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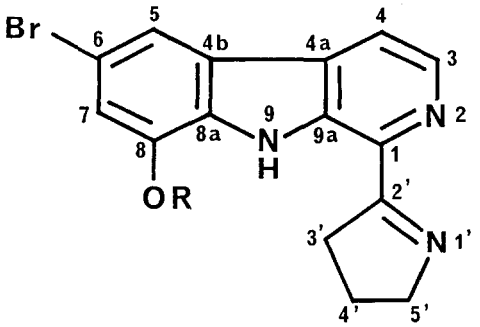
EUDISTOMIDIN-A, A NOVEL CALMODULIN ANTAGONIST FROM THE OKINAWAN TUNICATE EUDISTOMA GLAUCUS

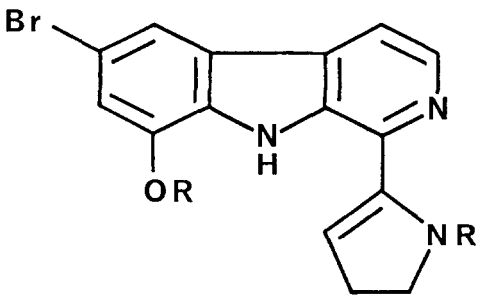
Jun'ichi Kobayashi*, Hideshi Nakamura, Yasushi Ohizumi
Mitsubishi-Kasei Institute of Life Sciences,
11 Minamiooya, Machida, Tokyo 194, Japan
and
Yoshimasa Hirata
Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468, Japan

Summary: Eudistomidin-A, a novel indole alkaloid having calmodulin-antagonistic activity has been isolated from the Okinawan tunicate Eudistoma glaucus and the structure determined to be 1 on the basis of the spectral data and chemical derivatization.

During our studies on bioactive metabolites from marine organisms[1-4], we have examined pharmacological and biochemical effects of extracts of various marine tunicates[5]. Recently, calmodulin antagonists have been very useful as tools for studying physiological functions of calmodulin[6], a ubiquitous Ca^{2+}-binding protein which acts as a major mediator regulating cellular function and a variety of cellular enzyme system. In this communication, we report the isolation and structure elucidation of eudistomidin-A (**1**), a novel calmodulin antagonist from the Okinawan tunicate Eudistoma glaucus.

The green colored colonial tunicate Eudistoma glaucus was collected at Ie Island, Okinawa, using SCUBA (-5 ~ -10 m), in July 1985. The methanol-toluene (3:1) extract of the tunicate was fractionated by monitoring inhibitory activities on calmodulin-activated brain phosphodiesterase. The extract was partitioned with toluene and water. The toluene soluble material was subjected to a silica gel column with hexane-ethyl acetate (2:1) to afford an active fraction, which was rechromatographed on a silica gel column with chloroform-methanol (85:15) to yield eudistomidin-A (**1**, 0.0003% wet weight)





1 ; R = H

2 ; R = Ac

3 ; R = Ac

as a yellow solid, mp. 225-230°C (decomp.).

The presence of a β-carboline ring containing bromo, hydroxy and alkyl substituents was suggested by UV maxima at 222(ε 33000), 254(17000) and 371(5500) nm[7] in MeOH; ^{13}C NMR (DMSO-d_6) signals at δ 144.4 (s, C-8), 138.0 (d, C-3), 136.1 (s, C-1), 133.7 (s, C-9a), 129.2 (s, C-4b), 128.2 (s, C-8a), 123.5 (s, C-4a), 116.8 (d, C-7), 115.8 (d, C-4), 115.1 (d, C-5) and 112.3 (s, C-6) ppm[8,9]; ^{1}H NMR (DMSO-d_6) signals for four aromatic protons at δ 8.44 (1H, d, J=5.2 Hz, H-3), 8.22 (1H, d, J=5.2 Hz, H-4), 7.98 (1H, d, J=1.8 Hz, H-5) and 7.08 (1H, d, J=1.8 Hz, H-7)[8,10]; and a fragment ion doublet at m/e 287 and 289 for $C_{12}H_6N_3OBr$ which could be assigned to a partial structure consisting of bromo-hydroxy-β-carboline (C-1 ~ C-9a) and an imino (C=N) group (N-1' and C-2'). The presence of the imino group was evident from the signal at δ 175.8 (C-2') in the ^{13}C NMR spectrum[11] and the bands at 1625 and 1605 cm^{-1} in the IR spectrum[12] of **1**. The position of the bromine at C-6 and the hydroxy group at C-8 was based upon comparison of the ^{1}H and ^{13}C NMR chemical shift data with those of 5-bromoindole or 7-methoxyindole derivatives[13,14] and the NOE enhancement (2%) of the H-7 signal of **1** when the OH group (δ 3.29, brs) or the indole NH (δ 11.2, brs) was irradiated.

In addition to the resonances assigned to the β-carboline protons in the ^{1}H NMR spectrum, signals were also visible at δ 4.23 (m, H-5'), 3.11 (m, H-3') and 1.99 (m, H-4'), which could be attributed to the methylene protons to generate a pyrroline ring[15] by the attachment to the C=N group at C-2' and N-1'. EIMS produced a molecular ion doublet at m/e 329, 331 and a fragment ion doublet at m/e 287, 289 corresponding to loss of $CH_2CH_2CH_2$ unit from $C_{15}H_{12}N_3OBr(M^+)$. The ^{13}C values of δ 34.7(C-3'), 21.2(C-4') and 61.9(C-5') were in good agreement with these assignments. Further confirmation of the relative positions of the hydroxy group at C-8 and the imino group in the

pyrroline ring was provided by the ^{1}H and ^{13}C NMR spectra of the two type of acetates **2**[16] and **3**[17], which were yielded in the ratio 1:1 by treatment of **1** with pyridine and acetic anhydride. N-Acetylation of pyrroline ring like the product **3** has been observed to other alkaloids containing a pyrroline ring[18]. The ^{13}C signal at C-8 of **2** was shifted to higher field (δ 144.4 → 138.9) by acetylation of the OH group while the H-7 of **2** shifted to lower field (δ 7.08 → 7.55).

