for 10 hr. The solvent was removed and the residue was washed with water and crystallized from benzene to give **30** (7.8 g., 78%), m.p. 123.5°.

Anal. Calcd. for $C_{11}H_{16}I_2N_2O_2S$: C, 26.75; H, 3.26; I, 51.35; N, 5.67. Found: C, 27.3; H, 3.05; I, 51.2; N, 5.4.

4-[Bis(2-bromoethyl)amino]acetanilide (22) was prepared from the corresponding chloro compound (**10**) and LiBr in 67% yield, m.p. 135–137° (from benzene).

Anal. Calcd. for $C_{12}H_{16}Br_2N_2O$: C, 39.6; H, 4.4. Found: C, 40.6; H, 4.67.

4-[Bis(2-iodoethyl)amino]acetanilide (28) was prepared from the corresponding bromo compound (**22**) and NaI in 75% yield, m.p. 145–146° (benzene–ethanol, 1:1).

Anal. Calcd. for $C_{12}H_{16}I_2N_2O$: C, 31.5; H, 3.51; I, 55.4. Found: C, 31.9; H, 3.2; I, 55.1.

***p*-[Bis(2-iodoethyl)amino]benzoic acid (27)** was prepared from the corresponding bromo compound (**21**) and NaI in 82% yield, m.p. 216° (from benzene).

Anal. Calcd. for $C_{11}H_{13}I_2NO_2$: C, 29.69; H, 2.94; I, 57.03; N, 3.15. Found: C, 29.68; H, 3.02; I, 56.83; N, 3.11.

N^4,N^4-Bis(2-iodoethyl)sulfanilamide (29) was prepared from the corresponding bromo compound (**24**) and NaI in 68% yield, m.p. 163–164° (benzene).

Anal. Calcd. for $C_{10}H_{14}I_2N_2O_2S$: C, 24.9; H, 2.94; I, 52.85; N, 5.83. Found: C, 25.3; H, 2.81; I, 52.75; N, 5.8.

Alkylation Rate Studies with 4-(*p*-Nitrobenzyl)pyridine.—The alkylating agents were dissolved in ethanol at a concentration of 0.2 μmole/ml. At least four 1-ml. aliquots of each solution were pipetted into a series of test tubes. To each test tube were then added 1 ml. of ethanol, 1 ml. of NBP solution [5% w./v. 4-(*p*-nitrobenzyl)pyridine in ethanol], and 1 ml. of 0.05 *M* potassium hydrogen phthalate buffer, pH 4.2. Another series of 4–8 test tubes (to be used as respective "blanks" for each reaction time) were prepared in the same way but replacing the alkylating agent solution with 1 ml. of ethanol.

The contents of each tube were mixed thoroughly, and the tubes were placed in the water bath maintained at constant temperature (80° for k_{80}' and 50° for k_{50}' determination). Individual test tubes (including one "blank" each time) were removed from the water bath at various intervals (*e.g.*, after 5, 10, 20, and 30 min., or after 20, 40, 60, 120, and 180 min., depending on the expected reactivity of the alkylating agents) and were cooled immediately by placing them into an ice–water bath. The contents of each test tube was then brought to a total volume of 5 ml. by the addition of alcohol and mixed thoroughly.

For determining the relative concentrations of the alkylation product formed at various times, the "blank" corresponding to a given reaction time was added to 0.6 ml. of a solution of 0.1 *N* KOH in 80% (v./v.) aqueous ethanol in a "matched" colorimeter tube, mixed for 20 sec. with a Vortex mixer and then immediately used for the adjustment of the colorimeter to "100% transmission" (zero absorbance) reading. Subsequently, the contents of the reaction tubes which had been heated for the same length of time were treated similarly, one by one, with the "alkali solution" and immediately read in the colorimeter. Before reading the next series of test tubes (heated for a different time period), the colorimeter was reset to "zero absorbance" with the use of the respective "blank." All readings were taken at 600 mμ in a Bausch and Lomb (Spectronic 20) spectrophotometer. The absorbance readings were plotted against time, and the slopes of the linear plots gave directly the k' values for the alkylating agents.

Biological Test Methods. Toxicity Determinations.—Male Holtzman rats, 180–200 g., and male Swiss mice, 22–26 g., were used. The animals were fed a pelleted diet (Purina Laboratory Chow) and tap water *ad libitum*. Animal quarters were maintained at a temperature of 23.3–24.4°. Compounds, dissolved or suspended in cottonseed oil, were administered by intraperitoneal injection to groups of 3 to 6 animals/dose level. All deaths within a 21-day period were recorded and approximate LD_{50} values were estimated graphically from per cent mortality/log dose plots.

Tumor Inhibition Studies.—Walker carcinosarcoma 256 was implanted subcutaneously in the flank region of male Holtzman rats using a trochar and cannula. A compound, dissolved or suspended in cottonseed oil, was injected intraperitoneally on the day following tumor implantation. Control animals received cottonseed oil only. The rats were killed 10 days later, and the tumors were dissected out and weighed. The ratio of the mean weight of treated tumors to the mean weight of control tumors (T/C) was determined and plotted against log dose. The therapeutic index was obtained from the ratio LD_{50}/ED_{90}, where ED_{90} is the dose corresponding to a T/C ratio of 0.1.

Acknowledgment.—The authors appreciate the able assistance of Mrs. Maureen Triggle, in the preparation of some of the compounds, and Mr. Paul L. Stanley, in the biological testing.

The Chemistry of Cephalosporins. IV. Acetoxyl Replacements with Xanthates and Dithiocarbamates

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The nucleophilic displacement of the acetoxyl group of synthetic cephalosporins by alkylxanthate salts and dialkyl- and dialkylaminoalkyldithiocarbamate salts is reported. A brief discussion of the biological activity of the products is included.

As indicated in a previous report,[1] one of the several points in naturally occurring cephalosporin C amenable to variation is the 3-acetoxymethyl function. Several changes in the acyloxy group of some synthetic cephalosporins were reported. The present paper concerns another type of variation of the 3-methyl group substitution. Abraham and co-workers[2] treated cephalosporin C with a series of pyridine derivatives and displaced the acetoxyl group, forming pyridinium derivatives which possessed enhanced antibiotic activity against gram-negative organisms. We have found that other nucleophilic reagents besides pyridine will displace the acetoxyl group and, in particular, that a ready displacement occurs with xanthates and dithiocarbamates which contain bivalent sulfur of high nucleophilicity. The cephalosporin chosen for modification was sodium 7-(2-thiopheneacetamido)-cephalosporanate (I)[3] because of its high intrinsic activity and ready availability.

Nucleophilic displacement with simple xanthates and dithiocarbamates was quite general. Derivatives of II, R = O-alkyl (**1–7**, see table numeration), were

(1) E. Van Heyningen, *J. Med. Chem.*, **8**, 22 (1965).

(2) C. W. Hale, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, **79**, 403 (1961).

(3) Keflin® (cephalothin, Lilly).

obtained with alkyl xanthate salts in yields of 40–50%. Generally, there were obtained somewhat lower yields of derivatives of simple N,N-dialkyldithiocarbamates [II, R = N< (**10–12**)]. Very low yields resulted in cases where hydroxyls were present in the alkyl groups (**13** and **14**), but the low yields reflected difficulty of isolation rather than some inherent lack of reactivity of hydroxylated dithiocarbamates. This was evident from the preparation of **16** from sodium N-methyl-N-sorbityldithiocarbamate in 52% yield where the isolation was relatively simple.

