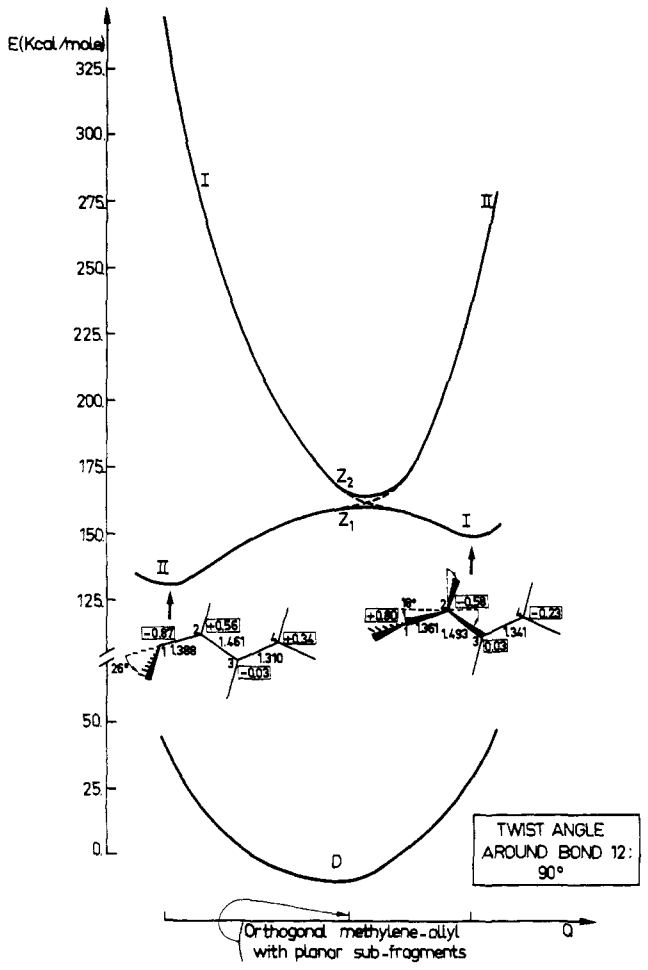

Figure 1. Potential surfaces for the lowest singlet states of 90° twisted methylene-allyl (minimal basis set). The coordinate Q is obtained by linear interpolation from the geometry of II to an intermediate geometry with planar subfragments, and from this intermediate geometry to the geometry of I. Bond lengths in this "half-way" skeleton are C_1C_2 = 1.375 Å, C_2C_3 = 1.475 Å, C_3C_4 = 1.325 Å (for this geometry II lies below I). Dotted lines show the avoided crossing between the configurations corresponding respectively to I and II.[7] Note that the diradical D is a *maximum*, or a near-maximum, along the coordinate for twisting around bond 12 (i.e., vertical excitation does not occur from D, but from the untwisted butadiene).

kcal/mol. Substitution by methyl at C-1 makes I, 1-methyl have a value of 0.1 kcal/mol. The energy difference between I and II has now decreased from 9.2 to 0.1 kcal/mol.[11] The terminal methyl group stabilizes the methylene positive center more than the allylic positive center, a reasonable result in view of the larger (0.80 at 1 in I vs. 0.34 at 4 in II) positive charge at the former center. If we *assume* that the methylene-allyl can in principle cyclize at 24 in *either* form I or form II, our results would indicate that: (a) unsubstituted butadiene cyclizes via II (allyl cation; disrotatory),[12] (b) 1-methylbutadiene cyclizes via both I and II (no stereospecificity), (c) 1,1-dimethylbutadiene cyclizes via I (allyl anion; conrotatory).[12] However, since minimal basis sets are notoriously inadequate for the energies of anions or anionic fragments (already the few extended basis set points appear to decrease the relative stability of II), and since also we have not reoptimized the geometries of I and II in the presence of the substituents, only the *qualitative trend* indicated by our results should be considered seriously.

This qualitative trend is *a smooth passage from disrotatory ring closure to conrotatory ring closure with increasing unsymmetrical terminal methyl (donor) substitution.* It would be interesting to test this prediction by investigating whether the observed conrotatory preference[13] in Dauben's pioneering experiment disappears in unsubstituted butadienes, using conveniently deuterium-labeled butadienes—or even better in a butadiene terminally substituted by an appropriate acceptor. Experimental confirmation would provide strong support for the zwitterionic nature of the primary intermediate and would reveal an extraordinary sensitivity of stereochemistry to substituents in photochemical reactions which proceed through zwitterionic intermediates.[14]

Acknowledgment. We thank R. Lefebvre for a stimulating suggestion.

