was neutralized with silver carbonate and filtered without suction to avoid any loss due to evaporation.

A portion (0.1 ml) of the filtrate was fractionated on paper, using solvent D, and a quantitative determination of 1-*O*-methyl-D-erythritol and 1,4-di-*O*-methylerythritol was carried out as described above. A 2-ml aliquot was found, by reference to the standard curves, to contain 13 μg of 1-*O*-methyl-D-erythritol and 60 μg of 1,4-di-*O*-methylerythritol. The molar ratio of mono- to di-*O*-methylerythritol as calculated from these results was 1:10.5 giving a value of 12.5 for the average chain length assuming that for every mole of 1-*O*-methyl-D-erythritol, there is also present 1 mole of 1,3-di-methylglyceritol which arises from the nonreducing ends. In a duplicate experiment, the molar ratio was found to be 1:11.5 thereby giving an average chain length of 13.5. The duplicate was carried out under the same conditionas used for the experiment except that the chromatogram was developed for 16–17 hr in solvent A in place of D. This change of irrigating solvent was necessitated by the fact that the 1-*O*-methyl-D-erythritol showed only a small movement from the origin when solvent D was used and, hence, it was contaminated with any impurities which likewise remained on the starting line of the chromatogram.

Determination of Chain Length of Clam Glycogen by Gas–Liquid Partition Chromatography.—An F and M gas chromatograph model 500 with thermoconductivity cell detector and a column packed with 20% silicone oil DC 500 (Dow Corning) on Teflon (polytetrafluoroethylene) (w/w) was employed. The operating conditions are described in the legend under Figure 2.

Standards (50–80 μg) of 1,3-di-*O*-methylglyceritol (VIII), methoxyacetaldehyde dimethylacetal (IX), 1,4-di-*O*-methylerythritol (X), and 1-*O*-methyl-D-erythritol (XI) in methanol solution (5–15 μl) were chromatographed individually as well as their synthetic mixtures. The retention time of each compound is recorded in Table V. This is the time in minutes between the point of appearance of methanol curve and the apex of the peak of the compound being tested. A mixture containing components X and XI was not resolved under these conditions.

Determination of the Molar Ratio of VIII, IX, and X in the Methanolysate of Methylated Hexahydric Alcohol from Methyl β-Lactoside.—The methylated hexahydric alcohol (about 10 mg) was methanolyzed by refluxing for 4.5 hr with 2% methanolic hydrogen chloride (1 ml). After neutralizing with silver carbonate, the solution was filtered (no suction), and an aliquot (10 μl) of the filtrate was subjected to gas chromatographic analysis. The gas chromatogram showed three peaks (Figure 3). The molar ratio of methoxyacetaldehyde dimethylacetal (IX), 1,3-di-*O*-methylglyceritol (VIII), and 1,4-di-*O*-methylerythritol (X) thus found was 2:0.95:0.76; theoretical, 2:1:1. Therefore, an appropriate correction for the components VIII and X was applied while computing their molar proportion in the methanolysis products of the methylated glycogen polyalcohol.

TABLE V

RETENTION TIMES OF THE STANDARDS RELATIVE TO METHANOL

Compound	Retention time, Min	Retention time, Sec
Methoxyacetaldehyde dimethylacetal	7	16
1,3-Di-*O*-methylglyceritol	8	48
1,4-Di-*O*-methylerythritol	13	6
1-*O*-Methyl-D-erythritol	14	0
1,4-Di-*O*-methylerythritol + 1-*O*-methyl-D-erythritol	12	12

Determination of the Chain Length of Clam Glycogen.—A solution (10–15 μl) of the methanolysis products of methylated glycogen polyalcohol (0.8–1.0 mg) in methanol (5–15 μl.) was injected into the column. The chain length (*cl*) is given by the number of moles of 1-*O*-methyl-D-erythritol and 1,4-di-*O*-methylerythritol plus 1 or by the number of moles of methoxyacetaldehyde dimethylacetal per mole of 1,3-di-*O*-methylglyceritol.

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Analogs of Firefly Luciferin. III[1]

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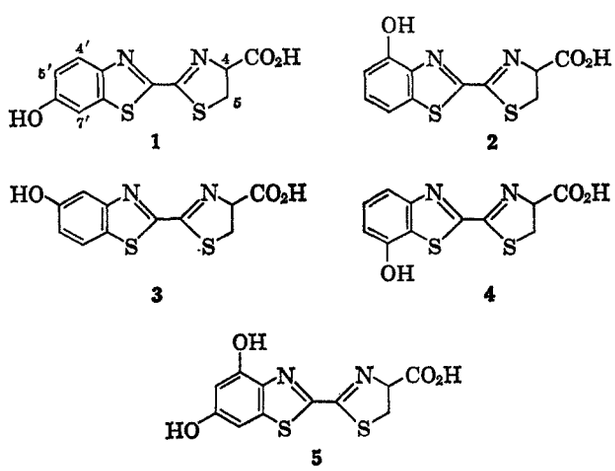
The arylhydroxy isomers of firefly luciferin (compounds **2–4**) and the derivative, 4-hydroxyluciferin (**5**), have been synthesized and tested. Only compound **5** proved active in the enzymatic assay. Physical properties of the luciferins are recorded.

