SYNTHETIC CEPHALOSPORINS

II. THE SYNTHESIS AND ORAL ACTIVITY OF 7-[*R*-2-AMINO-2-(3-CHLORO-4-HYDROXYPHENYL)ACETAMIDO]-3-METHYLTHIO-3-CEPHEM-4-CARBOXYLIC ACID AND RELATED COMPOUNDS†

KENJI SAKAGAMI, TADAHIRO WATANABE and SHUNZO FUKATSU

Central Research Laboratories, Meiji Seika Kaisha, Ltd.,
Morooka-cho, Kohoku-ku, Yokohama 222, Japan

HAJIME NITTA, MINORU HATANAKA* and TOSHIYASU ISHIMARU††

The Institute of Scientific and Industrial Research, Osaka University,
Mihogaoka, Ibaraki, Osaka 567, Japan

(Received for publication November 5, 1986)

A series of 3-methylthio-3-cephem-4-carboxylic acids were prepared to test their antibacterial activities, and 7-[*R*-2-amino-2-(3-chloro-4-hydroxyphenyl)acetamido]-3-methylthio-3-cephem-4-carboxylic acid was found to be a new orally active antibiotic.

The need still exists for development of new semisynthetic cephalosporins which exhibit potent, broad-spectrum, antibiotic activity, especially when they are administered orally. In the course of studies on orally active cephalosporins, we found that 7-[*R*-2-amino-2-(3-chloro-4-hydroxyphenyl)-acetamido]-3-methylthio-3-cephem-4-carboxylic acid showed excellent oral activity. The ED_{50} values of this compound against Gram-positive and Gram-negative bacteria were superior to those of cefroxadine[2] (CXD, **7a**). Herein reported is synthesis and biological activity of 7-[*R*-2-amino-2-(3-chloro-4-hydroxyphenyl)acetamido]-3-methylthio-3-cephem-4-carboxylic acid and related 3-methylthio-3-cephem-4-carboxylic acid derivatives.

