7-(α-AMINOPHENYLACETAMIDO)-3-AZIDOMETHYL-3-CEPHEM-4-CARBOXYLIC ACID

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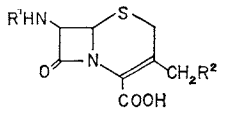
Since the discovery of cephalosporin C (**1**) modifications were introduced into the molecule to yield several important drugs. These changes were made at the 7 and 3 positions of the cephem nucleus.[1] Thus, the substitution of the D-aminoadipoyl group in **1** by a 2-thienylacetyl moiety yielded cephalothin.[2] Nucleophilic displacement of the acetoxy group in cephalothin yielded cephaloridine.[3] More recently, 7-aminocephalosporanic acid (7-ACA, **2**)[1,4] and 7-amino-3-desacetoxycephalosporanic acid (**3**)[5] were acylated with D-phenylglycine to yield cephaloglycin[6] and cephalexin[7], respectively. Both compounds had good activity against gram-negative bacteria and were orally absorbed. It is likely that these characteristics are associated with the presence of the D-phenylglycyl moiety.



Compd.	R^1	R^2
1	D-HOOCCH(NH_2)$CH_2CH_2CH_2CO$	CH_3COO
2	H	CH_3COO
3	H	H
4	D-$C_6H_5CH(NH_2)CO$	N_3
5	H	N_3
6	D-C_6H_5CH(NHBOC)CO	N_3

COCKER and coworkers[8] thoroughly investigated the nucleophilic displacement of the acetoxy group in N-acylated 7-ACA derivatives. Among the nucleophiles investigated was the azide ion. None of the compounds described by them had the D-phenylglycyl side chain. Our purpose was, therefore, to synthesize 7-(D-α-aminophenylacetamido)-3-azidomethyl-3-cephem-4-carboxylic acid (**4**)* and compare it with cephalexin.[7]

Chemistry

Compound **4** was prepared by coupling D-BOC-phenylglycine with 7-amino-3-azidomethyl-3-cephem-4-carboxylic acid (**5**)[9] *via* a mixed anhydride followed by removal of the protective BOC group. Racemization at the α-carbon of the amino acid was minimized or eliminated by using the conditions recommended by ANDERSON and coworkers.[10] We prepared the L-epimer of **4** (L-**4**) in order to estimate its concentration, if present, in the sample of **4**. The L-epimer could be detected by nmr since several of its absorptions were different from those of the D-epimer (see experimental). In addition, we wanted to compare the microbiological activity of **4** with that of L-**4**, since it is well known that cephalosporins derived from D-amino acids are considerably more active than their L-epimers.

Antimicrobial Activity

The *in vitro* minimum inhibitory concentrations (MICs) of **4** and L-**4** are compared with cephalexin in Table 1. The oral blood levels of **4** and cephalexin are compared in Table 2. The data indicate that **4** has good antimicrobial activity and is well absorbed orally in the mouse.

Experimental Section

Melting points were taken in capillaries on a Mel-Temp apparatus. Ir spectra were taken on a Beckman IR-5 Spectrophotometer. Nmr spectra were taken on Varian A-60 and HR-100 Spectrophotometers. Solvent evaporations were done under reduced pressure below 40°C.

* After this work was completed, a British Complete Patent Specification 16593/1968 (1969) of Glaxo Laboratories Ltd. was published describing the benzylamine salt of **6**. Compound **4** was also mentioned but no description was given of its preparation or properties.

Table 1*

Organism	Bristol No.	Cephalexin	4	L-4
Diplococcus pneumoniae	A-9585	1.3	0.3	5
Streptococcus pyogenes	A-9604	0.3	0.16	5
Staphylococcus aureus Smith	A-9537	1.3	0.6	10
S. aureus Smith+50 % serum	A-9537	2.5	1.3	>5
S. aureus BX 1633-2	A-9606	2	0.6	32
Salmonella enteritidis	A-9531	4	0.6	32
E. coli Juhl	A-15119	8	2	125
E. coli	A-9675	16	4	125
Klebsiella pneumoniae	A-9977	2	2	63
Klebsiella pneumoniae	A-15130	8	4	250
Proteus mirabilis	A-9900	2	1	32
P. morganii	A-15153	>250	125	>250
Pseudomonas aeruginosa	A-9843.A	>250	>250	>250
Serratia marcescens	A-20019	>250	>250	250