In continuation of our work on firefly luciferin[2] (**1**) and on its analogs,[1] we have prepared the three hydroxy positional isomers of luciferin (**2–4**) and also the dihydroxy analog **5**. (See Chart I.) The 4-hydroxy analog **2** was prepared by the stepwise procedure used in the original synthesis of luciferin,[2] whereas the other analogs were prepared using the modification of Seto, *et al.*[3]

"4-Hydroxyluciferin" (**2**).—Analog **2**, 2-(4-hydroxy-2-benzothiazolyl)-Δ²-thiazoline-4-carboxylic acid, was synthesized from *o*-anisidine. Condensation of this

CHART I

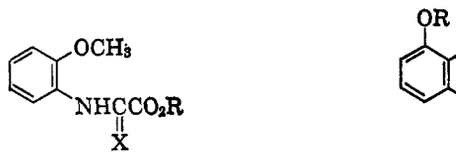


(1) (a) Paper I: E. H. White, H. Wörther, G. F. Field, and W. D. McElroy, *J. Org. Chem.*, **30**, 2344 (1965); (b) paper II: E. H. White, H. Wörther, H. Seliger, and W. D. McElroy, *J. Am. Chem. Soc.*, **88**, 2015 (1966).

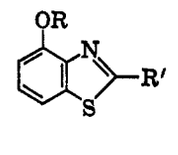
(2) E. H. White, F. McCapra, and G. F. Field, *ibid.*, **85**, 337 (1963).

(3) S. Seto, K. Ogura, and Y. Nishiyama, *Bull. Chem. Soc. Japan*, **36**, 331 (1963). This group of workers [*ibid.*, 173 (1963)] also prepared a tropolone analog of firefly luciferin.

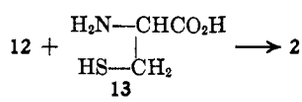
compound with ethyl oxalate gave ethyl N-(2-methoxyphenyl)oxamate (**6**), which was converted into thioamide acid **7** by treatment with phosphorus pentasulfide followed by hydrolysis; oxidation with potassium ferricyanide then yielded 4-methoxybenzothiazole-2-carboxylic acid (**8**). Esterification and treatment of the ester **9** with ammonia yielded amide **10**; this compound was dehydrated with phosphorus oxychloride to yield the nitrile **11**. Demethylation with pyridine hydrochloride then yielded 4-hydroxy-2-cyanobenzothiazole (**12**). The condensation of this compound with cysteine (**13**) yielded the 4-hydroxy analog of firefly luciferin (**2**).



