diethylaniline. Certainly molecular models indicate that if free rotation is preserved about all bonds in di-*n*-propyl and di-*n*-butyl groups considerable steric crowding occurs around the nitrogen atom, thus making solvation more difficult.

Acknowledgments.—For B. B. P. T. and I. L. the financial assistance for this work was provided by the Sir John Cass College. The carbon and hydrogen analyses were carried out by the Microanalytical Department of the Sir John Cass College.

[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY AND BIOLOGY, THE JOHNS HOPKINS UNIVERSITY, BALTIMORE 18, MD.]

The Structure and Synthesis of Firefly Luciferin[1]

BY EMIL H. WHITE, FRANK MCCAPRA AND GEORGE F. FIELD

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Luciferin from the common American firefly, *Photinus pyralis*, was shown to be D-2-(6-hydroxy-2-benzothiazolyl)-Δ^2-thiazoline-4-carboxylic acid (XXII). The structure was confirmed by a total synthesis as outlined in the text. L-Luciferin, DL-luciferin, dehydroluciferin and various related benzothiazoles were also synthesized.

A surprisingly large number of plant and animal species are bioluminescent.[2] The bioluminescence proper has been studied in only a few cases, however, despite the fact that interest in this subject dates back to the beginning of the scientific era with the work of Francis Bacon on luminous fungi and Robert Boyle on luminous bacteria.[3] From the systems that have been studied (these include the firefly *Photinus pyralis*, the crustacean *Cypridina hilgendorfii* and the bacterium *Photobacterium fischerii* as perhaps the best known examples),[2,4] the generalization can be drawn that light emission in bioluminescence is a result of the reaction of oxygen with an oxidizable substrate (a luciferin) catalyzed by an enzyme (a luciferase). In this paper we report the proof of structure and synthesis of luciferin from the common American firefly *Photinus pyralis*.[5] Firefly luciferin is at the present time the only luciferin of known structure.[6]

Luciferin.—Firefly luciferin[7] was first isolated in 1957 and a molecular formula of $C_{13}H_{14}N_2O_3S_2$ was assigned to it at that time.[4a] During the present work, since luciferin was difficult to purify and since very small quantities were available for investigation (we estimate that a total of 30 mg. was used for the structure determination), elementary analyses were not obtained for luciferin or for its degradation products with the one exception noted below. A molecular formula of $C_{11}H_8N_2O_3S_2$ was nevertheless assigned to luciferin during the present work, largely on the basis of indirect evidence from the degradation and from the analysis of a derivative of dehydroluciferin; a total synthesis of luciferin later showed that this formula was correct.

Luciferin is a pale-yellow, microcrystalline solid, m.p. 190° dec. It is difficult to recrystallize, it cannot be sublimed without decomposition, and it is unstable to acids, oxygen and light. Luciferin is stable in basic solutions free of oxygen, but in similar solutions containing oxygen it is rapidly oxidized to a derivative, dehydroluciferin. Spot tests on luciferin indicated the presence of a group that coupled readily with diazonium ions, and the absence of the thiol, thioketone, disulfide and basic nitrogen groups.

The ultraviolet spectrum of natural luciferin in water showed maxima at 265 mμ (broad, log ε 3.90) and 327 mμ (log ε 4.30). These bands were displaced in alkaline solutions to 283 mμ (log ε 3.88) and 383 mμ (log ε 4.27) with a pK_a for the change of 8.4. Similar values for the pK_a were obtained from shifts of electrophoretic mobility, fluorescence intensities and fluorescence excitation with pH.[4a,8] These results and the spot test data suggested that a phenolic group was present in luciferin.

Luciferin is highly fluorescent in solution, a fact in accord with its rigid and highly conjugated structure. In neutral solutions, luciferin fluoresces at 535 mμ (excitation at 327 mμ); in basic solutions it also fluoresces at 535 mμ (excitation at 385 mμ), but the emission intensity is about four times as great. Apparently the phenolate ion is the emitter in both cases, and the phenol which is excited in neutral or acid solutions loses a proton before it fluoresces.[9]

The infrared spectrum of luciferin showed, in particular, a broad band in the OH stretching region and a single band in the carbonyl region. A few samples of natural luciferin showed two carbonyl bands, but a later comparison with synthetic material indicated that the second band probably was due to some racemic luciferin in the samples. The low observed rotation ($[\alpha]_D$ −0.6°) and the abnormally low activity in the enzymatic assay of "highly purified" natural luciferin also suggested that our isolation procedure had led to partial racemization. The carbonyl band was assigned to a carboxyl group[4a] since luciferin reacted instantly with diazomethane to give an ester, and with ammonia to give a salt with a band in the infrared at 6.3 μ, a position characteristic of carboxylate ions. The three oxygen atoms of luciferin were therefore accounted for by the phenol and carboxylic acid groups.

Dehydroluciferin[10] was prepared by the oxidation of luciferin in basic solutions with either potassium ferricyanide or oxygen. Dehydroluciferin has also been isolated from firefly tails[4a] and it can be seen on paper chromatograms of the whole light organs of certain

(1) Presented in part before the Steroids and Other Natural Products Section of the Gordon Research Conferences, New Hampton School, New Hampton, N. H., August, 1961. A preliminary communication on this subject appeared in *J. Am. Chem. Soc.*, **83**, 2402 (1961).

(2) E. N. Harvey, "Bioluminescence," Academic Press, Inc., New York, N. Y., 1952.

(3) E. N. Harvey, "A History of Luminescence," The American Philosophical Society, Philadelphia, Pa., 1957.

(4) (a) B. Bitler and W. D. McElroy, *Arch. Biochem. and Biophys.*, **72**, 358 (1957); (b) W. D. McElroy and H. H. Seliger, "Light and Life," The Johns Hopkins Press, Baltimore, Md., 1961, p. 219; (c) Y. Haneda, *et al.*, *J. Cellular Comp. Physiol.*, **57**, 55 (1961); (d) W. D. McElroy, J. W. Hastings, V. Sonnenfeld and J. Coulombre, *J. Bacteriol.*, **67**, 402 (1954).

(5) The different species of fireflies apparently contain the same luciferin (enzymatic tests; see ref. 2). Our paper chromatographic results with single fireflies (males) supports this conclusion, at least insofar as the local species are concerned. The local species of fireflies are characterized in "The Fireflies of Delaware" by F. A. Mcdermott (Society of Natural History of Delaware, Wilmington, Del., 1958).

(6) A structure has been proposed for Cypridina luciferin by Y. Hirata, O. Shimomura and S. Eguchi (*Tetrahedron Letters*, **No. 5**, 4 (1959)); however, it is incorrect in part (private communication, O. Shimomura). Cypridina luciferin is an indole derivative and its structure is apparently quite different from that of firefly luciferin.

(7) Referred to as luciferin in the remainder of the paper.

(8) B. L. Strehler, "The Luminescence of Biological Systems," American Association for the Advancement of Science, Washington, D. C., 1955, p. 199.

(9) T. Förster, *Z. Elektrochem.*, **54**, 42 (1950).

(10) Previously called Oxyluciferin (ref. 4a).