Eudistomidin-A is the first calmodulin antagonist from marine origins. The values of the 50% inhibitory concentration of calmodulin-activated brain phosphodiesterase were 2 x 10^{-5} M for **1** and 3 x 10^{-4} M for W-7[6], a well-known calmodulin antagonist, indicating that **1** was about 15 times more potent than W-7. Eudistomidin-A appears to be biogenetically derived from tryptophan (N-2 ~ C-9a) and glutamate unit (C-1, N-1' and C-2' ~ C-5'). Similar β-carboline compounds, eudistomin A ~ Q, had been isolated from the Caribbean tunicate Eudistoma olivaceum[19,20]. A half of the β-carboline compounds contains a hydroxy group which is always substituted at C-6 in the benzenoid ring, whereas the hydroxy group of **1** is attached to C-8. Further chemical and pharmacological studies of eudistomidin-A and its related compounds are in progress.

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References

1. J. Kobayashi, Y. Ohizumi, H. Nakamura, T. Yamakado, T. Matsuzaki and Y. Hirata, Experientia **39**, 67 (1983).
2. Y. Ohizumi, A. Kajiwara, H. Nakamura and J. Kobayashi, J. Pharm. Pharmacol., **36**, 785 (1984).
3. H. Wu, H. Nakamura, J. Kobayashi, Y. Ohizumi and Y. Hirata, Tetrahedron Lett., **25**, 3719 (1984).
4. H. Nakamura, H. Wu, J. Kobayashi, M. Kobayashi, Y. Ohizumi and Y. Hirata, J. Org. Chem., **50**, 2494 (1985).
5. Y. Nakamura, J. Kobayashi, J. Gilmore, M. Mascal, K. L. Rinehart, Jr., H. Nakamura and Y. Ohizumi, J. Biol. Chem., in press.
6. Hidaka, T. Yamaki, T. Totsuka and M. Asano, Mol. Pharmacol., **15**, 55 (1978).

7. A. I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Pergamon Press, 1964, New York, p. 176.
8. T. A. Crabb, "Annual Reports on NMR Spectroscopy," Academic Press, 1982, New York, p. 138-192.
9. C. A. Coune, L. J. G. Angenot and J. Denoël, Phytochemistry, **19**, 2009 (1980).
10. U. M. G. Aguiar, R. B. Filho, O. R. Gottlieb, J. G. S. Maia, S. L. V. Pinho and J. R. De Sousa, Phytochemistry, **19**, 1859 (1980).
11. N. Naulet, M. L. Filleux, G. J. Martin and J. Pornet, J. Magn. Reson., **7**, 326 (1975).
12. L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," Chapman and Hall, 1975, London, p. 294-308.
13. R. S. Norton and R. J. Wells, J. Am. Chem. Soc., **104**, 3628 (1982).
14. J. Banerji, A. Chatterjee, D. J. Roy and J. N. Shoolery, Phytochemistry, **21**, 2765 (1982).
15. S. J. Love and J. A. Moore, J. Org. Chem., **33**, 2361 (1968).
16. **2**: HREIMS $C_{17}H_{14}O_2N_3Br$ (obs. 371.0261; calcd. 371.0252); ^1H NMR ($CDCl_3$) δ 10.8 (NH-9, brs), 8.54 (H-3, d, J=5.3 Hz), 8.15 (H-5, d, J=1.8 Hz), 7.94 (H-4, d, J=5.3 Hz), 7.55 (H-7, d, J=1.8 Hz), 4.29 (H-5', m), 3.32 (H-3', m), 2.50 (OAc, s) and 2.09 (H-4', m); ^{13}C NMR ($CDCl_3$) δ 176.7 (C-2', s), 168.3 ($O\underline{C}(=O)CH_3$, s) 138.9 (C-3, d), 138.9 (C-8, s), 136.7 (C-1, s), 135.4 (C-9a, s), 131.9 (C-4b, s) 128.4 (C-8a, s), 125.0 (C-4a, s), 123.8 (C-7, d), 122.1 (C-5, d), 116.2 (C-4, d), 111.8 (C-6, s), 62.3 (C-5', t), 34.9 (C-3', t), 21.9 (C-4', t) and 21.1 ($OC(=O)\underline{CH_3}$, q).
17. **3**: EIMS m/e 413 (M^+); ^1H NMR ($CDCl_3$) δ 9.14 (NH-9, brs), 8.47 (H-3, d, J=5.3 Hz), 8.03 (H-5, d, J=1.8 Hz), 7.77 (H-4, d, J=5.3 Hz), 7.50 (H-7, d, J=1.8 Hz), 5.82 (H-3', t, J=2.6 and 2.9 Hz), 4.22 (H-5', m), 2.82 (H-4', m) and 2.41 (NAc and OAc, s).
18. W. L. Fitch, P. M. Dolinger and C. Djerassi, J. Org. Chem., **39**, 2974 (1974).
19. J. Kobayashi, G. C. