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Chemiluminescence of 2-(6′-Hydroxy-2′-benzothiazolyl)-4-isopropylidene-Δ^2-thiazolin-5-one, a Byproduct Formed in the Chemiluminescence of a Firefly Luciferin Analogue

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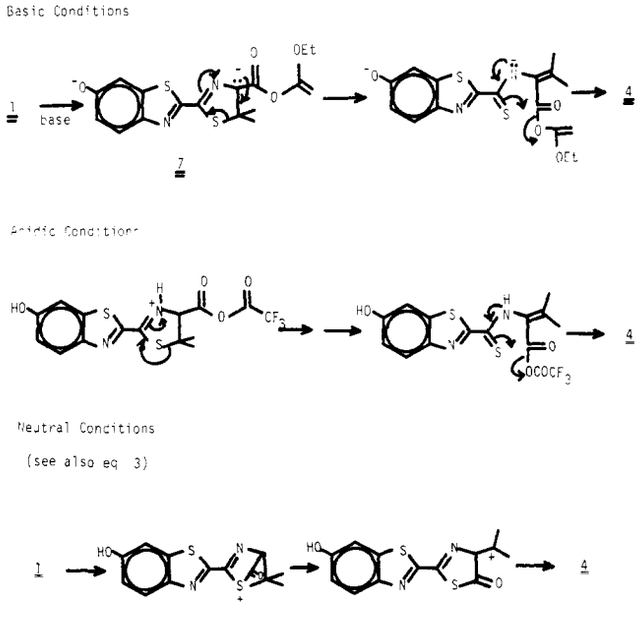
The structure of 2-(6′-hydroxy-2′-benzothiazolyl)-4-isopropylidene-Δ^2-thiazolin-5-one (**4**) is assigned to a byproduct formed in the chemiluminescence of esters of the dimethyl derivative of firefly luciferin (**3**). Compound **4** also proved to be chemiluminescent on reaction with potassium phenoxide and oxygen. Thiazolinecarboxylic acids and thiazolinones are apparently brought into equilibrium by base, and they share a common intermediate in the chemiluminescence reaction.

In studies dealing with the chemi- and bioluminescence of firefly luciferin we reported that the ethoxyvinyl ester of the 5,5-dimethyl derivative of luciferin (**1**) was chemiluminescent on treatment with base and oxygen and that three products were formed: 5,5-dimethyloxyluciferin (**2**) (formed in the excited state), 5,5-dimethylluciferin (**3**) (a hydrolysis product), and a compound analyzing for $C_{13}H_{10}N_2O_2S_2$ (**4**).[1-3] We had earlier proposed, on the basis of preliminary data, that the C_{13} compound was a thiazinone (structure **5**).[1] We now report, with additional evidence, that the C_{13} compound is the isomer 2-(6′-hydroxy-2′-benzothiazolyl)-4-isopropylidene-Δ^2-thiazolin-5-one (**4**).

The proof of structure rests largely on the elemental analysis ($C_{13}H_{10}N_2O_2S_2$) and the formation of acetone on ozonolysis. The mass spectrum showed a parent ion at *m/e* 290, the molecular weight corresponding to the formula given above. The methyl signals in the NMR spectrum, δ 2.45 and 2.51, were similar to the values reported for analogue **6** (δ 2.38 and 2.44).[4] The ultraviolet absorption at 373 nm (in Me_2SO) was consistent with structure **4** in that dimethylluciferin (**3**) absorbs at 330 nm and oxyluciferin **2** absorbs at 390 nm. The infrared absorption at 1660 cm^{-1} (KBr) is consistent with structure **4** (λ_{max} for compound **6** is 1686 cm^{-1} in $CHCl_3$).[4]

Scheme I



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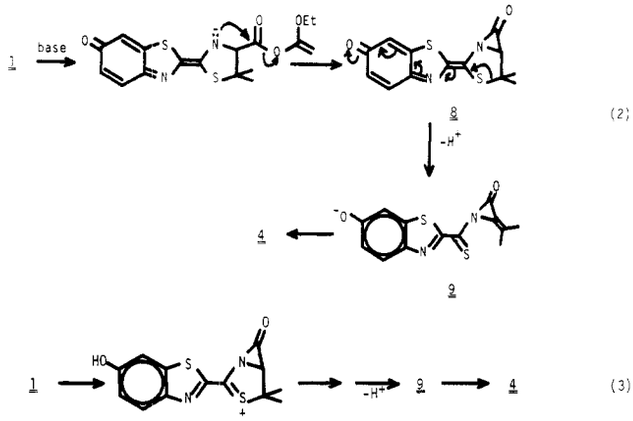
The isopropylidene compound (4) was formed under the basic conditions utilized in the chemiluminescence experiments (eq 1). It was also formed under acidic conditions in an attempted synthesis of the phenyl ester of dimethylluciferin (3) with a mixture of phenol and trifluoroacetic anhydride, and also under neutral conditions during attempted recrystallizations of compound 1. Suggested mechanisms for these transformations are given in Scheme I.

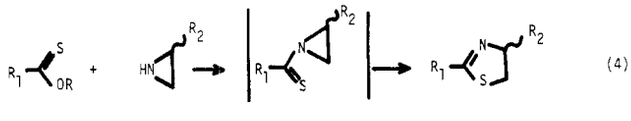
Other conceivable mechanisms involve ionic versions of vinylcyclopropane rearrangements (eq 2 and 3). The versions



(2)

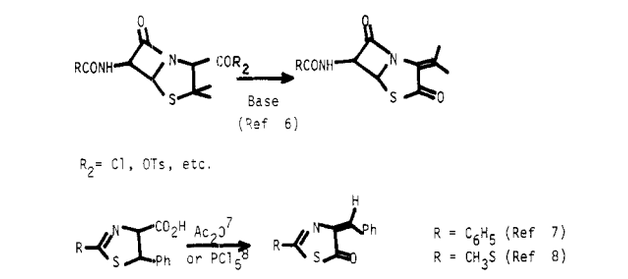
(3)

in eq 2 and 3 or the "neutral" rearrangement in Scheme I may account for the observations that the yield of 4 relative to that of 2 is somewhat greater at low base concentrations and the quantum yield of light emission is lower (the dinegatively charged anion 7 is required for chemiluminescence). Analogies for such rearrangements in heterocyclic compounds exist (eq 4).[5] Several analogies can be cited for the formation of compound 4.

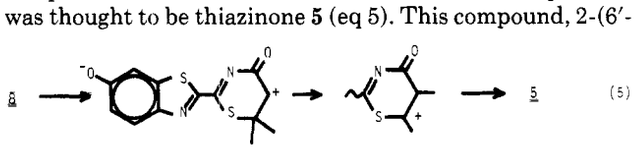


(4)

R_1, R_2 = alkyl or aryl

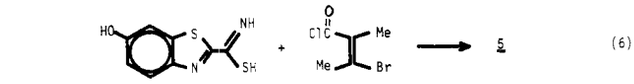


Thiazinones. In the early stages of this work, the C_{13} compound formed in the chemiluminescence of compound 1 was thought to be thiazinone 5 (eq 5). This compound, 2-(6′-



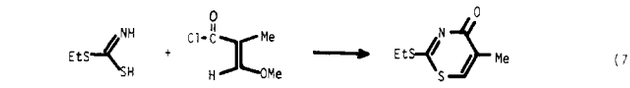
(5)

hydroxy-2′-benzothiazolyl)-5,6-dimethyl-4*H*-1,3-thiazin-4-one (5), was synthesized as shown in eq 6. The synthesis is



(6)

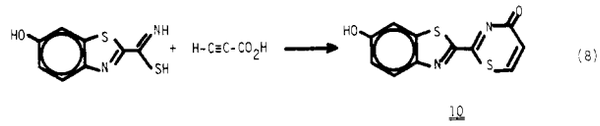
patterned after the preparation of the following thiazinone by Shaw and Warrener (eq 7).[9] The properties of isomers 4



(7)

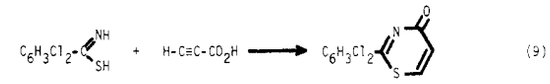
and 5 are similar, but the formation of acetone in the ozonolysis of 4 establishes its structure.

