74	1.01	16.29	22.42	78.7	58.0	80.5	63.4
FJ	6.47	6.57	0.92	1.32	10.06	16.59	37.1	56.6	37.8	62.1
TH	14.44	14.31	0.56	0.69	11.54	16.72	73.8	87.5	75.0	92.3
LA	13.17	12.20	0.62	0.64	17.11	19.47	66.2	64.1	68.8	67.2
HP	13.09	13.54	0.64	0.64	13.62	16.74	64.5	73.7	66.5	78.1
Mean	11.08	10.91	0.74	0.85	13.55	18.59	64.8	69.9	66.5	74.6
S. D.	3.19	3.30	0.17	0.27	2.73	2.34	14.5	12.4	14.9	12.4

Fig. 4. Mean serum levels of ampicillin and penicillanic acid sulfone after administration of 475 mg VD 1827, HCl to six fasting volunteers.

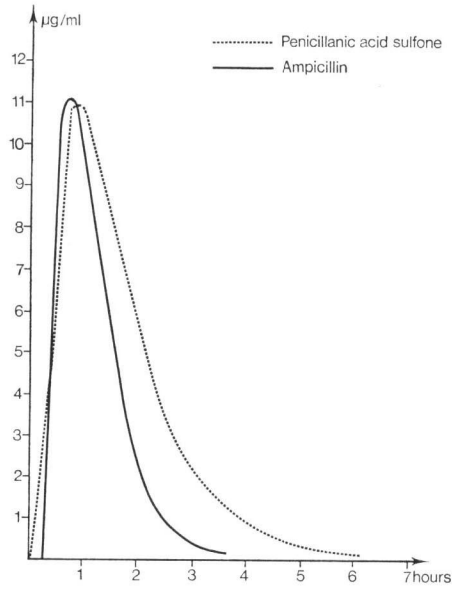
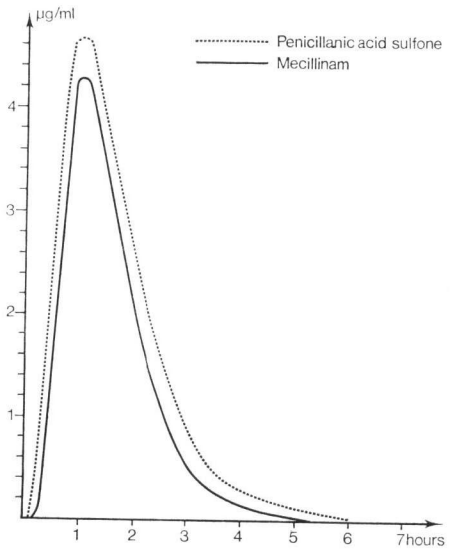


Fig. 5. Mean serum levels of mecillinam and penicillanic acid sulfone after administration of 260 mg VD 1825, HCl to eight fasting volunteers.



It is evident from these data that VD 1827 is rapidly absorbed from the gastrointestinal tract and delivered as ampicillin and penicillanic acid sulfone to the peripheral circulation resulting in mean peak serum levels of approximately 11 μg/ml for both components. In general, the peak values are reached less than 1 hour after administration of the drug.

That the two serum level *versus* time curves are almost coherent reflects that the serum half-lives of the two compounds are very similar. The fact that the urinary recoveries of both ampicillin and penicillanic acid sulfone are very close to the values reported to be excreted after parenteral administration of the two compounds (68% and 75%, respectively)[7,10] suggests that VD 1827 is almost completely absorbed from the gastrointestinal tract.

Table 3. Individual values of maximum serum concentrations (C_{max}) of mecillinam (A) and penicillanic acid sulfone (B), the time for their occurrence (T_{max}), the area under the serum level/time curves (AUC), and urinary excretions after oral administration of 260 mg of VD 1825, HCl to eight healthy, fasting volunteers.

