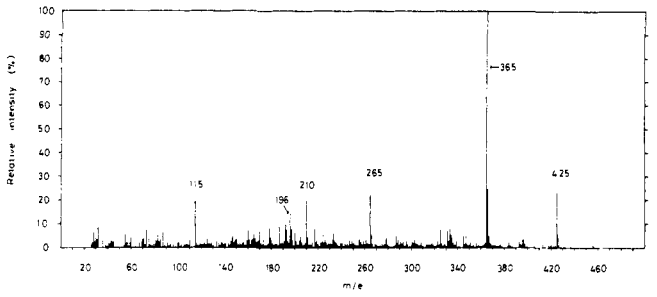

Figure 1. Mass spectrum of **5**.

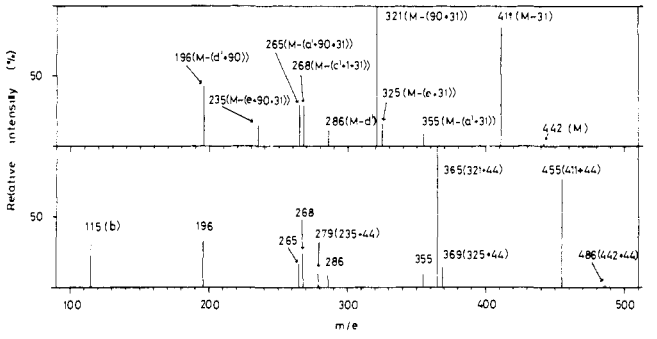

Figure 2. Prominent ions of the mass spectra of **15** (upper mass spectrum) and **6** (lower mass spectrum). The base peaks have been reduced to one-third of the original intensities.

Some information on functional groups in **2** was obtained by gas–liquid partition chromatographic analysis of various derivatives (see Table I). These experiments showed that the difference between the retention times of **5** and **6** (*C* 0.5) was identical with the difference between the retention times of **14** and **15** (*C* 0.5), thus indicating the presence of one hydroxyl group in the metabolite. The presence of two carboxyl groups in **2** was suggested by the finding that the difference between the retention times of **8** and **6** (*C* 1.2) was twice the difference between the retention times of **16** and **15** (*C* 0.6). In addition the mass spectra of **10** and **12** (obtained by reduction with borohydride and borodeuteride, respectively) showed base peaks [M − (3 × 60)] at *m/e* 306 (**10**) and 308 (**12**), thus indicating the presence of two keto groups in **2**.

Table I. *C* Values Found on Gas–Liquid Partition Chromatography (1% SE 30)

Derivative	**5**	**6**	**7**	**8**	**10**	**12**	**14**	**15**	**16**
C value[a]	24.5	24.0	24.0	25.2	25.2	25.2	21.6	21.1	21.7

[a] *Cf.* ref 5.

The mass spectrum of **5** is given in Figure 1. Prominent peaks are seen at *m/e* 425 (M − 31), 365 [M − (60 + 31)], 265 [M − (a + 60 + 31)], 210 [M − (c + 60)], 196 [M − (d + 60)], and 115 (b). The mass spectrum of **14** showed ions of high intensity at *m/e* 381 (M − 31), 321 [M − (60 + 31)], 265 [M − (a^1 + 60 + 31)], 210 [M − (c^1 + 60)], and 196 [M − (d^1 + 60)]. The fragmentation pattern of **5** was thus very similar to that of **14**, the only difference being the increase in 44 mass units of the fragments containing the carbomethoxy group of **5** instead of the methyl group of **14**.

