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SYNTHESIS OF N,O-DISUBSTITUTED 3-HYDROXY-METHYL-7-AMINOCEPHALOSPORANIC ACID

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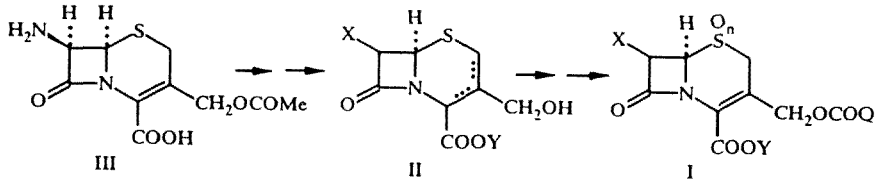
A method has been developed for the preparation of tert-*butoxycarbonylamino-3-hydroxymethylceph-2-em-4-carboxylic acid, which is an intermediate for the preparation of new antibiotic derivatives with antibacterial, anti-inflammatory, or antitumor activity.*

The variants of the preparation of 3-hydroxymethylcephalosporanic acid II through the transformation of 7-aminocephalosporanic acid (7-ACA, III) are a component part of multistep syntheses using this reagent for new biologically active acid derivatives I, which display antibacterial, anti-inflammatory, or antitumor activity depending on the structure of substituents *X*, *Y*, and *Q* and the oxidation state of the sulfur atom [1-3].



The solution of this problem involves the enzymatic or chemical hydrolysis of the acetoxymethyl group at $C_{(3)}$ of the cephemic system of the antibiotic or its alcoholysis using titanium isopropylate [4-6]. The latter reaction involves the prior isomerization of the double bond in cephalosporin from position Δ3 to Δ2 in order to prevent a side-reaction leading to formation of a heterocyclic lactone as the result of an intramolecular reaction of the hydroxymethyl and ether groups of the thiazine ring.

Our new synthetic scheme for the preparation of N,O-disubstituted 3-hydroxymethyl-7-aminocephalosporanic acid consists of BOC and ether protection of the amino and carboxyl groups, respectively, in 7-ACA (III) and subsequently cleavage of the acetyl group by titanium alcoholate.

The use of di-*tert*-butylpyrocarbonate and N,N'-dicyclohexyl-O-*tert*-butylisourea (V) for this purpose led to the unexpected formation of an isomeric mixture of the *tert*-butyl ester of 7-*tert*-butoxycarbonylaminocephalosporanic acid (VI) (the ratio of the Δ2 and Δ3 isomers is 85:15 as indicated by comparison of the integral intensities of the protons at $C_{(2)}$ in the PMR spectrum. The specific effect of V on the isomerization is noted only in combination with prior Boc protection of the amino group. Initial esterification and subsequent acylation of 7-ACA, in accord with the data of Durekheimer et al. [7], lead to VI with >95% of the Δ3 isomer.

The significant predominance of the Δ2 isomer made the planned step involving isomerization unnecessary and permits us to carry out the direct alcoholysis of the N,O-disubstituted aminocephalosporanic acid VI obtained due to esterification. This reaction is carried out in *tert*-butyl alcohol at reflux over 3-4 h. Chromatographic separation of the reaction mixture give the desired 3-hydroxymethylcephalosporin VII and lactone VIII. The formation of lactone VIII is made possible by the presence of the Δ3 isomer in starting ester VI. The PMR spectral data for these products are given in Table 1.

