SEMISYNTHETIC β-LACTAM ANTIBIOTICS. I. α-ISOCYANOBENZYL-PENICILLINS AND CEPHALOSPORINS

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The preparation of the new β-lactam antibiotics α-isocyanobenzyl-penicillins and cephalosporins from the corresponding amino derivatives and their microbiological properties are reported.

Ampicillin (Ia) and amoxycillin (Ib) are among the most effective broad spectrum antibiotics developed to date. The amino group in the side chain has been variously modified as in Ic[1], d[2], e[3], f[4], g[5] (Figure 1) in order to modulate the hydrophilic character which appears to be essential for Gram-negative activity[6]. Since amines can be regenerated from the corresponding isocyanides and in particular α-aminoacetic acids from α-isocyanoacetic acids[7], we thought it worthwhile to mask the amino group of Ia,b as isocyanide. Therefore we synthesized the α-isocyanobenzylpenicillins III and the α-isocyanobenzylcephalosporin analogue VI which are potentially "pro-drugs" of the parent antibiotics with improved in vivo performance.

The synthesis of the formamido derivatives IIa and IIb (Figure 1) was performed with formic anhydride[8] on a chilled aqueous solution of Ia,b, keeping pH about 7 with $NaHCO_3$. The pivaloyl ester IIc (Table 1) was prepared now in improved yield from IIa sodium salt and chloromethyl pivalate in DMF. The intermediates IIa...c are stable solids easily purified by crystallization; their pmr spectra show that the asymmetric center in the side chain was unaffected. Protection of IIa,b as trimethylsilyl esters followed by dehydration with $POCl_3$ and pyridine at $-60° \div -20°$ yielded, after mild hydrolysis, the isocyanides IIIa,b. Of course, the pivaloyl ester IIc was directly dehydrated to IIIc without silyl protection. Ir spectra of α-isocyanobenzylpenicillins show the characteristic β-lactam stretching absorption in the 1785-1780 cm^{-1} region and a further intense band at 2150-2130 cm^{-1} attributed to the stretching vibration of the isocyanide group. Pmr spectra of IIIa...c are consistent (splitting of the signals related to the protons in position 3,5,6 and gem-dimethyl) with epimerisation at the chiral carbon in the phenylacetic moiety during dehydration.

The same reaction sequence was also used to convert cephalexin (IV) into the corresponding isocyano derivative (VI). Formylation of IV gave V in 50% yield [mp 178-181 °C dec., from H_2O/Me_2CO; $[\alpha]_D^{20} = +58°$ (C = 0.5; $MeOH/Me_2CO$ 1:1)]. Subsequent dehydration of V afforded a 32% yield of the isocyanide VI as an epimeric mixture [mp 242-245°C dec., from $iPrOH/H_2O$; $[\alpha]_D^{20} = +166°$

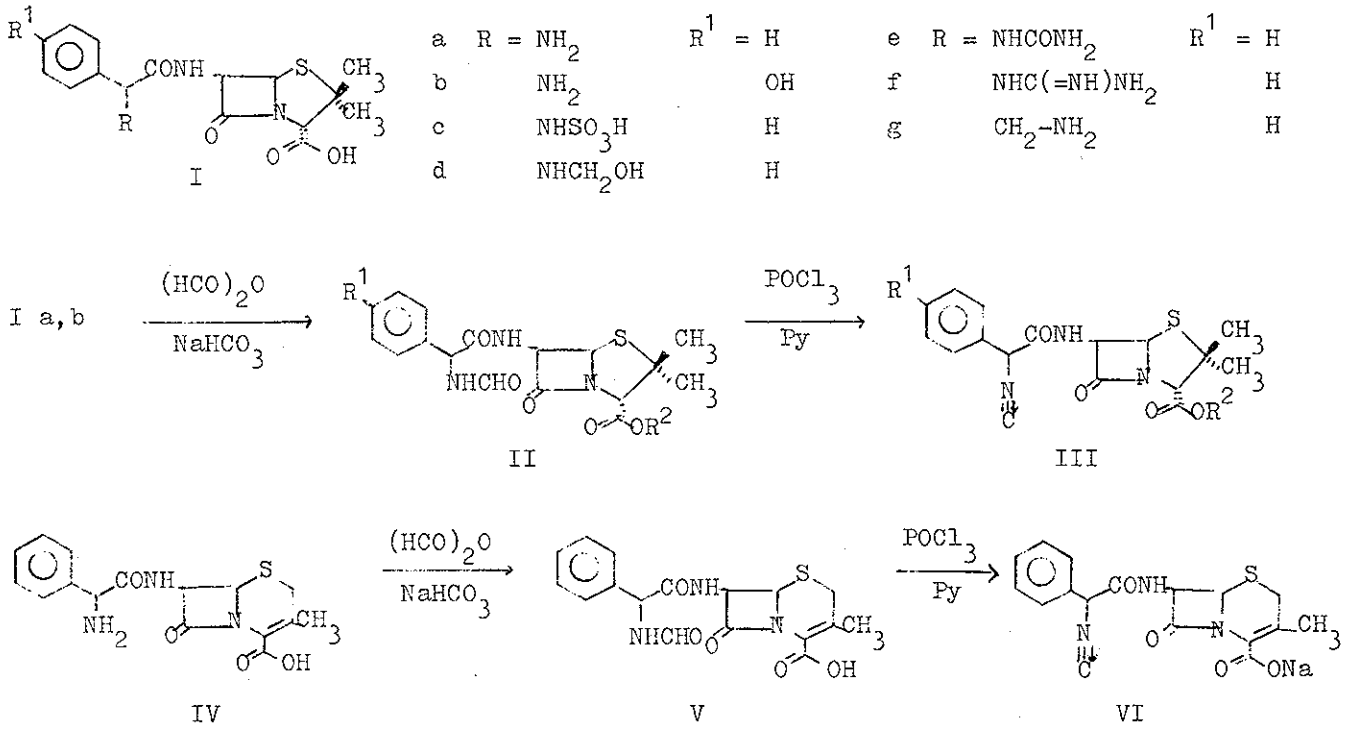
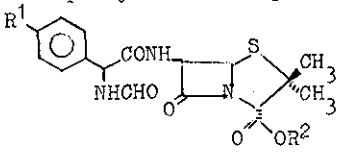

