

nucleophiles. Hudson⁹ reported kinetic data on trimethyl phosphate with a series of nucleophilic reagents. From these data the α has been calculated. From this α of 1.77 ± 0.05 , a value of E of 0.62 v. for $(\text{CH}_3\text{O})_2\text{P}(\text{O})\text{O}^-$ has been computed. The value is reasonable and indicates that the phosphate, tosylate, and sulfate ions have rather similar and poor nucleophilic properties.

In further papers in the series the use of the oxibase scale will be extended.

Acknowledgments. The author wishes to thank the National Institutes of Health (RH 00279-02) and the Walter Reed Army Institute of Research (DA 49-193) for grants supporting these investigations.

(9) R. F. Hudson and D. C. Harper, *J. Chem. Soc.*, 1356 (1958). An interesting paper by Hudson (*Chimia*, **16**, 173 (1962)) reports his views on the $\text{S}_{\text{N}}2$ reaction and a justification of the use of linear free-energy equations as (3). Another approach is reported (R. E. Davis, "Survey of Progress in Chemistry," Vol. 2, A. F. Scott, Ed., Academic Press Inc., 1964, pp. 189-238).

(10) Alfred P. Sloan Fellow, 1962-1966.

(11) The kinetic data used to compute α and β are (either from the literature or as a result of this study on CH_3OTs and $\text{TsOCH}_2\text{COONa}$): (a) bromoacetate: V. K. La Mer and M. E. Kammer, *J. Am. Chem. Soc.*, **57**, 2662 (1935); H. J. Backer and W. H. Van Mels, *Rec. trav. chim.*, **49**, 177, 363 (1930); see also ref. 5; (b) chloroacetate: *Rec. trav. chim.*, **49**, 457 (1930); H. M. Dawson and E. R. Pycocok, *J. Chem. Soc.*, 153 (1936); A. Slator and D. F. Twiss, *ibid.*, **95**, 93 (1909); (c) iodoacetate: C. Wagner, *Z. physik. Chem.*, **A115**, 121 (1925); H. J. Backer and W. H. Van Mels, *Rec. trav. chim.*, **49**, 177, 363, 457 (1930); (d) methyl bromide: A. Slator and D. F. Twiss, *J. Chem. Soc.*, **95**, 93 (1909); E. A. Moelwyn-Hughes, *Trans. Faraday Soc.*, **45**, 167, (1949); A. Slator, *J. Chem. Soc.*, **85**, 1286 (1904); (e) methyl iodide: E. A. Moelwyn-Hughes, ref. 11d; (f) methyl tosylate: A. Praetorius, *Monatsh.*, **26**, 1 (1905); R. E. Robertson, *Can. J. Chem.*, **31**, 589 (1953); C. G. Swain and C. R. Morgan, *J. Org. Chem.*, **29**, 2097 (1964); this study with $\log k/k_0 = 3.110$ for N_3^- , 2.774 for SCN^- , 3.873 for $\text{S}_2\text{O}_8^{2-}$, and 3.038 for OH^- in water at 25°. The values of α and β of methyl tosylate are nearly identical with those of ethyl tosylate (data of Dr. Nehring of this laboratory), also in water at 25°. The data on sodium tosylacetate, $\text{TsOCH}_2\text{COONa}$, were measured spectrophotometrically at 272 μ in dilute aqueous solution by Mr. William Blume at 35° and 50°, by R. E. D. at 25°. At 25° $\log k/k_0 = 2.247$ for I^- , 1.643 for Br^- , 1.711 for OH^- , 1.991 for SCN^- , 2.703 for $\text{S}_2\text{O}_8^{2-}$, and 2.810 for SO_3^{2-} . All of the data have been handled on the IBM 7094 using a multiple regression method assuming errors in all the values and the parameters. Errors in the log terms are ± 0.005 average. Enough data have been obtained at four or more ionic strengths to extrapolate the rate data to infinite dilution. This affects the tosylacetate data (an anion-anion reaction) much more than with methyl tosylate.

The E value of tosylate ion (0.49 ± 0.02 v.) can be estimated and compared with 0.59 v. for sulfate⁶ anion by the solubility product of silver sulfate and silver tosylate in water at 25°. Kinetically the two legate potentials of tosylate are consistent. Defining E with the methyl tosylate data places the α of tosylacetate on the line for $-\text{OOCCH}_2\text{-Y}$ derivatives.

Since one referee has expressed interest, we have made arrangements that the raw data of this paper and those in following papers and computer programs be microfilmed and placed in the Bureau of Documentation.

