Synthesis and microbiological action of new dipenicillins and dicephalosporins derived from asparagic acid

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A new synthetic method of some β-lactamic antibiotics containing an asparagic acid residue is described which is based on the acylation of the 6-aminopenicillanic acid (6-APA) and of the 7-aminodesacetoxycephalosporanic acid (7-ADCA), respectively, by acid dichlorides of the N-(o,m,p)-nitrobenzoyl asparagic acids at low temperatures in nonaqueous medium. The structure of the newly synthesized compounds has been elucidated by means of elemental analysis and spectral data (IR, UV, NMR) and their antibacterial activity has been evaluated.

The β-lactam antibiotics constitute the main class of the antibacterial pharmaceuticals applied in therapy. The discovery of 6-aminopenicillanic acid (6-APA) and subsequent synthesis of 7-aminodesacetoxycephalosporanic acid (7-ADCA)\(^1\) opened the way of chemical transformation of the side chain which resulted in a larger diversity of the penicillin and cephalosporin structures as well as of their antimicrobial and pharmaco-kinetics properties. A wide range of semi-synthetic antibiotics containing either 6-APA or 7-ADCA residue are mentioned in the literature as well as their formation by acylation with different organic compounds such as acid chlorides\(^2\)\(^-\)\(^3\), anhydrides\(^4\) and activated esters\(^5\)\(^-\)\(^6\).

In continuation of our work we intended to take the advantage of the presence of two carboxyl groups in the structures of o-, m- and p-nitrobenzoyl asparagic acids 1-3 previously synthesized\(^7\)\(^-\)\(^9\) to prepare the corresponding acid dichlorides to be used as acylating agents for 6-APA and 7-ADCA. The obtaining of dipenicillins and dicephalosporins by this method would possibly result in broadening of the bacterial spectrum of the antibiotics.

The acid dichlorides 4 - 6 were prepared from the corresponding acids by chlorination with either SOCl\(_2\) or PCl\(_3\) in toluene medium (Scheme I).

The reaction with SOCl\(_2\) was carried out with an excess of chlorinating agent (10-20%) with slight heating (40-45°C) for 2.5-3 hr, in the presence of dimethylformamide\(^10\). The chlorination with PCl\(_3\), in stoichiometric amount, was performed at 15.25°C for 30-60 min. The toluene was chosen as a reaction medium in order to remove easily by a low-pressure distillation the unreacted SOCl\(_2\) or PCl\(_3\) resulting from the reaction with PCl\(_3\). The crude dichlorides thus obtained were finally dissolved in dichloromethane and used for acylation without any previous purification.

The cephalosporanic and penicillanic acids were acylated in non-aqueous medium to avoid the dichloride hydrolysis (Scheme II).
The 6-APA was solubilized by its conversion into the triethylammonium salt which is soluble in 8-10% dichloromethane while the 7-ADCA was submitted to silification in dichloromethane for modifying the -COOH group.

The acid dichlorides dissolved in dichloromethane were added during 40-60 min to the solutions of 6-APA and 7-ADCA, respectively, previously cooled to 0-5°C. The acylation development was followed by TLC with the butyl acetate:acetic acid:butanol:methanol (80:40:45:5 by vol.) mixture as developer. The acylated compounds 7 - 12 were separated from the reaction medium by successive liquid-liquid extractions in dichloromethane-acetone at pH 2. The extracts were purified by heating with active carbon (AI type) followed by precipitation as sodium salt by means of sodium 2-ethyl hexanoate (either acetone or methanol solution) and filtration. The resulting precipitates were washed with acetone and then dissolved in water. The corresponding dipenicillins and dicephalosporins were finally obtained in their acid forms by adding 1N HCl solution, under stirring, to pH 2-3 at 0-5 °C. After filtration, the precipitates were washed with precooled water and dried under vacuum.
The structure of the newly obtained antibiotics was elucidated by spectral measurements\textsuperscript{11}. The UV absorption spectrum depends on the side chain nature as well as on the penam or cephem ring. Thus, the UV spectra of the dipenicillins 7 - 9 showed an absorption maximum at \( \lambda = 230 \pm 5 \text{ nm} \) due to the benzene ring in the structure of the starting compounds while in case of dicephalosporins a second band at \( \lambda = 260 \pm 5 \text{ nm} \) was also found attributable to the \( \pi \)-\( \pi \) conjugation in the cephem ring. The penam ring did not show characteristic absorption spectrum.

