Chemistry of Cephalosporin Antibiotics. VII. Synthesis of Cephaloglycin[1] and Some Homologs

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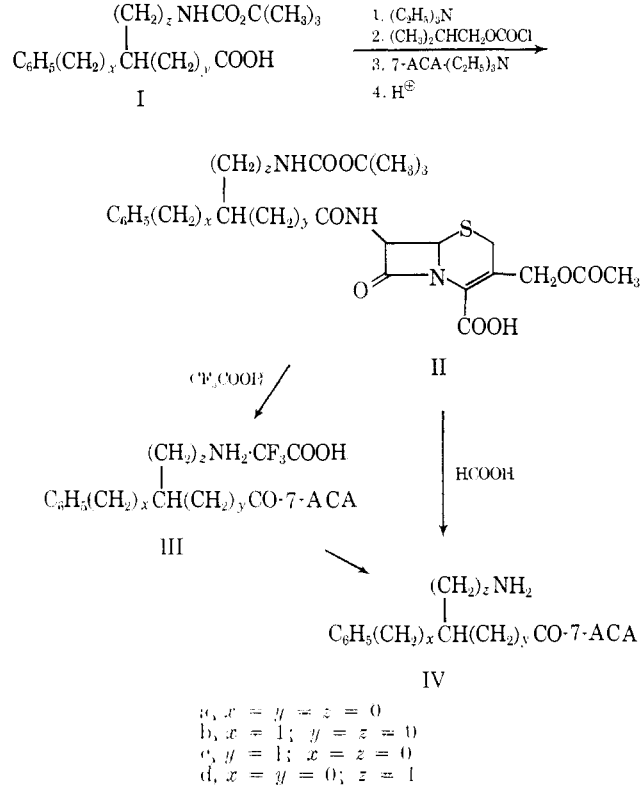
Methods of synthesis of cephaloglycin and the preparation of its epimer and several homologs are reported. Use of the Moore–Stein amino acid analyzer (Beckman Model 120B) in connection with this investigation is discussed. Biological activities of these new cephalosporins are described.

A number of acyl derivatives of 7-aminocephalosporanic acid[2] (7-ACA) and their antibacterial activities were described[3] earlier. This paper is concerned with the synthesis of D- and L-phenylglycine derivatives[3b] of 7-ACA and some homologs. The D-phenylglycine derivative, D-IVa, known generically as cephaloglycin, has a broad spectrum of antibacterial activity and has received a thorough biological evaluation by Wick and Boniece.[4]

Acylation of 7-ACA with an amino acid such as D-phenylglycine presents a problem common to peptide synthesis, *i.e.*, protection of the amino group by some function so that acylation is possible, followed by removal of the function in such a manner that the rest of the molecule is not affected. Difficulties with commonly used protecting groups, when applied to the synthesis of amino acid derivatives of penicillin, have recently been discussed by Ekström, *et al.*[5] These workers reported that the *N*-*t*-butoxycarbonyl group was not useful in the penicillin case since it could not be removed because of instability of the resulting penicillin derivatives in acid. Earlier we had found that this group could be used satisfactorily in the synthesis of cephaloglycin. Greater acid stability of the β-lactam system in cephalosporins permitted removal of the *t*-butoxycarbonyl substituent under acidic conditions with a minimum of undesirable side reactions. In fact, this method of synthesis is generally applicable to laboratory preparations of cephaloglycin analogs. The sequence of steps involved is shown in Scheme I.

Synthesis of the L epimer, L-IVa, was undertaken to see if antibacterial activity was affected by side-chain stereochemistry as is the case with the analogous penicillins.[6] Data in Table I show that an effect is observed. The activity of L-IVa is about 5% of that observed with D-IVa against *Staphylococcus aureus* 209 P. One may question whether activity of the L epimer is due, at least in part, to contamination with the more

SCHEME I



active D epimer which could be present as a result of racemization during the synthesis; incomplete resolution of the starting amino acid might be implicated also. From the biological assay (Table I), one may conclude that 5% is the maximum amount of D-IVa which could be present in L-IVa.

The possibility that D-IVa was contaminated with virtually inactive (biologically) L-IVa was of greater concern and could not be evaluated by biological assay alone. Measurement of optical rotation was not definitive because both derivatives have rotations of the same sign and are not sufficiently different in value (see Table II); the contribution of the side chain is overshadowed by that of the 7-ACA moiety. Nmr spectra were examined, but definitive measurements were not possible. Although a number of solvent systems for paper chromatography were tried, adequate separation of D-IVa and L-IVa was not obtained.

