Notes

Chemistry of Cephalosporin Antibiotics. XI. Preparation and Properties of Desacetylcephaloglycin and Its Lactone

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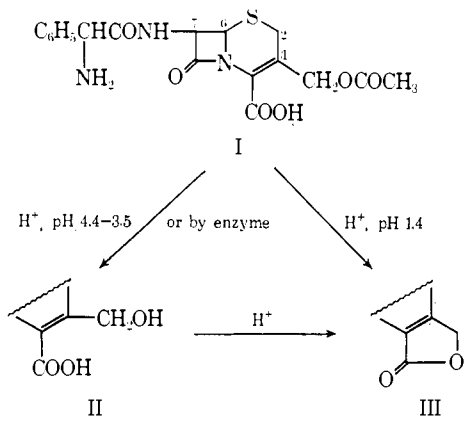
Previous publications[1] from these laboratories described the synthesis and the antibiotic activity of cephaloglycin (I).[2] In continuing investigations the stability of this antibiotic has been studied. In particular, attention has been directed toward the selective hydrolysis of cephaloglycin, since it has been shown that the desacetyl derivative of cephaloglycin is a metabolic product present in the blood and urine.[3] It was also shown to have antibacterial activity. For this reason it was necessary to establish the degree of therapeutic effectiveness of desacetylcephaloglycin (II) and to determine its role in the treatment of infections. Therefore, our attention and efforts were directed to the preparation and isolation of II in pure form.

Cleavage of the acetate group in cephalosporins has been successfully carried out by enzymatic hydrolysis.[4] The same group in Δ^2-cephalosporins was hydrolyzed with dilute alkali, since it has been reported that Δ^2 compounds are relatively more stable under alkaline conditions.[5]

It has been now found that, in an acidic medium, cephaloglycin is converted to desacetylcephaloglycin (II) and desacetylcephaloglycin lactone (III) (Scheme (I). By controlling the reaction conditions both products can be prepared simultaneously or separately. Thus the mild acidic hydrolysis at 30° for 96 hr (pH 4.4–3.5) gave II. If the hydrolysis was performed at a higher temperature (50°) and/or with a longer reaction time, lactone III was also produced. Desacetylcephaloglycin was separated from unreacted I and formed lactone III by chromatography on a cellulose column. Hydrolysis of I with HCl for 24 hr at 30° (pH 1.4) gave only III isolated as the hydrochloride. III can be made by lactonization of II with HCl. Desacetylcephaloglycin also could be prepared by enzymatic deacetylation of I according to the procedure described by Jansen, *et al.*[6]

SCHEME I



The course of reaction can be followed by tlc and nmr spectroscopy. The acetyl group in I showed a singlet at τ 7.9 which gradually disappeared as hydrolysis proceeded, and a new signal for the acetate anion was observed at τ 8.05.[7] Additional information which was useful in the identification of products was obtained from the signals due to the exocyclic CH_2 (see Table I). A tlc method was developed primarily to aid in identification and separation of mixtures. Column chromatography fractions were analyzed by tlc using cellulose plastic sheets. The leading spot having an R_f of 0.8 was identical with lactone III. The middle spot (R_f 0.7) was starting material I. The slow moving spot, desacetylcephaloglycin, had an R_f of 0.5. The separation on tlc cellulose plastic sheets was used as the basis for development of a method of isolation of the components from the reaction mixture by column chromatography. The crude mixture was chromatographed over cellulose. The column was developed with aqueous acetonitrile at 4°. The appropriate fractions were collected and, after removing the solvent, desacetylcephaloglycin was isolated as an amorphous powder and crystallized from aqueous acetonitrile.

Isolation of II presented considerable difficulty because of its chemical instability and physical properties. In a crystalline form desacetylcephaloglycin displayed good stability, but in solution stability was very dependent on pH and temperature. The lactone was formed easily with traces of acid or at higher temperatures. The free amino group in the lactone, because of its basic properties, is another source of instability. When the lactone was isolated as a free base, it polymerized rapidly. Most probably the lactone and/or lactam functions were attacked by the amino group forming a polymer. The lactone III was isolated in the form of a hydrochloride salt. Desacetylcephaloglycin (II) and the corresponding lactone (III) were characterized by ir, uv, and nmr spectra, elemental analyses, and optical rotations.