The use of metabolite **13** as reference in the mass spectrometric analysis is further shown in Figure 2 where prominent ions of the mass spectra of derivatives **6** and **15** are given. As can be seen, in the case of **6** the molecular ion as well as ions formed by elimination of 31, e + 31, 90 + 31, and e + 90 + 31 have *m/e* values which are 44 units above corresponding ions in the mass spectrum of **15**. In **6**, elimination of fragments containing the carbomethoxy group at C-16, *viz.*, a + 31, d, c + 1 + 31, a + 31 + 90, and d + 90, yielded ions with the same molecular weights as those formed by corresponding cleavages in **15**. The ion at *m/e* 115 in the mass spectrum of **6** corresponds to the charged methyl pentanoate radical (b) which is formed by cleavage between C-11 and C-12. The interpretation of the mass spectrum of **6** was supported by the mass spectrum of the deuterium-labeled derivative **7**, in which ions containing one or two O-methyloxime groups were shifted 3 or 6 mass units upward, respectively, and by the mass spectrum of the diethyl ester derivative **8**. In the latter case, cleavage between C-11 and C-12 yielded a charged ethyl pentanoate radical (b) which gave rise to a prominent ion at *m/e* 129.

The formation of **2** from prostaglandin E_2 apparently involves four sets of reactions, *viz.*, dehydrogenation of the alcohol group in the side chain,[3] reduction of the *trans* double bond,[3] two steps of β oxidation,[7] and ω oxidation.[8] Studies on quantitative aspects of the urinary excretion of **2** are in progress in our laboratory.

Acknowledgment. This work was supported by grants from the Swedish Medical Research Council (Project No. 13X-217) and by Knut and Alice Wallenbergs Stiftelse.

(7) Prostaglandin E_2 was mainly converted into tetranorprostaglandin E_1 when incubated with a preparation of rat liver mitochondria (*cf.* ref 5).

(8) It has recently been found that prostaglandin A_1 (15-hydroxy-9-ketoprosta-10,13-*trans*-dienoic acid) is converted into a mixture of 20-hydroxyprostaglandin A_1 and 19-hydroxyprostaglandin A_1 by preparations of liver microsomes from guinea pig and man (U. Israelsson, M. Hamberg, B. Samuelsson, and T. Scherstén, unpublished observations).

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Chemi- and Bioluminescence of Firefly Luciferin

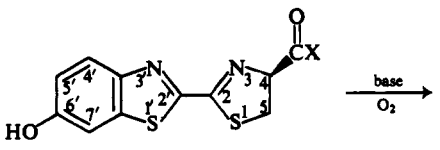
Sir:

We recently reported a red chemiluminescent emission of firefly luciferin[1] (eq 1) and showed that it was a reasonable model for the red bioluminescence observed *in vitro* at low pH; the keto anion III was identified as the light-emitting species.[1] The normal emission from the firefly *Photinus pyralis in vivo* (and *in vitro* at physiological pH) is yellow-green (ν_{max} 17,700 cm^{-1}).[2] We now report base concentration dependent shifts in color of the chemiluminescence emitted from model systems that support excited enzyme-bound dianion VI as the species emitting the yellow-green light.

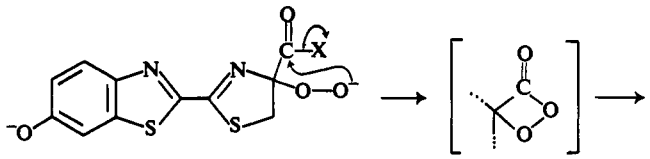
To investigate the need of enolizable hydrogens at

(1) T. A. Hopkins, H. H. Seilger, E. H. White, and M. W. Cass, *J. Am. Chem. Soc.*, **89**, 7148 (1967).

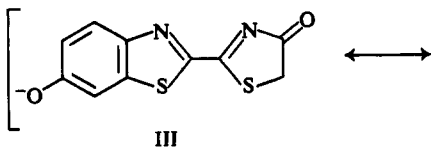
(2) H. H. Seliger and R. A. Morton in "Photophysiology," Vol. III, A. C. Geise, Ed., Academic Press, New York, N. Y., 1968, p 291.