a R = NH2 R1 = H
b NH2 OH
c NHSO3H H
d NHCH2OH H
e R = NHCONH2 R1 = H
f NHC(=NH)NH2 H
g CH2-NH2 H
I
I a,b
(HCO)2O
NaHCO3
II
POCl3
Py
III
IV
V
VI
R2 = H, Na, CH2OCOC(CH3)3

Figure 1

Table 1

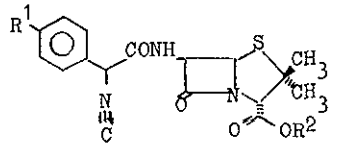
6-(R-α-Formamidophenylacetamido)penicillanic acids



II

Compound	R_1	R_2	Yield %	mp°C dec. (Crystn.solv.)	$[\alpha]_D^{20}$ deg. (C=0.5;MeOH)	Formula
a	H	H	31	142-144 (H_2O-Me_2CO)	+ 189	$C_{17}H_{19}N_3O_5S$ [a]
b	OH	H	40	150-153 (iPr_2O)	+ 146	$C_{17}H_{19}N_3O_6S$
c	H	$CH_2OCOC(CH_3)_3$	83	76-78 (Et_2O)	+ 150	$C_{23}H_{29}N_3O_7S$ [b]

6-(α-Isocyanophenylacetamido)penicillanic acids



III

a	H	Na	30	218-220 (iPrOH-MeOH)	+ 242	$C_{17}H_{16}N_3NaO_4S$
b	OH	Na	26	208-210 (iPrOH-H_2O)	+ 221	$C_{17}H_{16}N_3NaO_5S$
c	H	$CH_2OCOC(CH_3)_3$	36	54-56 (Et_2O-Petr.)	+ 197	$C_{23}H_{27}N_3O_6S$ [c]

a See ref. 9

b From IIa. In literature it was prepared by formylation of pivampicillin but not characterized. See ref. 10

c During completion of this manuscript, the synthesis of the ester IIIc was reported by T.H. Cronin. See ref. 10

Table 2

MIC values (μg/ml) and acid stabilities

	Bacteria	Ia	IV	IIIa	IIIb	IIIc	VI
GRAM +	Staph. aureus Smith	0.048	0.78	0.39	0.78	6.25	50
	Staph. aureus ATCC 6538/P	0.048	0.78	0.19	0.78	6.25	25
	Str. pyogenes ISM 68/241	0.012	0.78	0.19	0.19	0.39	12.5
	B. cereus ATCC 9364	0.012	0.78	0.19	0.19	0.39	25
	B. subtilis ATCC 6633	0.012	0.78	0.19	0.39	0.39	25
	S. lutea ATCC 9341	0.006	0.047	0.19	0.097	0.39	3.12
GRAM -	E. Coli 120	0.78	3.12	6.25	12.5	100	>100
	Salm. paratyphi ISM	3.12	3.12	12.5	6.25	50	>100
	Shi. dysenteriae NLTL 4837	0.78	4.25	12.5	12.5	100	>100
	P. aeruginosa ATCC 9027	>100	>100	>100	>100	>100	>100
% Activity after treatment with gastric juice		95	96	530	178	221	43

(C = 0.5; MeOH).

The minima inhibitory concentration (MIC) of compounds IIIa...c and VI against 16 strains of Gram-positive and Gram-negative bacteria was determined using the two fold serial dilution technique in brain-heart-infusion agar medium (Difco) plus 10% horse serum. The agar plates were inoculated with one drop of a diluted (1/25) overnight culture delivered by a multiple inoculating device and incubated for 18h at 37°C. The acid stability was tested in artificial gastric juice (USP XVIII) and values indicate the residual % of antimicrobial activity assayed by the microbiological agar-plate diffusion method. It is evident from the results listed in Table 2 that the activity of isocyano derivatives is lower than that of ampicillin and cephalexin. According to our prevision, we have found that activity of isocyanopenicillins IIIa...c is enhanced when tested in artificial gastric juice as a consequence of partial hydrolytic cleavage to the diastereo mixture of the parent antibiotics. This property was confirmed by a biochromatographic study. Surprisingly the same behaviour was not noticed for the cephalosporin analogue VI.

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