

- (8) C. S. Barnes, K. H. Pausacker, and C. I. Schubert, *J. Chem. Soc.*, 1381 (1949).

### Synthesis of Cephapirin† and Related Cephalosporins from 7-( $\alpha$ -Bromoacetamido)cephalosporanic Acid

Leonard B. Crast, Jr.,\* Robert G. Graham, and Lee C. Cheney

Research Division, Bristol Laboratories, Division of Bristol-Myers Company, Syracuse, New York 13201. Received July 5, 1973

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

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

Treatment of an aqueous-acetone suspension of 7-ACA (2) with bromoacetyl bromide in the presence of sodium bicarbonate afforded crystalline 7-( $\alpha$ -bromoacetamido)cephalosporanic acid (3) in 77% yield. The interaction of 3, 4-mercaptopyridine, and triethylamine in methylene chloride at room temperature produced 7-[ $\alpha$ -(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-[ $\alpha$ -(1-methyl-4-pyridylthio)acetamido]cephalosporanic acid betaine (5).<sup>‡,12</sup>

3-Mercaptopyridine<sup>13</sup> 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-[ $\alpha$ -(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.<sup>14</sup> Compound 4 and the product of unequivocal structure which they obtained by coupling 7-ACA (2) with  $\alpha$ -(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<sup>15</sup> 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<sup>16</sup> 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-[ $\alpha$ -(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<sup>17</sup> that 7-[ $\alpha$ -( $\alpha$ -formamidinothio)acetamido]cephalosporanic acid was not obtained from the reaction of 7-( $\alpha$ -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),<sup>†,18</sup> its 1-methyl betaine 5,<sup>‡,19</sup> and the class of substituted formamidinothioacetamidocephalosporanic acids<sup>20</sup> exemplified by 10 have disclosed the potent antimicrobial properties of these semisynthetic cephalosporins, some of which compare favorably with commercially important cephalothin (11).<sup>21,22</sup>

Some comparative minimal inhibitory concentrations (MIC's) data, expressed in  $\mu$ g/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  $\beta$ -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.<sup>20</sup>

### Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. The elemental analyses are within  $\pm 0.4\%$  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<sub>2</sub>O-TSP as the internal standard.

**7-( $\alpha$ -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<sub>3</sub> 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<sub>3</sub>PO<sub>4</sub>. 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<sub>3</sub>PO<sub>4</sub> 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<sub>12</sub>H<sub>13</sub>BrN<sub>2</sub>O<sub>6</sub>S) C, H, N, Br.

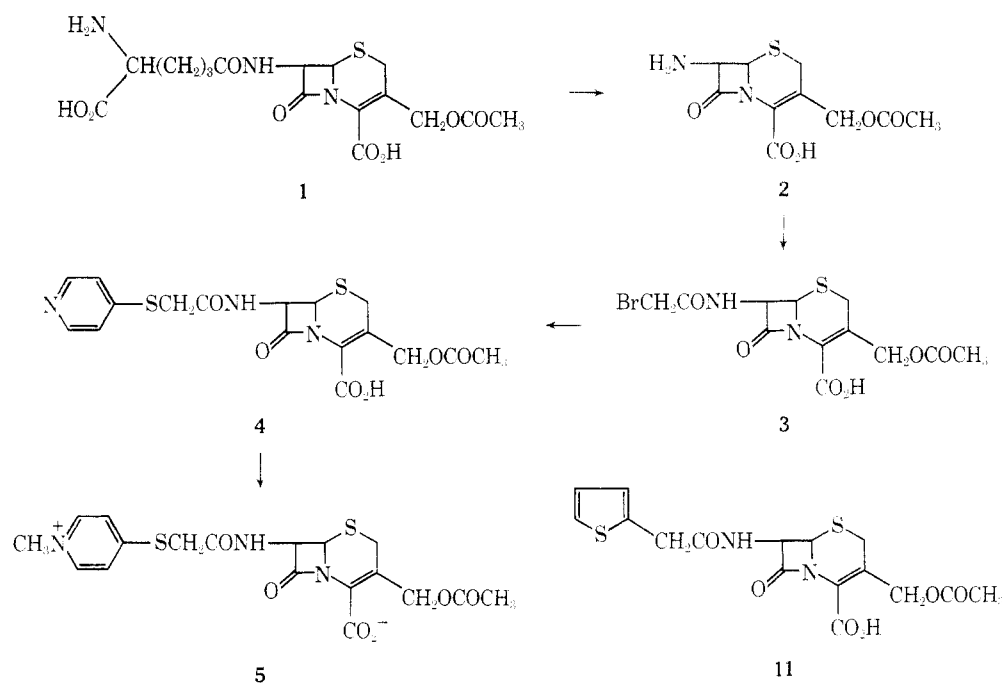
**7-[ $\alpha$ -(4-Pyridylthio)acetamido]cephalosporanic Acid (4).<sup>8</sup>** To a stirred suspension of 39.3 g (0.1 mol) of 3 in 500 ml of CH<sub>2</sub>Cl<sub>2</sub> was added 14 ml (0.1 mol) of triethylamine. To the resulting solution was added 11.1 g (0.1 mol) of 4-mercaptopyridine. A nearly 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<sub>2</sub>Cl<sub>2</sub>, 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<sub>2</sub>O + KHCO<sub>3</sub>)  $\delta$  7.7-8.6 (m, 4, -C<sub>6</sub>H<sub>4</sub>N), 4.2 (s, 2, -CH<sub>2</sub>-). *Anal.* (C<sub>17</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub> · 0.5C<sub>3</sub>H<sub>6</sub>O) C, H, N.