The parent thiazinone (10) was also synthesized (eq 8) since we wished to look for rearrangement products of firefly lu-



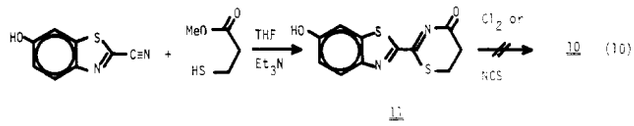
(8)

ciferin itself. The synthesis was patterned after the method of Daams[10] (eq 9) and also of Mushkalo and Yangol.[11] An



(9)

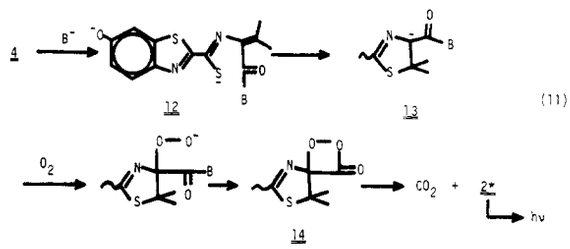
approach to the synthesis of thiazinone 10 via dihydrothiazinone 11 (eq 10) was unsuccessful because of the failure of the



(10)

oxidation step,[12] probably because of the oxidizability of the phenol ring and the heterocyclic functions.

Chemiluminescence. The Isopropylidene compound, 4, proved to be chemiluminescent on treatment with bases in the presence of oxygen. These conditions are the same as those used in the chemiluminescence of the luciferin ester (1) that produces 4,[1,3] but the isopropylidene compound reacts more slowly ($T_{1/2} \sim 1100$ s) than the luciferin ester ($T_{1/2} \sim 9$ s), permitting its accumulation in the reaction mixture. The only fluorescent compound formed in the chemiluminescence of compound 4 is oxyluciferin 2. A reaction mechanism for this conversion based on an analogous proposal for the chemiluminescence of firefly luciferin[1] is given in eq 11.

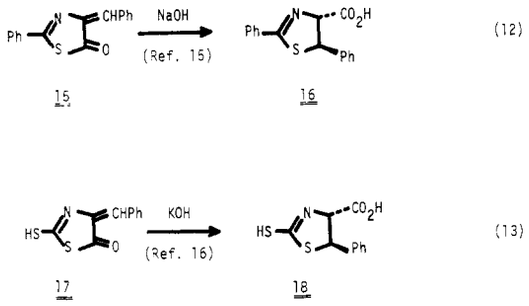


(11)

The quantum yield for the chemiluminescence of 4 is dependent on the nature of the base B. For B = phenoxide ion, QY $\sim 2.5 \times 10^{-2}$ and for B = hydroxide ion, QY $\sim 1.2 \times 10^{-4}$. The lower value for B = hydroxide ion presumably is a result of ionization of the carboxyl group in 12; the charged carboxylate group would slow the formation of intermediate 13 and effectively block dioxetanone formation (i.e., 14). An intermediate similar to 13 (B = adenosine monophosphate (AMP)) has been proposed for the bioluminescence of firefly luciferin.[13]

In dilute solutions (1.2×10^{-6} M), the chemiluminescence λ_{max} of 4 is 626 nm, a value close to that of the fluorescence of oxyluciferin (2) (631 nm)[1,3,14] and the chemiluminescence emission of the phenyl and AMP esters of 3.[1,3] The chemiluminescence of ester 1 occurs at 630 nm. In more concentrated solutions ($>2 \times 10^{-5}$ M) the wavelength of chemiluminescence of 4 shifts to 584 nm. An exiplex emission (from 2 + 4) may be involved since the addition of 4 (10^{-4} M) to a fluorescing solution of 2 (10^{-5} M) shifts the emission from 630 to 585 nm. Also, the addition of 4 to a chemiluminescing solution of ester 1 leads to a shift of the emission wavelength from 630 to 584 nm.