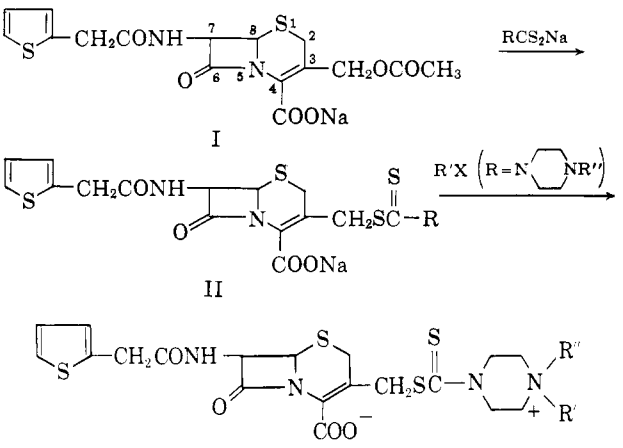
The usual procedure for synthesis of the above derivatives was to heat aqueous mixtures of the cephalosporanate salt and an alkali salt of the xanthate or dithiocarbamate in molar excess at slightly elevated temperatures for several hours. More vigorous reaction conditions were avoided because of hydrolysis of both the β-lactam ring and the acetoxyl group that occurred as competing side reactions. Although the sulfur derivatives so obtained were usually water soluble, they were fortunately less soluble in 50% saturated NaCl solution than either the starting cephalosporin or its hydrolysis products. Most of the derivatives were salted out as yellow glasses, but the crude products still contained traces of starting materials and various hydrolysis products. Many were purified quite readily because their salts were soluble in chloroform. The chloroform solution was washed repeatedly with 50% saturated NaCl solution, the impurities being extracted into the aqueous layer.

The procedure outlined above for the simple xanthates and dithiocarbamates did not yield derivatives when alkali salts of amino-substituted xanthates and dithiocarbamates were employed. Aminoalkylxanthate salts give strongly basic water solutions (pH >10) and if these were used, the β-lactam ring of the cephalosporin was quickly destroyed. In one instance, the alkalinity of the solution of sodium diethylaminoethylxanthate was reduced by formation of the zwitterion, but even then the product obtained did not possess the characteristic absorption of a β-lactam ring in the infrared and was biologically inactive.[3] Dialkylamino-substituted dithiocarbamates, however, did displace the acetoxy group and yielded the expected products, if the necessary pH adjustment to less than 8.0 was made. The alkali salts of those dithiocarbamates that contained rather weakly basic amino substituents, such as the N-alkylpiperazine derivatives, gave water solutions of an alkalinity low enough to be used without pH adjustment, while those with more strongly basic amine substituents required adjustment to a lower pH with HCl. In the latter cases products were not sodium salts but zwitterions (**29–31** and **34–36**), and in some instances these zwitterions were quite water insoluble and precipitated from the reaction mixture. Some of these zwitterions were soluble in chloroform and could be purified as described above.

The series of piperazine derivatives (II, R = N-$(CH_2CH_2)_2NR''$) that were prepared had alkyl groups R'' that varied from methyl to *n*-octyl (**17–23**) and adamantyl (**24**). The yields were consistently poor, less than 25%. Replacement of the alkyl substitution by phenyl (**25**) and hydroxyethyl (**26**) was made. One piperazine substituted with a carboxylate group (**28**) was also prepared.

The group of amine-substituted alkyl dithiocarbamates had structures II, R = $R'N(CH_2)_xNR_2''$, where R' was most often methyl, but also hydrogen or ethyl (see below for a discussion of structure); $(CH_2)_x$ was either ethylene or trimethylene; and NR_2'' was either a dialkyl or cyclic amine (**29–37**).

Betaines (III) were prepared from several of the N-alkylpiperazine-containing cephalosporin derivatives by reaction of the sodium salts II [R = $N(CH_2CH_2)_2$-



NR''] with methyl iodide (**38**, **39**, and **41**) or allyl bromide (**40**) in chloroform. There can be no doubt that the compounds prepared were the quaternary salts and not the isomeric esters. The esters would be soluble in organic solvents and would show the N-alkylpiperazine p*K* in titration and the carboxylate ester band in the infrared; the products obtained had the properties expected for structure III in solubility, titration, and spectrum. On the other hand, preparation of betaines by treatment of the 4-methylpiperazine derivative **17** [II, R = $-N(CH_2CH_2)_2NCH_3$] with less reactive alkyl halides, such as butyl or propyl bromide, in chloroform at room temperature or at reflux, was unsuccessful. From these limited data it would appear that a highly reactive alkyl halide is necessary for alkylations of these weakly basic materials. The state of aggregation in chloroform solution and the extent of solvation of the aggregates is probably also of significance.[4] Osmometric molecular weight determination for the N-methylpiperazine derivative **17** gave a value in dichloroethylene of 4000; the formula weight is 534. It is reasonable to suppose that all the N-alkylpiperazine derivatives are similarly aggregated and that aggregation would slow the rate of alkylation.

Only a few N-monoalkyldithiocarbamate derivatives (**8**, **9**, **31**, and **37**) were prepared because they proved to have low biological activity. Whether this low activity is a characteristic of monoalkylated derivatives or whether these compounds have different structures from the other derivatives could not be definitely established. Although infrared spectra of the monoalkylated derivatives were similar in β-lactam carbonyl intensity to that of the dialkylated dithiocarbamates, the ultraviolet spectra showed a deep trough in the 250-mμ region where the conjugated β-lactam system customarily absorbs. All attempts to obtain useful n.m.r. spectra of these salts, or of any dithiocarbamyl

(4) H. E. Zaugg, *J. Am. Chem. Soc.*, **82**, 2995 (1960).

derivative in this study for that matter, were unsuccessful, whether in deuteriochloroform, deuterium oxide, or hexadeuteriodimethylformamide. Although undoubtedly adequate concentrations of the salts were obtained, the spectra were of very low intensity and unsuited for analysis. Acids and esters gave similarly poor spectra. For example, the synthesis of methyl 7-benzamido-3-(N-methylaminothiocarbonylthiomethyl)-3-cephem-4-carboxylate, which contains a minimum of aliphatic protons, was attempted in the hope that its n.m.r. spectrum would be adequate. The product obtained, as all others, did not possess a useful spectrum. This phenomenon may be due to the state of aggregation in solution. As a consequence, no simple determination of the presence, absence, or location of the double bond in the monoalkylated dithiocarbamates was at hand, but it would appear probable that the double bond is either no longer present or shifted to the 2,3-position in the cephem ring.

All derivatives previously mentioned were prepared by nucleophilic displacement of the acetoxyl on a 7-acylaminocephalosporanic acid salt. An alternative route would be acylation of a 7-aminocephalosporanic acid derivative already containing the xanthate or dithiocarbamate group. Such a derivative was prepared from sodium ethyl xanthate and 7-aminocephalosporanic acid. The crude potassium 7-amino-3-ethoxythiocarbonylthiomethyl-3-cephem-4-carboxylate was then acylated with chloroacetyl chloride. The resulting compound probably could not have been prepared by nucleophilic displacement of the acetoxyl of the chloroacetylcephalosporanate because the chlorine would likewise have been displaced. When considered from the viewpoint of ease of isolation and purification of the respective intermediates in each route, the usual method above seems preferable for the general synthesis.