The IR spectrum shows an absorption maximum at \( \lambda = 1700 - 1900 \text{ cm}^{-1} \) characteristic of the carbonyl group in \( \beta \)-lactam which is different from the frequency characteristic of substituted amides (1500-1680 cm\(^{-1}\)) and carboxyl group (1400-1600 cm\(^{-1}\)).

The signals in the \( ^1\text{HNMR} \) spectrum of the reported compounds as discussed in experimental section showed the \( \beta \)-lactamic ring integrity, the presence of substituents and their steric position.

**Antibacterial activity**

The synthesized compounds were tested for their antibacterial activity by measuring the inhibition area on agar plates (diffusimetric method)\textsuperscript{12,13} with *Staphylococcus aureus*, *Sarcina lutea*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* as test germs.

The diameters of the inhibition areas given by the compounds 7 - 12 are presented in Table I. As can be seen in Table I, the penicillins under study show a wide action spectrum and their activity is comparable to that of carbenicillin. They are more active towards *Sarcina lutea* and *Staphylococcus aureus* and less active towards *Klebsiella pneumoniae* than carbenicillin. As regards the dcephalosporins they are almost inactive against *Pseudomonas aeruginosa* and active against the other germs.

The position of the \( -\text{NO}_2 \) group in the aromatic ring does not significantly influence the antibacterial activity. An activity intensification by the amino acid residue in the molecule might be assumed.

**Experimental Section**

IR spectra were recorded in KBr on a Perkin-Elmer 157 infrared spectrophotometer (\( \nu_{\max } \text{ in cm}^{-1} \)) and \( ^1\text{HNMR} \) spectra on a Varian XL-300 spectrometer using TMS as internal reference (chemical shifts in \( \delta \), ppm).

\( -(\text{Nitrobenzoyl}) \) asparagic acids 1 – 3. Compound 1 was prepared from asparagic acid and \( \alpha \)-nitrobenzoic acid chloride, in the presence of \( \text{NaHC}O_3 \); and 2 and 3 were prepared similarly following the literature methods\textsuperscript{9}.

Acid dichlorides 4 – 6. A suspension of \( N-(\alpha,\omega,\pi) \) nitrobenzoic acid (5mmoles) in toluene (15mL) was treated with PCls (10mmoles) at 10°C. After stirring the reaction mixture for about 60 min at the room temperature a clear solution resulted. The toluene and PCls were then removed by a low pressure distillation and the acid dichloride resulted in a 90% yield.

**Dipenicillin 7.** To a suspension of 6-APA (10mmoles) in dichloromethane (20mL) triethylamine (2.8mL) was added for the 6-APA dissolution. The resulting solution was cooled to 0-5°C and treated with 10\% \( N-(\alpha\text{-nitrobenzoyl}) \)asparagic acid dichloride (5mmoles) in dichloromethane. The mixture was stirred at low temperature for 90 min, then acetone (20mL) added at 0-5°C and finally the \( \rho \text{H} \) brought to 2 by means of \( 1\text{N HCl} \) solution. After separating the organic layer it was washed with cold water (20mL), dried to anhydrous state and treated with 26\% solution of sodium 2-ethylhexanoate in acetone (6.5mL). The resulting white precipitate was washed with acetone and then dissolved in water (30mL). The corresponding dipenicillin was finally obtained in its acid form as a white precipitate by adding \( 1\text{N HCl} \) solution, under stirring, to \( \rho \text{H} \) 2.5-3 at 0-5°C. After filtration, the precipitate was washed twice with precooled water (5-10°C) and dried under low pressure (yield \( 72\% \)). (Found: C, 47.92; H, 4.23; N, 12.10; S, 9.88. \( C_{23}H_{22}N_6O_7S_2 \) requires C, 48.69; H, 4.38; N, 12.17; S, 9.27\%); IR (KBr): 3240 (NH, amide), 1641 (CO,\( \beta \)-lactam), 1629 (CO,amide I), 1581(COO\(^{-}\) assym.), 1408(COO\(^{-}\) sym.), 1560 (NO\(_2\))

