Synthesis and structure-activity relationship of new cephalosporins modified at C-7 and C-4

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Abstract: The synthesis and antimicrobial activity of a series of cefaclor derivatives bearing phthalyl or tosylaminoacyl or dipeptidyl moieties attached to the α-amino group of the 7-phenylglycinamido acyl unit, or amino acid residues and their corresponding methyl esters linked to the carbonyl group on C-4 are described. Some compounds of this series were found to possess high activity against pseudomonas aeruginosa and other Gram-negative bacteria.

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Introduction:

In recent years, intensive research has been carried out in order to obtain modified cephalosporins with improved antimicrobial properties[1-11]. Many derivatives of 7-acylphenylacetamidocephalosporin have been synthesized and are found to possess enhanced activity toward gram-negative micro-organisms [12-19]. As far as we know, no cephal-osporins are known in which the α-amino group of 7-phenylglycinamido acyl moiety has been reacted with phthalyl or tosylaminoacyl or dipeptidyl units. On the other hand, some cephalosporin compounds having modifications on C-4 have shown consider-able biological activity[20-22].In continuation of our work on structure-activity relationship of amino acid derivatives (*SAR*)[23-27] , these facts prompted us to investigate the introduction of a new series of cefaclor compounds containing modified acyl chains at the C-7 or amino acid amide residues at the C-4 and to determine the effect of these modifications on the antibacterial activity of cefaclor against gram-positive and gram-negative bacteria including some strains of *Pseudomonas aeruginosa* and *proteus vulgaris* which are normally insensitive to some cephalosporin antibiotics [10].

Discussion:

Cefaclor methyl ester hydrochloride derivative (II) was easily prepared by treatment of a suspension of cefaclor (I) in absolute methanol with pure thionyl chloride. The reaction mixture was stirred for 3 hours in an ice bath. The isolated ester was obtained and secured in excellent yield.

The hydrazide derivative (III) was obtained by treatment of (II) with alc. hydrazine hydrate solution. The reaction mixture was kept at room temperature for 24 hours where the hydrazide separated out. The cefaclor hydrazide was isolated, purified and obtained in high yield.

Preparation of *N*-Tos-cefaclor (IV) was performed by the action of *p*-toluenesulphonyl chloride (tosyl chloride) on cefaclor (I) in an alkaline solution. To avoid the action of strong alkaline such as sodium hydroxide on cefaclor, it was found that the most suitable alkaline medium for this preparation consists of tetrahydrofuran, water and two molar equivalents of triethylamine. After sometime, the organic layer was removed and H_2O was added and then the desired product obtained by acidification. Facial preparation of *p*-nitophenyl ester of *N*-Tos-cefaclor derivative (V) was achieved through the action of 1 equivalent of *N, N*-dicyclohexylcarbodi-imide (DCC)[28] on a solution containing 1 equivalent each of *N*-Tos-cefaclor (IV) and *p*-nitrophenol in ethylacetate at -5°. The precipitated dicyclohexylurea (DCU) is removed by filtration and then the filtrate was evaporated to obtain the desired crude ester (V) which recrystallized several times from ethanol.

Phthalylamino acid derivatives of cefaclor methyl ester (VI-VIII) were synthesized using the acid chloride method [29]. Treatment of the phthalylamino acids with PCl_5 in dry benzene converts them into the corresponding phthalylaminoacyl chlorides. Interaction of the latter intermediates with cefaclor methyl ester hydrochloride (II) proceeds with the formation of the desired products (VI-VIII). The coupling reaction was performed in the presence of two molar equivalents of a base to liberate the free cefaclor and to neutralize HCl librated during the coupling reaction. Some new derivatives of phthalyl- and tosylamino acid cefaclor (IX-XIV) were prepared through coupling reaction between cefaclor (I) and phthalyl- or tosylaminoacyl chloride respectively

using the acid chloride method. The products were isolated, purified and obtained in high yield.

Using the same procedures applied in preparation of phthalylamino acid derivatives of cefaclor methyl ester (IX-XIV), we able to synthesize a new series of phthalyl- or tosyldipeptidyl cefaclor derivatives (XV-XX) via the reaction of cefaclor (I) with the requisite phthalyl- or tosyldipeptidyl chloride.

