The Removal and Displacement of the Thiazolidine Ring of Penicillin. I. 3-Acylaminoazetidinone and 3-Acylamino-4-phenylthioazetidinone[1]

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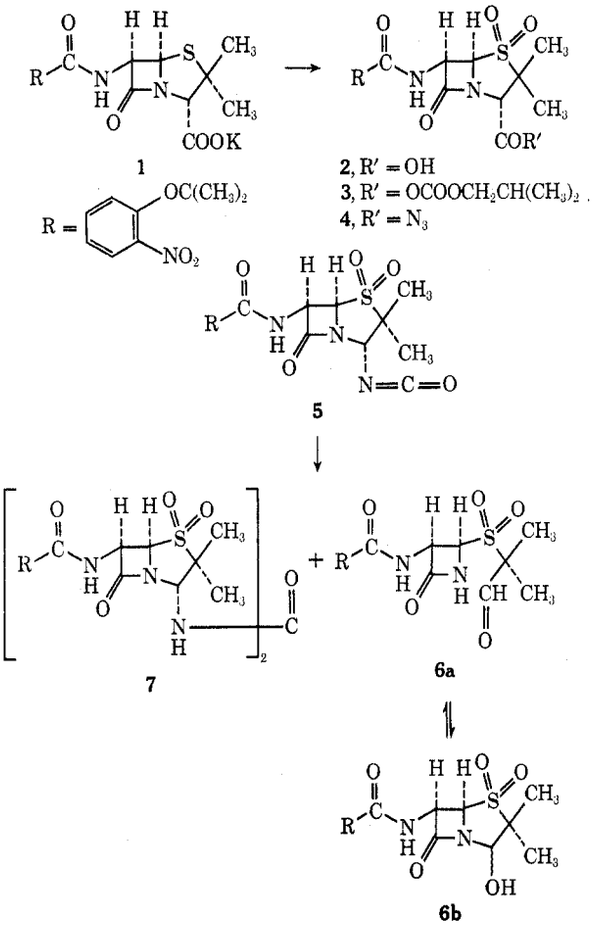
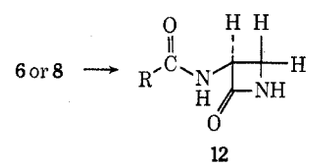
The thiazolidine ring in the penicillin **1** has been removed completely without opening or otherwise affecting the labile β-lactam ring. The key reaction is the removal of the sulfonyl side chain in the "aldehyde" **6** with excess potassium borohydride to afford **12**. Subsequently, the thiazolidine ring has been replaced by the phenylthio group (**9**).

A novel reaction of 4-sulfonyl-2-azetidinones derived from a penicillin has been discovered. Using this reaction, we have replaced and have removed completely the thiazolidine ring of a penicillin while the relatively more labile β-lactam ring was left intact. So far, this is one of the first reported examples of such a transformation. The thiazolidine ring in penicillins has been opened,[2] rearranged,[3] expanded,[4] and replaced.[5] However there appears to be no report of the complete removal of this ring.

Potassium 6-[2-methyl-2-(*o*-nitrophenoxy)propionamido]penicillanate[6] (**1**) was oxidized with potassium permanganate in neutral aqueous solution[7] to the corresponding sulfone, **2**. Compound **2** was then converted to the penamyl isocyanate **5** *via* the mixed anhydride **3** and the acid azide **4**, according to a modification of the method of Perron, *et al.*[8] Following the procedure of Sheehan and Brandt[2b] for the preparation of the 6-phthalimido analog, the acid hydrolysis of the isocyanate afforded 3-(2-methyl-2-(*o*-nitrophenoxy)-propionamido)-4-(1′-formyl-1′-methylethylsulfonyl)-2-azetidinone (**6**). This compound crystallized as a benzene solvate and appeared to exist mainly as the ring-closed form **6b**. Compound **6** slowly formed a 2,4-dinitrophenylhydrazone in aqueous alcoholic sulfuric acid. However, the infrared spectrum of a methylene chloride solution of **6** showed a hydroxyl stretching absorption and the nmr spectrum of a solution in deuteriochloroform contained a peak for the tertiary thiazolidine hydrogen at C-3 in **6b**. The urea **7** was obtained as a by-product in both the preparation of the isocyanate **5** and of the cyclic product (**6**). In contrast to the observations of Sheehan and Brandt[2b] and Heusler,[2h] the tautomeric aldehyde form of **6** (**6a**) was not detected by nmr and infrared analysis.



