Chemistry of Cephalosporin Antibiotics. XIII. Desacetoxycephalosporins. The Synthesis of Cephalexin[1] and Some Analogs

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A series of phenylglycine derivatives of 7-aminodesacetoxycephalosporanic acid has been prepared. The D-phenylglycine derivative, cephalexin, is shown to have a broad spectrum of biological activity and to be efficiently absorbed orally. Substituted phenyl and thienyl analogs are of about equal potency. A series of desacetoxycephalosporins not derived from amino acids is also discussed.

The cephalosporin antibiotics, members of the β-lactam class of antibiotics, are of interest because of their marked bactericidal activity and excellent stability to the action of acid and penicillinase.[2] Recently, Spencer, *et al.*,[3] of this laboratory reported the synthesis of several phenylglycyl-7-aminocephalosporanic acid derivatives. In addition to the abovementioned features, the parent compound, cephaloglycin, was characterized by a broad spectrum of activity and high potency against gram-negative organisms. Cephaloglycin also was well absorbed following oral administration to mice.[4] At least two of these characteristics, the activity against gram-negative organisms and the oral absorptivity, are associated with the phenylglycine side chain. It was of interest, therefore, to examine the effect the phenylglycyl group might have on the biological activity of cephalosporins derived from nuclei other than the cephaloglycin nucleus, 7-aminocephalosporanic acid. One nucleus that we have chosen to study is 7-aminodesacetoxycephalosporanic acid (7-ADCA).[5]

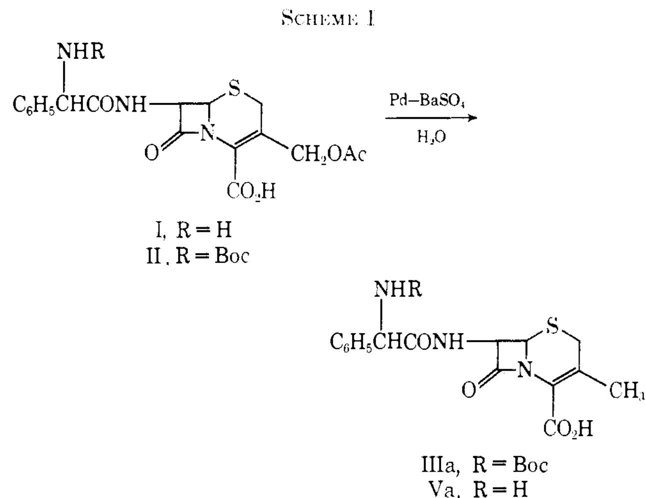
There were several logical synthetic routes to the desired derivatives, and we have examined two syntheses that are of general applicability. In the first method we cleaved the acetoxyl group from cephaloglycin (I) hydrogenolytically or, more satisfactorily, from N-*t*-butoxycarbonylcephaloglycin (II) to produce the desacetoxy analogs IIIa and Va as shown in Scheme I. The *t*-butoxycarbonyl (Boc) group was easily removed from IIIa with trifluoroacetic acid, and the reaction product was converted to desacetoxycephaloglycin (cephalexin, Va) by treatment with Amberlite LA-1 resin.

The second method was similar to that previously used to obtain cephaloglycin.[3] We prepared 7-ADCA, acylated it with Boc-protected D-phenylglycine employing a mixed anhydride synthesis, and then deblocked with trifluoroacetic acid as shown in Scheme II.

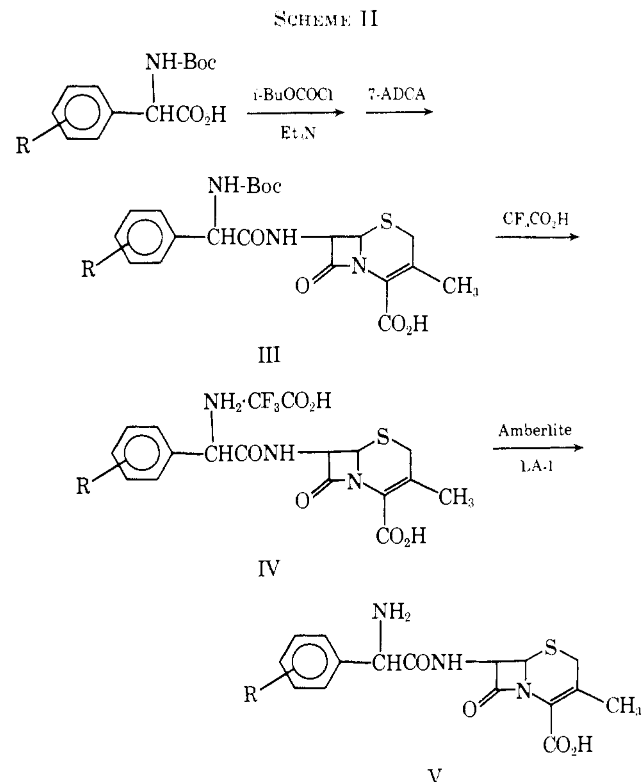
Several phenyl-substituted cephalexin analogs were prepared to study the effect of ring substituents on biological activity, as outlined in Scheme II.