Subject	C_{max} μg/ml		T_{max} hours		AUC μg/ml×hours		Urinary excretion %			
							0~6 hours		0~24 hours	
	A	B	A	B	A	B	A	B	A	B
DR	4.24	6.49	0.99	0.74	7.69	9.78	52.5	77.0	54.2	82.0
GK	4.46	2.65	1.30	1.09	6.19	5.56	51.7	72.0	53.1	75.0
KM	6.76	3.35	0.79	1.24	8.58	9.01	49.4	72.0	50.2	74.0
TH	5.38	6.34	0.77	0.93	6.16	8.87	60.3	76.0	61.2	78.0
FJ	2.18	3.81	1.38	1.29	4.02	8.42	47.6	68.0	48.0	69.0
MM	2.83	5.12	1.16	1.21	5.51	10.46	49.8	82.0	51.4	85.0
HP	4.21	4.46	0.76	0.74	5.90	6.68	50.8	73.0	52.5	76.0
LA	3.96	4.91	0.75	0.59	6.38	8.18	53.1	75.0	54.8	79.0
Mean	4.25	4.64	0.99	0.98	6.30	8.37	51.9	74.4	53.2	77.2
S. D.	1.41	1.36	0.26	0.27	1.37	1.59	3.8	4.2	3.9	5.0

A similar study was performed with VD 1825 (**4**). This drug was given in the form of its hydrochloride to a group of 8 healthy, fasting volunteers at a dose of 260 mg which is equimolar to 200 mg of pivmecillinam, HCl, an orally active pro-drug of mecillinam. The results of this study are presented in Table 3 and Fig. 5. It will be seen that also VD 1825 is rapidly absorbed and hydrolyzed giving rise to mean peak serum levels of 4.25 and 4.64 μg/ml of mecillinam and penicillanic acid sulfone, respectively, about 1 hour after dosing.

Here again, the urinary recoveries suggest an almost complete absorption. Thus, the recovery of mecillinam corresponds closely to the recoveries reported after i.v. and i.m. injection of a 200 mg dose of mecillinam (49% and 58%, respectively)[6], and the recovery of penicillanic acid sulfone to the value (75%) reported after i.v. and i.m. administration of a salt of this compound[7].

Discussion

With the preparation of the linked esters VD 1827 (**3**) and VD 1825 (**4**) we have demonstrated that it is possible to combine the β-lactam antibiotics ampicillin and mecillinam with the β-lactamase inhibitor penicillanic acid sulfone in single molecules which at the same time can function as pro-drug for both antibiotic and inhibitor. We have called this concept—which well may find application within other fields of medicinal chemistry—for "the mutual pro-drug principle".

Whereas ampicillin is incompletely absorbed and mecillinam and penicillanic acid sulfone very poorly absorbed when given by mouth, VD 1827 and VD 1825 seem to be almost completely absorbed from the gastrointestinal tract. A similar improvement in absorption of the three components can be achieved by converting them into their respective pivaloyloxymethyl esters[1,6,7]. However, the mutual pro-drugs VD 1827 and VD 1825 have obvious advantages over combinations of these simple pro-drugs. First of all because they ensure that antibiotic and inhibitor are absorbed at the same time and in a constant ratio. This in connection with the fact that the plasma half-lives of ampicillin and mecillinam are very similar to that of penicillanic acid sulfone means that antibiotic and inhibitor always will be present simultaneously in an appropriate ratio in the organism to provide optimal protection of the antibiotic against inactivation. Another advantage of "mutual pro-drugs" over combinations of simple pro-drugs is that therapeutically inactive pro-drug components such as for example pivalic acid are avoided.

A few examples of "mutual pro-drugs" have previously been described in the patent literature. Thus, amidinopenicillins such as mecillinam have been combined with ordinary penicillins like benzylpenicillin and ampicillin in linked esters of aldehyde hydrates in order to utilize the synergy often seen between these two classes of β-lactam antibiotics[11]. In a similar way ampicillin and probenecid have been combined with the aim of achieving prolonged blood levels of ampicillin[12]. However, none of these compounds seem to have been tested clinically.