The remnant of the original thiazolidine ring in **6** was removed completely by the action of a large excess of potassium borohydride in cold aqueous 2-propanol. The crystalline product was shown to be 3-[2-methyl-2-(*o*-nitrophenoxy)propionamido]-2-azetidinone (**12**) by



the following data. Lassaigne's test disclosed that there was no sulfur in the unknown compound. Secondly, the disappearance of the 1310-cm^{-1} sulfone band

(1) This work was assisted financially by Bristol Laboratories, Division of Bristol Myers Co., Syracuse, N. Y.

(2) (a) "The Chemistry of Penicillin," H. T. Clarke, T. R. Johnson, and R. R. Robonson, Ed., Princeton University Press, Princeton, N. J., 1949, p 243; (b) J. C. Sheehan and K. G. Brandt, *J. Amer. Chem. Soc.*, **87**, 5468 (1965); (c) J. C. Sheehan, U. S. Patent 3,487,079 (1969); *Chem. Abstr.*, **72**, 100688k (1969); (d) D. H. R. Barton, D. G. T. Greig, G. Lucente, P. G. Sammes, M. V. Taylor, C. M. Cooper, G. Hewitt, and W. G. E. Underwood, *Chem. Commun.*, 1683 (1970); (e) R. D. G. Cooper and F. L. José, *J. Amer. Chem. Soc.*, **92**, 2575 (1970); (f) K. Heusler and R. B. Woodward, German Patent 1,935,640 (1970); *Chem. Abstr.*, **72**, 100689m (1970); (g) S. Kukolja, *J. Amer. Chem. Soc.*, **93**, 6267 (1971); (h) K. Heusler, *Helv. Chim. Acta*, **55**, 388 (1972).

(3) S. Wolfe, J. C. Godfrey, C. T. Holdrege, and Y. G. Perron, *J. Amer. Chem. Soc.*, **85**, 643 (1963); J. P. Clayton, *J. Chem. Soc. C*, 2123 (1969).

(4) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, *J. Amer. Chem. Soc.*, **85**, 1856 (1963); **91**, 1401 (1969); R. B. Morin and B. G. Jackson, U. S. Patent 3,275,626 (1966); *Chem. Abstr.*, **65**, 20133d (1966); D. O. Spry, *J. Amer. Chem. Soc.*, **92**, 5006 (1970).

(5) J. C. Sheehan, U. S. Patent 3,487,074 (1969); *Chem. Abstr.*, **72**, 66933z (1969); I. Ager, D. H. R. Barton, G. Lucente, and P. G. Sammes, *Chem. Commun.*, 601 (1972).

(6) D. A. Johnson, C. A. Panetta, and R. R. Smith, *J. Org. Chem.*, **31**, 2560 (1966).

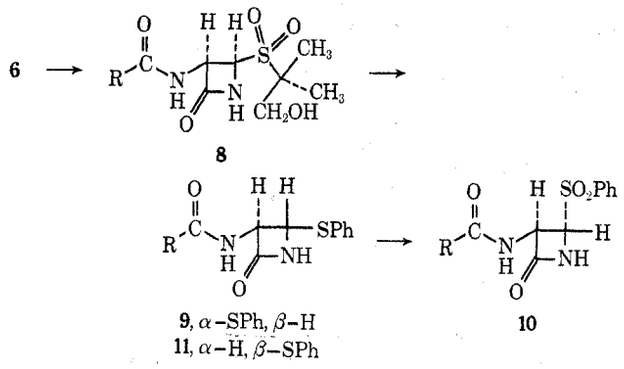
(7) D. A. Johnson, C. A. Panetta, and D. E. Cooper, *ibid.*, **28**, 1927 (1963).

(8) Y. G. Perron, L. B. Crast, J. M. Essery, R. R. Fraser, J. C. Godfrey, C. T. Holdrege, W. F. Minor, M. E. Neubert, R. A. Partyka, and L. C. Cheney, *J. Med. Chem.*, **7**, 483 (1964).

in the infrared spectrum and the shift of the β-lactam carbonyl absorption to a lower wavenumber added to the evidence that the 1-formyl-1-methylethylsulfonyl moiety had been cleaved from the β-lactam ring. Oxidation of the sulfur atom in penicillins to a sulfone has been reported to shift the β-lactam carbonyl absorption in the infrared region to higher wavenumbers.[7] Finally, elemental analyses and a molecular weight determination established the molecular formula and the nmr spectrum confirmed the structure assignment.

The desthioazetidinone **12** has also been prepared by the treatment of **6** with a large excess of lithium tri-*tert*-butoxyaluminum hydride in anhydrous tetrahydrofuran.