6, X = O; R = C_2H_5
7, X = S; R = H



8, R = CH_3; R′ = CO_2H
9, R = CH_3; R′ = CO_2CH_3
10, R = CH_3; R′ = $CONH_2$
11, R = CH_3; R′ = CN
12, R = H; R′ = CN



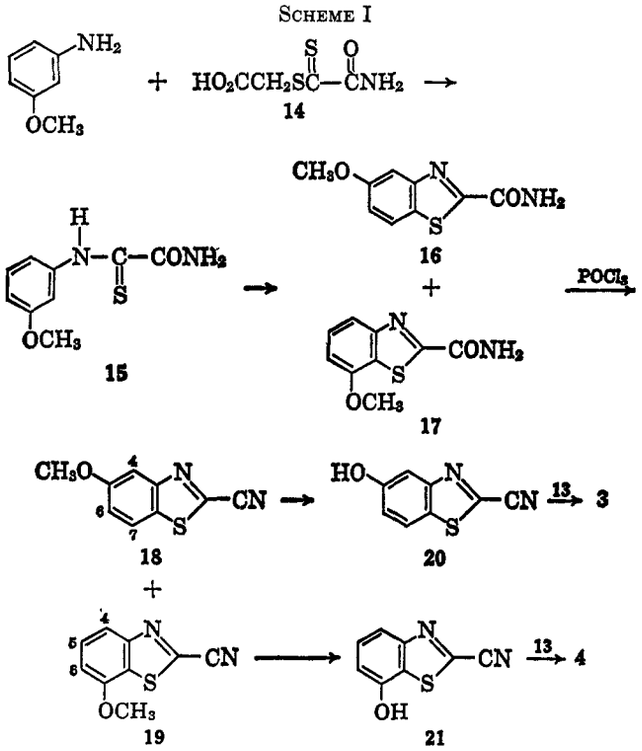
13

"**5- and 7-Hydroxyluciferins**" (**3 and 4**).—These compounds were prepared from 3-methoxyaniline by the method of Seto, *et al.*[3] (See Scheme I.) The condensation of 3-methoxyaniline with carbamoylthiocarbonylthioacetic acid (**14**)[3] yielded the thiooxanilamide **15**, which was oxidized with potassium ferricyanide to yield a mixture of amides **16** and **17**. Treatment of the amide mixture with phosphorus oxychloride and pyridine yielded the corresponding nitriles, **18** and **19**, which were separated and purified by chromatography on silica gel. The isomers were identified by means of their proton nmr spectra. The spectrum of compound **18** is of the AMX type in which A and X are not detectibly coupled; the spectrum resembles that of luciferin itself. The spectrum of compound **19** shows a more complex multiplet in which all of the protons are mutually coupled (Experimental Section). In this connection, concerning the peaks for the 4- and 7-protons, it appears that the sulfur and nitrogen atoms play secondary roles and that the methoxy group in this environment is a weak *shielding* group: *i.e.*, the 4- or 7-proton adjacent to the methoxy group is always at the higher field. In compound **18**, the 4-proton is at τ 2.35 and the 7-proton at τ 2.13; in the O-methyl ether of luciferin (O–Me of **1**), the 4-proton is at τ 2.00, and the 7-proton at τ 2.37;[1a] in compound **19**, the 4-proton is at τ 2.14. In support of the weak shielding of the methoxy group, we find that the 3-proton of 2-methoxy-4-nitroaniline in deuteriochloroform is a doublet at τ 2.8 (J = 2.2 cps) and the 5-proton is a doublet of doublets centered at τ 2.72 (J = 2.2 and 8.3 cps) (the 6-proton is a doublet at τ 3.81, J = 8.3 cps).

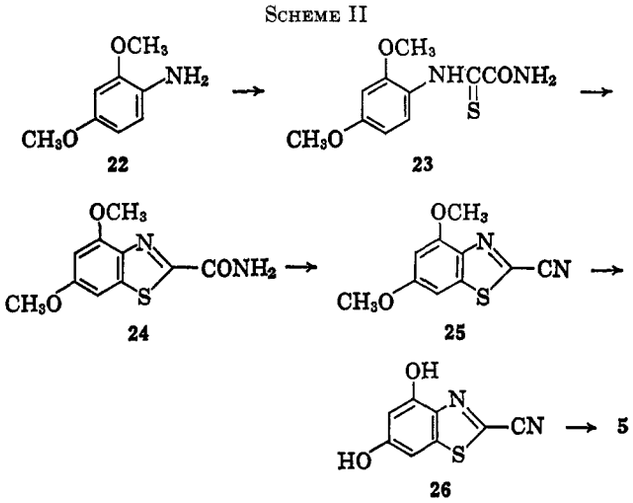
The nitriles were separately demethylated with pyridine hydrochloride and the hydroxynitriles **20** and **21** were condensed with cysteine to yield the luciferin analogs **3** and **4**. The infrared spectra of these compounds (in the 700–800- and 1600–2000-cm^{-1} regions) confirm the substitution patterns assigned.

4-Hydroxyluciferin (**5**).—This analog was prepared by a similar series of reactions (Scheme II).

SCHEME I



SCHEME II



The ultraviolet spectra of the analogs (Table I) show marked differences, although family relationships can be noted. The infrared spectra of the analogs in KBr were complex, and fewer similarities were noted than

TABLE I
ULTRAVIOLET ABSORPTION OF FIREFLY LUCIFERIN AND ANALOGS

Compd	Absorption bands, mμ (log ε), in 95% ethanol		
2	257 (4.06)	303 (4.12)	
3	229 (4.29)	294 (4.17)	350 (3.64)
1	269 (3.85)	330 (4.26)	
4	257 (4.28)	301 (4.16)	365 (sh)
5	269 (4.04)	324 (4.17)	360 (sh)

in the previous group.[1a] All of the compounds showed a strong band in the 11.2–11.5-μ region, however, and most of the spectra contained a strong band between 6.2 and 6.4 μ.

4-Hydroxyluciferin (**5**) tested positive in the *in vitro* luminescence (luciferase, ATP, Mg^{2+}, oxygen), but the emitted light was red rather than the yellowish green light obtained using the authentic luciferin. All the other analogs that we have reported here and elsewhere,[1] with the exception of the 6-amino analog, which also yielded red light, tested negative in light production.

Experimental Section

The ultraviolet spectra were measured in 95% ethanol.

DL-**Cysteine** (**13**).—The buffered solution was freshly prepared[1a] for each luciferin analog synthesis.

2-(4-Hydroxy-2-benzothiazolyl)-Δ^2-thiazoline-4-carboxylic Acid (**2**). **A. Ethyl N-(2-Methoxyphenyl)oxamate** (**6**).—Ethyl oxalate (111 g, 0.758 mole) and *o*-anisidine (68.5 g, 0.556 mole) were refluxed for 11 hr, during which time a precipitate formed. Ethanol (200 ml) was added and the mixture was brought to a boil and filtered. Cooling yielded white crystals of the product. Recrystallization from alcohol gave 87.5 g (0.392 mole, 70.5%) of oxamate **6**, mp 83.5–84.7° (lit.[4] mp 83.5).

B. N-(2-Methoxyphenyl)thiooxamic Acid (**7**).—Ethyl N-(2-methoxyphenyl)oxamate (130 g, 0.582 mole) was dissolved in 1300 ml of boiling xylene, and phosphorus pentasulfide (39 g, 0.175 mole) was added slowly to the refluxing solution. The solution gradually turned black. Refluxing was continued until the peak at 301 mμ in the ultraviolet spectrum of relactant was replaced by the 358-mμ peak of the product. The reaction mixture was then cooled and extracted with five 600-ml portions of 1 *N* sodium hydroxide. The basic extract was filtered and cooled to 2°. The crude N-(2-methoxyphenyl)thiooxamic acid was precipitated by making the solution strongly acidic with concentrated hydrochloric acid (pH 2). The yellow-orange precipitate was washed with cold water and dried. There was obtained 100 g (0.417 mole, 71.8%) of crude **7**. This material was used without purification in the next step.