Experimental

Melting points were determined with a Büchi-Tottoli apparatus and are uncorrected. ^{1}H-NMR spectra were measured at 100 MHz on a JEOL FX 100 spectrometer. The chemical shifts are given in ppm δ scale (TMS: $\delta=0.0$; HDO: $\delta=4.66$), J=coupling constants in Hz. IR spectra were obtained in KBr on a Perkin-Elmer Model 457 spectrophotometer, the main absorptions are given in cm^{-1}. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter. Dry-column chromatography was carried out on silica gel (Silica Woelm TSC) using *n*-hexane - ethyl acetate 3: 1 as developing solvent. Reversed phase HPLC analyses were performed on a LDC Model LC/SSUI liquid chromatograph (Laboratory Data Control) equipped with a LDC Model 1202 spectromonitor. Throughout the study, a 25 cm×4.6 mm I. D. stainless steel column packed with Lichrosorb RP 18 10 μ (E. Merck) was utilized and operated at room temperature. For the determination of the linked esters, the eluent was acetonitrile - 0.01 M aqueous diammonium hydrogen phosphate 60: 40 (VD 1827) or 70: 30 (VD 1825), the flow-rate 2 ml/min., and the detection wavelength 220 nm.

Ampicillin, mecillinam, and penicillanic acid sulfone were determined by using acetonitrile - 0.01 M aqueous diammonium hydrogen phosphate 12: 88 as eluent, the flow-rate being 1.5 ml/min. and the detection wavelength 205 nm. GLC analyses were performed on a Perkin-Elmer Model 990 gas chromatograph equipped with a flame-ionization detector. A 2 m×3.3 mm O.D. stainless steel column packed with 3% OV 210 on Diatomite CQ 100~200 mesh (J. J's Chromatography Ltd.) was used. The column temperature was 200°C, the injection port temperature 250°C, the detector temperature 275°C, and the carrier helium flow 30 ml/min.

Chloromethyl penicillanate (**6**)

To a stirred mixture of potassium penicillanate (**5**)[13] (23.8 g, 0.1 mol), potassium bicarbonate (30 g, 0.3 mol), and tetrabutylammonium hydrogen sulfate (3.4 g, 0.01 mol) in dichloromethane - water 1: 1 (200 ml) was added dropwise a solution of chloromethyl chlorosulfate (11.5 ml, 0.115 mol) in dichloromethane (40 ml) at such a rate that the temperature of the reaction was kept below 30°C. After stirring at room temperature for 30 minutes, the organic phase was separated and the aqueous phase extracted with dichloromethane (50 ml). The combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The residue was dissolved in ether (150 ml), insoluble material was filtered off (filter-aid), and the filtrate was evaporated *in vacuo* to give 22.5 g (90%) of chloromethyl penicillanate (**6**) as a colourless oil. ^{1}H-NMR ($CDCl_3$/TMS): 1.55 (s, 3H, 2-C$\underline{H}_3$), 1.87 (s, 3H, 2-C$\underline{H}_3$), 3.10 (dd, *J* 16 and 4, 1H, 6-$\underline{H}$), 3.62 (dd, *J* 16 and 2, 1H, 6-$\underline{H}$), 4.50 (s, 1H, 3-$\underline{H}$), 5.30 (dd, *J* 4 and 2, 1H, 5-$\underline{H}$), 5.77 (ABq, *J* 6.5, 2H, O$\underline{CH}_2$Cl).

Chloromethyl penicillanate 1,1-dioxide (**7**)

A. From penicillanic acid 1,1-dioxide (**2a**)

To a stirred suspension of penicillanic acid 1,1-dioxide (**2a**)[14] (46.6 g, 0.2 mol), sodium bicarbonate (63.8 g, 0.76 mol), and tetrabutylammonium hydrogen sulfate (6.8 g, 0.02 mol) in dichloromethane - water 1: 1 (400 ml) was added dropwise a solution of chloromethyl chlorosulfate (23 ml, 0.23 mol) in dichloromethane (50 ml) at such a rate that the temperature in the reaction mixture was kept below 30°C. After the addition was finished, the mixture was stirred at room temperature for 30 minutes. The organic phase was separated, dried ($MgSO_4$), evaporated to about 80 ml, and diluted with isopropanol (300 ml). Crystallization was induced by scratching, and the resulting suspension was concentrated *in vacuo* to remove the dichloromethane. After being kept in a refrigerator overnight, the crystals were filtered off, washed with isopropanol (6×20 ml) followed by ether (2×25 ml), and dried to afford 43.1 g

(76.5%) of chloromethyl penicillanate 1,1-dioxide (**7**), m.p. 95~97°C, $[\alpha]_D^{20}$ +227° (*c* 1, $CHCl_3$). Evaporation of the mother liquor *in vacuo* followed by dry-column chromatography of the residual oil on silica gel gave a further 2.2 g (3.9%) of **7**, m.p. 94~96°C.

