SEMISYNTHETIC β-LACTAM ANTIBIOTICS. III.[1] SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF α-(2-IMIDAZOLINYLAMINO) BENZYL-PENICILLIN AND -DESACETOXYCEPHALOSPORIN

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The preparation of the new β-lactam antibiotics α-(2-imidazolinylamino)benzyl-penicillin (I) and -desacetoxycephalosporin (II) via the appropriate phenylacetylchloride hydrochloride (VII) is reported. Their antibacterial activities against several micro-organisms have been determined in vitro.

The replacement of a benzylic proton of penicillin G with nitrogen containing moieties such as guanidino[2], ureido[3], 3-guanylureido[4] and 2-oxo-1-imidazolidincarboxamido[5] leads to an enhancement of Gram-negative antibacterial activity. As a part of our interest in the field of semisynthetic β-lactam antibiotics,[1,6] we synthesized the α-(2-imidazolinylamino)derivatives I

and II (Figure 1) in which the guanyl group is incorporated in the 2-imidazoline ring by an ethylene bridge.

The direct conversion both of ampicillin (III) and its triethylammonium salt or trimethylsilyl ester into the desired penicillin (I) by reaction with 2-methylthio-2-imidazoline (MTI)[7] or 2-chloro-2-imidazoline (CI)[8] failed to occur in a variety of experimental conditions. Thus we undertook the synthesis of the unknown intermediate R-α-[(2-imidazolin-2-yl)amino]phenylacetic acid (VI) in view of its condensation with 6-aminopenicillanic acid (6-APA) or 7-amino-3-methyl-3-cephem-4-carboxylic acid (7-ADCA).

A first attempt to obtain VI by reacting R-α-phenylglycine ethyl ester (IVb) with equimolar MTI.HI and KOH in MeOH, afforded only an optically inactive product, namely 2-phenyl-3-oxo-2,3,5,6-tetrahydro-1H-imidazo[1,2-a]imidazole (V) in 25% yield, mp 230° dec.: IR (mineral oil mull) 1735, 1698, 700 and 755 cm^{-1}; NMR (D_2O + CF_3COOH, ref. DSS) δ7.7 - 7.5 (5H, complex abs, Ph-H); 5.9 (1H, s, Ph-CH); 4.6 - 3.9 (4H, complex abs, CH_2-CH_2); mass spectrum; m/e 201 (M^+).

Otherwise the compound VI was obtained by refluxing R-α-phenylglycine (IVa) with a methanolic solution of an excess of MTI in the presence of catalytic amounts of sodium methoxide. The yield was 60% of VI[9] as a zwitterion: mp 253-254° (monohydrate from water); $[\alpha]_D^{20}$ = -177.4° (C=1; 1N HCl)[10]; IR (mineral oil mull) 3150, 2950, 1675 and 1580 cm^{-1}; NMR (D_2O + CF_3COOH,

ref. DSS) δ 7.5 (5H, s, Ph-H); 5.35 (1H, s, Ph-CH); 3.7 (4H, s CH_2-CH_2).

Activation of the carboxylic function of VI hydrochloride was performed with PCl_5 in CH_2Cl_2 at -60°/0°. After removal of $POCl_3$ under high vacuum at room temperature, compound VII (IR in CH_2Cl_2: 1795, 1760 and 1620 cm^{-1})[11] was not further purified, but directly reacted with 6-APA-trimethylsilyl ester in CH_2Cl_2 at -20°/0° in the presence of a slight excess of N,N-dimethylaniline. The following mild hydrolysis and precipitation with Et_2O from an isopropanol solution (pH 4.5) gave 60% yield of 6-{R-α-[(2-imidazolin-2-yl)amino]phenylacetamido}penicillanic acid (I): mp 183-185° dec; $[\alpha]_D^{20}$ = +162° (C=0.05; MeOH); IR (mineral oil mull) 3200, 1790, 1675 and 1600 cm^{-1}; NMR (DMSO-d_6; ref. TMS) δ 7.6 - 7.3 (5H, complex abs, Ph-H); 5.4 - 5.2 (3H, complex abs; PhCH, $C_{(6)}H$ and $C_{(5)}H$); 4.16 (1H, s, $C_{(3)}H$) 3.6 (4H, br s, CH_2-CH_2); 1.55 (6H, br s, gem CH_3); iodometric assay 93%; one spot in TLC.

With the same procedure, 7-{R-α-[(2-imidazolin-2-yl)amino]phenylacetamido}-3-methyl-3-cephem-4-carboxylic acid (II) was obtained in 30% yield mp 178-180° dec; $[\alpha]_D^{20}$ = +29.5° (C=0.05; H_2O); IR (mineral oil mull) 3200, 1775, 1670 and 1600 cm^{-1}; NMR (DMSO-d_6, ref. TMS) δ 9.53 (1H, d, $J_{NH,C_{(7)}H}$ = 8Hz, CONH); 7.6 - 7.3 (5H, complex abs, Ph-H); 5.75 (1H, dd, $J_{NH,C_{(7)}H}$ = 8Hz, $J_{C_{(7)}H,\ C_{(6)}H}$ = 4Hz, $C_{(7)}H$); 4.99 (1H, d, $J_{C_{(7)}H,\ C_{(6)}H}$ = 4Hz, $C_{(6)}H$); 3.60 (4H, br s, CH_2-CH_2); 3.46 and 3.32 (2H, ABq, J_{AB} =

18Hz, S-C$\underline{H}_2$); 2.01 (3H, s, C$\underline{H}_3$); one spot in TCL.