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On the Incorporation of Oxygen in the Conversion of 8,11,14-Eicosatrienoic Acid to Prostaglandin E₁

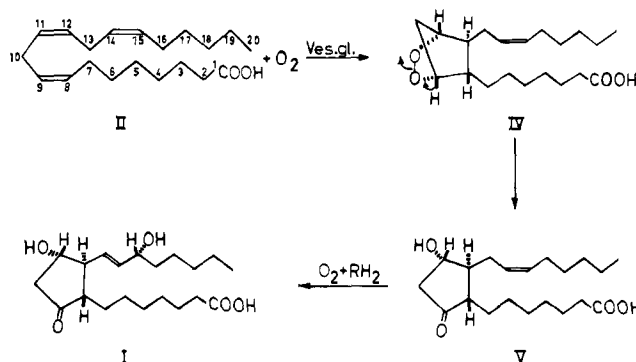
Sir:

The biosynthesis of prostaglandin E₁ (I) from 8,11,14-eicosatrienoic acid (II) involves incorporation of 3 atoms of oxygen.^{1,2} Two of these oxygens appear in

(1) D. A. van Dorp, R. K. Beerthuis, D. H. Nugteren, and H. Vonkeman, *Biochim. Biophys. Acta*, **90**, 204 (1964).

(2) S. Bergström, H. Danielsson, and B. Samuelsson, *Biochim. Biophys. Acta*, **90**, 207 (1964).

the hydroxyl groups at C-15 and C-11 and 1 in the keto group at C-9. Recent experiments demonstrated that the oxygens of the 2 hydroxyl groups are derived from molecular oxygen. However, the origin of the keto oxygen could not be established because of exchange of this oxygen with the aqueous media used for the incubation and isolation.³



The experiments described in this communication show that the oxygen atom of the keto group also originates in molecular oxygen and that it is in fact derived from the same molecule of oxygen as the oxygen atom of the hydroxyl group at C-11. These results indicate that a novel biological reaction is involved in the formation and oxygenation of the five-membered ring of the prostaglandins.

For the biosynthesis of prostaglandin E₁, [2-¹⁴C]8,11,14-eicosatrienoic acid was incubated with a preparation of vesicular gland for 10 min. in an atmosphere of ¹⁸O-labeled oxygen.³ Ice-cold ethanol containing NaBH_4 was then immediately added to the incubation mixture. In this way the oxygen of the keto group was protected from exchange by reduction to an alcohol.⁴ The ethyl ester of the resulting trihydroxy acid was converted into the trimethoxy derivative⁵ in order to minimize elimination of the oxygen functions during the electron impact. For determination of the origin of the oxygens of the ring, this derivative was oxidized by permanganate-periodate.⁵ The product was finally converted to the diethyl ester (III in Figure 1) so that, in the mass spectrometric analysis, eliminations involving the methoxy groups and the ester groups could be distinguished.

The upper m/e region of the mass spectrum⁶ of the reference sample of III, and of III formed in an atmosphere of ¹⁶O¹⁶O (43%), ¹⁶O¹⁸O (1%), and ¹⁸O¹⁸O (56%), is shown in Figure 1A and 1B, respectively. There were three fragments, which retained both oxygen atoms in the ring. These appeared at m/e 343 ($M - 15$ (CH_3)), 313 ($M - 45$ (OCH_2CH_3)), and 297 ($M - (45 + 1 + 15)$ ($\text{OCH}_2\text{CH}_3 + \text{H} + \text{CH}_3$)). In the spectrum of III derived from prostaglandin E₁ formed in the ¹⁸O atmosphere peaks were also seen at m/e 347

(3) R. Ryhage and B. Samuelsson, *Biochem. Biophys. Res. Commun.*, **19**, 279 (1965).

(4) The reduction gave mainly the 9 β -epimer (PGF_{1 β}); cf. S. Bergström, L. Krabich, B. Samuelsson, and J. Sjövall, *Acta Chem. Scand.*, **16**, 969 (1962). This epimer was isolated and used in the following experiments.

(5) S. Bergström, R. Ryhage, B. Samuelsson, and J. Sjövall, *ibid.*, **17**, 2271 (1963).

(6) The mass spectrometric analyses were carried out in conjunction with gas chromatography using the instrument described by R. Ryhage, *Anal. Chem.*, **36**, 759 (1964). The column (1% SE-30 on Gas Chrom P) was operated at 165° and about 5 μ g. of material was injected.