C. 4-Methoxybenzothiazole-2-carboxylic Acid (**8**).—The crude N-(2-methoxyphenyl)thiooxamic acid described above was dissolved in 1 *N* sodium hydroxide (1800 ml). The solution was filtered and then oxidized with potassium ferricyanide (480 g, 1.46 moles) by slowly dropping the thioamide solution into the ferricyanide dissolved in water (1200 ml), while keeping the temperature of the reaction mixture below 10°. A vile odor developed during the course of the reaction. The addition required about 4 hr. Fifteen minutes after the addition of the thioamide was completed, the precipitated salt of 4-methoxybenzothiazole-2-carboxylic acid was filtered off and washed with dilute sodium hydroxide. The mother liquor was saved and further product was precipitated from it by the addition of concentrated sodium hydroxide (100 ml) and by cooling the solution on ice. The salt that formed was filtered and washed with a small quantity of dilute sodium hydroxide. The two batches of salt were combined and dried in a desiccator. This sodium salt of **8** was dissolved in water and filtered. The solution was acidified slowly by the dropwise addition of concentrated hydrochloric acid; a small amount of sticky precipitate formed, which was removed. The remaining solution was acidified to pH 2, and the precipitate was collected and dried under vacuum. The solid weighed 54 g (0.258 mole) and represented a 54.5% yield of **8** based on oxamate **6**. This material (mp 102–105°) was used in the next step without further purification.

D. 2-Carbomethoxy-4-methoxybenzothiazole (**9**).—Crude 4-methoxybenzothiazole-2-carboxylic acid (54 g, 0.258 mole) was dissolved in methanol (2200 ml), and a solution of hydrochloric acid (anhydrous, 80 g) in methanol (800 ml) was added. After standing for 2 days at room temperature, the solution was cooled to −10° overnight and the precipitate that formed was collected. The yield was 28 g (0.126 mole, 48.6%) of **9**, mp 133–137.5°. This material was recrystallized from methanol to give 21.5 g (37.3%) of the pure ester. Further recrystallization raised the melting point to 137.5–138.5°: λ_{max} 249 mμ (ϵ 1.34 × 10^4), 293 (1.10 × 10^4), and 341 (3.70 × 10^3).

Anal. Calcd for $C_{10}H_9NO_3S$: C, 53.78; H, 4.24; N, 6.20; S, 14.50. Found: C, 53.79; H, 4.06; N, 6.27; S, 14.36.

E. 4-Methoxybenzothiazole-2-carboxamide (**10**).—2-Carbomethoxy-4-methoxybenzothiazole (24 g, 0.108 mole) was dissolved in methanol (480 ml) with heating and the solution was saturated with gaseous ammonia for 3 hr. During the saturation process the mixture was heated periodically to keep the methyl ester from precipitating. The mixture was cooled on ice and the precipitate was collected. There was obtained 19 g (9.12 mmoles, 85%) of **10**, mp 212–215°. Recrystallization from ethyl acetate raised the melting point to 214.4–215°: λ_{max} 237 mμ (ϵ 1.40 × 10^4), 288 (9.85 × 10^3), and 331 (3.34 × 10^3).

Anal. Calcd for $C_9H_8NO_2S$: C, 51.91; H, 3.87; N, 13.45; S, 15.40. Found: C, 51.83; H, 4.00; N, 13.59; S, 16.05 (15.56 on second trial).

F. 4-Methoxy-2-cyanobenzothiazole (**11**).—A solution of 4-methoxybenzothiazole-2-carboxamide (5.57 g, 26.7 mmoles) in phosphorus oxychloride (30 ml) was refluxed slowly for 50 min. The solution was cooled and the phosphorus oxychloride was evaporated under vacuum by use of a rotary evaporator. The remaining solid material was treated with a mixture of sodium bicarbonate (dilute solution), ice, and ether. Sufficient sodium bicarbonate was used to adjust the pH to 7. The ether layer was separated, washed with a small amount of water, and dried over sodium sulfate. The water layer and the remaining solid were extracted with three 150-ml portions of chloroform. The chloroform was washed with water and dried over sodium sulfate. The combined chloroform and ether solutions were filt red and evaporated to dryness. The solid that resulted was dissolved in ether and the solution was poured through a short column of alumina. The nitrile was eluted with ether and the recovered material was recrystallized from methanol. There was obtained 4.32 g (22.7 mmoles, 85%) of **11**, mp 127–130°. Recrystallization raised the melting point to 128.5–130°: λ_{max} 249 mμ (ϵ 1.51 × 10^3), 294 (1.19 × 10^4), and 345 (3.94 × 10^3).

Anal. Calcd for $C_9H_6N_2OS$: C, 56.82; H, 3.17; N, 14.73; S, 16.86. Found: C, 56.76; H, 3.38; N, 14.69; S, 17.04.

