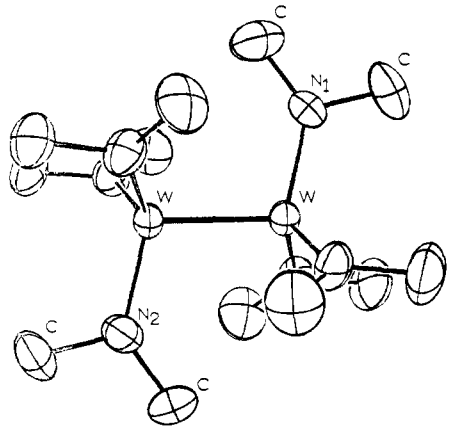

Figure 1.

$W(NMe_2)_6$ in 2:1 ratio based on tungsten is obtained. All attempts to separate pure $[W(NMe_2)_3]_n$ from this mixture by vacuum sublimation and fractional recrystallization have failed. Chromatographic techniques employing dehydrated Florisil also failed since $[W(NMe_2)_3]_n$ was selectively destroyed on the support.

Though pure $[W(NMe_2)_3]_n$ has not been isolated, spectroscopic properties of the mixture were informative with regard to the nature of $[W(NMe_2)_3]_2$. In the mass spectrum a strong parent ion, $W_2(NMe_2)_6^+$, and several other W_2-containing ions are observed. ^1H NMR studies reveal a single resonance at δ 3.44 ppm from TMS at room temperature and above, and two resonances with equal intensities at δ 2.46 and 4.39 ppm at $-40°$ and below. This behavior is directly analogous to that observed for $Mo_2(NMe_2)_6$ and corresponds to the temperature-dependent rate of proximal and distal methyl exchange.[9] Thus, all the physical data indicated the dinuclear, diamagnetic nature of $[W(NMe_2)_3]_n$ and thus suggested the presence of a W-W triple bond, rather than the presence of bridging dimethylamido ligands. Crystallographic examination of crystalline samples obtained from the $[W(NMe_2)_3]_2$-$W(NMe_2)_6$ mixture was then undertaken.

A crystalline sample, obtained from THF, contained crystals of pure $W(NMe_2)_6$[8] as well as single crystals having a mixed composition of $W(NMe_2)_6$ and $W_2(NMe_2)_6$ in a ratio of 1:2. The structure of this mixed species was solved and refined.[12] The unit cell contains two molecules of $W_2(NMe_2)_6$ with the tungsten atoms lying on a crystallographic threefold axis and a single molecule of $W(NMe_2)_6$ occupying a position of $\bar{3}$ symmetry. The $W(NMe_2)_6$ molecule shows crystallographic disorder arising from two orientations of the NMe_2 group about the W-N axis. The refined dimensions of this structure agree satisfactorily with those previously obtained for pure $W(NMe_2)_6$.[8]

The $W_2(NMe_2)_6$ molecules are ordered and have the structure shown in Figure 1. Important averaged dimensions are: W-W = 2.294 (1) Å, W-N = 1.96 (1) Å, N_1-C = 1.46 (2), Å, N_2-C = 1.45 (2) Å, and W-W-N = 103.9 (4)°; the WNC_2 groups are essentially planar with angles at the nitrogen atoms of 111 (1)° for C-N-C, 117 (1)° for distal W-N-C, and 132 (1)° for proximal W-N-C.

The structure is thus similar to that of $Mo_2(NMe_2)_6$[10] but has a metal-metal bond which is longer by 0.08 Å. The W-W triple bond is similar in length to the only other unbridged triple bond between metal atoms of the third transition series that has so far been reported,[13] viz., the Re≡Re bond in $Re_2Cl_5(CH_3SCH_2CH_2SCH_3)_2$ which has a length of 2.293 (2) Å. Further study of both of the $M_2(NMe_2)_6$ compounds is in progress.[14]

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(14) This work was supported by the National Science Foundation (Grant No. 33142X at TAMU and GP42691X at Princeton) and the donors of the Petroleum Research Fund, administered by the American Chemical Society, M.E. thanks the American Can Company for a fellowship.