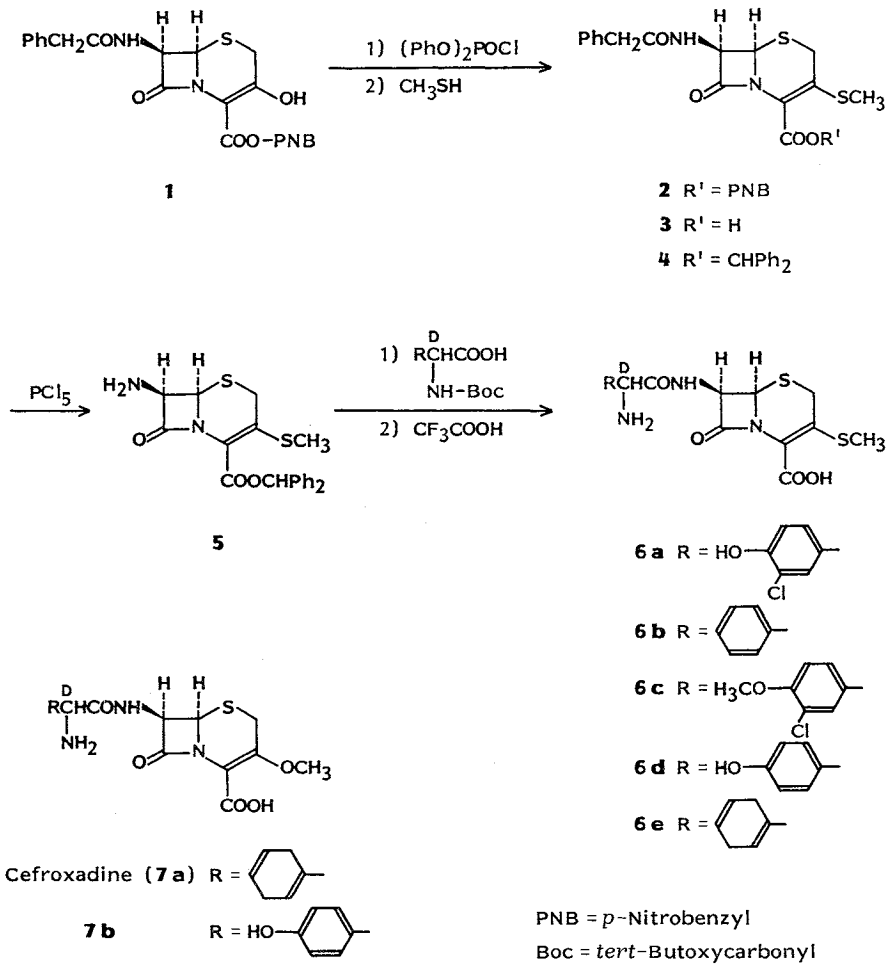
Chemistry

Although 3-methylthio-3-cephem-4-caboxylate have been prepared from 3-hydroxy-3-cephem-4-carboxylate (**1**) by treatment with *p*-toluenesulfonyl chloride followed by methylmercaptan[3,4], the products obtained were usually contaminated by considerable amounts of the Δ^2-isomers. We found that the pure **2** could be obtained by using diphenyl chlorophosphate instead of *p*-toluenesulfonyl chloride. In this way *p*-nitrobenzyl 7-phenylacetamido-3-hydroxy-3-cephem-4-carboxylate (**1**) was converted in an one-pot procedure into **2** without contamination of the Δ^2-isomer in 83% yield. The *p*-nitrobenzyl ester **2** was then transformed into benzhydryl ester **4** by zinc reduction followed by treatment of **3** with diphenyldiazomethane. Deacylation of the 7-substituent *via* imino ether and subsequent acylation with *N-tert*-butoxycarbonyl amino acids gave the protected cephalosporins. Removal of the protective groups afforded new cephalsoporins **6a**~**6e** (Scheme 1).

† See ref 1.

†† Present address: Faculty of Pharmaceutical Sciences, Tokushima-Bunri University, Yamashiro, Tokushima 770, Japan.

Scheme 1.



Biological Activity

The minimum inhibitory concentrations (MICs) of the new cephalosporins were tested against a series of Gram-positive and Gram-negative organisms using the 2-fold agar dilution method. The bacteria were cultivated in Müller-Hinton agar (BBL) and grown overnight at 37°C. One loopful (5 μl) of the 10^{-2} dilution (*ca.* 10^6 cfu/ml) of the suspension was inoculated with a microplanter (Sakuma Seisakusho, Tokyo, Japan) into 15 ml of the same agar containing serial 2-fold dilution of the test antibiotics. The MICs of the new cephalosporins are shown in Table 1. The activity of the 3-methylthio-3-cephem-4-carboxylic acids (**6a**~**6e**), except **6c**, can be characterized as comparable to or better than that of the 3-methoxy-3-cephem-4-carboxylic acids (**7a** and **7b**).

The *in vivo* antibacterial activities of these compounds were also tested using mice infected with Gram-positive and Gram-negative organisms. Male mice (*dd*Y-SLC strain, 4-weeks-old-age, weighting 19 to 20 g, 10 per a group) were used for the examinations. The mice were challenged intraperitoneally with 10^5 to 10^8 cfu/mouse of the bacteria which were suspended in 0.5 ml of saline containing 2.5% gastric mucin (Difco). The animals were treated orally and/or subcutaneously with the new

Table 1. *In vitro* MICs (μg/ml).

Organisms	6a	6b	6c	6d	6e	7a	7b	CEX
Staphylococcus aureus Smith	0.39	0.78	0.39	0.78	0.39	1.56	1.56	3.13
S. aureus 209P	0.20	0.39	0.20	0.39	0.20	0.78	1.56	1.56
Bacillus subtilis ATCC 6633	0.10	0.05	0.20	0.20	0.10	0.39	0.78	0.78
Escherichia coli NIHJ JC-2	3.13	3.13	12.5	3.13	3.13	6.25	6.25	12.5
E. coli K-12	3.13	3.13	50	6.25	3.13	6.25	6.25	12.5
E. coli ML 1629	6.25	6.25	100	6.25	6.25	12.5	12.5	12.5
E. coli RGN 823*	3.13	6.25	>100	6.25	3.13	6.25	6.25	12.5
E. coli RGN 238*	25	12.5	>100	50	25	12.5	50	12.5
Klebsiella pneumoniae PCI 602	3.13	3.13	>100	3.13	3.13	6.25	6.25	12.5
K. pneumoniae GN69**	12.5	3.13	50	6.25	12.5	6.25	6.25	6.25
Proteus vulgaris OX 19	0.78	1.56	1.56	1.56	0.78	6.25	6.25	25
P. rettgeri GN624**	>100	>100	>100	>100	>100	>100	>100	>100
Salmonella paratyphi-A	1.56	1.56	12.5	3.13	1.56	6.25	12.5	12.5
S. paratyphi B	1.56	1.56	3.13	3.13	1.56	6.25	6.25	6.25
Shigella flexneri 10·2a	1.56	0.78	12.5	1.56	1.56	3.13	6.25	6.25

* Penicillinase producing strain.
** Cephalosporinase producing strain.
CEX: Cephalexin.

