CHEMISTRY LETTERS, pp. 141-144, 1975. Published by the Chemical Society of Japan

SQUID BIOLUMINESCENCE II. ISOLATION FROM WATASENIA SCINTILLANS AND SYNTHESIS OF 2-(p-HYDROXYBENZYL)-6-(p-HYDROXYPHENYL)-3,7-DIHYDROIMIDAZO[1,2-a]PYRAZIN-3-ONE

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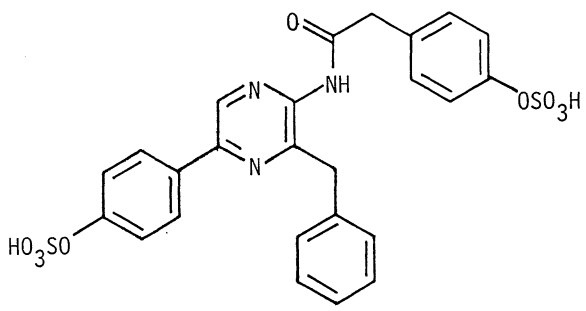
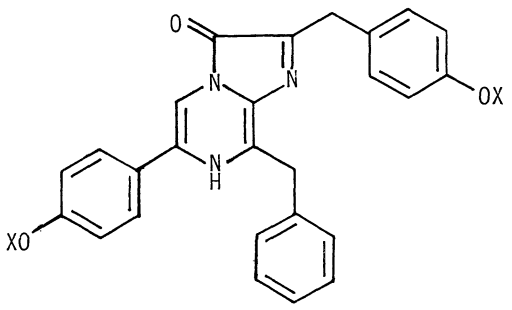
2-(p-Hydroxybenzyl)-6-(p-hydroxyphenyl)-3,7-dihydroimidazo-[1,2-a]pyrazin-3-one was isolated from the squid, Watasenia scintillans. Its structure was determined by synthesis. It is considered to be a precursor of hitherto unknown Watasenia luciferin or photoprotein.

In the previous paper[1] we reported the isolation from Watasenia scintillans (Japanese name: hotaru-ika) a fluorescent compound, Watasenia oxyluciferin (I), which is considered to be the light emitter by analogy from the proposed mechanisms for other bioluminescent systems.[2,3] It has not been clarified, however, whether the Watasenia bioluminescence system is the luciferin-luciferase type[2] or a kind of the photoproteins[3] since a complete bioluminescent system could not be extracted from the squid. Based on the structure of the oxyluciferin (I) the luciferin (or a chromophore if the system is a photoprotein type) could be IIb or a compound of the similar type.[1,2] As reported earlier,[4] compounds such



(I)



(IIa) X = H

(IIb) X = SO_3H

as II should be chemiluminescent in aprotic solvents such as dimethyl sulfoxide (DMSO) with or without a strong base. Therefore, if tissues of the squid contain II or a compound of the similar structure chemiluminescence might be able to demonstrate with the tissues even though we cannot isolate Watasenia luciferase (or Watasenia photoprotein). We found that lyophylized liver tissues of the squid shows strong chemiluminescence in DMSO, while the luminescence is scarcely observed from the tissues of other parts of the body. A chemiluminescent compound was isolated from the livers and its structure determined as IIa by synthesis. This compound is closely related to Renilla luciferin (partial structure was determined)[5] and also to the light-emitting chromophore in aequorin obtained from the luminous jellyfish Aequorea.[3] A plausible role of IIa in Watasenia bioluminescence presumed from its structure would be that after being transported from the liver to the photophores it is converted to its sulfate (possibly IIb)[6] which then acts as the luciferin[5] or combines further with a protein to form a photoprotein.[3]

Isolation and structure determination of IIa —— The contents taken out from lyophylized livers (200 g) of the squids were washed thoroughly with CH_2Cl_2 and MeOH-CH_2Cl_2 (1:15), and then extracted with methanol. The yellow-green methanol extracts were combined and evaporated to dryness. The residue was chromatographed on a silica gel column using MeOH-CH_2Cl_2 (1:3). The yellow-fluorescent fractions were evaporated and the residue was further separated on a tlc plate using MeOH-CH_2Cl_2 (1:7). Elution of the yellow band (Rf=0.69) gave yellow crystals (43 mg). Further tlc separations and crystallizations from methanol gave

