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References and Notes

(1) (a) P. Talalay and A. M. Benson in "The Enzymes", Vol. VI, 3d ed, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1972, Chapter 18; (b) F. H. Batzold, A. M. Benson, D. F. Covey, C. H. Robinson, and P. Talalay, "Advances in Enzyme Regulation", Vol XIV, G. Weber, Ed., Pergamon Press, New York, N.Y., 1976, pp 234–267.
(2) S. K. Malhotra and H. J. Ringold, *J. Am. Chem. Soc.*, **87**, 3228 (1965).
(3) (a) F. H. Batzold and C. H. Robinson, *J. Am. Chem. Soc.*, **97**, 2576 (1975); (b) *J. Org. Chem.*, **41**, 313 (1976).
(4) A. M. Benson and P. Talalay, personal communication.
(5) (a) C. Walsh, A. Schonbrunn, O. Lockridge, V. Massey, and R. Abeles, *J. Biol. Chem.*, **247**, 6004 (1972); (b) P. C. Holland, M. G. Clark, and D. P. Boxham, *Biochemistry*, **12**, 3309 (1973); (c) R. R. Rando and J. DeMairena, *Biochem. Pharmacol.*, **23**, 463 (1974); (d) P. Marcotte and C. Walsh, *Biochem. Biophys. Res. Commun.*, **62**, 677 (1975); (e) M. J. Jung and B. W. Metcalf, *ibid.*, **67**, 301 (1975).
(6) (a) K. Endo, G. M. Helmkamp, Jr., and K. Bloch, *J. Biol. Chem.*, **245**, 4293 (1970); (b) B. Morisaki and K. Bloch, *Biochemistry*, **11**, 309 (1972).
(7) (a) R. G. Carlson and D. E. Henton, *Chem. Commun.*, 674 (1969); (b) J. Meinwald and L. Hendry, *Tetrahedron Lett.*, 1657 (1969); (c) B. R. von Wartburg, H. R. Wolf, and O. Jeger, *Helv. Chim. Acta*, **56**, 1948 (1973).
(8) Correct C and H analyses were obtained for $C_{18}H_{22}O_3$.
(9) H. L. Carrell, J. P. Glusker, C. H. Robinson, F. H. Batzold, and D. F. Covey, Abstract PB8, American Crystallographic Association Winter Meeting, Clemson University, S.C., 1976.
(10) (4*R*)-5,10-Seco-19-norpregna-4,5-diene-3,10,20-trione (**5**): mp 122–124°, correct C and H analyses for $C_{20}H_{26}O_3$.
(11) (4*S*)-5,10-Seco-19-norpregna-4,5-diene-3,10,20-trione (**6**): MS *m/e* 314.1872, $C_{20}H_{26}O_3$ requires 314.1882).
(12) Crystalline Δ^5-3-ketosteroid isomerase[13] was used in all studies. Inactivation experiments were done at 26.5° in a total volume of 500 μl. The reaction vessel contained: 4.80 μM isomerase, 1.0 mM potassium phosphate buffer (pH 7.0) and compounds **1** through **6** at concentrations of 20 or 200 μM introduced in 1,4-dioxane (20 μl). Aliquots were removed at 1-, 2-, or 5-min intervals, diluted (as much as 1.5×10^6-fold in 1% neutral bovine serum albumin) and assayed for residual enzymatic activity in the presence of 57.8 μM Δ^5-androstene-3,17-dione (K_m = 340 μM)[1] by monitoring the appearance of the conjugated ketone chromophore at 248 nm in water.
(13) Kindly provided by Dr. P. Talalay.
(14) F. H. Batzold, personal communication.

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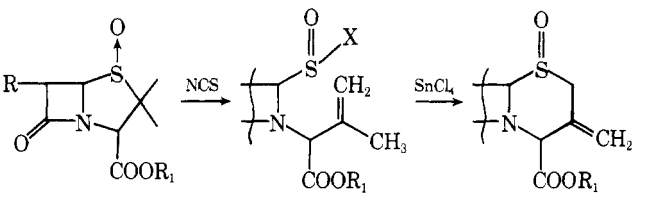
A Rearrangement of Penicillin Sulfoxides to 3-Methylenecephams via Sulfinyl Intermediates[1]

Sir:

The ring expansion of penicillin sulfoxides to deacetoxycephalosporins achieved by Morin and co-workers[2] is significant for two reasons: (a) it has provided the first direct chemical correlation between the penicillins and cephalosporins, and (b) it has afforded a practical method for preparing clinically important antibiotics containing deacetoxycephalosporin nucleus.[3] We have discovered a new oxidative ring expansion of penicillins yielding 3-methylenecepham sulfoxides. This new substance appears to be a very versatile intermediate for the synthesis of a wide variety of commercially significant cephalosporins. Namely, the highly desirable exomethylene functionality located at the 3-position offers the opportunity to functionalize that group and to make various 3-substituted cephalosporins.[4]

We have found that the sulfinyl halide, II (prepared from penicillin sulfoxide, I, and halogenating agents), can be cyclized to 3-methylenecepham sulfoxides, III, by means of Lewis acids.

