1-(2-Diethylaminoethyl)-4(5)-styryl-5(4)-nitroimidazole (I).—To 0.53 g of Na in 20 ml of ethanol was added 5 g of 4(5)-styryl-5(4)-nitroimidazole[12] in 100 ml of anhydrous EtOH (stirring). Solvent was removed under reduced pressure, and the resultant orange sodium salt was recrystallized from 2-propanol–petroleum ether (bp 40–60°). A mixture of 10 g of this sodium salt and 8.9 g of 2-diethylaminoethyl chloride in 50 ml of dry N,N-dimethylformamide (DMF) was refluxed for 90 min, cooled, and poured into water. This solid was collected and recrystallized from EtOH; mp 95–96°, yield 9.93 g (75%).

Anal. Calcd for $C_{17}H_{22}N_4O_2$: C, 64.95; H, 7.05; O, 10.18; N, 17.82. Found: C, 64.48; H, 7.17; O, 10.54; N, 17.45.

Chromatographic separation of the mixture on a column gave Ia, mp 67–68°, R_f 0.52, and Ib, mp 100–101°, R_f 0.74. See Table II.

1-(2-Pyrrolidinoethyl)-4(5)-styryl-5(4)-nitroimidazole (II).—This compound was prepared in the same way as I and recrystallized from 2-propanol; mp 112–113°, yield 11.78 (89.5%).

Anal. Calcd for $C_{17}H_{20}N_4O_2$: C, 65.37; H, 6.41; O, 10.24; N, 17.94. Found: C, 65.20; H, 6.51; O, 10.45; N, 17.75.

Chromatographic separation of the mixture on a column gave IIa, mp 115–116°, R_f 0.40, and IIb, mp 95–96°, R_f 0.65.

1-(2-Piperidinoethyl)-4(5)-styryl-5(4)-nitroimidazole (III) was prepared in the same way as I and recrystallized from EtOH; mp 110–111°, yield 8.25 g (60%).

Anal. Calcd for $C_{18}H_{22}N_4O_2$: C, 66.23; H, 6.79; O, 9.80; N, 17.16. Found: C, 66.07; H, 6.97; O, 9.73; N, 16.99.

Chromatographic separation of the mixture on a column gave IIIa, mp 88–89°, R_f 0.43, and IIIb, mp 116–117°, R_f 0.69.

1-(2-Morpholinoethyl)-4(5)-styryl-5(4)-nitroimidazole (IV) was prepared as described for I and recrystallized from EtOH; mp 127–128°, yield 8.99 g (65%).

Anal. Calcd for $C_{17}H_{20}N_4O_3$: C, 62.18; H, 6.14; O, 14.62; N, 17.06. Found: C, 61.86; H, 6.23; O, 14.79; N, 16.80.

Chromatographic separation of the mixture on a column gave IVa, mp 138–139°, R_f 0.32, and IVb, mp 140–141°, R_f 0.59.

2-Methyl-4(5)-styryl-5(4)-nitroimidazole.—A mixture of 15 g of 2,4(2,5)-dimethyl-5(4)-nitroimidazole,[13] 30 ml of benzaldehyde, and 2 ml of piperidine was heated (oil bath) to 140–150°. After cooling, the solid was collected and washed first with H_2O to remove the unreacted 2,4(2,5)-dimethyl-5(4)-nitroimidazole, then with EtOH to remove the colored material. Recrystallization from HOAc gave 10 g (41.4%) of 2-methyl-4(5)-styryl-5(4)-nitroimidazole, mp 245–246°.

Anal. Calcd for $C_{12}H_{11}N_3O_2$: C, 62.87; H, 4.84; O, 13.96; N, 18.33. Found: C, 62.98; H, 4.95; O, 14.07; N, 18.3.

1-(2-Diethylaminoethyl)-2-methyl-4(5)-styryl-5(4)-nitroimidazole (V).—To 3 g of the sodium salt of 2-methyl-4(5)-styryl-5(4)-nitroimidazole in 20 ml of dry DMF was added 2.29 g of 2-diethylaminoethyl chloride. The mixture was refluxed for 90 min, cooled, and poured into water; the solid was collected. Recrystallization from EtOH gave V, mp 93–94°, yield 2.74 g (70%).

Anal. Calcd for $C_{19}H_{24}N_4O_2$: C, 65.82; H, 7.36; O, 9.75; N, 17.06. Found: C, 65.75; H, 7.53; O, 9.98; N, 16.97.

The **sodium salt** was prepared as described for I.

1-(2-Pyrrolidinoethyl)-2-methyl-4(5)-styryl-5(4)-nitroimidazole (VI) was prepared as described for I; mp 100–101°, yield 2.84 g (73%).

Anal. Calcd for $C_{18}H_{22}N_4O_2$: C, 66.24; H, 6.79; O, 9.80; N, 17.16. Found: C, 65.71; H, 6.71; O, 9.84; N, 17.72.

1-(2-Piperidinoethyl)-2-methyl-4(5)-styryl-5(4)-nitroimidazole (VII) was prepared as described for I; mp 122–123°, yield 3.08 g (76%).

Anal. Calcd for $C_{19}H_{24}N_4O_2$: C, 67.04; H, 7.11; O, 9.40; N, 16.46. Found: C, 67.04; H, 7.22; O, 9.81; N, 16.28.

1-(2-Morpholinoethyl)-2-methyl-4(5)-styryl-5(4)-nitroimidazole (VIII) was prepared as described for I; mp 125–126°, yield 1.76 g (43%).

Anal. Calcd for $C_{18}H_{22}N_4O_3$: C, 63.14; H, 6.48; O, 14.02; N, 16.36. Found: C, 62.97; H, 6.52; O, 14.29; N, 16.20.

Acknowledgment.—The authors express their appreciation to Dr. E. Pella for microanalyses, to Dr. E. Dradi and to F. Confalonieri for nmr and infrared spectra, and to F. Casabuona and G. Longo for technical assistance.

(12) A. Windhaus and W. Langenbeck, *Ber.*, **56**, 683 (1923).

(13) A. Windhaus, *ibid.*, **42**, 758 (1909).

Penicillins and Cephalosporins from Isothiazolylacetic Acids

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A number of isothiazolylacetic acids have been synthesized including the three isomeric unsubstituted isothiazolylacetic acids, 4-chloro-3-isothiazolylacetic acid, 4-bromo-3-isothiazolylacetic acid, 3-methyl-4-isothiazolylacetic acid, and 3-methyl-5-isothiazolylacetic acid. The penicillins and cephalosporins prepared from these acetic acids by the activated ester method exhibit good antibacterial activity, particularly the unsubstituted derivatives. The antibacterial activity of the unsubstituted potassium 6-(isothiazolylacetamido)penicillanates against gram-negative microorganisms is considerably higher than that of benzylpenicillin.

Little recent information is reported in the literature on analogs of benzylpenicillin[1] (penicillin G). As part of a study on the effect of the aryl group on the antibacterial activity of heteroarylmethylpenicillins we synthesized several isothiazolylacetic acids[2] and from these the corresponding penicillins (I) and cephalosporins (II).

3-Isothiazolylacetic acids (III) were prepared from the 3-methylisothiazoles by a sequence involving bromination with N-bromosuccinimide, cyanation, methanolysis, and hydrolysis. The required 3-methylisothiazoles were obtained by reductive deamination of the corresponding 3-methyl-5-aminoisothiazoles.[3,4] 4- and 5-isothiazolylacetic acids (IV and V) were all successfully prepared from the corresponding carboxylic acids *via* the Arndt–Eistert synthesis[5]. Isothiazole-4-carboxylic acid could be prepared either by a permanganate oxidation of 4-methylisothiazole or by bromination of isothiazole, followed by cyanation and hydrolysis. Table I lists the various isothiazolylacetic acids prepared and their nmr parameters.

(1) For recent reviews on penicillins and related structures see: (a) F. P. Doyle and J. H. C. Nayler, *Advan. Drug Res.*, **1**, 1 (1964); (b) G. T. Stewart, "The Penicillin Group of Drugs," Elsevier Publishing Co., Amsterdam, 1965; (c) E. P. Abraham, *Quart. Rev.* (London), **21**, 231 (1967).

(2) For a recent review on isothiazole chemistry see: R. Slack and K. R. H. Wooldridge, *Advan. Heterocyclic Chem.*, **4**, 107 (1965).

(3) A. Adams and R. Slack, *J. Chem. Soc.*, 3061 (1959).

(4) D. Buttimore, D. H. Jones, R. Slack, and K. R. H. Wooldridge, *ibid.*, 2032 (1963).

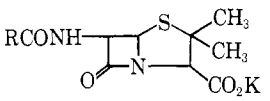
(5) W. E. Bachmann and W. S. Struve, *Org. Reactions*, **1**, 38 (1942).

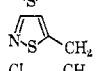
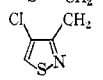
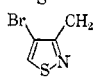
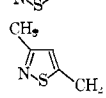
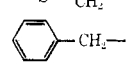
TABLE I
ISOTHIAZOLYLACETIC ACIDS

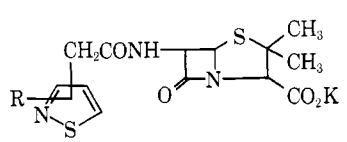
Compd	Isothiazole deriv	Mp, °C: Acid	Mp, °C: p-Nitrophenyl ester	Mp, °C: 2,4-Dinitrophenyl ester	Nmr absorption[a] (δ, τ): CH_3	CH_2	H^3	H^4	H^5	Formula	Neutr equiv: Calcd	Neutr equiv: Found	Analyses
IIIa[b]	3-CH_2CO_2H	130–132	82–85	Oil	...	6.18	...	2.68	1.08	$C_5H_5NO_2S$	143	143	C, H, N, S
IVa	4-CH_2CO_2H	117–119	73–75	100–102	...	6.38	1.54[d]	...	1.37[d]	$C_5H_5NO_2S$	143	143	C, H, N, S
Va[c]	5-CH_2CO_2H	153–155 dec	76–80	Oil	...	6.13	1.62	2.88	...	$C_5H_5NO_2S$	143	140	C, H, N, S
IIIb	4-Cl-3-CH_2CO_2H	109–110	93–97	...	...	6.22	...	...	1.25	$C_5H_4ClNO_2S$	177.6	177	C, H, N
IIIc	4-Br-3-CH_2CO_2H	108–109	...	88–90	...	6.18	...	...	1.11	$C_5H_4BrNO_2S$	222	210	H, N; C[e]
IVb	3-CH_3-4-CH_2CO_2H	160–161	...	80–81	7.64	6.47	...	...	1.44	$C_6H_7NO_2S$	157	149	C, H, N
Vb	3-CH_3-5-CH_2CO_2H	144–145 dec	...	...	7.67	6.22	...	3.15	...	$C_6H_7NO_2S$	157	163	C, H, N

[a] The nmr spectra were measured in D_2O from either the sodium or potassium salt. [b] Coupling constant $J_{4,5} = 5$ cps. [c] Coupling constant $J_{3,4} = 2$ cps. [d] The assignment of H^3 and H^5 is not certain. [e] C: calcd, 27.04; found, 26.60.

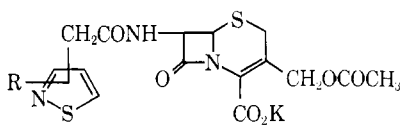
TABLE II
MINIMUM INHIBITORY CONCENTRATIONS (μg/ml) OF POTASSIUM 6-(ISOTHIAZOLYLACETAMIDO)PENICILLANATES



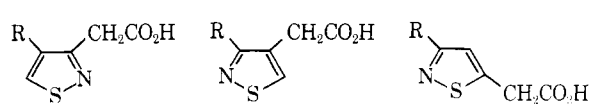
No.	R	Gram-positive microorganisms: *D. pneumoniae*	*S. pyogenes*	*S. aureus* Smith − serum	*S. aureus* Smith + serum	Gram-negative microorganisms: *E. coli*	*S. enteriditis*	*S. typhosa*	*K. pneumoniae*
1		0.016	0.008	0.016	0.031	25	0.8	1.6	1.6
2		0.008	0.008	0.031	0.031	12.5	0.125	1.6	1.6
3		0.004	0.004	0.016	0.016	12.5	0.125	1.6	1.6
4		0.016	0.008	0.016	0.125	50	3.1	12.5	25
5		0.031	0.016	0.031	0.125	100	6.2	25	50
6		0.008	0.008	0.031	0.031	25	0.8	3.1	3.1
7		0.004	0.004	0.016	0.031	25	0.8	6.2	6.2
		0.008	0.008	0.016	0.016	50	0.8	6.2	6.2
Ampicillin		0.008	0.008	0.031	0.031	6.2	0.125	1.6	1.6



I



II



IIIa, R = H
b, R = Cl
c, R = Br

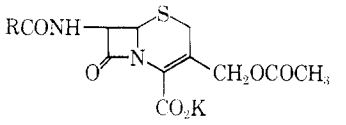
IVa, R = H
b, R = CH_3

Va, R = H
b, R = CH_3

p-Nitrophenyl- and 2,4-dinitrophenylisothiazolylacetic esters were readily obtained in high yields from the acetic acids and *p*-nitrophenol or 2,4-dinitrophenol using N,N′-dicyclohexylcarbodiimide as condensing agent. Triethylammonium 6-(isothiazolylacetamido)penicillanates and 7-(isothiazolylacetamido)cephalosporanates were obtained from the reaction between these activated esters and the triethylammonium salts of 6-aminopenicillinic acid (6-APA) and 7-aminocephalosporanic acid (7-ACA), respectively. The triethylammonium salts were converted into the potassium salts I and II by treatment of their methanolic solutions with potassium 2-ethylhexanoate. The *p*-nitrophenyl esters were sufficiently active for the preparation of the penicillins, except when an *o*-bromo or *o*-methyl substituent was present in the isothiazole ring, in which instances the dinitrophenyl esters had to be employed. Triethylammonium 7-aminocephalosporanate reacted very slowly with the *p*-nitrophenyl esters of the unsubstituted isothiazolylacetic acids, but also here the dinitrophenyl esters reacted smoothly.

Antibacterial Acitivity.—The compounds were tested

TABLE III
MINIMUM INHIBITORY CONCENTRATIONS (μg/ml) OF POTASSIUM 7-(ISOTHIAZOLYLACETAMIDO)CEPHALOSPORANATES



		S. aureus				Gram-negative microorganisms			
		Penicillin G sensitive		Penicillin G resistant					
No.	R	− serum	+ serum	− serum	+ serum	*E. coli*	*S. enteriditis*	*S. typhosa*	*K. pneumoniae*
8		0.125	0.25	0.8	0.8	50	1.6	12.5	12.5
9		0.125	0.4	0.8	0.8	50	1.6	3.1	6.2
10		0.125	0.25	0.8	1.6	25	1.6	3.1	6.2
11		0.25	0.4	0.8	0.8	100	3.1	25	25
		0.16	0.4	0.4	0.8	25	0.8	3.1	6.2

for antibacterial activity by Dr. A. Gourevitch and his associates in the Microbiology Department of Bristol Laboratories, Syracuse, N. Y., using published techniques.[6] Minimum inhibitory concentrations (MIC) were determined using a twofold serial dilution technique in heart infusion broth in the absence of serum and in the presence of 50% pooled human serum. The inocula were 10^4 dilutions of overnight cultures.

In Table II the MIC values of the penicillins **1–7** against several microorganisms are compared with the values for 6-(D-(−)-α-aminophenylacetamido)penicillanic acid (ampicillin) and penicillin G, obtained under the same conditions. It is evident that these isothiazole penicillins represent a class of highly active antibacterials. The unsubstituted compounds **1–3** exhibit considerably better activity against gram-negative organisms than the substituted compounds **4–7**, although the activity against gram-positive organisms is not much affected by substitution. The most active compounds, **2** and **3**, while showing approximately the same activity against gram-positive bacteria as penicillin G, are considerably more active against gram-negative bacteria and their activity compares favorably with that of ampicillin. The results of *in vivo* experiments were consistent with the high *in vitro* activity. When administered by the intramuscular route, the medium curative dose in mice for **1–3** against the Smith strain of *Staphylococcus aureus* was found to be 1.0, 0.35, and 0.6 mg/kg, respectively, compared to 0.3 mg/kg for ampicillin; for **2** and **3** the medium curative dose against *Klebsiella pneumoniae* was found to be 45 and 22.5 mg/kg, respectively, compared to 95 mg/kg for ampicillin. As expected, none of the compounds **1–7** showed any appreciable activity against penicillinase-producing strains of *Staphylococcus aureus*.

In Table III the MIC values of the isothiazole cephalosporins **8–11** against some representative microorganisms are compared with the values for cephalothin obtained under the same conditions. Again substitution of the isothiazole ring decreases the activity against gram-negative organisms (**11**). The unsubstituted compounds **8–10** exhibit an antibacterial activity very similar to cephalothin both *in vitro* and *in vivo;* however, none of the new cephalosporins shows a definite improvement over cephalothin.

(6) A. Gourevitch, G. A. Hunt, J. R. Lautinger, C. C. Carmack, and J. Lein, *Proc. Soc. Exptl. Biol. Med.*, **107**, 455 (1961).

Experimental Section[7,8]

5-Amino-4-chloro-3-methylisothiazole Hydrochloride.—A solution of 5-amino-3-methylisothiazole[9] (17.8 g, 0.156 mole) in a mixture of C_6H_6 (200 ml) and AcOH (20 ml) was cooled in an ice–salt mixture. With stirring Cl_2 was passed into the mixture at such a rate that the temperature did not exceed 10°. The addition of Cl_2 was stopped after the exothermic reaction had ceased (after approximately 30 min). The product was filtered off, washed with ether, and then dissolved in MeOH (75 ml). The methanolic solution was treated with decolorizing carbon, whereafter the hydrochloride was precipitated by the addition of ether, followed by cooling. The pale yellow product was filtered off and amounted to 21.0 g (73%).

4-Chloro-3-methylisothiazole.—A solution of $NaNO_2$ (14.5 g, 0.21 mole) in 60 ml of H_2O was added dropwise in 45 min to a stirred homogeneous mixture of 4-chloro-5-amino-3-methylisothiazole hydrochloride (33.0 g, 0.18 mole), concentrated H_2SO_4 (50 ml), and 85% H_3PO_4 (50 ml). The temperature of the reaction mixture was kept at 0–5°. The orange diazotization mixture thus obtained was added in portions with stirring to 250 ml of a 30% aqueous H_3PO_2 solution in which some Cu_2O was suspended. During the reduction the temperature of the reduction mixture was kept between 25 and 35°. When, during the deamination, the rate of N_2 evolution decreased, a small additional amount of Cu_2O was added. The resulting brown reaction mixture was cooled and made weakly alkaline by the careful addition of a 50% aqueous NaOH solution. This mixture was steam distilled until approximately 400 ml of distillate had been collected. The organic layer was taken up in ether and the aqueous layer was extracted with three 75-ml portions of ether. The combined ether solutions were dried, whereafter the ether was removed and the residue was distilled under reduced pressure. All material distilled as a colorless liquid at 59–61° (18 mm), and amounted to 9.7 g (55%). The compound has been reported to have bp 156–160° (atm pressure).[10]

4-Bromoisothiazole.—Bromine (500 g, 3.13 moles) was added dropwise over a 1-hr period to a stirred solution of isothiazole[11]

(7) All temperatures are uncorrected. Ir spectra were obtained on a Perkin-Elmer Infracord spectrophotometer Model 137, nmr spectra with a Varian Associates Model A-60 spectrometer (TMS). Microanalyses were performed by Drs. G. Weiler and F. B. Strauss, Microanalytical Laboratory, Oxford, England.

(8) As most of the experimental procedures have already been described in recent patents,[9] only new or improved experiments are reported here. Where analyses are indicated only by symbols of elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(9) R. Raap, R. U. Lemieux, and R. G. Micetich, U. S. Patents 3,268,523 and 3,271,407 (1966).

(10) M. P. L. Caton, D. H. Jones, R. Slack, and K. R. H. Wooldridge, *J. Chem. Soc.*, 446 (1964).

(200 g, 2.35 moles) in 1.5 l. of AcOH heated on a steam bath at 95°. When the addition was completed the mixture was heated on the steam bath for an additional 6.5 hr, whereafter it was left at room temperature overnight. The mixture was poured into 4 l. of ice–water. The aqueous layer was extracted with eight 150-ml portions of ether. To the combined organic layers was added 6 *N* aqueous NaOH, with cooling and stirring, until the aqueous layer was slightly alkaline. The aqueous layer was extracted with four 100-ml portions of ether followed by drying and removal of the ether by distillation. The residues of two batches were combined for a final fractional distillation under reduced pressure, which yielded 112.2 g of unreacted isothiazole, bp 30–39° (13 mm), and 154.6 g (28% based upon unrecovered starting material) of 4-bromoisothiazole, bp 57–60° (14 mm), mp 34–37°, lit.[10] mp 31–33°.