Amino acid methyl ester derivatives of *N*-Tos-cefaclor (XXI-XXV) were successfully prepared using the phosphorus oxychloride method[29]. The desired products were obtained upon treatment a mixture of *N*-Tos-cefaclor (IV) and an amino acid ester in anhydrous THF containing excess of TEA at –15°C, with $POCl_3$. This procedures leads to high yield products with high degree of purity prior to crystallization.

Finally, two *N*-Tos-cefaclor amino acid derivatives (XXVI, XXVII) were easily synthesized using the active ester method [30] that includes the aminolysis reaction of *N*-Tos-cefaclor *p*-nitrophenyl ester (V) as mediated compound by free amino acid in aqueous sodium carbonate solution at room temperature. Formation of the desired products proceeds with rapid liberation of the *p*-nitrophenol portion of the active ester moeity. The structure of compounds (II-XXVII) was confirmed on the bases of their elemental analysis, chromatographic studies, and spectral data (Table 1).The above reactions were summarized in scheme 1.

Table 1: The spectral data of the synthesized compounds (II-XXVII).

Compd.No.	Spectral Data
II	IR (ν in cm^{-1}): 3400-3200 (broad band of amine hydrochloride interfered with OH and NH bands), 3027 (CH, aromatic), 2952, 2926 (CH, aliphatic), 1762 (C=O, β-lactam), 1686 (amide I) 1560, 1515 (C=C, aromatic), 1242, (ester, -C-O) ans 698 (C-Cl). ^{1}HNMR at δ(ppm): 3.14 (s, 2H, $CH_{2\,(2)}$), 3.72 (s, 3H, OCH_3), 4.81 (d, 1H, $CH_{(6)}$), 4.93 (s, 1H, $CH_{(11)}$), 5.68 (d, 1H, $CH_{(7)}$), 6.77-7.30 (m, 5H, Ar-H), 8.43 (br., 2H, NH), 10.51 (br., 1H, NH_3^+ canceled with D_2O).
III	IR:3350-3243 (NH, NH_2), 3036 (CH, aromatic), 2991 (CH, aliphatic), 1753 (C=O, β-Lactam), 1682 ((C=O, amide I), 1610 (C=C, ethylenic), 702(C-Cl). Ms at m\e, (% abundance): molecular ion peak at m/e = 381 is compatible with its proposed structure with a base peak at m/e = 56 (100%) in support of the proposed structure. Other significant peaks were observed in the spectrum at m/e: 281 (3.2%), 224 (32.7%), 143 (29.5%), 99 (29%), 91 (35.7%), 70 (22.1%).