G. 4-Hydroxy-2-cyanobenzothiazole (**12**).—2-Cyano-4-methoxybenzothiazole (1.05 g, 5.51 mmoles) was treated with pyridine hydrochloride (18 g, 0.156 mole) under anhydrous conditions. The temperature was kept between 170 and 195° for 135 min. The mixture was cooled, dissolved in water (150 ml), and extracted with ethyl acetate (four 50-ml portions). The extract was filtered and 0.05 g of Norit A was added. After 1 hr, the mixture was filtered and the solvent was removed under vacuum. The treatment with Norit A was then repeated in chloroform. Recrystallization from chloroform gave 0.580 g (3.29 mmoles, 59.7%) of **12**, in the form of yellow needles, mp 159–161°. A small quantity was sublimed under high vacuum for analysis: mp 159.5–161°; λ_{max} 251 mμ (ϵ 16.2 × 10^3), 294 (11.0 × 10^3), and 353 (3.44 × 10^3).

Anal. Calcd for $C_8H_4N_2OS$: C, 54.53; H, 2.29; N, 15.90; S, 18.20. Found: C, 54.43; H, 2.45; N, 15.80; S, 18.38.

H. 2-(4-Hydroxy-2-benzothiazolyl)-Δ^2-thiazoline-4-carboxylic Acid (**2**).—Cysteine was prepared from 218 mg (0.906 mmole) of DL-cystine as described.[1a] Methanol (25 ml) was added to the aqueous solution of cysteine, followed by a solution of 2-cyano-4-hydroxybenzothiazole (0.354 g, 2.05 mmoles) in 25 ml of methanol (the reaction was carried out under nitrogen). The color of the solution changed rapidly during the addition from faint yellow to a deep orange. The mixture was allowed to react in darkness for 90 min. The reaction mixture was diluted with 40 ml of water, and the methanol was largely removed under vacuum. The remaining solution was extracted with ethyl acetate (100 ml), filtered, flushed with nitrogen to remove dissolved ethyl acetate, diluted to 130 ml with water, and acidified with 10% hydrochloric acid. There resulted a precipitate of yellow needles which was washed with water and dried under vacuum at 65°. There was obtained 0.477 g (0.170 mmole, 93.7%) of **2**, mp 169–171° dec. Recrystallization was effected from hot acetone to which an excess of cyclohexane was added (under nitrogen and in dim light), mp 171–172°. See Table I for the ultraviolet absorption; infrared, 745 and 780 cm^{-1} (KBr).

Anal. Calcd for $C_{11}H_8N_2O_3S_2$: C, 47.14; H, 2.85; N, 10.00; S, 22.88. Found: C, 47.18; H, 2.95; N, 9.85; S, 22.88.

(4) P. A. Petyunin and I. S. Berdinskiĭ, *Zh. Obshch. Khim.*, **21**, 1703 (1951).

2-(5-Hydroxy-2-benzothiazolyl)-Δ^2-thiazoline-4-carboxylic Acid (3) and 2-(7-Hydroxy-2-benzothiazolyl)-Δ^2-thiazoline-4-carboxylic Acid (4). A. 3-Methoxythiooxanilamide (15).—An alcoholic solution of carbamoylthiocarbonylthioacetic acid (14) was prepared starting with 15 g of trichloroacetamide by the method of Seto, *et al.*[3] The reagent was filtered and added to 8.26 g (67 mmoles) of freshly distilled 3-methoxyaniline. The amine dissolved and the mixture was allowed to rest at −2° for 2 days. There precipitated yellow needles, which were collected and washed successively with large quantities of water and then with some ice-cold alcohol. There was obtained 1.76 g (8.40 moles, 12.5%) of **15**, mp 127–129°. Recrystallization from methanol raised the melting point to 130.5–131°: λ_{max} 340 mμ (ε 9450).

Anal. Calcd for $C_9H_{10}N_2O_2S$: C, 51.42; H, 4.80; N, 13.33; S, 15.24. Found: C, 51.40; H, 4.99; N, 13.25; S, 15.40.

B. 2-Carbamoyl-5- (and 7-) methoxybenzothiazoles (16 and 17).—3-Methoxythiooxanilamide (780 mg, 3.7 mmoles), dissolved in a solution of sodium hydroxide (10%, 64 ml), was added over a period of 15 min to a well-stirred solution of 1.5 g (4.6 mmoles) of potassium ferricyanide in 32 ml of water. The mixture was stirred for an additional 30 min and the temperature was kept around 10°. The brown precipitate was filtered and washed carefully with water. There was obtained a mixture of the 5- and the 7-methoxy-2-carbamoylbenzothiazoles (485 mg, 2.32 mmoles, 62.8%, mp 173–202°). Part of this mixture was recrystallized from ethanol for analysis. The analytical sample melted at 175–200°.

Anal. Calcd for $C_9H_8N_2O_2S$: C, 51.93; H, 3.86; N, 13.46; S, 15.39. Found: C, 52.14; H, 3.98; N, 13.26; S, 15.15.

