Communications to the Editor

Esters of 6,7-Dimethyl-9-hydroxymethylisoalloxazine as Photodegradation Products of Riboflavin and Formylmethylflavin in Media Containing Fatty Acids[1]

Sir:

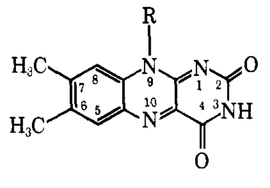
Both anaerobic and aerobic photodecompositions of riboflavin (RF) and formylmethylflavin (FMF) to lumichrome (LC) have not revealed any flavin intermediary in which only C-1′ would remain, though its existence is likely, at least in the case of FMF, because of the C_1 compounds found.[2]

A product fluorescing like a typical flavin was characterized by paper chromatography when riboflavin was photolyzed in a medium containing acetic acid.[9,10] It was designated 69 CX (I in the present paper). Possibly the same substance (compound "S")[11] was observed on thin-layer chromatograms when FMF was photolyzed on the plate in the presence of acetic acid. Incorporation of at least the C-2 atom of acetic acid into I during photolysis of riboflavin in an aqueous medium containing [^{14}C]acetate was demonstrated.[12]

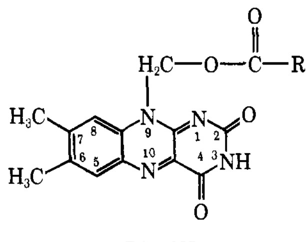
In our present experiments acetate and propionate buffers and higher acids in chloroform were used. Aerobic conditions were needed[13] for the appearance of I. I was formed from both RF and FMF. Photolysis of FMF in media containing propionic, butyric, heptanoic, and palmitic acids, respectively, leads to new products which behave like homologs on chromatograms. Compound I and its next higher (propionyl) homolog, II, were usually prepared in the following way. Solutions of FMF (5×10^{-4} *M*) in acetate and propionate buffers, respectively (acid and its sodium salt in 0.5 *M* concentration), were irradiated with a 150-W tungsten filament lamp from a 50-cm distance in an open vessel under frequent stirring for 8 (6)[15] hr. The depth of the solution layer was 2 cm. All the subsequent steps were carried out in subdued light. The solution was extracted by a half- (equal, twice)[15] volume of chloroform (benzene),[15] the organic layer was washed with water and concentrated under reduced pressure, and a small amount of ethanol was added. Crystals which appeared in the cold overnight were washed with ethanol and ether, dried *in vacuo*, and recrystallized from 1:1 ethanol–chloroform (ethanol–benzene)[15] and/or from boiling glacial acetic acid[16] followed by adjustment of the pH to 3–4 with solid Na_2CO_3. As a suitable purification step preparative tlc on silica gel was employed[17] followed by elution with ethanol. The melting points of I and II were 274–276 and 270–272° dec (uncorrected), respectively.

Both substances appeared to be relatively stable toward light but very sensitive in alkaline medium. Both alkaline and acid treatment converted them to lumichrome and formaldehyde (chromotropic acid method) in stoichiometric amounts. Gas chromatography, employed for the alkaline hydrolysate of II, demonstrated propionic acid. The rate constants for disappearance (at 33°) of I, leading to LC, calculated from the first-order decrease of optical density at 450 nm, were 1.25×10^{-1} min^{-1} in 0.05 *M* Na_2CO_3–NaB_4O_7 buffer (pH 10.6) and 9.0×10^{-3} min^{-1} in 1 *N* HCl.

Spectral characteristics of I and II include: uv and visible max (95% EtOH) 445, 350, 262, 224 and 445, 353, 268, 227, respectively; ir (KBr) of I peak at 1747 cm^{-1}, corresponding to ester C═O; nmr (Varian HA-100, CF_3COOH, TMS) for I δ 8.34 (s, 1, C-5 H),[18] 8.28 (s, 1, C-8 H),[18] 6.87 (s, 2, C-1′ H), 2.85 (s, 3, C-7 methyl H), 2.70 (s, 3, C-6 methyl H), 2.34 (s, 3, $COCH_3$); for II δ 8.33 and 8.30 (overlapping singlets, 2, C-5 H and C-8 H), 6.87 (s, 2, C-1′ H), 2.85 (s, 3, C-7 methyl H), 2.69 (s, 3, C-6 methyl H), 2.65 (q, 2, $COCH_2CH_3$), 1.24 (t, 3, J = 7.5 Hz, $COCH_2CH_3$); mass spectrum (MS-902 spectrograph) for I, *m/e* 314 (M^+), 284 ($M^+ - CH_2O$), 60 and 30 (corresponding to CH_3COOH and CH_2O fragments), remaining fragments are not directly relevant for the problem of structure determination; for II, *m/e* 328 (M^+) and other fragments analogous to those of I; accurate mass measurement at the I and II molecular peak positions gives *m/e* values of 314.1023 and 328.1159 which agree well with those of 314.1015 and 328.1171 calculated for $C_{15}H_{14}N_4O_4$ and $C_{16}H_{16}N_4O_4$, respectively.