Penicillins derived from D-α-amino acids show considerably more biological activity than their L epimers.[6] A similar effect has been found for cephalosporins.[3] Because of this relationship of stereo-

SCHEME I



SCHEME II



(1) Cephalexin is the generic name given to 7-(D-2-amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid.

(2) R. R. Chauvette, E. H. Flynn, B. G. Jackson, E. R. Lavagnino, R. B. Morin, R. A. Mueller, R. P. Pioch, R. W. Roeske, C. W. Ryan, J. L. Spencer, and E. Van Heyningen, *Antimicrobial. Agents Chemotherapy*, 687 (1962).

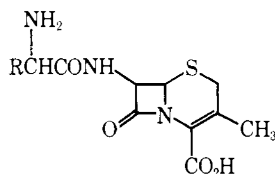
(3) J. L. Spencer, E. H. Flynn, R. W. Roeske, F. Y. Siu, and R. R. Chauvette, *J. Med. Chem.*, **9**, 746 (1966).

(4) W. E. Wick and W. S. Boniece, *Appl. Microbiol.*, **13**, 248 (1965).

(5) R. J. Stedman, K. Swered, and J. R. E. Hoover, *J. Med. Chem.*, **7**, 117 (1964).

(6) F. P. Doyle, G. R. Fosker, J. H. C. Nayler, and H. Smith, *J. Chem. Soc.*, 1440 (1962).

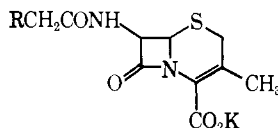
TABLE I
BIOLOGICAL ACTIVITIES OF AMINO ACID DERIVATIVES



No.	R	Cephaloglycin[a] assay	Resistant[b] *S. aureus*	Gram negative[c] N-9	N-26	X-26	X-68	*S. pyogenes*,[d] oral, mouse
Va	C_6H_5	490	3.5, 6.9	19.0	10.0	11.5	9.3	1.7
Vb	$2\text{-}C_4H_3S^e$	235	8.1, 11.6	18.9	15.6	15.3	12.2	5.2
Vc	$3\text{-}ClC_6H_4$	450	1.9, 3.6	>50.0	25.2	>50.0	17.0	3.9
Vd	$4\text{-}ClC_6H_4$	600	4.4, 6.1	>50.0	>50.0	>50.0	19.8	6.7
Ve	$3\text{-}BrC_6H_4$	470	1.3, 5.8	>50.0	>50.0	>50.0	14.5	7.7
Vf	$3\text{-}FC_6H_4$	210	7.2, 10.6	>50.0	18.8	22.2	15.4	5.1
Vg	$3\text{-}HOC_6H_4$	675	1.7, 3.4	20.9	11.4	14.6	8.8	1.7
Vh	$3\text{-}CH_3OC_6H_4$	363	4.1, 5.9	21.9	20.6	26.0	12.0	<1.2
Cephaloglycin		1000	1.0, 1.5	2.4	4.2	5.8	4.9	4.5

[a] Values are expressed as μg/mg of cephaloglycin activity against *S. aureus* 209P. [b] MIC in μg/ml by gradient plate assay. Each figure is the average value with four penicillin-resistant *S. aureus* strains. The first value is without, the second with, 25% human serum in the medium. [c] MIC value obtained with a gradient plate assay. [d] The values given are the minimum dose in mg/kg which afforded protection to 50% of the mice against *S. pyogenes* C203. The dose was given orally 1 and 5 hr postinfection. [e] C_4H_3S is thienyl.

