

SEMISYNTHETIC  $\beta$ -LACTAM ANTIBIOTICS. III<sup>1</sup> SYNTHESIS AND  
ANTIBACTERIAL ACTIVITY OF  $\alpha$ -(2-IMIDAZOLINYLAMINO)  
BENZYL-PENICILLIN AND -DESACETOXYCEPHALOSPORIN

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The preparation of the new  $\beta$ -lactam antibiotics  
 $\alpha$ -(2-imidazolinylamino)benzyl-penicillin (I) and -de-  
sacetoxycephalosporin (II) via the appropriate phenyl-  
acetylchloride hydrochloride (VII) is reported. Their  
antibacterial activities against several micro-organ-  
isms have been determined in vitro.

The replacement of a benzylic proton of penicillin G with  
nitrogen containing moieties such as guanidino<sup>2</sup>, ureido<sup>3</sup>, 3-  
guany lureido<sup>4</sup> and 2-oxo-1-imidazolidincarboxamido<sup>5</sup> leads to an  
enhancement of Gram-negative antibacterial activity. As a part  
of our interest in the field of semisynthetic  $\beta$ -lactam antibiot-  
ics,<sup>1,6</sup> we synthesized the  $\alpha$ -(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)<sup>7</sup> or 2-chloro-2-imidazoline (CI)<sup>8</sup> failed to occur in a variety of experimental conditions. Thus we undertook the synthesis of the unknown intermediate  $\underline{R}-\alpha\text{-(2-imidazolin-2-yl)amino}\gamma\text{-phenylacetic acid (VI)}$  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  $\underline{R}-\alpha\text{-phenylglycine ethyl ester (IVb)}$  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  $\text{cm}^{-1}$ ; NMR ( $\text{D}_2\text{O} + \text{CF}_3\text{COOH}$ , ref. DSS)  $\delta$  7.7 - 7.5 (5H, complex abs, Ph-H); 5.9 (1H, s, Ph-CH); 4.6 - 3.9 (4H, complex abs,  $\text{CH}_2\text{-CH}_2$ ); mass spectrum; m/e 201 ( $\text{M}^+$ ).

Otherwise the compound VI was obtained by refluxing  $\underline{R}-\alpha\text{-phenylglycine (IVa)}$  with a methanolic solution of an excess of MTI in the presence of catalytic amounts of sodium methoxide. The yield was 60% of VI<sup>9</sup> as a zwitterion: mp 253-254° (monohydrate from water);  $[\alpha]_{\text{D}}^{20} = -177.4^\circ$  (C=1; 1N HCl)<sup>10</sup>; IR (mineral oil mull) 3150, 2950, 1675 and 1580  $\text{cm}^{-1}$ ; NMR ( $\text{D}_2\text{O} + \text{CF}_3\text{COOH}$ ,

ref. DSS)  $\delta$  7.5 (5H, s, Ph-H); 5.35 (1H, s, Ph-CH); 3.7 (4H, s, CH<sub>2</sub>-CH<sub>2</sub>).