The minimal inhibitory concentration (MIC) of compounds I and II against 12 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[12] and incubated for 18 h at 37°. 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. From the results reported in Table 1 it appears that cephalosporin II versus cephalexine exhibits a negligible antibacterial activity. In the same table it can be observed that penicillin I possesses against Gram-positive bacteria a valuable activity comparable with that of ampicillin and BL-P 1654[4]. Surprisingly, compound I is poorly active against Gram-negative bacteria and particularly against Pseudomonas, which on the contrary is claimed to be very susceptible to BL-P 1654[4].

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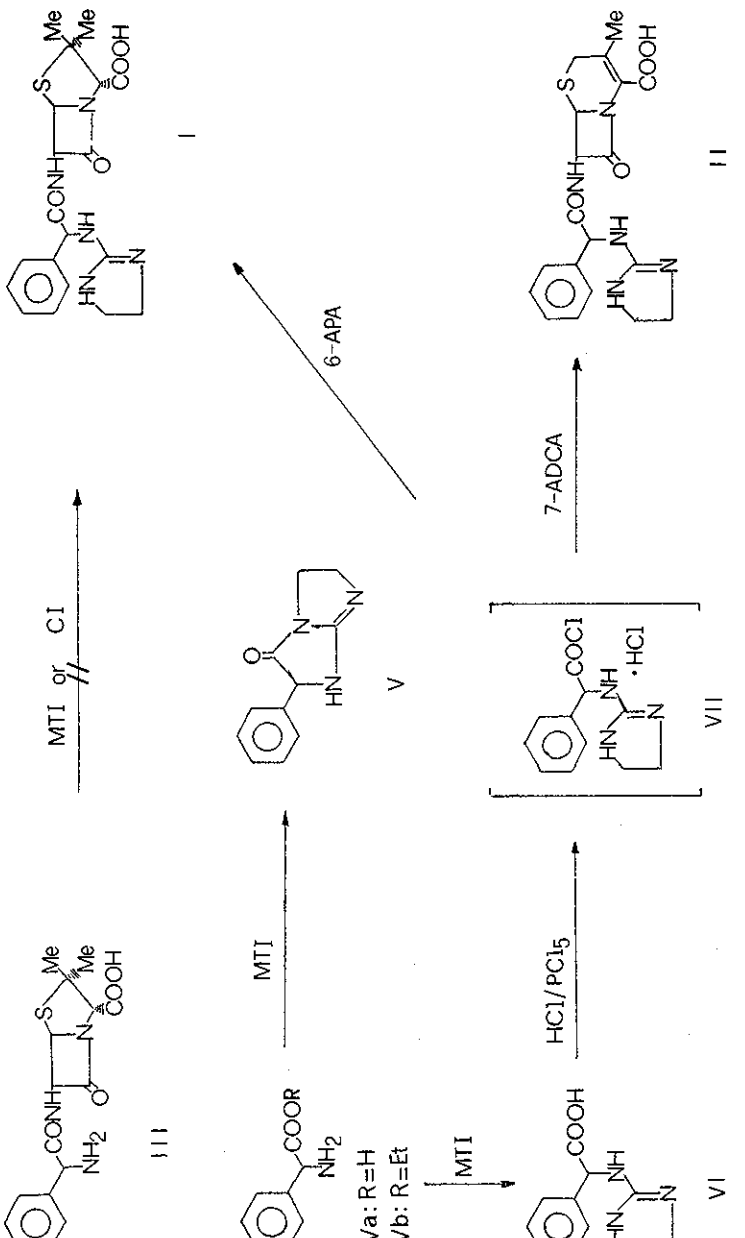
MTI or Cl
6-APA
MTI
HCl/PCl5
7-ADCA
IVa: R=H
IVb: R=Et
MTI : 2-methylthio-2-imidazoline
Cl : 2-chloro-2-imidazoline

Figure 1

TABLE 1

MIC values (μg/ml) and acid stabilities of compounds I and II

	Bacteria	I	Ampicillin	BL-P 1654*	II	Cephalexin
Gram +	Staph. aureus Smith	0.78	0.048	0.25	100	0.78
	Staph. aureus PCI (Pen. Resist.)	6.25	6.25	4	100	6.25
	Staph. aureus 39/II FBF (Pen. Resist.)	6.25	50	-	200	3.12
	Str. pyogenes ISM 68/241	0.097	0.012	0.015	100	0.39
	Str. faecalis ATCC 6057	1.56	0.78	3.3	>100	>100
	Dipl. pneumoniae ISM 68/67	0.048	0.012	0.063	25	1.56
Gram -	E. coli 120	50	0.78	2	>100	6.25
	Salm. paratyphi ISM	50	3.12	-	>100	6.25
	Shi. dysenteriae NCTC 4837	100	0.78	-	>100	3.12
	P. aeruginosa ATCC 9027	>100	>100	4	>100	>100
	Kl. pneumoniae ISM 68/67	>100	100	8	>100	6.25
	Neiss. meningitidis To A	0.024	0.006	0.25	50	0.048
% activity after treatment with gastric juice		100	95	-	-	96

* See ref. 4

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11 Mild hydrolysis of VII give VI hydrochloride $[\alpha]_D^{20} = -145°$

(C=1; H_2O) identical to an authentical sample, thus confirming the unchanged chirality of the carbon atom in VII.

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