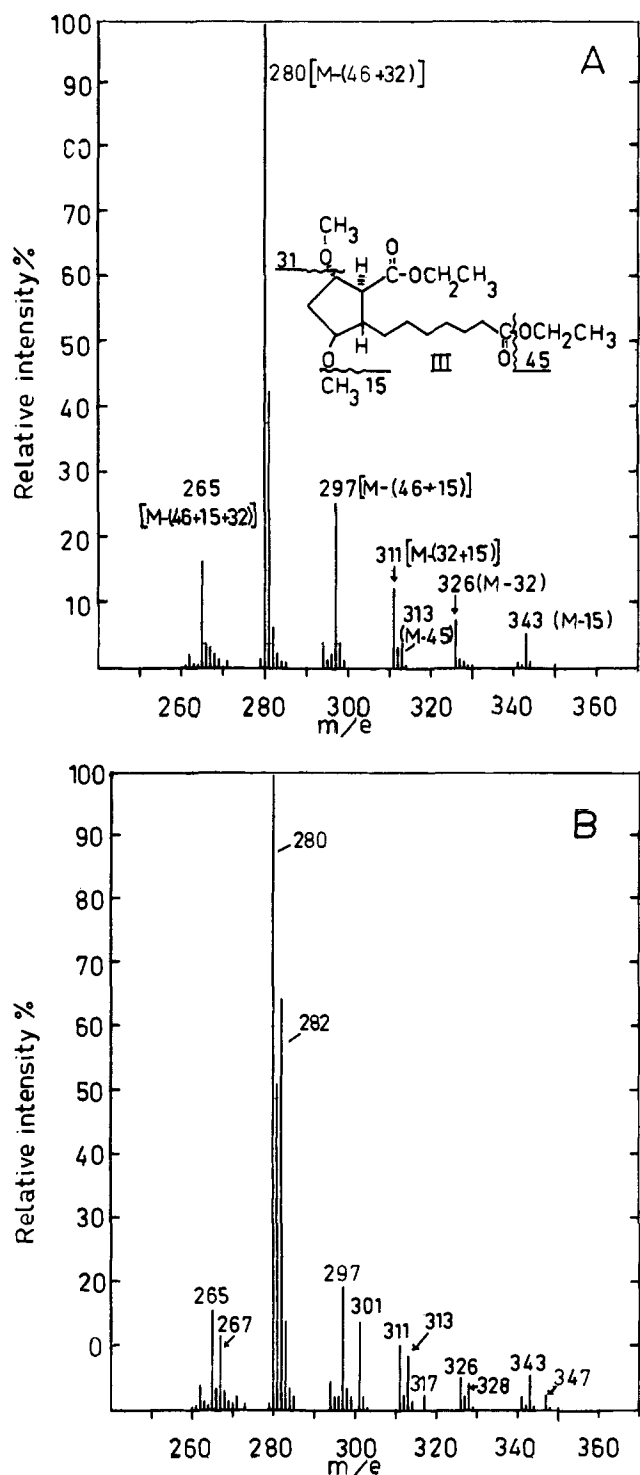


Figure 1. Mass spectra of (A) III and (B) III prepared from prostaglandin E₁ biosynthesized in ¹⁸O-labeled oxygen gas.

(343 + 4), 301 (297 + 4), and 317 (313 + 4), whereas the peaks at *m/e* 345 (343 + 2), 315 (313 + 2), and 299 (297 + 2) were very small. These findings demonstrate that both oxygen atoms of the ring originate in oxygen gas. Furthermore, if both oxygen atoms were derived from the same molecule of oxygen, the ratios between the intensities of the peaks at *m/e* 345 and 347, at 315 and 317, and at 299 and 301 should be 0.02, whereas the same ratios should be 1.5 if the oxygen atoms were derived from different molecules of oxygen. The values for the intensity ratios of *m/e* 345/347 and

of 299/301 calculated from the mass spectrum were 0.06 and 0.08, respectively.⁷

These findings clearly demonstrated that the oxygen atoms of the hydroxyl group at C-11 and of the keto group at C-9 originate in the same molecule of oxygen. The mechanistic implications of these findings are summarized below.⁸

The transformation involves two distinct reactions, *viz.*, a dioxygenase and a monooxygenase reaction. One possibility is that the dioxygenase reaction works by addition of oxygen across carbons 9 and 11 with concomitant ring closure between carbons 8 and 12 involving a conrotatory process.⁹ This view is also consistent with our finding that the hydrogen atoms at carbons 8, 11, and 12 remain in their original positions during this transformation.¹⁰

The dioxygenation reaction to some extent resembles, *e.g.*, the pyrocatecase-catalyzed conversion of catechol to *cis,cis*-muconic acid¹¹ and the photochemical formation of ascaridole.¹² The cleavage of the cyclic peroxide (IV) may either occur without net change in the state of oxidation, forming V, or by reduction, to give prostaglandin F_{1 α} .