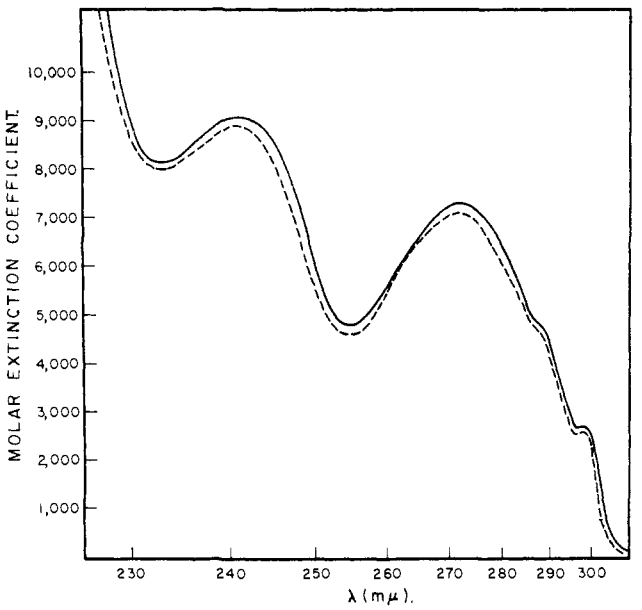


Fig. 1.—Ultraviolet spectra in 95% ethanol for: hydrolysis product of luciferin, ———; 6-hydroxybenzothiazole (III), - - - -.

species. It may be an artifact in these cases, however, since luciferin is very readily oxidized by air. Dehydroluciferin proved to be almost as difficult to purify as luciferin; crystallization on a small scale was unsuccessful and an attempted sublimation under high vacuum led to decarboxylation.

The infrared spectrum of dehydroluciferin is similar to that of luciferin. The ultraviolet spectrum, on the other hand, is shifted to longer wave lengths (λ_{max} 273 mμ (log ε 3.95), λ_{max} 350 mμ (log ε 4.38)) reflecting the presence of a longer chromophore than in luciferin. The most significant difference in properties, however, concerns the stability of dehydroluciferin, particularly to acid. Luciferin on treatment with dilute acid is rapidly degraded, whereas dehydroluciferin is completely stable to hot, concentrated hydrochloric acid. It seemed likely at this point that dehydroluciferin contained a thiazole nucleus, accounting for its stability and its inert nitrogen and sulfur, and that luciferin contained the corresponding thiazoline ring (thiazolines are readily hydrolyzed).[11]

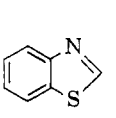
The Degradation and Proof of Structure of Luciferin.—The degradation of luciferin under mild conditions led to mixtures difficult to analyze on our scale of operations. The degradation under vigorous conditions, however, yielded small fragments, the identification of which led to the structure of luciferin.

The treatment of either luciferin or dehydroluciferin with Raney nickel in refluxing ethanol led to replacement of the characteristic ultraviolet absorptions of these compounds by a spectrum with absorption bands at 221, 270 and 277 mμ (*p*H 1). The spectrum was identical with that of *p*-aminophenol, which has absorption bands at 220, 272 and 278 mμ (*p*H 1). The spectrum was also very similar to that of *p*-(methylamino)-phenol and to the spectra reported for the *m*- and *o*-aminophenols,[12] and a decision concerning the substitution pattern could not be made at this point. That this compound was an aminophenol also seemed probable from the shifts of the absorption spectra with *p*H, and from its ready oxidation in air and its oxidation with ammoniacal silver nitrate.

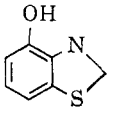
(11) R. B. Martin, S. Lowey, E. L. Elson and J. T. Edsall, *J. Am. Chem. Soc.*, **81**, 5089 (1959); R. B. Martin and A. Parcell, *ibid.*, **83**, 4830 (1961); H. A. Smith and G. Gorin, *J. Org. Chem.*, **26**, 820 (1961).

(12) H. E. Ungnade, "Organic Electronic Spectral Data," Vol. II, Interscience Publishers, Inc., New York, N. Y., 1960.

Treatment of luciferin with concentrated hydrochloric acid produced only two major fragments. On evaporation of the solution and sublimation of the residue, a volatile crystalline compound with a distinct ultraviolet spectrum was obtained (Fig. 1). The spectrum was similar to that of benzothiazole (I), but shifted to longer wave lengths, and in view of the bathochromic shift observed when the compound was dissolved in base, the product was considered to be a hydroxybenzothiazole. Of the various hydroxybenzothiazoles, only the 4- and 6-isomers have been reported and in neither case was the ultraviolet spectrum given. A sample of 4-hydroxybenzothiazole (II) was obtained[13] and shown to have a spectrum quite different from that of the degradation product (Table I). 6-Hydroxybenzothiazole (III) then was synthesized (from 6-nitrobenzothiazole[14] and also from 6-hydroxybenzothiazole

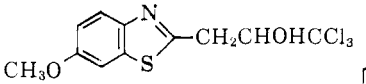


I

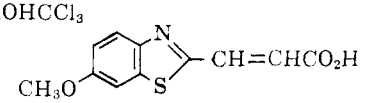


II

2-carboxylic acid) and shown to be identical in all respects with the degradation product (see Fig. 1). The source of the aminophenol in the Raney nickel experiment was now apparent since it is known that benzothiazoles are converted into the corresponding anilines by Raney nickel (either the primary or secondary amines are obtained depending on the reaction conditions[15]).



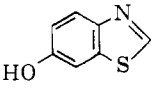
VI



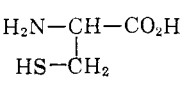
IX

Examination by paper chromatography of the water-soluble residue remaining after the acid hydrolysis of luciferin disclosed the presence of cysteine as the sole ninhydrin-positive material (excluding cystine, which is always present under these conditions). Confirmation of this finding was made by the conversion of the residue into alanine with Raney nickel and identification of the alanine by paper chromatography.

6-Hydroxybenzothiazole (III) and cysteine (IV) account for ten of the carbon atoms in luciferin. At this point it was still believed that the old analysis was



III



IV

correct and that luciferin contained thirteen carbons. With the assumption that one carbon was lost as carbon dioxide during the hydrolysis (luciferin → 6-hydroxybenzothiazole-2-carboxylic acid → 6-hydroxybenzothiazole (III)), two carbon atoms out of the thirteen remained to be placed. About the only location which remained for them was between the benzothiazole and cysteine moieties. The fact that luciferin absorbed one mole of hydrogen during catalytic hydrogenation to give a product with an ultraviolet spectrum practically identical with that of 6-hydroxybenzothiazole led us to believe at this point that an ethylenic linkage connected the two major parts of the molecule.[16] Model

(13) H. Erlenmeyer, H. Ueberwasser and H. M. Weber, *Helv. Chim. Acta.*, **21**, 709 (1938); H. Erlenmeyer and H. Ueberwasser, *ibid.*, **25**, 515 (1942). We thank Professor H. Erlenmeyer for a generous sample of this compound.

(14) W. A. Boggust and W. Cocker, *J. Chem. Soc.*, 355 (1949).

(15) D. Ivanov and Chr. Ivanov, *Compt. rend. acad. Bulgare Sci.*, **5**, No. 1, 13 (1953) [*Chem. Abstr.*, **49**, 4627 (1955)].