Bacillus subtilis and *Sarcina lutea* bioautographs for II and III displayed single, biologically active components. In the course of *in vitro* studies[3] desacetylcephaloglycin was found to have essentially the same broad-spectrum antibiotic activity as cephaloglycin[1c]

(1) (a) Paper X: J. W. Chamberlin and J. B. Campbell, *J. Med. Chem.*, **10**, 966 (1967); (b) J. L. Spencer, E. H. Flynn, R. W. Roeske, F. Y. Siu, and R. B. Chauvette, *ibid.*, **9**, 746 (1966); W. E. Wick and W. S. Boniece, *Appl. Microbiol.*, **13**, 248 (1965).

(2) Cephaloglycin is the generic name given to 7-[(D-2-amino-2-phenyl)-acetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid.

(3) Results will be published by W. E. Wick.

(4) (a) J. D'A. Jeffery, E. P. Abraham, and G. G. F. Newton, *Biochem. J.*, **81**, 591 (1961); (b) E. Van Heyningen, *J. Med. Chem.*, **8**, 22 (1965).

(5) J. D. Cocker, B. R. Cowley, J. S. G. Cox, S. Eardley, G. I. Gregory, J. K. Lazenby, A. G. Long, J. C. P. Sly, and G. A Somerfield, *J. Chem. Soc.*, 5015 (1965).

(6) E. F. Jansen, R. Jang, and L. R. MacDonnell, *Arch. Biochem.*, **15**, 415 (1947).

(7) (a) A. B. Taylor, *J. Chem. Soc.*, 7020 (1965); (b) J. D. Cocker, S. Eardley, G. I. Gregory, M. E. Hall, and A. G. Long, *ibid.*, 1142 (1966).

TABLE I

NMR SPECTRAL DATA FOR CEPHALOGLYCIN AND DERIVATIVES IN D_2O SOLUTIONS[a]

	Chemical shifts, τ values (J, Hz)						
Compd	2-H	3-CH_2	6-H	7-H	PhCH	$COCH_3$	Ph
I[b]	6.45 (18), 6.70 (18)	5.00 (13), 5.19 (13)	4.93 (4.6)	4.25 (5)	4.68	7.9	2.41
II[c]	6.47 (18), 6.70 (18)	5.80	4.98 (4.6)	4.36 (5)	4.80		2.55
III	6.24 (18), 6.46 (18)	4.98	4.85 (5)	4.14 (5)	4.68		2.45

[a] Containing Me_4Si as an internal reference. [b] With added DCl. [c] With added $NaHCO_3$.

TABLE II

BIOLOGICAL ACTIVITIES IN A GRADIENT PLATE TEST[a]

	MIC, $\mu g/ml$							
	Gram-negative organisms				Penicillin-resistant[b] *Staphylococcus aureus*			Cephaloglycin assay[c]
Compd	*Shigella sp.* (N-9)	*E. coli* (N-26)	*Klebsiella sp.* (N-26)	*Aerobacter sp.* (N-68)	(V-30)	(V-321)	(V-84)	
Cephaloglycin	2.1	3.6	3.6	3.9	2.3, 7.6	4.4, 8.8	2.5, 6.1	1000
Cephalexin	9.1	12.6	9.7	8.2	3.8, 6.7	3.7, 5.4	3.6, 4.2	480
Desacetylcephaloglycin	11.6	16.3	14.4	16.4	3.4, 5.5	2.8, 5.9	1.3, 3.0	825
Penicillin G	16.8	28.5	>50	34.5	>20	>20	>20	56

[a] C. W. Godzeski, G. Brier, and D. E. Pavey, *Appl. Microbiol.*, **11**, 122 (1963). The interpretation of these results should be done on a comparative basis only and requires use of an internal standard. [b] The minimum inhibitory concentrations in μg/ml; the first value is without, the second with, human serum. [c] The results are reported as μg/mg of cephaloglycin activity against *S. aureus* 209P.

and cephalexin.[8] Lactone III was considerably less active. The biological data are reported in Table II.