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(6) W. J. Hehre, W. A. Lathan, R. D. Ditchfield, M. D. Newton, and J. A. Pople, Program No. 236, QCPE, University of Indiana, Bloomington, Ind. Our calculations use the restricted Nesbet open-shell SCF method, together with a 3-by-3 configuration interaction for proper treatment of the diradical and zwitterionic singlet states.
(7) The double-well potential shown in the figure for the lowest excited *zwitterionic* state $^1Z_1$ should not be confused with the double-well potential obtained previously for an excited *diradical* state ^1D in highly polar solvents (L. Salem and W. D. Stohrer, *J. Chem. Soc., Chem. Commun.*, 140 (1975)). Here the origin of the double well is an avoided crossing between the two excited singlets,[8] whereas in the previous case the avoided crossing occurred between ground and excited singlet.
(8) J. Langlet and J. P. Malrieu, *Theor. Chim. Acta*, **33**, 307 (1974), point out that mixing of degenerate configurations in twisted butadiene may lead to local deformations of the nuclear geometry.
(9) The corresponding extended basis set values are 8.7 and 7.8 kcal/mol for the previously optimized geometries.
(10) J. Michl, *J. Mol. Photochem.*, **4**, 243, 257 (1972).
(11) On the same energy scale, I, 4-methyl lies at 13.2 kcal/mol and II, 1-methyl at 12.1 kcal/mol.
(12) It might be argued that in the Z state the allylic anion (or cation) is an *excited* anion (or cation) and should therefore undergo disrotatory, rather than conrotatory, closure. Consideration of the orbital occupancies in the allyl fragment shows that this is not the case. The excitation energy of the molecule is used exclusively for 1–2 twisting and for separating the two charges, thus lifting the diradical to an excited zwitterionic state (CH_2·, allyl· → CH_2^+, allyl$^-$).
(13) Conrotatory closure occurs in ethylidene-cyclooctene,[16a] which is equivalent to a 1,3,4-trialkyl derivative of butadiene. If we label the twisted double bond 12 (as in Figure 1), the substituted centers are 1, 3, and 4 for endocyclic twist and 1, 2, and 4 for exocyclic twist. We now *estimate* the relative stabilization of I and II by considering the net charges, in the *unsubstituted* system (Figure 1), at those positions which become alkyl-substituted. The stabilization of positive charges by the alkyl substituents is *optimized* in I by rotation about the endocyclic bond (the substituted centers bear charges +0.80, +0.03, −0.23), in II by rotation about the exocyclic bond (the substituted centers bear charges +0.56, +0.34, and −0.87). The larger net overall positive charge stabilized in I, and the poor interaction of a methyl group with a near-unit negative charge at a terminal carbon in II, can possibly make I the reactive species. We thank W. G. Dauben for a discussion of this case.
(14) Stereochemistry should also be temperature dependent (P. de Mayo, private communication, 1976).
(15) Deutsche Forschungsgemeinschaft Scholar.

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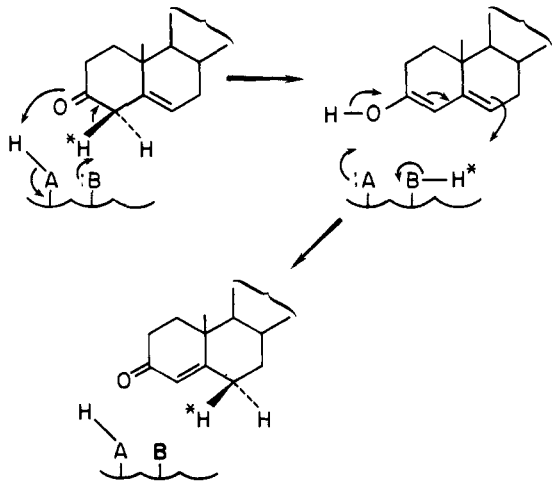
Conjugated Allenic 3-Oxo-5,10-secosteroids. Irreversible Inhibitors of Δ^5-3-Ketosteroid Isomerase