TABLE I

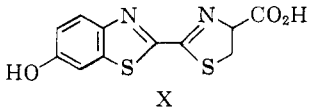
THE ULTRAVIOLET ABSORPTION OF CERTAIN BENZOTHIAZOLES[a]

Compound	Absorption bands [λ, mμ (log ε)]			
Benzothiazole (I)	250(3.74)		284(3.22)	296(3.15)
6-Hydroxybenzothiazole (III)	240(3.95)	272(3.85)	286(3.68)sh	298(3.42)sh
4-Hydroxybenzothiazole (II)	248(3.64)	264(3.51)sh		304(3.55)
	256(3.64)			
2-Methyl-6-methoxybenzothiazole (V)	240(4.00)sh	266.5(3.96)	286(3.62)sh	298(3.39)
Compound VI		271(4.01)	287(3.79)sh	298(3.58)
6-Methoxybenzothiazole-2-carboxylic acid (XIV)		260(3.86)		312(4.06)
6-Methoxybenzothiazole-2-carboxamide (XVI)	258(3.83)			312(4.18)
6-Hydroxybenzothiazole-2-carboxamide (VIII)	261(3.83)			317(4.14)
Luciferin (X)		269(3.85)		330(4.26)
Compound IX		267(3.79)		338(4.28)
Dehydroluciferin (XI)		273(3.95)		350(4.38)

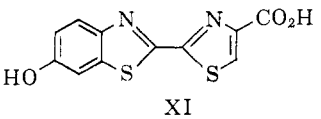
[a] The synthesis and properties of these compounds (except for I and II[13]) are given in the Experimental section.

compounds were prepared in efforts to locate the two carbon atoms. It soon became apparent that when the linking group extending from the benzothiazole was unsaturated (Table I, compound IX), the chromophore of the model was too extended relative to luciferin, and, conversely, when the linking group was saturated (Table I, compounds V and VI are suitable models), the chromophore was insufficiently extended. In 6-methoxybenzothiazole-2-carboxylic acid (XIV), on the other hand, the chomophore was of approximately the right length (see also compounds XVI and VIII, Table I). The data indicated, therefore, that the thiazoline ring was attached directly to the benzothiazole nucleus; there seemed to be no place to attach the "extra carbons" and a new elementary analysis was clearly required.

Since there appeared to be no possibility of purifying either luciferin or dehydroluciferin for analysis, a derivative of the rather stable dehydroluciferin was prepared. The remaining stocks of luciferin were oxidized and the dehydroluciferin formed was treated successively with diazomethane and acetic anhydride to give the methyl ester of acetyldehydroluciferin, which, unlike luciferin, could be purified by sublimation. Paper chromatograms showed that the product was essentially homogeneous. Analysis of the sublimate led to a formula of $C_{14}H_{10}N_2O_4S_2$ for the derivative,[17] and therefore to a formula of $C_{11}H_6N_2O_3S_2$ for dehydroluciferin. The most probable formula for luciferin based on these data and on the relationship of luciferin to dehydroluciferin then became $C_{11}H_8N_2O_3S_2$, and the problem of the two carbon atoms vanished. This C-11 formula, the hydrolysis evidence and the data of Table I, which indicated that in luciferin one double bond was in conjugation with the hydroxybenzothiazole system, led directly to structure X for luciferin and



X



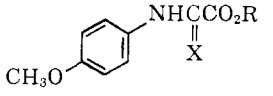
XI

to structure XI for dehydroluciferin.

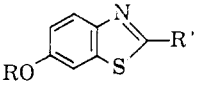
Confirmation of the structure assigned to luciferin was obtained at this point from an n.m.r. spectrum measured in perdeuterioacetone.[18] The aromatic region of the spectrum consisted of a doublet at 2.2 τ ($J_{4,5}$ = 10 c.p.s.), a doublet at 2.5 τ ($J_{5,7}$ = 3 c.p.s.), and a pair of doublets centered at 2.9 τ; these signals are assigned to protons 4, 7 and 5, respectively, in the hydroxybenzothiazole portion of the molecule. In the aliphatic region, an A_2X system was found (a triplet centered at 4.4 τ and a doublet at 5.6 τ, J = 10 c.p.s., partly masked by a peak from a contaminant) consistent with the proton distribution in the thiazoline portion of the molecule.

Firefly luciferin is, therefore, 2-(6-hydroxy-2-benzothiazolyl)-Δ^2-thiazoline-4-carboxylic acid. The total synthesis of luciferin and dehydroluciferin was next carried out to confirm the structures assigned and to elucidate the stereochemistry of luciferin.

The Synthesis of Luciferin.—The synthesis involves the construction of a suitably substituted benzothiazole followed by the addition of a thiazoline ring. This procedure defers the construction of the labile thiazoline ring to the last step, and it permits the ready synthesis of analogs of luciferin. The key intermediate, 2-cyano-6-hydroxybenzothiazole (XVIII), was synthesized starting with *p*-anisidine by an adaptation of the benzothiazole synthesis of Reissert.[19] *p*-Anisidine was condensed with ethyl oxalate to give ethyl N-(4-methoxyphenyl)-oxamate[20] (XII). This compound was converted into the thioamide with phosphorus pentasulfide and the thioamide was hydrolyzed without isolation to give N-(4-methoxyphenyl)-thioöxamic acid (XIII). Oxidation of compound XIII with alkaline ferricyanide produced 6-methoxybenzothiazole-2-carboxylic acid (XIV) (also prepared by the permanganate oxidation of compound IX). Treatment of the acid with diazomethane yielded the methyl ester XV which was condensed with ammonia to give the corresponding amide XVI. Dehydration of the amide with phosphorus



XII, X = O, R = C_2H_5
XIII, X = S, R = H



XIV, R = CH_3, R′ = CO_2H
XV, R = CH_3, R′ = CO_2CH_3
XVI, R = CH_3, R′ = $CONH_2$
XVII, R = CH_3, R′ = CN
XVIII, R = H, R′ = CN
XIX, R = C_2H_5, R′ = SO_2NH
XX, R = C_2H_5, R′ = CN
XXI, R = CH_3, R′ = $CSNH_2$

oxychloride then yielded 2-cyano-6-methoxybenzothiazole (XVII). Difficulty was encountered at the next stage in the synthesis since the nitrile group in compound XVII was quite reactive,[21] and attempts to remove the methyl group led to hydration of the nitrile group instead. Pyridine hydrochloride[22] proved

(16) The hydrogenation of luciferin probably resulted in saturation of the double bond in the thiazoline ring. The reduction of thiazolines to thiazolidines with aluminum amalgam has been reported by A. H. Cook and J. A. Elvidge, *J. Chem. Soc.*, 2362 (1949).

(17) This formula was confirmed by the mass spectrum, which showed a parent peak at 334 mass units. We thank Dr. R. I. Reed of Glasgow University for this determination.

(18) We thank Mr. Leroy F. Johnson of Varian Associates, Palo Alto, Calif., for this measurement.

(19) A. Reissert, *Ber.*, **37**, 3708 (1904).

(20) A. Piutti and R. Piccoli, *ibid.*, **31**, 330 (1898).

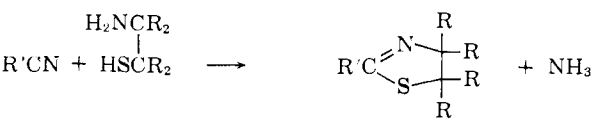
(21) D. D. Libman and R. Slack, *J. Chem. Soc.*, 2253 (1956).

(22) V. Prey, *Ber.*, **75**, 445 (1942).

to be a suitable reagent for the demethylation, however, and by its use 2-cyano-6-hydroxybenzothiazole (XVIII) was readily obtained.

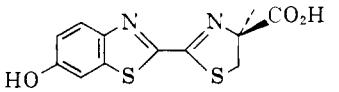
An alternate and more convenient synthesis of 2-cyano-6-hydroxybenzothiazole was developed later based on the report that benzothiazole-2-carboxylic acid could be prepared from the nitrile obtained by the reaction of potassium cyanide with benzothiazole-2-sulfonic acid.[23] In the present case, 6-ethoxybenzothiazole-2-sulfonamide[24] (XIX) was treated with potassium cyanide in dimethyl sulfoxide, and the nitrile formed (XX) was de-ethylated with pyridine hydrochloride by the procedure used for the methyl analog.

The thiazoline ring was added next by an adaptation of the method of Kuhn and Drawert[25] in which a nitrile is condensed with an aminothiol. The usual procedure for the synthesis of thiazolinecarboxylic acids, involving the reaction of an iminoether with an ester



of cysteine[26] followed by hydrolysis, was avoided since racemization would have occurred during the hydrolysis of the ester group.[27] The condensation of 2-cyano-6-hydrobenzothiazole with D-cysteine proceeded as expected in aqueous methanol and the reaction yielded a pale-ivory, crystalline material which was firefly luciferin.

