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Semisynthetic Penicillins

V.* α-(Ylideneimino-oxy)penicillins

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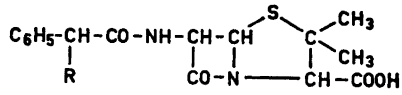
The synthesis and antibacterial properties of a series of α-(ylideneimino-oxy)penicillins are reported. The new penicillins were obtained by coupling α-(ylideneimino-oxy)carboxylic acid chlorides to 6-aminopenicillanic acid. Among the prepared penicillins, 6-[D(−)-α-(2-chlorobenzylideneimino-oxy)-α-phenylacetamido]penicillanic acid showed the best effect against *E. coli*.

Introduction of a hetero-substituent in the α-position of benzylpenicillin (penicillin G, I) greatly influences its properties, *e.g.* the acid stability [1] as well as its biological activity. The most interesting compound in this respect hitherto recorded is α-aminobenzylpenicillin (ampicillin, II) [2–4]



I R = H
II R = NH_2

which is considerably more active against Gram-negative bacteria than is penicillin G. This valuable widening of the activity range caused by the α-amino group prompted us to investigate if the same effect might be achieved with other nitrogen-containing substituents. As an attempt in this direction, we replaced the amino group in II by substituted amino-oxy moieties.

In the present paper, the synthesis and antibacterial properties of a series of α-(ylideneimino-oxy)penicillins (V, Scheme I) are reported.

The penicillins were prepared by acylation of 6-aminopenicillanic acid (6-APA) with Schiff bases of α-amino-oxycarboxylic acids of type III (Scheme

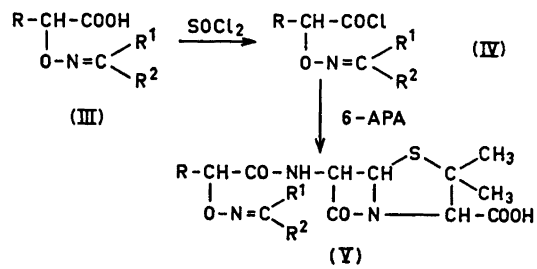
* Part IV. Undheim, K., Bamberg, P. and Sjöberg, B. *Acta Chem. Scand.* **19** (1965) 317.

I), described in a previous paper.[5] Various reactive acid derivatives can be used for acylation of 6-APA[6-8] and among these the acid chlorides are most convenient, if available. Most azomethine derivatives of amino acids are unstable;[9] furthermore, acid chlorides usually react easily with Schiff bases with addition to the carbon-nitrogen double bond.[10] However, as the acids of type III are much more stable than the corresponding azomethine derivatives of amino acids we considered it worthwhile to try the preparation of acid chlorides from III.

It was found that the carboxylic acids III are easily converted into their acid chlorides IV by warming them for about 30 min with excess of thionyl chloride. The crude acid chlorides were used directly in the next step without any further purification.

Acylation of 6-APA proceeded smoothly in neutral aqueous medium. After acidification of the reaction mixture, the penicillins were extracted with ether, unreacted 6-APA remaining in the aqueous phase as the hydrochloride. The penicillins were extracted with aqueous potassium bicarbonate from the ether and obtained in good yields as the potassium salts after freeze-drying. In order to obtain analytically pure material, some of the penicillins were converted into their isopropylamine salts which were recrystallized from organic solvents. In these cases, the penicillins were obtained as fine colourless needles or platelets.

Scheme I.

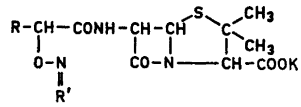


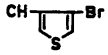
The penicillins were tested for their stability in acidic media and for penicillinase resistance. Compared to penicillin G (I) the compounds were found to be about thirty times more stable at pH 2 and slightly more resistant to *Bacillus cereus* penicillinase. The antimicrobial activity was determined in serial dilution tests on agar plates.[11] The activities against *Escherichia coli* and *Staphylococcus aureus*, Oxford, are presented in Table 1.

