PREPARATION OF Δ^3-7α-PHENYLACETAMIDODESACETOXY-CEPHALOSPORANIC ACID

P. G. CLAES, G. DECOSTER, L. A. KERREMANS and H. VANDERHAEGHE

Rega Institute and Pharmaceutical Institute, University of Leuven, B–3000 Leuven, Belgium

(Received for publication April 14, 1979)

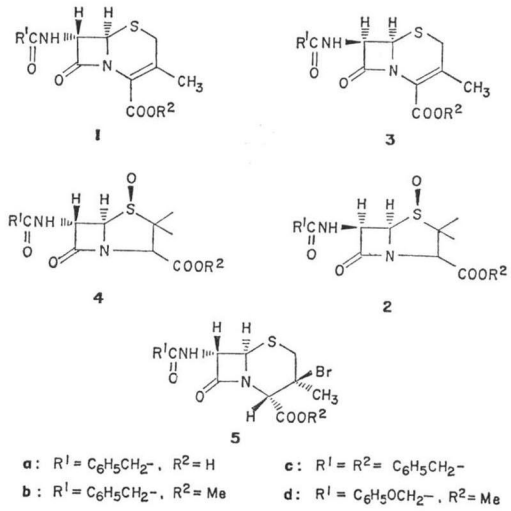
Δ^3-7α-Phenylacetamidodesacetoxycephalosporanic acid was prepared by ring expansion of 6-*epi*-benzylpenicillin-*S*-sulfoxide, using *N*,*O*-bis(trimethylsilyl)acetamide (BSA) as silylating and dehydrating agent and α-picoline/α-picoline hydrobromide as catalyst. In some experiments 7α-phenylacetamido-3β-bromo-3α-methylcepham-4α-carboxylic acid was obtained as a side product. 7-Epimers in the desacetoxycephalosporanic series were also prepared by base-catalyzed epimerization of the benzyl 7β-(*p*-nitrobenzylideneimino)desacetoxycephalosporanate and of the *S*-sulfoxide of natural methyl 6-phenylacetamidodesacetoxycephalosporanate. In both reactions 1,5-diazabicyclo(4.3.0)non-5-ene (DBN) was used as epimerization catalyst.

The present report deals with the preparation of Δ^3-7α-phenylacetamidodesacetoxycephalosporanic acid (**1a**), which was needed in the course of a study of the substrate specificity of *Escherichia coli* penicillin acylase[1]. 7-Epimers in the cephalosporanic acid series have been prepared by acid-catalysed ring expansion of 6-*epi*-penicillins[2,3], by epimerization of cephalosporanic acid derivatives with a natural configuration[4~6], and also by total synthesis[7]. Only the first two methods are investigated in this study in view of the preparation of **1a**. Ring expansion will be considered first.

The conversion of penicillin sulfoxides (**2**) into Δ^3-desacetoxycephalosporins (**3**) by an acid-catalysed ring expansion was originally reported by MORIN *et al.*[8] Reviews on the chemical interconversion of β-lactam antibiotics were published by COOPER *et al.*[9,10] The ring expansion was also described for 6-*epi*-penicillins[2,3], as mentioned earlier. Since the rearrangement of a free acid of penicillin sulfoxide into desacetoxycephalosporanic acid proceeds with extensive decarboxylation[8], the reaction is limited to esters. Thus procedures, described upto 1974, did not permit the preparation of a free acid or an alkali salt of Δ^3-desacetoxycephalosporins in a single step. In 1975 DE KONING *et al.*[11] reported a procedure in which the rearrangement is carried out on the free acid of benzylpenicillin sulfoxide (**2a**), using *N*,*O*-bis(trimethylsilyl)acetamide (BSA) as silylating- and dehydrating agent and α-picoline and its hydrobromide as catalyst. By this procedure Δ^3-7α-phenylacetamidodesacetoxycephalosporanic acid (**3a**) is obtained in a single step in high yield (78%). Another one-step procedure has been described by MIKOLAJCZYK *et al.*[12]

The procedure described by DE KONING *et al.* was applied for the preparation of **1a**. It should be mentioned that the starting material for this compound, the free acid of 6-*epi*-benzylpenicillin-*S*-sulfoxide[13] (**4a**), is a monohydrate (as shown by NMR). Therefore it is necessary to increase the ratio BSA-penicillin from ~3 : 1 (as described in the original method) to ~4 : 1. It was also observed that the ring expansion of **4a** is faster than that of the epimer with natural configuration **2a**. After heating for 60~90 minutes at 100°C no starting sulfoxide (**4a**) was found to be present in the reaction mixture and **1a** was formed in a 60% yield. Prolonged heating of **4a** up to 6 hours, as described for the preparation

of **3a**, gave a complex mixture which consisted mainly of more polar degradation products. Neither the free acid of **1a** nor its K or Na salt could be obtained in a crystalline state. Therefore the crude **1a** was purified by column chromatography on silica gel and isolated as its Na-salt. This purification however proceeded with considerable loss of **1a**. Spectral data of **1a** and of its methyl ester **1b** are in agreement with the proposed structure.