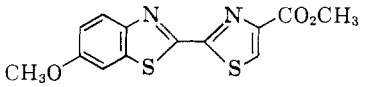
The synthetic and natural luciferins gave identical infrared, ultraviolet and fluorescence spectra and, in addition, the two luciferins exhibited identical activities in the *in vitro* enzymatic production of light.[28] This synthesis confirms the structure arrived at by the degradation of luciferin and, in conjunction with the absolute configuration of D-cysteine,[29] permits the assignment of expression XXII to firefly luciferin.



XXII

L-Luciferin and DL-luciferin also were synthesized by the method outlined. The L-luciferin proved to be enzymatically active for pyrophosphate turnover (in the reaction with ATP), but it was totally inactive as far as light production was concerned.[28]

The Synthesis of Dehydroluciferin.—Dehydroluciferin was prepared by a straightforward thiazole synthesis.[30] 2-Cyano-6-methoxybenzothiazole (XVII) was converted into 6-methoxybenzothiazole-2-thiocarboxamide (XXI) by treatment with hydrogen sulfide, pyridine and triethylamine.[31] The thioamide was condensed with methyl bromopyruvate and the dimethyldehydroluciferin (XXIII) formed was treated with boiling, concentrated hydrobromic acid to give synthetic dehydroluciferin (XI), identical in all respects with natural dehydroluciferin.



XXIII

Natural Products Related to Luciferin.—Compounds with structures related to that of firefly luciferin have not been reported to date. Few natural products containing the thiazoline nucleus have been isolated, presumably because of the ease with which thiazolines are hydrolyzed.[11] Only two natural products, bacitracin A[32] and one form of coenzyme A,[33a] have been reported to contain this ring system although there is evidence that the "masked" thiol groups in many proteins are bound up in thiazoline rings.[33b] The thiazole ring system is more common; it is present in thiamine (as a thiazolium salt)[30] and it has been reported in the degradation products of the antibiotics bottromycin,[34] micrococcin[35] and thiostreptone.[36] The related thiazolidine ring is found in the various penicillins.[30]

This work was supported in part by the National Institutes of Health and in part by funds from the Atomic Energy Commission, the National Science Foundation and the National Institutes of Health granted to W. D. McElroy.

Experimental[37]

Infrared spectra were measured on a Perkin–Elmer spectrophotometer, Model 21, and ultraviolet spectra were measured on a Beckman DK-2 spectrophotometer (the solvent used for the ultraviolet spectra was uniformly 95% ethanol). The purity of non-polar materials was monitored by thin layer chromatography (apparatus from Research Specialties Co., Richmond, Cal.) and that of polar substances by paper chromatography using the descending technique (except where noted); a mixture of 95% ethanol (70 parts) and 1 *M* aqueous ammonium acetate adjusted to *p*H 7.5 (30 parts) was usually used to develop the chromatograms of luciferin and related compounds. Most of our compounds were highly fluorescent and ultraviolet light was used to locate the spots on paper.

Natural Luciferin.—Firefly luciferin was isolated by the procedure reported earlier,[4a] except that the following changes were made to increase the recovery and purity of luciferin: (1) the dry firefly tails were pulverized in a Waring blender before they were extracted with acetone. (2) Luciferin was not eluted from the partition column; after the dehydroluciferin was eluted, the column was cut into sections and luciferin was extracted from the appropriate one with dilute aqueous base (preferably free of oxygen). The luciferin was normally obtained as a grayish powder. The ultraviolet and n.m.r. spectra are given in the text, and the infrared spectrum and other physical data for the natural material can be found in ref. 4a. Luciferin was readily destroyed by light, especially in aprotic solvents; the product mixture, which was complex, contained decarboxydehydroluciferin.

Treatment of Luciferin with Base.—Luciferin (1 mg.) was dissolved in sodium hydroxide solution (1 *N*, 5 ml.) and the solution was divided between two glass tubes. One tube was successively frozen and thawed under high vacuum and then sealed. The other was left open to the air. Both tubes were then heated in boiling water for 4 hours and the contents sampled. Paper chromatography using Whatman Grade No. 3 and development with aqueous ethanol buffered by ammonium acetate established that while the closed tube showed no increase in dehydroluciferin content (traces were invariably present in solutions of luciferin) the open tube, in contrast, contained no luciferin. Examination of the two solutions by ultraviolet spectroscopy confirmed that an almost quantitative conversion of luciferin to dehydroluciferin had occurred in the open tube.

(23) German Patent 613,067 (1934) (*Chem. Zentr.*, **106**, II, 2582 (1935)).

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(28) H. H. Seliger, W. D. McElroy, E. H. White and G. F. Field, *Proc. Natl. Acad. Sci. U. S.*, **47**, No. 8, 1129 (1961).

(29) A summary of the evidence leading to this assignment is given by J. P. Greenstein and M. Winitz, "The Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, N. Y., 1961, Vol. 1, p. 52. Using the convention of Cahn, Ingold and Prelog [*Experientia*, **12**, 81 (1956)] we are here concerned with (S) cysteine and (S) luciferin.

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(36) M. Bodanszky, J. T. Sheehan, J. Fried, N. J. Williams and C. A. Birkhimer, *J. Am. Chem. Soc.*, **82**, 4747 (1960).

(37) Analyses by the Scandinavian Microanalytical Laboratory, Box 25, Herlev, Denmark, and the Galbraith Laboratories, Inc., Nashville, Tenn.

Oxidation of Luciferin.—Luciferin (0.9 mg.) was dissolved in ethanol (1 ml.) and an aqueous solution of potassium ferricyanide (1 *N*, 0.5 ml.) mixed with sodium hydroxide solution (4 *N*, 1 drop) was added. Aliquots were taken and spotted on Whatman Grade No. 3 paper; development was with the ethanol–ammonium acetate solution. Inspection of the chromatograms under ultraviolet light showed that all the luciferin had disappeared in 30 minutes. The major product was dehydroluciferin identified by its R_f value, fluorescence spectrum and, after extraction into ethyl acetate and removal of solvent, by its ultraviolet spectrum. Traces of other fluorescent compounds were observed but in quantities too small for study.

Dehydroluciferin.—Crude luciferin (23 mg.) was dissolved in 1 *N* sodium hydroxide solution (50 ml.) and the solution was boiled in air until examination by paper chromatography showed the absence of luciferin (8 hours). The solution was acidified with concentrated hydrochloric acid (to *p*H 3) and extracted with four 50-ml. portions of ethyl acetate. Partition chromatography, as described by McElroy and Bittler,[4a] gave dehydroluciferin (15 mg.) in the form of ill-defined needles. The physical properties of this material are given in the text and in ref. 4a. Further purification was achieved by conversion to the methyl ester of acetyldehydroluciferin.

The Stability of Dehydroluciferin to Hydrolysis.—Dehydroluciferin (1 mg.) was dissolved in concentrated hydrochloric acid and the solution was boiled for 13 hours. No change was observed. A further amount (1 mg.) was treated with concentrated hydrochloric acid (1 ml.) in a sealed tube at approximately 120° for 3 days. The material was recovered unchanged.

Decarboxydehydroluciferin.—Dehydroluciferin (1.5 mg.) was heated under vacuum at 150° for 6 hours. The light yellow sublimate was dissolved in ethanol and chromatographed on Whatman Grade No. 3 paper. A bright blue fluorescent spot (R_f 0.9) accompanying that of dehydroluciferin was found. Pyrolysis of the sublimate at 200° showed an almost quantitative conversion to this new product, which from the infrared and ultraviolet spectra ($\lambda\lambda_{max}$ 274 and 350 mμ) was shown to be decarboxydehyroluciferin.