The conversion of compound 4 to 13 is in effect the reverse of the conversion of compound 1 to 4, implying that the thiazoline carboxylic acid ring system of 1 and 13 and the thiazolinone ring system of 4 can be brought into equilibrium with



base. The conversions of **15** to **16** and **17** to **18**, cited from the literature, are analogies for the conversion of **4** to **13** (eq 11).

Experimental Section

Instrumentation. Melting points were taken with a Thomas-Hoover capillary melting point apparatus or a microscope hot stage and are uncorrected. Elemental analyses were performed by Galbraith Laboratories (Knoxville, Tenn.). Proton magnetic resonance spectra were measured on a JEOL MH-100 or a Varian HA-100 instrument and values are reported relative to tetramethylsilane (Me_4Si). Photometric determinations were made by measuring the output of EMI 9558B or 1P21 photomultiplier-photometers exposed to the reacting solution. The values obtained were corrected for phototube spectral response. Quantum yields are relative to luminol[17] and are ±0.5%. Thin-layer chromatography was performed using 20 × 20 cm Eastman plates coated with cellulose or silica gel.

Materials. Potassium phenoxide solutions were made immediately before use by dissolving freshly sublimed potassium *tert*-butoxide and a half-molar excess of phenol in dry Me_2SO. Phosphoric acid (100%) was prepared by a literature method.[18] Tetrahydrofuran was freshly distilled from $LiAlH_4$ prior to use. The following compounds were synthesized according to literature procedures: 2-cyano-6-hydroxybenzothiazole,[2] ethoxyacetylene,[19] 2-(6′-hydroxy-2′-benzothiazolyl)-5,5-dimethyl-Δ^2-thiazoline-4-carboxylic acid (5,5-dimethylluciferin),[20] 6-hydroxybenzothiazole-2-thiocarboxamide,[21] *trans*-2-methyl-3-bromo-2-butenoic acid (β-bromoangelic acid).[22]

Ethoxyvinyl 2-(6′-Hydroxy-2′-benzothiazolyl)-5,5-dimethyl-Δ^2-thiazoline-4-carboxylate (1) (5,5-Dimethylluciferin Ethoxyvinyl Ester). 5,5-Dimethylluciferin (308 mg, 1 mmol) was dissolved in dry freshly distilled THF (20 mL) under dry nitrogen. Ethoxyacetylene (1.0 mL, 793 mg, 11 mmol) was added via a syringe. Mercuric acetate (6 mg, 0.02 mmol) was added and the solution was stirred at room temperature and monitored by TLC (1:1 ethyl acetate–benzene on silica gel). After 32 h the reaction mixture was poured into ether (50 mL) and the mixture was extracted three times with 5% sodium bicarbonate and once with water. The ether layer was dried (sodium sulfate), filtered, and evaporated to give 354 mg (94%) of 5,5-dimethylluciferin ethoxyvinyl ester: mp 255–256 °C dec; IR (nujol) 3150 (broad), 1785, 1680, 1375, and 1220 cm^{-1}; UV λ_{max} (95% EtOH) 335 (16 200), 267 nm (7900); NMR (Me_2SO-d_6) δ 10.18 (s, 1), 7.96 (d, 1, J = 8.5 Hz), 7.46 (d, 1, J = 2.5 Hz), 7.08 (d of doublets, 1, J = 8.5, 2.5 Hz), 5.24 (s, 1), 3.95 (t, 2, J = 8.0 Hz), 3.95 (m, 2), 1.76 (s, 3), 1.48 (s, 3), 1.26 (t, 3, J = 8.0 Hz). Anal. Calcd for $C_{17}H_{18}N_2S_2O_4$: C, 53.97; H, 4.76; N, 7.41; S, 16.93. Found: C, 53.76; H, 4.61; N, 7.31; S, 16.85.

An attempted recrystallization of this ester from acetone/hexane resulted in the production ~10% of the isopropylidene compound, **4**.

