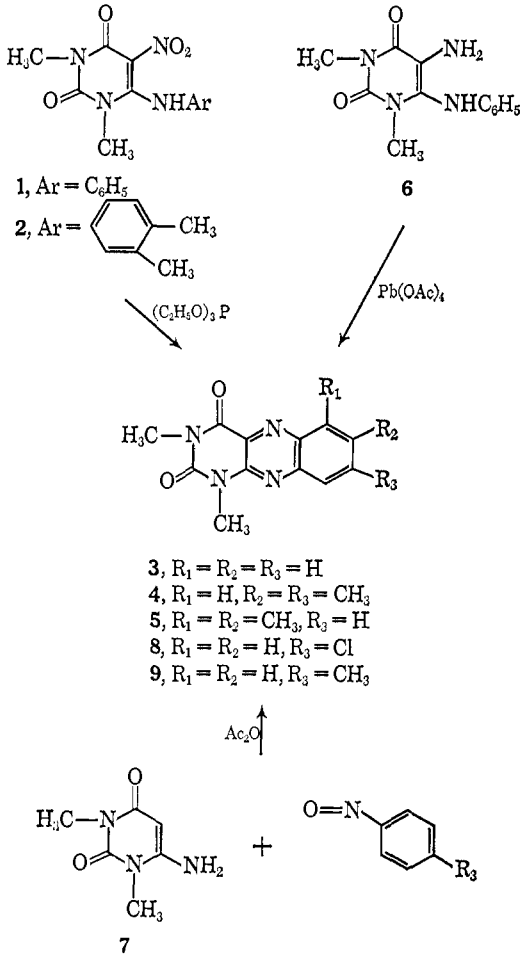
methyl-5-nitro-6-anilinouracil (**1**), mp 200.1°, in excess triethyl phosphite under N_2 for **2** hr, removal of volatiles by partial evaporation under a vigorous stream of N_2, and dilution with ethanol gave 1,3-dimethylalloxazine (**3**),[14] mp 243.3°[8] (30%). It is of considerable interest that the major product of this reaction was 1,3-dimethyl-6-anilinouracil, mp 187.7°.[8] To our knowledge, this is the first example of *de*nitration in the pyrimidine series. Similarly, heating 1,3-dimethyl-5-nitro-6-(3,4-xylidino)uracil (**2**), mp 212–214°, in triethyl phosphite for 7.5 hr gave a mixture of 1,3,7,8-tetramethylalloxazine (**4**), mp 253–254°[15] (14%), and 1,3,6,7-tetramethylalloxazine (**5**), mp 273.3°, along with the product of denitration, 1,3-dimethyl-6-(3,4-xylidino)uracil, mp 233.6°.

Method B. 1,3-Dimethylalloxazine (**3**) was prepared in 61% yield by portionwise addition of 1.5 moles of lead tetraacetate to a refluxing ether suspension of 1,3-dimethyl-5-amino-6-anilinouracil (**6**), mp 160.3°, followed by filtration and washing with water. The same conversion could be effected in lower yield (48%) by heating an intimate mixture of **6** with lead dioxide at 220°.



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Method C. Refluxing 1 equiv of 1,3-dimethyl-6-aminouracil (**7**) with 2 equiv of nitrosobenzene, *p*-chloronitrosobenzene, or *p*-nitrosotoluene in acetic anhydride for 15 min, followed by dilution with water, gave 1,3-dimethylalloxazine (**3**), 52%, 1,3-dimethyl-8-chloroalloxazine (**8**), mp 251.0° (68%), and 1,3,8-trimethylalloxazine (**9**) mp 251.7° (49%). This latter compound was identical with the product of previously undetermined structure (1,3,6- or 1,3,8-trimethylalloxazine, mp 252–253°) prepared by nitrosation of 1,3-dimethyl-6-(*p*-toluidino)uracil.[8]

Applications of these procedures to the preparation of other condensed pyrazine heterocycles are in progress.

(14) Satisfactory microanalytical and spectral data were obtained for all compounds reported.

(15) (a) P. Hemmerich, B. Prijs, and H. Erlenmeyer, *Helv. Chim. Acta.*, 43, 372 (1960); R. Kuhn and H. Rudy, *Chem. Ber.*, 67, 1826 (1934).

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Correlation between the Photochemistry and the Mass Spectra of Pyruvic Acid and Isopropyl Pyruvate[1,2]

Sir:

We wish to report an interesting correlation between the mass spectral behavior and photochemistry of both pyruvic acid and its isopropyl ester. Although processes which are general in photolyses have long been known to have analogs in mass spectral fragmentations,[1] *the cases reported here are examples of unusual behavior of two molecular ions which are paralleled by unusual behavior of two corresponding n,π* excited states.* Such an observation is significant in that it provides evidence for the validity of attempts to interrelate the mass spectrometry and photochemistry of organic molecules.