Biological Evaluation.—The compounds were evaluated in five different tests. The penicillin G test was the usual disk–plate assay against *Staphylococcus aureus*, and in it the greatest number of these derivatives failed to follow the penicillin G curve. Values recorded are either for a range, if the curves were highly divergent (higher values were at lower dilutions), or a single value if the slopes of the curves were similar. The penicillin-sensitive *S. aureus* test was obtained as one of a spectrum of representative organisms in a minimum inhibitory concentration (MIC) test by agar serial dilution and is included for comparison with the penicillin-resistant test, performed without and with human serum (20%). The former was the usual plate test with *S. aureus* 3055 while the latter was a gradient plate test,[5] with a penicillinase-producing strain of *S. aureus*, V-30. The oral mouse test was in *Streptococcus pyogenes* (C 203) infected mice and dosage was by oral administration (gavage), 1 and 5 hr. post infection with 2000–6000 times the LD_{50} inoculum (i.p.). The dose listed is the effective dose in prolonging life in 50% of the mice.[6] Results for gram-negative organisms were obtained in gradient plates.

Table I is, in general, self-explanatory, although some brief deductions will be noted. Most of the derivatives possess a fair degree of gram-positive activity. Those in which activity is low are compounds with long alkyl chains (**23**) or with polyhydroxy groups (**14** and **16**) or carboxylate groups (**28**) in the substituent group. In the dithiocarbamates, two substituents on the amide nitrogen are necessary for good activity; those with one substituent on the nitrogen may not be strictly comparable, however, because of their dubious structure (**8**, **9**, **31**, and **37**). Quaternized or protonated amino groups impart enhanced gram-positive activity (**29**–**41**).

Activity against penicillin-resistant *S. aureus* is generally good in the absence of serum, but added serum necessitated a 10-fold or more increase in antibiotic concentration and also caused "skip zones" in the gradient plate test in most instances. Those derivatives which are not so noticeably affected by added serum are those with charged amino groups, either quaternized or protonated at pH 7 (**29**–**41**).

The mouse protection test gives a value which is probably a function of oral absorption and gram-positive activity. Since the derivatives are mostly of the same order of gram-positive activity, the degree of absorption is of major significance in comparisons. The smaller xanthates and dithiocarbamates are more readily absorbed than the larger piperazine derivatives. It is somewhat surprising, at first glance, that charged and water-insoluble zwitterions and quaternaries are apparently quite well absorbed; this may only illustrate lack of serum effects on these compounds, and oral absorption could well be of the same order or less than the less polar compounds.

Gram-negative activity is relatively poor in the xanthates (**1**–**7**), being about the order of penicillin G. The simple dithiocarbamates (**10**–**12**) are only slightly better. The piperazine-containing dithiocarbamates (**17**–**28**) show much better activity, and the best compound of the series (**17**) is in this group. The zwitterionic derivatives (**29**–**36**) are half as active as the piperazino compounds (**17**–**28**) as a group, while the quaternized piperazine derivatives (**38**–**41**) are less effective than their precursors.

The biological data for penicillin G and cephalothin are included at the end of Table I for comparison.

Experimental[7]

Preparation of Xanthates.—The method of Drawert, *et al.*, was used.[8] Crushed KOH (0.1 mole) was dissolved in 40–100 ml. of the alcohol, warming gently if necessary, and then cooling to 25°. Carbon disulfide (0.1 mole) was added dropwise, slowly, with stirring, and the solution was stirred for a further hour. Ether was added and the precipitated xanthate was filtered. The xanthates could be recrystallized either from ethyl acetate or from methanol by dilution with ether to cloudiness. In this way the potassium salts of ethyl-,[9] *n*-propyl-,[9] isopropyl-,[9] *n*-butyl-,[9] *n*-hexyl-,[9] cyclopentyl-,[10] cyclohexyl-,[9] and diethylaminoethylxanthates were prepared.

Preparation of Dithiocarbamates.—The method of Bögemann[11]

(5) C. W. Godzeski, G. Brier, and D. E. Pavey, *Appl. Microbiol.*, **11**, 122 (1963).

(6) W. E. Wick, F. Streightoff, and D. H. Holmes, *J. Bacteriol.*, **81**, 233 (1961).

(7) The melting points were obtained on a Fisher-Johns melting point apparatus and are uncorrected. The ultraviolet spectra were determined in ethanol, the infrared spectra in chloroform or Nujol mull. The titrations were determined in 66% aqueous dimethylformamide using a glass electrode and a standard calomel half-cell.

(8) F. Drawert, K. H. Reuther, and F. Born, *Chem. Ber.*, **93**, 3064 (1960).

(9) W. F. Whitmore and E. Lieber, *Anal. Chem.*, **26**, 1985 (1954).

(10) M.p. >290°. *Anal.* Calcd. for $C_8H_9KOS_2 \cdot 0.5H_2O$: C, 34.42; H, 4.81. Found: C, 34.87; H, 4.51.

(11) M. Bögemann, "Methoden der Organischen Chemie (Houben-Weyl)," Vol. 9, Georg Thieme Verlag, Stuttgart, 1955, p. 826.

TABLE I

BIOLOGICAL ACTIVITIES[a]

No.[b]	Pen. G assay,[c] units/mg.	*S. aureus* MIC,[d] γ/ml.	Pen. resist. *S. aureus*,[e] γ/ml.	*S. pyogenes* oral-mouse[f] ED_{50}, mg./kg. × 2	Gram-negative organisms (MIC, γ/ml.)[g] *Shigella* sp. (N-9)	*E. coli* (N-26)	*Aerobacter* sp. (X-68)	*Klebsiella* sp. (X-26)
1	598	0.05	0.7->10	15.6	78	83	67	6
2	650	0.025	1.3-13.3	10.4	70	84	80	5
3	540	0.05	0.7->10	33	126	129	>200	9
4	610	0.05	0.3-35	13.1	101	108	94	8
5	192	0.20	0.4-55	41.5	>200	>200	108	14
6	550	0.05	0.4-14.3	9.5	99	107	102	6
7	580	0.025	0.7->50	20.8	92	108	116	6
8[h]	56	0.4	1.5-1.4	...	>200	42	42	9
9[h]	77-93	...	1.2-3.8	>41	>200	61	36	11
10	160	0.10	0.6->20	10.4	84	78	134	13
11	280	0.10	0.8->20	10.4	22	44	40	4
12	147	0.2	0.8->50	22.9	64	75	115	16
13	165-240	0.1	0.4->1	72.0	42	23	33	12
14	90	0.2	1->1	>41	124	54	42	18
15	390	0.05	0.2->10	21	>200	>200	>200	13
16	74	0.4	2.4->10	>41	141	88	76	72
17	350-400	0.025	0.4->1.0	11.4	2	3	4	2
18	330-430	...	0.5->1.0	>41	12	4.5	6	2.8
19	435	0.05	0.4->10	>41	26	16	22	5
20	350	0.5	0.5->10	>41	46	14	26	5
21	370-460	0.05	1.0->10	>41	15	24	28	4.5
22	360-530	0.05	0.6->10	>41	40	34	72	5
23	46-130	0.1	0.3->20	>41	>100	>100	>100	8
24	270-370	<0.025	0.3->10	>41	>100	>100	>100	14
25	270	<0.025	0.4->10	>41	>100	>100	59	33
26	260	0.1	0.4->1	19.4	14	6	13	6
27	260-360	...	0.2->1	>41	18	7	7	7
28	30	0.05	0.7->10	>41	58	41	14	20
29	232	0.4	2.4-6.8	7.6	110	54	33	18
30	800	0.05	0.3-1.0	7.3	60	22	27	5
31[h]	<15	3.13	25	>41	>200	>200	>200	>200
32	700	...	0.8->1.0	24.4	118	58	55	11
33	195-250	0.1	1.6->1.0	...	95	23	48	16
34	500	0.048	0.7->1.0	9.1	100	39	57	16
35	500	0.048	0.8-0.4	7.3	88	16	37	10
36	...	...	...	>41	...	...	...	...
37[h]	34	0.78	6.7-...	>41	>200	>200	>200	>200
38	620	0.05	0.4-0.2	1.64	37	7	16	10
39	530	0.05	0.5-0.4	2.6	131	18	24	7
40	700	0.1	0.8-0.4	4.6	88	12	16	6
41	460	0.05	0.4-0.3	4.3	106	19	28	7
42[i]	325	0.02	0.5->1	41.5	11	8	5	6
43[j]	1600	0.01	77-108	1.0	32	42	42	109