**7-[ $\alpha$ -(1-Methyl-4-pyridylthio)acetamido]cephalosporanic Acid Betaine (5).<sup>12</sup>** 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-[ $\alpha$ -(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 ( $D_2O$ )  $\delta$  4.3 (5.3,  $CH_3N^+$ ). *Anal.* ( $C_{18}H_{19}N_3O_6S_2 \cdot H_2O$ ) C, H, N.

**7-[ $\alpha$ -(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.<sup>12</sup> 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. Triethyl-

amine (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-[ $\alpha$ -(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  $\alpha$ -Heterosubstituted Acetamidocephalosporanic Acids**

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

<sup>a</sup>Betaine structure instead of free acid. <sup>b</sup>Benzylpenicillin-resistant strain.

7-[ $\alpha$ -(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<sup>15</sup> in 200 ml of  $\text{CH}_2\text{Cl}_2$  was cooled to  $-5^\circ$  while being saturated with dry  $\text{HCl}$  gas. To this suspension was added 17.85 g (0.086 mol) of  $\text{PCl}_5$  and the mixture stirred 1 hr at  $-5^\circ$  and 2 hr at  $0^\circ$ . The solids were collected by filtration, washed well with dry  $\text{CH}_2\text{Cl}_2$ , and dried under vacuum over  $\text{P}_2\text{O}_5$ . The yield was 9.3 g whose ir spectra had a carbonyl (acid chloride) at  $1785\text{ cm}^{-1}$  as opposed to the carbonyl on the starting acid hydrochloride of  $1710\text{ cm}^{-1}$ . 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  $\text{CH}_2\text{Cl}_2$  was added 8.1 ml (0.058 mol) of TEA and 5.3 ml of *N,N*-dimethylaniline. The resulting solution was cooled to  $0^\circ$  and 7.6 ml (0.06 mol) of trimethylchlorosilane in 30 ml of  $\text{CH}_2\text{Cl}_2$  was added dropwise. After 5 min at  $0^\circ$  the solution was refluxed for 30 min and cooled to  $-5^\circ$ , 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%  $\text{NaOH}$ . 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%  $\text{NaOH}$  under a layer of 150 ml of ether. The product crystallized and after 10 min stirring was cooled at  $0^\circ$  for 30 min. The product was collected by filtration, washed with water and then acetone, and air-dried. After drying 18 hr over  $\text{P}_2\text{O}_5$  the yield was 7.01 g (59%), mp  $192^\circ$ . *Anal.* ( $\text{C}_{17}\text{H}_{18}\text{N}_4\text{O}_6\text{S}\cdot\text{H}_2\text{O}$ ) H, N; C: calcd 48.11; found, C, 48.56.

7-[ $\alpha$ -(1,3-Diethylformamidino-2-thio)acetamido]cephalosporanic Acid (10).<sup>11</sup> 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  $\text{CH}_2\text{Cl}_2$  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  $\text{CH}_2\text{Cl}_2$ , air-dried, and vacuum-dried over  $\text{P}_2\text{O}_5$ . The yield was 3.05 g (68%), mp  $130^\circ$ . *Anal.* ( $\text{C}_{17}\text{H}_{24}\text{N}_4\text{O}_6\text{S}_2$ ) C, H, N.