Conversion of V to prostaglandin E₁ (I) involves isomerization of a *cis*- Δ^{14} double bond to a *trans*- Δ^{13} double bond and introduction of a hydroxyl group at C-15. This reaction is analogous to the lipoxidase-catalyzed reaction of polyunsaturated fatty acids¹³ except that an electron donor should be required here for the formation of the alcohol.

Whether or not the introduction of the oxygens at C-9 and C-11 precedes the introduction of oxygen at C-15 requires further studies. The proposed scheme is consistent with our findings that in homogenates of lung tissue, where arachidonic acid is transformed into both prostaglandin E₂ and prostaglandin F_{2 α} , no interconversion of these prostaglandins occurs.¹⁴

Another mechanism, which is in agreement with both the ¹⁸O experiments and the ³H tracer experiments,¹⁰ involves the formation of a hydroperoxide at C-11 with isomerization of the double bond to Δ^{12} -position. Attack by the hydroperoxide at C-9 and a concerted reaction involving ring closure, isomerization of the Δ^{12} -double bond, and introduction of the hydroxyl group at C-15 would then yield the cyclic peroxide having the Δ^{13} -double bond and the hydroxyl group at C-15.

Acknowledgments. This research was supported by the Swedish Medical Research Council. The author is

(7) These ratios were calculated from the original spectra after correction of the "+2 peaks." The correction involved subtraction of the normal isotope peak arising from the parent peak. No value is given for the ratio of *m/e* 315/317 because of the complex origin of *m/e* 313 (normal isotope peak of *m/e* 311, 311 + 2 (from ¹⁸O), and M - 45). Since the enzyme preparation contained endogenous prostaglandin E₁, the ratios between the parent peak and the "+2" and "+4" peaks" have no simple quantitative relationship with the isotopic composition of the oxygen gas.

(8) The author is grateful to Drs. D. Arigoni and E. J. Corey for helpful discussions during the course of these experiments.

(9) R. B. Woodward and R. Hoffmann *J. Am. Chem. Soc.*, **87**, 395 (1965).

(10) D. Klenberg and B. Samuelsson, *Acta Chem. Scand.*, in press.

(11) O. Hayaishi, Plenary Sessions of the Sixth International Congress of Biochemistry, Vol. 33, 1964, p. 31.

(12) G. Schenk and K. Ziegler, *Naturwissenschaften*, **32**, 157 (1944).

(13) A. L. Tappel in "The Enzymes," Vol. 8, P. D. Boyer, H. Lardy, and K. Myrbäck, Ed., Academic Press Inc., New York, N. Y., 1963.

(14) E. Ånggård and B. Samuelsson, *J. Biol. Chem.*, in press.

greatly indebted to Dr. R. Ryhage for the mass spectro-metric analyses.

Bengt Samuelsson

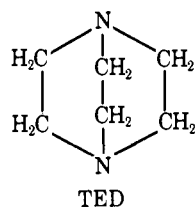
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The Triethylenediamine Cation Radical

Sir:

A recent study of the crystal structure of 1,4-diaza-bicyclo[2.2.2]octane¹ (or triethylenediamine, TED) showed that the nitrogen atoms are only 2.5 Å. apart.



This fact, along with the spatial disposition of the nitrogen unshared electron pairs in orbitals whose axes coincide with the C_{3v} symmetry axis, led us to consider the possibility of observing the electron spin resonance (e.s.r.) spectrum of the cation radical, TED⁺; this possibility has now been realized.

allowed to flow slowly (*ca.* 0.2 ml. min.⁻¹) over a platinum gauze electrode in a flat quartz cell which was placed directly in the microwave cavity. The potential of the platinum electrode was +1 v. vs. aqueous s.c.e. and the steady-state current was *ca.* 100 μa.

The spectrum has been assigned on the basis of a coupling constant of 16.96 ± 0.08 gauss for two equivalent ¹⁴N nuclei and a coupling constant of 7.34 ± 0.08 gauss for twelve equivalent protons. A computed spectrum using these constants, a line width of 0.4 gauss, and a Lorentzian line shape is shown below the experimental spectrum in Figure 1.

The observation of equivalent nitrogen atoms establishes that electron transfer between the nitrogen atoms occurs at a rate greater than the observed hyperfine splitting, *i.e.*, >4 × 10⁷ c.p.s. While electron transfer may occur through spatial overlap of nitrogen orbitals, the possibility of participation of the ethylene bridge carbon atoms cannot be ignored. In this context, previous observation of the anion radicals of paracyclophanes³ and adamantane and hexamethylenetetramine⁴ is noteworthy.