TABLE II
BIOLOGICAL ACTIVITIES OF NONAMINO DESACETOXYCEPHALOSPORINS



No.	R	Pen. G[a] assay	Resistant[b] *S. aureus*	Gram negative[c] N-9	N-26	X-26	X-68	*S. pyogenes*[d] oral, mouse
VIa	$4\text{-}ClC_6H_4$	46	0.8, 1.0	>200	>200	>200	>200	>41.5
VIb	$3\text{-}BrC_6H_4$	113	0.5, 0.8	>200	>200	42.7	>200	26.0
VIc	$3\text{-}NO_2C_6H_4$	68	1.0, 4.6	>50	>50	>50	>50	14.3
VId	$4\text{-}NO_2C_6H_4$	43	1.4, 3.8	>50	>50	>50	>50	<1.2
VIe	$3\text{-}CF_3C_6H_4$	146	0.7, 4.8	>50	>50	36.0	>50	16.9
VIf	$4\text{-}NCC_6H_4$	50	8.1, 11.4	>50	>50	>50	>50	26.0
VIg	$4\text{-}CH_3SC_6H_4$	55	12.1, 15.1	>50	>50	>50	>50	>41.5
VIh	$3\text{-}ClC_6H_4S$	315	0.3, 0.9	>50	>50	24.2	>50	18.2
VIi	$4\text{-}NCC_6H_4S$	85	5.9, 6.1	>50	>50	30.0	>50	26.0
VIj	$2\text{-}C_8H_5O^e$	134	0.3, 0.7	>200	>200	44.2	>200	33.0
Cephalothin		310	0.5, 1.0	11.5	15.2	2.4	9.2	41.5

[a] Disk-plate assay for penicillin G. [b-d] See corresponding footnotes in Table I. [e] C_8H_5O is benzofuranyl.

chemistry and biological activity, it was important to know the relative amounts of D and L epimers present in the preparations of the present study.

The Moore–Stein amino acid analyzer, which was previously used to establish the stereochemical purity of zwitterions of the cephaloglycin type,[3] is also a useful analytical tool for the desacetoxycephalosporins of the cephalexin type. DL mixtures of the N-Boc-phenylglycine derivatives may be used as starting materials (Scheme II). After coupling with 7-ADCA and treatment with trifluoroacetic acid, the salts (IV) were shown by Moore–Stein analysis to contain equal amounts of the D and L epimers. This was not surprising because these salts did not crystallize but were merely precipitated from solution with ether. The zwitterions (V) obtained after treatment with Amberlite LA-1 resin were again analyzed by the Moore–Stein method. In most cases crystalline products obtained in the first crop were shown to contain more than 50% of the D epimer. This material was used for biological testing without further purification (Table I). In several instances the first crop was shown to contain more than 50% of the less active L epimer. In these instances, work-up of the filtrates produced material enriched in the active D epimer and, therefore, suitable for biological testing. DL mixtures of Boc-phenylglycines were used as starting materials with two exceptions. The D isomer of phenylglycine is commercially available; the D isomer of Boc-3-hydroxyphenylglycine was obtained conveniently by crystallization of its quinine salt (after removal of much of the L isomer as the cinchonine salt). The trifluoroacetate salts (IVa and IVg) and zwitterions (Va and Vg) obtained from these side chains were shown by Moore–Stein analysis to be pure D epimers.

Analysis of the nmr spectrum of these compounds confirms the Moore–Stein data. A 5-cps difference consistently separated the signal attributed to the 3-

