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Synthesis of Cephapirin† and Related Cephalosporins from 7-(α-Bromoacetamido)cephalosporanic Acid

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Since the structural elucidation of cephalosporin C (1),[1,2] the discovery of ingenious and remarkably efficient chemical methods[3-6] for its N-deacylation has rendered the novel fused dihydrothiazine-β-lactam nucleus, 7-aminocephalosporanic acid (7-ACA, **2**), readily available for the synthesis of semisynthetic cephalosporins which now complement the penicillins in chemotherapeutic interest.[7]

The purpose of this report is to describe a convenient and versatile synthesis of cephapirin (**4**)[8] and related α-heterosubstituted acetamidocephalosporanic acids by nucleophilic displacement of the bromine atom in 7-(α-bromoacetamido)cephalosporanic acid (**3**)[9] with mercapto- and aminopyridines[8,10] and various 1,3-disubstituted and trisubstituted thioureas.[11]

Treatment of an aqueous–acetone suspension of 7-ACA (**2**) with bromoacetyl bromide in the presence of sodium bicarbonate afforded crystalline 7-(α-bromoacetamido)-cephalosporanic acid (**3**) in 77% yield. The interaction of **3**, 4-mercaptopyridine, and triethylamine in methylene chloride at room temperature produced 7-[α-(4-pyridylthio)acetamido]cephalosporanic acid (cephapirin, **4**) in 82% yield (Scheme I).

A stirred suspension of **4** and *N,N*-diisopropylethylamine in aqueous acetone reacted with methyl iodide at ambient temperature to provide a fair yield of 7-[α-(1-methyl-4-pyridylthio)acetamido]cephalosporanic acid betaine (**5**).‡[12]

3-Mercaptopyridine[13] and commercially available 2-mercaptopyridine were converted by procedures analogous to the one used for the preparation of **4** into the corresponding cephapirin isomers **6** and **7**, respectively (Table I). Chemical properties and spectral data observed for cephapirin (**4**), **6**, and **7** were in accord with the assigned structures. However, the remote possibility remained that the tertiary nitrogen atom instead of the thiol function in 4-mercaptopyridine had preferentially undergone reaction with **2** to form 7-[α-(4-thiopyridon-1-yl)acetamido]cephalosporanic acid rather than the thioether **4**. This question was definitely settled by the independent synthesis of cephapirin by Silvestri and Johnson.[14] Compound **4** and the product of unequivocal structure which they obtained by coupling 7-ACA (**2**) with α-(4-pyridylthio)acetyl chloride hydrochloride were identical.

In contrast to the behavior of 4-mercaptopyridine, 4-aminopyridine reacted with **3** to form the betaine **8** as the only product isolated. Since thiols are generally stronger nucleophiles than the corresponding amines, this result was not unexpected. Compound **8** was reported by other investigators[15] after the completion of our work. Their method of synthesis was analogous to our own.

In order to obtain the desired amino analog **9** of cephapirin (**4**), *N*-(4-pyridyl)glycine[16] was converted into its acid chloride hydrochloride and coupled in an anhydrous medium with silylated 7-ACA to obtain a 59% yield of **9**.

7-[α-(1,3-Diethylformamidino-2-thio)acetamido]cephalosporanic acid (**10**) is a representative of a series of this class of cephalosporins prepared by displacement of the bromine atom in **3** by various acyclic and cyclic 1,3-disubstituted and trisubstituted thioureas in methylene chloride in the presence of a tertiary amine.

Other investigators have reported[17] that 7-[(α-formamidinothio)acetamido]cephalosporanic acid was not obtained from the reaction of 7-(α-chloroacetamido)-cephalosporanic acid with thiourea in aqueous solution. The products isolated were 7-ACA and pseudothiohydantoin formed by intramolecular displacement of the *N*-chloroacetyl group by thiourea.

Structure–Activity Relationships. Microbiological reports relating to cephapirin (**4**),†[18] its 1-methyl betaine **5**,‡[19] and the class of substituted formamidinothioacetamidocephalosporanic acids[20] exemplified by **10** have disclosed the potent antimicrobial properties of these semisynthetic cephalosporins, some of which compare favorably with commercially important cephalothin (**11**).[21,22]

Some comparative minimal inhibitory concentrations (MIC's) data, expressed in μg/ml, are given in Table I. The MIC's were determined by the standard twofold broth dilution method after overnight incubation at 37°. Although all of the compounds shown have broad-spectrum activity, **10** and **5** stand out in overall activity. Crystalline **10** is unstable in aqueous solutions and, to a lesser degree, in the dry state. In general, these compounds derived from the substituted thioureas had good resistance toward β-lactamases produced by cephalothin-sensitive strains of *Klebsiella pneumoniae* and *Escherichia coli* but were found to be somewhat more toxic to animals than the other cephalosporins reported in this paper.[20]

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. The elemental analyses are within ±0.4% of theoretical values unless stated. The ir spectra were recorded on a Beckman IR-9 spectrometer. The nmr spectra were run on a Varian A-60 spectrometer at a sweep width of 500 Hz with Silanor-D_2O–TSP as the internal standard.