* The MIC values are in μg/ml and were determined by the two-fold tube dilution method, essentially as described by A. GOUREVITCH and coworkers.[12] In the present experiments nutrient broth (Difco) was used as the test medium for all organisms except for *D. pneumoniae* and *S. pyogenes*. These two organisms were evaluated in a 1 : 1 mixture of nutrient broth and Antibiotic Assay broth (BBL) supplemented with 5 % human serum.

Table 2. Oral mouse blood levels*

Minutes after administration	4	Cephalexin
30	5.63 ; 9.43	8.48 ; 8.51
60	3.5 ; 4.03	4.9 ; 6.03
120	1.55 ; 0.7	1.14 ; 2.0
210	0.73 ; 0～0.38**	0～0.20** ; 0.68

* Compounds administered at the level of 20 mg/kg. Results are in μg/ml and are the average of 8 mice per experiment.
** Range of values.

7-Amino-3-azidomethyl-3-cephem-4-carboxylic Acid (**5**)[9]. 7-ACA (13.6 g) was dissolved in 300 ml of H_2O by adding 4.2 g of $NaHCO_3$. After adjusting to pH 6.5 with 10 % NaOH, the solution was filtered through Celite. To this was added a solution of 16.25 g of NaN_3 in 50 ml of H_2O. The solution was stirred at 50°C for 19 hours at pH 6.5～6.7. The cooled (25°C) solution was acidified to pH 3 with concentrated HCl. The mixture was stirred for 1 hour in ice, the solid collected by filtration and dried *in vacuo* giving 6.8 g, 54 %, of **5***. The ir and nmr spectra were consistent. Yields ranged from 30～55 % in other experiments.

3-Azidomethyl-7-(D-α-*t*-butoxycarboxamidophenylacetamido)-3-cephem-4-carboxylic Acid (**6**). To a vigorously stirred solution of 20.08 g of D-BOC-phenylglycine[6] in 500 ml of THF, protected from moisture at −15°C, was added 8.08 g of N-methylmorpholine and 11 g of isobutyl chloroformate all at once. After stirring for 3 minutes, an ice-cold solution of 20.4 g of **5** and 8.08 g of N-methylmorpholine in 500 ml of H_2O was added at a rate to keep the temperature at 0～3°C. The solution was allowed to warm to room temperature during 1 hour. After the THF was evaporated, 200 ml of H_2O and 500 ml of EtOAc were added and the cooled mixture was stirred and acidified to pH 2 with 42 % H_3PO_4. The layer was separated and the extraction repeated. The combined organic layers were washed (H_2O), dried ($MgSO_4$) and the solvent evaporated to a small volume. This solution was added dropwise to 2,500 ml of cyclohexane with stirring. The solid was collected by filtration and air dried. A tlc (Me_2CO: AcOH, 97 : 3) showed the presence of D-BOC-phenylglycine. The cyclohexane treatment was repeated until D-BOC-phenylglycine could not be detected by tlc; 28.13 g, 69.4 %, $[\alpha]_D^{26}$ +13.2° (*c* 0.82, Me_2CO).

7-(D-α-Aminophenylacetamido)-3-azidomethyl-3-cephem-4-carboxylic Acid (**4**). A solution of 10 g of **6** in 250 ml of HCOOH was stirred at room temperature for 2 hours. The excess HCOOH was evaporated at 26～28°C (5 mm). The residue was treated thrice with toluene and evaporated to dryness each time. The residue was triturated with a mixture of 300 ml of H_2O and 300 ml of EtOAc. The solid was filtered, triturated with Me_2CO and dried; 5.7 g. An additional 0.46 g was obtained from the aqueous phase after washing it successively with EtOAc and Et_2O and concentrating to a small

* The preparation of this compound should be carried out in a well ventilated hood, since HN_3 (as well as NaN_3) is extremely toxic. Early symptoms of exposure are sinus congestion and and a throbbing headache. In order to decompose the HN_3 present in the final filtrate, the solution was cooled in ice, strongly acidified with HCl and treated slowly with solid $NaNO_2$ until a persistent coloration was obtained with starch-iodine paper.

volume.