When compound **6** was treated with a smaller excess of potassium borohydride than was used in the preparation of **12** and the reaction time was limited to 2 min, the corresponding alcohol, **8**, was produced in quantitative yield. 3-[2-Methyl-2-(*o*-nitrophenoxy)-propionamido]-4-(2′-hydroxy-1′,1′-dimethylethylsulfonyl)-2-azetidinone (**8**) was easily characterized from elemental analysis and spectroscopic data.



The reaction of **6** with a large excess of potassium borohydride was examined using 20-cm long silica gel-coated glass plates. Starting at zero time and at 5-min intervals thereafter, aliquots of the reaction solution were acidified and spotted on the plates. The resultant chromatograms showed the alcohol **8** to be essentially the sole product during the first 5 min. Thereafter, the concentration of **12** increased while that of the alcohol decreased until 45 min, at which time it disappeared completely. These results have been interpreted to indicate that the alcohol **8** is an intermediate in the transformation of **6** to **12**.

We have replaced successfully the 2-hydroxy-1,1-dimethylethylsulfonyl side chain with the phenylthio group by the reaction of **8** with benzenethiol in aqueous ethanol for 25 min at pH 9.0 and room temperature. The product, 3-[2-methyl-2-(*o*-nitrophenoxy)propionamido]-4-phenylthio-2-azetidinone (**9**), was consonant with the observed elemental analysis and spectral properties. The β-lactam carbonyl absorption in the infrared spectrum shifted to a lower wavenumber than that observed for the alcohol **8**, and the sulfonyl band was absent. The nmr spectrum confirmed the presence of a benzene solvate in the crystalline structure of **9**. The coupling constants of the signals centered at 4.95 and 4.70 ppm indicate that the β-lactam hydrogens are cis in the alcohol **8**[9] and trans in the phenylthioazetidinone **9**.[10]

(9) G. F. H. Green, J. E. Page, and S. E. Staniforth, *J. Chem. Soc.*, 1595 (1965).

The replacement of the sulfonyl side chain with the phenylthio group also occurred when **6** was treated with sodium thiophenoxide in *N*,*N*-dimethylformamide. However, the reaction was slower and the yield lower than when the alcohol **8** was employed under similar conditions. The product obtained from either precursor was the same (**9**) in all respects.

The filtrates from **9** always contained, in low yield, a second reaction product which had a smaller R_f value on silica gel coated plates. This product was never obtained completely free of **9**, but it was assumed to be the 4 epimer of **9**, **11**.

The phenylthioazetidinone **9** was easily oxidized to 3-[2-methyl-2-(*o*-nitrophenoxy)pripionamido]-4-phenylsulfonyl-2-azetidinone (**10**) by treatment with potassium permanganate in aqueous acetic acid. The coupling constants of the β-lactam hydrogens (2.0 cps) of **10** indicate that it retains the same trans configuration of the unoxidized precursor, **9**.

The phenylsulfonyl group was replaced by the phenylthio group on the β-lactam ring in a reversal of the above oxidation experiment. The *trans*-phenylsulfonylazetidinone **10** was treated with benzenethiol in a manner similar to that used with the alcohol **8**. The product consisted of two components according to thin layer chromatography. Only one of these was isolated in a pure form and crystallized. It was identical with the phenylthioazetidinone **9** with respect to melting point and R_f value.

Experimental Section[11]

6-[2-Methyl-2-(*o*-nitrophenoxy)propionamido]penicillanic Acid Sulfone (2).—A stirred solution of 111 g (0.24 mol) of potassium 6-[2-methyl-2-*o*-nitrophenoxy)propionamido]penicillanate[6] in 1200 ml of cold water was treated with the dropwise addition of a solution of 79.0 g (0.5 mol) of potassium permanganate, 25.9 ml of 85% phosphoric acid, and 2 l. of water until the permanganate color persisted (required about 20 min and about 1/2 of the permanganate solution). During the above addition, the pH was maintained at 6.0–6.5 by the addition of 10% sodium hydroxide or 10% phosphoric acid. Sodium bisulfite was added in order to destroy excess permanganate and the mixture was filtered through a layer of Standard Super-Cel. Ethyl acetate (1 l.) was added to the filtrate and the resultant mixture was chilled in an ice bath while the pH was adjusted to 2.0 with 6 *N* hydrochloric acid. Two more extractions were made with ethyl acetate (700 and 300 ml) and the combined extracts were washed with water and with aqueous sodium chloride solution. The organic solution was dried over anhydrous magnesium sulfate and concentrated to a volume of 300 ml under reduced pressure. The sulfone **2** crystallized from the concentrate. The mixture was diluted with 2 l. of petroleum ether (bp 37–50°) and stirred for 30 min. The crystalline product was collected by filtration and washed with petroleum ether to yield 96.5 g (88.3%) of a homogeneous solid: tlc R_f 0.3 (benzene/acetone/acetic acid, 60:35:5); mp 153.0–154.0°; ir (CH_2Cl_2) 3370 (NH), 1815 (β-lactam), 1755 (carboxyl), 1690

