and then an equivalent amount of calcium chloride solution was added to precipitate desoxycholate. The supernatant solution was separated after centrifugation, and the enzyme was precipitated from 30% ammonium sulfate solution. The precipitate obtained upon centrifugation (37,000*g*, 20 min) was taken up in cold phosphate buffer (pH 7.4) and centrifuged at 100,000*g* for 3 hr. The clear supernatant liquid was removed by pipet and assayed for total protein and activity in the anaerobic 2,3-oxidosqualene–β-amyrin conversion. The particle-free solution had a specific activity in cyclization to β-amyrin (per mg of protein) approximately 12 times that of the original microsomes.

Studies are continuing on the details of the enzymic formation of β-amyrin and other triterpenes.

Acknowledgment. We are pleased to acknowledge the expert advice and assistance of Dr. P. D. G. Dean. This work was supported by the National Science Foundation and the National Institutes of Health.

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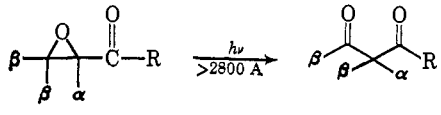
Photochemical Rearrangement of α,β-Epoxy Ketones. An Elaboration of the Mechanism

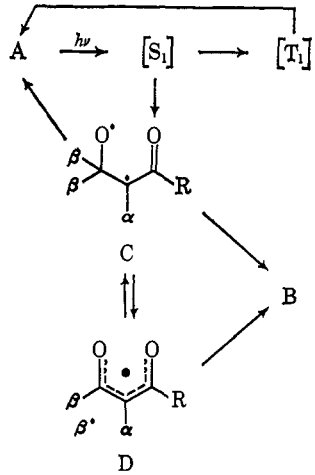
Sir:

The photorearrangement of α,β-epoxy ketones to β-diketones (eq 1) is characterized by an unusual order for the migrational aptitudes of various β groups (*e.g.*, $RCH_2 \gg C_6H_5$).[1,2] Suggestions concerning the



(1)

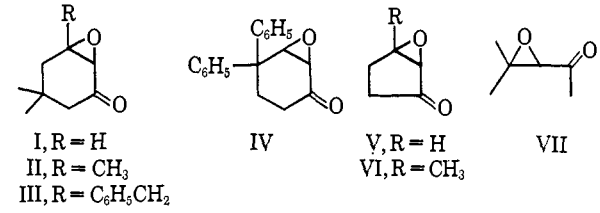
A B



(2)

mechanism of this transformation have been advanced,[1-4] and our recent work in this area has disclosed certain features of the rearrangement that support the elaboration of these views presented in eq 2.

The formation of intermediate C from a singlet [S_1] or triplet [T_1] excited state is theoretically reasonable,[5] and the reactions leading to this species constitute the true "photochemistry" of these compounds. Inasmuch as the epoxy ketone rearrangements are not sensitive to the presence of oxygen or changes in the solvent, and since the addition of known triplet quenchers (piperylene and 2,5-dimethyl-2,4-hexadiene were used in concentrations ranging from 0.1 to 9.0 *M*) did not affect the rate or course of rearrangement (specifically shown for II, III, VII, and VIII), *intermediate C appears to be formed predominantly from the initially produced (S_1) state.* Furthermore, acetophenone (0.5 *M*) did not function as a sensitizer for the rearrangement of II (0.3 *M*) in acetonitrile solution. The low quantum yield observed for some of these rearrangements (*e.g.*, *ca.* 0.03 for the conversion of VII to 3-methylpentane-2,4-dione) may indicate poor efficiency for the [S_1] → C transformation, or an unfavorable competition between rearrangement and oxirane ring formation from C. A preliminary study involving reduction of [T_1] by tri-*n*-butylstannane suggests that in the case of VII both factors are important.[6]