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Received December 23, 1974

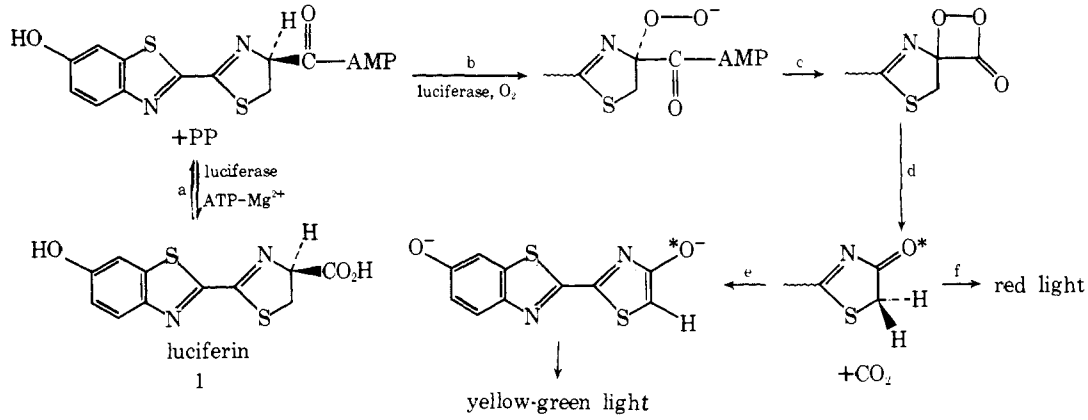
Modification of Firefly Luciferase with a Luciferin Analog. A Red Light Producing Enzyme

Sir:

Light production in the firefly appears to involve the sequence of reactions outlined in Chart I, as determined by in vitro studies.[1,2] Luciferase activity is measured by light emission, and in the normal assay (pH 7.9) the enzyme produces the familiar yellow-green light emission.[2] Proton losses occur at steps b and e, and basic residues on the enzyme are presumably involved in these ionizations. If two different bases are involved, it should be possible, in principle, to block the second one (Chart I, step e) to form an enzyme capable of only red light emission (process f, Chart I).[3] In the presence of protons (pH 5.5 to ~7) and heavy metals (acting reversibly) firefly luciferase produces red light,[4] and possibly these species block the second site by reducing its basicity. We now report on the use of an inhibitor of luciferase that produces a red light emitting enzyme by alkylation.

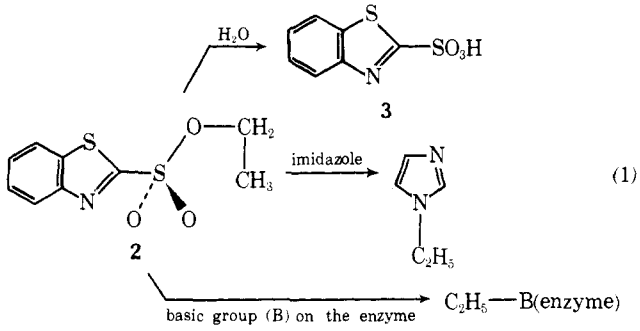
Enzymes have been chemically modified with reagents that range from those that bear little resemblance to native substrates to those that are patterned after the substrate.[5] Inhibitors in the latter category are more likely to modify the active site of the enzyme.[6] In most cases of derivatization, a major part of the reactive substrate becomes attached to the enzyme. The appending of a large group to the active site probably accounts for the observation that most modified enzymes prepared in this way are inactive.[7,8] Within the past few years, the use of inhibitors that deliver a small group to the enzyme has been reported. Methyl 4-

Chart I



nitrobenzenesulfonate was found to transfer a methyl group to histidine-57 of chymotrypsin,[9] and trimethyloxonium salts were used to derivatize acetylcholinesterase[10] (methyl group transfer almost certainly occurred). In both cases, the derivatized enzyme was inactive indicating either that an essential functional group was blocked or else that the specificity of the enzyme was altered to such a degree that the remaining activity was not detected with the usual test reagents.[11] In the present study, an inhibitor similar in shape to the natural substrate, luciferin (**1**), was used to direct a small group (C_2H_5) onto the active site of the enzyme, leaving it active, but modifying its chemical properties.