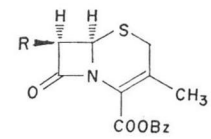


a: $R^1 = C_6H_5CH_2-$, $R^2 = H$ c: $R^1 = R^2 = C_6H_5CH_2-$
b: $R^1 = C_6H_5CH_2-$, $R^2 = Me$ d: $R^1 = C_6H_5OCH_2-$, $R^2 = Me$

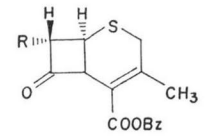
In some preparations of **1a**, which were carried out with BSA of inferior quality (partially hydrolyzed to *N*-trimethylsilylacetamide), a crystalline side product was isolated in a yield of 5~10%. The compound was obtained in a 20% yield when the rearrangement was carried out with a ratio BSA-**4a** of 3: 1. It was identified as 7α-phenylacetamido-3β-bromo-3α-methylcepham-4α-carboxylic acid (**5a**). The structure is based on elemental analysis and spectral data obtained for **5a** and for its methyl and benzyl ester (**5b** and **5c**). The NMR spectra showed a coupling constant of 14.5 Hz for the geminal CH_2, which is a typical value for a halocepham structure[14,15] (the ^2J value for an isomeric halogenopenam is about 12 Hz[14,15]). The assumed β-bromo configuration is based on mechanistic considerations discussed by KUKOLJA *et al.*[16] and the reaction is supposed to be similar to that described by KAMIYA *et al.*[17] These authors obtained a mixture of a 2-chloromethylpenam and **3c** upon treatment of **2c** with pyridine-HCl and pyridine in tetrachloroethane. The cepham structure proposed for **5a** is not in disagreement with the experiment described by KAMIYA *et al.*, since it is known[16,17] that 2-bromopenams easily rearrange into the more stable 3-bromocephams.

Another approach for the preparation of **1a** is the epimerization of esters of **3a** and of its sulfoxides. It should be mentioned that epimerization at C-7 of cephalosporanic acid derivatives has not been studied to the same extent as that of penicillins. To our knowledge epimerization in the former series has been described only in three reports[4~6]. One of the problems encountered during base-catalyzed epimerization of cephalosporanates and desacetoxycephalosporanates is the isomerization of the double bond[18]. It was found, that a treatment of **3a** or its esters with BSA/1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in the conditions described for the epimerization of penicillin[19] afforded a mixture of Δ^3 and Δ^2 isomers in a ratio of 4: 1. No 7-epimers were found to be present in the reaction mixture.

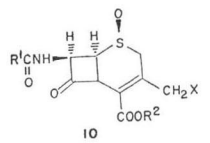
It has been reported by KIM *et al.*[6] that a SCHIFF base of the benzhydryl ester of 7β-aminocephalosporanic acid can be epimerized under certain reaction conditions without migration of the double bond. Thus a method similar to that described by KIM *et al.* was investigated in view of the preparation of **1c**. A solution of the SCHIFF base **7** in CH_2Cl_2 was epimerized at room temperature for one minute in the presence of 0.25 equivalent of DBN. The nmr spectrum showed that the reaction mixture consisted of **7** and its epimer **8** in a ratio of about 4: 6. Decomposition of the SCHIFF bases according to FIRESTONE *et al.*[5], followed by phenylacetylation, gave a mixture of **3c** and **1c** from which the latter was isolated by column chromatography in a 25% yield (based on **7**). Compound **1c** was identical in all aspects to that obtained by reaction of **1a** with phenyldiazomethane. Similar results were obtained when



6 R = NH_2-
7 R = O_2N-C_6H_4-CH=N-



8 R = NH_2-
9 R = O_2N-C_6H_4-CH=N-



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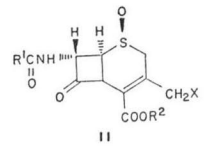

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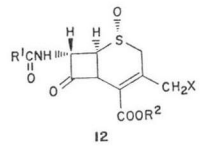

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a: R^1 = $C_6H_5CH_2$-, R^2 = Me, X = H
b: R^1 = 2-Thienyl-CH_2-, R^2 = H, X = OCOCH$_3$
c: R^1 = 2-Thienyl-CH_2-, R^2 = Me, X = OCOCH$_3$
d: R^1 = $C_6H_5OCH_2$-, R^2 = Me, X = H

7 was epimerized with diisopropyl ethyl amine in tetrahydrofuran at 0°C as described in the original procedure of KIM *et al.* No migration of the double bond was observed in both experiments.