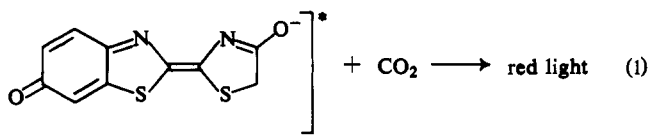
I



II

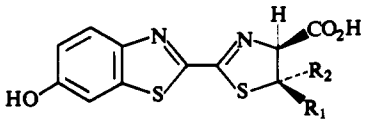


III



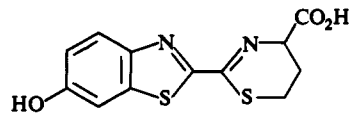
(X = C_6H_5O, Cl, AMP, and other conjugate bases of strong acids)

position 5, we synthesized *cis*- and *trans*-5-methylluciferin (IVb, c) and homoluciferin (V).[3] Whereas IVd and V (as



IV

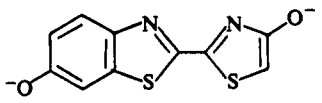
a, $R_1 = R_2 = H$
b, $R_1 = CH_3$; $R_2 = H$
c, $R_1 = H$; $R_2 = CH_3$
d, $R_1 = R_2 = CH_3$



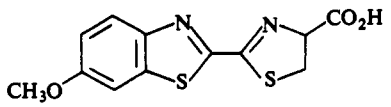
V

adenylates) do not react with *P. pyralis* luciferase to produce bioluminescence, IVb, c (as adenylates) lead to yellow-green light emission, the same as observed for natural luciferin (IVa).

The phenyl ester of IVa, when treated with small amounts of potassium *t*-butoxide in dimethyl sulfoxide (DMSO), yields the same red chemiluminescence ($\bar{\nu}_{max}$ 15,850 cm^{-1} ± 1%; FWHM[4] = 1700 cm^{-1} ± 10%) as luciferyl adenylate.[1] The keto anion III appears to be the light emitter in both cases. Additional base leads to simultaneous red and green chemiluminescence, and large amounts of base lead exclusively to a strong yellow-green emission ($\bar{\nu}_{max}$ 18,000 cm^{-1} ± 1%; FWHM = 2050 cm^{-1} ± 10%). The phenyl esters of IVb, c showed the same base-dependent colors of chemiluminescence. The fluorescence of spent reaction mixtures of IVa–c consists of a single yellow-green emission band ($\bar{\nu}_{max}$ 18,000 cm^{-1} ± 1%; FWHM = 2300 cm^{-1} ± 5%). This base dependence of the chemiluminescence suggests that the red emitting form III is acidic in the excited state and that the base abstracts a proton before light emission occurs.[5] Since the model compound, 2-phenyl-Δ^2-thiazolin-4-one, has been reported to exist predominately as the enol in neutral DMSO solutions,[6] we attribute the yellow-green chemiluminescence of the phenyl esters of IVa–c, and the yellow-green fluorescence of the products to the excited state dianions VIa–c.



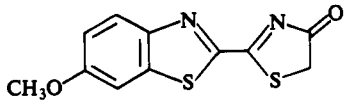
VI



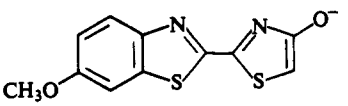
VII

Consistent with this view is the finding that the non-enolizable analog IVd yields only red chemiluminescence, independent of base concentration. Only a red chemiluminescence is emitted by the phenyl ester of homoluciferin (V). A slow rate of proton loss compared to the rate of decay of the excited-state keto anion is expected in this case since the enolate formed is nonaromatic.