Decarboxydehydroluciferin was also prepared by the photochemical decomposition of L-luciferin in dimethyl sulfoxide. Chromatography of the complex reaction mixture yielded 26% of this product, m.p. 272° dec., $\lambda\lambda_{max}$ 350 mμ (ε 21,400), and 273 mμ (ε 6,100).

Anal. Calcd. for $C_{10}H_6N_2OS_2$: C, 51.26; H, 2.58; N, 11.96; S, 27.37. Found: C, 51.42; H, 2.60; N, 12.10; S, 27.00.

Action of Raney Nickel on Luciferin.—Luciferin (2.7 mg.) was dissolved in hot ethanol (2 ml.), and freshly prepared Raney nickel[38] (W2, approx. 20 mg.) was added in portions. The mixture was refluxed and aliquots were taken at intervals. Each aliquot was filtered and evaporated to dryness. The residue was taken up in absolute ethanol and the ultraviolet spectrum was determined. After the addition of a further 20 mg. of Raney nickel and a total reaction time of 6 hours, the ultraviolet spectrum had ceased to change. The ethanolic solution had two broad maxima, at 241 and 299 mμ.

After evaporation of the ethanol, the residue was dissolved in ether and extracted into 1 *N* hydrochloric acid. This acid solution had $\lambda\lambda_{max}$ 221, 276, 282 (sh) mμ. This spectrum was identical with that of *p*-aminophenol in 1 *N* HCl, and both solutions showed, furthermore, the same shifts with *p*H. The acid solution (0.02 ml.) was spotted on Whatman Grade No. 3 paper in the usual way, and developed with aqueous ethanol buffered with 0.1 *M* ammonium acetate. Spraying with ammoniacal silver nitrate (2%) gave a black spot at R_f 0.85, a value close to that found for *p*-methylaminophenol.

It was noticed that all solutions of this product rapidly darkened on exposure to air and the ultraviolet spectrum became poorly defined.

Hydrolysis of Luciferin.—Luciferin (2 mg.) was dissolved in concentrated hydrochloric acid (2.5 ml.) and the solution was refluxed. Aliquots were taken from time to time and evaporated to dryness. The residue was dissolved in absolute ethanol and the ultraviolet absorption was measured. In 20 minutes the spectrum of luciferin ($\lambda\lambda_{max}$ 263, 327 mu) had changed to the spectrum of hydroxybenzothiazole ($\lambda\lambda_{max}$ 271, 287 (sh), 297mμ). Refluxing a portion for a further 12 hours produced no further change. After 30 minutes of refluxing, the hydrochloric acid was removed *in vacuo* and the solid residue was taken up in water. Paper chromatography of this solution using the ascending technique on Whatman Grade No. 3 paper and eluting with ethanol–ammonia–water = 90:5:5, 1-butanol–acetic acid–water = 60:15:25, and phenol–water = 80:20, in three separate runs showed the presence of cysteine. This was confirmed by parallel runs with authentic material. No other ninhydrin-positive material (other than cystine) was found.

The residue from the acid hydrolysis was submitted to vacuum sublimation, giving white needles, m.p. 190–191°; the m.p. of a mixture with synthetic 6-hydroxybenzothiazole was undepressed. A comparison with synthetic material by thin-layer chromatography in two different solvent systems (acetone–benzene (1:3) and 2-propanol–methanol (8:1)) also showed that the compound was 6-hydroxybenzothiazole. The ultraviolet spectrum in 95% ethanol was complex: $\lambda\lambda_{max}$ 240 mμ (log ε 3.96), 272 mμ (log ε 3.87), 287 mμ (log ε 3.69) (sh) and 298 mμ (log ε 3.43) (sh) (Fig. 1). The spectrum showed two peaks at 251 and 306 mμ in strong base and two peaks at 251 mμ and 298 mμ in strong acid, with the long wave length peak being the more intense in both cases.

The Methyl Ester of Acetyldehydroluciferin.—Dehydroluciferin (15 mg.) was dissolved in methanol (10 ml.) by warming, and the solution was added to an excess of diazomethane in ether (500 ml.). The ether was rapidly removed under vacuum to give the crude methyl ester. This was treated with acetic anhydride (10 ml.) containing 1 drop of pyridine and the mixture was left for 2 hours at room temperature. Evaporation of the acetic anhydride and pyridine under vacuum followed by sublimation at 100° (10^{-6} mm.) gave the methyl ester of acetyldehydroluciferin as needles, m.p. 190–195°.

Anal. Calcd. for $C_{14}H_{10}N_2O_4S_2$: C, 50.28; H, 3.01; N, 8.38. Found: C, 50.72; H, 3.33; N, 8.66.

The mass spectrum[17] of acetyldehydroluciferin methyl ester showed as the major fragment a mass of weight 291, corresponding to the loss of the acetyl group (CH_3CO). The parent peak occurred at a mass number of 334, in agreement with the formula weight derived from the analysis.

The Hydrogenation of Luciferin.[16,39]—Luciferin (9 mg.) in 95% ethanol (5 ml.) was catalytically reduced in a micro apparatus with hydrogen and 50 mg. of 10% palladium-on-charcoal. In 2 hours, 1.1 molar equivalents of hydrogen was absorbed and the hydrogenation had stopped. Values ranging from 0.9 to 1.1 molar equivalents were also obtained from similar hydrogenations performed on 0.5-mg. quantities in a Warburg apparatus. The product of the reaction gave an ultraviolet spectrum very similar to that of the benzothiazoles (Fig. 1) with maxima at 247, 278, 289 and 299 mμ in 95% ethanol, 257 and 315 mμ in basic solutions, and 257 and 307 mμ in acidic solutions. Dihydroluciferin was rather unstable and it was not possible to characterize it.

6-Hydroxybenzothiazole (III).—6-Methoxybenzothiazole-2-carboxamide (XVI, 5 g., 24 mmoles) was heated under reflux with concentrated hydrobromic acid (70 ml.) for 4 hours. After the solution was cooled to 5°, the solid was filtered off, dissolved in water (25 ml.), and precipitated as the free base by the addition of sodium bicarbonate. The solid was recrystallized from aqueous ethanol to give 3.03 g. (20 mmoles, 83%) of 6-hydroxybenzothiazole, m.p. 191–192.5°. For analysis it was recrystallized from an ethyl acetate–ligroin mixture and sublimed; m.p. 190.5–191.5°; $\lambda\lambda_{max}$ 298 (ε 2,600), 286 mμ (sh) (ε 4,800), 271.5 mμ (ε 7,100) and 240 mμ (ε 8,900).

Anal. Calcd. for C_7H_5NOS: C, 55.60; H, 3.33; N, 9.27; S, 21.21. Found: C, 55.53; H, 3.44; N, 9.23; S, 21.49.

6-Hydroxybenzothiazole was also synthesized from 6-nitrobenzothiazole by the method of Boggust and Cocker[14]; the yield in this case was very low.

6-Hydroxybenzothiazole-2-carboxamide (VIII).—2-Cyano-6-methoxybenzothiazole (XVII, 2.25 g., 12 mmoles) was heated with pyridine hydrochloride (21 g.) at 190–220° for 0.5 hr. After the reaction mixture had cooled, it was dissolved in 1 *N* sodium hydroxide (250 ml.) and the solution was extracted with two 50-ml. portions of chloroform. The aqueous solution was cooled and then acidified with concentrated hydrochloric acid. The precipitate was collected and then recrystallized from ethanol to give tan crystals (1.6 g.). Treatment with charcoal and two recrystallizations from ethanol gave a sample (0.48 g., 2.5 mmoles, 21%), m.p. 268–274°, which was sublimed for analysis; m.p. 268–273°, $\lambda\lambda_{max}$ 317 mμ (ε 13,700) and 261 mμ (ε 6,700).