The inhibitor used, ethyl 2-benzothiazolesulfonate (**2**),[12] was expected to be an ethylating agent by analogy to the chemistry of sulfonate esters,[13] and it was shown to be one by the reaction with imidazole (eq 1). It is highly reactive, decomposing in water with a half-life of about 12.5 min at 25° and pH 7.9.



(1)

Reversible inhibition of luciferase by compound **2** on short exposures (<30 sec) was found to be competitive with respect to luciferin (Table I), but noncompetitive with respect to ATP-Mg^{2+}. The K_i of **2** (2.3×10^{-6} *M*, Table I) indicates that it competes effectively with luciferin ($K_m = 3 \times 10^{-5}$)[14a] for the luciferin active site.[14] The hydrolysis product of **2**, 2-benzothiazole sulfonic acid (**3**), is also competitive with luciferin, but it is less efficient than **2** ($K_i = 1.4 \times 10^{-5}$ *M*, Table I). The formation of sulfonic acid **3** was taken into consideration in the inhibition studies, and luciferin concentrations were increased in some assays to offset its presence.

Compound **2** inhibits firefly luciferase irreversibly on longer exposures. When the ethyl ester was incubated with luciferase in 0.05 *M* sodium phosphate buffer, pH 7.9, the overall enzyme activity decreased with time (Figure 1). This inhibition is accompanied by a shift of the emission wavelength from the yellow-green end of the spectrum

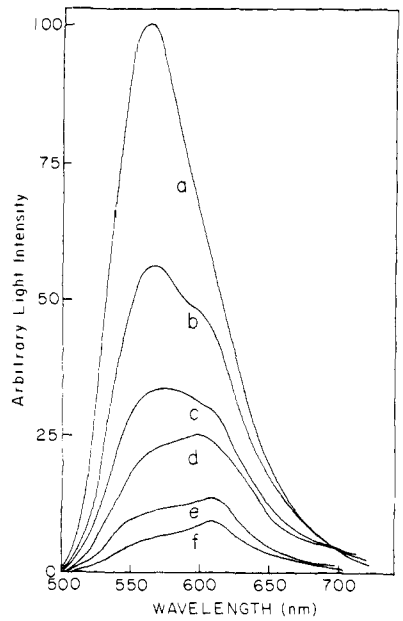


Figure 1. Bioluminescence emission spectra of luciferase modified by ethyl 2-benzothiazolesulfonate (**2**) (measured at pH 7.9 and curves corrected for the spectral response of the Hitachi R446 phototube employed): a, unmodified enzyme (final concentration = 9.9×10^{-8} *M*); b, enzyme (final concentration = 9.9×10^{-8} *M*) 10 min after addition of **2** (final concentration = 6.85×10^{-6} *M*); c, modified enzyme after 30 min; d, modified enzyme after 60 min (Additional ester was added to the enzyme-**2** solution after 65 min of incubation so that final concentration of **2** = 1.26×10^{-5} *M*.); e, the modified spectrum after 80 min total incubation time; f, modified enzyme after 155 min.

Table I. Inhibition constants (K_i) of Luciferin Analogs and Benzothiazole Derivatives

Inhibitor	$10^6 K_i$(*M*)	pH
Luciferin (1)	30[a]	7.9
Ethyl 2-benzothiazolesulfonate (2)	2.3[b]	7.9
2-Benzothiazole sulfonic acid (3)	14[b]	7.9
Benzothiazole	25[c]	7.7
Dehydroluciferin	1.0[c]	7.7
TPCK	250[d]	7.9

[a] This value is the reported K_m for 1.[14a] [b] Determined graphically from Lineweaver–Burk plots. [c] Reference 14b. [d] Reference 14a.

(~560 nm) to the red end (~610 nm) (Figure 1). The inhibition rate was decreased in the presence of luciferin (**1**) (Table II), suggesting that the action of the inhibitor (**2**) was directed toward the luciferin active site of the enzyme.