7-(α-Bromoacetamido)cephalosporanic Acid (**3**). To a vigorously stirred suspension of 200 g (0.731 mol) of 7-aminocephalosporanic acid (7-ACA, **2**) in 2 l. of water and 750 ml of acetone was added 200 g (2.38 mol) of $NaHCO_3$, in portions, to prevent excessive foaming. The resulting solution was cooled to 3° and 200 g (0.99 mol) of bromoacetyl bromide was added in portions over a 10-min period. After 1 hr of stirring with the cooling bath removed, the pH was adjusted to 4 with 40% H_3PO_4. Decolorizing carbon (40 g, Darko-KB) was added and after 15 min the slurry was filtered through a Celite pad, with suction, and the pad washed with 600 ml of water. The combined filtrates were then brought to pH 2 with 40% H_3PO_4 and after stirring for 1 hr in an ice bath the crystalline product was filtered, washed well with cold water, and air-dried. The yield was 200 g (77%). An analytical sample was obtained by recrystallization from acetone–water, mp 173°. *Anal.* ($C_{12}H_{13}BrN_2O_6S$) C, H, N, Br.

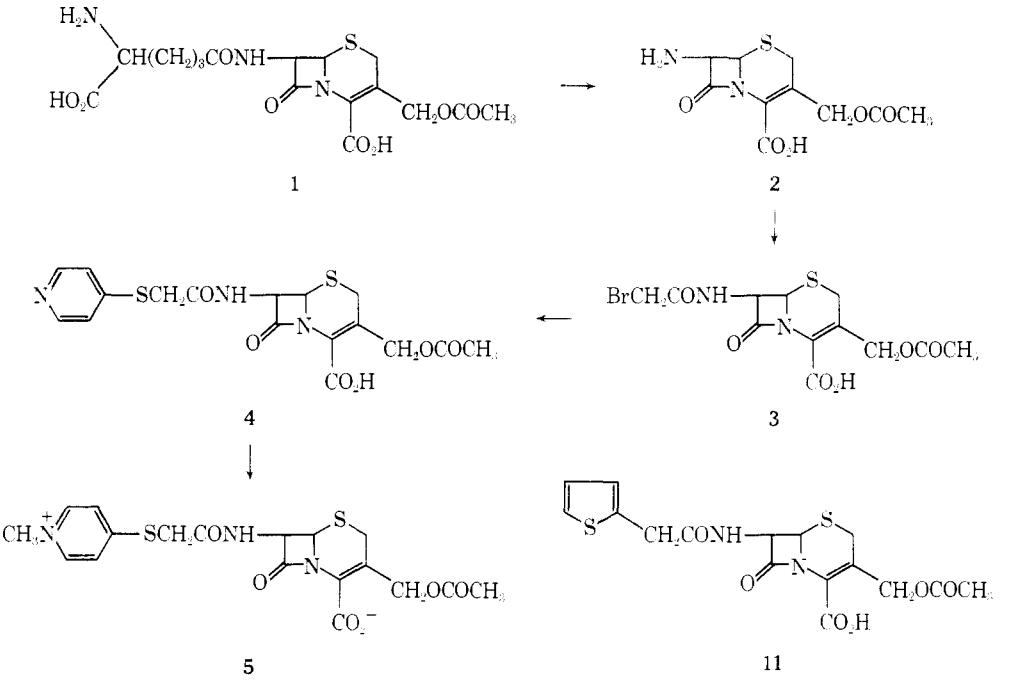
7-[α-(4-Pyridylthio)acetamido]cephalosporanic Acid (**4**).[8] To a stirred suspension of 39.3 g (0.1 mol) of **3** in 500 ml of CH_2Cl_2 was added 14 ml (0.1 mol) of triethylamine. To the resulting solution was added 11.1 g (0.1 mol) of 4-mercaptopyridine. A nearly clear solution resulted with precipitation of the product beginning after a few minutes. After stirring 4 hr the crystalline product was filtered off, washed well with CH_2Cl_2, and air-dried. The white product weighed 35 g (82%). Recrystallization from boiling acetone–water (1:1) gave an analytical sample, mp 155°. Nmr indicated 0.5 mol of acetone of solvation: nmr (D_2O + $KHCO_3$) δ 7.7–8.6 (m, 4, $-C_6H_4N$), 4.2 (s, 2, $-CH_2-$). *Anal.* ($C_{17}H_{17}N_3O_6S_2 \cdot 0.5C_3H_6O$) C, H, N.