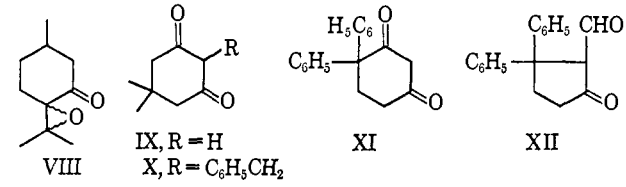


I, R = H
II, R = CH_3
III, R = $C_6H_5CH_2$

IV

V, R = H
VI, R = CH_3

VII

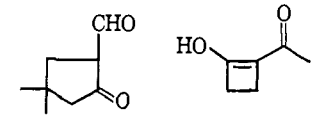


VIII

IX, R = H
X, R = $C_6H_5CH_2$

XI

XII



XIII XIV

A recent report[7] concerning the thermal decomposition of β-methyl-β-phenyl-β-peroxypropiolactone noted a fivefold preference for methyl migration *vs.* phenyl and suggested a 1,3-diradical intermediate similar to C. The implication that such a species can be generated by nonphotochemical pathways is supported by the isomerization and rearrangement of pulegone oxide diastereoisomers (VIII) at 200°.[1] A rationalization of the abnormal migrational aptitudes found in rearrangements proceeding from intermediate C is achieved by assuming that the migrating group must have radical characteristics. This feature can be incorporated in a fragmentation (two-step) mechanism involving the caged radical pair D, or in a single step path having a transition state resembling D in certain respects.[8]

(1) C. K. Johnson, B. Dominy, and W. Reusch, *J. Am. Chem. Soc.*, **85**, 3894 (1963).
(2) H. E. Zimmerman, B. R. Cowley, C. Y. Tseng, and J. W. Wilson, *ibid.*, **86**, 947 (1964).
(3) O. Jeger, K. Schaffner, and H. Wehrli, *Pure Appl. Chem.*, **9**, 55 (1964).
(4) H. Wehrli, C. Lehmann, P. Keller, J. Bonet, K. Schaffner, and O. Jeger, *Helv. Chim. Acta*, **49**, 2218 (1966).
(5) H. E. Zimmerman, *Advan. Photochem.* 1, 183 (1963).
(6) This aspect of our investigations will be developed fully in a subsequent paper and should lead to a rough assignment of relative rates for the various steps in eq 2.
(7) F. D. Greene, W. Adam, and G. Knudsen, Jr., *J. Org. Chem.*, **31**, 2087 (1966).
(8) A detailed discussion of these rearrangements with particular emphasis on stereochemistry has been presented by the Zürich research group.[3,4] Their arguments are in favor of a synchronous mechanism.

This view of the reaction led us to anticipate an especially high migrational aptitude for benzyl and benzhydryl β substituents, and this has been confirmed by experiments with III and IV. Thus, irradiation[9] of a 0.025 *M* solution of IV in ether for 3.5 hr gave roughly 50% conversion to a 1:4 mixture of XI and XII.[10] The sole rearrangement product obtained from a similar irradiation of III proved to be X (*ca.* 20%), but this was accompanied by significant quantities of fragmentation products (*e.g.*, IX, dibenzyl and α-benzylethyl ethyl ether). Since X was essentially unreactive under equivalent reaction conditions, the formation of the latter products suggests that intermediate C is in this case at least partly diverted to the radical pair D, and that the relatively stable benzyl radical escapes the solvent cage.

Other photochemical transformations recently established in our laboratory are: I to IX (*ca.* 40%) and XIII (*ca.* 4%); VI to XIV (*ca.* 40% by a technique involving continuous extraction of the product); and V to cyclopentane-1,3-dione and an unidentified base-soluble substance.

The rearrangements described in this communication together with previous findings[1-4] support the following rough order for the migrational aptitude of β substituents: benzhydryl and benzyl > hydrogen > methylene > methyl ≫ phenyl. The position of hydrogen in this listing argues against a general fragmentation mechanism for the rearrangement, since hydrogen atoms are not normally produced in preference to alkyl radicals. Also, the formation of a strained four-membered ring (XIV) in the photolysis of VI[11] leads us to prefer a single step or synchronous route for rearrangement from C. If a radical pair (*i.e.*, D) was an intermediate in this rearrangement, the formation of a six-membered heterocyclic system or possibly fragmentation with loss of ethylene would seem to provide attractive alternate reaction modes. Products of this type were not found.

Acknowledgment. This work was supported in part by Grant GP-02025 from the National Science Foundation. We thank Dr. Peter Wagner for helpful and stimulating discussions.

(9) These experiments employed a 450-w mercury lamp (Hanovia) equipped with a Corex filter.

(10) The compounds described in this paper were identified by a combination of carbon and hydrogen analysis, infrared, nmr, and mass spectroscopy, chemical degradation or derivative formation, and direct comparisons with authentic materials when possible.

(11) Wehrli, *et al.*,[4] have reported the first example of rearrangement to a cyclobutanone derivative.