Finally the preparation of **11a** was attempted by epimerization of the *S*-sulfoxide **10a** (**11a** can be easily converted into **1b** by reduction of the sulfoxide function[20]). It has been reported by SASSIVER *et al.*[4] that the 9-fluorenyl ester of **10b** can be epimerized by heating in DMSO in the presence of 0.2 equivalent of triethylamine. Isomerization of the double bond does not occur during this reaction, because Δ^3-cephem sulfoxides are thermodynamically more stable than Δ^2-cephem sulfoxides[20]. Since the yield of the 7-epimer is not given in the original paper, the epimerization was repeated using the methylester **10c** as a starting material. After column chromatography the 7-epimer **11c** was isolated in a 19%, **10c** in a 16.5% yield. When **10a** is treated in a similar way, no 7-epimer was found to be present. Therefore a stronger base such as DBN (1 equivalent) was taken as epimerization catalyst. To avoid extensive degradation, the epimerization was carried out at 0°C in a 3: 1 mixture of CH_2Cl_2–DMSO. Preliminary experiments, in which the progress of the reaction was monitored by tlc, showed that a maximum amount of the 7-epimer was obtained after about 45 minutes in the case of the cephalosporanate **10c**, and 3 hours for the desacetoxycephalosporanate **10a**. Thus the epimerization of **10a** proceeds more slowly than that of **10c**. This is undoubtly related to a lower acidity of H-7 in **10a**, which may be caused by the nature of the side chain, or by the absence of the acetoxy function. The fact that the epimerization of the parent **10d***, with a phenoxyacetyl side chain, proceeds about three to four times faster than that of **10a** (as estimated by tlc monitoring of epimerization experiments conducted under identical conditions) seems to indicate, that the difference is rather due to the nature of the side chain. The epimerizations described above were also conducted on a preparative scale. The yields observed after column chromatography of the reaction mixtures were 20% epi, 23% natural, starting from **10c**, and 13% epi, 46% natural, starting from **10a**. It should be noted that a considerable amount of the starting material or its 7-epimer is degraded during these epimerizations. Thus the ratios of epi: natural configurations does not necessarily give equilibrium mixture.

The epi-sulfoxide **11a** was also prepared by oxidation of **1b** with *m*-chloroperbenzoic acid. The reaction gave two sulfoxides in a ratio 1: 2, which can be differentiated by tlc. The major component, which showed the lowest Rf value, was isolated in a crystalline state. It was found to be identical in all aspects to the sulfoxide which was formed during the epimerization of the *S*-sulfoxide **10a**. Thus the configuration of the two sulfoxides, obtained by oxidation of **1b**, is established; the more polar be-

* **10d** was obtained by oxidation of **1d**[8] with *m*-chloroperbenzoic acid using the reaction conditions described for the preparation of **10a**.

ing the *S*- and the less polar the *R*-sulfoxide.

The experiments presented in this paper show that both epimerization procedures are less convenient for the preparation of **1a** or is esters than ring-enlargement of the sulfoxide of 6-*epi*-benzylpenicillin.

Experimental

Melting points were determined with a Büchi-Tottoli apparatus and are uncorrected. Solvents were evaporated under reduced pressure below 30°C, unless otherwise stated. Tlc was performed on silica gel F-254 plates (Merck) using the following solvent systems: I, C_6H_6 - Me_2CO - HOAc (60: 38: 2); II, C_6H_6 - Me_2CO (80: 20); III, C_6H_6 - Me_2CO (90: 10); IV, CH_2Cl_2 - Me_2CO (80: 20). Spots were located by UV-illumination and exposure to iodine vapour. Ir spectra were determined on a Perkin-Elmer 257 spectrometer. Mass spectra were recorded on a AEI MS 12 apparatus and nmr spectra on a Varian XL-100 or a Hitachi-Perkin Elmer R24 apparatus with tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSSA) as internal standard.

Δ^3-7α-Phenylacetamidodesacetoxycephalosporanic acid (**1a**) (Na-salt)

To a suspension of 3.15 g (8.56 mmol) 6-*epi*-benzylpenicillin sulfoxide (**4a**) monohydrate[13] in 95 ml of dioxane was added 9.8 ml (39.6 mmol) BSA, 18 ml (18.56 mmol) of α-picoline and 1.53 ml (8.87 mmol) of a 5.8 M solution of α-picoline hydrobromide in CH_2Cl_2. The reaction mixture was refluxed for 90 minutes, cooled to room temperature and poured into 400 ml of ice-water. EtOAc (250 ml) was added and the pH of the stirred mixture was adjusted to pH 7, by addition of NaOH. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined EtOAc layers were extracted with 75 ml of a 0.75 M potassium phosphate buffer (pH 7). To the combined aqueous layer and the phosphate buffer was added 500 ml EtOAc, the pH of the stirred mixture was adjusted to pH 2 by addition of HCl and the organic layer was separated. After a second extraction with EtOAc, the two organic layers were combined, dried (Na_2SO_4) and evaporated under reduced pressure yielding 2.52 g of the crude **1a** (free acid) as a yellow oil. Tlc showed a main spot at Rf 0.39 (solvent system I). The purity estimated by conversion of an aliquot into its methyl ester (by reaction with CH_2N_2, see following section) was 75%. After correction for purity the yield is about 60%.