4-Cyanoisothiazole.—A mixture of 4-bromoisothiazole (77.3 g, 0.47 mole), CuCN (63.0 g, 0.70 mole), and DMF (150 ml) was heated under reflux for 45 min. After cooling, the reaction mixture was added to a solution of NaCN (84 g, 1.7 moles) in 250 ml of H_2O, with vigorous stirring. When the mixture had reached room temperature again, it was extracted with four 150-ml portions of ether. The combined ether extracts were washed with 150 ml of 10% aqueous NaCN, followed by drying and treatment with decolorizing carbon. The solid residue obtained after removal of the ether was washed with 100 ml of cold hexane to give 27.0 g (53%) of pale yellow plates, mp 94–96°, lit.[12] mp 87°.

Isothiazole-4-carboxylic Acid.—4-Cyanoisothiazole (28.6 g, 0.26 mole) was dissolved in 125 ml of concentrated H_2SO_4. The resulting solution was heated at 60° for 16 hr, whereafter it was cooled and carefully diluted with 65 ml of H_2O. The mixture was cooled in ice and under vigorous stirring a solution of $NaNO_2$ (26.0 g, 0.39 mole) in 60 ml of H_2O was added dropwise in 1.5 hr. When the addition was completed the reaction mixture was heated at 55–60° for 15 min, then it was poured into 300 ml of ice–water. The white solid was filtered off and dissolved in 200 ml of warm EtOAc. The filtrate was extracted with three 75-ml portions of EtOAc. The combined EtOAc solutions were dried and concentrated to a volume of approximately 150 ml, followed by the addition of 100 ml of hexane and cooling. The product was collected by filtration; yield 30.0 g (90%) of white solid, mp 162–164°, lit.[3] mp 162°. The material was identical with that prepared by a permanganate oxidation of 4-methylisothiazole.[9]

Isothiazole-4-carbonyl Chloride.—A mixture of isothiazole-4-carboxylic acid (82.0 g, 0.635 mole) and $SOCl_2$ (250 ml) was heated under reflux for 2 hr. The excess $SOCl_2$ was removed and the residue was distilled *in vacuo* to give 88.0 g (94%) of colorless liquid, bp 59.5–60° (2.5 mm).

4-Isothiazolyl Diazomethyl Ketone.—A solution of isothiazole-4-carbonyl chloride (14.3 g, 0.097 mole) in 20 ml of ether was added carefully to an ice-cold 0.4 *M* ethereal CH_2N_2 solution (500 ml). The mixture was left at room temperature overnight, whereafter it was concentrated to dryness leaving 15.0 g (101%) of solid residue. Without further purification, the material was used for the next step. A small sample was recrystallized from benzene–hexane to give fine yellow needles, mp 57–59°.

Methyl 4-Isothiazolylacetate.—A mixture of isothiazolyl 4-diazomethyl ketone (119 g, 0.73 mole) and 400 ml of MeOH was heated under reflux in the presence of freshly prepared Ag_2O. The extent of the reaction could be followed by the disappearance in the infrared spectrum of the diazo band at 2100 cm^{-1} and the emergence of the ester CO band at 1740 cm^{-1}. A total amount of Ag_2O of approximately 20 g was added in portions over 9 hr. The MeOH was removed under reduced pressure and ether was added to the residue. The suspended solid was filtered off and the filtrate was washed once with 1 *N* HCl. The residue obtained after drying and removal of the ether was distilled *in vacuo* to give 80.0 g (70%) of product, bp 71–73° (0.2 mm). The ethyl ester, bp 80–82° (0.3 mm), could be prepared in the same manner by using EtOH as solvent (68% yield). A reflux time of 2 hr was sufficient in this instance.

Activated Esters. General Procedure.—Isothiazolylacetic acid (10 mmoles) and *p*-nitrophenol or 2,4-dinitrophenol (10 mmoles) were dissolved in anhydrous EtOAc, dioxane, or THF (30 ml) and the solution was cooled in ice. N,N′-Dicyclohexylcarbodiimide (10 mmoles) was added with stirring, whereafter the reaction mixture was left at room temperature for 1–2 hr. The N,N′-dicyclohexylurea was filtered off and washed with some EtOAc. The combined filtrate and washings were concentrated under reduced pressure, leaving the activated ester as a solid or thick wax, which generally crystallized on scratching. The esters thus obtained (Table I) were sufficiently pure for the next step.