B. From chloromethyl penicillanate (**6**)

To a stirred solution of chloromethyl penicillanate (**6**) (20 g, 0.08 mol) in isopropanol (100 ml) was added 30% hydrogen peroxide (20 ml), followed by 0.5 M aqueous sodium tungstate-(VI) (5 ml). After a few minutes, the temperature in the reaction mixture rose to about 60°C, whereafter it slowly decreased. The mixture was left at room temperature overnight, the resulting suspension was cooled to 0~5°C, and the crystalline precipitate was filtered off, washed with isopropanol (2×20 ml), and dried to afford 17.2 g (76.3%) of chloromethyl penicillanate 1,1-dioxide (**7**), m.p. 96~97°. Evaporation of the mother liquor *in vacuo* and subsequent dry-column chromatography of the residual oil on silica gel gave a further 2.0 g (8.9%) of **7**, m.p. 95~96°C.

Iodomethyl penicillanate 1,1-dioxide (**8**)

A solution of chloromethyl penicillanate 1,1-dioxide (**7**) (56.4 g, 0.2 mol) and sodium iodide (45 g, 0.3 mol) in acetone (150 ml) was stirred at room temperature for 18 hours. The resulting suspension was cooled to 0~5°C and its apparent pH-value was adjusted from 3.0 to 7.2 by addition of saturated aqueous sodium bicarbonate with stirring. After decolourization by titration with 0.5 M aqueous sodium thiosulfate, water (150 ml) was added dropwise to the stirred mixture to precipitate colourless crystals. The crystals were filtered off, washed with acetone - water 1: 1 (2×20 ml), isopropanol (2×20 ml), and ether (2×20 ml), and dried to yield 62.8 g (84.2%) of iodomethyl penicillanate 1,1-dioxide (**8**), m.p. 100~102°C, $[\alpha]_D^{20}$ +232° (*c* 1, $CHCl_3$).

Potassium 6β-[N-(1-methoxycarbonylpropen-2-yl)-D-α-amino-α-phenylacetamido]-penicillanate (**9**)

To a suspension of potassium carbonate (76.0 g, 0.55 mol) in dimethylformamide (625 ml) was added methyl acetoacetate (108 ml, 1.0 mol) and ampicillin trihydrate (201.5 g, 0.5 mol), and the mixture was stirred for 2 hours at room temperature followed by 4 hours at 0~5°C. After addition of ether (2.5 liters) and stirring for 5 minutes, the mixture was left for 30 minutes at 0~5°C, whereupon the solvent phase was decanted. The residue thus obtained was washed two additional times by decantation with ether (1 liter) as described before and finally dissolved in acetone (1.25 liters). Insoluble material was removed by filtration (filter aid), and the filtrate was diluted with isopropanol (1.25 liters) and seeded. On further dilution with isopropanol (2 liters) and concentration of the mixture *in vacuo* to about 2 liter, precipitation of a colourless crystalline product occurred. After being kept overnight at 0~5°C, the crystals were filtered off, washed with isopropanol (2×150 ml) followed by ether (2×250 ml), and dried to give 219.1 g (90.0%) of **9**, $[\alpha]_D^{20}$ +256° (*c* 1, H_2O)

VD 1827, HCl (**3**, HCl)