The crude **4** was purified *via* its TsOH salt in the following manner. To a suspension of 19.1 g of crude **4** in 200 ml of H_2O was added dropwise a solution of 11.41 g of $TsOH \cdot H_2O$ in 40 ml of H_2O. A gummy precipitate formed immediately. The mixture was heated on a steam bath, then cooled to room temperature and the solution decanted. The remaining solid was extracted once more with H_2O. The combined extracts were stirred for 20 minutes with 2.8 g carbon, warmed to 50°C and filtered through Celite. The filtrate was concentrated until crystallization started and was chilled. The product was collected and air dried; 12.7 g. An additional 0.7 g was obtained by further concentrating the mother liquor. The material was recrystallized by dissolving 13 g of the TsOH salt in 250 ml of H_2O at 60°C, carbon treating and concentrating to a small volume ; 10 g. The ir and nmr spectra were consistent for the TsOH salt of **4**. *Anal.* Calcd. for $C_{23}H_{24}N_6O_7S_2 \cdot {}^1/_2 H_2O$: C, 48.49; H, 4.42; N, 14.76; H_2O, 1.58. Found: C, 48.28; H, 4.78; N, 15.10; H_2O, 1.96.

Pure **4** was obtained by dissolving 32.5 g of the TsOH salt in 325 ml of H_2O at 70°C. The solution was cooled and separated from oily material. The solution was heated to 50~55°C and adjusted to pH 4.2 with NEt_3. Crystallization of **4** started immediately. The mixture was left at room temperature for 1 ${}^1/_2$ hour and then ${}^1/_2$ hour at 0°C. The solid was collected, air dried and finally dried over P_2O_5; 14.9 g of needles, mp 240~250°C, $[\alpha]_D^{24}$ +97.4° (*c* 0.5, 0.1N HCl); ir (KBr) 2100 (N_3), 1770 (β-lactam), 1690, 1535 (CONH), 1600 (COO^-) and 705 cm^{-1} (C_6H_5); nmr (D_2O and trace of DCl ; chemical shifts in δ relative to TPS ; splitting pattern, *J*, and identification of H given in brackets), 7.54 (s, C_6H_5), 5.77 (d, 4.5; 7-H), 5.10 (d, 4.5 ;6-H), 5.28 (s, PhCHN), 4.43, 3.93 (2 d's, 14 ; CH_2N_3), 3.62, 3.36 (2 d's, 18 ; 2-H); *Anal.* Calcd. for $C_{16}H_{16}N_6O_4S$: C, 49.46 ; H, 4.15; N, 21.63. Found: C, 49.59; H, 4.34; N, 21.72.

L-Epimer of **4**. The coupling was carried out as described for **4** between L-BOC-phenylglycine[6] and **5**. The intermediate L-**6** was purified *via* its K salt, obtained by adding a solution of K 2-ethylhexanoate in *n*-BuOH to crude L-**6** in EtOAC. Purified L-**6** (10 g), $[\alpha]_D^{25}$ +56.2° (*c* 0.5, Me_2CO) was added with good stirring to 50 ml of CF_3COOH at 10°C. After 15 minutes the solution was poured into an ice-cold mixture of Skellysolve B and anhydrous Et_2O (2 : 1). The CF_3COOH salt was collected and dried over P_2O_5; 6.0 g. A solution of the CF_3COOH salt in 180 ml of H_2O and 50 ml of Amberlite LA-1 resin[6] in toluene* and 50 ml of toluene was stirred at room temperature for 1 ${}^1/_2$ hour. Et_2O was added and the layers were separated. The organic layer was extracted with H_2O and the washing added to the aqueous phase. The aqueous solution was washed 8 times with Et_2O, filtered through Celite and concentrated. At a volume of 50 ml crystallization started. When completed, the solid was collected and dried over P_2O_5 giving 1.25 g of crystalline L-**4**; mp>180°C (dec.) $[\alpha]_D^{25}$ +98.6° (*c* 0.5, 0.1N HCl); ir consistent and the same as for **4**; nmr 7.54 (s, C_6H_5), 5.56 (d, 4.5; 7-H), 5.18 (d, 4.5; 6-H), 5.29 (s, PhCHN), 4.49, 4.06 (2 d's, 14; CH_2N_3), 3.75, 3.53 (2 d's, 18; 2-H); *Anal.* Calcd for $C_{16}H_{16}N_6O_4S \cdot H_2O$: C, 47.28; H, 4.46; N, 20.68; S, 7.89. Found: C, 47.48; H, 4.33; N, 19.92; S, 7.67. An additional crop of 0.75 g was obtained by concentrating the mother liquor.