C. 2-Cyano-5-methoxybenzothiazole (18) and 2-Cyano-7-methoxybenzothiazole (19).—The crude mixture of amides (5- and 7-methoxy-2-carbamoylbenzothiazoles, 1.020 g, 4.9 mmoles), obtained under B, was dissolved in pyridine (20 ml) with slight warming. The solution was cooled to −10° and phosphorus oxychloride (1.2 ml, cooled to 5°) was added during a period of 15 min with occasional cooling. The mixture was allowed to stand for 2 hr. Cyclohexane (150 ml) was added and the slurry was cautiously treated with water (100 ml). The aqueous phase was extracted with two additional 100-ml fractions of cyclohexane. The solvent was evaporated leaving a mixture of **18** and **19**. The mixture (0.850 g) was dissolved in a minimum of ethyl acetate, SiO_2 (25 g) was added, and the solvent was removed under vacuum. This mixture was placed on the top of a column of silica gel (300 g, 30 × 3.5 cm) prepared in petroleum ether (bp 35–55°). Ethyl acetate–petroleum ether mixtures (1:200 and 1:100) served as eluents. There was eluted a greenish fluorescent band followed immediately by a blue fluorescent band.

All fractions having a melting point of 103–108° (from the greenish fluorescent band) were collected to give **19** (358 mg, 1.88 mmoles, 38.5%). Recrystallization from cyclohexane raised the melting point to 108–109°: λ_{max} 218 mμ (ε 37,895), 250.5 (16,670), 294 (7567), and 339 (1869); nmr (CCl_4), singlet at τ 5.90 (OCH_3), pair of doublets at τ 2.96 (J = 1.5 and 7.5 cps, probably 6-H), pair of doublets at τ 2.14 (J = 1.3 and 8.4 cps, probably 4-H), and a multiplet of three peaks at τ 2.28, 2.40, and 2.54 (5-H).

Anal. Calcd for $C_9H_6N_2OS$: C, 56.82; H, 3.15; N, 14.73; S, 16.86. Found: C, 56.80; H, 3.29; N, 14.70; S, 16.96.

All the fractions in the chromatogram having a melting point of 95–98° (from the blue fluorescent band) were collected to give **18** (272 mg, 1.43 mmoles, 29.2%). Recrystallization from cyclohexane raised the melting point to 97.5–99°: λ_{max} 225 mμ (ε 19,800), 286 (10,300), and 340 (1940); nmr (CCl_4), singlet at τ 6.03 (OCH_3), doublet at τ 2.13 (J = 9.5 cps, 7-H), doublet at τ 2.35 (J = 2.4 cps, 4-H), and a pair of doublets at τ 2.71 (J = 9.5 and 2.4 cps, 6-H); infrared (CCl_4), broad band at 860 cm^{-1}; the second band characteristic of a 1,2,4-trisubstituted benzene ring was masked by the CCl_4 absorption.

Anal. Calcd for $C_9H N_2OS$: C, 56.82; H, 3.15; N, 14.73; S, 16.86. Found: C, 56.64; H, 3.30; N, 14.83; S, 16.69.

D. 2-Cyano-7-hydroxybenzothiazole (21).—2-Cyano-7-methoxybenzothiazole (358 mg, 1.88 mmoles) was treated with pyridine hydrochloride (14.8 g, 128 mmoles) for 6 hr at 175°. During the reaction, a shift in the ultraviolet spectrum from 293 to 297.5 mμ was observed (ultraviolet in ethanol containing 1 drop of hydrochloric acid). The mixture was partitioned between 50 ml of water and 50 ml of ethyl acetate, and the aqueous layer was extracted again with three 50-ml portions of ethyl acetate. The extract was dried over sodium sulfate, filtered, and then taken to dryness along with 10 g of silica gel. The mixture was placed on the top of a column of silica gel (100 g, 19 × 3.3 cm) and elution was effected with 5 and 10% ethyl acetate–petroleum ether mixtures. A slightly blue, fluorescent main band was eluted which yielded 241 mg (1.37 mmoles, 72.7%) of 2-cyano-7-hydroxybenzothiazole, mp 222–224°. Recrystallization from a chloroform–ethyl acetate–cyclohexane mixture gave yellow plates: mp 226–227.5 dec; λ_{max} 252 mμ (ε 20,436), 297 (9259), and 347 (2352); infrared, 715, 780, 1740, 1830, and 1910 cm^{-1} (1,2,3-trisubstituted benzene ring).

Anal. Calcd for $C_8H_4N_2OS$: C, 54.53; H, 2.28; N, 15.90; S, 18.20. Found: C, 54.25; H, 2.43; N, 15.71; S, 18.46.

E. 2-Cyano-5-hydroxybenzothiazole (20).—2-Cyano-5-methoxybenzothiazole (272 mg, 1.43 mmoles) was treated with pyridine hydrochloride (14.2 g, 124 mmoles) at 175° for 7 hr. The reaction mixture was partitioned between 50 ml of water and 50 ml of ethyl acetate. The extraction was repeated with four 50-ml portions of ethyl acetate. The extract was dried with sodium sulfate, 10 g of silica gel was added, and the solvent was removed under vacuum. This mixture was slurried onto the top of a column of silica gel (100 g, 20 × 3.4 cm). Ethyl acetate (5%) in petroleum ether eluted the main band, which after washing with cyclohexane gave 161 mg (0.92 mmole, 64%) of **20**: mp 193.5–194.0°; λ_{max} 227 mμ (ε 18,900), 287 (10,562), and 351 (3620).

Anal. Calcd for $C_8H_4N_2OS$: C, 54.53; H, 2.28; N, 15.90; S, 18.20. Found: C, 54.29; H, 2.40; N, 15.75; S, 18.33.