IV	IR:3278 (broad bands, OH, NH), 3060 (CH, aromatic) 2973 (CH, aliphatic), 1782 (C=O, β-lactam), 1723 (C=O, acid), 1654, 1542 (amide I and II), 1348, 1162 (S=O), 814 (*p*-disubstituted benzene), 696 (C-Cl, sharp). ^{1}H-NMR: 2.32 (s, 3H, CH_3), 3.2 (s, 2H, $CH_{2(2)}$), 5.01 (s, 1H, $CH_{(6)}$), 5.17 (d, 1H, $CH_{(11)}$), 5.46 (d, 1H, $CH_{(7)}$), 7.1-7.8 (m, 9H, Ar-H), 8.50 (2H,-CONH,-SO_2NH), 9.87 (s,1H,COOH canceled with D_2O).
V	IR : 3248 (NH), 3058 (CH, aromatic), 2934 (CH, aliphatic), 1776 (C=O, β-lactam), 1678 (C=O, amide I), 1514 (C=C, aromatic), 1334, 1158 (SO_2), 698 (C-Cl). ^{1}H-NMR : 2.34 (s, 3H, CH_3), 3.11 (s, 2H, $CH_{2(2)}$), 4.98 (s, 1H, $CH_{(6)}$), 5.21 (d, 1H, $CH_{(11)}$), 5.64 (d, 1H, $CH_{(7)}$), 6.88-8.08 (m, 9H, Ar-H), 8.62 (s,1H, SO_2NH).
VI	IR: 3299 (NH), 3010, (CH, aromatic), 2952 (CH, aliphatic), 1773, 1719, 1651 (C=O of β-lactam, C=O of phthalyl, ester, and amide I respectively), 698, (C-Cl).
VII	IR: 3381, 3286 (broad bands, NH), 1776 (C=O, β-lactam) 1711 (C=O, ester), 1643 (amide I).
VIII	MS: 657 (M-1, 0.07%), 626 (0.07%), 599 (0.08%), 466 (0.17 %), 383 (0.21%), 295 (2.75%), 205 (17.61%), 148 (100%), 91 (37.92%).
IX	^{1}H-NMR: 3.16 (s, 2H, $CH_{2(2)}$), 3.89 (s, 2H, CH_2CO), 5.07 (d, 1H, $CH_{(6)}$), 5.20 (s, 1H, $CH_{(11)}$), 5.58 (d, 1H, $CH_{(7)}$), 6.94-7.83 (m, 9H, Ar-H)], 8.62 (s,1H, CONH).
X	IR: 3292 (broad bands NH, OH), 3051 (CH-aromatic), 2951 (CH-aliphatic), 1772 (C=O, β-lactam), 1716 (C=O, anhydride), 1696 (C=O, carboxylic & amide), 1636 (COO^-, carboxylate), 1540 (C=C, aromatic), 1386 (COO^-, (sym)), 720 (C-Cl).
XI	IR: 3272 (broad bands NH, OH), 3046 (CH-aromatic), 2984 (CH-aliphatic), 1772 (C=O, β-lactam), 1750, 1698 (C=O, carboxylic & amide), 1636 (COO, carboxylate), 1540 (C=C, aromatic), 1386 (COO^-, (sym)), 704 (C-Cl).
XII	IR: 3304, 3275 (broad bands of NH, OH), 3054 (CH-aromatic), 2975 (CH-aliphatic), 1784 (C=O, β-lactam), 1716 (C=O, acid), 1640, 1540(amide I and II), 1368 (COO^-, (sym)), 1328,1160 (S=O), 822 (*p*-disubstituted benzene), 672 (C-Cl sharp).