The crude free acid (2.52 g) was purified by column chromatography on 200 g of silica gel (70~230 mesh), using benzene - acetone - HOAc, 70: 30: 0.5 as an eluant. Fractions containing **1a** were pooled and evaporated under reduced pressure. The residue was dissolved in EtOAc, H_2O was added and the stirred suspension was adjusted to pH 7 with NaOH. The aqueous layer was concentrated and freeze-dried yielding 1.05 g (2.96 mmol, 34% based on **4a**) on the Na-salt of **1a**, $[\alpha]_D^{25}$ +78.3° (*c* 1, H_2O). IR (KBr), ν_{max} 1750 (β-lactam), 1665 (amide), 1600, 1400 (COO^-) cm^{-1}. NMR (100 Mc, D_2O, DSSA) δ 1.88 (Me), 3.1 and 3.44 (d, J=18 Hz, CH_2), 3.64 (s, CH_2CO), 4.7 and 4.8 (d, J=2Hz, H-6 and H-7, one of these doublets is hidden under the HOD signal at 4.7), 7.3 (C_6H_5) ppm. UV $\lambda_{max}^{H_2O}$ 258 nm (ϵ 8030).

Methyl Δ^3-7α-phenylacetamidodesacetoxycephalosporanate (**1b**)

A suspension of 1.35 g of the crude free acid of **1a** (described in the previous section) in 15 ml CH_2Cl_2 was treated with a slight excess of CH_2N_2 in ether. The reaction mixture was evaporated and the residual oil was crystallized from CH_2Cl_2-ether, yielding 910 mg of **1b**, mp 142~144°C (dec.). $[\alpha]_D^{25}$ +23.5° (*c* 1, acetone). IR (KBr) ν_{max} 1770 (β-lactam), 1740 (ester), 3280, 1660 (amide) cm^{-1}. NMR ($CDCl_3$-DMSO-d_6, 4: 1) δ 2.02 (s, CH_3), 3.18 and 3.45 (AB pattern, CH_2), 3.52 (s, CH_2CO), 3.7 (s, $COOCH_3$), 4.6 (d, J=1.8 Hz, H-6), 4.85 (dd, J=1.8 and 8 Hz, H-7), 7.25 (s, C_6H_5) and 8.9 (d, J=8 Hz, NH) ppm. UV λ_{max}^{MeOH} 264 nm (ϵ 7430). MS *m/e* 346 (M^+). Tlc (system II) Rf 0.57.

Evaporation of the filtrate and column chromatography of the residue on silica gel (solvent system benzene - acetone 90: 10) afforded another 155 mg of the methyl ester. This brings the total amount of this compound to 1.065 g (3.06 mmol), which means that the starting material is at least 75% pure.

Benzyl Δ^3-7α-phenylacetamidodesacetoxycephalosporanate (**1c**)

A suspension of 1.5 g of crude free acid of **1a** in 15 ml CH_2Cl_2 was treated with a slight excess of

$C_6H_5CHN_2$ in ether. Purification by column chromatography on 50 g silica gel using benzene-acetone 90: 10 as a solvent system and crystallization for CH_2Cl_2-Et_2O afforded 925 mg (2.19 mmol) of **1c**, mp 158~162°C (dec.). $[\alpha]_D^{25}$ +36.5° (*c* 1, acetone). IR (KBr) ν_{max} 1780 (β-lactam), 1740 (ester), 3200, 1650 (amide) cm^{-1}. NMR ($CDCl_3$) δ 2 (s, CH_3), 3.1 and 3.3 (AB pattern, J=18 Hz, CH_2), 3.5 (s, CH_2CO), 4.54 (d, J=1.8 Hz, H-6), 4.81 (dd, J=1.8 and 7 Hz, H-7), 6.85 (d, J=7 Hz, NH), 7.25 and 7.3 (C_6H_5) ppm. MS *m/e* 422 (M^+). Tlc (solvent system II) Rf=0.71.

