The crude solid was recrystallized from acetone-benzene (1:3), 2.6 g., m.p. 147°.[3,4] A mixture of this compound and an authentic sample of oxindole-3-acetic acid[5] showed no depression of the melting point. The compound in ethanol had a maximum absorption in the ultraviolet at 249 mμ (ϵ = 8,860) with a shoulder at 270–280 mμ (ϵ = 1,500). Calcd. for $C_{10}H_9NO_3$: C, 62.8; H, 4.7; N, 7.3. Found: C, 62.8; H, 4.98; N, 7.28.

As further proof of identity, a portion of the tryptamine transformation product was converted in hot 6% HCl to 3,4-dihydroquinolone-4-carboxylic acid.[2,3]

A two-dimensional paper chromatogram of oxindole-3-acetic acid, [first solvent, 2-propanol, H_2O, ammonium hydroxide (sp. gr. 0.90) (200: 20:10); second solvent, BuOH, glacial acetic acid, H_2O (120:30:50)] sprayed with Ehrlich reagent, gave a yellow-green spot which became intensely blue-green on standing.

In order to learn more about the substrate specificity of the indole oxidase in *Hygrophorus conicus*, various β-substituted indoles were incubated with cultures of this organism for one week. The culture fluids then were extracted and papergrams made of equal volumes of the concentrated extracts. The extent to which oxindole-3-acetic acid was produced was judged visually from the intensity of the color formed after spraying the papergram with Ehrlich reagent. A list is given of these β-substituted indoles arranged in order of the degree of their conversion to oxindole acetic acid: tryptamine, indoleacetic acid, 1-(3′-indolyl)-3-butanone, tryptophol, N-methyltryptamine, tryptophan, N,N-dimethyltryptamine, indolepropionic acid, N-acetyltryptophan, and 1-(3′-indolyl)-3-butanol. The last two compounds showed no evidence of conversion to oxindole-3-acetic acid.

Acknowledgement.—It is with pleasure that the author acknowledges the help of Dr. E. C. Schuytema in carrying out the fermentations.

(3) P. L. Julian, J. C. Printy, R. Ketcham and R. Doone, *J. Am. Chem. Soc.*, **75**, 5305 (1953).

(4) W. B. Lawson and B. Witkop, *J. Org. Chem.*, **26**, 263 (1961).

(5) The author is indebted to Dr. Bernhard Witkop, National Institutes of Arthritis and Metabolic Diseases, Bethesda, Md., for supplying authentic samples of oxindole-3-acetic acid and of 3,4-dihydroquinolone-4-carboxylic acid.

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## THE STRUCTURE AND SYNTHESIS OF FIREFLY LUCIFERIN

Sir:

Light emission in the American firefly *Photinus pyralis* has been shown to involve the interaction of magnesium ion, oxygen, ATP, the enzyme luciferase, and the oxidizable substrate luciferin.[1] We now wish to report the structure and synthesis of firefly luciferin.[2] Structure I was deduced from

(1) Pertinent references and an outline of the isolation and partial characterization of firefly luciferin have been given elsewhere by B. Bitler and W. D. McElroy, *Arch. of Biochem. and Biophys.*, **72**, 358 (1957).

(2) The luciferin from a luminous ostracod, *Cypridina hilgendorfi*, has been assigned a tentative structure by Y. Hirata, O. Shimomura,