Summary

The syntheses of the D and L epimers of 7-(α-aminophenylacetamido)-3-azidomethyl-3-cephem-4-carboxylic acid are described and their antimicrobial activity is compared with that of cephalexin. The D-epimer is active and orally absorbed.

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References

1) Morin, R.B.; B.G. Jackson, E.H. Flynn & R.W. Roeske: Chemistry of cephalosporin antibiotics. I. 7-Aminocephalosporanic acid from cephalosporin C. J. Am. Chem. Soc. 84 : 3400~3401, 1962

* Toluene, instead of MIBK (ref. 6) was used in the preparation of the resin solution.

2) CHAUVETTE, R. R.; 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 & E. M. VAN HEYNINGEN: Chemistry of cephalosporin antibiotics. II. Preparation of a new class of antibiotics and the relation of structure to activity. J. Am. Chem. Soc. 84 : 3401~3402, 1962

3) SPENCER, J. L.; F. Y. SIU, B. G. JACKSON, H. M. HIGGINS & E. H. FLYNN: Chemistry of cephalosporin antibiotics. IX. Synthesis of cephaloridine. J. Org. Chem. 30 : 500~501, 1967

4) FECHTIG, B.; H. PETER, H. BICKEL & E. VISCHER: Modifikationen von Antibiotika. II. Über die Darstellung von 7-Aminocephalosporansäure. Helv. Chim. Acta 51 : 1108~1119, 1968

5) STEDMAN, R. J.; K. SWERED & J.R.E. HOOVER: 7-Aminodesacetoxycephalosporanic acid and its derivatives. J. Med. Chem. 7 : 117~119, 1964

6) SPENCER, J. L.; E. H. FLYNN, R. W. ROESKE, F. Y. SIU & R. R. CHAUVETTE: Chemistry of cephalosporin antibiotics. VII. Synthesis of cephaloglycin and some homologs. J. Med. Chem. 9 : 746~750, 1966

7) RYAN, C. W.; R. L. SIMON & E. M. VAN HEYNINGEN: Chemistry of cephalosporin antibiotics. XIII. Desacetoxycephalosporins. Synthesis of cephalexin and some analogs. J. Med. Chem. 12 : 310~313, 1969

8) COCKER, J. D.; B. R. COWLEY, J. S. G. COX, S. EARDLY, G. I. GREGORY, J. K. LAZENLY, A. G. LONG, J. C. P. SLY & G. A. SOMERFIELD: Cephalosporanic acids. II. Displacement of the acetoxy-group by nucleophiles. J. Chem. Soc. 1965 : 5015~5031, 1965

9) WETHERILL, L. A.; W. GRAHAM & M.J. COVIL: Brit. Patent 1, 104, 938, 1968

10) ANDERSON, G. W.; J. E. ZIMMERMAN & F. M. CALLAHAN: A reinvestigation of the mixed carbonic anhydride method of peptide synthesis. J. Am. Chem. Soc. 89 : 5012~5017. 1967

11) GOUREVITCH, A.; G. A. HUNT, J. R. LUTTINGER, C. C. CARMACK & J. LEIN: Activity of α-phenoxyalkyl penicillins against sensitive and resistant staphylococci. Proc. Soc. Exptl. Biol. Med. 107 : 455~458, 1961