The fact that phenylglycine, 7-ACA, and cephaloglycin react with ninhydrin led to investigation of the Moore–Stein amino acid analyzer as an analytical device. The method proved to be an excellent technique for measurement of the above materials. Peaks could be

(1) Cephaloglycin is the generic name given to 7-(D-α-aminophenylacetamido)cephalosporanic acid, D-IVa. The D or L before the formula number indicates the configuration of compounds Ia–c and that of the side chain for IIa–d, IIIa–b, and IVa–c.

(2) R. B. Morin, B. G. Jackson, E. H. Flynn, and R. W. Roeske, *J. Am. Chem. Soc.*, **84**, 3400 (1962).

(3) (a) B. Loder, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, **79**, 408 (1961); (b) 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, *J. Am. Chem. Soc.*, **84**, 3401 (1962); (c) 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).

(4) W. E. Wick and W. S. Boniece, *Appl. Microbiol.*, **13**, 218 (1965).

(5) B. Ekström, A. Gomez-Revilla, R. Mollberg, H. Thelin, and B. Sjöberg, *Acta Chem. Scand.*, **19**, 281 (1965).

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

TABLE I

BIOLOGICAL ACTIVITIES[a]

Structure	Cephaloglycin[b] assay	Resistant[c] *S. aureus*	Gram[d] negative	Mouse[e] protection
D-IVa	1000	1.0, 1.5	2	4.5
L-IVa	42	0.7, >10	>50	>41
D-IIIb	150	11, >20	>50	>41
L-IIIb	55	12, >20	>50	>41
D-IVc	1700	1, 1	16	>41
L-IVc	725	2, 3	>50	>41
DL-IIId	270	9, 12	>50	Not done
L-IVd[f]	<63	>20, >20	>50	Not done
D-IVd[f]	550	6, 8	>50	Not done
Cephalothin[g]	3000	0.4, 1.0	8	41

[a] See ref 10. [b] Values are expressed as μg/mg of cephaloglycin activity against *S. aureus* 209P using the paper disk agar diffusion plate method. [c] Values, expressing the minimum inhibitory concentrations in μg/ml, are the results of gradient plate assay. The first value is without, and the second value with, 25% human serum in the medium; each figure is the average value with four penicillin-resistant (>50 μg/ml) *S. aureus* strains. The MIC values for methicillin in this test average about 2–3 μg/ml. [d] Values given were obtained with a gradient plate assay in the absence of serum; the figure is the mean MIC value against seven representative gram-negative organisms.[3c] [e] The values given are the dose of drug in mg/kg administered orally 1 and 5 hr post-infection with 250–600 LD_{50}'s of *S. pyogenes* C-203. Values were calculated by the method of Reed and Muench.[4] [f] The configurational assignment is on the basis of biological activity. [g] See ref 3b.

identified with standards of each of these substances. In addition, the epimer of cephaloglycin, L-IVa, showed a shorter retention time on the column and thus was separated satisfactorily from D-IVa. Using this procedure, purity of both compounds could be determined with regard to three possible impurities inherent in the synthetic procedure.

A serious impediment to the preparation of cephaloglycin analogs free of epimeric impurities was the necessity of resolving the side-chain amino acids. Use of the racemic amino acids is undesirable since relative proportions of the epimers in the product might vary. (Such variation might be anticipated since 7-ACA contains two carbon atoms with fixed asymmetry.) If the separation of epimers in the Moore–Stein analysis proved to be a general phenomenon, the analysis would provide a method of checking a final product for the presence of both epimers, in addition to determining quantitatively the amount of unreacted starting materials.