Table 2. Therapeutic effect of **6a**, **6b**, **6d** and cephalexin (CEX) against experimental infections with *Escherichia coli* No. 29 in mice.

Compound	Challenge dose (cfu/mouse)	MIC (μg/ml)	ED_{50} (mg/kg)	
			po	sc
6a	8.3×10^5	1.56	1.85 (0.65~5.5)[a]	3.65 (1.3~10.5)[a]
CEX	8.3×10^5	3.13	17.70 (14.75~21.24)	23.85 (13.18~43.17)
6b	8.3×10^5	1.56	9.25 (7.28~11.74)	13.75 (7.5~26.0)
CEX	8.3×10^5	3.13	17.70 (14.75~21.24)	23.85 (13.18~43.17)
6d	7.0×10^5	1.56	14.0 (8.0~25.0)	NT
CEX	7.0×10^5	3.13	34.5 (19.0~62.0)	NT

[a] In parentheses: 95% confidence limit.
NT: Not tested.

cephalosporins immediately after infection. The number of surviving mice were recorded 1 week after infection. The median effective dose (ED_{50}) was calculated by the method of LITCHFIELD-WILCOXON. The data of the ED_{50} values of the new cephalosporins against *Escherichia coli* No. 29 are shown in Table 2 and compared with that of cephalexin (CEX) in separate tests. All of the compounds **6a**, **6b** and **6d** are superior to CEX. Particularly, the compound **6a** bearing chloro substituent at the *meta*-position of the phenyl group showed the best oral activity among these cephalosporins. The effectiveness of **6a** was further represented by comparison of the ED_{50} values against several Gram-positive and Gram-negative bacteria with those of CXD (**7a**) (Table 3). The serum

Table 3. Therapeutic effect of **6a** and **7a** (cefroxadine ((CXD)) against experimental infections in mice (po).

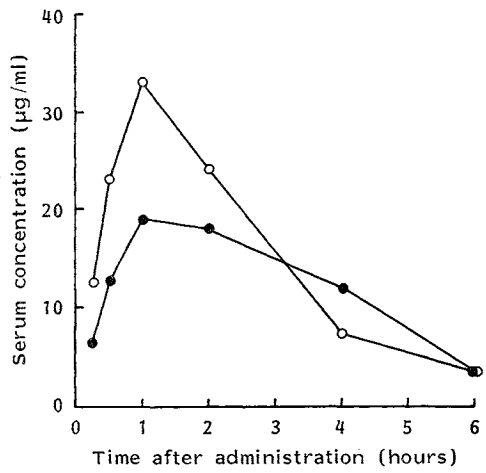
Organisms	Challenge dose (cfu/mouse)	Sample	MIC (μg/ml)	ED_{50} (mg/kg)
Staphylococcus aureus 209P JC-1	5.0×10^5	**6a**	0.20	2.75 (1.05~7.2)[a]
		CXD	1.56	12.5 (5.95~26.25)
Escherichia coli No. 29	2.4×10^5	**6a**	1.56	3.7 (2.0~6.5)
		CXD	6.25	12.0 (6.5~23.0)
E. coli A-0022	1.7×10^6	**6a**	1.56	17.5 (11.0~28.0)
		CXD	3.13	48.5 (16.0~145.5)
Klebsiella pneumoniae PCI 602	1.6×10^5	**6a**	1.56	35.0 (23.5~52.5)
		CXD	3.13	65.0 (47.5~89.0)
K. pneumoniae GN69	8.0×10^7	**6a**	3.13	85.0 (36.0~200.5)
		CXD	3.13	1,000
Salmonella enteritidis No. 11	1.2×10^5	**6a**	1.56	275.0 (112.0~674.0)
		CXD	3.13	380.0 (181.0~798.0)

[a] In parentheses: 95% confidence limit.