Regarding *Staph. aureus* the figures are rather uniform. With two exceptions they all lie within one dilution step from the value 0.13 μg/ml; a figure which is appreciably higher than that observed for penicillin G (M.I.C.* = 0.01 μg/ml). However, against *E. coli* several of the compounds possess the same or even better activity than penicillin G (M.I.C. = 25 μg/ml). If either R or the ylidene group in V (Scheme I) is aliphatic (Vs, Vt, Vu, Vaa; Table 1) the activity

* M.I.C. = minimum inhibitory concentration.

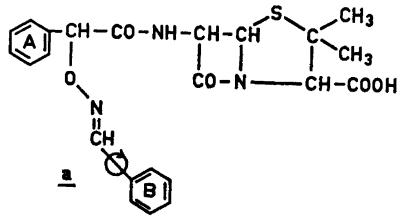
Table 1.



No.	R	R′	Yield %	Purity %	Activity (μg/ml) *E.coli*	*Staph.aureus*
Va	C_6H_5	CH-2-ClC_6H_4	76	100	12.5	0.13
Vb	C_6H_5	CH-2-BrC_6H_4	72	75	12.5	0.25
Vc	3-C_4H_3S	CH-2-ClC_6H_4	65	50	12.5	0.13
Vd	C_6H_5	CH-2,6-$Cl_2C_6H_3$	67	90	12.5	0.13
Ve	C_6H_5	CH-2,4-$Cl_2C_6H_3$	81	76	12.5	0.13
Vf	C_6H_5	CH-2-$CH_3C_6H_4$	67	90	25	0.25
Vg	C_6H_5	CH-2-$CH_3OC_6H_4$	85	77	25	0.13
Vh	C_6H_5		56	85	25	0.13
Vi	C_6H_5		67	90	25	0.13
Vj	C_6H_5		55	62	25	0.13
Vk	C_6H_5	CHC_6H_5	53	93	62.5	0.13
Vl	C_6H_5	CH-2-FC_6H_4	62	78	62.5	0.06
Vm	C_6H_5	CH-4-ClC_6H_4	87	104	62.5	0.06
Vn	C_6H_5	CH-2-OH-C_6H_4	79	52	62.5	0.13
Vo	C_6H_5	CH-2-OH-5-ClC_6H_3	61	73	62.5	0.13
Vp	C_6H_5		44	69	62.5	0.25
Vq	C_6H_5		31	72	62.5	0.13
Vr	C_6H_5		42	81	62.5	0.13
Vs	C_6H_5	$CHCCl_3$	45	69	62.5	0.06
Vt	C_6H_5	$C(CH_3)CH_2Cl$	62	80	62.5	0.13
Vu	C_6H_5	$C(CH_3)_2$	76	92	62.5	0.13
Vv	C_6H_5	$C(C_6H_5)_2$	74	83	62.5	0.63

Vw	C_6H_5	CH-3-ClC_6H_4	78	76	125	0.25
Vx	C_6H_5	CH-4-$NO_2C_6H_4$	84	81	125	0.06
Vy	2-ClC_6H_4	CH-2-ClC_6H_4	64	82	125	0.13
Vz	4-ClC_6H_4	CH-2-ClC_6H_4	52	69	125	0.13
Vaa	CH_3	CH-2,6-$Cl_2C_6H_3$	88	66	>250	0.06
Vab	C_6H_5	CH-4-$N(CH_3)_2C_6H_4$	82	66	>250	0.63

is rather low, the only exception being the cyclohexylidene compound (Vj, M.I.C. = 25 μg/ml), but if R and R^1 are aromatic or heteroaromatic the compounds may show good activity.



