アマクサアメフラシ内臓からのピロフェオフォルバイドaおよびbの単離

誌名	日本水産學會誌
ISSN	00215392
著者	小林, 優 神田, 扶由子 神谷, 久男
巻/号	57巻10号
掲載ページ	p. 1983-1983
発行年月	1991年10月

Short Paper

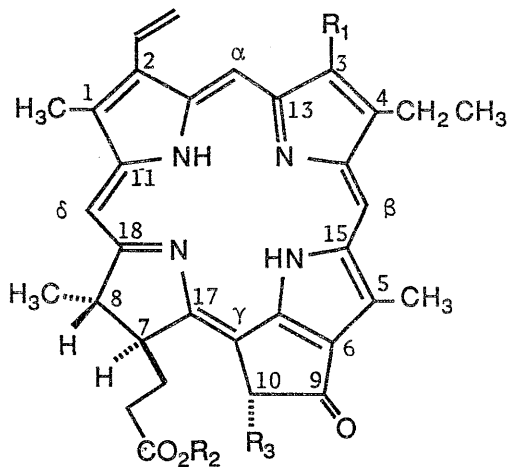
Occurrence of Pyropheophorbides *a* and *b* in the Viscera of the Sea Hare *Aplyria juliana*

Masaru Kobayashi,*1 Fuyuko Kanda,*1
and Hisao Kamiya*2

(Received March 7, 1991)

Pyropheophorbide *a* (**1a**) is the causative agent of severe photosensitive disease, caused by the ingestion of the viscera of the abalone *Haliotis discus* Hannai.[1] Identification of **1a** in marine invertebrates has not been reported for nearly 30 years, till the recent discovery of **1a** and its derivatives from several bivalves.[2] We report here the occurrence of fairly large amounts of **1a** and pyropheophorbide *b* (**2a**) in the sea hare *Aplysia juliana*. These observations suggest that such chlorophyll metabolites are rather widely distributed in marine mollusks. Sea hares are not commonly consumed by human beings but the complex and as yet unknown food chain in marine circumstances may transfer these photodynamic substances to sea foods. This is the first example of **2a** found in marine invertebrates. Even though **2a** and its methyl ester (**2b**) have been known long since, there are no reliable ^{1}H- and ^{13}C-NMR spectral data available for their identification in other sources. We recorded those of **2b** in this report simultaneously.