To test this method, 7-ACA was acylated with DL-phenylalanine (DL-α-amino-β-phenylpropionic acid) *via* the *t*-butoxycarbonyl-protected derivative to yield IVb. When the crystalline product was subjected to Moore–Stein analysis, there was obtained only one major peak and several very minor ones. It appeared that in this case the two epimers D-IVb and L-IVb had the same retention time and no separation of the epimers was obtained, or that work-up of the reaction mixture resulted in a separation. The derivatives of 7-ACA (D-IVb and L-IVb) corresponding to D-phenylalanine and L-phenylalanine, respectively, were prepared then, in like manner according to Scheme I, from pure D and L enantiomorphs. A crystalline product resulted from D-phenylalanine, but the L epimer was amorphous. When run on the Moore–Stein columns, D-IVb corresponded in position to the product from DL-phenyl-

TABLE II

CRYSTALLINE MATERIALS

Structure	Mp °C dec	$[\alpha]^{25}D$,[a] deg	Ultraviolet λ_{max}, mμ (ε)	Titration[b] pKa′	AMW (FMW)	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S
D-IIa	105–110		258 (7200)	5.1	498 (514)	$C_{23}H_{27}N_3O_8S_2 \cdot 0.5H_2O$	53.69	5.48	8.17		53.53	5.75	8.05	
D-IVa	190–195	+72	260 (7350)	4.4, 7.1	420 (423)	$C_{18}H_{19}N_3O_6S \cdot H_2O$	51.05	5.00	9.92	7.57	50.84	5.31	9.83	7.54
VI	165–115		265 (16,310)	4.7	619 (594)	$C_{26}H_{24}N_4O_{10}S \cdot 0.5H_2O$	52.61	4.24	9.44		52.30	4.48	9.44	
L-IVa	190–195	+100	260 (8240)	4.6, 7.1	440 (423)	$C_{18}H_{19}N_3O_6S \cdot H_2O$	51.05	5.00	9.92	7.57	51.48	5.26	10.07	7.63
D-IVb	180–185	+56	260 (7650)	4.6, 7.3	426 (428)	$C_{19}H_{21}N_3O_6S \cdot 0.5H_2O$	53.26	5.18	9.81	7.48	53.54	4.99	9.81	7.48
D-IVc	240–250	+83	258 (8570)	4.6, 8.1	420 (419)	$C_{19}H_{21}N_3O_6S$	54.40	5.05	10.02	7.65	54.02	5.62	10.24	7.57
L-IVc	230–240	+80	258 (6750)	4.5, 8.2	421 (419)	$C_{19}H_{21}N_3O_6S$	54.40	5.05	10.02	7.65	54.11	5.89	9.94	7.14
D-IVd	Not done	Not done	260 (7820)	4.4, 8.4	452 (437)	$C_{19}H_{21}N_3O_6S \cdot H_2O$	52.16	5.30			52.24	5.37		
L-IVd	Not done	Not done	259 (8850)	4.4, 8.2	430 (428)	$C_{19}H_{21}N_3O_6S \cdot 0.5H_2O$	53.26	5.18			53.61	5.48		

[a] Optical rotations were determined as a 1% solution in 50% aqueous acetonitrile. [b] The titrations are in 66% aqueous dimethylformamide; the formula molecular weight (FMW) includes solvate and is listed after the apparent molecular weight (AMW). [c] The Karl Fischer moisture determination of 4.41% is in agreement with a monohydrate. A hemihydrate and a sesquihydrate were also obtained by varying the drying conditions.

alanine while L-IVb showed a shorter retention time. It was evident that separation of epimers had occurred in the work-up of the product from DL-phenylalanine, specifically in the conversion of IIIb to IVb. Mother liquors from IVb showed a high content of L-IVb.

Application of the Moore–Stein technique was successful also when applied to the β-amino-β-phenylpropionic acid (β-aminohydrocinnamic acid) derivative of 7-ACA. In this case, comparison of products from synthesis with the L- and the D-amino acids with that obtained using DL acid showed that separation of epimers occurred during isolation of IIc (see Experimental Section).

Derivatives of 7-ACA with β-amino-α-phenylpropionic acid (α'-aminohydratropic acid) were prepared, employing the DL acid as starting material. The trifluoroacetate salt, DL-IIId, showed two peaks by Moore-Stein analysis and was separated into two crystalline components during conversion to the zwitter ion IVd. Each of these corresponded to one of the component peaks on the Moore–Stein column.