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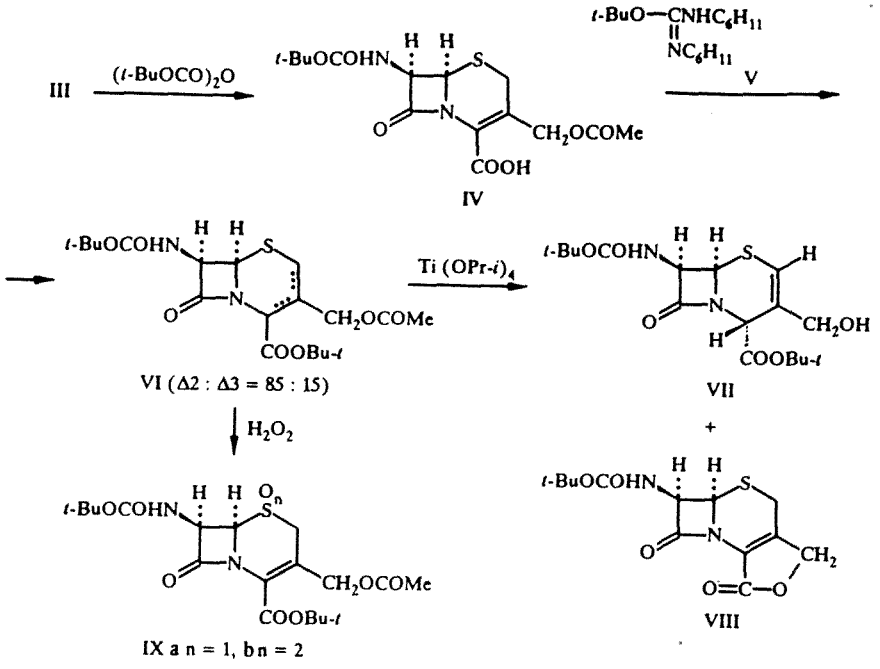

The displacement of the double bond from position Δ2 to Δ3 in VI was carried out by the hydrogen peroxide oxidation of the sulfur atom in the heterocyclic system to give sulfoxide IXa or sulfone IXb. Analysis of the PMR spectra of these compounds indicates formation of a specific hydrogen bond between the sulfoxide group oxygen and amide group proton in IXa, supported by the significant upfield shift of the proton signal (6.24 ppm, Table 1). Attempts to carry out the hydrolytic cleavage of the acetyl group in N,O-disubstituted esters VI (Δ3 isomer) and IXb using sodium hydroxide in aqueous methanol at −20°C (according to the procedure given by Christensen [4]) led to the formation of mostly lactone VIII and many decomposition products lacking a β-lactam ring.

The IR spectra of the products synthesized are given in Table 2. The synthesis of VI, IXa, and IXb, which are N-Boc-O-*tert*-butyl-protected 7-ACA with different oxidation states of the sulfur atom, permitted us to identify the vibrational frequencies for the SO and SO_2 groups at 1370-1040 cm^{-1}, which is complicated by the presence of several bands at the same position but differing in intensity.

EXPERIMENTAL

The melting points of the products were determined on a Boetius microheating table. The PMR spectra were taken on a Bruker WH-90 spectrometer in $CDCl_3$ and DMSO-d_6 with TMS as the internal standard. The IR spectra were taken on a Perkin−Elmer 580B spectrometer. A Carlo Erba 1108 instrument was used for the elemental analysis. The reaction course and purity of the products were monitored by thin-layer chromatography on Silufol UV-254 plates. Kieselgel (0.063-0.230) was used for the preparative column chromatography. The spectral indices are given in Tables 1 and 2.

The elemental analysis data for VI (Δ2 isomer), VII, IXa, and IXb for C, H, and N corresponded to the calculated values.

7-*tert*-Butoxycarbonylaminocephalosporanic acid (IV). A suspension consisting of 2.0 g (7.4 mmoles) 7-ACA (III), 2.0 ml triethylamine, and 10 ml CH_2Cl_2 was stirred until a clear solution was formed. The solvent was evaporated at reduced pressure and the residue was dissolved in 10 ml CH_2Cl_2. A sample of 4.8 g (21 mmoles) di-*tert*-butylpyrocarbonate was added to this solution and the mixture was stirred for 24 h at room temperature, monitoring the reaction by thin-layer chromatography with 1:1 toluene-ethyl acetate as the eluent. The solution was then washed with 5% hydrochloric acid and evaporated at reduced pressure. The residue was dissolved in 30 ml ethyl acetate, mixed with 30 ml water, and neutralized by adding aqueous ammonium hydroxide to pH 8. The aqueous phase was removed, cooled to from 0 to −5°C, mixed with 30 ml ethyl acetate, and acidified by adding 5% hydrochloric acid. The organic phase was removed, washed with water, dried over anhydrous Na_2SO_4, and evaporated at reduced pressure. The residue was subjected to chromatography on a kieselgel column with 1:1