An inhibitor not closely related to luciferin, TPCK (L-1-chloro-4-phenyl-3-(*p*-tolysulfonamido)-2-butanone),[14,15] inhibits the enzyme (Table II),[16] but with no shift in the

Table II. Effect of Inhibitors on Firefly Luciferase Activity

Inhibitor	[Inhibitor]/[luciferase][a]	% activity of enzyme[b,f]
Control[c]	----	100
Ethyl 2-benzothiazolesulfonate (2)	30	48
	40	44
2 + luciferin (1)[d]	40	87
	200[e]	33
2-Benzothiazole sulfonic acid (3)	57	96
Methyl 4-nitrobenzenesulfonate	39	91
TPCK	40	14

[a] Recrystallized twice.[17] [b] From the initial flash of light emitted.[18] The light reaction was initiated by rapid injection of 0.2 ml of 0.02 *M* ATP into a solution of 2.0 ml of 0.05 *M* sodium phosphate buffer (pH 7.9), 0.1 ml of 0.1 *M* $MgSO_4$, 0.1 ml of ca. 1.2–5.8 × 10^{-3} *M* luciferin, and 0.015 ml of 1.6 × 10^{-5} *M* incubated luciferase solution (inhibitors added in THF, THF–*tert*-butyl alcohol (1:4), or methanol; concentration 0.5%). [c] Enzyme plus the appropriate organic solvent (0.5%). [d] Luciferin (1) was present in the luciferase–ester (2) solution ([1]/[2] = 2.9). [e] Not all initially soluble. [f] Percent activity of enzyme remaining after 1 hr incubation at 22°.

emission wavelength. Either this reagent attacks the first base or the enzyme becomes totally inactivated by attachment of a large group to the second base. The simple methylating agent methyl 4-nitrobenzenesulfonate[9] inhibits the enzyme, but slowly (Table II).

Our working hypothesis for these results is that the second basic center on the enzyme (Chart I, step e) displaces 2-benzothiazole sulfonate ion from the ethyl group of bound **2** faster than does the first basic center (Chart I, step b). Alkylation of the first center totally inhibits the enzyme, whereas alkylation of the second center modifies the enzyme so that red light is produced over the entire pH range from 6 to 8.6. A control experiment has shown that neither the observed shift of the emission wavelength to the red nor the irreversible inhibition of the enzyme are produced by 2-benzothiazole sulfonic acid (**3**) (Table II). Difference ultraviolet spectra of the inhibited enzyme and native luciferase do not show a benzothiazole chromophore indicating that compound **2** is not acting as a sulfonating agent. Work is in progress on the use of C-14 labeled **2** to identify the amino acid(s) being modified.

Acknowledgment. We thank Dr. William S. Morrison for help in the synthesis of compound **2**, Mrs. Reiko T. Lee for helpful information about firefly luciferase, Dr. H. H. Seliger for his generous gift of firefly tails, and the U.S. Public Health Service (Grant GM 19488) for its financial support.

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Tetracobalt Carbonyls in Solution

Sir:

We wish to report carbon-13 NMR studies of the two types of skeletal structures found for tetracobalt clusters. These observations demonstrate the dependence of carbonyl scrambling mechanisms upon the type of bridging carbonyl group.

The "butterfly" structures of the $RCCRCo_4(CO)_{10}$ series[1] presented the first skeletal type. These complexes have crystalline structures containing two asymmetrically bridging CO groups[2] (eq 1). The ^{13}C NMR[3] of (**1**), R = CO_2CH_3, and (**2**), R = Ph, were recorded (Table I) over a temperature range. At ca. −90° three resonances were observed. As the temperature is raised these signals broaden and eventually (−20°) coalesce to a broad resonance at ca. 203 ppm. Interaction with the cobalt nucleus (vide infra) maintains this broadening. It is apparent that at temperatures above ca. −70° CO scrambling over the entire Co_4 skeleton is occurring. In the region −104 to −70°, we believe the spectra are best interpreted in the following manner.

In the absence of tautomerism, five carbonyl resonances (one bridging and four terminal) are expected. Thus selective equilibration is occurring. The signal at lowest field is of correct intensity and shift to be due to the average of one bridging and one terminal environment, the remaining pair of signals resulting from averaged terminal carbonyl sites. Carbonyls CO_b (unsymmetric bridge) and CO_c equilibrate