[a] Explanations of the various assays are in the Discussion section. Data of this type are only relatively comparable with other testing systems. [b] The numbers correspond to those assigned to compounds in Tables III–VI. [c] This is the usual disk–plate assay for penicillin G. [d] Disk–plate assay for minimum inhibitory concentration (MIC). [e] Type V-30. The first value is without, the second with human serum. [f] The value of the ED_{50} (effective curative dose in 50% of the animals) is twice the value indicated; this latter is the dose given 1 and 5 hr. post infection. [g] Gradient plate assay. [h] The structures of these compounds are equivocal. [i] Cephalothin. [j] Penicillin G.

was used. A solution of sodium hydroxide (0.2 mole) in 35 ml. of water was cooled to 0° and mixed with the amine (0.2 mole). Then carbon disulfide (0.2 mole) was added dropwise and the mixture was stirred 1 hr. The dithiocarbamates with nonbasic side chains precipitated and were filtered, air dried, and recrystallized from ethyl acetate or methanol–ether. The simpler dithiocarbamates so prepared as the sodium salts were made from the following amines: methylamine,[12] *n*-propylamine,[13] N,N-diethylamine,[9] piperidine,[12] α-pipecoline,[14] N-methyl-N-β-hydroxyethylamine,[15] N,N-bis(β-hydroxyethyl)amine,[16] N-methylaniline,[17] and N-methyl-N-sorbitylamine.[18] The yields were from 60–80%.

Some of the basic dithiocarbamates also precipitated but others were soluble in the reaction mixture. In every case, several volumes of acetone were added to complete or effect precipitation of the product. The air-dried products were generally recrystallized from ethyl acetate or acetone–water. They were light yellow to white materials; the basic ones, particularly, possessed disagreeable odors. The ultraviolet spectra were typical[19] and, while the amino groups of the basic dithiocarbamates could be titrated, the dithiocarbamate group decomposed readily on acidification below about pH 5. The basic dithiocarbamate salts gave solutions with pH values 2 units

(12) L. Compin, *Bull. soc. chim. France*, **27**, 464 (1920).

(13) M. Delépine, *Compt. rend.*, **144**, 1126 (1907).

(14) S. S. Livshits and N. V. Preobrazhenskii, *J. Gen. Chem. USSR*, **17**, 1706 (1947).

(15) M.p. 73–76°. *Anal.* Calcd. for $C_4H_8NNaOS_2$: C, 27.73; H, 4.65; N, 8.09. Found: C, 27.50; H, 4.82; N, 8.27.

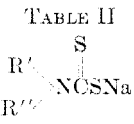
(16) H. L. Klöpping and G. J. M. VanderKerk, *Rec. trav. chim.*, **70**, 935 (1951).

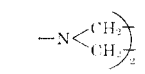
(17) M.p. 130–133°. *Anal.* Calcd. for $C_8H_8NNaS_2$: C, 46.80; H, 3.92; N, 6.82. Found: C, 45.34; H, 3.97; N, 6.57.

(18) M. Hunt, U. S. Patent 2,379,965 (1945); *Chem. Abstr.*, **39**, 5411 (1945).

(19) H. P. Koch, *J. Chem. Soc.*, 401 (1949).

TABLE II



R′ (and R″)[a]	R″	M.p., °C.	Yield,[b] %	Formula[c]	C, % Calcd.	C, % Found	H, % Calcd.	H, % Found	N, % Calcd.	N, % Found
$CH_3N(CH_2CH_2)_2$		>290 dec.	45.5[d]	$C_6H_{11}N_2NaS_2$	36.34	36.43	5.59	5.62	14.13	13.78
$C_2H_5N(CH_2CH_2)_2$		232–235 dec.	76[e]	$C_7H_{13}N_2NaS_2 \cdot 0.5H_2O$	37.98	38.32	6.37	6.20	12.65	12.02
n-$C_3H_7N(CH_2CH_2)_2$		265–268 dec.	80[f]	$C_8H_{15}N_2NaS_2$	42.45	42.28	6.68	6.84	12.38	12.35
i-$C_3H_7N(CH_2CH_2)_2$		262–266 dec.	61.7[g]	$C_8H_{15}N_2NaS_2 \cdot 0.5H_2O$	40.82	40.92	6.85	6.64	11.90	11.57
n-$C_4H_9N(CH_2CH_2)_2$		248–250 dec.	63[d]	$C_9H_{17}N_2NaS_2$	44.97	44.82	7.12	7.14	11.65	11.60
n-$C_5H_{11}N(CH_2CH_2)_2$		254–258 dec.	85[f]	$C_{10}H_{19}N_2NaS_2$	47.21	47.34	7.52	7.75	11.01	10.80
n-$C_8H_{17}N(CH_2CH_2)_2$		224–227 dec.	58[h]	$C_{13}H_{25}N_2NaS_2 \cdot H_2O$	49.65	49.58	8.59	8.51	8.91	8.63
$C_{10}H_{15}N(CH_2CH_2)_2$[i]		267–269 dec.	50[j]	$C_{15}H_{23}N_2NaS_2 \cdot 2H_2O$	50.82	51.08	7.68	7.20	7.90	7.21
$C_6H_5N(CH_2CH_2)_2$		325–328 dec.	83[f]	$C_{11}H_{13}N_2NaS_2$	50.74	50.85	5.03	5.22	10.70	10.59
$HOCH_2CH_2N(CH_2CH_2)_2$		293–294 dec.	75[j]	$C_7H_{14}N_2NaOS_2$	36.82	36.54	5.73	5.89	12.27	11.97
		374–378 dec.	68[k]	$C_6H_8N_2Na_2S_4 \cdot H_2O$	23.98	24.44	3.35	3.52	9.32	9.47
$(CH_3)_2NCH_2CH_2$	CH_3	75–77	55[d]	$C_6H_{13}N_2NaS_2 \cdot H_2O$	33.01	32.79	6.93	6.93	12.83	13.12
$(C_2H_5)_2NCH_2CH_2$	CH_3	80–82	93[f]	$C_8H_{17}N_2NaS_2 \cdot H_2O$	39.00	39.69	7.77	7.52	11.37	11.57
$(C_2H_5)_2NCH_2CH_2$	H	138–150	78[f]	$C_7H_{15}N_2NaS_2 \cdot H_2O$	36.18	36.41	7.38	8.07	12.06	11.70
$(n$-$C_3H_7)_2NCH_2CH_2$	CH_3	...	96[l]	$C_{10}H_{21}N_2NaS_2 \cdot H_2O$	43.76	43.84	8.44	8.67	10.20	10.50
$O(CH_2CH_2)_2NCH_2CH_2$	CH_3	230–233 dec.	15.9[j]	$C_8H_{15}N_2NaOS_2 \cdot H_2O$	36.90	36.63	6.67	6.58	10.76	10.38
$(C_2H_5)_2NCH_2CH_2$	C_2H_5	78–79 dec.	76[f]	$C_9H_{19}N_2NaS_2 \cdot H_2O$	41.36	41.30	8.11	8.05	10.72	10.83
$(C_2H_5)_2NCH_2CH_2CH_2$	CH_3	77–79 dec.	96[f]	$C_9H_{19}N_2NaS_2$					11.56	11.66
$CH_2(CH_2CH_2)_2N(CH_2)_3$	CH_3	115–119	29[j]	$C_{10}H_{19}N_2NaS_2$	44.08	44.50	7.70	7.69	10.28	10.24
$C_6H_5N(CH_3)CH_2CH_2$	H	131–135	59[f]	$C_{10}H_{13}N_2NaS_2$	48.36	48.05	5.27	5.43	11.28	11.56