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<thead>
<tr>
<th>Compd</th>
<th>Antibacterial activity</th>
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<tr>
<td></td>
<td><em>S. aureus</em></td>
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<tr>
<td>7</td>
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<td>12</td>
<td>36</td>
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<td>Carbenicillin</td>
<td>30</td>
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* Diameter of inhibition zone is expressed in mm

Table I — Antibacterial activity of compounds 7-12*
washed with acetone (50mL) and then dissolved in water (50mL). The obtained solution was treated with active carbon and acidified to pH 2.2-2.5 at 0-5°C with 1N HCl solution. The precipitate was filtered, washed with cold water (25mL) and dried under vacuum, yield 76% (Found: C, 47.92; H, 4.15; N, 12.25; S, 8.85. C27H26N6O11S2 requires C, 48.06; H, 3.89; N, 12.46; S, 9.49%). IR (KBr): 3300 (NH, amide), 1890 (CO, β-lactam), 1690 (CO, amide I), 1535 (CO, amide II), 1640 (COO' asym.), 1405 (COO' sym.), 1580 (NO2 asym.), 1350 (NO2 sym.), 880 (substituted aromatic ring). 1HNMR (MeOD): δ 1.8 (m, 3H, CH3), 1.9 (m, 3H, CH3), 2.1 (d, 2H, CH2), 4.0 (m, 1H, CH), 5.2 (dd, 1H, CH), 5.40 (dd, 1H, CH), 6.0 (m, 2H, CH2), 6.10 (m, 2H, CH2), 6.5 (dd, 1H, CH), 6.6 (dd, 1H, CH), 9.1 (t, 1H, CHAr), 9.3 (d, 1H, CHAr), 9.4 (d, 1H, CHAr), 9.5 ppm (d, 1H, CHAr).

Dipenicillin 11. It was synthesized from the N-(m-nitrobenzoyl)asparaginic acid dichloride 5 (25mnoles) as described for 10 (Found: C, 48.5; H, 4.23; N, 11.92; S, 10.25. C27H26N6O11S2 requires C, 48.06; H, 3.89; N, 12.46; S, 9.49%). IR (KBr): 3320 (NH, amide), 1892 (CO, β-lactam), 1680 (CO, amide I), 1520 (CO, amide II), 1615 (COO' asym.), 1420 (COO' sym.), 1570 (NO2 asym.), 1378 (NO2 sym.), 870 (substituted aromatic ring). 1HNMR (MeOD): δ 1.85 (m, 3H, CH3), 1.92 (m, 3H, CH3), 2.15 (d, 2H, CH2), 3.90 (m, 1H, CH), 5.10 (dd, 1H, CH), 5.4 (dd, 1H, CH), 6.05 (m, 2H, CH2), 6.15 (m, 2H, CH2), 6.45 (dd, 1H, CH), 6.68 (dd, 1H, CH), 9.1 (t, 1H, CHAr), 9.2 (d, 1H, CHAr), 9.35 (d, 1H, CHAr), 9.5 ppm (s, 1H, CHAr).

Dipenicillin 12. The acylation was carried out with N-(p-nitrobenzoyl)asparaginic acid dichloride 6 (25mnoles) leading to the final product in a 78% yield (Found: C, 47.85; H, 4.45; N, 11.90; S, 8.80. C27H26N6O11S2 requires C, 48.06; H, 3.89; N, 12.46; S, 9.49%). IR (KBr): 3280 (NH, amide), 1890 (CO, β-lactam), 1684 (CO, amide I), 1527 (CO, amide II), 1680 (COO' asym.), 1415 (COO' sym.), 1575 (NO2 asym.), 1365 (NO2 sym.), 840(substituted aromatic ring). 1HNMR (MeOD): δ 1.75 (m, 3H, CH3), 1.95 (m, 3H, CH3), 2.20 (d, 2H, CH2), 4.10 (m, 1H, CH), 5.15 (dd, 1H, CH), 5.35 (dd, 1H, CH), 5.95 (m, 2H, CH2), 6.15 (m, 2H, CH2), 6.40 (dd, 1H, CH), 6.55 (dd, 1H, CH), 9.1 (d, 2H, CHAr), 9.4 ppm (d, 2H, CHAr).

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