Anal. Calcd. for $C_8H_6N_2O_2S$: C, 49.47; H, 3.11; N, 14.43; S, 16.51. Found: C, 49.31; H, 3.23; N, 14.36; S, 16.27.

1,1,1-Trichloro-2-hydroxy-3-(6-methoxy-2-benzothiazolyl)-propane (VI) was prepared from chloral and 2-methyl-6-methoxybenzothiazole by the method of Ried and Gross.[40] After treatment with charcoal and two recrystallizations from ethanol, the yield of compound VI was 40%. An analytical sample prepared by further recrystallization had m.p. 158–159°, $\lambda\lambda_{max}$ 298 mμ (ε 6,800) and 271 mμ (ε 10,000).

Anal. Calcd. for $C_{11}H_{10}Cl_3N_2O_2S$: C, 40.45; H, 3.07; N, 4.29; S, 9.82. Found: C, 40.57; H, 3.10; N, 4.27; S, 10.15.

3-(6-Methoxy-2-benzothiazolyl)-acrylic Acid (IX).—Compound VI (0.5 g., 14.7 mmoles) was added to a solution of potassium hydroxide (0.8 g.) in 10 ml. of 95% ethanol, and the solution was heated on the steam-bath for 1 hour.[41] A white precipitate formed during the reaction and the mixture set to a solid mass on

(38) R. Mozingo, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 181.

(39) We thank Dr. Robert S. Schutz for this experiment.

(40) W. Ried and R. M. Gross, *Ber.*, **90**, 2646 (1957).

(41) This is essentially the method of W. Ried and H. Keller, *ibid.*, **89**, 2578 (1956).

cooling on ice. It was acidified with acetic acid and diluted with water (10 ml.). The product was collected, washed with water and recrystallized from ethanol to give yellow needles of 3-(6-methoxy-2-benzothiazolyl)-acrylic acid (IX) (0.24 g., 10.3 mmoles 70%). An analytical sample prepared by further recrystallization from ethanol had m.p. 235–240° dec., $\lambda\lambda_{max}$ 338 mμ (ε 19,100) and 267 mμ (ε 6,100).

Anal. Calcd. for $C_{11}H_9NO_3S$: C, 56.16; H, 3.85; N, 5.96; S, 13.63. Found: C, 56.17; H, 3.95; N, 5.98; S, 13.75.

Ethyl N-(4-Methoxyphenyl)-oxamate (XII).—Ethyl oxalate (40 ml., 0.23 mole) was dried by azeotropic distillation with benzene (120 ml.) and ethanol (120 ml.). The remainder of the solvent was removed at the water-pump, and the remaining ethyl oxalate was heated to 120°. *p*-Anisidine (22.5 g., 0.183 mole) was added, and the mixture was heated in an open flask to 180° for 5 minutes. After the mixture had been cooled overnight in the refrigerator, the solid was collected, washed with a small amount of cold ethanol, and extracted with boiling 95% ethanol (300 ml.). The insoluble N,N'-bis-(4-methoxyphenyl)-oxamide was filtered off and the filtrate concentrated to 150 ml. Cold water was added to the hot solution to cloudiness. On cooling, the product crystallized out; yield 28 g. (58%, 0.106 mole), m.p. 107–109° (lit.[20] 108–109°).

N-(4-Methoxyphenyl)-thioöxamic Acid (XIII).—Ethyl N-(4-methoxyphenyl)-oxamate (28 g., 0.106 mole) was dissolved in boiling xylene (560 ml.), and phosphorus pentasulfide (8.4 g., 0.038 mole) was added slowly to the refluxing solution. The solution gradually turned black. Reflux was continued until the peak at 283 mμ in the ultraviolet spectrum of the starting material was replaced by the 330 mμ peak of the product; 40 minutes reflux was generally sufficient. The reaction mixture was then cooled and extracted with five 200-ml. portions of 1 *N* sodium hydroxide. The basic extracts were filtered, cooled to 0°, and acidified with concentrated hydrochloric acid. The orange-yellow precipitate was collected and washed with cold water. This material was used without further purification for the oxidation to the benzothiazole.

The ammonium salt was formed by dissolving the crude free acid (1 g.) in absolute ethanol (80 ml.), filtering, saturating the solution with gaseous ammonia and collecting the yellow precipitate (0.82 g.). The precipitate was recrystallized three times from aqueous ethanol containing ammonium hydroxide to give 0.49 g. of the pure salt, m.p. 170–180° dec., λ_{max} 333 mμ (ε 10,200).

Anal. Calcd. for $C_9H_{12}N_2O_3S$: C, 47.35; H, 5.30; N, 12.27; S, 14.05. Found: C, 47.42; H, 5.40; N, 12.22; S, 14.06.

6-Methoxybenzothiazole-2-carboxylic Acid (XIV).—Crude N-(4-methoxyphenyl)-thioöxamic acid (from 30 g. (0.113 mole) of ethyl N-(4-methoxyphenyl)-oxamate) was dissolved in 1 *N* sodium hydroxide (500 ml.) and oxidized with potassium ferricyanide (135 g. in 340 ml. of water) by the slow addition of the thioamide to the ferricyanide solution, keeping the temperature of the reaction mixture below 10°. A vile odor developed during the course of the reaction. Fifteen minutes after the addition of the thioamide was complete, the precipitated salt of 6-methoxybenzothiazole-2-carboxylic acid was filtered off and washed with water and dilute sodium hydroxide. The sodium salt was converted to the free acid by slurrying it in water (600 ml.) and acidifying to *p*H 1 with concentrated hydrochloric acid. After the mixture was thoroughly stirred, the free acid was collected and washed with water. The yield of crude acid was 18 g. (0.086 mole, 76%, from ethyl N-(4-methoxyphenyl)-oxamate). Crude 6-methoxybenzothiazole-2-carboxylic acid (0.8 g.) was dissolved in methanol (60 ml.) and the solution was filtered. Sodium hydroxide (30 ml. of 1 *N*) was added and the precipitated sodium salt (0.63 g.) was collected. A sample recrystallized several times from aqueous methanol had m.p. 257–262° dec. An analytical sample of the free acid was prepared by dissolving the recrystallized sodium salt (0.1 g.) in warm water (5 ml.), filtering, and precipitating the free acid with 1 *N* hydrochloric acid (2 ml.); m.p. 105–108° dec., $\lambda\lambda_{max}$ 312 mμ (ε 11,500) and 260 mμ (ε 7,200).

Anal. Calcd. for $C_9H_7NO_3S$: C, 51.66; H, 3.37; N, 6.69; S, 15.32. Found: C, 51.60; H, 3.49; N, 6.63; S, 15.40.

2-Carbomethoxy-6-methoxybenzothiazole (XV).—(a) Crude 6-methoxybenzothiazole-2-carboxylic acid (14 g., 0.067 mole) from the oxidation step was slurried in cold methanol (300 ml.) and ethereal diazomethane was added with continuous stirring until a slight excess was present. The reaction mixture was then allowed to stand on ice for 15 minutes. After removal of ether and excess diazomethane under vacuum, the ester was precipitated by the addition of water (*ca.* 500 ml.) to the methanol solution. The product was collected, treated with decolorizing charcoal in methanol, and recrystallized from methanol to give 6.2 g. (0.028 mole, 40%) of the methyl ester, m.p. 140–142°. (b) Crude 6-methoxybenzothiazole-2-carboxylic acid (68.8 g.) was dissolved in methanol (5 l.) and anhydrous hydrogen chloride (135 g.) in methanol (500 g.) was added. After standing 2 days at room temperature, the solution was cooled to −10° overnight and the ester (34 g., 0.153 mole, 40%) was collected. After two recrystallizations from methanol, the melting point was 142–142.8°. An analytical sample was prepared by sublimation; $\lambda\lambda_{max}$ 320 mμ (ε 14,600), 263 mμ (ε 6,970) and 260 mμ (ε 6,970).