2-(6′-Hydroxy-2′-benzothiazolyl)-4-isopropylidene-Δ^2-thiazolin-5-one (4) from 5,5-Dimethylluciferin, Phenol, and Trifluoroacetic Anhydride. Trifluoroacetic anhydride (4.5 g, 21 mmol) was added dropwise to 5,5-dimethylluciferin (**3**) (77 mg, 0.25 mmol) and phenol (240 mg, 2.5 mmol) with stirring under N_2 at 0 °C. The mixture was then stirred at room temperature for 1 h. The reaction mixture was evaporated to dryness to remove excess anhydride, trifluoroacetic acid, and phenol to give a quantitative yield of 2-(6′-trifluoroacetyl-2′-benzothiazolyl)-4-isopropylidene-Δ^2-thiazolin-5-one (trifluoroacetyl derivative of **4**): NMR ($CDCl_3$) δ 8.17 (d, 1, J = 8 Hz), 7.86 (d, 1, J = 2 Hz), 7.37 (d of d, 1, J = 8, 2 Hz), 2.55 (s, 3), 2.48 (s, 3); IR ($CHCl_3$) 1805, 1690 cm^{-1}. This product was dissolved in ethyl acetate (50 mL) and the solution was washed twice with water. The organic layer was dried (Na_2SO_4) and evaporated to give 66 mg (92%) of compound **4**: mp 250 °C dec; IR (KBr) 3400 (broad), 1660, cm^{-1}; NMR (Me_2SO-d_6) δ 8.12 (d, 1, J = 8 Hz), 7.81 (d, 1, J = 2 Hz), 7.32 (d of d, 1, J = 8, 2 Hz), 2.51 (s, 3), 2.45 (s, 3); UV λ_{max} (95% EtOH) 373 (17 300), 315 (6500), 280 (sh) (7100), 265 (10 900), 257 nm (10 400); λ_{max} (95% EtOH + base) 442 (21 000), 310 (sh) (6600), 285 (8200), 250 nm (9400); λ_{max} (Me_2SO + KOH) 473 (35 200), 320 nm (9400); fluorescence λ_{max} (Me_2SO + potassium phenoxide) 510 nm (λ_{exc} 468 nm); mass spectrum, m/e 290 (30), 195 (43), 194 (46), 149 (100).

Anal. Calcd for $C_{13}H_{10}N_2O_2S_2$: C, 53.78; H, 3.47; N, 9.65. Found: C, 53.80; H, 3.37; N, 9.57.

Reaction of 5,5-Dimethylluciferin Ethoxyvinyl Ester (1) with Potassium Phenoxide and Oxygen. 5,5-Dimethylluciferin ethoxyvinyl ester (**1**) (20 mg, 5.3 × 10^{-2} mmol) was placed in a small sealed reaction vessel equipped with a stopcock, magnetic stirring bar, and a septum. Freshly distilled dry Me_2SO (2 mL) was added through the septum and the solution was stirred for 1 min and then degassed by two freeze–pump–thaw cycles. Oxygen (1 atm) was introduced into the reaction vessel and the solution was stirred at room temperature. A freshly prepared potassium phenoxide solution (10 molar excess) was then injected into the vessel through the septum to initiate the reaction and the resulting reaction mixture was stirred for 5 min. The reaction mixture was then frozen in liquid nitrogen for subsequent analysis.

The dark red reaction mixture was thawed and an aliquot (150 μL) was transferred to the bottom of a microsublimer. The solution was acidified with a microdrop of 1 N HCl, the sublimer was evacuated, and the condenser was cooled with dry ice–acetone to remove Me_2SO and excess phenol. The residue was dissolved in 95% EtOH and the solution was applied to a cellulose-coated TLC plate. The plate was eluted with 1:1 MeOH/H_2O. Products separated (followed by R_f and color of band under fluorescent light) were: 5,5-dimethylluciferin (**3**) (R_f 0.88, blue), 5,5-dimethyloxyluciferin (**2**) (0.73, yellow), unknown degradation product (0.54, blue-green), isopropylidene compound **4** (0.19, green). The yields were: **3**, 12%; **2**, 30%; and **4**, 44% (when a 1/1 ratio of phenoxide/**1** was used, the yields were 17, 13, and 53%, respectively). The identities of the products were verified and the yields were determined by scraping the appropriate bands, eluting with 95% EtOH, and analyzing the organic solutions by UV spectroscopy. A close comparison of the IR and mass spectra of the isolated materials with those of authentic samples verified the structure assignments. The blue-green fluorescent compound, produced in low yields, absorbs in the UV at 320 nm and is probably a thiazoline ring-opened material.