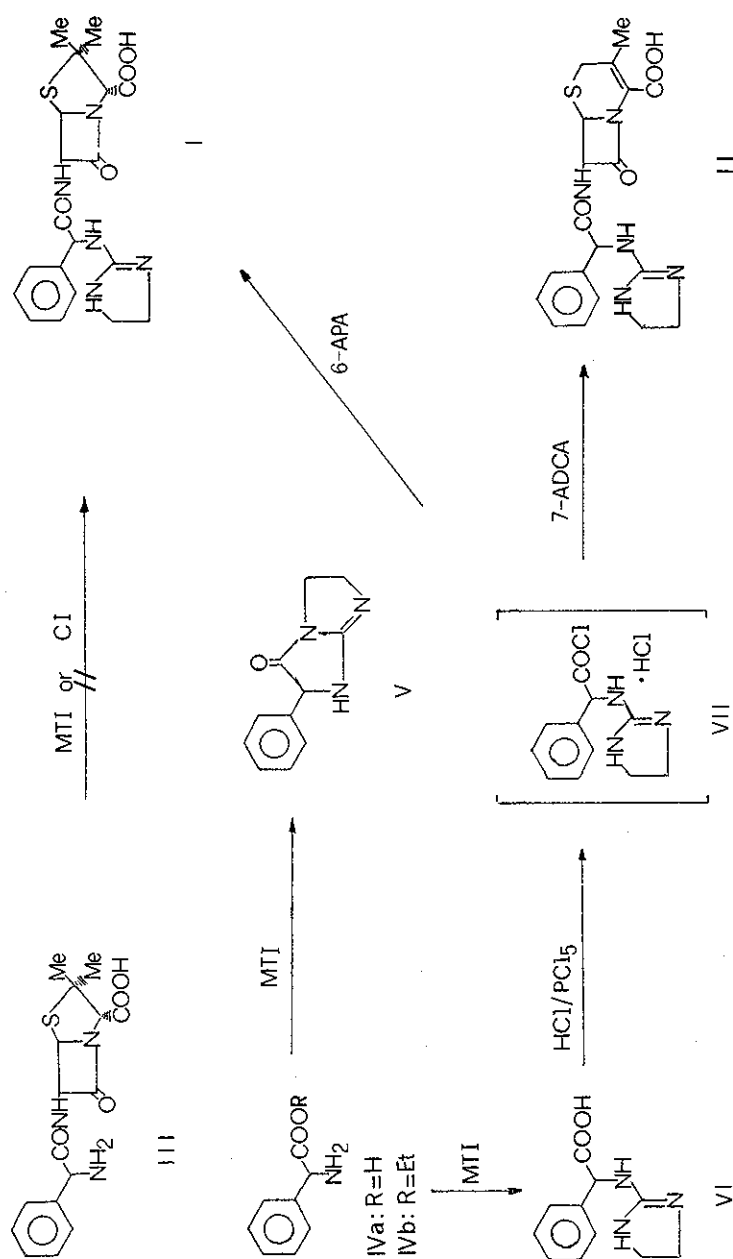
Activation of the carboxylic function of VI hydrochloride was performed with PCl<sub>5</sub> in CH<sub>2</sub>Cl<sub>2</sub> at -60°/0°. After removal of POCl<sub>3</sub> under high vacuum at room temperature, compound VII (IR in CH<sub>2</sub>Cl<sub>2</sub>: 1795, 1760 and 1620 cm<sup>-1</sup>)<sup>11</sup> was not further purified, but directly reacted with 6-APA-trimethylsilyl ester in CH<sub>2</sub>Cl<sub>2</sub> at -20°/0° in the presence of a slight excess of N,N-dimethylaniline. The following mild hydrolysis and precipitation with Et<sub>2</sub>O from an isopropanol solution (pH 4.5) gave 60% yield of 6-{R- $\alpha$ -[2-imidazolin-2-yl]amino-7-phenylacetamido}penicillanic acid (I): mp 183-185° dec;  $\angle_{\alpha_D}^{20} = +162^\circ$  (C=0.05; MeOH); IR (mineral oil mull) 3200, 1790, 1675 and 1600 cm<sup>-1</sup>; NMR (DMSO-d<sub>6</sub>; ref. TMS)  $\delta$  7.6 - 7.3 (5H, complex abs, Ph-H); 5.4 - 5.2 (3H, complex abs, PhCH, C<sub>(6)</sub>H and C<sub>(5)</sub>H); 4.16 (1H, s, C<sub>(3)</sub>H) 3.6 (4H, br s, CH<sub>2</sub>-CH<sub>2</sub>); 1.55 (6H, br s, gem CH<sub>3</sub>); iodometric assay 93%; one spot in TLC.

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

18Hz, S-CH<sub>2</sub>); 2.01 (3H, s, CH<sub>3</sub>); one spot in TLC.

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<sup>12</sup> 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 cephalaxine 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<sup>4</sup>. 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<sup>4</sup>.

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MTI : 2-methylthio-2-imidazoline  
 Cl : 2-chloro-2-imidazoline

Figure 1

TABLE 1

MIC values ( $\mu\text{g/ml}$ ) and acid stabilities of compounds I and II

	Bacteria	I	Ampicillin	BL-P 1654*	II	Cephalexin
+ Gram	<u>Staph. aureus</u> Smith	0.78	0.048	0.25	100	0.78
	<u>Staph. aureus</u> PCI (Pen. Resist.)	6.25	6.25	4	100	6.25
	<u>Staph. aureus</u> 39/11 FBF (Pen. Resist.)	6.25	50	-	200	3.12
	<u>Str. pyogenes</u> ISM 68/241	0.097	0.012	0.015	100	0.39
	<u>Str. faecalis</u> ATCC 6057	1.56	0.78	3.3	>100	>100
	<u>Dipl. pneumoniae</u> ISM 68/67	0.048	0.012	0.063	25	1.56
- Gram	<u>E. coli</u> 120	50	0.78	2	>100	6.25
	<u>Salmon. paratyphi</u> ISM	50	3.12	-	>100	6.25
	<u>Shi. dysenteriae</u> NCTC 4837	100	0.78	-	>100	3.12
	<u>P. aeruginosa</u> ATCC 9027	>100	>100	4	>100	>100
	<u>Kl. pneumoniae</u> ISM 68/67	>100	100	8	>100	6.25
	<u>Neiss. meningitidis</u> 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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- 10 The optical purity was undetermined.
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(C=1; H<sub>2</sub>O) identical to an authentic sample, thus confirming the unchanged chirality of the carbon atom in VII.

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