7-[α-(1-Methyl-4-pyridylthio)acetamido]cephalosporanic Acid Betaine (**5**).[12] To a stirred suspension of 42.3 g (0.1 mol) of **4** in 50 ml of water and 600 ml of acetone at 22° was added 12.9 g

†Cephapirin is the nonproprietary name for 7-[α-(4-pyridylthio)acetamido]cephalosporanic acid (**4**), code number BL-P1322; CEFADYL is the trade name of Bristol Laboratories for this compound.

‡The code number for **5** is BL-S217.

Scheme I

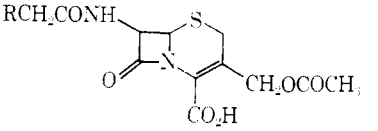


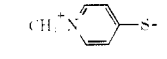
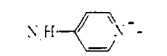
(0.1 mol) of *N,N*-diisopropylethylamine. To the resulting clear solution was added 14.2 g (0.1 mol) of methyl iodide and the solution seeded after 30 min. After 6 hr the product was collected by filtration and washed with acetone–water (12:1) and finally with acetone. The yield was 20 g (44%); mp 160°; nmr (D_2O) δ 4.3 (5.3, CH_3N^+). *Anal.* ($C_{18}H_{19}N_3O_6S_2 \cdot H_2O$) C, H, N.

7-[α-(**3-Pyridylthio**)**acetamido**]**cephalosporanic Acid (6).** To a stirred solution of 3.93 g (0.01 mol) of **3** and 1.68 g (0.02 mol) of $NaHCO_3$ in 50 ml of water was added 1.11 g (0.01 mol) of 3-mercaptopyridine.[12] Ten minutes later the solution was extracted once with 50 ml of ethyl acetate. The aqueous phase was stirred under a layer of ethyl acetate (50 ml) while being acidified to pH 2 with 40% H_3PO_4. The aqueous phase was separated and extracted with 3 g (0.0075 mol) of Aerosol OT (dioctyl sodium sulfosuccinate) in 50 ml of MIBK. The MIBK extract was dried briefly over Na_2SO_4, filtered, and concentrated to about 25 ml. Triethylamine (1 ml, TEA) was added and the resulting precipitate was collected by filtration and washed with MIBK and ether. Air-dried it weighed 550 mg. From the aqueous mother liquor was obtained 455 mg of crystalline material on standing 4 hr. An analytical sample was obtained by recrystallization from hot 2-propanol–water (50%), mp 166°. *Anal.* ($C_{17}H_{17}N_3O_6S_2$) C, H, N.

7-[α-(**2-Pyridylthio**)**acetamido**]**cephalosporanic Acid (7).** To a stirred solution of 3.93 g (0.01 mol) of **3** and 1.68 g (0.02 mol) of $NaHCO_3$ in 50 ml of water was added 1.11 g (0.01 mol) of 2-mercaptopyridine. After 1 hr at 22°, the pH was adjusted to 2.5 with 40% H_3PO_4 under a layer of 50 ml of ethyl acetate. The ethyl acetate layer was washed with 25 ml of water and dried 15 min over Na_2SO_4, filtered, and concentrated under reduced pressure to a volume of *ca.* 20 ml. A total of 1.7 g of crystalline product was obtained after standing 2 hr. The yield was 1.45 g (34%), mp 140°. *Anal.* ($C_{17}H_{17}N_3O_6S_2 \cdot H_2O$) C, H, N.

Table I. Antibacterial Activity of α-Heterosubstituted Acetamidocephalosporanic Acids



No.	R	*D. pneumoniae*	*Str. pyogenes*	*S. aureus* Smith	*S. aureus* BX 1633[b]	*E. coli* Juhl	*K. pneumoniae*	*Sal. enteriditis*
4		0.062	0.062	0.25	0.8	12.5	3.1	0.3
5[a]		0.004	0.004	0.3	0.3	2	2	1
6		0.062	0.016	0.031	0.2	25	3.1	0.8
7		0.5	0.062	0.125	0.4	100	50	12.5
8[a]		0.008	0.008	0.13	2	16	8	8
9		0.004	0.004	0.16	0.3	32	16	2.5
10		0.04	0.04	0.6	0.3	1	0.5	0.3
11	Cephalothin	0.08	0.08	0.16	0.8	12.5	6.2	0.13

[a]Betaine structure instead of free acid. [b]Benzylpenicillin-resistant strain.

