CHEMISTRY LETTERS, pp. 259-262, 1977. Published by the Chemical Society of Japan

SQUID BIOLUMINESCENCE IV. ISOLATION AND STRUCTURAL ELUCIDATION OF WATASENIA DEHYDROPRELUCIFERIN

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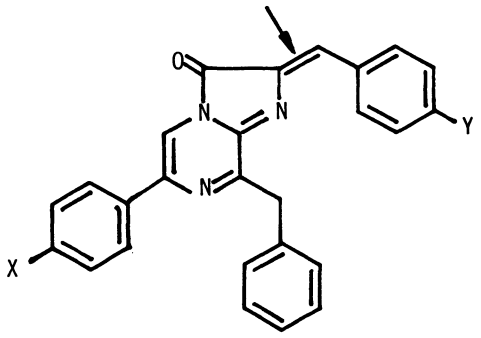
Watasenia dehydropreluciferin was isolated from livers of Watasenia scintillans. Its structure was determined as Ia rather than Ib. Sodium borohydride reduction of Ia gave Watasenia preluciferin (IV).

Previously we reported the isolation of Watasenia preluciferin[1] from the liver, and Watasenia luciferin[2] and W. oxyluciferin[3] from the arm photophores of the luminous squid, Watasenia scintillans (Japanese name: hotaru-ika). In-vitro bioluminescence of this squid, however, has not been demonstrated yet. In our continuous effort for isolation of substances related to the bioluminescence, we could isolate Watasenia dehydropreluciferin from livers of the squids as described in this communication.

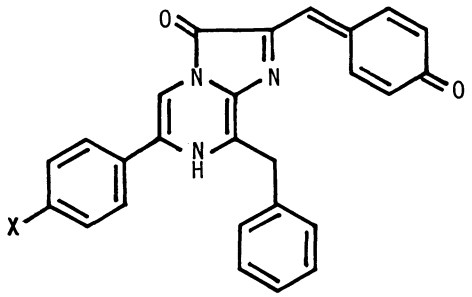
Isolation of W. dehydropreluciferin (I) from the squids. —— The contents taken out from lyophylized livers (70 g from ca 180 specimens) of the squids were thoroughly washed with oxygen-free CH_2Cl_2 and then extracted with oxygen-free methanol. The extracts were chromatographed on a silica gel column by elution with methanol-CH_2Cl_2 (1:7). Dark-colored fractions were collected and evaporated to give a solid. The subsequent yellow-colored fractions contained W. preluciferin (IV) (22.5 mg) as described previously.[1] The solid was further separated twice by TLC [Merck Kieselgel 60 F_{254}; acetone-CH_2Cl_2 (1:10) and ethyl ether-n-hexane (5:1)]. Elution of the red fluorescent band gave a dark-red solid (3.3 mg). Mass spectrum of this compound showed a strong peak at m/e 421, which is two mass units less than M^+ of W. preluciferin (IV).[1] When reduced with $NaBH_4$, this compound was converted to IV. These results indicated that the dark-red compound is a dehydro derivative of IV. The structure was finally determined as I by comparison of UV-VIS and mass spectra, and Rf values on TLC [MeOH-CH_2Cl_2 (1:10) Rf 0.57; ethyl ether-n-hexane (5:1) Rf 0.27; ethyl ether Rf 0.52] with synthetic I described below.