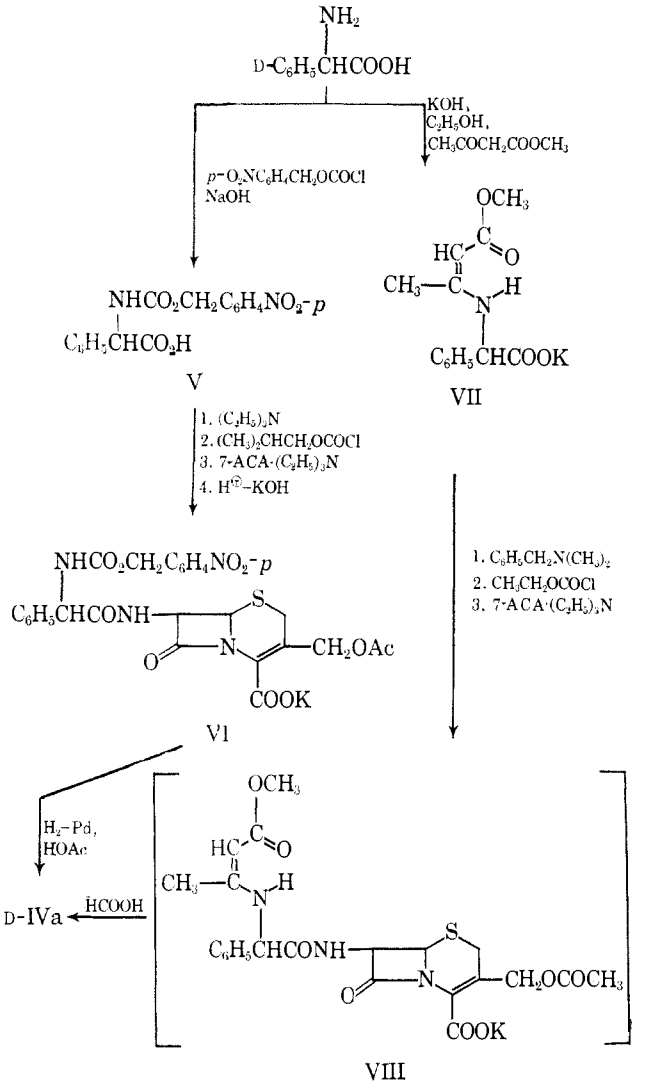
It can be concluded that the Moore–Stein procedure is a useful one for analyzing cephalosporin derivatives which possess an amino-containing substituent as a 7-acyl group. It is further evident that composition of products obtained starting with DL-amino acids may not represent a 1:1 ratio of the D and L epimers.

The use of the N-benzyloxycarbonyl group (and its removal by catalytic hydrogenolysis) in the synthesis of aminopenicillins has been described,[6] and some of its limitations have been discussed.[5] The method was used successfully in the synthesis of cephaloglycin, but better results were obtained using the N-*p*-nitrobenzyloxycarbonyl group as described by Gish and Carpenter.[7] The steps involved are shown in Scheme II.

β-Dicarbonyl compounds have been used to protect amino functions during peptide synthesis; their application to α-aminobenzylpenicillin has been reported.[8] An analogous procedure was applied to the synthesis of cephaloglycin (Scheme II). The protected intermediate VIII was not isolated prior to hydrolysis with formic acid to produce cephaloglycin. This method provided a means of converting 7-ACA to cephaloglycin in one step without isolation or purification of intermediates.

Cephaloglycin has been reported to be unstable in a bacteriological medium,[4] the degree of stability being pH dependent. Absorption in the ultraviolet (at 260 mμ) by cephalosporin derivatives is attributed to conjugation in the β-lactam dihydrothiazine fused-ring system[9] and is dependent on integrity of the β-lactam ring. As measured by changes in intensity at this wavelength, the pH of maximum stability appeared to be at 4.5, the isoelectric point of cephaloglycin. Loss of absorption at this pH in phosphate buffered solution was about 5%/day while at pH 6 the loss was about 6%/hr. The percentage drop correlated with loss in antibacterial activity over the range which was studied.

SCHEME II



Biological evaluation of these compounds is summarized in Table I.[10] In general, none of the homologs nor the L epimer possesses the breadth of activity exhibited by cephaloglycin. It may be noted that L-IVc and D-IVc exhibit activity of a useful range on penicillin-resistant *S. aureus*. Significant gram-negative activity is shown only by D-IVc, and this is considerably less than that of cephaloglycin. The mouse-protection results (last column of Table I) probably represent a composite of two important factors, *i.e.*, oral absorbability and sensitivity of the organism to the agent. Although other variables may intervene (rates of excretion, serum inactivation, etc.), an ED_{50} value of >40 mg/kg is generally assumed by us to be correlated with lack of oral therapeutic utility. Most therapeutically useful agents give values under this figure using comparable test conditions.