[a] The first 11 compounds are piperazines. [b] Footnotes to yields indicate solvents used for recrystallization. [c] The samples of high melting point were dried on a block for 1–3 min. at 135–140° before analysis. [d] Water–acetone. [e] Ethyl acetate–petroleum ether (35–60°). [f] Ethyl acetate. [g] Ethanol–petroleum ether (60–71°). [h] THF–benzene. [i] 4-Adamantylpiperazine, supplied by K. Gerzon and E. Krumkalns. [j] Methanol–ether. [k] Methanol. [l] Chloroform.

higher than their amino group pK_a values. Thus, sodium N-methyl-N-dimethylaminoethyldithiocarbamate when titrated in 66% aqueous dimethylformamide had an initial pH of 10.5, a pK_a of 8.8, decomposed at pH 5, and showed an apparent molecular weight of 215 (calcd. 218). The yields and physical constants of the basic dithiocarbamates are listed in Table II.

Nucleophilic Displacement of the Acetoxyl Group of Sodium 7-(2-Thiopheneacetamido)cephalosporanate. A. Neutral Dithiocarbamates and Xanthates.—The general procedure described was employed with only slight variations for all the compounds listed in Table III. A 500-mg. (0.0012-mole) sample of sodium 7-(2-thiopheneacetamido)cephalosporanate and an equimolar amount of either the potassium xanthate or sodium dithiocarbamate was dissolved in 10 ml. of water and heated at 40–45° in a thermostated oil bath for 24 hr. Usually, the solution at the end of this time was clear; however, the product could be precipitated as a yellow glass by addition of an equal volume of saturated NaCl solution and chilling for several hours. The supernatant solution was decanted from the yellow, insoluble sodium salt and this glassy salt was dissolved in 25–50 ml. of chloroform. This chloroform solution was washed about ten times with successive 12–15 ml. portions of 50% saturated NaCl solution. At times, especially towards the end of the washings, troublesome emulsions were formed. These would separate with long standing, but it was found to be more convenient to perform the extractions in 125-ml. separatory funnels that were spun briefly at low speed in a centrifuge. The emulsions were readily broken by this technique. The washing could be followed by qualitative ultraviolet spectra of the wash solutions: disappearance of the spectrum for starting material and appearance of the spectrum of product indicated when washing was complete.

The chloroform solution was evaporated to half volume or less and then diluted with ether and chilled. The sodium salt separated as a fine powder which was centrifuged and dried *in vacuo*. Xanthate and simple dithiocarbamate derivatives are listed in Table III. The melting points of the compounds in Tables III–VI are unsharp, accompanied with decomposition, vary depending on the temperature of the block when first applied, and so are difficult to duplicate. The ultraviolet spectra, titrations, and analyses were considered as sufficient to characterize the derivatives. The titrations and spectra determinations for all the derivatives in this paper were performed on materials dried in a vacuum desiccator over KOH pellets, while elemental analyses were performed on samples dried for several minutes on a block at 100–135°. Some of the materials, particularly the basic derivatives (see below), tenaciously bound water which could not be removed without decomposing the products. The amount of water in the samples used for physical characterization may be more than that indicated by analysis, although, as a rule, the titration values corroborate the analytical results. All of the products in this report had infrared spectra consistent with expectations and gave one spot against *Bacillus subtilis* in a bioautograph of paper chromatograms, which were developed with ethyl methyl ketone saturated with water.

Several of the compounds of Table III could not be purified as indicated above. Their purifications are detailed below.

Because of its insolubility in chloroform, the derivative **16** from sodium N-methyl-N-sorbityldithiocarbamate was precipitated from its water solution at pH 2, filtered, redissolved in water at pH 7, and again reprecipitated at pH 2. The acid after thorough washing and digestion in water was analytically pure.

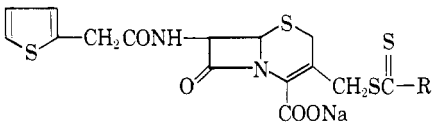
The derivative **8** from sodium N-methyldithiocarbamate was likewise chloroform insoluble. Recrystallization from methanol–2-propanol failed to give a pure product, so a concentrated water solution of the sodium salt was treated with concentrated cobaltous chloride solution. The pink precipitate was centrifuged, washed with water, recentrifuged, and then acidified to pH 2 in water. The acid was extracted into ethyl acetate and back extracted into water at pH 5.5. The water was evaporated in a rotating evaporator and the dried residue was recrystallized from methanol–2-propanol.

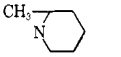
The derivative **14** from sodium N,N-bis(β-hydroxyethyl)dithiocarbamate was converted to the acid at pH 2, extracted into ethyl acetate, and obtained as a solid by evaporation. After trituration with chloroform the solid was dissolved in Methyl Cellosolve and methanol (3:1) and adjusted to pH 6.5 with 1 *N* NaOH. The residue from evaporation of the solvent was recrystallized from methanol–2-propanol.

The reaction mixture from sodium N-*n*-propyldithiocarbamate yielded a large amount of insoluble solid that did not contain β-lactam absorption. The filtrate contained the product and it was isolated by acidification to the acid that was then converted into the sodium salt **9**.