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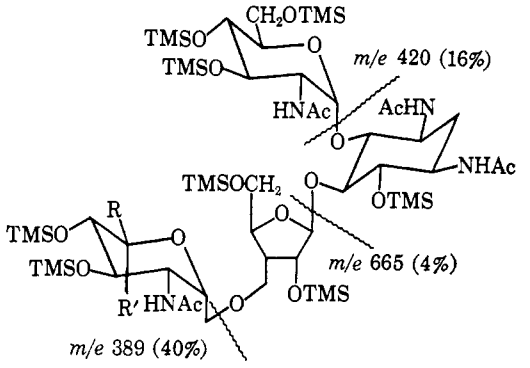
Mass Spectrometric Studies on Aminocyclitol Antibiotics

Sir:

We wish to report preliminary results of a mass spectrometric investigation of the antibiotics paromomycin[1] and paromomycin II[2] (**1a** and **1b**, respectively, TMS and Ac = H), members of the diaminocyclitol (deoxystreptamine) class of antibiotics.[2-4]

The above-mentioned antibiotics, together with the model compounds derived from them and from the neomycins by various degradative reactions,[2-4] were investigated in the form of their N-acetyl-O-trimethylsilyl derivatives. The use of the trimethylsilyl (TMS) blocking groups was found advantageous in the study of these molecules of low volatility. The N-acetyl and N-acetyl-3-*d* derivatives **1–6** (TMS = H) were prepared by selective N-acetylation of the respective free bases with acetic anhydride in methanol. The chromatographically homogeneous solids[5] (compounds **2**, **4–6**, TMS = H, were obtained crystalline) were then subjected to silylation with trimethylsilyl chloride and hexamethyldisilazane in dry pyridine.[6] The trimethylsilyl ethers **1–6** thus obtained were nonhygroscopic white solids which had the infrared and nmr spectral properties expected for N-acetyl-O-trimethylsilyl derivatives.

A minute molecular ion peak is present in the mass spectrum[7] of the N-acetyl-O-trimethylsilyl derivative of paromomycin II (**1b**). A peak of 2.5% intensity (relative to *m/e* 73, $(CH_3)_3Si^+$) at *m/e* 1386 is characteristic of the loss of a methyl radical from a trimethylsilyl group.[8] Relatively intense peaks are also present at *m/e* 665 (4.0%), 420 (16.0%), and 389 (40.0%) from cleavages of glycosidic bonds as indicated in structure **1**. Other fragments in the spectrum can be accounted for by further fragmentations of the molecular ion and of these fragment ions.



1a, R = H; R′ = CH_2NHAc
b, R = CH_2NHAc, R′ = H
(M − CH_3, *m/e* 1386 (2.5%)

(1) (a) T. H. Haskell, J. C. French, and Q. R. Bartz, *J. Am. Chem. Soc.*, 81, 3480 (1959); (b) T. H. Haskell and S. Hanessian, *J. Org. Chem.*, **28**, 2598 (1963).

(2) K. L. Rinehart, Jr., "The Neomycins and Related Antibiotics," John Wiley and Sons, Inc., New York, N. Y., 1964; S. Tatsuoka and S. Horii, *Proc. Japan Acad.*, **39**, 314 (1963).

(3) J. D. Dutcher, *Advan. Carbohydrate Chem.*, **18**, 259 (1963).

(4) S. Hanessian and T. H. Haskell, "The Carbohydrates," Vol. 2, W. Pigman and D. Horton, Ed., Academic Press Inc., New York, N. Y., in press.

(5) Thin layer chromatography of the N-acetates was carried out on glass plates coated with Avirin (a product of American Viscose Corp., Marcus Hook, Pa.) and the compounds were detected by spraying lightly with a 1% solution of potassium permanganate in 1 *N* sulfuric acid or by exposure to iodine vapors.

(6) In a typical experiment, 0.1 g of N-acetyl derivative dissolved in 5 ml of dry pyridine was treated with 1 ml of trimethylsilyl chloride and 3 ml of hexamethyldisilazane at room temperature. After 1–2 hr the mixture was evaporated to dryness, the residue was suspended in benzene, and the soluble portion was evaporated to dryness and used as such.

(7) The mass spectra of compounds **2–6** were obtained from an Atlas CH4 mass spectrometer, using a vacuum-lock direct-inlet system. The authors wish to thank Dr. W. J. McMurray, Yale University School of Medicine, for obtaining the spectrum of compound **1b** on an AEI MS9 mass spectrometer.

(8) A. G. Sharkey Jr. R. A. Friedel, and S. H. Langer, *Anal. Chem.*, 29, 770 (1957).