Table 4. Acute toxicity of **6a** and cephalexin (CEX) in mice.

Compound	Dose (g/kg)	Administration route	Mortality
6a	5	po	0/5
CEX	2	po	1/5

Fig. 1. Serum levels in dogs after oral administration (beagle dog, 20 mg/kg, $n=4$).
○ **6a**, ● cephalexin.



concentration-time curve of **6a** in male beagle dogs after oral administration is illustrated in Fig. 1. The serum concentrations were determined by the paper-disc agar diffusion method using *Micrococcus luteus* 9341 as the test organism. The serum levels of **6a** within 3 hours after administration was higher than that of CEX. When the acute toxicity of **6a** in male mice (Jcl-ICR strain, 4-weeks-old-age, weighting 20 ± 0.5 g) was compared with that of CEX, the compound **6a** was better tolerated after oral administration (Table 4).

These data indicate that 7-[*R*-2-amino-2-(3-chloro-4-hydroxyphenyl)acetamido]-3-methylthio-3-cephem-4-carboxylic acid (**6a**) is a new orally active cephalosporin.

Experimental

Melting points were uncorrected. IR spectra were recorded on a Jasco-IR-1 spectrometer. NMR spectra were determined with tetramethylsilane as an internal standard on either a Hitachi R-600 or R-900 spectrometer, chemical shifts being given in ppm unit.

p-Nitrobenzyl 7-Phenylacetamido-3-methylthio-3-cephem-4-carboxylate (**2**)

To a solution of *p*-nitrobenzyl 7-phenylacetamido-3-hydroxy-3-cephem-4-carboxylate (**1**, 5.6 g) in dry acetonitrile (40 ml) containing diisopropylethylamine (2.4 ml), diphenyl chlorophosphate (2.6 ml) was added dropwise at −20°C. After stirring for 30 minutes at −20 to 10°C, the mixture was

cooled at −30°C, treated with diisopropylethylamine (2.4 ml) and methylmercaptan (3 g) and then stirred for 2 hours at −30 to −20°C. The solid was collected by filtration and dried *in vacuo* to yield **2** (4.95 g, 83%): MP 231°C (dec); IR (Nujol) cm^{-1} 3230, 1775, 1705, 1650; ^1H NMR (DMSO-d_6 - $CDCl_3$) δ 1.99 (3H, s), 3.61 (2H, s), 3.68 (2H, s), 5.03 (1H, d, J=4.6 Hz), 5.73 (2H, s), 5.64 (1H, dd, J=4.6 and 7.8 Hz), 7.29 (5H, s), 7.63 (2H, d, J=8.2 Hz), 8.20 (2H, d, J=8.2 Hz), 8.83 (1H, d, J=7.8 Hz).

Anal Calcd for $C_{23}H_{21}N_3O_6S_2$: C 55.30, H 4.23, N 8.41, S 12.84.
Found: C 55.60, H 4.24, N 8.28, S 12.83.

7-Phenylacetamido-3-methylthio-3-cephem-4-carboxylic Acid (3)

To a solution of **2** (2.0 g) and DL-mandelic acid (6.0 g) in DMF (15 ml), zinc powder (1.54 g) was added at once at room temp. After stirring at 50°C for 3 hours, the mixture was cooled and the insoluble material was removed by filtration. The filtrate was evaporated *in vacuo*, and the residue was taken into EtOAc (50 ml). The EtOAc solution was washed with 5% HCl and then brine, and extracted with aq sodium bicarbonate solution. The extract was washed with EtOAc and then acidified with 5% HCl. The precipitate was collected by filtration and dried *in vacuo* to yield **3** (1.0 g, 70%): MP 197~198°C (dec); IR (Nujol) cm^{-1} 3500, 3280, 1770, 1640; ^1H NMR (DMSO-d_6) δ 2.33 (3H, s), 3.57 (2H, s), 3.67 (2H, s), 5.01 (1H, d, J=4.7 Hz), 5.56 (1H, d, J=4.7 and 8.2 Hz), 7.25 (5H, s), 9.01 (1H, d, J=8.2 Hz).