(10) (a) E. J. Corey and A. M. Felix, *J. Amer. Chem. Soc.*, **87**, 2518 (1965); (b) K. D. Barrow and T. M. Spotswood, *Tetrahedron Lett.*, 3325 (1965); (c) H. B. Kagan, J. Basselier, and J. Luche, *ibid.*, 941 (1964); (d) I. McMillan and R. J. Stoodley, *ibid.*, 1205 (1966).

(11) Melting points were determined with a Kofler hot-stage microscope. Infrared spectra were recorded on a Perkin-Elmer Model 237 recording spectrophotometer. The nmr spectra were recorded on a Varian A-60. Microanalytical data were supplied by Dr. S. M. Nagy and his associates and by Midwest Microlab, Inc., Indianapolis, Ind. The thin layer chromatograms were run on silica gel G coated (250 μ) glass microscope slides (25 × 75 mm) except where exact R_f values are given, in which case 20-cm plates were used. The zones were detected as yellow areas on a purple background after spraying with a 0.5% aqueous potassium permanganate solution. Mass spectra were recorded on a Hitachi Perkin-Elmer RMU-6 mass spectrometer at 70 eV.

(amide I), 1525 (amide II), 1505 and 1340 (nitro), and 1310 cm^{-1} (sulfone); nmr (acetone-d_6) δ 8.06 (d, 1, NH), m centered at 7.32 (4, aromatic), 6.01 (dd, 1, $J_1 = 4$, $J_2 = 10$ Hz, H-6), 4.84 (d, 1, $J = 4$ Hz, H-5), 4.46 (s, 1, H-3), 1.52, 1.37 (singlets, 12, methyl).

Anal. Calcd for $C_{18}H_{21}N_3O_9S$: C, 47.45; H, 4.64; N, 9.23; S, 7.04. Found: C, 47.65; H, 4.83; N, 9.69; S, 7.65.

2,2-Dimethyl-6-[2-methyl-2-(*o*-nitrophenoxy)propionamido]-1,1-dioxo-3-penamyl Isocyanate (5).—A molecular sieve (type 4A) dried solution of 2.15 g (4.71 mmol) of the penicillin sulfone **2** in 12 ml of tetrahydrofuran was cooled to −5°, and 0.66 ml (0.48 g, 4.71 mmol) of triethylamine and 0.618 ml (0.644 g, 4.71 mmol) of isobutyl chloroformate were added consecutively. After stirring for 50 min at −5°, a cold solution of 0.31 g (4.71 mmol) of sodium azide in 5 ml of water was added dropwise during a 5–15-min period. Cold water (40 ml) was added and the resultant mixture was extracted with three portions (40, 15, and 5 ml) of benzene. The benzene solution was dried over molecular sieves (type 4A) and anhydrous magnesium sulfate and the filtrate was heated to reflux for 20 min. The solvent was removed under reduced pressure, leaving 1.28 g (60%) of a yellow glass. Crystallization from benzene gave a solvated solid with a diffuse melting point. Recrystallization from chloroform–benzene gave a sharp melting point (131–133°): $[\alpha]^{25}D$ 12.7° (*c* 1, $CHCl_3$); ir (Nujol) 3570 (water), 3395 (NH), 2260 (thiocyanate), 1810 (β-lactam), 1695 (amide I), 1520 (amide II), 1510 and 1360 (nitro), 1320 (sulfone), and 1250 cm^{-1} (phenoxy); nmr (acetone-d_6) δ 8.25 (d, 1, NH), m centered at 7.5 (4, aromatic), 6.10 (dd, 1, $J_1 = 4.5$, $J_2 = 11$ Hz, H-6), 5.37 (s, 1, H-3), 5.9 (d, 1, $J = 5$ Hz, H-5), 1.63, 1.50, 1.45 (singlets, 12, CH_3).

Anal. Calcd for $(C_{18}H_{20}N_4O_8S)_2 \cdot H_2O$: C, 46.90; H, 4.58; N, 12.12; S, 6.95. Found: C, 46.73; H, 4.37; N, 11.85; S, 7.02.