To a stirred solution of potassium 6β-[N-(1-methoxycarbonylpropen-2-yl)-D-α-amino-α-phenylacetamido]penicillanate (**9**) (276.8 g, 0.57 mol) in dimethylformamide (1 liter) was added at 5°C iodomethyl penicillanate 1,1-dioxide (**8**) (186.6 g, 0.5 mol). After stirring for 15 minutes at 5~10°C, the reaction mixture was poured into an icecold mixture of ethyl acetate (4 liters) and saturated aqueous calcium chloride (2 liters) with stirring. The organic layer was separated, washed with saturated aqueous calcium chloride (2×500 ml), filtered, and evaporated to about 1 liter *in vacuo*. To the concentrate containing the intermediate **10** was added water (500 ml) and *n*-butanol (500 ml) whereupon the amino-protecting group in **10** was removed hydrolytically at pH 2.5 by dropwise addition of 4 N hydrochloric acid with stirring. After the addition was finished, ether (1 liter) and water (500 ml) were added to the stirred mixture, the aqueous phase was separated, and the organic phase extracted with water (800 ml). The combined aqueous extracts were washed with ether (1 liter), sodium chloride (640 g) and dichloromethane (2 liters) were added, and the mixture was stirred for 15 minutes. The organic phase was separated, the aqueous phase extracted with dichloromethane (1 liter), and the combined organic extracts were dried ($MgSO_4$) and evaporated to about 600 ml under reduced pressure. To the concentrate was added 2-butanone (200 ml), the mixture was kept in a refrigerator overnight, and the resulting crystals were filtered off, washed with 2-butanone (2×100 ml) followed by ether (2×200 ml), and dried to give 250.2 g (79.2%) of **3**, HCl; undefined m.p. (decomposition at 150~170°C); $[\alpha]_D^{20}$ +218° (*c* 1, H_2O). IR:

1785 (β-lactam and ester, overlapping), 1690 (amide I). ^{1}H-NMR (D_2O): 1.31 (s, 6H, 2-C$\underline{H}_3$), 1.37 (s, 3H, 2-C$\underline{H}_3$), 1.49 (s, 3H, 2-C$\underline{H}_3$), 3.45 (m, 2H, 6-$\underline{H}$), 4.54 and 4.55 (2s, overlapping, 2H, 3-H), 4.95 (m, 1H, 5-$\underline{H}$), 5.21 (s, 1H, C$\underline{H}$NH$_3^{\oplus}$), 5.46 (s, 2H, 5-$\underline{H}$ and 6-$\underline{H}$), 5.90 (bs, 2H, OC$\underline{H}_2$O), 7.46 (s, 5H, C$_6\underline{H}_5$).

VD 1825, HCl (**4**, HCl)

To a stirred suspension of mecillinam (**11**) (130.2 g, 0.4 mol) in dimethylformamide (800 ml) was added water (20 ml), potassium bicarbonate (40 g, 0.4 mol), and, after cooling to 5°C, iodomethyl penicillanate 1,1-dioxide (**8**) (152 g, 0.4 mol). The mixture was stirred vigorously for 2.5 hours at 5~7°C whereby an almost clear, colourless solution was obtained. After dilution with icecold ethyl acetate (2 liters), the mixture was washed with ice-water (2×1 liter), and the combined washings were re-extracted with icecold ethyl acetate (600 ml). To the combined organic extracts was added ice-water (1.6 liters), and the pH of the aqueous phase was adjusted to 3.0 by addition of 6 N hydrochloric acid with stirring. The aqueous phase was separated, the ethyl acetate layer was re-extracted with water (400 ml), and to the combined aqueous extracts were added dichloromethane (1 liter) and saturated aqueous sodium chloride (1 liter) with stirring. The dichloromethane layer was separated, the aqueous phase extracted with another portion of dichloromethane (400 ml), and the combined organic extracts were dried ($MgSO_4$) and evaporated to about 500 ml under reduced pressure. To the concentrate was added ethanol (80 ml) and 2-butanone (2 liters), whereupon the resulting mixture was concentrated to about 1 liter *in vacuo*.

This concentrate was diluted with 2-butanone (1 liter), crystallization was induced by seeding and scratching, and the mixture was left at room temperature for 3~4 hours. After being kept in the refrigerator overnight, the crystals were filtered off, washed with 2-butanone (2×100 ml), followed by ether (2×100 ml), and dried to give 187.6 g (77.2%) of **4**, HCl; undefined m.p. (decomposition at 160~170°C); $[\alpha]_D^{20}$+238° (*c* 1, H_2O). IR: 1800 (β-lactam), 1768 (ester), 1685 (protonated amidine). ^{1}H-NMR (D_2O): 1.42 (s, 3H, 2-C$\underline{H}_3$), 1.50 (s, 3H, 2-C$\underline{H}_3$), 1.56 (s, 3H, 2-C$\underline{H}_3$), 1.68 (s, 3H, 2-C$\underline{H}_3$), 1.5~2.0 (m, 8H, (C$\underline{H}_2$)$_4$), 3.3~3.8 (m, 6H, C$\underline{H}_2$NC$\underline{H}_2$ and 6-$\underline{H}$), 4.66 (s, 1H, 3-$\underline{H}$), 4.70 (s, 1H, 3-$\underline{H}$), 5.03 (m, 1H, 5-$\underline{H}$), 5.46 (d, *J* 4, 1H, 6-$\underline{H}$), 5.60 (d, *J* 4, 1H, 5-$\underline{H}$), 5.97 (s, 2H, OC$\underline{H}_2$O), 7.98 (s, 1H, C$\underline{H}$=N).