Benzhydryl 7-Phenylacetamido-3-methylthio-3-cephem-4-carboxylate (4)

To a solution of **3** (1.82 g) in Me_2CO (20 ml), a solution of diphenyldiazomethane (1.45 g) in hexane was added, and the mixture was stirred for 5 hours at room temp and evaporated *in vacuo*. The residue was washed with hexane - isopropyl ether, and recrystallized from Me_2CO - MeOH to give crystals (2.4 g): MP 162~163°C (dec); IR (Nujol) cm^{-1} 3230, 1780, 1700, 1650; ^1H NMR ($CDCl_3$) δ 1.99 (3H, s), 2.91 (1H, d, J=16.8 Hz), 3.38 (1H, d, J=16.8 Hz), 3.64 (2H, s), 4.95 (1H, d, J=4.3 Hz), 5.62 (1H, dd, J=4.3 and 8.6 Hz), 6.86 (1H, s), 7.2~7.33 (16H, m); field desorption mass spectrometry (FD-MS) m/z 530 (M+H).

Benzhydryl 7-Amino-3-methylthio-3-cephem-4-carboxylate (5)

To a solution of **4** (2.65 g) in dichloromethane (50 ml), pyridine (4 ml) and phosphorus pentachloride (3.2 g) was added at −30°C and the mixture was stirred for 3 hours at 0~5°C. The mixture was cooled at −30°C and treated with MeOH (15 ml). After stirring for 1 hour at 0~5°C, the mixture was poured into brine (40 ml), adjusted to pH 1.5~2.0 with diluted ammonia solution and stirred for 1 hour at 0~5°C. The precipitate was collected by filtration, washed with water and then EtOAc, and dried *in vacuo* to yield **5** as the hydrochloride (2.25 g): MP 203~205°C (dec); IR (Nujol) cm^{-1} 1780, 1760, 1700; ^1H NMR ($CDCl_3$) δ 2.44 (3H, s), 3.73 (1H, d, J=16 Hz), 4.13 (1H, d, J=16 Hz), 5.08 (1H, d, J=4.3 Hz), 5.28 (1H, d, J=4.3 Hz), 6.90 (1H, s), 7.20~7.88 (11H, m).

7-[*R*-2-Amino-2-(3-chloro-4-hydroxyphenyl)acetamido]-3-methylthio-3-cephem-4-carboxylic Acid (6a)

To an ice-cooled solution of **5** (290 mg), *R*-2-*tert*-butoxycarbonylamino-2-(3-chloro-4-hydroxyphenyl)acetic acid[5] (221 mg) and 1-hydroxybenzotriazole (108 mg) in dichloromethane (10 ml), dicyclohexylcarbodiimide (165 mg) was added and the mixture was stirred for 3 hours at 0°C. The mixture was diluted with EtOAc and filtered. The filtrate was washed in turn with 2.5% HCl, aq sodium bicarbonate solution and brine, and then evaporated *in vacuo*. The remaining residue was chromatographed on silica gel with EtOAc - benzene (1 : 5) to give the acylated compound (200 mg): MP 145~146°C (dec); IR (Nujol) cm^{-1} 3390, 3300, 1790; ^1H NMR ($CDCl_3$) δ 1.41 (9H, s), 2.05 (3H, s), 3.23 (2H, d, J=2.8 Hz), 4.95 (1H, d, J=4.3 Hz), 5.27 (1H, d, J=6.7 Hz), 5.55~5.91 (2H, m), 6.89~7.50 (14H, m).

The product was treated with anisole (0.5 ml) and TFA (3 ml) at 0°C for 30 minutes. The mixture was evaporated *in vacuo* and the residue was triturated in isopropyl ether to give a white powder. The solid was dissolved in 95% EtOH (0.5 ml) and the solution was treated at 0°C with triethylamine (10 mg). After the mixture was stirred for 1 hour at 0°C, the precipitate was collected by

filtration, washed with 95% EtOH and dried *in vacuo* to give white crystals (35 mg): MP 147~150°C (dec); IR (Nujol) cm^{-1} 1760, 1690; ^1H NMR (D_2O - HCl) δ 2.35 (3H, s), 3.35 (1H, d, J=17.6 Hz), 3.71 (1H, d, J=17.6 Hz), 5.15 (1H, d, J=4.3 Hz), 5.23 (1H, s), 6.02 (1H, d, J=4.3 Hz), 7.05~7.59 (3H, m).