Treatment of the penicillin sulfoxide I (R = Ft, $R_1 = CH_3$) with NCS (1 equiv, 70 min) in refluxing CCl_4 gave in almost



I II III

R = phthalimido (Ft), phenoxyacetamido
R_1 = CH_3, *p*-nitrobenzyl (pNB)
NCS = *N*-chlorosuccinimide

quantitative yield a mixture of the sulfinyl chlorides II which are epimeric at sulfur.[5] Similarly, when the penicillin sulfoxide having an amide side chain (I, R = phenoxyacetamido, R_1 = pNB) was refluxed in toluene with NCS (1 equiv, 90 min), the corresponding sulfinyl chloride II (X = Cl) was obtained:[6] NMR ($CDCl_3$) δ 1.93 (s, 3, CH_3), 4.58 (s, 2, side chain CH_2), 5.17 (m, 3, olefinic methylene and -C*H*COOpNB), 5.35 (s, 2, ester CH_2), 5.61 (d, 1, J = 5.0 Hz, azetidinone H), 6.2 (dd, 1, J = 5.0 and 8.0 Hz), and 6.9–8.3 (m, 9, ArH); *m/e* 374.

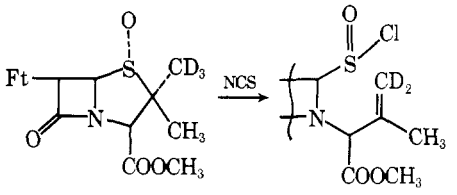
Ring closure of II (R = Ft, $R_1 = CH_3$, X = Cl) with $SnCl_4$[7] (1 equiv, CH_2Cl_2, 22°, 1–2 h) gave a mixture of the *R* and *S* sulfoxides III in the ratio of ca. 2:1, separable by chromatography on silica gel (eluent: 20% EtOAc/$CHCl_3$).[8] The *R* sulfoxide III melts at 201–202° (CH_2Cl_2/cyclohexane): NMR ($CDCl_3$) δ 3.62 and 4.12 (ABq, J = 14 Hz, C_2–H), 3.85 (s, 3, OCH_3), 4.88 (d, 1, J = 4.5 Hz, C_6–H), 5.25 (br s, 1, C_4–H), 5.58 (m, 2, CH_2=), 5.97 (d, 1, J = 4.5 Hz, C_7–H), and 7.84 Hz (m, 4 ArH). The *S* sulfoxide was isolated as colorless foam: NMR ($CDCl_3$) δ 3.63 (s, 2, C_2–H), 3.82 (s, 3, OCH_3), 4.90 (d, 1, J = 4.5 Hz, C_6–H), 5.32 (s, 1), 5.46 (br s, 1, C_4–H), 5.64 (d, 1, J = 4.5 Hz, C_7–H), 5.77 (s, 1), and 7.84 Hz (m, 4, ArH); *m/e* 374.[9]

Both *R* and *S* sulfoxides III after reduction with PBr_3 (1 equiv, DMF, 0–5°, 0.5 h)[10] gave the same methyl 7-phthalimido-3-methylenecepham (75%): mp 194–196.5° (EtOAc); NMR ($CDCl_3$) δ 3.38 and 3.63 (ABq, 2, J = 14 Hz, C_2–H), 3.80 (s, 3, OCH_3), 5.32 (m, 3), 5.46 (d, 1, J = 4.5 Hz, C_6–H), 5.67 (d, 1, J = 4.5 Hz, C_7–H), and 7.83 Hz (m, 4, ArH); *m/e* 358.

While the cyclization of II with the phthalimido side chain resulted in the formation of *R* and *S* sulfoxides III, a similar cyclization with the phenoxyacetamido compound II yielded only the *S* sulfoxide III. Thus, the treatment of II (R = phenoxyacetamido, R_1 = pNB, X = Cl)[11] with $SnCl_4$ (1 equiv of toluene, 2 h, 22°) gave III:[8] mp 194–196° (EtOAc); NMR ($CDCl_3$) δ 3.5 and 3.75 (ABq, 2, J = 15 Hz, C_2–H), 4.55 (s, 2, side chain CH_2), 4.83 (d, 1, J = 4.5 Hz, C_6–H), 5.3 (s, 2, ester CH_2), 5.33 (s, 1), 5.5 (s, 1), 5.78 (s, 1), 6.02 (dd, 1, J = 4.5 and 9.0 Hz), and 6.9–8.3 (m, 9, ArH). Reduction of this sulfoxide with PBr_3 (1 equiv, DMF, 22°, 1 h) gave *p*-nitrobenzyl 7-phenoxyacetamido-3-methylenecepham-4-carboxylate identical with an authentic sample.[12]