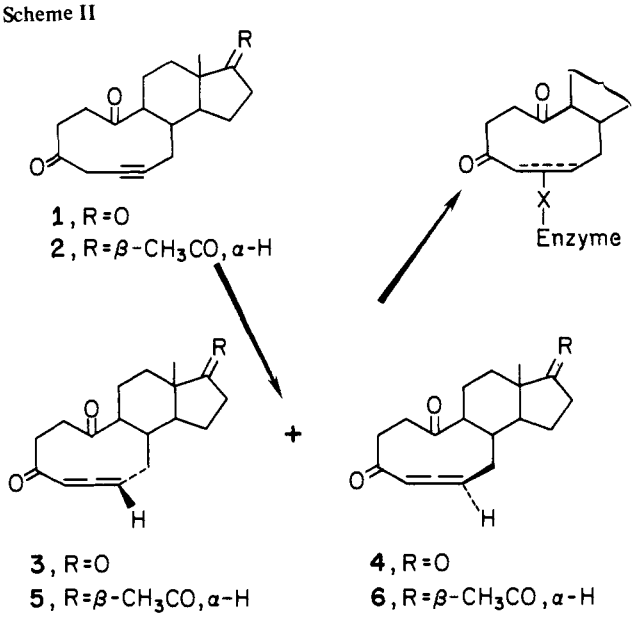
Sir:

The enzyme Δ^5-3-ketosteroid isomerase[1] (EC 5.3.3.1) from *Pseudomonas testosteroni* converts C_{19} and C_{21} Δ^5-3-ketosteroids to the corresponding Δ^4-3-ketosteroids. The proposed mechanism[1,2] involves enolization with removal of the axial 4β-hydrogen followed by ketonization of the $\Delta^{3,5}$-dienol with axial reprotonation at C-6. The hydrogen transfer from C-4

Scheme I



Scheme II



to C-6 is intramolecular (Scheme I). This reaction when carried out by mammalian Δ^5-3-ketosteroid isomerases is a key step in the biosynthesis of steroid hormones.

The rapid irreversible inhibition of bacterial Δ^5-3-ketosteroid isomerase by the mechanism-based inhibitors **1** and **2** has been reported from this laboratory.[3] A recent experiment with tritium-labeled **1** indicated[4] that a single steroid molecule binds to each subunit of the enzyme. It was proposed[3] that the β,γ-acetylenic ketone grouping in compounds **1** and **2** is converted to a conjugated allenic ketone via enzymatic enolization. Covalent modification of the enzyme should then occur by Michael addition of an adjacent nucleophilic amino acid residue to the allenic ketone (Scheme II).

We report here the synthesis and characterization of the allenic ketones derived from compounds **1** and **2** and their inhibitory effects on bacterial Δ^5-3-ketosteroid isomerase. Although enzyme-generated allenes have been postulated as alkylating agents in a number of cases,[5] the allenic compound has been synthesized and studied only in the original example described by Bloch et al.[6]

Isomerization of **1** with triethylamine in dioxane at room temperature for 30 min yielded a 7:3 mixture (relative ratio as determined by HPLC) of the isomeric allenic ketones (4*R*)-5,10-secoestra-4,5-diene-3,10,17-trione (**3**) and (4*S*)-5,10-secoestra-4,5-diene-3,10,17-trione (**4**). We also found that a mixture of allenes **3** and **4** in similar proportions was generated under these conditions starting from either of the pure allenic ketones. Compounds **3** and **4** were isolated by HPLC in yields of 50 and 10%, respectively, based on acetylenic ketone **1** and had the expected spectroscopic properties.[7] The more abundant isomer **3**[8] had: mp 141–143°; ir ($CHCl_3$) 1945, 1735, 1710, 1680 cm^{-1}; NMR ($CDCl_3$) δ 0.98 (s, 3, CH_3, C-18), 5.32 (m, 1, $J_{6\beta,4}$ = 6 Hz, $J_{6\beta,7\beta}$ = 6 Hz, $J_{6\beta,7\alpha}$ = 12 Hz, $H_{6\beta}$), 5.58 (m, 1, $J_{4,6\beta}$ = 6.0 Hz, J = 4.2 Hz, J = 1.8 Hz, H_4); uv (CH_3CN) λ_{max} 222 nm (ϵ 12 000); MS *m/e* 286 (M^+). Allenic ketone **4** had similar properties: ir ($CHCl_3$) 1945, 1740, 1710, 1675 cm^{-1}; NMR ($CDCl_3$) δ 0.98 (s, 3, CH_3, C-18), 5.61 (overlapped m, 1, $H_{6\alpha}$), 5.69 (overlapped m, 1, H_4); uv (CH_3CN) λ_{max} 223 nm (ϵ 9400); MS *m/e* 286.1576 ($C_{18}H_{22}O_3$ requires 286.1569). The assignment of stereochemistry at C-6, originally based on NMR data, has been unambiguously established by x-ray diffraction studies of **3**.[9] Isomerization of **2** under identical conditions gave a 7:3 mixture of **5**[10] and **6**.[11] Isolated yields and spectroscopic properties of **5** and **6** were closely similar to those reported for **3** and **4**.