Experimental Section

Compounds II and III decomposed on heating, displaying no definite melting points. All evaporations were performed at below 30° in a rotary vacuum evaporator. The spectra were taken on a Varian Associates Model HA-60 spectrometer. The uv spectra were determined in EtOH or H_2O on a Cary Model 14 recording spectrometer. The ir spectra were obtained in Nujol or KBr. The behavior was followed using cellulose plastic sheets with fluorescent indicator (Brinkmann Instruments, Inc.). The solvent system was 75% (v/v) aqueous MeCN. The bioautography was performed according to the techniques described by J. D'A. Jeffery, *et al.*,[9a] using cellulose plastic sheets. Column chromatographic separations were made using microcrystalline cellulose. All titrations were carried out in 66% aqueous DMF using a glass electrode and a standard calomel half-cell. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

7-[(D-2-Amino-2-phenyl)acetamido]-3-hydroxymethyl-3-cephem-4-carboxylic Acid (II). A. By Enzymatic Hydrolysis.—To a suspension of 2.64 g (0.006 mole) of I in 160 ml of H_2O was added 40 ml of a solution of citrus acetyl esterase.[4a,6,9] The mixture was stirred at 34° for 2.5 hr while 0.5 *N* NaOH was added at such a rate that the pH of the solution was kept between 5.2 and 5.5. After that the solution was concentrated *in vacuo* to 40 ml and diluted with 40 ml of MeCN, and the pH of the solution was adjusted to 5.2 with AcOH. This solution was applied to a cellulose column (4 × 100 cm). The column was developed with 75% aqueous MeCN at 4° and 24-ml fractions were collected by an automatic fraction collector in the interval of 20.5 min. The progress of the separation was followed by tlc. Uv light and ninhydrin spray were used to visualize the spots. Fractions 1–40 were only solvent. Fractions 45–58 contained I and traces of lactone III, and fractions 63–77 contained the desired II. The latter fractions were combined, and the solvent evaporated. The almost colorless, amorphous solid residue was II which weighed 1.17 g, after drying. It was crystallized from H_2O after the addition of MeCN to cloudiness and chilling as well-formed, colorless, long blades. This product displayed a single biologically active spot on bioautograph and one spot on a cellulose plastic sheet as visualized by uv or detected with ninhydrin spray; λ_{max} 260 mμ (ϵ 8400); ν_{max} 5.67 (β-lactam C=O), 5.90 (amide C=O), and 6.27 μ (COO^-); pK_a = 4.9 and 7.6; apparent molecular weight of 401 (calcd 399.4); $[\alpha]^{25}D$ +102° (*c* 1.0, 50% aqueous MeCN). For nmr see Table I. *Anal.* ($C_{16}H_{17}N_3O_5S \cdot 2H_2O$) C, H, N, S.

B. By Boric Acid or AcOH Hydrolysis.—In 100 ml of H_2O 155 mg of boric acid (or 3–5 drops of AcOH) was dissolved, and 1.0 g of 7-[(D-2-amino-2-phenyl)acetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid (I) was added. The mixture (pH 4.41 was stirred at 30° for 96 hr, and the cloudy liquid was filtered. The filtrate (pH 3.5) was concentrated *in vacuo* to 30 ml and diluted with 30 ml of MeCN, and the solution was put on a cellulose column. Separation on the column was as described under method A. Fractions 59–65 containing the desired 3-hydroxymethyl compound II were combined, the solvent was evaporated, and the residue was dried to give 110 mg of a product which after crystallization was identical with that prepared *via* method A.