7α-Phenylacetamido-3β-bromo-3α-methylcepham-4α-carboxylic acid (**5a**)

The monohydrate of **4a** (2.86 g, 7.77 mmol) was reacted in dioxane with BSA (6.98 ml, 28.2 mmol), α-picoline (16.5 ml, 16.91 mmol), and α-picoline hydrobromide (8 mmol) in the conditions described for the preparation of **1a**. The reaction mixture was worked up as described for **1a** and residual oil was crystallized from 35 ml EtOAc yielding 640 mg (1.55 mmol, 20% yield), mp 134~136°C (dec.). $[\alpha]_D^{25}$ −44° (*c* 1, acetone). Tlc (solvent system I) Rf=0.39. IR (KBr) ν_{max} 1780 (β-lactam), 1750, 1720 (COOH), 3250, 1660 (amide) cm^{-1}. NMR ($CDCl_3$-DMSO-d_6 4: 1) δ 1.92 (s, CH_3) 2.66 and 3.6 (d, J=14 Hz, CH_2), 3.5 (s, CH_2CO), 4.65 (s, CHCOOH), 4.8 (dd, J=1.5 and 7 Hz, H-7), 4.95 (d, J=1.5 Hz, H-6), 7.25 (s, C_6H_5), 8.38 (d, 7 Hz, NH), 9.45 (br.s, COOH) ppm.

Evaporation of the filtrate and reaction of the residual oil with CH_2N_2 afforded 500 mg (1.4 mmol, 18% yield) of crystalline **1b**.

Methyl 7α-phenylacetamido-3β-bromo-3α-methylcepham-4α-carboxylate (**5b**)

A suspension of **5a** (100 mg, 0.24 mmol) in CH_2Cl_2 was treated with a solution of CH_2N_2 in ether. The residue obtained after evaporation of the reaction mixture was crystallized from CH_2Cl_2-Et_2O yielding 80 mg (0.19 mmol, 78%) **5b**, mp 159~160°C (dec.). $[\alpha]_D^{25}$ −49.5° (*c* 1, acetone). IR (KBr) ν_{max} 1775 (β-lactam), 1740 (ester), 3260, 1655 (amide) cm^{-1}. NMR ($CDCl_3$-DMSO-d_6 4: 1), δ 1.9 (s, CH_3), 3.00 and 3.5 (d, J=15 Hz, CH_2), 3.5 (s, -CH_2CO), 3.76 (s, $COOCH_3$), 4.75 (s, CH-$COOCH_3$), 4.80 (dd, J=1.5 and 8 Hz, H-7), 4.93 (d, J=1.5 Hz, H-6), 7.25 (s, C_6H_5), 8.75 (d, J=8 Hz, NH) ppm. Tlc (system II) Rf=0.57.

Benzyl 7α-phenylacetamido-3β-bromo-3α-methylcepham-4α-carboxylate (**5c**)

A suspension of 1.09 g (2.64 mmol) of the **5a** in CH_2Cl_2 was treated with a slight excess of phenyldiazomethane in Et_2O. The residue obtained after evaporation of the reaction mixture was crystallized from CH_2Cl_2-Et_2O yielding 900 mg **5c** (1.79 mmol, 67%), mp 127~128°C (dec.). $[\alpha]_D^{25}$ −48.3° (*c* 1, acetone). IR (KBr) ν_{max} 1780 (β-lactam), 1740 (ester), 3280, 1655 (amide) cm^{-1}. NMR ($CDCl_3$), δ 1.75 (s, CH_3), 2.6 and 3.45 (d, J=15 Hz, CH_2), 3.56 (s, CH_2CO), 4.8 (s, CH-COOBz), 4.8 (dd, J= 1.5 and 7 Hz, H-7), 5.00 (d, J=1.5 Hz, H-6), 5.15 (AB pattern, $CH_2C_6H_5$), 6.65 (d, J=7Hz, NH), 7.25 and 7.31 (s, C_6H_5) ppm. MS *m/e* 502 (M^+), 422 (M^+−HBr). Tlc (solvent system II) Rf=0.78.

Elemental analysis. Calcd. for $C_{23}H_{23}BrN_2O_4S$: C, 54.87; H, 4.61; N, 5.57.
Found : C, 54.85; H, 4.77; N, 5.51.