7-(*R*-2-Amino-2-phenylacetamido)-3-methylthio-3-cephem-4-carboxylic Acid (**6b**)[8]

By the use of the procedure described above for **6a**, this compound was prepared from **5** and D-*N*-*tert*-butoxycarbonylphenylglycine. IR (Nujol) cm^{-1} 1760, 1690; ^1H NMR (D_2O - HCl) δ 2.32 (3H, s), 3.42 (1H, d, J=17.5 Hz), 3.58 (1H, d, J=17.5 Hz), 5.10 (1H, d, J=4.3 Hz), 5.20 (1H, s), 5.70 (1H, d, J=4.3 Hz), 7.38~7.70 (5H, m).

7-[*R*-2-Amino-2-(3-chloro-4-methoxyphenyl)acetamido]-3-methylthio-3-cephem-4-carboxylic Acid (**6c**)

By the use of the procedure described above for **6a**, this compounds was prepared from **5** and *R*-2-*tert*-butoxycarbonylamino-2-(3-chloro-4-methoxyphenyl)acetic acid. IR (Nujol) cm^{-1} 1760, 1685; ^1H NMR (D_2O - HCl) δ 2.35 (3H, s), 3.22 (1H, s, J=18 Hz), 3.68 (1H, d, J=18 Hz), 3.69 (3H, s), 5.22 (1H, d, J=4.3 Hz), 5.30 (1H, s), 5.56 (1H, d, J=4.3 Hz), 7.10~7.75 (3H, m).

7-[*R*-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methylthio-3-cephem-4-carboxylic Acid (**6d**)

By the use of the procedure described above for **6a**, this compound was prepared from **5** and *R*-2-*tert*-butoxycarbonylamino-2-(4-hydroxyphenyl)acetic acid. IR (Nujol) cm^{-1} 1760, 1690; ^1H NMR (D_2O - HCl) δ 2.36 (3H, s), 3.36 (1H, d, J=18 Hz), 3.66 (1H, d, J=18 Hz), 5.13 (1H, d, J=4.3 Hz), 5.26 (1H, s), 5.58 (1H, d, J=4.3 Hz), 7.00 (2H, d, J=8.6 Hz), 7.45 (2H, d, J=8.6 Hz).

7-[*R*-2-Amino-2-(1,4-hexadiene-1-yl)acetamido]-3-methylthio-3-cephem-4-carboxylic Acid (**6e**)

By the use of the procedure described above for **6a**, this compound was prepared from **5** and *R*-2-*tert*-butoxycarbonylamino-2-(1,4-hexadiene-1-yl)acetic acid. IR (Nujol) cm^{-1} 1780, 1700; ^1H NMR (D_2O - HCl) δ 2.36 (3H, s), 2.38 (4H, br s), 3.08 (1H, d, J=16.2 Hz), 3.35 (1H, d, J=16.2 Hz), 4.30 (1H, s), 4.80 (1H, d, J=4.3 Hz), 5.05 (1H, d, J=4.3 Hz), 5.34 (2H, br s), 5.76 (1H, br s).

References

1) SAKAGAMI, K.; T. MISHINA, T. KURODA, M. HATANAKA & T. ISHIMARU: Synthetic cephalosporins. The synthesis and antibacterial activities of 7-[2-(2-amino-1,3,4-thiadiazol-5-yl)acetamido]cephalosporins. J. Antibiotics 36: 1205~1210, 1983
2) ZAK, O.; W. A. VISCHER, C. SCHENK, W. TOSCH, W. ZIMMERMANN, J. REGÖS, E. R. SUTER, F. KRADOLFER & J. GELZER: CGP 9000: A new orally active, broad-spectrum cephalosporin. J. Antibiotics 29: 653~655, 1976
3) SCARTAZZINI, R.; P. SCHNEIDER & H. BICKEL: Nere β-Lactam-Antibiotika. Über die Funktionalisierung der Cephem-3-Stellung mittles Schwefel oder Stickstoff. Helv. Chim. Acta 58: 2437~2450, 1975
4) SCARTAZZINI, R. & H. BICKEL: New orally active cephalosporins. Heterocycles 7: 1165~1188, 1977
5) CRAST, L. B., Jr. (Bristrol-Myers): Antibacterial 7-amino-cephalosporanic and decephalosporanic acid derivatives. U.S. 3,489,751, Jan. 13, 1970