The viscera of *A. juliana* (1.83 kg), collected in July 1988 at Okirai Bay, Iwate prefecture, was extracted with MeOH and MeOH-$CHCl_3$ (1:1) to give 183 g of the extract. It was subjected to Folch's partition[3] to yield crude lipids (58.7 g). This was partitioned with a mixture of hexane-MeOH-H_2O (20:10:2) to hexane (46.56 g) and MeOH (10.8 g) extracts. Chromatography of the MeOH extract over a column of silica gel, with a gradient mixture of ethyl acetate-hexane and with 3% MeOH in $CHCl_3$, gave a 3:1 mixture of **1a** and **2a** (total 225 mg). Treatment of a portion (130 mg) of this mixture with ethereal diazomethane afforded a mixture of the methyl esters **1b** and **2b**. This was submitted, in portions, to silica gel column chromatography with hexane-acetone (8:2) giving **1b** (71 mg) and **2b** (29 mg), in this order. The molecular formulae of **1b** and **2b** were confirmed as $C_{34}H_{36}O_3N_4$ and $C_{34}H_{34}O_4N_4$, respectively, by high-resolution EI mass spectroscopy. The ^{1}H- and ^{13}C-NMR spectra of **1b** were identical to those reported for methyl pyropheophorbide *a*.[2,4-6] Compound **2b** showed no distinct mp but sintered at *ca.* 245°C. Its ^{1}H- and ^{13}C-NMR data showd close similarity to those of **1b**, except that the 3a-methyl signal of **1b** (^{13}C-NMR, δ 10.5; ^{1}H-NMR, δ 3.25, 3H, s) was converted to that of an aldehyde (^{13}C-NMR, δ 186.5; ^{1}H-NMR, δ 11.15, 1H, s). The ^{13}C-NMR signals of C-α (δ 96.5), C-4a (18.8), C-4b (16.9), and C-β (103.2) in **1b** were slightly shifted to δ 100.1, 18.0, 18.7, and 105.1, respectively, in **2b**. These deviations ($\Delta\delta$ 176.0, 3.6, -0.8, 1.8, and 1.9 ppm) are identical to those found between methyl pheophorbide *a* (**3**) and methyl pheophorbide *b* (**4**) ($\Delta\delta$ 175.2, 4.0, -0.9, 1.8, and 1.9 ppm),[5] and indicate clearly that the original minor component **2a** is pyropheophorbide *b*. The ^{1}H- and ^{13}C-NMR, and UV spectral data of **2b** were as follows: ^{1}H-NMR (400 MHz in $CDCl_3$, 0.0082 mol/*l*) δ: −1.57 and 0.51 (each 1H, br s, NH), 1.81 (3H, t, J=7.5 Hz, 4b-H_3), 1.83 (3H, d, J=7.5 Hz, 8a-H_3), 3.39 (3H, s, 1a-H_3), 3.63 and 3.64 (each 3H, s, 5a-H_3 and OMe), 4.04 (2H, br q, J=7.5 Hz, 4a-H_2), 4.27 (1H, m, 7-H), 4.47 (1H, dq, J=2.0, 7.5 Hz, 8-H), 5.08 and 5. 24 (each 1H, d, J=20.0 Hz, 10-H_2), 6.22 (1H, dd, J=11.0, 1.0 Hz, 2b *E*)-H), 6.37 (1H, dd, J=18.0, 1.0 Hz, 2b (*Z*)-H, 8.01 (1H, dd, J=18.0, 11.0 Hz, 2a-H), 8.25 (1H, s, δ-H), 9.58 (1H, s, β-H), 10.34 (1H. s, α-H), 11.15 (1H, s, 3a-H). ^{13}C-NMR (22.5 MHz, in $CDCl_3$, *ca.* 0.07 mol/*l*) δ: C-α (100.1, d), C-β (105.1, d), C-γ (105.1, s), C-δ (92.6, d), C-1a, 5a (11.4, 11.6, each q), C-2a (128.2, d), C-2b (122.5, t), C-3a (186.5, d), C-4a (18.0, t), C-4b (18.7, q), C-7 (51.4, d), C-7a (30.8, t), C-7b (29.2, t), C-7c (173.0, s), OMe (51.5, q), C-8 (49.7, d), C-8a (22.7, q), C-9 (195.3, s), C-10 (47.5, t), C-17 (162.5, s), C-18 (172.6, s). Other unassignable signals, δ: 130.6, 130.9, 131.3, 135.9, 136.5, 136.9, 142.4, 145.6, 149.3, 157.7 (each s. two singlet carbons are overlapped by other signals and are not detected). UV (EtOH) nm (s) 436 (76000), 530 (6100), 598 (5200), 654 (16000).



1a: $R_1 = CH_3$, $R_2 = H$, $R_3 = H$
1b: $R_1 = CH_3$, $R_2 = CH_3$, $R_3 = H$
2a: $R_1 = CHO$, $R_2 = H$, $R_3 = H$
2b: $R_1 = CHO$, $R_2 = CH_3$, $R_3 = H$
3: $R_1 = CH_3$, $R_2 = CH_3$, $R_3 = CO_2CH_3$
4: $R_1 = CHO$, $R_2 = CH_3$, $R_3 = CO_2CH_3$

References

1) J. Tsutsumi and Y. Hashimoto: *Agr. Biol. Chem.*, **28**, 467–470 (1964) and references cited therein.
2) K. Sakata, K. Yamamoto, H. Ishikawa, N. Watanabe, H. Etoh, A. Yagi, and K. Ina: *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu*, **1990**, 57–64.
3) J. Folch, M. Lees, and G. H. S. Stanley: *J. Biol. Chem.*, **226**, 497–509 (1957).
4) K. M. Smith, D. A. Goff, R. J. Abraham: *Org. Magn. Reson.*, **22**, 779–783 (1984).
5) K. M. Smith and J. F. Unsworth: *Tetrahedron*, **31**, 367–375 (1975).
6) V. Wray, U. Jurgens, and H. Brockmann Jr.: *Tetrahedron*, **35**, 2275–2283 (1979).

*1 Faculty of Pharmaceutical Sciences, Hokkaido University, Kita, Sapporo, Hokkaido 060, Japan (小林優, 神田扶由子: 北海道大学薬学部).
*2 School of Fisheries Sciences, Kitasato University, Sanriku, Kesen, Iwate 022-01, Japan (神谷久男: 北里大学水産学部).