Benzyl Δ^3-7β-phenylacetamidodesacetoxycephalosporanate (**3c**)

A solution **3a**[21] (3.32 g, 10 mmol) in 50 ml anhydrous DMF, containing Et_3N (1.4 ml, 10 mmol) and benzyl bromide (1.19 ml, 10 mmol), was stirred for 4 hours at room temperature. The reaction mixture was poured under stirring into ice-water and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed (with 5% $NaHCO_3$ and water), dried (Na_2SO_4) and evaporated to a light yellow oil, which was crystallized from Et_2O yielding 3.16 g (75%) of **3c**, mp 166.5~167°C. $[\alpha]_D^{25}$ +95° (*c* 1, acetone). IR (KBr) ν_{max} 1770 (β-lactam), 1725 (ester), 3285, 1660 (amide) cm^{-1}. NMR ($CDCl_3$, TMS) δ 2.05 (s, Me), 3.15 and 3.35 (d, J=17 Hz, CH_2), 3.55 (s, CH_2CO), 4.85 (d, J=4.5 Hz, H-6), 5.20 (s, OCH_2), 5.70 (dd, J=4.5 and 7.5 Hz, H-7), 6.90 (d, J=7.5 Hz, NH), 7.25 and 7.30 (s, C_6H_5) ppm. MS *m/e* 422 (M^+).

Benzyl Δ^3-7β-aminodesacetoxycephalosporanate (**6**)

The phenylacetyl side chain of **3c** (2.11 g, 5 mmol) was cleaved using the reaction conditions described by CHAUVETTE *et al.*[21] for the parent *p*-methoxybenzylester (imino chloride method). The title compound was isolated as its *p*-toluenesulfonic acid salt, mp 149~150°C (dec.), in a 50% yield. IR (KBr) ν_{max} 3100 (NH_3^+), 1790 (β-lactam), 1725 (ester) cm^{-1}. NMR (DMSO-d_6, TMS) δ 2.08 (s, CH_3),

2.28 (s, $C_6H_4CH_3$), 3.50 (s, CH_2), 5.10 (br.s., OCH_2, H-6 and H-7), 7.23 (s, C_6H_5), 7.03 and 7.53 (d, J= 8 Hz, C_6H_4) ppm.

Benzyl Δ^3-7β-(*p*-nitrobenzylidene)desacetoxycephalosporanate (**7**)

A suspension of the *p*-toluenesulfonic acid salt of **6** (4.76 g, 10 mmol) in H_2O–Et_2O was adjusted to pH 7.5~8 with 1 N NaOH. The ethereal solution was dried (Na_2SO_4) and evaporated to dryness. The residual oil was taken up in 100 ml anhydrous benzene, treated with *p*-nitrobenzaldehyde (1.5 g, 10 mmol) and refluxed for 2 hours under a water separator. After evaporation of the solvent, **7** was crystallized from benzene-ether yielding 3.49 g (80%), mp 174~176°C (dec.). IR (KBr) ν_{max} 1770 (β-lactam), 1730 (ester), 1640 (-CH=N-) cm^{-1}. NMR ($CDCl_3$, TMS) δ 2.1 (s, CH_3), 3.20 and 3.50 (d, J= 18 Hz, CH_2), 5.12 (d, J=4.5 Hz, H-6), 5.25 (s, OCH_2), 5.4 (dd, J=4.5 Hz and 1.5 Hz, H-7), 7.30 (C_6H_5), 7.90 and 8.22 (d, J=8 Hz, C_6H_4), 8.7 (d, J=1.5 Hz, -CH=) ppm. MS *m/e* 437 (M^+).

Epimerization of **7** in the presence of DBN

To a stirred solution of **7** (3.49 g, 8 mmol) in 60 ml CH_2Cl_2 was added DBN (0.248 ml, 2 mmol). The reaction mixture was stirred for one minute at room temperature and poured into ice-water (40 ml) containing 2 mmol HOAc. The organic layer was separated, washed with water (3×30 ml), dried (Na_2SO_4), and concentrated, yielding 3.4 g of a crude mixture of **7** and **9** in a ratio of 4: 6 (determined by NMR). Following signals observed in the spectrum ($CDCl_3$, TMS) of this mixture were assigned to **9**: δ 2.10 (s, CH_3), 3.20 and 3.50 (d, J=18 Hz, CH_2), 4.85 (dd, J=1.2 Hz and 1.2 Hz, H-7), 4.90 (d, J= 1.2 Hz, H-6), 5.25 (s, OCH_2), 7.30 (s, C_6H_5), 7.90 and 8.22 (d, J=8 Hz, C_6H_4), 8.51 (d, J=1.2 Hz, CH= N) ppm.

Treatment of the reaction mixture with dinitrophenylhydrazine and *p*-toluenesulfonic acid monohydrate in ethanol, following a procedure described by FIRESTONE *et al.*[5], afforded a mixture of **6** and **8**. Solutions of Et_3N (0.585 ml, 8 mmol) and phenylacetyl chloride (1.24 g, 8 mmol) in CH_2Cl_2 (15 ml each) were added gradually (in 1 hour) to a cooled (0°C) solution of **6** and **8** in CH_2Cl_2 (50 ml). After storage for 2 hours at 0°C the CH_2Cl_2 solution was washed (with 0.05N HCl, 5% $NaHCO_3$ and H_2O) dried and evaporated, yielding a mixture of **3c** and **1c**. Tlc (system III) of the mixture showed two main spots (Rf=0.33 and 0.17). The more polar one was assigned to **1c**. Separation of **3c** and **1c** was obtained by column chromatography on 100 g silica gel using a gradient of CH_2Cl_2 to CH_2Cl_2-acetone (98: 2). This afforded 800 mg **3c** and 850 mg **1c** (2.01 mmol). Physical constants and spectral data of **1c** were identical to those given in a previous section.