(Vk)

Using α-(benzylideneimino-oxy)benzylpenicillin (Vk, M.I.C. = 62.5 μg/ml) as a reference it can be seen that higher activity is obtained only if ring B is substituted in the 2-position by rather bulky groups. Thus, the fluoro and the hydroxy compounds (Vl, Vn, Vo) have the same activity as Vk, but the methyl and methoxy compounds (Vf, Vg, M.I.C. = 25 μg/ml) are about twice and the chloro and bromo compounds (Va, Vb, Vd, Ve, M.I.C. = 12.5 μg/ml) five times as active as Vk. Inspection of a "Catalin" molecular scale model reveals that the 2-fluoro and 2-hydroxy substituents do not hinder to any appreciable extent the free rotation of the benzene ring around bond *a* while this is the case with the other substituents. Though, in all compounds, except the 2,6-dichloro derivative (Vd), the nucleus B may be brought into coplanarity with the azomethine group. The high activity of compounds carrying large *o*-substituents in ring B indicates that it is essential for the activity that ring B should not lie in the plane formed by the C=N—O— moiety.

From the results it can be seen that in Vk both ring A and B may be replaced with heteroaromatic ring systems (Vc, Vp, Vq, Vr) without any loss in activity. When ring B is a thiophene nucleus vicinal bromo substituents will also enhance the activity (Vh, Vi). Finally it is found that substitution of ring A causes loss of activity (Vy, Vz).

In order to investigate in which way the stereochemistry of the penicillins affects the biological activity, we decided to study the two epimeric penicillins obtainable by acylation of 6-APA with L(+) and D(−)-α-(2-chlorobenzylidenei-

Table 2.

Penicillin	$[\alpha]_D^{20}$ (acetone/water 3:2)	Activity (μg/ml) *E.coli*	*Staph.aur.*
D-Va	+ 113.5°	6.25	0.06
L-Va	+ 175.5°	> 250	0.25
"DL"-Va	+ 144.0°	12.5	0.13

mino-oxy)phenylacetic acid [5] (III, R = C_6H_5, R^1 = H, R^2 = 2-chlorophenyl), respectively. The optically pure acids were converted into the acid chlorides by warming at 50° with thionyl chloride in dry ethylene chloride. Reacting the acid chlorides with 6-APA in the usual manner yielded the epimeric penicillins D-Va and L-Va (Table 2).

Table 2 shows the specific rotations and antibacterial activities of the epimeric compounds and of the penicillin obtained from racemic side-chain acid. The peniclins D-Va and L-Va were recrystallized as their isopropylamine salts from methanol until the rotation remained constant. The specific rotation of the isopropylamine salt of the DL compound remained the same after one crystallization and was found to have the mean value of the rotations of the D- and L-penicillin (Table 2). Penicillin L-Va, obtained from L(+)-α-(2-chlorobenzylideneimino-oxy)phenylacetic acid showed very low activity against *E. coli* while D-Va, derived from the D(+)-acid, possessed double the activity compared to that of DL-Va.

The differences in activity noted between the epimeric forms of penicillins are, according to our experience, usually of the order of one or two dilution steps. Thus, against the same *E. coli* strain as used in the tests reported here we have previously found [12] for the corresponding D- and L-epimers of α-aminobenzylpenicillin (II) M.I.C. values of 1.25 and 6.25 μg/ml, respectively.

Table 3.

No.	Formula	IR-Absorption cm^{-1}	Recryst. solvent		Analysis			
Va	$C_{26}H_{31}ClN_4O_5S$	1785	methanol	found	C 57.1	H 5.87	N 9.65	
				calc.	57.09	5.70	10.23	
L-Va	$C_{26}H_{31}ClN_4O_5S$	1795	methanol	found	C 57.04	H 5.68	N 9.84	
				calc.	57.09	5.70	10.23	
D-Va	$C_{26}H_{31}ClN_4O_5S$	1780	methanol	—				
Vg	$C_{27}H_{34}N_4O_6S$	1785	methanol	found	C 59.51	H 6.28	N 10.09	
				calc.	59.7	6.27	10.32	
Vh	$C_{24}H_{29}BrN_4O_5S_2$	1780	methanol/ether	found	C 48.17	H 4.89	N 9.53	Br 13.39
				calc.	48.2	4.85	9.37	13.38
Vu	$C_{22}H_{32}N_4O_5S$	1780	methanol/ether	found	C 56.62	H 7.09	N 12.08	
				calc.	56.7	6.9	12.05	