	^{1}H-NMR: 2.31 (s, 3H, CH_3), 3.12 (s, 2H, $CH_{2(2)}$), 4.95 (d, 1H, $CH_{(6)}$), 5.26 (s, 1H, $CH_{(11)}$), 5.60 (d, 1H, $CH_{(7)}$), 7.04-7.93 (m, 9H, Ar-H), 8.62 (s,1H, CONH), 9.34 (s,1H, SO_2NH canceled with D_2O).
XIII	IR: 3292 (broad bands, OH, NH), 3048 (CH, aromatic), 2971 (CH, aliphatic), 1772(C=O, β-lactam), 1636, 1540 (amide I and II), 1320, 1156 (S=O), 816 (*p*-disubstituted benzene), 698 (C-Cl).
XIV	IR: 3292 (broad bands, NH, OH), 3056, 3031 (CH, aromatic), 2982 (CH, aliphatic), 1772 (C=O, β-lactam), 1716 (C=O, acid), 1636, 1540 (amide I and II), 1361 (S=O), 720 (=CH, aromatic), 693 (C-Cl).
XV	IR:3304 (broad bands, OH and NH), 3065 (CH, aromatic), 2928 (CH, aliphatic), 1776, 1728, 1648 (C=O of β-lactam, C=O of COOH and amide respectively), 698 (C-Cl sharp). ^{1}H-NMR: 3.16 (s, 2H, $CH_{2\,(2)}$), 3.86 (s, 2H, CH_2), 4.35 (s, 2H, CH_2), 5.08 (d, 1H, $CH_{(6)}$), 5.62 (s, 1H, $CH_{(11)}$), 5.68 (d, 1H, $CH_{(7)}$), 7.29-7.91 (m, 9H, Ar-H), 9.04, 9.06, 9.39, 9.42 (4H, 3NH, OH).
XVI	MS: 637 (M-2, 12.50 %), 615 (11.11 %), 591 (16.67 %), 572 (18.06 %), 451 (22.22 %), 345 (33.33 %), 288 (38.89 %), 233 (47.42 %), 163 (54.17 %), 97 (50.00 %), 51(8.11 %).
XVII	IR: 3290 (broad bands NH, OH), 3031, 1540 (CH-, and C=C aromatic), 2943(CH-aliphatic), 1792,1775 (phthalyl C=O, β-lactam C=O), 1654 (amide I), 1386 (COOH Sym), 700 (C-Cl Sharp).
XVIII	IR: 3321 (broad bands, OH, NH), 3011 (CH, aromatic), 2958 (CH, aliphatic), 1771 (C=O, β-lactam), 1716 (C=O, acid), 1683, 1558 (amide 1 and II), 1396, 1116 (S=O).
XXII	IR: 3342 (NH), 3062 (CH-aromatic), 2975, 2909 (CH-aliphatic), 1774 (C=O, β-lactam), 1728 (C=O, ester) 1663, 1542 (amide I), 1376, 1160 (S=O), 816 (*p*-disubstituted benzene), 700 (C-Cl, sharp). ^{1}H-NMR:2.37 (s, 3H, CH_3), 3.07(d, 2H, CH_2), 3.37 (t, 2H, CH_2CO), 3.74(s,3H,OCH_3), 4.05(t,2H, CH_2NH), 5.02(d, 1H, $CH_{(6)}$), 5.26(s, 1H, $CH_{(11)}$), 5.50(d, 1H, $CH_{(7)}$), 6.88-7.72 (m, 9H, Ar-H), 8.48, 9.74 (s, NHCO, and SO_2NH canceled by D_2O).
XXIII	IR: 3284 (NH), 3060 (CH, aromatic), 2982, 2932 (CH, aliphatic) 1782 (C=O, β-lactam), 1671, 1548 (amide I and II), 1348, 1161 (S=O), 814 (*p*-disubstituted benzene).
XXIV	IR: 3254 (NH), 3066 (CH, aromatic), 2973, 2954 (CH, aliphatic) 1758 (C=O, β-lactam), 1740 (C=O, ester) 1656, 1543 (amide I and II), 1618, 1508 (C=C, aromatic), 1350, 1164 (S=O), 814 (*p*-disubstituted benzene), 700 (C-Cl).
XXV	IR: 3282 (NH), 3061 (CH, aromatic), 2972 (CH, aliphatic), 1782 (C=O, β-lactam), 1723 (C=O, ester) 1654, 1542 (amide I and II), 1602 (C=C, aromatic), 1312, 1160 (S=O), 831 (*p*-disubstituted benzene), 696 (C-Cl sharp). ^{1}H-NMR: 1.92 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 5.18(s, 1H, $CH_{(11)}$), 5.57 (d, 1H, $CH_{(7)}$), 6.81-7.89 (m, 13H, Ar-H), 8.28 (s,1H,NHCO-), 9.59 (s,1H,-SO_2NH canceled by D_2O).
XXVI	IR: 3282 (broad bands, OH, NH), 3062 (CH, aromatic), 2932 (CH, aliphatic), 1768-1654 (C=O), 1514 (C=C, aromatic), 1334 (SO_2), 698 (C-Cl, sharp). ^{1}H-NMR : 2.2 (s, 3H, CH_3), 2.95 (d, 2H, CH_2), 3.3 (s, 2H, $CH_{2(2)}$), 4.1 (t, 1H, CH), 4.9 (d, 1H, $CH_{(6)}$), 5.5 (d, 1H, $CH_{(11)}$), 5.9 (d, 1H, $CH_{(7)}$), 6.72-7.92 (m, 13H, Ar-H), 11.43 (s, 1H, COOH), canceled by D_2O].
XXVII	IR: 3297 (broad bands, OH, NH), 3061 (CH, aromatic), 2957, 2926 (CH, aliphatic), 1776 (C=O of β-lactam), 1736 (C=O, acid), 1681 (amide I), 1525 (C=C, aromatic), 1329, 1159 (SO_2), 699 (C-Cl, ,sharp).