RF, R = ribityl (carbons 1′–5′)
FMF, R = CH_2CHO
LC, R = H (in position 1)



I, R′ = CH_3
II, R′ = CH_2CH_3

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(1) (a) In this paper N-9-substituted 6,7-dimethylisoalloxazines will be termed flavins. (b) Chromatography of Riboflavin Decomposition Products. VII. For part VI, see: J. Cerman and I. M. Hais, *J. Chromatogr.*, **36**, 393 (1968).

(2) In the case of formylmethylflavin (which is the photoproduct of RF[3,4]) proofs for one-carbon[5] and indirect evidence (including the analogy with 9-hydroxyethylisoalloxazines[6-8]) for both C_1[5] and C_2[6-8] fragments are available.

(3) E. C. Smith and D. E. Metzler, *J. Amer. Chem. Soc.*, **85**, 3285 (1963).

(4) W. L. Cairns and D. E. Metzler, *ibid.*, **93**, 2772 (1971).

(5) E. Knobloch, *Biochim. Biophys. Acta*, **141**, 19 (1967).

(6) M. Halwer, *J. Amer. Chem. Soc.*, **73**, 4870 (1951).

(7) M. M. McBride and D. E. Metzler, *Photochem. Photobiol.*, **6**, 113 (1967).

(8) W. M. Moore, J. T. Spence, F. A. Raymond, and S. D. Colson, *J. Amer. Chem. Soc.*, **85**, 3367 (1963).

(9) S. Svobodová, I. M. Hais, and J. V. Koštíř, *Chem. Listy*, **47**, 205 (1953).

(10) I. M. Hais and K. Macek, Ed., "Paper Chromatography," Academic Press, New York, N. Y., 1963, p 664

(11) G. E. Treadwell, W. L. Cairns, and D. E. Metzler, *J. Chromatogr.*, **35**, 376 (1968).

(12) See ref 1b.

(13) Participation of singlet-state oxygen in the reaction (*cf.* ref 14) may be considered. Quenching experiments are in progress.

(14) P. S. Song and T. A. Moore, *J. Amer. Chem. Soc.*, **90**, 6507 (1968).

(15) The data in parentheses apply to the production of II.

(16) Only in the case of I because of transacylation of II to I in acetic acid.

(17) In $CHCl_3$–MeOH (9:1) I and II were the only flavins with R_f values (about 0.45 and 0.49) higher than that of LC (about 0.41). The R_f data in a number of solvent systems will be published separately.

(18) Resolution improved on addition of D_2O.

The structures of acetate and propionate of 6,7-dimethyl-9-hydroxymethylisoalloxazine (see formulas) are compatible with all these data for compounds I and II. Hydroxymethylflavin and its derivatives have not been described before.

The formation of I and II from FMF under our conditions would involve a step in which one carbon is lost, although the mechanism is likely to differ from that of the anaerobic photodegradation in which formaldehyde was demonstrated.[5]

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Interpretation of the Pseudocontact Model for Nuclear Magnetic Resonance Shift Reagents. I. The Agreement Factor, *R*

Sir:

Controlled alteration of nmr spectra by addition of chelates of lanthanide ions has been a source of increasing excitement since Hinckley's initial report.[1] The phenomenon responsible for the signal dispersion has been discussed in terms of a contact (spin delocalized) shift, a pseudocontact (anisotropic) shift, or a combination of these two effects.[2,3] Many of the recent applications have been qualitative. In addition, graphical[3] and analytical[4,5] methods have been used to treat experimental data in a more quantitative sense. We are engaged in a systematic exploration of the applicability of the McConnell–Robertson[6] version of the pseudocontact interaction to the complete interpretation of the lanthanide-induced proton chemical shifts (LIS's)[7] in rigid oxygenated bicyclic molecules. Our approach, while similar to that employed by other workers,[4,5] offers so many advantages that we present this preliminary account.