7-[(D-2-Amino-2-phenyl)acetamido]-3-hydroxymethyl-3-cephem-4-carboxylic Acid Lactone Hydrochloride. A. By Hydrochloride Acid Starting from I.—A solution of 10.9 g (0.04 mole) of I in 200 ml of 1 *N* HCl was stirred at 30° for 24 hr, then 75 ml of 2 *N* NaOH was added, and the mixture was evaporated *in vacuo* to dryness. The residue was extracted with 100 ml of dry MeOH. To separate the NaCl completely the alcoholic solution of the hydrochloride salt was evaporated to dryness and again extracted with 50 ml of MeOH. The extract was concentrated to give 14.0 g of hydrochloride salt, which was recrystallized from 45 ml of EtOH. The solution was kept at 4° overnight, the precipitate was filtered and immediately triturated with dry ether to yield 4.0 g of slightly yellow powder. To the filtrate 10 ml of *i*-PrOH was added, and the precipitate was filtered and triturated with dry ether to furnish 3.8 g of a yellow powder. Both crops showed a single biologically active component on bioautographs and were combined. The yield was 51%. An analytical sample was recrystallized from a mixture of ethanol and ether. The product decomposed at 195–210°; pK_a' = 6.76; apparent molecular weight 394 (calcd 381); λ_{max} 255 mμ (ϵ 6800) in EtOH; ν_{max} 5.6 (β-lactam C=O), 5.72 (lactone C=O), and 5.95 μ (amide C=O); $[\alpha]^{25}D$ +60° (*c* 1, 50% MeCN). For nmr see Table I. Bioautographs and tlc on cellulose plastic sheets indicated one spot material. *Anal.* ($C_{16}H_{15}N_3O_4S \cdot HCl$) C, H, Cl, S; N: calcd, 11.01; found, 10.28.

B. By HCl Starting from II.—A solution of 3 ml of 0.1 *N* HCl containing 100 mg of 7-[(D-2-amino-2-phenyl)acetamido]-3-hydroxymethyl-3-cephem-4-carboxylic acid was kept at 30° for 80 min and then evaporated to dryness. The residue was crystallized from EtOH–Et_2O to give a product identical with the compound described in method A.

(8) Cephalexin is the generic name given to 7-[(D-2-amino-2-phenyl)-acetamido]-3-methyl-3-cephem-4-carboxylic acid; *cf.* W. E. Wick, *Appl. Microbiol.*, **15**, 765 (1967).

(9) The author wishes to thank Mr. D. Fukuda *for* the preliminary work on the enzymolysis.

Acknowledgment.—The author is grateful to W. E. Wright for valuable discussions; to G. Maciak and his staff for the microanalyses; to H. Boaz, D. O. Woolf, L. A. Spangle, and their associates for spectral data and titrations; to W. E. Wick and J. L. Ott for biological data; to C. T. Pugh for bioautography; and to A. I. Ellis for technical assistance.

Cassaine Analogs. V.[1] A Distant Analog of Cassaine

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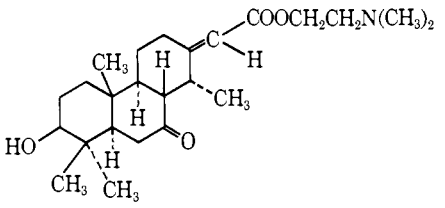
Extending our efforts in the search for a cardiac stimulant, we have prepared another analog related to the *Erythrophleum* alkaloid cassaine (**1**).[2] We felt



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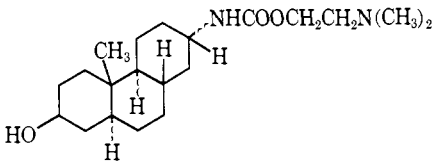
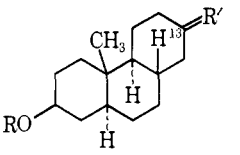
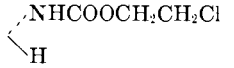
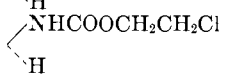
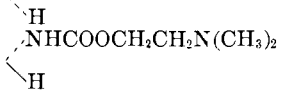
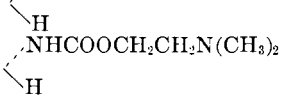


2

that if the group $=CHCOOCH_2CH_2N(CH_3)_2$ of **1** were replaced by the equatorially oriented $-NHCOOCH_2CH_2N(CH_3)_2$, perhaps we could obtain a cardiac stimulant having a much longer duration of activity. The ease of *in vivo* hydrolysis of the ester group might be related to duration of activity. It was hoped that the equatorial configuration of the side chain would approximate the configuration demanded by the double bond of the natural product and that the unshared electron pair on the nitrogen might substitute for the electron character of the double bond. In order to test this hypothesis, we have prepared the carbamate **2**.