***N,N'*-Bis[2,2-dimethyl-6-[2-methyl-2-(*o*-nitrophenoxy)propionamido]-1,1-dioxo-3-penamyl]urea (7).**—In some preparations of the penamyl isocyanate **5**, the glassy yellow product did not completely dissolve in hot benzene. When this happened, the insoluble material was separated and crystallized from ethyl acetate: mp 198–200°; ir (Nujol) 3620 and 3540 (water), 3360 and 3390 (NH), 1810 (β-lactam), 1700–1865 (amide I and urea), 1550 (amide II), 1510 and 1350 (nitro), 1330 (sulfone), and 1255 cm^{-1} (phenoxy).

Anal. Calcd for $C_{35}H_{42}N_8O_{15}S_2 \cdot H_2O$: C, 46.90; H, 4.94; N, 12.50; S, 7.14. Found: C, 46.98; H, 4.72; N, 12.31; S, 6.83.

3-[2-Methyl-2-(*o*-nitrophenoxy)propionamido]-4-(1'-formyl-1'-methylethylsulfonyl)-2-azetidinone (6).—A solution of 13.9 g (0.0292 mol) of the penamyl isocyanate **5** in 370 ml of tetrahydrofuran was added dropwise during a 3.5-hr period to a stirred solution of 29.2 ml of 1 *N* hydrochloric acid in 300 ml of water and 300 ml of tetrahydrofuran. The resultant solution was stirred for 40 min and extracted with 3 × 370 ml of methylene chloride. The extract was washed with 2 × 250 ml of water and dried. Removal of the solvent under reduced pressure afforded a yellow glass, which crystallized from 70 ml of benzene. The total yield of two crystalline fractions was 12.8 g (86.4%). Two recrystallizations from chloroform–benzene gave an analytical sample: mp 87°, resolidified and melted again at 125–127°; $[\alpha]^{25}D$ 77.8° (*c* 1, $CHCl_3$); tlc R_f 0.5 (benzene–ethyl acetate, 1:1); ir (Nujol) 3370 (NH), 1800 (β-lactam), 1680 (amide II), 1525 (amide II), 1510 and 1360 (nitro), 1310 (sulfone), and 1245 cm^{-1} (phenoxy); ir (CH_2Cl_2) 3525 cm^{-1} (OH); nmr ($CDCl_3$) δ 8.30 (d, 1, NH), m centered at 7.35 (10, aromatic and solvate), 6.05 (dd, 1, $J_1 = 4.5$, $J_2 = 10$ Hz, H-6), 4.90 (d, 1, $J = 4$ Hz, H-5), 5.32 (d, 1, $J = 5.5$ Hz, H-3), 4.65 (d, 1, $J = 5.5$ Hz, OH), 1.60, 1.40 (singlets, 12, methyl); mass spectrum *m/e* 292 [M − $SO_2C(CH_3)_2CHO$].

Anal. Calcd for $C_{17}H_{21}N_3O_8S \cdot C_6H_6$: C, 54.65; H, 5.38; N, 8.31; S, 6.34. Found: C, 54.59; H, 5.35; N, 8.38; S, 6.23.

A sample of **6** was treated with 2,4-dinitrophenylhydrazine in aqueous, alcoholic sulfuric acid for 2 hr at room temperature. Yellow crystals separated and were collected, mp 197–200°, tlc R_f 0.4 (benzene–ethyl acetate, 1:1). The infrared spectrum showed bands in the proper region for NH stretching, β-lactam, amide, nitro, and sulfone absorptions.

3-[2-Methyl-2-(*o*-nitrophenoxy)propionamido]-2-azetidinone (12). A. Using Potassium Borohydride.—A cold solution of 0.10 g (0.198 mmol) of **6** in 6.0 ml of 2-propanol was added in two or three portions to a stirred solution of 0.056 g (1.04 mmol) of potassium borohydride[12] in 5.0 ml of 2-propanol and 5.0 ml of water at 5°. The solution was stirred in an ice bath for 25 min, the pH was adjusted to 7, and 25 ml of water was added. The resultant solution was extracted with 3 × 20 ml of methylene chloride and the extracts were combined, washed with 2 × 15 ml of water, and dried over anhydrous magnesium sulfate. Removal of the solvent under reduced pressure left a colorless glass (62.7 mg) which slowly crystallized from a mixture of benzene and petroleum ether, 8.6 mg. Two recrystallizations of another sample afforded material suitable for analysis: mp 151.5–152.5°; $[\alpha]^{25}D$ −34.0° (*c* 0.7, $CHCl_3$); tlc R_f 0.34–0.35 (ethyl acetate); qualitative tests following sodium fusion[13] showed no sulfur present; ir (Nujol) 3325 and 3250 (NH), 1770 (β-lactam), 1670 (amide I), 1545 (amide II), and 1515 and 1340 cm^{-1} (nitro); nmr (acetone-d_6) δ 8.5–7.1 (m, 5, aromatic and NH), 5.1 (broad m, 1, H-3), 3.4 (m, 2, H-4), 1.65, 1.58 (singlets, 6, CH_3); mass spectrum *m/e* 264 (M − CH_2NH_2), 250 (M − CONH).