Anal. Calcd. for $C_{10}H_9NO_3S$: C, 53.79; H, 4.06; N, 6.27; S, 14.36. Found: C, 53.78; H, 4.14; N, 6.08; S, 14.44.

6-Methoxybenzothiazole-2-carboxamide (XVI).—Methyl ester XV (8 g., 0.036 mole) was dissolved in boiling methanol (160 ml.). The hot solution was saturated with anhydrous ammonia and kept hot for 0.5 hour, during which time the amide precipitated out. After cooling, the amide (7 g., 0.036 mole, 100%) was collected. An analytical sample was prepared by two recrystallizations from pyridine and two from acetic acid; m.p. 258–260° dec., $\lambda\lambda_{max}$ 312 mμ (ε 15,100) and 258 mμ (ε 6,800)

Anal. Calcd. for $C_9H_8N_2O_2S$: C, 51.91; H, 3.87; N, 13.45; S, 15.40. Found: C, 51.98; H, 3.77; N, 13.30; S, 15.52.

2-Cyano-6-methoxybenzothiazole (XVII).—6-Methoxybenzothiazole-2-carboxamide (6.2 g., 0.03 mole) was treated with phosphorus oxychloride (30 ml.), and the mixture was heated under gentle reflux until all the amide had dissolved (*ca.* 15 minutes was required). Heating was continued for 3 additional minutes (longer reaction times gave a darker product). The solution was cooled slightly and the excess phosphorus oxychloride was removed as completely as possible with a rotary evaporator. The residue was cooled on ice and the residual phosphorus oxychloride was decomposed with 10% sodium bicarbonate solution (after a little ether was added to prevent foaming). The *p*H of the slurry was adjusted to 6 and the nitrile was extracted into chloroform. The chloroform solution was dried over magnesium sulfate and evaporated to dryness, and the crude nitrile was purified by chromatography on acid-washed alumina. A chloroform solution of the crude nitrile (4 g.) was evaporated onto alumina (50 g.) and the mixture was added to the top of a column of alumina (300 g.) in petroleum ether. Elution with ether–petroleum ether mixtures gave the nitrile (3.2 g., 16.8 mmoles, 56%), which, after recrystallization from isoöctane, melted at 129–131°, $\lambda\lambda_{max}$ 320 mμ (ε 17,000) and 263 mμ (ε 8,000).

Anal. Calcd. for $C_9H_6N_2OS$: C, 56.82; H, 3.15; N, 14.73; S, 16.86. Found: C, 56.85; H, 3.34; N, 14.69; S, 17.00.

2-Cyano-6-hydroxybenzothiazole (XVIII).—Pyridine hydrochloride was formed from dry pyridine (25 g.) and anhydrous hydrogen chloride (11.5 g.) and protected from atmospheric moisture. It was heated to 200° and 2-cyano-6-methoxybenzothiazole (2 g., 11.5 mmoles) was added slowly. After 0.5–1 hour at this temperature, the reaction mixture was cooled on ice and decomposed with 10% sodium carbonate solution, taking care that the solution did not become strongly alkaline. The crude hydroxynitrile was filtered off and recrystallized from aqueous ethanol to yield 1.15 g. of XVIII (6.5 mmoles, 62%). For further purification, the hydroxynitrile was dissolved in ethanol (75 ml.) and the solution was passed through a column composed of charcoal (3 g.) and Celite (5 g.). The column was then washed with more ethanol (150 ml.). The eluates were combined and concentrated to 50 ml. Water was added to precipitate the nitrile, which was filtered, dried, and recrystallized from ethanol–water or ethyl acetate. An analytical sample was prepared by four recrystallizations from ethanol; m.p. 212–215° dec., $\lambda\lambda_{max}$ 322 mμ (ε 16,100) and 263 mμ (ε 8,000).

Anal. Calcd. for $C_8H_4N_2OS$: C, 54.53; H, 2.28; N, 15.90; S, 18.20. Found: C, 54.44; H, 2.36; N, 15.45, 15.63; S, 18.33.

2-Cyano-6-ethoxybenzothiazole (XX).—6-Ethoxybenzothiazole-2-sulfonamide (XIX) (10 g., 0.038 mole) [$\lambda\lambda_{max}$ 304 mμ (4.08) (sh), 297 mμ (4.10), and 252.5 mμ (3.93)] and potassium cyanide (5 g., 0.077 mole) were combined in dimethylsulfoxide (1000 ml.) and the mixture was heated at 125° for 2.5 hours with continuous stirring. The peak at 320 mμ in the ultraviolet spectrum of the *product* was used to monitor the reaction. The reaction mixture was cooled and diluted with ice and water (1000 ml.). The precipitate was collected, washed with water, and dissolved in chloroform; the solution was then dried with sodium sulfate. Removal of the solvent left 6.4 g. of a residue which was absorbed on alumina by evaporating a mixture of alumina (85 g.) and a solution in chloroform to dryness. The mixture of compound and alumina was placed on the top of a column made up of 400 g. of Merck acid-washed alumina and the product was eluted with 1:9 ether–petroleum ether. Recrystallization from isooctane yielded 4.6 g. (22.5 mmoles, 58%) of white needles, m.p. 107.5–108.5°. The analytical sample, prepared by recrystallization from isoöctane and sublimation, had a m.p. of 108–108.2°; $\lambda\lambda_{max}$ 320 mμ (ε 17,300), 263 mμ (ε 7,500) and 259 mμ (ε 7,400).

Anal. Calcd. for $C_{10}H_8N_2OS$: C, 58.80; H, 3.95; N, 13.72; S, 15.70. Found: C, 58.68; H, 4.03; N, 13.43; S, 15.72.

Conversion of 2-Cyano-6-ethoxybenzothiazole to 2-Cyano-6-hydroxybenzothiazole.—2-Cyano-6-ethoxybenzothiazole (2 g., 0.0098 mole) was added to pyridine hydrochloride (28 g.) maintained at 185° and the solution was kept at 185–195° for 2 hours. The reaction was followed by removing aliquots periodically and

using the shift in the ultraviolet spectrum in alkali to determine the extent of dealkylation. The mixture was cooled, diluted with water, and the precipitate which formed was filtered off. The precipitate was dissolved in 40 ml. of ethanol and the solution was run through a column containing charcoal (2 g.) and Celite (4 g.). The column was washed with 150 ml. of ethanol and the total eluate was evaporated. Recrystallization of the residue from aqueous ethanol yielded 0.54 g. (3.6 mmoles, 31%) of compound XVIII in the form of white platelets, m.p. 212–214°.