Experimental Section

All cephalosporin derivatives were subjected to antibacterial assays and the reactions were followed by bioautographic techniques. The solvent system for paper chromatography was generally 70% aqueous propanol. Compounds were detected by

(7) F. H. Carpenter and D. T. Gish, *J. Am. Chem. Soc.*, **74**, 3818 (1952); D. T. Gish and F. H. Carpenter, *ibid.*, **75**, 5872 (1953).

(8) E. Dane, F. Dreis, P. Konrad, and T. Dockner, *Angew. Chem. Intern. Ed., Engl.*, **1**, 658 (1962); E. Dane and T. Dockner, *Angew. Chem.*, **76**, 342 (1964).

(9) G. C. Barrett, V. V. Kane, and G. Lowe, *J. Chem. Soc.*, 783 (1964); E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **79**, 377 (1961).

(10) Data for comparison are found in ref 3b,c and 4 as are references to description of methods. Results of these tests should be interpreted on a comparative basis only and require use of an internal standard for accuracy.

plating on *Bacillus subtilis* seeded agar plates. All of the cephalosporins decomposed on heating, showing no definite melting point. Some products were not obtained analytically pure; many of the crystalline compounds contained solvate of crystallization. Physical–chemical methods of identification were used including ultraviolet spectra in water or ethanol, infrared spectra in Nujol mulls, nmr spectra in D_2O or trifluoroacetic acid, and titrations in 66% aqueous dimethylformamide using a glass electrode and a standard calomel half-cell (see Table II). All evaporations were performed at room temperature or below with a rotary evaporator under reduced pressure. The amino acid analysis was carried out on a Beckman Model 120B amino acid analyzer which uses resin chromatography *à la* Moore and Stein.

7-(α-Aminophenylacetamido)cephalosporanic Acid Zwitter Ion (IVa), D-α-t-Butoxycarboxamidophenylacetic Acid (Ia).—Reaction of D-phenylglycine[11] with *t*-butoxycarbonyl azide[12] was carried out by the method of Schwyzer[13] to give the protected amino acid[5] in good yield.

7-(D-α-t-Butoxycarboxamidophenylacetamido)cephalosporanic Acid (D-IIa).—A solution of 12.6 g (0.05 mole) of D-α-*t*-butoxycarboxamidophenylacetic acid and 6.9 ml (0.05 mole) of triethylamine in 200 ml of tetrahydrofuran (THF) was cooled to −10°. While stirring, 6.5 ml (0.05 mole) of isobutyl chloroformate was added and the temperature was maintained at −10° for 10 min. A cold solution of 7-ACA[2] triethylamine salt, prepared by slowly adding 6.9 ml (0.05 mole) of triethylamine to a suspension of 13.6 g (0.05 mole) of 7-ACA in 180 ml of cold 50% aqueous THF, was added with stirring to the mixed anhydride solution.

The mixture was stirred at 5° for 1 hr and at room temperature for 1 additional hr. THF was evaporated and the residue was dissolved in a mixture of 300 ml of water and 100 ml of ethyl acetate. The organic layer was discarded and the aqueous layer was cooled to 5°, 300 ml of fresh ethyl acetate was added, and the mixture was acidified to pH 3.0 with 10% HCl. The mixture was filtered to remove unreacted 7-ACA, and the ethyl acetate was separated. The aqueous layer was extracted again with 150 ml of ethyl acetate. The combined organic extracts were washed with 100 ml of water and dried ($MgSO_4$). After evaporation the residue was triturated with a mixture of 15 ml of ether and 50 ml of petroleum ether (60–71°) until the oily residue soldified. The product, 26.0 g, 74% yield, could be further purified by dissolving in ether and reprecipitating by adding petroleum ether (60–71°). However, the product was used in subsequent steps without further purification.

7-(D-α-Aminophenylacetamido)cephalosporanic Acid Zwitter Ion, Cephaloglycin (D-IVa). A. By Formic Acid Hydrolysis.—A solution of 25.5 g of the protected derivative, D-IIa, in 700 ml of 50% aqueous formic acid was warmed at 40° for 3.5 hr. Solvent was evaporated, and the residual syrup was stirred with 200 ml of ethyl acetate until it solidified. Stirring was continued while 200 ml of ether was added, then for an additional 15 min to give 18.0 g of amorphous material.