Anal. Calcd for $C_{13}H_{15}N_3O_5$: C, 53.24; H, 5.16; N, 14.33; mol wt, 293. Found: C, 52.98; H, 5.14; N, 14.03; mol wt (in dioxane freezing), 285, 289.

B. Using Lithium Tri-*tert*-butoxyaluminum Hydride.—A solution of 0.20 g (0.396 mmol) of **6** in 10 ml of dry tetrahydrofuran was stirred at −5° while a solution of 1.02 g (4.0 mmol) of lithium tri-*tert*-butoxyaluminum hydride[12] in 20 ml of dry tetrahydrofuran was added in two portions. After the solution was stirred cold for 30 min, 1.0 ml of ethanol was added. Almost all of the tetrahydrofuran was evaporated and the residue was treated with 20 ml of benzene and anhydrous magnesium sulfate. The mixture was filtered through Standard Super-Cel and the filtrate was concentrated *in vacuo* to a yellow solid, 49.7 mg. Crystallization from benzene and petroleum ether gave 5.2 mg. The infrared spectrum of this substance was identical with that of the desthioazetidinone **12**.

3-[2-Methyl-2-(*o*-nitrophenoxy)propionamido]-4-(2'-hydroxyl-1',1'-dimethylethylsulfonyl)-2-azetidinone (8).—A cold (5°) solution of 0.117 g (2.16 mmol) of potassium borohydride[12] in 100 ml of water and 100 ml of methanol was added in one portion to a stirred and cooled (5°) solution of 2.19 g (4.34 mmol) of **6** in 100 ml of methanol. After exactly 2.0 min, the pH was adjusted to 2.0 (6 *N* hydrochloric acid) and 450 ml of water was added. The mixture was extracted with 3 × 120 ml of methylene chloride and the organic solution was washed with 2 × 90 ml of water and dried. The methylene chloride was evaporated and the residual glass, which was entirely the alcohol **8** according to thin layer chromatography, was obtained in almost quantitative yield, 1.86 g (99.6%). Recrystallization from benzene gave an analytical sample: mp 176.0–176.5°; $[\alpha]^{25}D$ 54.1° (*c* 1, $CHCl_3$); tlc R_f 0.21–0.23 (ethyl acetate); ir (CH_2Cl_2) 3560 (OH), 3365 (NH), 1810 (β-lactam), 1695 (amide I), 1525 (amide II), 1515 and 1345 (nitro), and 1290 cm^{-1} (sulfone); nmr ($CDCl_3$) δ 6.9–8.1 (m, 6, NH and aromatic), 5.9 (dd, 1, $J_1 = 5$, $J_2 = 10$ Hz, H-6), 5.15 (d, 1, $J = 5$ Hz, H-5), 3.75 (d, 2, $J = 7$ Hz, CH_2), 3.5 (broad s, 1, OH), 1.65, 1.57, 1.39, 1.31 (singlets, 12, methyl); mass spectrum *m/e* 292 [M − $SO_2C(CH_3)_2CH_2OH$].

Anal. Calcd for $C_{17}H_{23}N_3O_8S$: C, 47.50; H, 5.39; N, 9.78; S, 7.47. Found: C, 47.64; H, 5.49; N, 9.60; S, 7.38.

3-[2-Methyl-2-(*o*-nitrophenoxy)propionamido]-4-phenylthio-2-azetidinone (9). A. From the Alcohol 8.—A solution of 0.22 g (0.50 mmol) of the alcohol **8** in 20 ml of ethanol and 10 ml of water was treated with 0.051 ml (0.055 g, 0.50 mmol) of benzenethiol and the pH was adjusted to and maintained at 9.0–9.5 (1 *N* sodium hydroxide). Nitrogen was bubbled through the solution. After 26 min, 65 ml of water was added and the resultant mixture was extracted with 3 × 40 ml of methylene chloride. The organic solution was washed with 2 × 25 ml of water and dried. The solvent was evaporated and the residual oil was crystallized from 5 ml of benzene to yield 0.102 g (51%) of product. Recrystallization from benzene gave a solvated solid with diffuse melting point. Recrystallization from chloroform–benzene gave a sharp melting point (81.5–83°); $[\alpha]^{25}D$ −98.3° (*c* 1, $CHCl_3$); ir (CH_2Cl_2) 3395 (NH), 1795 (β-lactam), 1695 (amide I), 1530 (amide II), and 1515 and 1345 cm^{-1} (nitro); nmr ($CDCl_3$) δ 6.8–7.9 (m, benzene solvate, aromatic and NH), 4.95 (d, 1, $J = 2$ Hz, H-5), 4.7 (dd, 1, $J_1 = 2$, $J_2 = 8$ Hz, H-6), 1.54, 1.58 (singlets, 6, methyl); mass spectrum *m/e* 401 (M^+).