(Ia) X = Y = OH

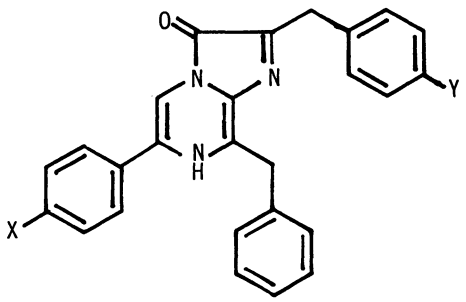
(IIa) X = OCH_3, Y = OH

(III) X = OCH_3, Y = H



(Ib) X = OH

(IIb) X = OCH_3



(IV) X = Y = OH

(V) X = OCH_3, Y = OH

(VI) X = OCH_3, Y = H

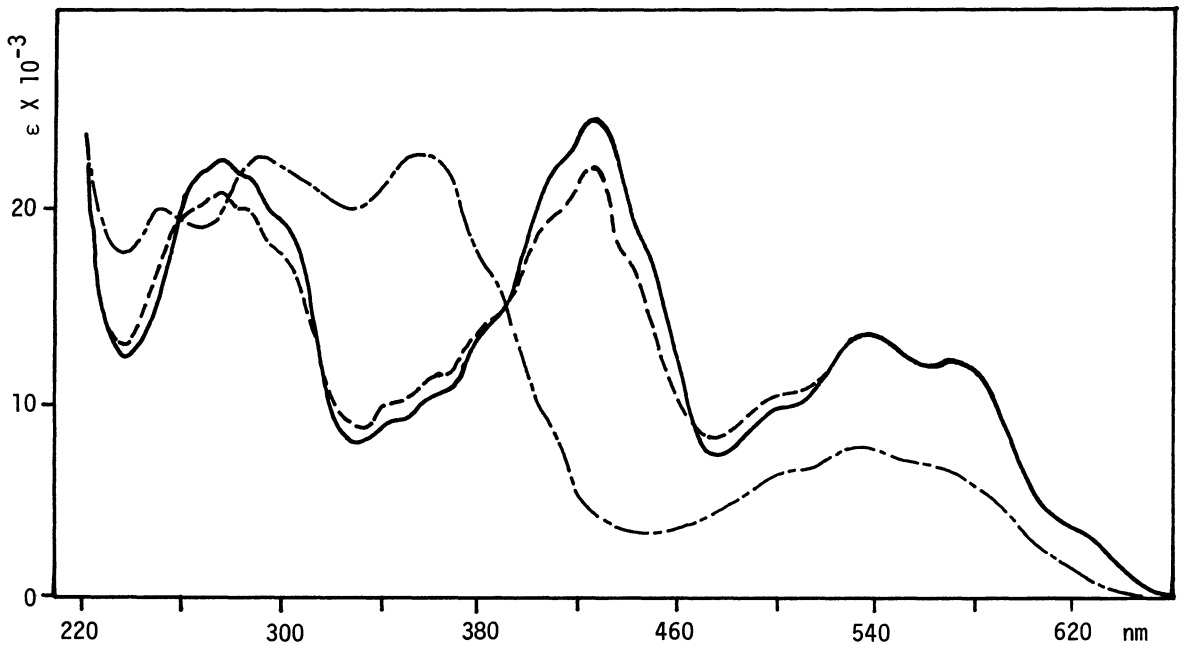


FIG. 1. UV-VIS SPECTRA IN ETHANOL

(I) ——— (II) - - - - - (III) —·—·—

Synthesis of W. dehydropreluciferin (I). —— To an ice-cooled solution of W. preluciferin (IV)[1] (100 mg) in a mixture of abs. ethanol and ether was added MnO_2[4] (1 g), and the mixture was stirred in an ice-bath for 2 hr. After filtration, the filtrate was evaporated under vacuum to dryness and the residue chromatographed on a silica gel column using acetone-CH_2Cl_2 (1:10) as eluant. The crude product from the earlier fractions was crystallized from ether to give I as dark-red crystals (73 mg),[8] decomp. p. ca 250°; MS (m/e) 421 (M^+, base peak), 393, 261; NMR (ppm in DMSO-d_6) 4.31 (2H,s), 6.76 (2H, $A_2'X_2'$, J=8Hz), 6.93 (2H, $B_2'Y_2'$, J=8Hz), 7.1-7.6 (6H,m), 7.75 (2H, $A_2'X_2'$, J=8Hz), 7.87 (1H,s), 8.28 (2H, $B_2'Y_2'$, J=8Hz), 9.56 (1H,br,s,OH); IR (cm^{-1} in KBr) 3300br, 1685, 1570br,s, 1510s, 1440, 1400, 1250br,s, 1155s; λ_{max}^{EtOH} nm (ε) 274 (22500), 425 (24400), 536 (13600), 574 (12500); $\lambda_{max}^{EtOH-NaOH}$ 295 (7800), 546 (25500), 587 (23400), 629 (17600). It gives no light with Oplophorus luciferase,[5] which catalizes luminescence of preluciferin (IV), nor in dimethyl sulfoxide, in which IV gives light. Sodium borohydride reduction of I in ethanol afforded IV (yield 93%).

From the later fractions of the chromatography was recovered IV (18 mg).

Structure of W. dehydropreluciferin (I). —— Two tautomeric structures, Ia and Ib, are possible for I. Comparison of UV-VIS spectra of I and its derivatives, II[6] and III[7], (Fig. 1) indicates that these compounds have the same chromophore and hence I must have structure Ia (in ethanol). This conclusion is further supported by analysis of its NMR spectrum (in DMSO). Thus, two pairs of $A_2'X_2'$ patterns show the normal ortho coupling constants (8 Hz) of p-substituted benzenes. Configuration of the extended double bond (indicated by an arrow in Ia) is not determined, but the configuration in Ia would be sterically more favorable.

In the case of firefly, firefly dehydroluciferin has been considered to act some important role on the bioluminescence.[9] The role of W. dehydropreluciferin in the bioluminescence of the squid is not known and further investigation is necessary.

Acknowledgments —— We thank the Japan Society for the Promotion of Science for financial support, and Mr. K. Aoyanagi for his help on collection of the squids.

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6) II was synthesized from V (mp 165-171°C)by MnO_2 oxidation, yield 79%, mp 247-249°C,[8] MS (m/e) 435 (M^+, base peak), 407, 291; λ_{max}^{EtOH} nm (ε) 274 (20900), 425 (22000), 535 (13200), 574 (19500); IR (cm^{-1} in KBr) 3200br, 1680, 1565br,s, 1500, 1440, 1400, 1280s, 1252s, 1155s; NMR (ppm in pyridine-d_5) 371 (3H,s), 446 (2H,s), 7.05 (2H, $\underline{A}_2'X_2'$, J=8Hz), 7.1-7.5 (6H,m), 7.88 (1H,s), 7.95 (2H, $A_2'\underline{X}_2'$, J=8Hz), 8.50 (2H, $B_2'\underline{Y}_2'$). Sodium borohydride reduction of II afforded methylated preluciferin (V) (yield 77%).

7) III was synthesized from VI (mp 100-105°C)by MnO_2 oxidation, yield 94%, mp 208-210°C, MS (m/e) 419 (M^+, base peak); λ_{max}^{EtOH} nm (ε) 252 (18900), 295 (25000), 355 (21800), 536 (7600); NMR (ppm in $CDCl_3$) 3.81 (3H,s), 4.34 (2H,s), 6.88 (2H, $\underline{A}_2'X_2'$, J=8Hz), 7.1-7.6 (m), 7.69 (2H, $A_2'\underline{X}_2'$, J=8Hz), 8.23 (2H,m).

8) Satisfactory elemental analyses were obtained.

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(Received January 14, 1977)