B. Basic Dithiocarbamates.—The procedure outlined above was varied slightly for many of the aminodithiocarbamates. The water solutions of the alkylpiperazinedithiocarboxylate

TABLE III



No.	R	Yield, %	Ultraviolet λ_{max}, mμ (ϵ)	Titration[a] pK_a'	AMW, FMW	Formula	Calcd., % C	H	N	Found, % C	H	N
1	OC_2H_5	48.5[b]	223 (19,200), 284 (20,200)	5.0	457, 498	$C_{17}H_{17}N_2NaO_5S_4 \cdot H_2O$	40.95	3.87	25.7[c]	40.52	3.87	26.17[c]
2	O-*n*-C_3H_7	45.5[d]	226 (17,560), 284 (17,820)	5.1	485, 494	$C_{18}H_{19}N_2NaO_5S_4$	43.71	3.87	5.66	43.82	3.80	5.49
3	O-*i*-C_3H_7	44.5[b]	227 (17,550), 285 (17,550)	5.05	485, 494	$C_{18}H_{19}N_2NaO_5S_4$	43.71	3.87	5.66	43.08	4.51	6.16
4	O-*n*-C_4H_9	36.0[d]	226 (17,180), 285 (17,280)	5.07	502, 508	$C_{19}H_{21}N_2NaO_5S_4$	44.86	4.16	5.51	44.60	4.28	5.66
5	O-*n*-C_6H_{13}	40.5[d]	229 (17,800), 284 (14,400)	5.05	565, 536	$C_{21}H_{25}N_2NaO_5S_4$	46.99	4.69	5.22	47.08	4.72	5.39
6	O-	37.0[d]	228 (18,180), 285 (17,100)	5.05	515, 520	$C_{20}H_{21}N_2NaO_5S_4$	46.13	4.06	5.38	46.39	4.07	5.39
7	O- 	40.0[d]	230 (18,500), 286 (17,900)	5.02	537, 534	$C_{21}H_{23}N_2NaO_5S_4$	47.17	4.33	5.24	46.95	4.35	5.28
8	$NHCH_3$[e]	65.0	237 (11,800), 270 (16,900)	4.8	475, 465	$C_{16}H_{16}N_3NaO_4S_4$	41.27	3.46	9.03	41.21	3.64	8.88
9	NH-*n*-C_3H_7[e]	...[d]	235 (14,200), 273 (15,310)	4.60	530, 512	$C_{18}H_{20}N_3NaO_4S_4 \cdot H_2O$	42.25	4.33	8.21	42.07	4.02	7.64
10	$N(C_2H_5)_2$	31.0	235 (18,350), 271 (19,200)	5.15	578, 507	$C_{19}H_{22}N_3NaO_4S_4$	44.95	4.36	8.28	45.17	4.47	8.08
11		45.0[d]	230 (18,000), 273 (23,600)	5.12	525, 519	$C_{20}H_{22}N_3NaO_4S_4$	46.22	4.26	8.09	45.98	4.44	8.28
12		28.0[d]	234 (17,360), 272 (17,900)	5.15	545, 533	$C_{21}H_{24}N_3NaO_4S_4$	47.26	4.53	7.87	47.46	4.78	7.63
13	$N(CH_3)CH_2CH_2OH$	11.5[b]	233 (17,800), 271 (20,200)	5.0	503, 509	$C_{18}H_{20}N_3NaO_5S_4$	42.42	3.95	8.25	42.62	3.98	8.13
14	$N(CH_2CH_2OH)_2$	6.8[b]	235 (15,000), 270 (11,750)	5.0	542, 540	$C_{19}H_{22}N_3NaO_6S_4$	42.28	4.10	7.79	42.54	3.90	7.53
15	$N(CH_3)C_6H_5$	15.4[d]	239 (19,900), 270 (20,700)	5.05	538, 542	$C_{22}H_{20}N_3NaO_4S_4$	48.78	3.72	7.76	50.03	4.42	8.05
16	$N(CH_3)CH_2(CHOH)_4CH_2OH$	52.5[f]	236 (25,700), 270 (6,900)	5.05	670, 598	$C_{22}H_{30}N_3O_9S_4$	43.47	4.80	6.91	43.39	4.97	7.06

[a] The titrations are in 66% DMF–water; the apparent molecular weight (AMW) is listed before the formula molecular weight (FMW). [b] Recrystallized from methanol–2-propanol. [c] Sulfur analysis. [d] Recrystallized from chloroform–ether. [e] Compounds **8** and **9** have equivocal structure, see Discussion. [f] See Experimental; this compound is the acid.

salts are not strongly basic and were used as such in the condensation reaction. pH values of water solutions of the other basic dithiocarbamates, however, exceeded 8.5 (some had an initial pH near 11–12) and the solutions were adjusted with 1 *N* HCl to pH 7.5–8.0. As recorded above, the usual reaction temperature and duration was 40° for 24 hr. In some cases equal success was obtained by heating at 70° for 2–3 hr.; this was true of **24**, **28**, and **33**.

The piperazine derivatives were isolated as sodium salts but all the others were obtained as zwitterions. The zwitterions were also precipitated from the reaction mixture with an equal volume of saturated NaCl solution just as the sodium salts. Most were soluble in chloroform and were purified and isolated as described above. Derivatives from piperazine dithiocarboxylates are listed in Table IV, those from open-chain diamines in Table V.

Several products required special preparation and isolation procedures. The product **29** from sodium N-dimethylaminoethyl-N-methyldithiocarbamate required several pH adjustments to pH 8 in the initial few hours of reaction at room temperature. After 48 hr. at room temperature, the product started to precipitate. After another 24 hr., it was filtered and purified by solution in the minimum amount of hot water and subsequent evaporation to small volume in a rotating evaporator.

The derivative **30** from sodium N-diethylaminoethyl-N-methyldithiocarbamate was obtained by heating the pH adjusted solution for several days at 40°. A white precipitate of the zwitterion separated. It was purified by solution in water at the boiling point, chilling, adding an equal volume of methanol, and then collecting and drying the gelatinous precipitate *in vacuo* over KOH pellets.

In most of the reactions employing piperazinedithiocarboxylates a solid formed which did not possess the cephalosporin nucleus (infrared). It was removed before adding the NaCl solution to precipitate the product.

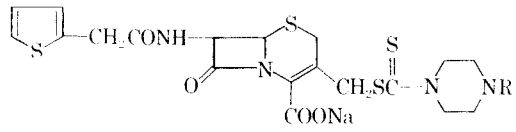
Sodium 4-adamantylpiperazinedithiocarboxylate is insoluble in water, and the reaction was therefore run in 4:1 dimethylformamide–water at 70° for 2 hr. An equal volume of saturated NaCl solution precipitated the product which was dissolved in methanol to free it from salt. The residue from evaporation was recrystallized from DMF–ether to give **24**.

The disodium salt of 4-methyl-2-carboxypiperazinedithiocarboxylic acid was used in the crude state. The aqueous reaction mixture was acidified to pH 8 and heated at 70° for 2 hr. When nothing precipitated on addition of saturated NaCl solution, the diacid was precipitated at pH 2. This acid was redissolved in dilute NaOH (pH 6.5). The solid obtained by evaporation was crystallized from chloroform–ether to yield **28**.

Purification of the chloroform-insoluble product (**26**) from sodium 4-(β-hydroxyethyl)piperazinedithiocarboxylate was effected by precipitating the zwitterion at pH 5.5, washing with water, and resolution with 1 *N* NaOH and water to pH 7.2. The solid from evaporation was recrystallized from methanol–2-propanol.