A 10.0-g sample of this product was stirred with a mixture of 50 ml of water and 50 ml of 25% Amberlite LA-1 (acetate form)[14] in isobutyl methyl ketone for 2 hr. The material, which was initially soluble in this mixture of solvents, crystallized as a hydrate during extraction. After being collected by filtration, it was washed with a mixture of 20 ml of water and 20 ml of isobutyl methyl ketone, then, 120 ml of isobutyl methyl ketone in three portions, and finally two 40-ml portions of ethyl acetate; yield 3.6 g (32%).

B. *Via* Trifluoro Acid Cleavage.—To 10.0 ml of anhydrous trifluoroacetic acid which had been cooled to 5° was added 1.0 g of D-IIa with stirring. Gas evolution was noted, and the solution was stirred for 3 min after the bubbling had ceased. Pouring the reaction solution into anhydrous ether afforded the trifluoroacetate salt D-IIIa. This was converted to the zwitter ion, D-IVa, by slurrying with water and Amberlite LA-1 as in method A to furnish a product identical with that obtained in this preceding method.

C. *Via* Hydrogenolysis of N-*p*-Nitrobenzyloxycarbonyl Protecting Group.—A solution of 6.6 g (0.020 mole) of V (mp 175–176°, obtained from D-phenylglycine and *p*-nitrocarbobenzoxy chloride) and 2.7 ml (0.02 mole) of triethylamine in 60 ml of THF was cooled in an ice–alcohol bath while 2.7 g (0.02 mole) of isobutyl chloroformate in 20 ml of THF was added dropwise with stirring. After stirring in the cold for 20 min, the mixed anhydride was treated with a solution of 5.4 g (0.02 mole) of 7-ACA and triethylamine (2.0 g, 0.02 mole) in 20 ml of water. The reaction mixture was refrigerated overnight and diluted with 500 ml of water. After evaporation of THF, the aqueous solution was acidified to pH 2.5 in the presence of ethyl acetate. The product, which was in the ethyl acetate solution, was extracted into cold water by treatment with 1.0 *N* KOH to pH 6.8. After filtration and evaporation, the residue was crystallized by dissolving in 600 ml of methanol and diluting with an equal volume of 2-propanol to yield 3.3 g of VI.

A solution of 0.425 g (0.00068 mole) of VI in 20 ml of glacial acetic acid was added to 1.0 g of 10% Pd–C. A fine stream of hydrogen was passed through the mixture for 2 hr with stirring at room temperature. After removal of catalyst by filtration, the solvent was evaporated and the by-products were removed under high vacuum leaving 200 mg of crude product. Purification as described under method A afforded material which was identical with this previously described compound.

D. *Via* β-Dicarbonyl Protecting Group.—A solution of 2.1 ml (0.022 mole) of ethyl chloroformate in 70 ml of acetone containing a catalytic amount (3 drops) of N,N-dimethylbenzylamine was cooled to −10°. With stirring, 6.4 g (0.022 mole) of VII[8] (D enantiomorph) was added. The temperature rose to −2°, but the reaction mixture was cooled to −10° where it was maintained for 20 min.

A solution of 6.3 g (0.023 mole) of 7-ACA was prepared by suspending the 7-ACA in 30 ml of water, cooling, and adding triethylamine to pH 7.5, then adding 30 ml of acetone. This solution was added to the above mixed anhydride with stirring. After the addition, the mixture was stirred for 1 hr while the temperature was maintained at 0°. The mixture was then filtered and the acetone was evaporated.

The resulting aqueous solution of VIII was shaken with a mixture of 75 ml of isobutyl methyl ketone and 10 ml of formic acid. The mixture was again filtered rapidly through a pad of filter aid. The filtrate was stirred at 0° for 1 hr and the solid was collected after overnight refrigeration. This product, 3.3 g (35% yield), was identical with that prepared *via* methods A, B, and C.

7-(L-α-t-Butoxycarboxamidophenylacetamido)cephalosporanic acid (L-IIa) was prepared in 49% yield from L-α-*t*-butoxycarboxamidophenylacetic acid[11–13] and 7-ACA in the same manner as the D epimer (D-IIa).

7-(L-α-Aminophenylacetamido)cephalosporanic Acid Trifluoroacetic Acid Salt (L-IIIa).—To 50 ml of cold anhydrous trifluoroacetic acid was added 10.0 g of L-IIa, while stirring and cooling in an ice bath. The solid dissolved and gas was evolved. After stirring for 15 min, the solution was poured into 500 ml of cold, anhydrous ether. The precipitate was collected, washed with ether, and vacuum dried, yielding 4.0 g.