Whereas the nitrogen atom in most nitrogenous cation radicals is located in a plane with the three atoms bonded to it, this is not the case for TED⁺ where a pyramidal distortion exists.⁵ Recent theoretical calculations⁶ of *a*_N for the NH₃⁺ cation yielded values of 20.9 gauss and 66 gauss for planar and

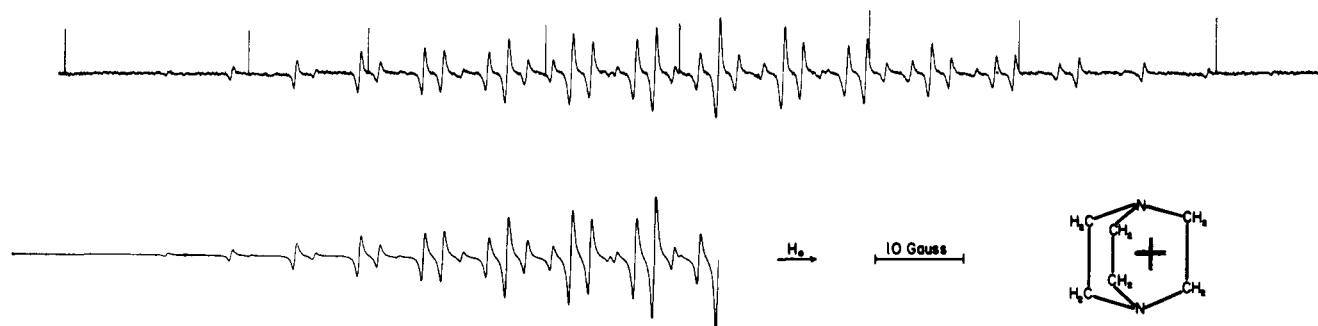


Figure 1. First-derivative electron spin resonance spectrum of the cation radical of triethylenediamine; experimental spectrum given in upper portion and computed spectrum given in lower portion.

A preliminary investigation of the electrochemistry² of TED provided the basis for electrochemical generation of TED⁺. The half-wave potential for electro-oxidation of TED on a rotating platinum electrode in acetonitrile solution is +0.68 v. vs. aqueous s.c.e. This electrode process is established as involving one electron by comparison of the limiting current constant of 4.5 ± 0.3 μa. mM⁻¹ for TED with the value of 4.9 μa. mM⁻¹ for the known one-electron oxidation of iodide ion on the same electrode under identical stirring conditions. Cyclic voltammetry using a platinum electrode in an unstirred solution established that the life-time of the cation radical is limited to several seconds.

The e.s.r. spectrum of TED⁺ shown in the upper portion of Figure 1 was obtained by electrooxidation of a 4 mM solution of TED in acetonitrile which was

pyramidal models, respectively. Since the experimental⁷ value for the nitrogen coupling constant for NH₃⁺ is 19.5 gauss, the calculated value supports the suggestion that the radical is planar. The fact that *a*_N for two nitrogen nuclei in TED⁺ is almost 17 gauss probably reflects enhancement of the nitrogen coupling constant by the pyramidal configuration about the nitrogen atom.

Examination of the ultraviolet spectrum of gaseous TED showed an intense line, ε = 1000 l./mole cm., at 2510 Å. with rich vibronic structure decreasing to shorter wave lengths. The emission spectrum, obtained by electron impact in a high-power radiofrequency field, showed 19 discrete lines between 2730

(1) G. S. Weiss, A. S. Parker, E. R. Nixon, and R. E. Hughes, *J. Chem. Phys.*, **41**, 3759 (1964).

(2) The electrochemistry of TED was briefly studied in aqueous solution by A. Julliard, *J. Electroanal. Chem.*, **1**, 10 (1959).

(3) S. I. Weissman, *J. Am. Chem. Soc.*, **80**, 6462 (1958). Details of electron transfer in the paracyclophane anion radicals have been examined by H. M. McConnell, *J. Chem. Phys.*, **35**, 508 (1961).

(4) K. W. Bowers, G. J. Nolfi, Jr., and F. D. Greene, *J. Am. Chem. Soc.*, **85**, 3707 (1963).

(5) Since the C-N-C bond angle in TED is 108.9°, it is reasonable to assume approximately the same bond angle in TED⁺.

(6) G. Giacometti and P. L. Nordio, *Mol. Phys.*, **6**, 301 (1963).

(7) T. Cole, *J. Chem. Phys.*, **35**, 1169 (1961).