TABLE III

DESACETOXYCEPHALOSPORINS

No.	D epimer, %	Ultraviolet λ_{max}, mμ (ε)	Titration pK$_a'$	Titration AMW (FMW)	Formula	Analyses
Va	100	260 (7750)	5.2, 7.3	365 (365)	$C_{16}H_{17}N_3O_4S$	N, H; C[a]
Vb	86[b]	234 (12,700) 260 (7300)	5.3, 6.8	410 (371)	$C_{14}H_{15}N_3O_4S_2 \cdot H_2O$	N, H; C[c]
Vc	57	261 (6660)	5.3, 6.8	468 (399)	$C_{16}H_{16}ClN_3O_4S \cdot H_2O$	C; H,[d] N[e]
Vd	60	260 (6900)	5.4, 7.2	464 (399)	$C_{16}H_{16}ClN_3O_4S \cdot H_2O$	C, H, N
Ve	75	261 (7130)	5.3, 7.0	550 (444)	$C_{16}H_{16}BrN_3O_4S \cdot H_2O$	C, H, N
Vf	58	263 (8000)	5.3, 7.0	456 (365)	$C_{16}H_{16}FN_3O_4S$	C, H, N
Vg	98	264 (7100)	5.3, 7.3, 12.7	438 (399)	$C_{16}H_{17}N_3O_5S \cdot 2H_2O$	C, H, N
Vh	67[b]	263 (8050)	5.4, 7.4	440 (413)	$C_{17}H_{19}N_3O_3S \cdot 2H_2O$	H; C,[f] N[g]
VIa		262 (7460)	5.8	462 (423)	$C_{16}H_{14}ClKN_2O_4S \cdot H_2O$	C, H, N
VIb		260 (7430)	5.5	520 (467)	$C_{16}H_{14}BrKN_2O_4S \cdot H_2O$	C, H, N
VIc		264 (13,220)	5.8	490 (448)	$C_{16}H_{14}KN_3O_6S \cdot CH_3OH$	H, N; C[h]
VId		257 (17,480)	5.8	423 (433)	$C_{16}H_{14}KN_3O_6S \cdot H_2O$	C, N; H[i]
VIe		260 (8290)	5.6	453 (439)	$C_{17}H_{14}F_3KN_2O_4S$	C, H, N
VIf		233 (22,600) 262 (7950)	5.6	384 (413)	$C_{17}H_{14}KN_3O_4S \cdot H_2O$	C, H, N
VIg		256 (18,400)	5.6	635 (417)	$C_{17}H_{17}KN_2O_4S_2$	C, H, N
VIh		255 (13,500)	5.7	455 (497)	$C_{16}H_{14}ClKN_2O_4S_2 \cdot (CH_3)_2CHOH$	H, N; C[j]
VIi		219 (15,550) 272 (18,900)	5.6	450 (463)	$C_{17}H_{14}KN_3O_4S_2 \cdot 2H_2O$	C, H, N
VIj		247 (20,050) 274 (8030) 282 (6100)	5.8	462 (428)	$C_{18}H_{15}KN_2O_5S \cdot H_2O$	C, H, N

[a] C: calcd, 55.31; found, 54.73. [b] This material is from the second crop; the first crop of product contained less than 50% of the D isomer. [c] C: calcd, 45.28; found, 44.29. [d] H: calcd, 4.56; found, 5.24. [e] N: calcd, 10.51; found, 9.44. [f] C: calcd, 49.39; found, 49.93. [g] N: calcd, 10.17; found, 11.24. [h] C: calcd, 45.62; found, 45.19. [i] H: calcd, 3.68; found, 4.15. [j] C: calcd, 45.91; found, 45.43.

methyl group of the D epimer (appearing at 125 cps) from that of the L epimer (at 130 cps). Differences were also noted in other parts of the spectrum, notably the signals due to the protons at positions 6 and 7 of the β-lactam ring.

The biological activities of preparations which contained 50% or more of the D epimer are summarized in Table I. The biological tests performed on these compounds have revealed interesting features resulting from the combination of a phenylglycyl residue with the 7-ADCA nucleus. Several 7-ADCA derivatives have already been reported.[5] Their *in vitro* microbiological activities against gram-negative and gram-positive organisms appear to be less than 20% of the activities of the corresponding acetoxyl analogs. However, the phenylglycyl group imparts to cephalexin 50% or more of the activity of its acetoxyl analog, cephaloglycin, against gram-positive organisms and 20–50% of its activity against gram-negative organisms. Apparently cephalexin retains a broad antibacterial spectrum similar to that of cephaloglycin.

An especially noteworthy feature results from the combination of the phenylglycyl group with the desacetoxycephalosporin nucleus: cephalexin is very efficiently absorbed from the gastrointestinal tract. This may be inferred from the very small amount (ED_{50} = 1.7 mg/kg, see Table I) needed to protect mice from death due to a *Streptococcus pyogenes* infection. Wick has reported[7] an average peak blood level of 19 μg/ml after oral administration to mice. Griffith and Black have shown[8] that humans absorb approximately 90% of orally administered doses of cephalexin leading to average peak blood levels of 17 μg/ml after a 500-mg dose.