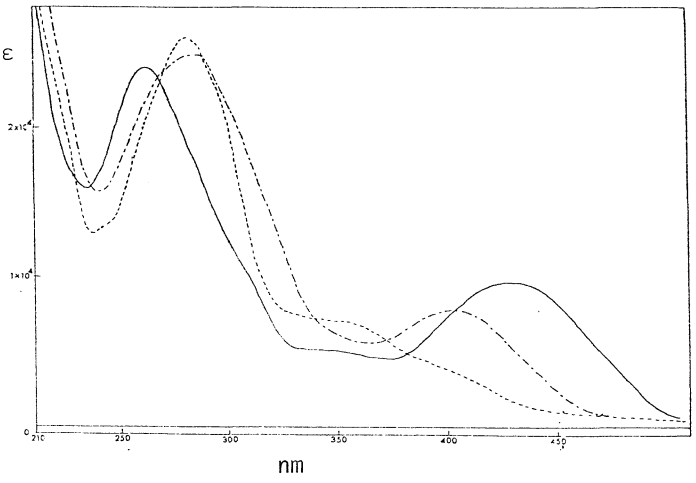


Fig. 1
UV Spectra of
Natural and Synthetic IIa

——— in MeOH
------ in MeOH + HCl
------ in MeOH + NaOH

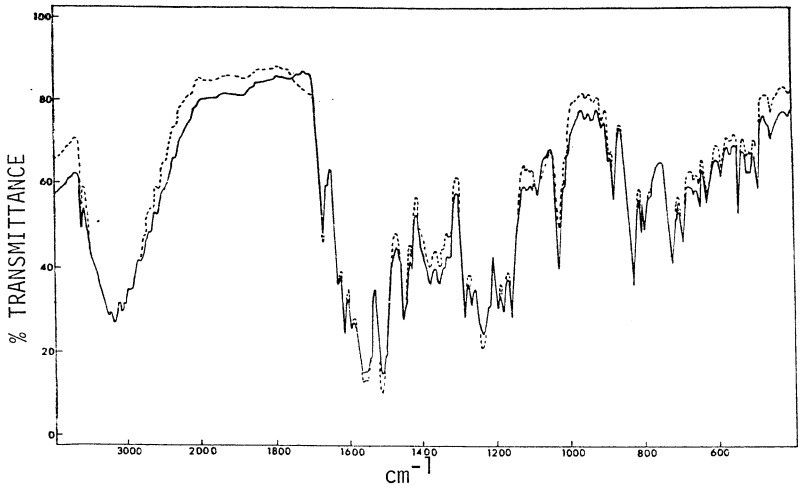


Fig. 2
IR Spectra of IIa

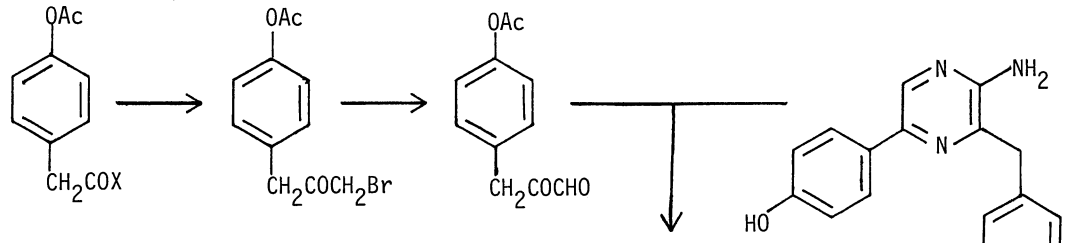
----- Natural
——— Synthetic
(KBr disc)

orange-yellow prisms, mp 175-178°C (dec) (15 mg): M^+ 423; δ(CD_3OD) 4.08 (2H,s), 4.41 (2H,s), 6.69 (2H, $\underline{A}_2'X_2'$, J=8 Hz), 6.68 (2H, $\underline{A}_2'X_2'$, J=8 Hz), 7.1-7.9 (m); uv and ir, see Figs. 1 and 2. This compound was identified as IIa by comparison of mp, Rf values on tlc plates, and the spectral data (uv, ir, nmr and mass) with the authentic sample synthesized by the route described below.

Synthesis of IIa —— p-Acetoxyphenylacetic acid (III) was treated with $SOCl_2$ to give p-acetoxyphenylacetyl chloride (IV), which was converted to p-acetoxyphenyl bromomethyl ketone (V) (mp 86°C) in 68% yield by treatment with an excess of diazomethane followed by the addition of HBr in ether. The bromoketone (V) (3.5 g) was warmed in pyridine (15 ml) at 80°C for 2 hr and the solution was evaporated in vacuo to dryness. After being washed with dry ether the residue was dissolved in water (50 ml) and to this solution was added p-nitrosodimethylaniline·HCl (2.5 g) dissolved in water. The mixture was treated with 10% NaOH (20 ml) at 10°C, when orange-brown solid precipitated. The solid was collected and mixed with 10% H_2SO_4 (100 ml) in a mortor to make a slurry. The solid was again collected by filtration giving a crude p-acetoxybenzylglyoxal (VI) (1.8 g, 70%): M^+ 206; δ(in DMSO-d_6 it exists in the enolic form) 2.28 (3H,s), 6.36 (1H, s), 7.17 (2H, $\underline{A}_2'X_2'$, J=8 Hz), 7.89 (2H, $\underline{A}_2'X_2'$, J=8 Hz), 9.28 (1H,s), 9.86 (1H,s,O$\underline{H}$).

2-Amino-3-benzyl-5-(p-hydroxyphenyl)pyrazine (VII)[7] (350 mg) and the glyoxal (VI) (260 mg) were dissolved in ethanol (20 ml) and the solution was mixed with water (2 ml) and 3% ethanolic HCl (4 ml).[8] The mixture was heated at 80°C for 3 hr and then evaporated to dryness in vacuo. The residue was chromatographed

on a silica gel column using CH_3OH-CH_2Cl_2 (1:10). IIa was obtained as orange-yellow prisms (from methanol), mp 175-178°C (dec); yield 336 mg (63%): M^+ 423 (Found: C, 70.70; H, 5.38; N, 9.06%. $C_{26}H_{23}O_4N_3 \cdot H_2O$ requires: C, 70.75; H, 5.25; N, 9.52%); nmr in CD_3OD is superimposable with that of natural IIa; uv and ir, see Figs. 1 and 2.



(III) X = OH
(IV) X = Cl
(V) (VI) (IIa) (VII)

Acknowledgements —— We thank the Toray Science Foundation for the grant and Mr. K. Aoyanagi, Namerikawa-shi, Toyama, for his help on collection of the squids.

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(Received December 9, 1974)