Allenic ketones **3** and **5** were stable in the 1.0 mM potassium phosphate buffer (pH 7.0) used for enzyme inactivation studies. In contrast, the allenic ketones **4** and **6** were not stable under these conditions and their disappearance followed pseudo-first-order kinetics, with half-lives of 37 and 44 min, respectively. The products generated from **4** failed to inactivate Δ^5-3-ketosteroid isomerase. Incubation[12] of Δ^5-3-ketosteroid isomerase with the allenic ketones **3–6** (20 or 200 μM) gave the expected pseudo-first-order rates of inactivation.[3] Half-lives of enzyme inactivation at 200 μM concentrations of acetylenic ketone **1** and of the derived allenes **3** and **4** were 564, 540, and 660 s, respectively. Similarly compounds **2, 5,** and **6** gave $t_{1/2}$ values of 152, 168, and 333 s. The rapid enzymatic conversion of the acetylenic ketones **1** and **2** to their derived allenic ketones **3, 4** and **5, 6,** respectively, accounts for the closely similar rates of inactivation observed for both types of ketones. This facile conversion is not surprising in view of the extraordinarily high turnover rate of this enzyme (8.76×10^6 molecules of Δ^5-androstene-3,17-dione per minute per dimer[1b]). Incubation of allenic ketone **3** with isomerase in the presence of 19-nortestosterone, a competitive inhibitor[1] of this enzyme, afforded significant protection against inactivation.[14] This result is consistent with the postulated inactivation of isomerase by the allenic ketones at the active site.

HPLC analysis showed that the allenic ketones were indeed generated enzymatically from the acetylenic ketones. Acetylenic ketone **1** (200 μM) was incubated with isomerase (4.80 μM) and after 2 min an aliquot was injected directly on a high pressure liquid chromatograph. An 8:2 (relative ratio) mixture of allenic ketones **3** and **4,** respectively, was observed accounting for 94% of the initially incubated acetylenic ketone. Analysis of an exactly comparable experiment with **2** showed that 90% of the acetylenic ketone could be accounted for as a 9:1 (relative ratio) mixture of **5** and **6**. No allenic ketones were found in control incubations without enzyme.

In summary, we have synthesized and characterized the allenic ketones which were postulated to be enzyme generated inhibitors of Δ^5-3-ketosteroid isomerase. We have shown that these compounds are generated by the enzyme and are indeed powerful irreversible inhibitors of the enzyme. Evidence has been presented on the stoichiometry and active site-directed nature of the inactivation. Further studies on the interconversions of the acetylenic ketones and the derived isomeric allenic ketones as well as the effects of these compounds on steroid hormone biosynthesis are in progress.

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Weisenberger for the high resolution mass spectra. This work was supported in part by Grant No. CA-16418 awarded by the National Cancer Institute, Department of Health, Education and Welfare, as well as U.S. Public Health Service Grants AM-15918 and GM-01183.

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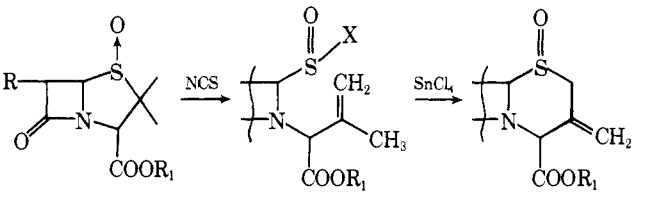
A Rearrangement of Penicillin Sulfoxides to 3-Methylenecephams via Sulfinyl Intermediates[1]

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



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):