Antimicrobial screening results

Antibiogram susceptibility and resistance of twenty seven semi-synthetic antibiotics from cefaclor: Twenty seven semi-synthetic antibiotics or derivatives synthesized from cefaclor were applied during this study against the growth of the six bacterial and three unicellular and multicellular fungal strains by paper disc diffusion method [31,33]. All antimicrobial activities of the new synthesized derivatives or semi-synthetic antibiotics were compared with the activities of the starting cefaclor antibiotic. It was clear from the results recorded in tables (2) and (3) that cefaclor (I) exhibited susceptibility or activity against only four tested microorganisms only viz. Bacillus subtilis NCTC 10400 (32mm), Staphylococcus aureus ATCC 25923 (36mm), Escherichia coli ATCC 25922 (23mm) and Candida albicans (35mm) represented Gram-positive bacilli, Gram-positive staphylococci, Gram-negative short rods and unicellular fungi respectively.

Cefaclor derivatives or semi-synthetic antibiotics IV, XII, XIII, XIV, and XVI were highly active against all tested Gram-positive and Gram-negative bacterial growth respectively. The previously mentioned five cefaclor derivatives exhibited broad spectrum antibacterial activity against the tested bacterial strains. Cefaclor derivatives or semi-synthetic antibiotics XII, XIII and

XIV were highly active against all tested bacterial strains i.e. Bacillus subtilis NCTC 10400 (35, 30 and 34 mm), Staphylococcus aureus ATCC 25923 (40, 32 and 40 mm), Enterococcus faecalis NCTC 821(33, 29 and 33 mm), Escherichia coli ATCC 25922 (34, 30 and 35 mm), Proteus vulgaris NCTC 4175 (30, 27 and 32 mm), Pseudomonas aeruginosa ATCC 10415 (29, 25 and 30 mm) respectively. Cefaclor derivatives or semi-synthetic antibiotics IV and XVI were moderately active against all tested bacterial strains i.e. Bacillus subtilis NCTC 10400 (26 and 27 mm), Staphylococcus aureus ATCC 25923 (28 and 30 mm), Enterococcus faecalis NCTC 821(23 and 26 mm), Escherichia coli ATCC 25922 (25 and 28 mm), Proteus vulgaris NCTC 4175 (20 and 28 mm), Pseudomonas aeruginosa ATCC 10415 (20 for IV) respectively. Five semi-synthetic antibiotics synthesized from cefaclor IX, XI, XV XVII and XXI exhibited weak to moderately activity against some tested bacterial strains i.e., Bacillus subtilis NCTC 10400 (20,15,21,29 and 16 mm), Staphylococcus aureus ATCC 25923 (26 ,20, 25, 21 and 19 mm), Enterococcus faecalis NCTC 821(15 and 15 mm for IX and XVII respectively) and Escherichia coli ATCC 25922 (19, 14, 17, 20 and 13 mm) , Proteus vulgaris NCTC 4175 and Pseudomonas aeruginosa ATCC 10415 were resistant of previously mentioned five derivatives. Unfortunately the remaining semi-synthetic antibiotics were found to be antibacterial inactive. Cefaclor (I) , (XII) and XIII were highly active against unicellular fungi i.e. Candida albicans (36 35 and 33 mm respectively), while other five semi-synthetic antibiotics IX, X, XIV, XVII and XXIII were exhibited low activity (25, 27 , 25, 24 and 22 mm) respectively in comparable with standard cefaclor (Table 3). All semi-synthetic cefaclor antibiotics were found to be completely inactive with all tested filamentous fungi viz. Aspergillus niger and Aspergillus flavus. Results recorded in table (4) showed concentration (*MIC*, mg/ml) of the most active compounds. This study showed that :

1) Esterification and hydrazinolysis, reactions of cefaclor completely destroyed the antimicrobial activity of cefaclor.

2) Introduction of tosylaminoacyl moieties in combination with cefaclor (XII, XIII and XIV) and *N*-Tos-cefaclor (IV) improved and verified the antibacterial activity of cefaclor especially against the strains of gram-negative bacteria.

3) The remaining derivatives of cefaclor resulting from its reactions with phthalylaminoacyl- , dipeptidyl- , amino acid methyl esters or free amino acids decreased or canceled, in most cases the biological activity of cefaclor.

Table 2. In-vitro antimicrobial activities of some synthestic cefaclor derivatives (I-XXVII).

Compd No.	Mean diameter of inhibition zone(mm)					
	B.subtilis NCTC10400	S.aureus TCC25923	E.faecalis NCTC 821	E.coli ATCC25922	P.vulgaris NCTC4175	P.aeruginosa ATCC10425
I	32	36	0	23	0	0
II	0	0	0	0	0	0
III	0	0	0	0	0	0
IV	26	28	23	25	20	20
V	0	0	0	0	0	0
VI	0	0	0	0	0	0
VII	0	0	0	0	0	0
IX	20	26	15	19	0	0
X	0	0	0	0	0	0
XI	15	20	0	14	0	0
XII	35	40	33	34	30	29
XIII	30	32	29	30	27	25
XIV	34	40	33	35	32	30
XV	21	25	0	17	15	0
XVI	27	30	26	28	28	0
XVII	29	21	15	20	0	0
XXI	16	19	0	13	0	0
XXII	0	0	0	0	0	0
XXIII	0	0	0	0	0	0
XXIV	0	0	0	0	0	0
XXV	0	0	0	0	0	0
XXVI	0	0	0	0	0	0
XXVII	0	0	0	0	0	0

Table 3. In-vitro antifungal activities of some synthetic cefaclor derivatives (I-XXVII).

