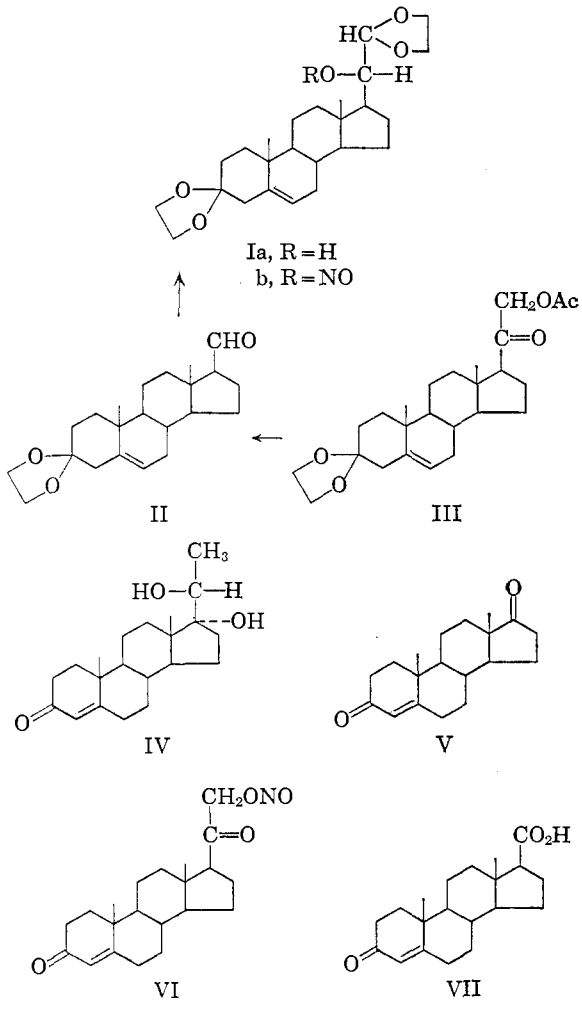
nitrosyl chloride in pyridine and photolyzed in benzene for a one-hour period, by means of a 200-watt mercury lamp, at 20°. The resulting material had unexpected bands in the infrared spectrum at 3.69 and 5.83 μ, and its elementary analysis was in agreement with the formula $C_{22}H_{32}O_3$.



It was therefore assigned structure II, the 20-aldehyde.[4]

This assignment was buttressed by correlation with a compound of unequivocal structure. 3,3-Ethylenedioxy-21-acetoxy-5-pregnen-20-one[5] (III) was converted to the mixture of 20,21-diols with lithium aluminum hydride and subsequent oxidative cleavage with potassium periodate in dioxane[6] gave an ethylenedioxy-aldehyde identical with II.

Similarly, an attempt to extend the nitrite irradiation method that had led to the preparation of 18-nitriloprogesterone[3c] to the corresponding 17α-hydroxy analog led to an unexpected result: Treatment of 17α,20β-dihydroxy-4-pregnen-3-one[7] (IV) with nitrosyl chloride led to an ill-defined dinitrite[8] which, upon irradiation, gave rise to 4-androstene-3,17-dione (V).

Finally, during the study of the behavior of 21-nitrites, a representative member (21-desoxycorticosterone nitrite, VI, (m.p. 126–127° dec., $[\alpha]_D$ 157, ϵ_{241} 17,200, λ^{Nujol} at 5.81, 6.02, 6.10 and 6.19 μ) was irradiated in benzene. One of the conversion products isolated proved to be the known 3-keto-4-etiocholenic acid (VII).

It would appear, then, that in the special case where a nitrite is vicinally substituted by an oxygen-bearing moiety, photolytic oxidative fission occurs in competition with, and perhaps to the exclusion of, intramolecular hydrogen abstraction. The neighboring group may be a ketone, an alcodol or an acetate, and the full scope of the reaction is under investigation.[9]

(4) A similar observation in the corresponding 11-oxygenated series by Dr. J. M. Beaton of the Research Institute for Medicine and Chemistry stimulated this present systematic study. We thank Dr. Beaton for informing us of his results.

(5) R. Antonucci, S. Bernstein, R. Lenhard, N. J. Sax and J. H. Williams, *J. Org. Chem.*, **17**, 1369 (1952).

(6) Periodic acid in methanol gave mixtures, presumably by *trans*-ketalization at C-3 and partial conversion to the acetal at C-20.

(7) J. Romo, M. Romero, C. Djerassi and G. Rosenkranz, *J. Am. Chem. Soc.*, **73**, 1528 (1951).

(8) An attempt to prepare a mononitrite gave mixtures.

(9) A number of simple aliphatic 1,2-glycol dinitrites have been observed to undergo similar oxidative fission on pyrolysis; *cf.* L. P. Kuhn and L. DeAngelis, *J. Am. Chem. Soc.*, **76**, 328 (1954).

SCHERING CORPORATION
BLOOMFIELD
NEW JERSEY
A. L. NUSSBAUM
C. H. ROBINSON
E. P. OLIVETO

IMPERIAL COLLEGE
LONDON, ENGLAND
D. H. R. BARTON

RECEIVED APRIL 5, 1961

THE FORMATION OF OXINDOLE ACETIC ACID FROM INDOLES BY A BASIDIOMYCETE

Sir:

During a study of the metabolism of indoles by Basidiomycetes in submerged culture, a compound was found in the spent beers of *Hygrophorus conicus* which gave a blue-green spot on a papergram sprayed with Ehrlich reagent. This compound, which was produced from both tryptamine and indole-3-acetic acid in good yields, was isolated and proven to be oxindole-3-acetic acid. No isolation of oxindole-3-acetic acid from a natural source has been reported, although Klämbt[1] published chromatographic evidence for its presence in an extract of maize.

The high yield of oxindoleacetic acid from tryptamine reported here indicates that the indoleacetic acid oxidase produced by *Hygrophorus conicus* differs from that of *Omphalia flavida*,[2] which converts indoleacetic acid to 3-methyloxindole.

Two 7-l. fermentors, each containing 5 l. of 4% malt extract and 2.5 g. of tryptamine, were inoculated with growing mycelium of *Hygrophorus conicus*. The fermentation was carried out at 27° and an aeration rate of 3 l./min. for one week. The beer was filtered, concentrated to 1.5, acidified (*p*H 2), and extracted with ethyl acetate. The desired compound was removed from the concentrated ethyl acetate solution with 5% $NaHCO_3$ and then taken back into ether. When the ether was removed *in vacuo* crystals formed (3.16 g.).

(1) H. Klämbt, *Naturwissenschaften*, **23**, 649 (1959).

(2) P. M. Ray and K. V. Thimann, *Arch. Biochem. Biophys.*, **64**, 175 (1956).

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.

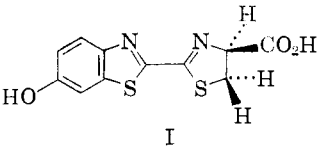
ABBOTT LABORATORIES
NORTH CHICAGO, ILLINOIS D. J. SIEHR

RECEIVED FEBRUARY 20, 1961

THE STRUCTURE AND SYNTHESIS OF FIREFLY LUCIFERIN

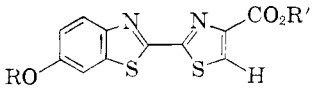
Sir:

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



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 *p*H 1) practically identical with the spectra of the aminophenols[3] (found for *p*-aminophenol, λ_{max} 220, 272 and 278 mμ at *p*H 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 = CH_3CO, R′ = CH_3

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

(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, 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.