In view of this, the great difference between the values for D- and L-Va is remarkable, and in direct contrast to the differences of activity against *Staph. aureus*, which appears to be "normal". Pfeiffer [13] has postulated that the lower the effective dose of a compound, the greater will be the difference in the biological activity of its optical isomers. This is generally found to be true.[14,15] Applying Pfeiffer's postulate, one would expect the difference in activity against *E. coli* between D- and L-α-aminobenzylpenicillin to be greater than that between D- and L-Va. Since Va is more active against *Staph. aureus* than against *E. coli* the activity difference of the epimers should be greater for the former than for the latter organism. However, in both cases the opposite is true.

EXPERIMENTAL

The penicillins Va—Vab (Table 1) were prepared according to a standard method, of which a typical example is given. All compounds showed an IR absorption band in the region between 1765—1795 cm^{-1}, characteristic for a β-lactam system.

α-(Benzylideneimino-oxy)benzylpenicillin (Vk, Table 1). α-(Benzylideneimino-oxy)-phenylacetic acid (2,55 g, 0.01 mole) and thionyl chloride (5 ml) were refluxed for 30 min, the excess thionyl chloride was removed *in vacuo*, 20 ml of dry benzene added and evaporated. The last procedure was repeated twice, the residue was dissolved in 20 ml of dry ether and added dropwise to an ice-cold solution of 6-APA (2.16 g, 0.01 mole) in N NaOH (10 ml). During the reaction, the pH of the mixture was maintained at 6.5 by addition of N NaOH (10 ml).

After stirring for an additional 30 min at 0—5°, the aqueous phase was separated, washed with ether, acidified to pH 2, and the penicillin extracted with ether. After extraction from the latter with N $KHCO_3$ solution, the potassium salt of the penicillin was isolated by freeze drying, yielding a white powder (2.6 g, 53 %) with a purity of 93 %, as determined by the hydroxylamine method [16] using penicillin G as standard. The infrared spectrum showed an absorption at 1765 cm^{-1}.

The penicillins Va, g, h, and u were purified by crystallization in the following manner: About 1 g of freeze-dried penicillin potassium salt was dissolved in 50 ml of water, chilled to 0°, acidified to pH 2 with 2 N H_2SO_4 and the precipitated penicillin extracted with ether. The ether was washed several times with saturated NaCl-solution and dried. The penicillin was precipitated with 50 % isopropylamine-acetone, filtered, washed thoroughly with dry ether and recrystallized 2 to 3 times from suitable organic solvents. Analytical data, IR absorption and recrystallization solvents are listed in Table 3.

D- and L-Epimers of α-(2-chlorobenzylideneimino-oxy)benzylpenicillin (D-Va and L-Va, Table 2). D(—) and L(+)-α-(2-chlorobenzylideneimino-oxy)phenylacetic acid,[5] respectively, (2 g, 0.0069 mole), 8 ml of dry ethylene chloride and thionyl chloride (1 ml, 0.014 mole) were warmed to 50° for 2 h. The solution was concentrated *in vacuo* to about half of its volume and 5 ml of dry benzene added. The mixture was reevaporated to half of its volume, and more benzene was added. This was repeated 3 times. After the last evaporation the residue was dissolved in 20 ml of dry ether, the solution added dropwise to 6-APA (3 g, 0.014 mole) in N NaOH (14 ml), and the reaction mixture processed as described above.

2 g (55 %) of L-Va and 1.9 g (53 %) of D-Va were obtained as crude products. The penicillins were converted into their isopropylamine salts as described above. The isopropylamine salts were recrystallized from methanol to constant rotation (3 times) affording fine colourless needles.

Rotations and activities against *Staph. aureus* and *E. coli* are given in Table 2. For analysis and IR absorption, see Table 3.

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