Compd. No.	Mean diameter of inhibition zone(mm)		
	Candida albicans	Aspergillus niger	Aspergillus flavus
I	35	0	0
II	0	0	0
IX	25	0	0
X	27	0	0
XI	0	0	0
XII	35	0	0
XIII	33	0	0
XIV	25	0	0
XVI	0	0	0
XVII	24	0	0
XXIII	22	0	0

Table 4. Minimum inhibitory concentration (*MIC*) of most active cefaclor derivatives.

Comp.No.	Minimum inhibitory concentration (*MIC*) (mg/ml)					
	B.subtilis NCTC10400	S.aureus TCC25923	E.faecalis NCTC 821	E.coli ATCC25922	P.vulgaris NCTC4175	P.aeruginosa ATCC10425
XII	0.005	0.003	0.01	0.015	0.02	0.02
XIII	0.007	0.005	0.019	0.022	0.027	0.03
XIV	0.006	0.004	0.017	0.02	0.025	0.024
XVI	0.01	0.007	0.017	0.021	0.027	0.027

Experimental

Melting points were uncorrected and measured on electric melting point apparatus SMPI. Purity of compounds was checked by thin layer chromatography (TLC) on plastic sheets coated with silica gel 60 (Merck) and developed with n-butanol: acetic acid: water (4: 1: 1) using Iodine-potassium Iodide (20%) and benzidine solutions as spraying agents, and also detected under UV lamp. The infrared, IR, spectra (ν max, $cm^{-1)}$ were taken in KBr discs using FTIR-2000 instrument. The Nuclear Magnetic Resonance, ^{1}HNMR spectra were measured in DMSO-d_6 or $CDCl_3$ using FX90Q Fourier Transform NMR spectrometer. The mass spectra were performed using Shimad Zu-GC-MS-QP 1000 EX using the direct inlet system. Elemental analyses were carried out at Microanalytical Unit, Faculty of Science, and Cairo University. The biological activities were measured in Department of Potany, Faculty of Science, Al-Azhar University, and Cairo, Egypt.

1. Cefaclor (I) was supplied from Cairo Pharmaceutical and Chemical Industrial Company, Cairo, Egypt.

2. Synthesis of cefaclor methyl ester hydrochloride (II).

Cefaclor (I, 0.01 mol) was added to 100 ml of abs. methanol and the mixture was cooled in an ice bath at (0- 5°C) then (0.011 mol.) of pure thionyl chloride was added dropwise. The temperature of the reaction was kept below (5°C) during the addition of thionyl chloride. The reaction was stirred for additional 3 hrs at 5-10°C, kept overnight at room temperature and then the solvent was removed in vacuo. Methanol was added and re-evaporated several times.

3. Synthesis of cefaclor hydrazide (III).

To a solution of cefaclor methyl ester hydrochloride (II**,** 0.01 mole) in 100 ml abs. methanol was added (0.015 mol) of hydrazine hydrate and the solution was left for 24 hrs at room temperature. The hydrazide compound was filtered and recrystallized from ethanol.

4. Synthesis of *N*-Tos-cefaclor (IV).

A solution of *p*-tosylchloride (0.01 mol) in 10 ml of THF was added over a period of 30 min. to a stirred, cooled suspension of cefaclor (I, 0.01 mol) in a mixture of 8 ml. of water, 4 ml. of THF, and triethylamine (0.011 mol). The mixture was stirred for an additional 45 min. at room temperature, concen-trated under reduced pressure and 10 ml of water was then added. The solution obtained was washed with ether and the product precipitates on acidification of the aqueous layer with dil. HCl. The crude product was recrystallized from aqueous ethanol.