I
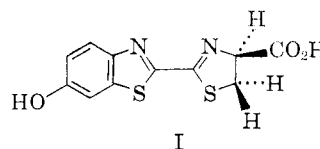
several observations: (a) preliminary microanalysis and molecular weight determination indicated the formula $C_{13}H_{12}N_2O_3S_2$; work with a luciferin derivative (luciferin itself is difficult to purify) later led to a revision of this formula (part i); (b) spot tests indicated the absence of NH, SH, and S–S bonds; (c) color tests and the effect of base on the ultraviolet absorption, on the fluorescence emission, and on the electrophoretic mobility indicated the presence of a phenolic hydroxyl group (pK_a ca. 8); (d) chemical behavior and the infrared spectra of luciferin and its ammonium salt showed that the molecule contained a carboxyl group; (e) the ultraviolet spectrum ($\lambda\lambda_{max}$ 263 and 327 mμ, log ϵ 3.90 and 4.30) was similar in position to that of 2-methyl-5-phenyl-4-styryl-thiazole ($\lambda\lambda_{max}$ 260 and 315 mμ, log ϵ 4.1 and 4.3)[3] and in shape to that of 2-phenylbenzothiazole ($\lambda\lambda_{max}$ 256 and 297 mμ, log ϵ 3.90 and 4.29)[3]; (f) Raney nickel desulfurization yielded a substance with an ultraviolet spectrum ($\lambda\lambda_{max}$ 221, 270, and 277 mμ at pH 1) practically identical with the spectra of the aminophenols[3] (found for p-aminophenol, λ_{max} 220, 272 and 278 mμ at pH 1); (g) hydrolysis with hydrochloric acid yielded cysteine (identified through paper chromatography) and a sublimable compound with an ultraviolet spectrum ($\lambda\lambda_{max}$ 271, 287 (sh), 297 (sh) in EtOH; 251, 298 in acid; 251, 306 mμ in base) similar to that of benzothiazole ($\lambda\lambda_{max}$ 250, 284 (sh), 294 (sh) in EtOH; 236, 277 mμ in acid) (this compound was later shown to be 6-hydroxybenzothiazole); (h) the n.m.r. spectrum in perdeuterioacetone indicated a 1, 2, 4 distribution of three protons on an aromatic ring; (i) oxidation with oxygen or ferricyanide yielded dehydroluciferin, a compound which had been isolated earlier from the lanterns of the firefly.[1] The ultraviolet spectrum of dehydroluciferin ($\lambda\lambda_{max}$ 267 and 348 mμ), its stability to hydrolysis, and its mode of synthesis suggested that this derivative is the thiazole analog, IIa, of luciferin.[4]
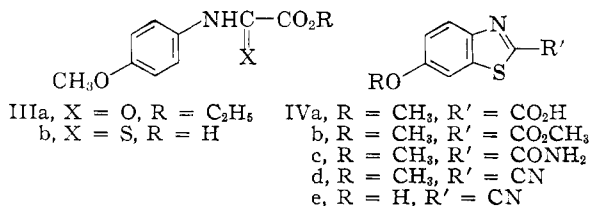

IIa, R = R′ = H
b, R = CH₃CO, R′ = CH₃
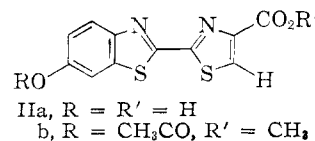
Esterification and acylation of dehydroluciferin yielded an acetoxymethyl derivative IIb which was purified more easily than luciferin. The analysis of this derivative (C, 50.72; H, 3.33; N, 8.66. Calcd. for $C_{14}H_{10}N_2O_4S_2$: C, 50.28; H, 3.01; N, 8.38) led to formula $C_{11}H_8N_2O_3S_2$ for

and S. Eguchi (*Tetrahedron Letters*, No. 5, 4 (1959)). It bears no resemblance to firefly luciferin.

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

(4) Structure IIa for dehydroluciferin has been confirmed by synthesis; condensation of bromopyruvic acid with the thioamide corresponding to amide IVc and subsequent demethylation gave a product identical in all respects to dehydroluciferin.

luciferin and, along with the degradative evidence, secured structure I; confirmation of this structure was achieved by a total synthesis of luciferin.

p-Anisidine was condensed with ethyl oxalate to give amide IIIa,[5] which, by reaction with phosphorus pentasulfide and then base, was converted into thioamide IIIb (m.p. 134–138° dec.); oxida-