7-(L-α-Aminophenylacetamido)cephalosporanic Acid Zwitter Ion (L-IVa).—The above product (L-IIIa) (4.0 g) was stirred with 30 ml of water and 30 ml of 25% Amberlite LA-1 (acetate form) in isobutyl methyl ketone at room temperature for 15 min, then at 5° for an additional 45 min. A crystalline product formed, was collected by filtration, and washed in the same manner as was the D epimer.

7-(α-Amino-β-phenylpropionamido)cephalosporanic Acid Zwitter Ion (IVb), 7-(DL-α-t-Butoxycarboxamido-β-phenylpropionamido)cephalosporanic Acid (IIb).—The procedure described for D-IIa was followed and the product was obtained in 68% yield starting with DL-α-*t*-butoxycarboxamido-β-phenylpropionic acid[15] and 7-ACA. The product, which was used in subsequent steps without further purification, had an ultraviolet maximum at 260 mμ (ε 6770), and titration gave a pK_a of 4.8 with an apparent molecular weight of 540 (calcd 519).

(11) The resolved phenylglycine use in the early part of this investigation was obtained by the method of E. Fisher and O. Weichhold [*Ber.*, **41**, 1286 (1908)] *via* the N-formyl derivative of DL-phenylglycine (Eastman). Both isomers are now available from Ott Chemical Co.

(12) *t*-Butoxycarbonyl azide is now commercially available from Aldrich Chemical Co.

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

(14) Amberlite LA-1 is a high molecular weight, water-insoluble, liquid secondary amine, commercially available from Rohm and Haas Co. The acetate form used in this investigation was prepared as follows. To 1.0 l. of Amberlite LA-1 and 3.0 l. of isobutyl methyl ketone was added 120 ml of glacial acetic acid and the solution was stirred for 5 min. After stirring with 800 ml of water for 25 min, the organic layer was separated for use.

(15) The starting amino acid was obtained from Nutritional Biochemicals Corp. both as the racemic mixture and as the resolved material. These were converted to *t*-butoxycarbonyl derivatives by the procedure of Schwyzer.[13]

7-(DL-α-Amino-β-phenylpropionamido)cephalosporanic Acid Trifluoroacetic Acid Salt (IIIb).—Cleavage of the protecting group and isolation of the salt was carried out as described for the L-phenylglycine derivative. This product was obtained in 62% yield, had an ultraviolet maximum at 259 mμ (ε 8650), and titration gave pK_a values of 4.6 and 7.4 with an apparent molecular weight of 536 (calcd 533).

7-(D-α-Amino-β-phenylpropionamido)cephalosporanic Acid Zwitter Ion (D-IVb). From the DL salt.—A 3.0-g sample of the above DL salt (IIIb) was stirred with 10 ml of water and 20 ml of 25% Amberlite LA-1 (acetate form) in isobutyl methyl ketone at room temperature for 15 min, then at 5° for 30 min. The crystalline solid (0.9 g) was collected and washed in the same manner as cephaloglycin.

B. From D-Phenylalanine.—The above procedures were repeated starting with D-phenylalanine.[15] The yields and quality of products were comparable except that the yield of crystalline zwitter ion, D-IVb, from the trifluoroacetic acid salt was twice that obtained starting with DL-phenylalanine.

7-(L-α-Amino-β-phenylpropionamido)cephalosporanic Acid Zwitter Ion (L-IVb).—The above procedures were repeated starting with L-phenylalanine.[15] The yields and quality of products were comparable through the trifluoroacetic acid salt (L-IIIb). Attempted conversion to the zwitter ion failed to produce crystalline material, and only poor quality, amorphous material could be isolated.

The crude material from evaporation of the aqueous portion of the filtrate from IVb (from the DL salt) showed an enrichment of the L epimer.

7-(β-Amino-β-phenylpropionamido)cephalosporanic Acid Zwitter Ion (IVc), 7-(L- and D-β-t-Butoxycarboxamido-β-phenylpropionamido)cephalosporanic Acid (IIc).—The usual coupling procedure (*e.g.*, as for D-IIa) with 11.0 g of DL-β-*t*-butoxycarboxamido-β-phenylpropionic acid[16] and 11.3 g of 7-ACA was used except for the following changes. During final concentration of the ethyl acetate solution containing the product, a solid precipitate formed. When the volume was approximately 25 ml, this solid (X) was collected. (Subsequent investigation showed that this material was the same as that obtained starting from D-β-amino-β-phenylpropionic acid.)