Methyl Δ^3-7β-(2-thienylacetamido)cephalosporanate *S*-sulfoxide (**10c**)

Treatment of a CH_2Cl_2 solution of the sulfoxide **10b*** with a slight excess of CH_2N_2, yielded **10b**, mp 210~213°C (crystals from EtOH), $[\alpha]_D^{25}$ +167.5° (*c* 1, DMSO). IR (KBr) ν_{max} 1785 (β-lactam), 1735 (acetate, ester), 1655, 1540 (amide), 1040 (S=O) cm^{-1}. NMR (DMSO-d_6, TMS, 100 Mc) δ 2.00 (s, $COCH_3$), 3.6 and 3.88 (d, J=19 Hz, SCH_2), 3.78 (s, CH_2 side chain), 3.82 (s, OCH_3), 4.6 and 5.1 (d, J=12 Hz, CH_2OAc), 4.88 (d, J=5 Hz, H-6), 5.8 (dd, J=5 Hz and 9 Hz, H-7), 6.8 to 7.4 (m, thienyl protons), 8.35 (d, J=9 Hz, NH) ppm. MS *m/e* 426 (M^+).

Epimerization of methyl Δ^3-7β-(2-thienylacetamido)cephalosporanate *S*-sulfoxide (**10c**)

(a) With triethylamine in DMSO

A solution of **10c** (212.5 mg, 0.5 mmol) in 10 ml DMSO containing 0.1 mmol triethylamine was heated at 50°C for 48 hours. The cooled reaction mixture was poured into 20 ml water, adjusted to pH 3 with HCl and extracted three times with CH_2Cl_2. The combined organic layer was washed several times with water (to eliminate most of the DMSO), dried and evaporated to an oil. Tlc (solvent system IV) showed the presence of the starting material (Rf=0.61) of its 7-epimer (Rf=0.25) and of more polar degradation products. Separation by column chromatography on a silica gel column (solvent system CH_2Cl_2 - acetone, 80: 20) afforded 35 mg (16.5%) of the crystalline starting material and 40 mg (19%) of the crystalline 7-epimer (**11c**), mp 162~163.5°C (dec.) (crystallized from CH_2Cl_2-Et_2O) $[\alpha]_D^{25}$ +105° (*c* 1, CH_2Cl_2). IR (KBr) ν_{max} 1780 (β-lactam), 1750, 1725 (acetate, ester), 1680, 1520 (amide),

* Prepared by oxidation of the sodium salt of cephalothin with sodium metaperiodate, using the procedure described by COCKER *et al.*[22]

1030 (S=O) cm^{-1}. NMR (DMSO-d_6, TMS) δ 2.0 (s, $COCH_3$), 3.64 and 3.88 (d, J=18 Hz, SCH_2), 3.76 (s, CH_2 side chain), 3.8 (s, OCH_3), 4.54 and 4.88 (d, J=12 Hz, CH_2OAc), ~4.82 (m, H-6 and H-7), 6.8 to 7.4 (m, thienyl protons), 9.08 (d, J=8 Hz, NH) ppm. MS *m/e* 426 (M^+).

(b) With DBN in CH_2Cl_2-DMSO

To a cooled (0°C) solution of **10c** (1 g, 2.35 mmoles) in 100 ml CH_2Cl_2 and 30 ml DMSO was added 0.29 ml (2.35 mmol) DBN. The dark brown solution was stirred for 45 minutes at 0°C, diluted with CH_2Cl_2, washed (2 N HOAc and H_2O), dried and evaporated. Column chromatography as described in the previous section afforded 230 mg of **10c** (23%) and 200 mg **11c** (20%). Both compounds were isolated in a crystalline state. A marked increase of the polar degradation products was observed, when the reaction was carried out for more than 60 minutes.