D-Luciferin.—D-Cystine (364 mg., 1.51 mmoles) was dissolved in liquid ammonia (70 ml.) and reduced with sodium; small pieces were added until the blue color persisted for 10 minutes, at which time the excess was destroyed with ammonium chloride. The ammonia was allowed to evaporate in a current of nitrogen. The residue was dissolved in 10 ml. of water saturated with nitrogen and the *p*H of the solution was adjusted to approximately 8 with 1 *N* hydrochloric acid (8 ml.) and 1 *N* sodium hydroxide (3 ml.). This solution of cysteine was added to a solution of 2-cyano-6-hydroxybenzothiazole (503 mg., 2.88 mmoles) in 20 ml. of methanol saturated with nitrogen and the mixture was kept under nitrogen in a flask of low-actinic glass for 0.5 hour. The precipitate which formed first redissolved. The solution was filtered from traces of the precipitate that remained, acidified with 1 *N* hydrochloric acid (4 ml.) and allowed to stand overnight at 4°. Filtration yielded 0.743 g. (2.65 mmoles, 94%) of luciferin in the form of yellow needles, m.p. 189–190.5° dec. Paper chromatography showed this material to be contaminated with a blue-green fluorescent material with R_f 0.84. A portion of the crude product (83 mg.) was recrystallized rapidly from 7 ml. of methanol to give a sample (44 mg., 50%), m.p. 189.5–190° dec., $[\alpha]^{22}D$ −36° (*c* 1.2, dimethylformamide) which contained only a trace of impurities as shown by paper chromatography. See Table I for the ultraviolet spectrum. The infrared, ultraviolet and fluorescence spectra of synthetic D-luciferin were identical with those of natural luciferin; a comparison of the paper chromatographic behavior and the enzymatic reactivity[28] also showed that the two luciferins were identical.

Anal. Calcd. for $C_{11}H_8N_2O_3S_2$: C, 47.14; H, 2.85; N, 10.00; S, 22.88. Found: C, 47.36; H, 3.13; N, 9.96; S, 22.96, 22.97.

The L- and DL-luciferins were prepared directly from L- and DL-cysteine in a similar fashion.

6-Methoxybenzothiazole-2-thiocarboxamide (XXI).—2-Cyano-6-methoxybenzothiazole (1 g., 5.3 mmoles) was dissolved in a mixture of pyridine (20 ml.) and triethylamine (0.75 ml.), and hydrogen sulfide was bubbled through the solution for 3 hours. The solution was then diluted with water (20 ml.) and cooled on ice. The precipitate was collected and washed with water to give thionamide XXI (1.13 g., 5 mmoles, 95%), m.p. 197–198°. A sample was purified by three recrystallizations from methanol; m.p. 196–198°; $\lambda\lambda_{max}$ 354.5 mμ (ε 13,700), 318 (sh) (ε 9,600), 270 mμ (ε 7,600) and 265 mμ (ε 6,200).

Anal. Calcd. for $C_9H_8N_2OS_2$: C, 48.20; H, 3.56; N, 12.49; S, 28.60. Found: C, 48.25; H, 3.54; N, 12.44; S, 28.68.

Dimethyldehydroluciferin (XXIII).—6-Methoxybenzothiazole-2-thiocarboxamide (2.0 g., 9.0 mmoles), methyl bromopyruvate (5.4 g., 30 mmoles) and absolute methanol (200 ml.) were stirred together at room temperature for 22 hours and then heated under reflux for 17 hours. The mixture was cooled to 0° and filtered to yield 2.48 g. (7.9 mmoles, 88%) of the condensation product, m.p. 233–235.5°. For analysis, it was recrystallized three times from acetone and sublimed *in vacuo;* m.p. 233–235°, $\lambda\lambda_{max}$ 348 mμ (ε 23,400) and 274 mμ (ε 9,600).

Anal. Calcd. for $C_{13}H_{10}N_2O_3S_2$: C, 50.96; H, 3.29. Found: C, 50.96; H, 3.27.

Dehydroluciferin (XI) —Dimethyl dehydroluciferin (0.69 g., 2.2 mmoles) was heated under reflux with concentrated hydrobromic acid (25 ml.) for 1.5 hours. The solution was cooled and poured into 100 ml. of water. The dark olive-colored precipitate (0.56 g.) was collected and then treated in dimethylformamide (5 ml.) with 50 mg. of charcoal. The treated extracts were heated, diluted with 10 ml. of water, and cooled slowly. Filtration yielded the crude product, which was treated again with charcoal to yield a light greenish-yellow solid weighing 0.35 g. This material was crystallized from a dimethylformamide–water mixture, then dissolved in methanol containing aqueous sodium hydroxide and precipitated with dilute hydrochloric acid. The product, dehydroluciferin, was obtained as a yellow, crystalline solid (0.13 g., 0.47 mmole, 20%) melting at 315–321° dec. and showing a single spot on paper chromatography; $\lambda\lambda_{max}$ 350 mμ (ε 24,000), 275 mμ (ε 8,800) and 271 mμ (ε 8,800).

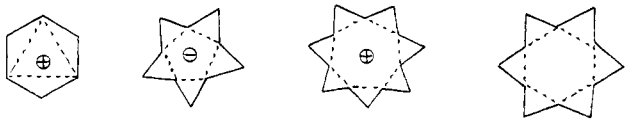
Anal. Calcd. for $C_{11}H_6N_2O_3S_2$: C, 47.47; H, 2.17; N, 10.07; S, 23.04. Found: C, 47.40; H, 2.34; N, 10.19; S, 22.84.

COMMUNICATIONS TO THE EDITOR

THE HEXAHOMOBENZENE PROBLEM. TETRACYCLO-[9.1.0.0^{3,5}0^{7,9}]DODECANE

Sir:

Evidence was reported recently in favor of a non-classical trishomocyclopropenyl structure (I) for the 3-bicyclo[3.1.0]hexyl cation.[1] Further, it was suggested that the homo-relationship of this ion to the cyclopropenyl cation might be generalized to include homo-counterparts of other aromatic molecules.[1,2] On this basis, conceivable homoaromatic species are the pentahomocyclopentadienide and heptahomotropylium ions II and III, and even the neutral hexahomobenzene IV was considered as a possibility.



I II III IV

In the course of synthetic approaches to systems with the spacing and stereochemistry of cyclopropane rings required to test these ideas, a shortcut to "hexahomobenzene" presented itself when *cis-cis-cis*-1,4,7-cyclononatriene[3] became available to us. Thus, we now can report the preparation and properties of the pertinent compound, *cis*-tetracyclo[9.1.0.0^{3,5}O^{7,9}]dodecane.

When *cis-cis-cis*-1,4,7-cyclononatriene[3] (V) was subjected to the Simmons–Smith reaction,[4] employing 10 equivalents of methylene iodide and a 12 hour reaction time, vapor phase chromatography indicated complete disappearance of the starting material. The reaction mixture contained a number of products, but one predominated quite largely (80–90%). Conventional work-up afforded an oil which solidified on cooling. Purification by fractional melting and sublimation at 65° and 60 mm. yielded thin white needles, m.p. 54–57°. The vapor phase chromatogram of this material on a column of XF1150 Nitrile Silicone (F and M Scientific Corp., Avondale, Pa.) indicated it to be a 97:3 mixture of two materials. Vapor phase chromatographic separation of the major component yielded material, m.p. 60–62°. The C,H-analysis and molecular weight of this material are consistent with the formula $C_{12}H_{18}$ for a *tris*-methylene adduct of the original cyclononatriene.

Anal. Calcd. for $C_{12}H_{18}$: C, 88.82; H, 11.18; mol. wt., 162. Found: C, 88.61; H, 11.11; mass spectral[5] parent peak, 162.

As regards the structure of the $C_{12}H_{18}$ hydrocarbon, its infrared and proton magnetic resonance spectra show it to be saturated and to possess distinct cyclopropane and "ordinary" alicyclic methylene groups as

(1) S. Winstein and J. Sonnenberg *J. Am. Chem Soc.*, **83**, 3235 3244 (1961).

(2) S. Winstein, *ibid.*, **81**, 6524 (1959).

(3) P. Radlick and S. Winstein, *ibid.*, **85**, 344 (1963).

(4) (a) H. E. Simmons and R. D. Smith, *ibid.*, **81**, 4256 (1959); (b) R. D. Smith and H. E. Simmons, *Org. Syn.*, **41**, 72 (1961).

(5) We are indebted to Dr. Richard Teeter of the California Research Corporation for the mass spectrum determination.