Preparation of theBetaines, 7-(2-Thiopheneacetamido)-3-(4-alkyl-4-methylpiperazinium-1-thiocarbonylthiomethyl)-3-cephem-4-carboxylate.—All four quaternary piperazinium com-

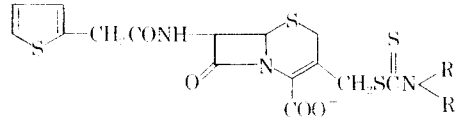
TABLE IV

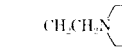
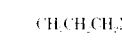


No.	R	Yield, %	Ultraviolet λ_{max}, mμ (ε)	Titration[a] pK_a'	AMW, FMW	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
17	CH_3	23.5[b]	272 (21,600)	4.6, 6.1	582, 534	$C_{20}H_{23}N_4NaO_4S_4$	44.92	4.33	10.48	44.77	4.50	10.28
18	C_2H_5	6.1[b]	242 (16,550), 270 (19,500)	. . . . .		$C_{21}H_{25}N_4NaO_4S_4$	45.97	4.59	10.21	46.06	4.65	10.18
19	n-C_3H_7	25.2[b]	270 (22,200)	4.4, 6.2	580, 562	$C_{22}H_{27}N_4NaO_4S_4$	46.95	4.83	9.96	47.09	4.79	9.91
20	i-C_3H_7	13.3[b]	240 (18,200), 270 (20,600)	4.5, 6.5	600, 563	$C_{22}H_{27}N_4NaO_4S_3$	46.95	4.83	9.96	46.90	4.99	9.55
21	n-C_4H_9	23.2[b]	271 (21,600)	4.5, 6.25	625, 578	$C_{23}H_{29}N_4NaO_4S_4$	47.89	5.06	9.71	47.61	5.25	9.48
22	n-C_5H_{11}	14.2[b]	270 (19,700)	4.4, 6.2	632, 609	$C_{24}H_{31}N_4NaO_4S_4 \cdot H_2O$	47.41	5.42	9.20	47.06	5.36	9.12
23	n-C_8H_{17}	13.5[c]	272 (12,570)	4.35, 6.12	676, 633	$C_{27}H_{37}N_4NaO_4S_4$	51.24	5.89	8.85	52.16	5.91	8.77
24	$C_{10}H_{15}$[d]	4.1[e]	274 (15,900)	4.6, 7.0	735, 655	$C_{29}H_{35}N_4NaO_4S_4$	53.18	5.38	8.56	53.11	5.59	8.67
25	C_6H_5	10.1[b]	270 (21,600)	4.9, 8.8	635, 597	$C_{25}H_{25}N_4NaO_4S_4$	50.31	4.22	9.38	51.27	4.72	9.96
26	CH_2CH_2OH	9.6[f]	235 (16,500), 270 (20,500)	4.4, 5.8	596, 565	$C_{21}H_{25}N_4NaO_5S_4$	44.66	4.46	9.92	44.88	4.40	9.98
27		[f,g]	. . .	4.8, 7.3	1086, 955	$C_{34}H_{32}N_6Na_2O_8S_6$	42.75	3.37	8.80	42.58	3.61	8.75
28		[h]	6.6[b]	234 (15,400), 272 (17,300)	4.7, 6.85	540, 635	$C_{21}H_{22}N_4Na_2O_6S_4 \cdot 2H_2O$	39.62	4.09	8.80	39.70	4.11	8.25

[a] The titrations are in 66% DMF–water; the apparent molecular weight (AMW) is listed before the formula molecular weight (FMW). [b] Recrystallized from chloroform–ether. [c] Recrystallization from chloroform–petroleum ether (b.p. 60–71°). [d] Adamantyl-1-. [e] Recrystallized from DMF–ether. [f] Recrystallized from methanol–2-propanol. [g] Derived from disodium 1,4-bis(dithiocarbamoyl)piperazine. [h] Derived from disodium 4-methyl-2-carboxypiperazine-1-dithiocarboxylate.

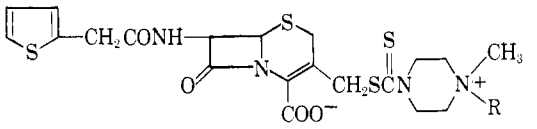
TABLE V



No.	R	R′	Yield, %	Ultraviolet λ_{max}, mμ (ε)	Titration[a] pK_a'	AMW, FMW	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
29	CH_3	$CH_2CH_2N(CH_3)_2 \cdot H^+$	24.2[b]	244 (18,500), 270 (20,590)	4.65, 7.5	546, 515	$C_{20}H_{26}N_4O_4S_4$	46.67	5.09	10.89	46.70	5.29	10.60
30	CH_3	$CH_2CH_2N(C_2H_5)_2 \cdot H^+$	18.0[c]	270 (20,000)	4.72, 7.9	592, 542	$C_{22}H_{30}N_4O_4S_4$	48.68	5.57	10.32	48.39	5.77	10.14
31[d]	H	$CH_2CH_2N(C_2H_5)_2 \cdot H^+$	40.5[c]	235 (9,000), 278 (17,350)	3.5, 7.95	585, 564	$C_{21}H_{28}N_4O_4S_4 \cdot 2H_2O$	44.66	5.71	9.92	44.55	5.92	10.11
32	CH_3	$CH_2CH_2N(n\text{-}C_3H_7)_2 \cdot H^+$	5.4[c]	Insoluble	4.6, 7.6	593, 570	$C_{24}H_{34}N_4O_4S_4$	50.50	6.00	9.82	50.40	5.90	9.84
33	CH_3		41.4[c]	272 (17,600)	4.35, 5.55	662, 579	$C_{29}H_{27}N_7NaO_5S_5$	45.05	4.70	9.68	44.83	4.60	9.63
34	C_2H_5	$CH_2CH_2N(C_2H_5)_2 \cdot H^+$	30.0[c]	Insoluble	4.75, 7.88	560, 556	$C_{23}H_{32}N_4O_4S_4 \cdot 1.5H_2O$	47.31	6.04	9.60	47.52	5.98	9.82
35	CH_3	$CH_2CH_2CH_2N(C_2H_5)_2 \cdot H^+$	21.0[c]	269 (20,100)	4.8, 9.05	553, 556	$C_{23}H_{32}N_4O_4S_4 \cdot H_2O$	48.05	5.96	9.74	47.86	5.49	10.06
36	CH_3		11.8[c]	270 (14,000)	4.75, 8.75	603, 605	$C_{24}H_{32}N_4O_4S_4 \cdot 2H_2O$	47.65	5.99	9.26	47.48	5.51	9.03
37[d]	H	$CH_2CH_2N(CH_3)C_6H_{11} \cdot Na^+$	55[c]	237 (42,100), 274 (15,230)	4.50	678, 585	$C_{23}H_{25}N_7NaO_5S_5$	49.29	4.31	9.58	49.36	4.38	9.37

[a] The titrations are in 66% DMF–water; the apparent molecular weight (AMW) is listed before the formula molecular weight (FMW). [b] Recrystallized from water–acetone. [c] Recrystallized from chloroform–ether. [d] The structures of **31** and **37** are equivocal; see Discussion. [e] Recrystallized from water–methanol.