Penicillins and Cephalosporins. General Procedure.—6-APA or 7-ACA (10 moles) and Et_3N (20 mmoles) were shaken with CH_2Cl_2 (20 ml) until the mixture was homogeneous. The mixture was cooled in ice and the activated ester was added in one portion with stirring. The reaction mixture was allowed to come to room temperature and usually left overnight. The extent of the reaction was determined by periodically obtaining an infrared spectrum of the mixture and comparing the relative intensities of the bands at approximately 1770, 1690, and 1600 cm^{-1}, due to the β-lactam, amide, and carboxlate carbonyl, respectively. When the reaction was complete, excess dry ether was slowly added with scratching. The solid or oily precipitate was again taken up in some CH_2Cl_2 and reprecipitated with ether. Next it was dissolved in the minimum amount of dry MeOH, followed by the addition of a 2.5 *M* solution of potassium 2-ethylhexanoate in *n*-BuOH (5 ml). The potassium penicillanate or cephalosporanate was precipitated by the addition of dry ether. The solid precipitate was again taken up in some MeOH and reprecipitated with ether. It was finally dried *in vacuo* over P_2O_5 for 24 hr. The over-all yield from the acetic acid was 50–80%. The purity was estimated from the thin layer chromatogram, the ir and the nmr spectrum. In every instance the purity was 90% or more. As an example, a large-scale preparation of potassium 6-(5-isothiazolylacetamido)penicillanate follows.

Potassium 6-(5-Isothiazolylacetamido)penicillanate.—To an ice-cold solution of 5-isothiazolylacetic acid (42.9 g, 0.30 mole) and *p*-nitrophenol (45.9 g, 0.33 mole) in anhydrous THF (500 ml) was added N,N′-dicyclohexylcarbodiimide (61.9 g, 0.30 mole) with stirring. The reaction mixture was cooled in ice for 45 min. The N,N′-dicyclohexylurea was filtered off and washed with EtOAc (200 ml). The combined filtrate and washings were concentrated to dryness to give a red-colored residue, which crystallized slowly. A solution of triethylammonium 6-aminopenicillanate was prepared by shaking a mixture of 6-APA (56.2 g, 0.26 mole), Et_3N (72.7 g, 0.72 mole), and CH_2Cl_2 (450 ml) for 1 hr at room temperature. This solution was added to the crude *p*-nitrophenyl ester, cooled in ice. The reaction mixture was cooled in ice for 1 hr, then left at room temperature overnight. A small amount of insoluble material was filtered off and the triethylammonium penicillanate was precipitated by the addition of ether (approximately 1 l.) The material, which crystallized upon scratching, was redissolved in the minimum amount of CH_2Cl_2 and reprecipitated by the addition of ether. It was then dissolved in 150 ml of MeOH and treated with 130 ml of a 2.4 *M* solution of potassium 2-ethyl hexanoate in *n*-BuOH. The potassium penicillanate crystallized from the solution upon careful addition of ether, with scratching. The product was recrystallized twice from a MeOH–ether mixture and finally kept *in vacuo* over P_2O_5 for 24 hr, to give 50.0 g (51%, based on 6-APA) of pale brown solid. The ir spectrum (Nujol) contained sharp bands at 1765, 1670, and 1605 cm^{-1}, ascribed to the β-lactam, amide, and carboxylate carbonyl, respectively. The nmr spectrum (in D_2O) shows one-proton doublets (coupling constant J = 1.8 cps) τ 1.53 and 2.73, respectively, due to the protons in the 3 and 4 position of the isothiazole nucleus, a two-proton signal at 4.42 for the two β-lactam hydrogens, a sharp one-proton signal at 5.71 for the proton next to the carboxylate group, a sharp two-proton signal at 5.88 for the methylene group, and two three-proton singlets at 8.38 and 8.47 for the *gem*-dimethyl group.

Anal. Calcd for $C_{13}H_{14}KN_3O_4S_2$: C, 41.14; H, 3.72; K, 10.30; N, 11.07; S, 16.90. Found: C, 40.44; H, 3.78; K, 10.23; N, 10.90; S, 17.17.

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(11) (a) F. Wille, L. Capeller, and A. Steiner, *Angew. Chem. Intern. Ed. Engl.*, **1**, 335 (1962); (b) R. Raap, *Can. J. Chem.*, **44**, 1324 (1966).

(12) F. Hübenett, F. H. Flock, W. Hansel, H. Heinze, and H. Hofmann, *Angew. Chem. Intern. Ed. Engl.*, **2**, 714 (1963).