CHEMISTRY LETTERS, pp. 417-418, 1987.

Chemical Studies on Myctophina Fish Bioluminescence

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A new type of masked Watasenia preluciferin was isolated from the liver of myctophina fish and its structure was determined as Watasenia preluciferyl β-D-glucopyranosiduronic acid.

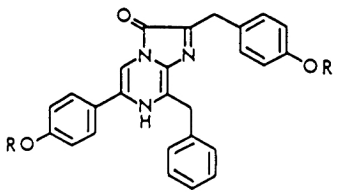
Watasenia preluciferin (WPL) (1), first isolated from the squid Watasenia scintillans,[1] is a compound playing a key role in the light-emitting process of various bioluminescent marine organisms[2] such as squids, shrimps, coelenterates, and fish. In the case of myctophina fish, WPL 1 was isolated either from the liver of Neoscopelus microchir (Japanese name; sango-iwashi)[3] or from a pair of big nasal photophores of Diaphus elucens (Japanese name; suito-hadaka).[4] It is of interest that not the free form, but rather the bound form of 1 posessing an unknown molecule was present in the fish liver.

The present paper reports the structure of this new bioluminescent substance to be watasenia preluciferyl β-D-glucopyranosiduronic acid (2). The methanol extracts obtained from the lyophilized livers of Diaphus elucens (3 g, 50 individuals) were chromatographed on a sephadex LH-20 column with MeOH. The fractions giving chemiluminescence in DMSO-t-BuOK were combined and rechromatographed on a column of Sephadex LH-20 using acetone-MeOH (1:1) to give a crude chemiluminescent substance, which was successively separated on a silica gel TLC using AcOEt-acetone-MeOH-H_2O (6:2:1:1), acetone-CH_2Cl_2-MeOH (1:2:1), and 90% MeCN as solvents (Rf value: 0.70, 0.80, and 0.50, respectively) giving rise to the pure compound 2 (ca. 0.5 mg). The UV spectrum of 2 (275 nm in MeOH) was very close to that of Renilla luciferyl sulfate (3).[5] The ^{1}H-NMR spectrum[6] of 2 also indicated the presence of the partial structure of 1 as an enol ether form with a sugar-like moiety. Acid hydrolysis of 2 with 1% HCl-MeOH (rt, 5 min) followed by extraction with AcOEt afforded an aglycone identical to WPL 1 in all respects whereas the other fragment remained in the aqueous phase. This hydrophilic counterpart could not be characterized in the usual way. Its structure was deduced as D-glucuronic acid, since light was emitted on adding a solution of β-glucuronidase in phosphate buffer (pH 7.6) to an aqueous solution of 2 containing 3% NaCl extracts of flesh of the fish. The structure of this luminescent substance is thus considered to be 2, as confirmed by the following synthesis.

O,O-Diacetyl WPL (4), prepared from 1 according to our previous report,[5] was treated with methyl (2,3,4-tri-O-acetyl-α-D-glucopyranosyl bromide)-uronate in the presence of silver trifrate and tetramethylurea in dry CH_2Cl_2 (rt, 2 h) to

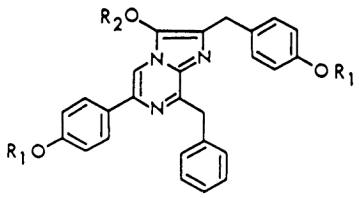
give pentaacetylluciferyl glucuronide methyl ester (5) in 42% yield. Heating 5 in MeOH containing 10 equiv. of NaOH under reflux for 2 min resulted in the removal of all protecting groups to give the desired luciferyl glucuronide 2 in 79% yield. The chromatographical (TLC, HPLC(ODS/30% CH_3CN)) and spectral (UV, ^{1}H-NMR, Mass(negative SIMS)) properties of synthetic 2 were identical with those of the natural product.

Glucuronide 2 was also found in the liver of Diaphus coeruleus (Japanese name: Hadaka-iwashi), a representative luminous fish in Japan, and various other Myctophina fish such as Diaphus suborbitalis, Benthosema fibulata, and Myctophum asperum. In contrast to glucuronide 2 in liver, free WPL 1 was found, for example, in the photophores of Diaphus coeruleus as well as Diaphus elucens (characterized by in vitro bioluminescence). From the data presented above, glucuronide 2, a masked form of 1, is possibly synthesized from luciferin 1 in the liver and conveyed to the photophores to be hydrolysed to the free form 1 (luciferin). It would be of interest to make a comparison of this new type of bioluminescence system with that in Renilla mülleri[5] or Watasenia scintillans.[7] The further characterization of luminous substances in the photophores of other Myctophina fishes is now in progress.



1 : R=H

4 : R=Ac



2 : R_1=H, R_2=1-β-D-glucopyranosyl-uronic acid

3 : R_1=H, R_2=SO_3H

5 : R_1=Ac, R_2=methyl (2,3,4-tri-O-acetyl-1-β-D-glucopyranosyl)-uronate

References

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(Received November 28, 1986)