TABLE 1. Spectral Indices of Cephalosporins

Compound	Isomer	PMR spectrum, δ, ppm, J, H_2										
		Solvent	*t*-BuOCO	COOBu-*t*	$OCOCH_3$	SCH_2	C_4—H	CH_2O	C_6—H	C_7—H	NH	SCH=
IV	Δ3	DMSO-d_6	1,40 (9H, s)		2,04 (3H, s)	3,42, 3,64 (ABq, *J* = 16)		4,68, 4,97 (1H, ABq, *J* = 16)	5,04 (1H, *J* = 5)	5,40, 5,51 (1H, d. d, *J* = 5, *J* = 10)	7,93 (1H, d, *J* = 10)	
IV	Δ2	DMSO-d_6	1,46 (9H, s)		1,98 (3H, s)		4,82 (1H, s)	4,71, 5,00 (2H, ABq, *J* = 17)	5,04 (1H, *J* = 5)	5,40, 5,51 (1H, d. d, *J* = 5, *J* = 10)	7,93 (1H, d, *J* = 10)	6,68 (1H, s)
VI	Δ3	DMSO-d_6	1,42 (9H, s)	1,46 (9H, s)	2,00 (3H, s)	3,44, 3,68 (ABq, *J* = 18)		4,57, 4,93 (2H, ABq, *J* = 14)	5,06 (1H, *J* = 5)	5,44, 5,53 (1H, d. d, *J* = 5, *J* = 8)	7,97 (1H, d, *J* = 8)	
VI	Δ2	DMSO-d_6	1,37 (9H, s)	1,42 (9H, s)	2,00 (3H, s)		4,82 (1H, s)	4,53, 4,71 (2H, ABq, *J* = 13)	5,13 (1H, *J* = 4)	5,44...5,28 (1H, m)	7,93 (1H, d)	6,71 (1H, s)
VII	Δ2	$CDCl_3$	1,44 (9H, s)	1,46 (9H, s)			4,95 (1H, s)	4,22 (2H, s)	5,11...5,49 (3H, m)			6,31 (1H, s)
VIII	Δ3	DMSO-d_6	1,38 (9H, s)			3,77 (2H, s)		5,06 (2H, s)	5,11 (1H, *J* = 5)	5,60, 5,69 (1H, d, d, *J* = 5, *J* = 8)	8,06 (1H, d, *J* = 8)	
IXa	Δ3	DMSO-d_6	1,42 (9H, s)	1,48 (9H, s)	2,00 (3H, s)	3,57, 3,95 (ABq, *J* = 19)		3,57, 3,95 (2H, ABq, *J* = 19)	4,91 (1H, *J* = 4)	5,63, 5,74 (1H, d. d, *J* = 4, *J* = 10)	6,24 (1H, d, *J* = 10)	
IXb	Δ3	DMSO-d_6	1,41 (9H, s)	1,47 (9H, s)	2,00 (3H, s)	3,45, 4,22 (ABq, *J* = 17)		4,55, 4,22 (2H, ABq, *J* = 17)	4,82 (1H, *J* = 4)	5,40, 5,51 (1H, d. d, *J* = 4, *J* = 9)	8,06 (1H, d, *J* = 9)	

TABLE 2. IR Spectra of IV-IX

Compound	Isomer	IR spectrum, ν, cm^{-1}
IV	Δ3	1790 (β-lactam), 1760, 1740, 1720 (OCOMe, *t*-BuOCONH, COOH)
VI	Δ3	1810 (β-lactam), 1770, 1760, 1720 (OCOMe, *t*-BuOCONH, COOBu-*t*)
VI	Δ2	1780 (β-lactam), 1740, 1720 (OCOMe, *t*-BuOCONH, COOBu-*t*)
VII	Δ2	1780 (β-lactam), 1720, 1700 (*t*-BuOCONH, COOBu-*t*)
VIII	Δ3	1780 (β-lactam), 1720 (*t*-BuOCONH, γ-lactone
IXa	Δ3	1790 (β-lactam), 1740, 1720 (OCOMe, *t*-BuOCONH, COOBu-*t*), 1045 (S=O)
IX b	Δ3	1790 (β-lactam), 1740, 1720 (OCOMe, *t*-BuOCONH, COOBu-*t*), 1160, 1370 (O=S=O)

toluene–ethyl acetate as the eluent. The fractions with R_f 0.84 were combined and evaporated to give 2.0 g (73%) IV (Δ3 isomer) with spectral indices (Tables 1 and 2) identical to literature data [8].