(12) Obtained from Metal Hydrides, Inc., Beverly, Mass.

(13) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th ed, Wiley, New York, N. Y., 1956, pp 57–58.

Anal. Calcd for $C_{19}H_{19}N_3O_5S \cdot 0.5C_6H_6$: C, 60.00; H, 5.03; N, 9.54; S, 7.28. Found: C, 60.22; H, 4.92; N, 9.33; S, 7.20.

B. From the Aldehyde 6.—A solution of 0.51 g (1.0 mmol) of **6**, 0.13 g (1.0 mmol) of sodium thiophenoxide,[14] and 1.0 ml of *N,N*-dimethylformamide was stirred for 40 min. Water (25 ml) was added and the resulting mixture was extracted with 3 × 10 ml of methylene chloride. The methylene chloride solution was washed with 2 × 7 ml of water and dried. The solvent was evaporated and residual oil was chromatographed on a silicic acid (100 mesh) column with ethyl acetate. The effluent was separated into a homogeneous fraction which crystallized from a mixture of benzene and petroleum ether after a seed of the phenylthioazetidinone **9** was added, 87.6 mg (19.9%), tlc R_f was identical with that of **9** in ethyl acetate.

C. From the *trans*-Phenylsulfonylazetidinone 10.—A stirred solution of 0.284 g (0.656 mmol) of *trans*-phenylsulfonylazetidinone **10** (see below for preparation), 0.067 ml (0.072 g, 0.66 mmol) of benzenethiol, 20 ml of ethanol, and 10 ml of water was prepared under nitrogen. The pH was held at 9.0 (1 *N* sodium hydroxide) for 40 min. Water (65 ml) was added and the mixture was extracted with 3 × 40 ml of methylene chloride. The organic solution was washed with 2 × 25 ml of water, dried, and evaporated under reduced pressure. The residue (85.3 mg) contained two components according to thin layer chromatography (benzene–ethyl acetate, 1:1). Only one of these was obtained pure by chromatography on a 2-mm thick silica gel coated plate (Brinkmann Instruments) (same solvent as above). Crystallization from benzene gave 8.9 mg, mp 76.0–78.5°; tlc R_f was the same as that of the phenylthioazetidinone **9**.

(14) Prepared from benzenethiol and sodium methoxide in anhydrous methanol. Addition of ether caused separation of the product.

***trans*-3-[2-Methyl-2-(*o*-nitrophenoxy)propionamido]-4-phenylsulfonyl-2-azetidinone (10).**—A solution of 0.88 g (5.6 mmol) of potassium permanganate in 8.4 ml of water was added dropwise over a 10-min period to a solution of 1.23 g (2.8 mmol) of phenylthioazetidinone **9** in 20 ml of 80% acetic acid. The resultant mixture was stirred for 45 min, and 30% hydrogen peroxide was added until all color was discharged. Water (100 ml) was added and the mixture was extracted with three portions (80, 40, 40 ml) of methylene chloride. The organic solution was washed with 2 × 20 ml of water and dried. The methylene chloride was removed and the residual oil was slowly crystallized from benzene. This was recrystallized from benzene–ethanol (4:1) to yield the pure trans isomer, **10**: 0.33 g; mp 158.0–159.0°; $[\alpha]^{25}D$ −32.5° (*c* 1, $CHCl_3$); tlc R_f 0.59 (benzene–ethyl acetate, 1:1); ir (CH_2Cl_2) 3395 (NH), 1815 (β-lactam), 1695 (amide I), 1525 (amide II), 1150 and 1335 (nitro), and 1300 cm^{-1} (sulfone); nmr (acetone-d_6) δ 6.9–8.7 (m, 11, aromatic and amide), 5.0–5.4 (dd overlapped by d, 2, J_1 of dd = 2 Hz, H-5 and H-6), 1.52, 1.49 (singlets, 6, methyl).

Anal. Calcd for $C_{19}H_{19}N_3O_7S$: C, 52.65; H, 4.42; N, 9.70; S, 7.37. Found: C, 52.41; H, 4.47; N, 9.56; S, 7.43.