Methyl Δ^3-7β-phenylacetamidodesacetoxycephalosporanate (**3b**)

Compound **3b** was prepared by methylation of the free acid **3a**. Physical constants and spectral data were identical to those described by BARTON *et al.*[23]

Methyl Δ^3-7β-phenylacetamidodesacetoxycephalosporanate *S*-sulfoxide (**10a**)

To a stirred and ice-cooled solution of **3b** (346 mg, 1 mmol) in 15 ml $CHCl_3$ was added gradually *m*-chloroperbenzoic acid (1 mmol) dissolved in 15 ml $CHCl_3$. The reaction mixture was kept at 0°C for 2 hours, washed (with 10% $NaHCO_3$ and water), dried (Na_2SO_4) and evaporated. Crystallization of the residual oil from acetone afforded 282 mg **10a** (0.78 mmol, 78% yield), mp 211~213°C (dec.). $[\alpha]_D^{25}$ +182.5° (*c* 1, DMSO). IR (KBr) ν_{max} 1780 (β-lactam), 1735 (ester), 1660, 1530 (amide), 1030 (S=O) cm^{-1}. NMR (DMSO-d_6, TMS), δ 2.04 (s, CH_3), 3.62 and 3.68 (d, CH_2), 3.78 (s, OCH_3), 4.86 (d, J=4.5 Hz, H-6), 5.76 (dd, J=4.5 Hz and 9 Hz, H-7), 7.30 (s, C_6H_5), 8.30 (d, J=9 Hz, NH) ppm.

Epimerization of methyl Δ^3-7β-phenylacetamidodesacetoxycephalosporanate *S*-sulfoxide (**10a**)

To a cooled (0°C) solution **10a** (362 mg, 1 mmol) in 30 ml CH_2Cl_2 and 10 ml DMSO was added 0.12 ml (1 mmol) DBN. The reaction mixture was stirred for 3 hours at 0°C, diluted with CH_2Cl_2, washed (2 N HOAc and H_2O), dried and evaporated to an oil. Tlc (solvent system I) showed the presence of the starting material (Rf=0.55) of its 7-epimer (Rf=0.26) and more polar degradation product. Separation on silica gel column (solvent system CH_2Cl_2-acetone, 80:20) gave 165 mg (46%) crystalline **10a** and 45 mg (13%) crystalline **11a**, mp 173~175°C (dec.) (crystals from CH_2Cl_2-Et_2O); $[\alpha]_D^{20}$ +225° (*c* 1, DMSO). IR (KBr) ν_{max} 1780 (β-lactam), 1735 (ester), 1680, 1535 (amide), 1030 (S=O) cm^{-1}. NMR ($CDCl_3$-DMSO-d_6, 3:1, TMS) δ 2.00 (s, CH_3), 3.53 (s, CH_2SO), 3.54 (s, CH_2 side chain), 3.85 (s, CH_3 ester), 4.65 (d, J=2 Hz, H-6), 5.08 (dd, J=2 Hz and 8 Hz, H-7), 7.25 (s, C_6H_5), 8.9 (d, J=8 Hz, NH) ppm. MS *m/e* 362 (M^+).

Methyl Δ^3-7α-phenylacetamidodesacetoxycephalosporanate *S* and *R*-sulfoxide (**11a** and **12a**)

To a cooled (0°C) and stirred solution of **1b** (519 mg, 1.5 mmol) in 12 ml CH_2Cl_2 was gradually added *m*-chloroperbenzoic acid (316 mg, 85% pure, 1.56 mmol) in 6 ml CH_2Cl_2. The solution was stirred for 1 hour at 0°C, washed (with 10% $NaHCO_3$ and water), dried (Na_2SO_4) and evaporated. The reaction product showed two spots on tlc (solvent system I) in a ratio of about 1:2. The minor component, which showed the highest Rf value was considered to be the *R*-sulfoxide (**12a**), the more polar one the *S*-sulfoxide (**11a**). Column chromatography on 20 g silica gel, using CH_2Cl_2 - acetone 90:10 as solvent system, afforded 80 mg of the *R*-sulfoxide and 200 mg of the *S*-sulfoxide. The *R*-sulfoxide (**12a**) was isolated as an oil $[\alpha]_D^{20}$ +14° (*c* 1, DMSO). IR (KBr) ν_{max} 1775 (β-lactam), 1730 (ester), 1650, 1535 (amide), 1030 (S=O) cm^{-1}. NMR ($CDCl_3$, TMS) δ 2.1 (s, CH_3), 3.56 (s, CH_2SO), 3.6 (s, CH_2 side chain), 3.84 (s, CH_3 ester), 4.5 (d, J=2 Hz, H-6), 4.96 (dd, J=2 and 7 Hz, H-7), 7.00 (d, J=7 Hz, NH), 7.25 (s, C_6H_5) ppm. MS *m/e* 362 (M^+).

Spectral data were identical to those described in a previous section.

Acknowledgments

We are indebted to the Belgian Fonds voor Wetenschappelijk Geneeskundig Onderzoek (FWGO) for financial support, to Dr. S. TOPPET for the determination of the 100Mc spectra and to Dr. G. JANSSEN for mass spectral determination. We thank Gist-Brocades N. V., Delft, The Netherlands, for a supply of 7-ADCA used

in this work.

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