The efficient absorption must be attributable to both the phenylglycine side chain and the lack of any substituent other than hydrogen on the 3-methyl group. In the "3-acetoxymethylcephalosporin" series the only 7-acyl function permitting a significant amount of oral absorption is the phenylglycyl group. Examination of cephalosporins in which the acetoxy has been displaced by functions other than hydrogen reveals no substituent that contributes high absorptivity to the cephalosporin,[9] although some derivatives are apparently more efficiently absorbed than their parent acetoxyl analogs.[9b]

One might question if the presence of a 3-methyl group in a cephalosporin is sufficient to provide high absorptivity regardless of which 7-acyl function it contains. A series of 7-acyldesacetoxycephalosporins was prepared, and a qualitative estimate of their absorption after oral administration was made by comparing their *in vitro* antibiotic potency with their ED_{50} values in mice infected with *Streptococcus pyogenes*. Although the series is not very extensive, only one compound, the 7-*p*-nitrophenylacetyl derivative, showed any significant degree of absorption (Table II). The removal of the acetoxyl group from a cephalosporin does not of itself, evidently, afford high absorptivity. Our conclusion must be, therefore, that the combination of a phenylglycyl side chain and a 3-methyl group in a cephalosporin has resulted

(7) W. E. Wick, *Appl. Microbiol.*, **15**, 765 (1967).

(8) R. S. Griffith and H. R. Black, *Antimicrobial Agents Chemotherapy*, in press.

(9) (a) E. Van Heyningen, *J. Med. Chem.*, **8**, 22 (1965); (b) E. Van Heyningen and C. N. Brown, *ibid.*, **8**, 174 (1965); (c) J. L. Spencer, F. Y. Siu, E. H. Flynn, B. G. Jackson, M. V. Sigal, H. M. Higgins, R. R. Chauvette, S. L. Andrews, and D. E. Block, *Antimicrobial Agents Chemotherapy*, 573 (1966).

in a propitious combination of features that leads to efficient oral absorption.

Substitution of the phenyl ring of cephalexin with certain substituents does not appear to modify significantly the antibacterial properties of cephalexin. The activities of the 3-hydroxy and 3-methoxy (Vg and Vh, Table I) derivatives are comparable to the activity of cephalexin. The other compounds either are of lower potency or do not have the broad spectrum of activity of the parent compound. Oral absorption, as judged from ED_{50} values, appears to be similar to that of cephalexin.

Experimental Section[10]

N-*t*-Butoxycarboxamidophenylacetic Acids.—D-Phenylglycine is commercially available. 2-Thienylglycine, 3-chlorophenylglycine, 4-chlorophenylglycine, 3-bromophenylglycine, 3-fluorophenylglycine, 3-hydroxyphenylglycine, and 3-methoxyphenylglycine were prepared by the method of Doyle, *et al.*[6] The amino acids were converted to their N-*t*-butoxycarbonyl derivatives as described by Schwyzer, *et al.*[11]

D-2-*t*-Butoxycarboxamido-2-(3-hydroxyphenyl)acetic Acid.—A solution of 10.5 g (0.039 mole) of DL-2-*t*-butoxycarboxamido-2-(3-hydroxyphenyl)acetic acid and 11.6 g (0.039 mole) of cinchonine was prepared in 100 ml of warm EtOAc. After cooling overnight, the crystalline solid was collected and washed with EtOAc. The filtrate, which contained the D-(−) isomer, was treated with dilute HCl to obtain the free acid. After removal of solvent, 4.6 g of noncrystalline oil was obtained, $[\alpha]_D$ −100.8°. This partially resolved acid (0.017 mole) and 5.4 g (0.017 mole) of quinine were dissolved in 150 ml of EtOAc. After overnight cooling, the crystalline quinine salt was collected and recrystallized from 125 ml of EtOAc, yielding 6.8 g of D-2-*t*-butoxycarboxamido-2-(3-hydroxyphenyl)acetic acid quinine salt. The salt was treated with dilute HCl and extracted with EtOAc, yielding after evaporation 2.5 g of the noncrystalline D-(−) isomer, $[\alpha]_D$ −129° (c 1.04, EtOH). This amorphous solid was used to prepare the 7-ADCA derivative.

7-(2-Butoxycarboxamidophenylacetamido)-3-methyl-3-cephem-4-carboxylic Acids.—Reaction of D-2-*t*-butoxycarboxamido-2-phenylacetic acid and D-2-*t*-butoxycarboxamido-2-(3-hydroxyphenyl)acetic acid was accomplished using the mixed anhydride procedure described by Spencer, *et al.*[3] Reactions using unresolved acids were also conducted in this way. Uv and titration data confirmed the structure of these compounds. They were used in subsequent steps without further purification.