7-[α-(4-Pyridylamino)acetamido]cephalosporanic Acid (9). (a) *N*-(4-Pyridyl)glycyl Chloride Dihydrochloride. A stirred suspension of 10 g (0.065 mol) of *N*-(4-pyridyl)glycine[15] in 200 ml of CH_2Cl_2 was cooled to −5° while being saturated with dry HCl gas. To this suspension was added 17.85 g (0.086 mol) of PCl_5 and the mixture stirred 1 hr at −5° and 2 hr at 0°. The solids were collected by filtration, washed well with dry CH_2Cl_2, and dried under vacuum over P_2O_5. The yield was 9.3 g whose ir spectra had a carbonyl (acid chloride) at 1785 cm^{-1} as opposed to the carbonyl on the starting acid hydrochloride of 1710 cm^{-1}. The crude acid chloride was used for the acylation.

(b) **Coupling.** To a suspension of 8.16 g (0.03 mol) of 7-ACA (2) in 150 ml of dry CH_2Cl_2 was added 8.1 ml (0.058 mol) of TEA and 5.3 ml of *N,N*-dimethylaniline. The resulting solution was cooled to 0° and 7.6 ml (0.06 mol) of trimethylchlorosilane in 30 ml of CH_2Cl_2 was added dropwise. After 5 min at 0° the solution was refluxed for 30 min and cooled to −5°, and the crude *N*-(4-pyridyl)glycyl chloride hydrochloride added in portions over a 30-min period. The cooling bath was then removed and the mixture allowed to come to room temperature over a 2-hr period. To this mixture was added 150 ml of water and the pH adjusted to 1.8 with 20% NaOH. The slurry was then filtered and the aqueous layer separated from the filtrate. The aqueous solution was stirred 15 min with 2 g of decolorizing carbon (Darko-KB) and filtered and the pH adjusted to 3 with 20% NaOH under a layer of 150 ml of ether. The product crystallized and after 10 min stirring was cooled at 0° for 30 min. The product was collected by filtration, washed with water and then acetone, and air-dried. After drying 18 hr over P_2O_5 the yield was 7.01 g (59%), mp 192°. *Anal.* ($C_{17}H_{18}N_4O_6S \cdot H_2O$) H, N; C: calcd 48.11; found, C, 48.56.

7-[α-(1,3-Diethylformamidino-2-thio)acetamido]cephalosporanic Acid (10).[11] To a stirred solution of 3.93 g (0.01 mol) of 3 and 1.4 ml (0.01 mol) of TEA in 50 ml of CH_2Cl_2 and 10 ml of acetone was added 1.32 g (0.01 mol) of *N,N'*-diethylthiourea (Eastman). The slightly turbid solution was filtered and after stirring for 30 min the crystalline precipitate was collected by filtration, washed well with CH_2Cl_2, air-dried, and vacuum-dried over P_2O_5. The yield was 3.05 g (68%), mp 130°. *Anal.* ($C_{17}H_{24}N_4O_6S_2$) C, H, N.

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Additions and Corrections

1972, Volume 15

Raymond D. Kimbrough, Jr.: Synthesis and Oral Hypoglycemic Activity of *N*-(*p*-Deuteriomethylbenzenesulfonyl)-*N'*-*n*-butylurea, Deuterium-Substituted Tolbutamide.

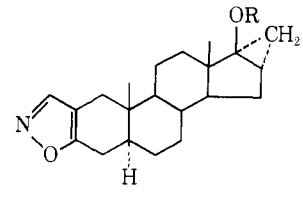
Page 409. Reference to prior work on this subject by R. U. Lemieux, K. F. Sporek, I. O'Reilly, and E. Nelson, *Biochem. Pharmacol.*, 7, 31 (1961), was omitted. The results published are in agreement with the prior definitive work of Lemieux, *et al.*

T. Kametani, M. Ihara, T. Suzuki, T. Takahashi, R. Iwaki, H. Takei, N. Miyake, M. Yoshida, Y. Hasegawa, and H. Kitagawa: Studies on the Syntheses of Heterocyclic Compounds. 459. Synthesis of Rescinnamine-Like Compounds as Antihypertensive Agents.

Page 686. In Table I, R_2 of compound **12** and R_3 of compound **13** should be $OCO_2C_2H_5$.

Kenneth E. Fahrenholtz, Kenneth P. Meyers, and R. W. Kierstead: Cycloprop[16α,17α]androstanes.

Page 1057. Structure **23** should be corrected to read



Page 1058. Footnote *a* in Table II should be changed from $p < 0.0001$ to $p < 0.001$.