Recrystallization: A solution of **4**, HCl (100 g) in dichloromethane (100 ml) was diluted with ethanol (200 ml), and the dichloromethane was removed by evaporation to about 150~200 ml at reduced pressure. 2-Butanone (200 ml) was added, and the mixture was concentrated to about 200 ml *in vacuo*. After dilution of the concentrate with further 2-butanone (600 ml) and subsequent filtration, the clear filtrate was seeded, crystallization was induced by scratching, and the mixture was left at room temperature for 3~4 hours before being kept in a refrigerator overnight. The crystals were filtered off, washed with 2-butanone (4×50 ml) followed by ether (4×50 ml), and dried to afford 84 g of essentially pure **4**, HCl; $[\alpha]_D^{20}$+242° (*c* 1, H_2O). Concentration of the mother liquor to about 100 ml *in vacuo* afforded another crop (12 g) of crystalline **4**, HCl.

Hydrolysis *In Vitro*

Freshly prepared aqueous solutions of VD 1827, HCl and VD 1825, HCl, respectively, were diluted to final concentrations of 200 μg/ml with either an appropriate 0.067 M buffer solution (pH 2~6, citrate; pH 7~8, phosphate) or a suitable amount of the biological fluid followed by 0.067 M phosphate buffer, pH 7.4. The resulting test solutions were immediately placed in a water bath at 37°C, and aliquots were withdrawn at proper intervals and assayed by reversed phase HPLC.

Absorption and Excretion in Humans

Groups of healthy volunteers were dosed orally with 475 mg of VD 1827, HCl (equimolar to 250 mg of anhydrous ampicillin) and 260 mg of VD 1825, HCl (equimolar to 200 mg of pivmecillinam, HCl), respectively. The drugs were given in gelatine capsules on an empty stomach. Blood samples were drawn at 0.5, 1, 2, 4, and 6 hours after the administration of the compounds, and serum was separated from the blood specimens by centrifugation. The urine was collected from 0 to 6 and 6 to 24 hours.

(a) Microbiological assay. Serum and urine samples were assayed for ampicillin and mecillinam by the usual agar plate method using as test organisms *Sarcina lutea* ATCC 9341 and *Escherichia coli* Leo Strain HA2, respectively, and standard preparations of ampicillin and mecillinam as reference compounds. It should be noted that penicillanic acid sulfone neither exhibited intrinsic activity (MIC> 100μg/ml) nor synergistically enhanced the activity of ampicillin or mecillinam against the test organisms.

(b) GLC assay. To the serum sample (1.0 ml) was added internal reference solution (1.0 ml of 0.5×10^{-4} M aqueous potassium 6,6-dibromopenicillanate), water (2 ml), and 1 N hydrochloric acid (0.25 ml). The resulting mixture was twice extracted with ethyl acetate (3 ml) by gently shaking followed by centrifugation. The combined organic extracts were shaken vigorously with 0.67×10^{-2} M phosphate buffer, pH 6.3 (4 ml). The aqueous layer was separated, acidified with 1 N hydrochloric acid (0.5 ml), and extracted with ethyl acetate (6 ml). The ethyl acetate extract was evaporated *in vacuo* and the residue methylated with excess ethereal diazomethane. After removal of the solvent in a stream of nitrogen at room temperature, the residual material was dissolved in ethyl acetate (30 μl) and a sample (2 μl) injected into the gas chromatograph. Urine samples were worked up in a similar way using 3.15×10^{-4}M aqueous potassium 6,6-dibromopenicillanate (1.0 ml) as internal reference solution.

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