Slurrying the residue from evaporation of the filtrate from X with ether furnished 4.7 g of product (Z) that was shown by subsequent investigation to correspond to the derivative from L-β-amino-β-phenylpropionic acid.

7-(D-β-Amino-β-phenylpropionamido)cephalosporanic Acid Trifluoroacetic Acid Salt (D-IIIc). A. From DL Starting Material.—The first crop (X) from the preceding experiment was cleaved and the salt was isolated in 85% yield in the usual manner (see L-IIIa).

B. From D-β-Amino-β-phenylpropionic Acid.—The *t*-butoxycarbonyl derivative of D-β-amino-β-phenylpropionic acid[16] was prepared, coupled with 7-ACA, and cleaved to the trifluoroacetic acid salt by the foregoing methods. This material was the same as that obtained from X in procedure A above.

7-(D-β-Amino-β-phenylpropionamido)cephalosporanic Acid Zwitter Ion (D-IVc).—A 1.5-g sample of the above resolved salt (D-IIIc) from A was stirred with 5.0 ml of water and 10 ml of 25% Amberlite LA-1 (acetate form) in isobutyl methyl ketone for 15 min at room temperature, then for 30 min at 5°. No precipitate was formed. The layers were separated, and the aqueous layer was evaporated to dryness. The residue was stirred with water and isobutyl methyl ketone; the water layer was separated and diluted with acetonitrile. A crystalline product resulted and was collected after overnight refrigeration.

7-(L-β-Amino-β-phenylpropionamido)cephalosporanic Acid Trifluoroacetic Acid Salt (L-IIIc). A. From DL Starting Material.—The second crop of 7-(β-*t*-butoxycarboxamido-β-phenylpropionamido)cephalosporanic acid (Z) was cleaved with trifluoroacetic acid to give the salt in 80% yield by the previously described method.

B. From L-β-Amino-β-phenylpropionic Acid.—This compound was prepared by the same procedure as the D isomer and was the same as that obtained from Z in A.

7-(L-β-Amino-β-phenylpropionamido)cephalosporanic Acid Zwitter Ion (L-IVc.)—The salt (L-IIIc) from B was stirred with water and LA-1 as previously described to obtain a crystalline product, L-IVc.

7-(β-Amino-α-phenylpropionamido)cephalosporanic Acid Zwitter Ion (IVd), 7-(DL-β-Amino-α-phenylpropionamido)cephalosporanic Acid Trifluoroacetic Acid Salt (DL-IIId).—The *t*-butoxycarbonyl-protected derivative, DL-IId, was prepared in 42% yield starting with 1.75 g of DL-β-*t*-butoxycarboxamido-α-phenylpropionic acid.[16] This was cleaved and isolated as the trifluoroacetic acid salt (DL-IIId) in 63% yield.

7-(D- and L-β-Amino-α-phenylpropionamido)cephalosporanic Acid Zwitter Ion (D-IVd and L-IVd).—To 2.5 ml of water was added 500 mg of the above salt, DL-IIId. The mixture was stirred until only a small amount of gummy material remained undissolved. The solution after being decanted was stirred with 4.0 ml of 25% Amberlite LA-1 (acetate form) in isobutyl methyl ketone for 15 min at room temperature and finally for 30 min at 5°. A gelatinous precipitate, which remained in the organic phase, separated during this time. Both the precipitate and organic phase remained behind when the mixture was filtered through a sintered-glass funnel so the filtrate consisted only of the aqueous fraction. It was evaporated to dryness; the residue was dissolved in 2.0 ml of 50% acetonitrile, and additional acetonitrile was added slowly until the solution became turbid. After standing overnight at 5°, 37 mg of crystalline product was obtained and shown to be one epimer which was designated L-IVd on the basis of biological activity.

The other epimer, designated D-IVd on the basis of biological data, was obtained from the thick organic layer. This material was extracted into two 5-ml portions of water. After washing with isobutyl methyl ketone, the water was evaporated. During evaporation a white crystalline precipitate formed; when the volume reached about 2.0 ml, this product (81 mg) was collected.

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