F. 2-(7-Hydroxy-2-benzothiazolyl)-Δ^2-thiazoline-4-carboxylic Acid (4).—Cystine (68 mg, 0.565 mmole) was converted to cysteine in 5 ml of water as described.[1a] Methanol (5 ml, oxygen free) was added. 2-Cyano-7-hydroxybenzothiazole (105 mg, 0.596 mmoles) was dissolved in 10 ml of methanol and added to the cysteine solution. After 90 min, 150 ml of water was added, the pH of the solution was brought to 8.5, and the mixture was extracted with 50 ml of ethyl acetate. The solution was acidified and the luciferin analog was extracted into 80 ml of ethyl acetate. The extract was dried over sodium sulfate and the solvent was evaporated to a final volume of about 5 ml. The compound was precipitated by the addition of cyclohexane. There was obtained 140 mg (0.499 mmole, 88.2%) of **4**: mp 191–193° dec (fast heating), 185–189° dec (slow heating); ultraviolet, Table I; infrared, 717 and 780 cm^{-1} (1,2,3-trisubstituted benzene ring).

Anal. Calcd for $C_{11}H_8N_2O_3S_2$: C, 47.14; H, 2.85; N, 10.00; S, 22.88. Found: C, 46.90; H, 3.01; N, 10.03; S, 22.68.

G. 2-(5-Hydroxy-2-benzothiazolyl)-Δ^2-thiazoline-4-carboxylic Acid (3).—Cystine (53 mg, 0.22 mmole) was converted into cysteine in 5 ml of water as described.[1a] Methanol was added (5 ml, oxygen free). 2-Cyano-5-hydroxybenzothiazole (81 mg, 0.46 mmole) was dissolved in 10 ml of methanol and added to the cysteine solution. The mixture was allowed to stand for 3.5 hr. Water was added (50 ml) and the pH of the solution was adjusted to about 8.5. The solution was extracted with 30 ml of ethyl acetate. The aqueous layer was acidified and the luciferin analog was extracted into ethyl acetate. This extract was dried over sodium sulfate and the ethyl acetate was evaporated to a small volume. **3** was precipitated by the addition of cyclohexane. There was obtained 101 mg (0.36 mmole, 81.7%) of the compound: mp 201–203°; ultraviolet, Table I; infrared, 840 and 885 cm^{-1} (1,2,4-trisubstituted benzene ring).

Anal. Calcd for $C_{11}H_8N_2O_3S_2$: C, 47.14; H, 2.85; N, 10.00; S, 22.88. Found: C, 47.10; H, 2.95; N, 10.09; S, 23.04.

2-(4,6-Dihydroxy-2-benzothiazolyl)-Δ^2-thiazoline-4-carboxylic Acid (5). A. 2,4-Dimethoxythiooxanilamide (23).—Using the method of Seto, *et al.*,[3] a solution of carbamoylthiocarbonylthioacetic acid (14) was prepared in aqueous alcohol starting with 1.85 g of trichloroacetamide. The freshly prepared reagent (filtered) was added to a solution of 2,4-dimethoxyaniline (0.946 g, 0.617 mmole, freshly distilled under vacuum) in 5 ml of ethanol (flushed with nitrogen) and 5 ml of water (flushed with nitrogen). The reaction mixture was allowed to stand for 14 hr. The precipitated yellow needles were collected and washed thoroughly with water. There was obtained 0.722 g (3.0 mmoles, 48.6%) of **23**, mp 182–184°. For analysis, the material was recrystallized from ethanol: mp 185–186°; λ_{max} 233 mμ (ε 11,600), 252 (sh) (8220), and 370 (12,795).

Anal. Calcd for $C_{10}H_{12}N_2O_3S$: C, 49.98; H, 5.00; N, 11.66; S, 13.35. Found: C, 49.68 (49.85); H, 5.03 (5.20); N, 11.38 (11.55); S, 13.39.

B. 2-Carbamoyl-4,6-dimethoxybenzothiazole (24).—Potassium ferricyanide (9.0 g, 27.4 mmoles) was dissolved in 20 ml of

water and the solution was cooled to about 10°. A solution of 2,4-dimethoxythiooxanilamide (0.501 g, 2.08 mmoles) in 12% aqueous potassium hydroxide (45 ml) was added slowly to the solution of potassium ferricyanide with stirring. Almost immediately, a yellowish precipitate formed and a vile odor developed. After the addition was complete, the mixture was stirred for an additional 30 min. The yellowish precipitate was collected, washed thoroughly with water, and dried at 65° under vacuum. There was obtained 0.382 g (1.605 mmoles, 77.0%) of **24**, mp 254–256°. For analysis, the product was recrystallized twice from ethanol. There resulted white crystals: mp 258.5–259.5°; λ_{max} 259 mμ (ε 10,867) and 310 (11,480).

Anal. Calcd for $C_{10}H_{10}N_2O_3S$: C, 50.41; H, 4.23; N, 11.76; S, 13.46. Found: C, 50.44 (50.60); H, 4.27 (4.43); N, 11.53 (11.73); S, 13.54.