**Acknowledgments.** The authors wish to thank Miss Delores Gibbs for valuable technical assistance. We also wish to thank Mr. R. M. Downing and Miss Elizabeth A. Ragan for the microanalyses, Mr. D. V. Whitehead and Mr. A. L. Vulcano for the spectral data, and Dr. M. Mis-

iek and Mr. K. L. DenBleyker for the microbiological data.

## References

- (1) E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **79**, 377 (1961).
- (2) D. C. Hodgkin and E. N. Maslem, *ibid.*, **79**, 393 (1961).
- (3) R. B. Morin, B. G. Jackson, E. H. Flynn, and R. W. Roeske, *J. Amer. Chem. Soc.*, **84**, 3400 (1962).
- (4) R. B. Morin, B. G. Jackson, E. H. Flynn, R. W. Roeske, and S. L. Andrews, *ibid.*, **91**, 1396 (1969).
- (5) B. Fechtig, H. Peter, H. Bickel, and E. Vischer, *Helv. Chim. Acta*, **51**, 1108 (1968).
- (6) H. W. O. Weissenburger and M. G. van der Hoeven, U. S. Patent 3,575,970 (1971).
- (7) E. H. Flynn, Ed., "Cephalosporins and Penicillins Chemistry and Biology," Academic Press, New York, N. Y., 1972.
- (8) L. B. Crast, Jr., U. S. Patent 3,422,100 (1969).
- (9) L. B. Crast, Jr., South African Patent 6,707,783 (1968); *Chem. Abstr.*, **70**, 68389 (1969).
- (10) L. B. Crast, Jr., U. S. Patent 3,647,789 (1972).
- (11) L. B. Crast, Jr., U. S. Patent 3,646,025 (1972).
- (12) L. B. Crast, Jr., U. S. Patent 3,631,027 (1971).
- (13) H. M. Wuest and E. H. Sakal, *J. Amer. Chem. Soc.*, **73**, 1210 (1951).
- (14) H. H. Silvestri and D. A. Johnson, U. S. Patent 3,503,967 (1970).
- (15) H. Bickel, J. Mueller, R. Bosshardt, H. Peter, and B. Fechtig, *German Offen.* 2,048,436 (1971); *Chem. Abstr.*, **75**, 20417t (1971).
- (16) D. Masaki and M. Mitsuo, *Bull. Chem. Soc. Jap.*, **33**, 1150 (1960).
- (17) J. D. Cocker, B. R. Cowley, J. S. G. Cox, S. Eardley, G. I. Gregory, J. K. Lazenby, A. G. Long, J. C. P. Sly, and G. A. Somerfield, *J. Chem. Soc.*, 5015 (1965).
- (18) D. R. Chisholm, F. Leitner, M. Misiek, G. E. Wright, and K. E. Price, *Antimicrob. Ag. Chemother.*, **244** (1970).
- (19) R. E. Buck, F. Leitner, and K. E. Price, *ibid.*, **1**, 67 (1972).
- (20) M. Misiek, T. A. Pursiano, L. B. Crast, F. Leitner, and K. E. Price, *ibid.*, **1**, 54 (1972).
- (21) R. R. Chauvette, 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. C. Spencer, and E. Van Heyninge, *J. Amer. Chem. Soc.*, **84**, 3402 (1962).
- (22) E. H. Flynn, U. S. Patent 3,218,318 (1965).

## 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.

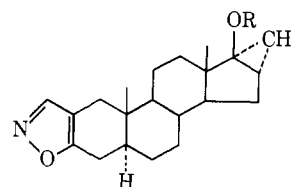
Page 409. Reference to prior work on this subject by R. U. Lemieux, K. F. Sporek, I. O'Reilly, and E. Nelson, *Biochem. Pharmacol.*, **7**, 31 (1961), was omitted. The results published are in agreement with the prior definitive work of Lemieux, *et al.*

**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  $\text{OCO}_2\text{C}_2\text{H}_5$ .

**Kenneth E. Fahrenholtz, Kenneth P. Meyers, and R. W. Kierstead:** Cycloprop[16 $\alpha$ ,17 $\alpha$ ]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  $p < 0.001$ .