IIIa, X = O, R = C$_2$H$_5$　IVa, R = CH$_3$, R' = CO$_2$H
   b, X = S, R = H　　b, R = CH$_3$, R' = CO$_2$CH$_3$
               c, R = CH$_3$, R' = CONH$_2$
               d, R = CH$_3$, R' = CN
               e, R = H, R' = CN

tion of IIIb with basic ferricyanide yielded 6-methoxy-2-benzothiazolecarboxylic acid (IVa, m.p. 109–111° dec.). This acid was esterified with diazomethane and the methyl ester (IVb, m.p. 142–143°) was condensed with ammonia to give the corresponding amide (IVc, m.p. 255–257°). Dehydration of amide IVc with phosphorus oxychloride yielded nitrile IVd (m.p. 130–131°), which was demethylated with pyridine hydrochloride to give 2-cyano-6-hydroxybenzothiazole (IVe, m.p. 212–215° dec.). The reaction of this nitrile with D-cysteine[6] in aqueous methanol yielded firefly luciferin (I) in the form of pale yellow needles, m.p. 196° dec., $[\alpha]^{24}$D $-29°$ (dimethylformamide), $\lambda\lambda_{max}$ 268 and 327 mμ, log ϵ 3.88 and 4.27. Analysis gave: C, 47.11; H, 2.91; N, 9.89; S, 22.60. Calcd. for C$_{11}$H$_8$N$_2$O$_3$S$_2$: C, 47.14; H, 2.85; N, 10.00; S, 22.88. Synthetic luciferin and natural luciferin gave identical infrared, ultraviolet, and fluorescence spectra and, in addition, the two luciferins exhibited identical activities in the *in vitro* enzymatic production of light.[7]

Satisfactory analyses and other physical data were obtained for the compounds cited in the synthesis section of this paper; the scale of operations precluded analysis of the compounds identified in the degradative work.

We thank Miss Joan Friedland for assistance, and the National Science Foundation for partial support of this work.

(5) A. Piutti and R. Piccoli, *Ber.*, **31**, 330 (1898).

(6) The luciferin prepared from L-cysteine was enzymatically inactive.

(7) H. H. Seliger, W. D. McElroy, E. H. White and G. F. Field, *Proc. Nat. Acad. Sci., U. S.*, in press (1961).

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IRONTRICARBONYL-HEXATRIENE COMPLEX[1]

Sir:

Many iron carbonyl complexes have been prepared with cyclic unsaturated hydrocarbons, but relatively few have been reported with non-cyclic alkenes.[2-10]

We wish to communicate here the synthesis and identification of a new iron carbonyl complex with *trans*-1,3,5-hexatriene as the ligand.

Iron dodecacarbonyl (3 g., 6 mmoles) and *trans*-1,3,5-hexatriene[11] (6 g., 75 mmoles) were refluxed on a steam-bath under dry nitrogen for about 3 hours. The color changed from green to orange-yellow during the reaction. The products were distilled in high vacuum and the unreacted hexatriene removed from the distillate. An orange-red oily material thus obtained was heated at 160–170° under dry nitrogen atmosphere and the volatile material removed. The viscous liquid remaining again was distilled in high vacuum and the portion distilling at about 100° collected; yield 1.2–1.8 g. $\sim 30\%$—based on Fe$_3$(CO)$_{12}$.

The final product is an oily orange-red liquid, solidifying at about $-70°$ and is assigned the formula Fe(CO)$_3$(C$_6$H$_8$)$_2$. *Anal.* Found: C, 60.04; H, 5.72; Fe 18.32. Calcd.: C, 60.07; H, 5.35; Fe, 18.62. The molecular weight as determined by the cryoscopic method was 303.

An infrared spectrum of the compound showed carbonyl bands at about 1970 cm.$^{-1}$ (very strong) and 2045 cm.$^{-1}$ (very strong and broad). The vinyl CH out of plane and CH$_2$ out of plane deformation frequencies of the *trans*-hexatriene[12] appeared in the spectrum of the complex at 998 cm.$^{-1}$ and at 896 cm.$^{-1}$, respectively.

Obviously this was not the only complex formed during the reaction but it was the one that could be isolated in a rather pure form. An orange-yellow light material that distilled at 160–170° seemed to be another complex formed during the reaction. The molecular weight of this material determined again by the cryoscopic method was consistently in the region of 194. However, the carbon and hydrogen analyses varied significantly. Absorption from two carbonyl stretching frequencies and also vinyl groups could be identified in the infrared spectrum of this compound. It is possible that this product can be formulated as Fe(CO)$_2$(C$_6$H$_8$). More work on the structure and identification of the products of this reaction is in progress.

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