A similar run carried out in the absence of oxygen (vacuum) yielded principally the isopropylidene compound (**4**) (55%) along with dimethylluciferin (**3**) (11%) and dimethyloxyluciferin (**2**) (1%).

Ozonolysis of 2-(6′-Hydroxy-2′-benzothiazolyl)-4-isopropylidene-Δ^2-thiazolin-5-one (4). Compound **4** (12.4 mg, 4.27 × 10^{-2} mmol) was dissolved in 1 mL of absolute methanol. The solution was cooled to −30 °C by a methanol–dry ice bath and ozone was bubbled through the yellow solution. The solution became colorless and the bubbling was stopped when the solution turned blue (~10 min). Nitrogen was passed through the solution to displace the ozone and a few drops of a saturated potassium iodide solution in methanol/acetic acid were added. The color of the reaction mixture turned purple. A few drops of a sodium sulfite solution was added to discharge the purple color and 2,4-dinitrophenylhydrazine reagent was added. The precipitate which formed was collected by centrifugation (26 mg, 3.8 × 10^{-2} mmol, 89%). This crude material was analyzed by TLC and was found to contain mostly acetone 2,4-dinitrophenylhydrazone. The crude material was heated to 70 °C under vacuum (10 μm) in a sublimer. The yellow material which sublimed had an R_f on TLC identical to that of authentic acetone 2,4-dinitrophenylhydrazone. TLC: 8:2 ethyl acetate–benzene on silica gel; R_f 0.65 (R_f of authentic acetone DNP 0.65); 8:2 ethyl acetate–benzene on alumina; R_f 0.81 (R_f of authentic acetone DNP 0.81); 1:1 methanol–H_2O on cellulose, R_f 0.76 (R_f of authentic acetone DNP 0.76).

The melting point of the sublimed material on a microscope hot stage was 127–130 °C. Authentic acetone DNP gave a mp of 126–130 °C (lit.[23] mp 130 °C). A control run was made duplicating the above operations except that compound **4** was omitted. No acetone 2,4-dinitrophenylhydrazone was detected in the control run by TLC. In addition a control run was made duplicating the above conditions using 2,4,4-trimethyl-2-pentene (5.8 mg, 5.17 × 10^{-2} mmol) as the substrate to verify the susceptibility of an isopropylidene group to ozonolysis under the above conditions. Acetone 2,4-dinitrophenylhydrazone was verified as the product of the reaction by TLC.

***trans*-2-Methyl-3-bromo-2-butenoyl Chloride (β-Bromoangelic Acid Chloride).** β-Bromoangelic acid (4.7 g, 26 mmol) was added slowly to phosphorous trichloride (2 mL) at 50 °C and the mixture was heated at 60–65 °C for 2.5 h. The supernatant liquid was decanted from the syrupy layer of phosphoric acid. Distillation of the decanted layer under reduced pressure (50 °C (15 mm)) yielded 4.1 g (85%) of 3-bromoangelic acid chloride: bp 55 °C (20 mm); NMR

($CDCl_3$) δ 2.20 (s), 2.55 (s); IR ($CHCl_3$) 1740 and 1775 cm^{-1} (C=O).

2-(6′-Hydroxy-2′-benzothiazolyl)-5,6-dimethyl-4*H*-1,3-thiazin-4-one (5). 6-Hydroxybenzothiazole-2-thiocarboxamide[3] (100 mg, 0.476 mmol) and β-bromoangelic acid chloride (93.6 mg, 0.476 mmol) were dissolved in dry THF (1 mL) in a Pyrex tube. The tube was sealed under vacuum and it was heated to 85 °C for 24 h. The precipitate obtained was filtered and purified by fractional sublimation to give 20 mg (14%) of compound **5**: mp >250 °C; IR (KBr) 1610 cm^{-1}; mass spectrum, *m/e* 290 (59), 176 (20), 149 (58), 114 (91), 86 (100); UV (95% EtOH) λ_{max} 383, 273 nm and with base 490, 300, 250 nm. Anal. Calcd for $C_{13}H_{10}N_2O_2S_2$: C, 53.78; H, 3.47. Found: C, 53.80; H, 3.50.