Photolysis of pyruvic acid in the vapor phase[3] and in aqueous solution[4] yields acetaldehyde and CO_2, and acetoin, respectively. The reaction has been proposed to involve an n,π* state which forms an uncommon five-membered transition state.[5] The latter collapses to CO_2 and methylhydroxycarbene which then rearranges to acetaldehyde. From Table I it can be seen that the analogous process occurs in the mass

Table I. Partial Monoisotopic Mass Spectra (75 ev) of Pyruvic Acid and Pyruvic Acid-OD[a]

CH_3COCO_2H		CH_3COCO_2D[b]	
%	Ion	%	Ion
4.2	$C_3H_4O_3$	4.2	$C_3H_3DO_3$
16	CHO_2	22	CDO_2
3.4	C_2H_4O	6.7	C_2H_3DO
100	C_2H_3O	5.8	C_2H_2DO
		100	C_2H_3O

[a] Empirical formulas were determined by exact mass measurement on a CEC 21-110B mass spectrometer. Inlet system and source were maintained below 70° to avoid thermal decomposition. [b] Prepared by injecting a solution of pyruvic acid in a ten-volume (~40 mole) excess of D_2O into the spectrometer previously equilibrated with D_2O. Relative abundances corrected to 100% d_1.

(1) Part II in this series; see N. J. Turro, D. C. Neckers, P. A. Leermakers, D. Seldner, and P. D'Angelo, *J. Am. Chem. Soc.*, 87, 4079 (1964) for part I.

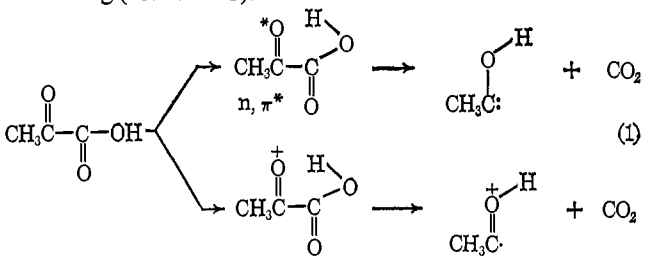
(2) The authors gratefully acknowledge generous support from the Air Force Office of Scientific Research (Grant AFOSR 1000-66) and the National Science Foundation (Grant NSF-GP-4280) at Columbia University, and the National Institutes of Health (Grant GM 12755) at Purdue University.

(3) G. F. Vesley and P. A. Leermakers, *J. Phys. Chem.*, 68, 2364 (1964).

(4) P. A. Leermakers and G. F. Vesley, *J. Am. Chem. Soc.*, 85, 3776 (1963).

(5) α-Keto acids exist as proton chelates, even in the gas phase: A. Schellenburger, W. Beer, and G. Dehme, *Spectrochim. Acta*, 21, 1345 (1965).

spectrometer to yield the ion fragments C_2H_4O and C_2H_3OD from pyruvic acid and pyruvic acid-OD, respectively. Since hydrogen rearrangement *via* a five-membered transition state is uncommon in the mass spectra of carbonyl compounds,[6] the analogy between the photolysis and mass spectral decomposition is striking (reaction 1).



(1)

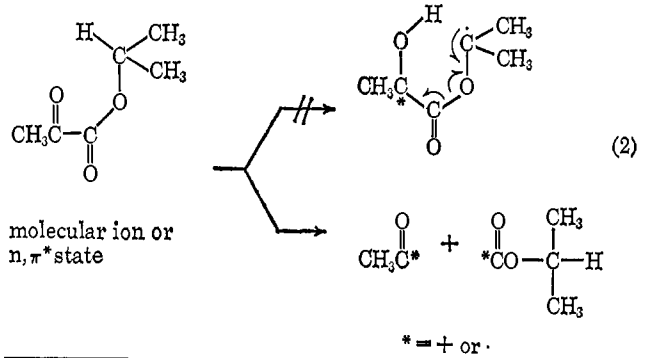
The photolysis of $CH_3COCO_2CH(CH_3)_2$ (**1**) in the vapor phase[7] or in benzene solution[8] yields acetone and carbon monoxide as major products and only a low yield of acetaldehyde. Leermakers[8] has explained these and related results by proposing a mechanism involving α cleavage as the primary photochemical act followed by loss of CO rather than the intuitively reasonable type II photorearrangement. The mass spectral behavior of the molecular ions of **1** and $CH_3COCO_2CD(CH_3)_2$ (**2**), shown in Table II, appears to

Table II. Partial Monoisotopic Mass Spectra (75 ev) of **1** and **2**[a]