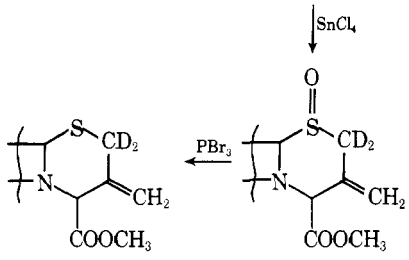
From a mechanistic point of view it was of interest to know which carbon of the intermediate sulfinyl halide participates in the formation of the S–C bond during the cyclization process. The rearrangement was repeated with the deuterated compound IV, the stereochemistry of which has been previously established.[13] In IV, deuterium is incorporated only in the α-methyl group and consequently after treatment of IV with NCS (1 equiv, 30 min, Cl_2CHCH_2Cl, 114°) the sulfinyl chloride V, with the methylene group being more than 95% deuterated, was obtained. Ring closure of V to VI was achieved with $SnCl_4$ (1 equiv, CH_2Cl_2, 22°, 50 min). A mixture of the *R* and *S* sulfoxides of VI was isolated and immediately reduced with PBr_3 (1 equiv, DMF, 0–5°, 35 min) to methyl 2-dideuterio-3-methylene-7-phthalimidocepham-4-carboxylate (VII):

mp 198–201° (CH_2Cl_2/cyclohexane); NMR ($CDCl_3$) δ 3.80 (s, 3, CO_2CH_3), 5.32 (m, 3, exomethylene and C_4–H), 5.46 (d, 1, J = 4.5 Hz, C_6–H), 5.67 (d, 1, J = 4.5 Hz, C_7–H), and 7.03 (m, 4, ArH). The NMR spectrum exhibited only a very small (less than 5%) signal for protons at the C_2 position while the signal for the 3-exomethylene group was of normal intensity, indicating selective incorporation of the CD_2 group into the 2-position.[14]



IV

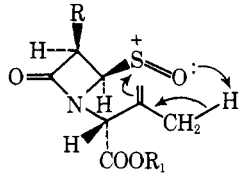
V



VII

VI

A sulfinium cation VIII is a probable intermediate in the ring closure of sulfinyl chlorides II with Lewis acids, and the mechanism can be visualized as an intramolecular ene reaction.[15]



VIII

It seemed that if the mechanism does involve the intermediacy of a sulfinium cation VIII that any other derivative of the sulfinic acid capable of forming such an intermediate might also be used as a starting material for the synthesis of 3-methylene cephams. In fact, using Bronsted acids (e.g., H_2SO_4, H_3PO_4, CH_3SO_3H), we have been able to cyclize the sulfinic acid (II, X = OH), and some of its derivatives such as esters (II, X = OCH_3), thiol esters (II, X = S-*i*-Pr), amides (II, X = NHPh), imides (II, X = succinimido), and hydrazides (II, X = N(COOR)NH(COOR)). These reactions will be described at a later date in a full paper [16]

Future publications from these laboratories will demonstrate the utilization of 3-methylenecephams in the preparation of various 3-substituted cephalosporins.