5. Synthesis of *N*- Tos-cefaclor *p*-nitophenyl ester (V).

A solution of *N*-cefaclor **(**IV, 0.01mol) and *p*-nitro-phenol (0.01 mol) in ethyl acetate (25 ml) was stirred and cooled in an ice-bath to -5°C and dicyclohexyl-carbodiimide (DCC; 0.01mol) was then added, in a few portions. The reaction mixture was stirred at 0°C for 3 hrs and the left to stand at room temperature for overnight. The precipitated dicyclohexylurea (DCU) was removed by filtration and the filtrate was evaporated under reduced pressure. The residual material was dissolved in ethylacetate and few drops of gl. AcOH was added and the solution left for 24 hrs at room temperature. The solution was filtered again to remove the precipitated DCU crystals. The filtrate was re-evaporated under vacuum and the residual material was purified by recrystallization many times from 95% ethanol.

6. General procedure for Synthesis of Phthalyl amino acid derivatives of cefaclor methyl ester (VI-VIII).

A solution of phthalylaminoacyl chloride of (0.001 mol) in 10 ml of THF was added over a period for 30 min. to a stirred, cooled suspension of cefaclor methyl ester hydrochloride (II, 0.0011 mol), previously stirred and treated with triethylamine (0.0022 mol) in a mixture of 20 ml THF and 5 ml DMF. The mixture was stirred for an additional 3 hrs at room temperature and then poured into crushed iced-water to extract triethylamine HCl. The crude product that precipitated, removed by filtration, washed with cold water and then recrystallized from ethanol.

7. General procedure for Synthesis of Phthalyl- or tosylamino acid derivatives of cafaclor (IX-XIV).

To a cold solution of cefaclor (I, 0.001 mol) in 20 ml of THF and 5 ml of DMF containing triethylamine (0.001 mol) was slowly added a solution of phthalyl- or tosylaminoacyl chloride (0.001 mol) in 10 ml of THF. The reaction mixture was stirred for 1 hr at 5°C and 3 hrs at room temperature, and then poured into crushed ice water. The precipitated crude products were isolated, dried and purified by recrystallization from the proper solvent.

8. General procedure for Synthesis of Phthalyl- or tosyldipeptide cefaclor derivatives (XV-XX).

A solution of phthalyl- or tosyldipeptidyl chloride (0.001 mol) in 20 ml of THF was added dropwisely over a period of 30 min. to a stirred, cooled suspen-sion of cefaclor (I, 0.001) in 20 ml of THF containing triethylamine (0.0011 mol). The remaining procedure was the same for that used for preparation of (IX-XIV). The isolated dipeptide derivatives were recrystallized from the proper solvent.

9. General procedure for Synthesis of *N*-Tos-cefaclor amino acid methyl ester derivatives (XXI-XXV).

A mixture of *N*-Tos-cefaclor (IV, 0.002 mol), an amino acid methyl ester hydrochloride (0.0022 mol) and triethylamine (0.0022 mol) suspended in 20 ml. of anhydrous THF was cooled to -15°C with shaking for 15 minutes. The mixture was then treated with purified phosphorus oxychloride (0.003 mol) and directly thereafter (0.004 mol) of triethylamine was added. After the reaction mixture has stood for 1 hr. at -15°, 20 ml. of water was added and the mixture evaporated in vacuo in order to the remove tetrahydrofuran. The residual material was treated with 20 ml. of water, extracted two times with 20 ml. portion of ethyl acetate, and the combined ethyl acetate extracts were washed three times each with 5 ml. portions of water, with several portions of 5% sodium bicarbonate solution, and finally with water. After being dried with anhydrous sodium sulfate, the ethyl acetate fraction was concentrated to dryness at room temperature. The residual compounds were recrystallized from the proper solvent.

10. General procedure for Synthesis of *N*-Tos-cefaclor amino acids (XXVI and XXVII).

A solution of an amino acid (0.001 mol) in Na_2CO_3 solution (0.002 mol) was treated portionwisely with a solution of *N*-Tos-cefaclor *p*-nitrophenyl ester (V, 0.001 mol) in THF. The mixture was stirred at room temperature for 3 hrs, and then the mixture was poured into crushed ice water, and acidified with dil. hydrochloric acid. The resulting precipitate was filtered, washed many times with 10 ml portions of water, dried and then purified by recrystallization from the proper solvent.

References:

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