2-(6′-Hydroxy-2′-benzothiazolyl)-4*H*-1,3-thiazin-4-one (10). 6-Hydroxybenzothiazole-2-thiocarboxamide (100 mg, 0.476 mmol) was added all at once to propiolic acid (~4 mL) under N_2 at room temperature. The mixture turned a red color and became homogeneous. After ca. 0.5 h a precipitate began to form. The reaction was monitored by UV spectroscopy, the λ_{max} shifting from 360 to 380 nm as the reaction progressed. After 6 h the reaction mixture was centrifuged. The precipitate was washed several times by stirring with small portions of ethanol, followed by centrifugation and pipetting off the supernatants. On sublimation of the solids at 170 °C and 10 μm of pressure a yellow material deposited on the cold finger. This material (λ_{max} 320, 255 nm) was discarded. Material was collected after the ratio of the 380 and 325 nm peaks became constant. The residue was 2-(6′-hydroxy-2′-benzothiazolyl)-4*H*-1,3-thiazin-4-one: 58 mg (47%); mp 250 °C; IR (KBr) 3100 (broad), 1615 cm^{-1}; UV λ_{max} (95% EtOH) 385, 275 nm and with base 490, 300, and 250 nm; NMR (Me_2SO-d_6) δ 8.35 (d, 1, J = 10.5 Hz), 8.05 (d, 1, J = 8.0 Hz), 7.64 (d, 1, J = 2.5 Hz), 7.26 (d of d, 1, J = 2.5, 8.0 Hz), 6.70 (d, 1, J = 10.5 Hz); mass spectrum, *m/e* 262 (56), 176 (100), 149 (54), 86 (72). Anal. Calcd for $C_{11}H_6N_2S_2O_2$: C, 50.36; H, 2.29; N, 10.68; S, 24.42. Found: C, 50.46; H, 2.40; N, 10.44; S, 24.63.

2-(6′-Hydroxy-2′-benzothiazolyl)-5,6-dihydro-4*H*-1,3-thiazin-4-one (11). 6-Hydroxy-2-cyanobenzothiazole[3] (100 mg, 0.568 mmol) was dissolved in ca. 0.5 mL of freshly distilled dry THF; methyl 3-mercaptopropionate (732 mg, 6.11 mmol) was added. The solution was then deaerated by bubbling dry nitrogen through the solution. A 4:1 triethylamine–acetic acid mixture (20 microdrops) was added. The solution was refluxed and the reaction was monitored by UV spectroscopy.

The reaction was stopped after 6 h when the 490-nm peak began to decrease relative to a peak at 300 nm. The reaction mixture was evaporated to give an orange oil which was dissolved in THF and applied to six preparative silica gel TLC plates. The plates were eluted with a 4:1 ethyl acetate–benzene mixture. The corresponding bands from each TLC plate were scraped off, combined, and eluted with 95% ethanol. The yellow fluorescent band (red when basic) at R_f ~0.40 yielded 2-(6′-hydroxy-2′-benzothiazolyl)-5,6-dihydro-4*H*-1,3-thiazine-4-one (62.5 mg, 42%). The material was purified by removing volatiles at 10 μm and 180 °C. The residue had the following properties: IR (KBr) 3270 (broad), 1655 cm^{-1}; UV λ_{max} (95% EtOH) 385, 278 and with base 490, 335, 295 nm; mass spectrum, *m/e* 264 (35), 236 (100), 194 (21), 176 (75), 149 (31).