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References and Notes

(1) Azetidinone antibiotics. 17. Paper 16. S. Kukolja, M. R. Gleissner, A. I. Ellis, D. E. Dorman, and J. W. Paschal, *J. Org. Chem.*, **41**, 2276 (1976).
(2) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, *J. Am. Chem. Soc.*, **85**, 1896 (1963); **91**, 1401 (1969).
(3) C. W. Ryan, R. L. Simon, and E. M. Van Heyningen, *J. Med. Chem.*, **12**, 310 (1969).
(4) R. R. Chauvette and P. A. Pennington, *J. Am. Chem. Soc.*, **96**, 4986 (1974); R. Scartazzini and H. Bickel, *Helv. Chim. Acta*, **57**, 1919 (1974); **58**, 2437 (1975); and ref 12 in this paper.
(5) S. Kukolja and S. R. Lammert, *Angew. Chem., Int. Ed. Engl.*, **12**, 67 (1973). In addition to NCS, other halogenating agents have been also used successfully, e.g., *N*-bromosuccinimide, *N*-chlorophthalimide, *N*-chloro-*N*-methyl-*p*-toluenesulfonamide, *N,N*-dichlorourethane, *tert*-butyl hypochlorite.
(6) When 6-amidopenicillin sulfoxides are employed in this rearrangement the best yields are obtained when the reaction is carried out in the presence of acid scavengers.[16]
(7) The other Lewis acids, such as $TiCl_4$, $AlCl_3$, $ZnCl_2$, $ZnBr_2$, $SbCl_5$, $HgCl_2$, $FeCl_3$, and $ZrCl_4$, were also successfully used for a ring closure of II.
(8) A rearrangement of I to III via the sulfinyl chloride (II) can be performed in two separate steps. However, it is preferable to carry out these two steps as a one pot reaction. For example, (a) a mixture of 18.8 g of methyl 6-phthalimidopenicillanate 1-oxide and 6.7 g of NCS in 1 l. of CCl_4 was refluxed for 70 min, cooled to ca. 25°, and then 6 ml of $SnCl_4$ was added and the reaction mixture was stirred for 45 min. After workup, 18.4 g of a mixture of *R* and *S* sulfoxides of the corresponding 3-methylenecephams was obtained. (b) p-Nitrobenzyl 6-phenoxyacetamidopenicillanate 1-oxide (6.0 g), 1.8 g of NCS, and 500 ml of toluene were refluxed for 90 min, cooled to ca. 50°, and then 1.8 g of $SnCl_4$ was added and the reaction mixture stirred for 90 min. After workup, 2.16 g of *p*-nitrobenzyl 7-phenoxyacetamido-3-methylenecepham-4-carboxylate 1-oxide was obtained.
(9) All new compounds gave good mass spectral or elemental analyses.
(10) G. V. Kaiser, R. D. G. Cooper, R. E. Koehler, C. F. Murphy, J. A. Webber, I. G. Wright, and E. M. Van Heyningen, *J. Org. Chem.*, **35**, 2430 (1970).
(11) Compounds with the other side chains and acid protective groups were also cyclized under a similar condition. The details will be published in a full paper.[16]
(12) R. R. Chauvette and P. A. Pennington, *J. Org. Chem.*, **38**, 2994 (1973).
(13) R. D. G. Cooper, *J. Am. Chem. Soc.*, **92**, 5010 (1970).
(14) Mass spectral analysis gave the following deuterium distribution in the C_2 position: d_0, 2.2%; d_1, 25.5% d_2, 72.3% ± 2%.
(15) H. M. R. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, **8**, 556 (1969); J. A. Marshall, N. H. Andersen, and P. C. Johnson, *J. Org. Chem.*, **35**, 189 (1970); K. B. Sharpless and T. Hori, *ibid.*, **41**, 176 (1976); P. E. Peterson, R. Brockington, and M. Dunham, *J. Am. Chem. Soc.*, **97**, 3517 (1975).
(16) S. Kukolja, S. R. Lammert, M. R. Gleissner, A. I. Ellis, J. L. Pfeil, T. S. Chou, J. R. Burgtorf, W. A. Spitzer, T. Goodson, I. G. Wright, M. A. Schmoll, R. R. Chauvette, and P. A. Pennington, unpublished results.

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5-Iodoacetamidomethyl-2′-deoxyuridine 5′-Phosphate. A Selective Inhibitor of Mammalian Thymidylate Synthetases

Sir:

In 1959 Baker proposed a unique approach to the control of cancer by drugs that are designed to detect differences between cancerous and normal cells.[1] The theory is based on the fact that, although an enzyme derived from different sources catalyzes the same transformation, the primary structure of the enzyme may vary with the source.[2] This variation in primary structure, in all probability, will not alter the structure of the active site; however, it is reasonable to assume tertiary structural differences will be detectable in other portions of the various isozymes.

We wish to report evidence for the first example of selective irreversible inhibition of thymidylate synthetase (EC 2.2.2.6).[3] 5-Iodoacetamidomethyl-2′-deoxyuridine 5′-phosphate (I), a competitive inhibitor of thymidylate synthetases obtained from calf thymus and Ehrlich ascites tumor, is an irreversible inhibitor of the tumor, but, not the thymus enzyme. It is proposed that the tertiary structural differences existing near the active site of the two enzymes are such that the alkylating portion of I is excluded from the reactive nucleophile in the thymus enzyme. 5-Fluoro-2′-deoxyuridine 5′-phosphate is an irreversible inhibitor of thymidylate synthetase; however, there is no evidence that it is isoenzyme specific.[3]

5-Iodoacetamidomethyl-2′-deoxyuridine 5′-phosphate (I) was prepared from the corresponding nucleoside[4] by selective chemical phosphorylation using phosphorus oxychloride according to the procedure of Yoshikawa and co-workers.[5] Iodoacetamide (II) used was a recrystallized sample of a preparation obtained from Aldrich Chemical Co.

Thymidylate synthetase was extracted from fresh calf thymus according to the method of Jenny and Greenberg[6] through the ammonium sulfate fractionation step. Ehrlich ascites tumor