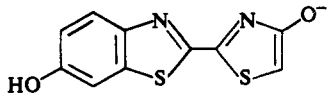
Other possible light emitters have been eliminated from consideration by experiments such as the following. The phenyl ester of VII, which serves as a model for chemiluminescence when the phenolic group is un-ionized, upon treatment with a small amount of *t*-butoxide in DMSO yields a low-intensity blue chemiluminescence ($\bar{\nu}_{max}$ 21,400 cm^{-1} ± 1%; FWHM = 2300 cm^{-1} ± 10%) attributable to emission from the neutral product VIII. Higher base concentrations produce a weak orange chemiluminescence attributable to species IX. The fluorescence of compound VIII[7] has been found to be blue in neutral



VIII



IX



X

(3) Structures follow from elemental analysis, infrared, ultraviolet, and nmr spectra. The nmr spectra of the *cis* isomer IVb (perdeuterioacetone), τ 4.76 (d, 1, J = 9 Hz, $CHCO_2H$), and *trans* IVc (perdeuterioacetone), τ 5.00 (d, 1, J = 6 Hz, $CHCO_2H$), prove the configurations.

(4) FWHM = full band width between half-maximum intensity points of the spectrum.

(5) Th. Forster, *Z. Elektrochem.*, **61**, 340 (1957); A. Weller, *Progr. Reaction Kinetics*, **1**, 187 (1961).

(6) S. Gronowitz, B. Mathiasson, R. Dahlbom, B. Holmberg, and K. A. Jensen, *Acta Chem. Scand.*, **19**, 1215 (1965).

(7) Prepared by reacting ethyl thioglycolate and 2-cyano-6-methoxybenzothiazole in 1,2-dimethoxyethane.

DMSO; upon addition of base, the fluorescence shifts to orange ($\bar{\nu}_{max}$ 16,900 cm^{-1} ± 1%; FWHM = 3075 cm^{-1} ± 5%). Thus, X is not a likely emitter because of the low chemiluminescence efficiency observed and the spectral properties of IX.

Normal solvent shifts in fluorescence emission peaks are not accompanied by appreciable changes in FWHM.[8] If we postulate that the enzyme-bound excited-state product is only in a different effective dielectric from DMSO, we can correlate the yellow-green bioluminescence ($\bar{\nu}_{max}$ 17,700 cm^{-1}; FWHM = 2050 cm^{-1})[2] with emission from the dianion VIa in DMSO ($\bar{\nu}_{max}$ 18,000 cm^{-1}; FWHM = 2050 cm^{-1}).[9]

The very high base concentrations required to produce enolization of the excited keto anion (and thus yellow-green chemiluminescence in DMSO solutions) requires that in yellow-green bioluminescence the enolization must be enzyme catalyzed (as is initial anion formation at C-4). This might occur through C-5 proton extraction by one of the sulfhydryl groups (as an anion) at the active site.[10] Protonation of the mercaptide ion and also chelation by Zn^{2+}, Cd^{2+}, or Hg^{2+} would therefore explain the red bioluminescence observed in acidic solutions and in the presence of heavy metal ions.[11]

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(8) W. O. McClure and G. M. Edelman, *Biochemistry*, **5**, 1908 (1966).
(9) The wavelength differences measured for various species of fireflies, all of which utilize luciferin (IVa), can similarly be accounted for by slight differences in hydrogen bonding and hydrophobicity at the active site.[2]
(10) M. DeLuca, G. W. Wirtz, and W. D. McElroy, *Biochemistry*, **3**, 935 (1964).
(11) H. H. Seliger and W. D. McElroy, *Proc. Natl. Acad. Sci. U. S.*, **52**, 75 (1964).

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Stereochemistry at Trivalent Nitrogen. V. Origin of Sulfur–Nitrogen Torsional Barriers in N-Sulfenylsulfonamides[1]

Sir:

Although the barriers to rotation about C–C single bonds are known to be low,[2,3] evidence has accumulated recently that substantial torsional barriers obtain about bonds between atoms which bear nonbonded valence electrons. Thus far, the systems studied using dynamic nmr spectroscopy[2] include sulfenamides,[4,5] aminophosphines,[6] disulfides,[7] trialkylhydroxylamines,[1] and the α-sulfinylcarbanion.[8,9]

One factor which has been implicated in these barriers is the coulombic repulsion between vicinal pairs of nonbonding valence electrons.[10] A theoretical study of the torsional barrier in an α-sulfinylcarbanion [$HS(O)CH_2$] has been performed.[8] The calculated transition states for torsion are ones in which the lone pairs of electrons on sulfur and carbon are *syn* and *anti* periplanar and the ground states are ones in which the dihedral angles between the nodal planes of the two filled nonbonding valence orbitals are close to 90°. This study also indicated a dependence on dihedral angle of the bond order of the S–C bond, although sulfur d orbitals were not involved. We report, here, experimental evidence which indicates that factors in addition to electron repulsion must be involved in determining the height of the torsional barrier in some sulfenamides.