Attempted Oxidation of 2-(6′-Hydroxy-2′-benzothiazolyl)-5,6-dihydro-4*H*-1,3-thiazin-4-one (11). *N*-Chlorosuccinimide Method. 2-(6′-Hydroxy-2′-benzothiazolyl)-5,6-dihydro-4*H*-1,3-thiazin-4-one (0.518 mg, 1.96×10^{-3} mmol) was dissolved in ca. 0.5 mL of freshly distilled glyme. A solution of *N*-chlorosuccinimide (0.400 mg, 3×10^{-3} mmol) in glyme was added at room temperature and the reaction mixture was stirred under N_2. The reaction was followed by TLC (4:1 ethyl acetate–benzene on silica gel) and by liquid chromatography (LC) (12 ft corasil II; 3:1 hexanes–glyme). No compound with a UV spectrum or retention time consistent with the desired product was obtained. **Cl_2 Method:** The reaction operations were similar to the above methods except a Cl_2–glyme solution was used as the source of chlorine. One equivalent of Cl_2 was added to the dihydrothiazine–glyme solution at −78 °C in the dark.[12] No compound with a UV spectrum or retention time consistent with the desired product was obtained.

Chemiluminescence of the Isopropylidene Compound 4. A solution of **4** in dimethyl sulfoxide (2 mL, $M = 2.6 \times 10^{-5}$) was saturated with oxygen. The addition of 0.1 mL of a 1.4×10^{-2} M solution of potassium phenolate at 25 °C led to red light emission, λ_{max} 585 ± 10 nm (fwhm 1480 cm^{-1}) with a half-life of ~1100 s. The addition of 3 molar equiv of oxyluciferin **2** (fluorescence λ_{max} ~630 mm) led to emission at 624 nm. The quantum yield for **4** determined with the luminol standard[17] was 0.025 einstein/mol. At 55 °C the half-life was ~120 s, and the initial emission at 585 nm shifted to 603 nm as the light intensity dropped to the point that spectra could no longer be measured. For 1.2×10^{-6} M solutions at 55 °C, the emission occurred at 626 ± 20 nm; shortly after the first half-life, the intensity had dropped to a point where spectra could no longer be measured. The fluorescence spectrum for the 25 °C run showed λ_{max} at 508 nm initially (λ_{exc} 468 nm) (4 fluoresces at 510 nm) which shifted to 633 nm after standing overnight. The spent reaction mixture showed one spot on silica gel TLC (R_f 0.86; 1:1 benzene–ethyl acetate) corresponding to oxyluciferin **2**.

Chemiluminescence of Ester 1. A solution of **1** in oxygen saturated dimethyl sulfoxide (3.5×10^{-5} M; 3 mL) glowed very weakly; strong red light emission at λ_{max} 633 nm occurred when 0.1 mL of a solution of 1.4×10^{-2} M potassium phenolate in dimethyl sulfoxide was added at 25 °C (half-life = ~9 s). The spent reaction mixture showed two spots on TLC carried out as described above; they were identified as oxyluciferin **2** and isopropylidene compound **4** on the basis of the R_f's (0.86 and 0.54, respectively; the R_f for ester **1** is 0.68).

Chemiluminescence of Ester 1 in the Presence of Isopropylidene Compound 4. Dimethyl sulfoxide solutions of **1** ($M = 3.51 \times 10^{-5}$) and **4** ($M = 2.72 \times 10^{-5}$) were mixed in volume ratios of 3:1, 1:1, and 1:3, corresponding to mol ratios of 3.9, 1.3, and 0.43. To 4 mL of these solutions was added 0.1 mL of 1.4×10^{-2} M potassium phenolate solutions in the same solvent. The λ_{max} of the chemiluminescent emissions were 605, 599, and 584 nm, respectively.

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Registry No.—**1**, 54495-41-9; **3**, 66069-71-4; **4**, 66069-72-5; **4** trifluoroacetyl derivative, 66069-73-6; **5**, 66069-74-7; **10**, 66069-75-8; **11**, 66069-76-9; ethoxyacetylene, 927-80-0; *trans*-2-methyl-3-bromo-2-butenoyl chloride, 66069-77-0; β-bromoangelic acid, 35057-99-9; 6-hydroxybenzothiazole-2-thiocarboxamide, 36727-08-9; propiolic acid, 471-25-0; 6-hydroxy-2-cyanobenzothiazole, 939-69-5; methyl 3-mercaptopropionate, 2935-90-2.

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