Registry No.—**1**, 10514-63-3; **2**, 37696-07-4; **5**, 37696-08-5; **6b**, 37696-09-6; **6** 2,4-DNPH, 37696-10-9; **7**, 37818-75-0; **8**, 37696-11-0; **9**, 37755-01-4; **10**, 37755-02-5; **12**, 37696-12-1.

Synthesis of 6-Methylthiopenicillins and 7-Heteroatom-Substituted Cephalosporins

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A number of 6α-methylthiopenicillins and 7α-methylthiocephalosporins have been prepared from intermediates obtained by methylthiolation of Schiff bases of 6-aminopenicillanic acid esters and 7-aminocephalosporanic acid esters. Fluorination with perchloryl fluoride gave a 7α-fluorocephalosporin Schiff base that could be solvolyzed to 7α-methoxy- and 7α-methylthiocephalosporin intermediates. The same 7α-methoxycephalosporin Schiff base intermediate could be obtained by mercuric acetate catalyzed methanolysis of the corresponding 7α-methylthio Schiff base. Reaction of a 7α-methylthiocephalosporin with mercuric acetate in methanol gave a mixture of 7α- and 7β-methoxycephalosporins from which pure 7β-methoxy epimer could be isolated. The same reaction with methanol replaced by dimethoxyethane or acetic acid yielded a 7α-acetoxycephalosporin. Nuclear Overhauser studies performed on the 7-substituted cephalosporins led to assignments of configuration at C-7, which were supported by single-crystal X-ray analysis of 7α-methylthio-7-phenylacetamidodeacetoxycephalosporanic acid *tert*-butyl ester.

Previous investigations have shown that neither the introduction of a 7α-methyl group into a cephalosporin nor of a 6α-methyl group into a penicillin results in improved antimicrobial activity.[1,2] Similar results were found when the 7(6)-α-methyl substituents were replaced by α-acetyl groups.[3] As part of the biological study of cephalosporins and penicillins possessing substituents at the C-7(6) position, it seemed reasonable for us to examine the effects of electron-withdrawing substituents other than acetyl. Heteroatom substituents were an obvious choice; thus, we report now our synthesis of 7-acetoxy-, 7-methoxy-, and 7-methylthiocephalosporins and 6-methylthiopenicillins.[4] Key compounds and the general synthetic schemes are outlined below (1).

The methylthio group was introduced stereospecifically into the 7 position of the Δ^3-cephem nucleus by two routes. Using a one-step method, the anion of the benzaldehyde Schiff base (I) of 7-aminodeacetoxycephalosporanic acid *tert*-butyl ester, prepared by using 1 equiv of KO-*t*-Bu in dimethoxyethane at −20°, was methylthiolated with methyl methanethiolsulfonate ($CH_3SSO_2CH_3$)[5] or methylsulfenyl chloride (CH_3SCl)[5] to give the crystalline 7α-methylthio Schiff base II in 40% yield. In an alternative procedure, the anion of the Schiff base I was fluorinated, using perchloryl fluoride, to give the 7α-fluoro Schiff base III, which could be solvolyzed with methanethiol under acidic conditions to the 7α-methylthio Schiff base II. Schiff bases ob-

(1) E. H. W. Böhme, H. E. Applegate, B. Toeplitz, J. E. Dolfini, and J. Z. Gougoutas, *J. Amer. Chem. Soc.*, **93**, 4324 (1971).

(2) R. A. Firestone, N. Schelechow, D. B. R. Johnston, and B. G. Christensen, *Tetrahedron Lett.*, 375 (1972).

(3) E. H. W. Bohme, H. E. Applegate, J. B. Ewing, P. T. Funke, M. S. Puar, and J. E. Dolfini, *J. Org. Chem.*, **38**, 230 (1973).

(4) During these studies, there appeared reports of 7-methoxycephalosporins obtained from fermentations[4a,b] and by an elegant synthesis[4c] of these and related analogs: (a) R. Nagarajan, L. D. Boeck, M. Gorman, R. L. Hamill, C. E. Higgens, M. M. Hoehn, W. M. Stark, and J. G. Whitney, *J. Amer. Chem. Soc.*, **93**, 2308 (1971); (b) S. Karady, S. H. Pines, L. M. Weinstock, F. E. Roberts, G. S. Brenner, A. M. Hoinowski, T. Y. Cheng, and M. Sletzinger, *ibid.*, **94**, 1410 (1972); (c) L. D. Cama, W. J. Leanza, T. R. Beattie, and B. G. Christensen, *ibid.*, **94**, 1408 (1972).

(5) I. B. Douglass, *J. Org. Chem.*, **24**, 2004 (1959).