The hydroxyl group of the starting material **3** was protected as its *t*-butyl ether derivative **4**[3] while the necessary transformations were made at C-13. The oxime **5** of **4** was reduced chemically (Na, EtOH), a procedure which allows assignment of the major product as the equatorial (α) amine **6** and the minor product as the axial (β) amine **7**. Not being crystalline, these amines were characterized by their conversion in the next step of the sequence to crystalline 2-



3, R = H; R′ = O
4, R = *t*-C_4H_9; R′ = O
5, R = *t*-C_4H_9; R′ = NOH
6, R = *t*-C_4H_9; R′ = 
7, R = *t*-C_4H_9; R′ = 
8, R = *t*-C_4H_9; R′ = 
9, R = *t*-C_4H_9; R′ = 
10, R = *t*-C_4H_9; R′ = 
11, R = F_3CCO; R′ = 

chloroethyl carbamates **8** and **9**. Reaction of **8** with dimethylamine afforded the dimethylaminoethyl carbamate **10** which was characterized as its hydrochloride salt. The *t*-butyl ether group in **10** was cleaved by treatment with trifluoroacetic acid; the compound isolated was the trifluoroacetate **11**. This ester was hydrolyzed with methanolic NH_4OH at room temperature to give 2-dimethylaminoethyl *dl*-N-(1,2,3,4,4aα,4b,5,6,7,8,8aα,9,10,10aβ-tetradecahydro-7β-hydroxy-4bβ-methylphenanthr-2α-yl)carbamate (**2**) in good yield.

Biological Testing.[4]—Compound **2**, upon intravenous administration in the dog at a dose level of 4 mg/kg, produced an increase of 30% in ventricular contractile force, accompanied by a blood pressure drop of 25%.[5] Ouabain at a dose level of 0.03 mg/kg or cassaine (**1**) at a dose level of 0.04 mg/kg produces an increase in ventricular contractile force of 20% without concomitant lowering of blood pressure.[4]

Experimental Section[6]

***dl*-7β-*t*-Butoxy-3,4,4aα,4b,5,6,7,8,8aα,9,10,10aβ-dodecahydro-4bβ-methyl-2(1H)-phenanthrone 2-Oxime (5).**—A solution of 6 g (25 mmoles) of hydroxyphenanthrone **3**[7] in 150 ml of CH_2Cl_2 was treated with 1 ml of BF_3 etherate and 1 ml of anhydrous H_3PO_4. The latter was prepared by the addition of a calculated amount of P_2O_5 to 85% H_3PO_4. Isobutene (150 ml) was added to the solution at 10°. The solution was shaken in the Parr shaker for 5 hr. The reaction mixture was poured into 200 ml of 2 *N* NH_4OH, the layers were separated, and the aqueous layer was washed with 200 ml of CH_2Cl_2. The organic layers were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure to yield an oil that was chromatographed on 300 g of silica gel. Ether eluted 4.7 g of ***dl*-7β-*t*-butoxy-3,4,4aα,4b,5,6,7,8,8aα,9,10,10aβ-dodecahydro-4bβ-methyl-2(1H)-phenanthrone (4)** as an oil that would not crystallize. The oil,

(1) For paper IV see R. L. Clarke, S. J. Daum, P. E. Shaw, T. G. Brown, Jr., G. E. Groblewski, and W. V. O'Connor, *J. Med. Chem.*, **10**, 593 (1967).

(2) See F. Erjavec and Š. Adamic, *Arch. Intern. Pharmacodyn.*, **155**, 251 (1965); E. L. McCawley, *Alkaloids*, **5**, 101 (1955), and references therein.

(3) H. C. Bayerman and G. J. Heiszwolf, *Rec. Trav. Chim.*, **84**, 203 (1965).

(4) For details of the experimental methods of evaluation see R. L. Clarke, S. J. Daum, P. E. Shaw, T. G. Brown, Jr., G. E. Groblewski, and W. V. O'Connor, *J. Med. Chem.*, **10**, 582 (1967).

(5) We wish to thank Mr. William V. O'Connor for the biological testing.

(6) All melting points are corrected. Ir spectra were recorded on a Perkin-Elmer Infrared spectrophotometer, Model 21. The silica gel used for column chromatography (100–200 mesh) was obtained from the Davison Co., Baltimore, Md. Silica gel G, purchased from Brinkmann Instruments, Inc., was used for thin layer chromatography. Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values.

(7) S. J. Daum, P. E. Shaw, and R. L. Clarke, *J. Org. Chem.*, **32**, 1427 (1967).