7-(2-Amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic Acids.—The N-protected derivatives described above were treated with cold CF_3CO_2H to cleave the Boc group. The CF_3CO_2H salts were then precipitated by the addition of Et_2O. Physical data (nmr, uv, and titration) were consistent for the structures of these compounds, and they were subjected to Moore–Stein amino acid analysis. The CF_3CO_2H salts prepared from D-*t*-butoxycarboxamidoacetic acids [2-phenyl and 2-(3-hydroxyphenyl)] were shown by the Moore–Stein technique and by nmr (single peak at 125 cps) to contain 2% or less of the less active L epimer.

$CF_3CO_2^-$ salts prepared from DL-*t*-butoxycarboxamidophenylacetic acids were shown by Moore–Stein analysis and by an nmr spectrum (equal peaks at 125 and 130 cps) to contain essentially equal amounts of D and L epimers.

The CF_3CO_2H salts were converted to zwitterions by treatment with Amberlite LA-1 resin as described in the following example.

7-(D-2-Amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic Acid (Va).—To a solution of 0.5 g of IVa in 2 ml of H_2O was added 4 ml of 25% Amberlite LA-1 (acetate form) in MIBK. After stirring and cooling for 1 hr, the white solid was collected, washed (H_2O, MIBK), and dried *in vacuo;* yield 0.24 g.

Hydrogenolysis of 7-(D-2-Amino-2-phenylacetamido)cephalosporanic Acid (I).—A solution of 20.0 g (0.049 mole) of 7-(D-2-amino-2-phenylacetamido)cephalosporanic acid in 200 ml of H_2O was prepared by adjusting the solution to a pH of 8.4 with 20% NaOH solution. The solution was hydrogenated at 4 kg/cm² for 1 hr at room temperature using 70 g of 5% Pd–$BaSO_4$. After filtration of the catalyst, the solution was acidified to pH 1 (CF_3CO_2H) and extracted three times with MIBK. The combined MIBK solution was evaporated to dryness. The residue was triturated with dry Et_2O, and the solid was collected. This crude $CF_3CO_2^-$ salt was converted to the zwitterion by the method previously described for the preparation of Va, yielding 4.9 g of product. The white crystals were shown by uv, titration, and Moore–Stein analysis to be Va containing about 5% of unreduced acetoxy compound.

Hydrogenolysis of 7-(D-2-*t*-Butoxycarboxamido-2-phenylacetamido)cephalosporanic Acid (II).—A solution of the Na salt of II (25 g, 0.05 mole) and 5 g of $NaHCO_3$ in 1 l. of H_2O was hydrogenated at 4 kg/cm² using 50 g of 5% Pd–$BaSO_4$. After 1 hr an additional 25 g of catalyst was added, and the hydrogenation continued for 30 min. After filtration the filtrate was layered with EtOAc and cooled while concentrated HCl was added to obtain a mixture with a pH of 2.5. The EtOAc layer was washed (H_2O), dried ($MgSO_4$), and evaporated to dryness. The crude IIIa (18.7 g) was dissolved by the addition of 100 g of cold CF_3CO_2H. After 15 min the solution was evaporated to dryness, the residue was slurried with Et_2O, and the solid was collected and washed (Et_2O). The crude CF_3CO_2H salt (16.9 g) was converted to Va as previously described, yielding 8.22 g of Va. This material was shown by Moore–Stein analysis to be of 99% purity.

Preparation of Nonamino Desacetoxycephalosporins (VI). General Procedure.—To an ice-cold solution of 2.1 g (0.01 mole) of 7-ADCA in 50 ml of H_2O in 50 ml of Me_2CO containing 2.0 g of $NaHCO_3$ was added 0.01 mole of the acid chloride of the side chain being used. After 1 hr the solution was layered with EtOAc, and the mixture was adjusted to pH 2 with concentrated HCl. The EtOAc was dried ($MgSO_4$) and evaporated to dryness. The residue (usually crystalline) was dissolved in EtOH or EtOH–CH_3OH. The K salt was then precipitated by the addition of a solution of KOAc in EtOH. The salt was recrystallized from MeOH–EtOH or MeOH–*i*-PrOH (see Table III).

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(10) Uv spectra were obtained in H_2O, ir spectra on Nujol mulls. Nmr spectra were taken on a Varian Associates Model HR-60 spectrometer in D_2O, D_2O–DCl, or D_2O–CF_3CO_2H. The amino acid analyses were performed on a Beckman Model 120B amino acid analyzer. Titrations were performed in 66% DMF.

(11) R. Schwyzer, P. Sieber, and H. Keppler, *Helv. Chim. Acta*, **42**, 2622 (1959).