The chromatographic fraction with R_f 0.92 obtained upon chromatography is a mixture of the Δ2 and Δ3 isomers of IV with readily identifiable PMR signals (see Table 1).

***tert*-Butyl ester of 7-*tert*-butoxycarbonylaminocephalosporanic acid (VI, Δ2 isomer, $C_{19}H_{28}N_2O_7 \cdot 0.25C_6H_{14}$).** A suspension consisting of 6.9 g (18 mmoles) IV (Δ3 isomer) in 100 ml CH_2Cl_2 and 100 mmoles N,N'-dicyclohexyl-O-*tert*-butylisourea (V) (as a mixture obtained from 20.6 g dicyclohexylcarbodiimide, 7.4 g *tert*-butyl alcohol, and 0.2 g CuCl in 100 ml CH_2Cl_2 maintained over 48 h) was stirred at room temperature for 24 h. The reaction mixture was filtered, washed with 5% hydrochloric acid, 5% aq. sodium bicarbonate, and water, dried over anhydrous sodium sulfate, and evaporated at reduced pressure to give 4.8 g (61%) VI as an 85:15 mixture of Δ2 and Δ3 isomers, which was used in the subsequent alcoholysis. A sample of 0.3 g of this product was subjected to chromatography on a kieselgel column using 1:2 hexane–ethyl acetate as the eluent. The fractions with R_f 0.95 were combined and evaporated to give 0.15 g VI (Δ2 isomer) with mp 75-76°C. The spectral indices of this compound are given in Table 1.

***tert*-Butyl ester of 7-*tert*-butoxycarbonylamino-3-hydroxyceph-2-em-4-carboxylic acid (VII, $C_{17}H_{26}N_2O_6S \cdot 0.25C_6H_{14}$).** A solution of 4.3 g (10 mmoles) VI as an 85:15 mixture of the Δ2 and Δ3 isomers, 4.3 g (15 mmoles) titanium isopropylate (IV), and 50 ml *tert*-butyl alcohol was heated at reflux for 6 h. The reaction end-point was determined using thin-layer chromatography with 1:1 hexane–ethyl acetate as the eluent. The reaction mixture was evaporated and the residue was dissolved in 100 ml ethyl acetate. The solution obtained was washed with 5% hydrochloric acid, 5% aq. sodium bicarbonate, and water, dried over anhydrous sodium sulfate, and evaporated at reduced pressure. The residue was subjected to chromatography on a kieselgel column using 1:1 hexane–ethyl acetate as the eluent. The fractions with R_f 0.58 were combined and evaporated to give 1.5 g 6-*tert*-butoxycarbonylamino-5a,6-dihydro-3H,7H-azeto[2,1-*b*]furo[3,4-*d*]thiazine-1,7-dione (VIII) as indicated by spectral data (Tables 1 and 2).

***tert*-Butyl ester of 7-*tert*-butoxycarbonylaminocephalosporanic acid sulfoxide (IXa, $C_{19}H_{28}N_2O_8S$).** A sample of 15 ml 25% hydrogen peroxide and 30 mg sodium tungstate were added to a solution of 2.0 g (4.7 mmoles) VI as a mixture of Δ2 and Δ3 isomers in 20 ml acetonitrile. The mixture was stirred at room temperature for 48 h, diluted with 50 ml ethyl acetate, washed with water, dried over anhydrous sodium sulfate, and evaporated at reduced pressure. The residue was subjected to chromatography on a kieselgel column using 1:1 hexane–ethyl acetate as the eluent. The fractions with R_f 0.4 were combined and evaporated to give 0.3 g (14%) IXb, mp 87-89°C. The spectral characteristics of this compound are given in Tables 1 and 2.

***tert*-Butyl ester of 7-*tert*-butoxycarbonylaminocephalosporanic acid sulfone (IXb, $C_{19}H_{28}O_9S \cdot 0.25CH_3CN$)** was obtained analogously to IXa by the oxidation of XI. The fractions with R_f 0.24 were combined and evaporated to give 1.4 g (64%) IXb, mp 97-99°C. The spectral indices of this sample were identical to those reported by Durekheimer et al. [7].

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