TABLE VI



No.	R	Yield, %	Ultraviolet λ_{max}, mμ (ϵ)	Titration[a] pK_a'	Titration[a] AMW, FMW	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
38	CH_3	48.0	269 (20,400)	4.58	515, 526	$C_{21}H_{26}N_4O_4S_4$	47.88	4.97	10.64	47.79	5.42	10.43
39	n-C_3H_7	26.4[b]	273 (23,000)	4.6	555, 554	$C_{23}H_{30}N_4O_4S_4$	49.79	5.45	10.10	49.67	5.55	9.82
40	$CH_2CH{=}CH_2$	66.3[b]	271 (21,650)[c]	4.6	559, 552	$C_{23}H_{28}N_4O_4S_4$	49.97	5.10	10.14	49.72	5.21	9.96
41	n-C_4H_9	25.0[b]	271 (22,900)	4.6	578, 568	$C_{24}H_{32}N_4O_4S_4$	50.67	5.67	9.85	50.56	5.64	9.89

[a] The titrations are in 66% DMF–water; the actual molecular weight (AMW) is listed before the formula molecular weight (FMW). [b] Recrystallized from dimethylformamide–tetrahydrofuran–water. [c] Water solution.

pounds in Table VI were prepared in the same way. The N-alkylpiperazinodithiocarboxylate derivative of the cephalosporin was prepared as described above except that the chloroform solution was washed only twice with the salt solution and then evaporated and the residue was dried. The crude sodium salts so obtained were weighed and used without further purification.

The crude sodium salt (about 0.01 mole) was dissolved in 100 ml. of dry chloroform, and a solution of 0.0105 mole of methyl iodide or allyl bromide in 10 ml. of chloroform was added. The mixture was allowed to stand at room temperature in a stoppered flask for 4–7 days with occasional shaking. There was a gradual precipitation of solid, starting within a few days of initial mixing. The cream-colored precipitate was filtered and air dried. It was then triturated in water to remove coprecipitated sodium iodide. The product was quite water insoluble and separated as a yellow taffy.

The product was recrystallized from a small amount (25–35 ml./g.) of dimethylformamide by warming gently and adding sufficient water until the cloudiness cleared. Then tetrahydrofuran (5–10 vol.) was added and the turbid mixture was cooled. The precipitated product was centrifuged, washed with ether, and vacuum dried.

The dimethyl betaine was more insoluble than the other three and could not be dissolved in DMF. It was therefore triturated in warm DMF, filtered, washed with water, then ether, and dried.

All the betaines gave a single spot in paper chromatography (ethyl methyl ketone as developing solvent, *B. subtilis* as the organism for the bioautograph).

Sodium 7-Chloroacetamido-3-ethoxythiocarbonylthiomethyl-3-cephem-4-carboxylate. A. Potassium 7-Amino-3-ethoxythiocarbonylthiomethyl-3-cephem-4-carboxylate.—7-Aminocephalosporanic acid[20] (5.2 g., 0.0175 mole) was dissolved in 125 ml. of water by adjusting the solution to pH 8 with 1 *N* KOH. Then 3.1 g. (0.019 mole) of potassium ethyl xanthate was added and the mixture was heated at 40° for 60 hr. After being chilled for several hours, the mixture was filtered and the product was dried *in vacuo* (1.23 g.). The material was not purified further; the infrared spectrum was consistent with the required structure and the ultraviolet spectrum showed maxima at 284 and 246 mμ [λ_{max} 284 mμ (ϵ 16,820)].

B. Acetylation with Chloroacetyl Chloride.—The above crude xanthate (1.0 g., *ca.* 0.0028 mole) was dissolved in 50 ml. of water and 50 ml. of acetone, 0.925 g. (0.011 mole) of sodium bicarbonate was added, and the mixture was cooled in an ice bath. Then a solution of 630 mg. (0.0056 mole) of chloroacetyl chloride in 10 ml. of acetone was dropped in during 0.5 hr. After a further 0.5 hr., the mixture was concentrated in a rotating evaporator to remove the acetone. The water solution (pH 5) was layered with ethyl acetate and quickly adjusted to pH 2 with 1 *N* HCl. The ethyl acetate layer was washed twice with cold water and then extracted with water adjusted to pH 6.5 with 1 *N* NaOH. The water solution was evaporated to dryness in a rotating evaporator. The residue, after thorough drying *in vacuo*, was dissolved in warm methanol, a small insoluble fraction was removed, and the solution was diluted with 2-propanol and chilled. The crystalline product was isolated and dried. Its ultraviolet spectrum showed a maximum at 284 mμ (ϵ 15,360). The infrared spectrum was consistent with the required structure. In the usual bioautograph of a paperchromatogram, there was one spot. Titration gave a pK_a of 5.1 (mol. wt., 440; calcd. 385).

Anal. Calcd. for $C_{13}H_{14}ClN_2O_5$: C, 36.06; H, 3.25; N, 6.47. Found: C, 35.95; H, 3.27; N, 6.21.

Attempted Preparation of Methyl 7-Benzamido-3-methylaminothiocarbonylthiomethyl-3-cephem-4-carboxylate. A. Acylation of 7-Aminocephalosporanic Acid (7-ACA).—The acylation was performed as indicated for the chloroacetylation above. From 7.78 g. of 7-ACA and 3.83 g. of benzoyl chloride there was obtained 6.68 g. (58.7%) of pure sodium 7-benzamidocephalosporanate. It was recrystallized from methanol by dilution with 2-propanol, m.p. 240° dec., after turning violet at 145°.

Anal. Calcd. for $C_{17}H_{15}N_2NaO_5S$: C, 51.25; H, 3.79; N, 7.03. Found: C, 51.19; H, 3.87; N, 7.15.

B. Nucleophilic Displacement with Sodium N-Methyldithiocarbamate.—The usual method was employed starting with 6.0 g. of sodium 7-benzamidocephalosporanate. There was obtained 5.36 g. of crude product. It was insoluble in chloroform and was recrystallized from hot methanol by the addition of 2-propanol to give 2.55 g.; in the ultraviolet, λ_{max} 270 mμ (ϵ 18,000), 226 (14,690). Titration gave a pK_a of 4.6 (mol. wt., 470; calcd. 477).

Anal. Calcd. for $C_{17}H_{16}N_3NaO_5S_3$: C, 45.83; H, 3.61; N, 9.43. Found: C, 45.54; H, 3.74; N, 9.41.

C. Methylation with Diazomethane.—One gram of the previous product was dissolved in water, acidified to pH 2, and extracted with ethyl acetate. The ethyl acetate solution, after drying (Na_2SO_4), was treated in the cold with excess diazomethane in ether. A precipitate formed. After 0.5 hr., the solution was evaporated to remove the diazomethane and then diluted with ethyl acetate and extracted with water adjusted to pH 6.3 with 1 *N* NaOH. The organic layer was washed with water and evaporated. The residue was recrystallized from ethyl acetate–petroleum ether (b.p. 60–71°), m.p. 132–135°. The yield was 300 mg. The ultraviolet spectrum showed maxima at 235 mμ (ϵ 12,450) and 270 (16,200).

Anal. Calcd. for $C_{18}H_{19}N_3O_4S_3$: C, 49.41; H, 4.37; N, 9.60. Found: C, 49.23; H, 4.49; N, 9.52.

An n.m.r. spectrum of this product was very indistinct, the absorption peaks barely rising above the base-line "noise." The positions of the O-methyl and benzene ring protons could be distinguished, but not those of the single protons.

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