We have investigated electronic effects on the barriers to conformational interchange in three series of N-arenesulfenyl-N-isopropylarenesulfonamides (**1**, **2**, and **3**).[11] In each case, the coalescence of resonances from diastereotopic isopropyl methyl protons is associated with a degenerate racemization reaction, which has as its rate-determining step torsion about the sulfenyl sulfur–nitrogen bond.[4,5,12] The coalescence temperature (T_c) was measured, the approximate rate at T_c calculated, and the free energy of activation (ΔG^*) at T_c obtained using the Eyring equation.[13] The relevant data are presented in Table I.

The magnitude of the effect of electronegativity was determined by obtaining linear least squares fit of experimental results to the free energy form of the Hammett equation:[14,15] $\Delta G^* = -2.3R\rho' + \Delta G^*_o$, where $\rho' = T\rho$. The data for series **1** fitted better when the point for **1***** was omitted (Figure 1, Table II). The correlation coefficients obtained for **1** and **2** fall within the range judged to be acceptable correlations.

(1) Part IV: M. Raban and G. W. J. Kenney, Jr., submitted for publication.
(2) G. Binsch in "Topics in Stereochemistry," Vol. 3, E. L. Eliel and N. L. Allinger, Ed., Interscience Publishers, New York, N. Y., Chapter 2.
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(5) J. M. Lehn and J. Wagner, *Chem. Commun.*, 1298 (1968).
(6) A. H. Cowley, M. J. S. Dewar, and W. R. Jackson, *J. Am. Chem. Soc.*, **90**, 4185 (1968).
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(8) S. Wolfe, A. Rauk, and I. G. Csizmadia, *J. Am. Chem. Soc.*, **89**, 5710 (1967); A. Rauk, S. Wolfe, and I. G. Csizmadia, *Can. J. Chem.*, **46**, 113 (1968).
(9) H. Kloosterziel, *Chem. Commun.*, 1330 (1968).
(10) L. Pauling, *Proc. Natl. Acad. Sci. U. S.*, **35**, 495 (1949).
(11) All compounds were prepared by reaction of the appropriate sulfenyl chloride with the lithium salt of the appropriate sulfonamide in ether.[12] All new compounds had elemental analyses and nmr spectra in accord with their structures.
(12) M. Raban, G. W. J. Kenney, Jr., J. M. Moldowan, and F. B. Jones, Jr., *J. Am. Chem. Soc.*, **90**, 2985 (1968).
(13) Although rate constants calculated in this way are only approximate, the resultant free energies of activation are themselves quite accurate. In some sulfenamides, ΔG^* was calculated using the approximate rate expression at T_c and also using complete line-shape analysis with comparable results (M. Raban, G. W. J. Kenney, and F. B. Jones, Jr., unpublished results).
(14) For reviews dealing with linear free energy relationships see: (a) H. Van Bekkum, P. E. Verkade, and B. M. Wepster, *Rec. Trav. Chim.*, **78**, 815 (1959); (b) P. R. Wells, *Chem. Rev.*, **63**, 171 (1963); (c) H. H. Jaffé, *ibid.*, **53**, 191 (1963).
(15) Linear least squares analysis furnished the constant ρ' rather than ρ since each of the values of ΔG^* is obtained at a different temperature. Comparison with other reactions is facilitated by the calculation of a hypothetical $\rho_{300} = \rho'/300$.