C. 2-Cyano-4,6-dimethoxybenzothiazole (25).—2-Carbamoyl-4,6-dimethoxybenzothiazole (0.375 g, 1.57 mmoles) was dissolved in 20 ml of pyridine, and 4.5 ml of phosphorus oxychloride was added slowly within 5 min. The solution became warm and turned red in color; it was allowed to stand for 2 hr. After this time a heavy precipitate had formed. The mixture was diluted with 50 ml of ethyl acetate and cooled to 5°; 50 ml of water was then carefully added. The aqueous phase was extracted a second time with ethyl acetate (100 ml); the ethyl acetate layer was washed with 5 ml of water, dried with sodium sulfate, and then treated with a small amount of Norit A. The solvent was removed under vacuum and the yellowish residue was dried over concentrated sulfuric acid under vacuum to constant weight. There was obtained 0.309 g (1.41 mmoles, 90%) of **25**, mp 138.5–140.5° (some sintering at 135°). For analysis, part of this material was sublimed under high vacuum (bath temperature 100–125°) and recrystallized from cyclohexane containing a few drops of chloroform. There resulted white needles: mp 141–142°; λ_{max} 262 mμ (ε 12,465), 312 (12,815), and 338 (8504).

Anal. Calcd for $C_{10}H_8N_2O_2S$: C, 54.53; H, 3.66; N, 12.72; S, 14.56. Found: C, 54.74; H, 3.72; N, 12.91; S, 14.28.

D. 2-Cyano-4,6-dihydroxybenzothiazole (26).—2-Cyano-4,6-dimethoxybenzothiazole (0.915 g, 4.15 mmoles) was treated with pyridine hydrochloride (20.8 g, 208.0 mmoles) at 170–180° for 3 hr under anhydrous conditions. Aliquots were removed several times and the ultraviolet absorption was measured in ethanol containing a small quantity of hydrochloric acid. A peak at 309 mμ shifted to 315 and 350 mμ during the course of the reaction. The reaction mixture was dissolved in 150 ml of water and extracted with four 50-ml portions of ethyl acetate. The ultraviolet absorption of the last extract was very weak. The volume of the combined extracts was brought to exactly 250 ml, and 25 ml was taken for a first test on chromatography. This 25-ml portion was evaporated to dryness with silica gel (2.2 g) and the mixture was placed on the top of a column of silica gel (30 g) prepared with petroleum ether. Ethyl acetate in petroleum ether (1:4) served as the eluent, 50-ml fractions being taken. Fraction 4 contained a blue fluorescent band having λ_{max} at 313 and 350 mμ in ethanol. Addition of 1 drop of sodium hydroxide solution (10%) shifted the absorption to λ_{max} 303 (very strong) and 388 mμ. The residue obtained on evaporation of this fraction was very small and therefore it was neglected. Fractions 8 and 11 showed absorption at λ_{max} 317 and 352 mμ, which shifted to λ_{max} 265, 288, 362, and 427 mμ in basic solution. On evaporation, there was obtained 42 mg of the correct product showing a nitrile band in the infrared and no carbonyl absorption. With pure ethyl acetate, there was eluted 33 mg of a yellow mixture (fractions 12–16) which showed both a nitrile and a carbonyl absorption (amide). Similarly the main fraction was chromatographed two times on silica gel to yield 444 mg (2.31 mmoles, 55.7%) of **26**. For analysis, this material was recrystallized from ethyl acetate: the material turns dark but does not melt to 340°; λ_{max} 265 mμ (ε 11,770), 316 (11,640), and 350 (7465).

Anal. Calcd for $C_8H_4N_2O_2S$: C, 49.99; H, 2.10; N, 14.58; S, 16.68. Found: C, 49.73; H, 2.06; N, 14.45; S, 16.61.

E. 2-(4,6-Dihydroxy-2-benzothiazolyl)-Δ²-thiazoline-4-carboxylic Acid (5).—DL-Cystine (180 mg, 0.75 mmole) was converted to cysteine in 10 ml of water as described.[1a] Methanol (10 ml, flushed with nitrogen) was added. The mixture was treated with 2-cyano-4,5-dihydroxybenzothiazole (313 mg, 1.63 mmoles) dissolved in 10 ml of methanol. On mixing, the faint yellow solution of the cyano compound changed to a deep orange. The mixture was allowed to react for 45 mins. Aliquots were removed after 3, 15, and 25 min, respectively, and they were checked by paper chromatography. Apparently the luciferin analog was generated right at the start of the reaction. The mixture was diluted with water (60 ml, flushed with nitrogen) and extracted two times with ethyl acetate (100-ml portions, flushed with nitrogen). Ethyl acetate (50 ml, flushed with nitrogen) was added and the solution was acidified to a pH of *ca.* 1. The extraction was repeated a second time (20 ml of ethyl acetate) and the combined extracts were washed with water (25 ml). The extract was dried over sodium chloride and filtered into a dustfree flask, and the solution was concentrated. After storage in a refrigerator overnight, 309 mg (1.04 mmoles, 70.5%) of **5** separated from the reaction mixture; an additional 66 mg could be recovered from the mother liquor, showing one single spot on paper chromatography. See Table I for the ultraviolet spectrum. **5** darkened at *ca.* 230°; the resin formed did not melt below 340°.

Anal. Calcd for $C_{11}H_8N_2O_4S_2$: C, 44.58; H, 2.72; N, 9.45; S, 21.65. Found: C, 44.73; H, 2.90; N, 9.78; S, 21.47.

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