For ease in calculation, a molecule is described with respect to an internal Cartesian coordinate system with oxygen at the origin (Figure 1). The lanthanide, L, is then moved incrementally over the surface of a sphere of radius *d*, the assumed L–O distance. The location of L on the sphere is described in terms of the two angles, ρ (the colatitude, measured from the positive *Z* axis) and ϕ (the azimuth, measured counterclockwise from the *X*–*Z* plane), as shown in Figure 1. At each lanthanide position, the variable term

$$(3\cos^2\theta_i - 1)/r_i^3$$

in the pseudocontact equation[6] is evaluated for all *i* protons. This set of numbers is then scaled by least squares against the relative observed shifts $(\Delta H/H)_{oi}$ to yield a set of calculated shifts $(\Delta H/H)_{ci}$. In order to assess the correspondence between the observed and calculated values, an agreement factor, *R*, is evaluated as

$$R = \left[\frac{\sum_i((\Delta H/H)_{oi} - (\Delta H/H)_{ci})^2 w_i}{\sum_i(\Delta H/H)_{oi}^2 w_i}\right]^{1/2}$$

We have used equal weighting factors, w_i, for the results in Table II. Hamilton has provided a comprehensive discussion of agreement factors of this form and their statistical interpretation.[8] The dependence of *R* on ρ and ϕ for a given distance, *d*, is conveniently displayed using contour lines on a map projection such as the Sanson–Flamsteed sinusoidal equal area projection.[9] Orientation of *endo*-norborn-5-en-2-ol as in Figure 2[10] and the resulting agreement factor plots of Figure 3 illustrate the method.

Table I shows minimum agreement values obtained

Table I. Minimum Agreement Factors Obtained for Oxygenated Hydrocarbons

Compound	*R*
cis-4-*tert*-Butylcyclohexanol[a]	0.043
trans-4-*tert*-Butylcyclohexanol[a]	0.081
Norcamphor	0.074
endo-Norborn-5-en-2-ol	0.034
Borneol[a,b]	0.081
Isoborneol[a,b]	0.050
Bicyclo[3.2.0]hept-3-en-2-one[c]	0.060
5-Methylbicyclo[3.2.0]hept-3-en-2-one[c]	0.080
Andamantan-2-ol[d]	0.032
Cyclooctatetraene dimer epoxide[e]	0.092

[a] P. V. Demarco, T. K. Elzey, R. B. Lewis, and E. Wenkert, *J. Amer. Chem. Soc.*, **92**, 5734 (1970). [b] This work. [c] Compound obtained from Professor R. L. Cargill, University of South Carolina. [d] G. H. Wahl and M. R. Peterson, *Chem. Commun.*, 1167 (1970). [e] M. R. Willcott, J. F. M. Oth, J. Thio, G. Plincke, and G. Schröder, *Tetrahedron Lett.*, 1579 (1971).

Table II. Comparison of Observed and Calculated Lanthanide-Induced Chemical Shifts for the System Eu(DPM)$_3$-*endo*-Norborn-5-en-2-ol

Proton	Obsd	Calcd	Proton	Obsd	Calcd
1	11.0	9.9	5	6.0	6.1
2	22.8	23.0	6	8.0	7.9
3-endo	15.2	15.2	7-syn	5.7	6.3
3-exo	10.2	10.1	7-anti	4.8	4.4
4	5.3	5.7			

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(1) C. C. Hinckley, *J. Amer. Chem. Soc.*, **91**, 5160 (1969).

(2) J. K. M. Sanders and D. H. Williams, *Tetrahedron Lett.*, 2813 (1971).

(3) C. C. Hinckley, M. R. Klotz, and F. Patil, *J. Amer. Chem. Soc.*, **93**, 2417 (1971).

(4) (a) The first account of computer simulation of a LIS proton spectrum is that for borneol–Pr(DPM)$_3$: F. A. Hart, J. Briggs, G. H. Frost, G. P. Moss, and M. L. Staniforth, *Chem. Commun.*, 1506 (1970). (b) Computer simulation of the ^{13}C LIS spectrum of isoborneol–Pr(DPM)$_3$ is also recorded: G. P. Moss, J. Briggs, F. A. Hart, and E. N. Randall, *ibid.*, 364 (1971).

(5) Another computer simulation of Eu(DPM)$_3$ induced proton chemical shifts for three compounds has been reported by S. Farid, A. Ateya, and M. Maggio, *ibid.*, 1285 (1971).

(6) H. M. McConnell and R. E. Robertson, *J. Chem. Phys.*, **29**, 1361 (1958).

(7) The descriptor LIS has been introduced into the literature by B. L. Shapiro, J. R. Hlubucek, G. R. Sullivan, and L. F. Johnson, *J. Amer. Chem. Soc.*, **93**, 3281 (1971).

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(9) J. A. Steers, "An Introduction to the Study of Map Projections," University of London Press, London, 1965.

(10) C. K. Johnson, ORTEP, ORNL-3794 (Revised), Oak Ridge National Laboratory, Oak Ridge, Tenn., 1960.