$CH_3COCO_2CH(CH_3)_2$ (**1**)		$CH_3COCO_2CD(CH_3)_2$ (**2**)	
%	Ion	%	Ion
0.4	$C_6H_{10}O_3$	0.5	$C_6H_9DO_3$
4.1	$C_4H_7O_2$	4.6	$C_4H_6DO_2$
4.4	C_2H_5O	3.6	C_2H_4DO
100	C_2H_3O	100	C_2H_3O

[a] Analysis conditions given in Table I, except that inlet and source temperatures were 165°. [b] Greater than 98% d_1. The sample was kindly donated by Professor P. A. Leermakers.

be quite analogous to the photochemical behavior of their n,π^* excited states. A striking feature of the mass spectra is the conspicuous absence of $C_3H_4O_2$, $C_3H_3DO_2$, C_2H_4O, and C_2H_3DO ions. The $C_3H_4O_2$ and $C_3H_3DO_2$ ions in the spectra of **1** and **2**, respectively, would correspond to products of the very general McLafferty rearrangement,[6] and these ions might be expected to decompose further to $C_2H_4O\cdot^+$ and $C_2H_3DO\cdot^+$ by analogy to reaction 1.[9] Indeed, the ions



(2)

$C_4H_7O_2^+$, $C_4H_6DO_2^+$, and $C_2H_3O^+$ serve as evidence of the importance of cleavage of the CO–CO bond (reaction 2).

Further studies of these and other systems are being pursued in order to understand better the chemistry of molecules ionized by electron impact and to determine how the structures and reactivities of electronically excited states and molecular ions may be correlated.

(6) F. W. McLafferty, "Interpretation of Mass Spectra," W. A. Benjamin, Inc., New York, N. Y., 1966, p 123 ff.
(7) P. A. Leermakers, M. E. Ross, G. F. Vesley, and P. C. Warren, *J. Org. Chem.*, 30, 914 (1965).
(8) P. A. Leermakers, P. C. Warren, and G. F. Vesley, *J. Am. Chem. Soc.*, 86, 1768 (1964).
(9) Also there are no $C_3H_5O_2^+$ ions which might be expected by analogy to the double hydrogen rearrangement common in esters.[6]

(10) Alfred P. Sloan Fellow, 1966–1968.
(11) National Institutes of Health Predoctoral Fellow, 1966–1967.

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On the Mechanism of Lanosterol Biosynthesis from Squalene 2,3-Oxide

Sir:

In connection with our continuing program concerned with the organic and biological chemistry of terpenoid terminal epoxides,[1] we wish now to present new findings and considerations which further delineate the role of squalene 2,3-oxide in the biosynthesis of lanosterol and therefore other members of the sterol class.

Although recent experiments in these laboratories[2] and elsewhere[3] indicated that ^{14}C-labeled squalene 2,3-oxide[1a] can be biosynthesized and also act as a natural triterpene source of lanosterol and cholesterol, the fate of *oxygen* in the original epoxide moiety was left unsettled. The following experiments elucidate this matter. Squalene 2,3-oxide-^3H,^{18}O (4150 dpm/μg, 30% ^{18}O by mass spectral comparison[4] of normal and ^{18}O-labeled oxides) was prepared from squalene-^3H (4350 dpm/μg) by the action of N-bromosuccinimide[1a] in 3:1 THF–water (30% ^{18}O-labeled) and was incubated anaerobically with washed microsomes of rat liver in 0.08 *M* potassium phosphate buffer, pH 7.4. The sterol fraction was isolated, and lanosterol (R_f, 0.43; 200,000 dpm) was separated by tlc on silica gel in 15% ethyl acetate–hexane. Purification by glpc of the trimethyl silyl ether on a 6 ft × 0.25 in. column of 5% Carbowax on Chromosorb W at 235° gave lanosterol trimethylsilyl ether (retention time relative to cholestane = 3.7) which on mass spectral analysis was found to contain 29% excess ^{18}O. The retention of the original epoxy oxygen as the 3β-hydroxyl group of lanosterol supports the mechanism proposed earlier[2,3] for the proton-initiated enzymic cyclization of squalene 2,3-oxide to lanosterol. Moreover, our inability to demonstrate any cofactor requirements for the microsomal enzyme system is also consistent with the proposed mechanism.

(1) Initial publications in this series: (a) E. E. van Tamelen and T. J. Curphey, *Tetrahedron Letters*, 121 (1962); (b) E. E. van Tamelen, A. Storni, E. J. Hessler, and M. Schwartz, *J. Am. Chem. Soc.*, 85, 3295 (1963).
(2) E. E. van Tamelen, J. D. Willett, R. B. Clayton, and K. E. Lord, *ibid.*, 88, 4752 (1966).
(3) E. J. Corey and W. E. Russey, *ibid.*, 88, 4750 (1966), have also recorded the biochemical conversion of squalene 2,3-oxide[1a] to sterols.
(4) K. Biemann, "Mass Spectrometry, Organic Chemical Applications," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp 204–205.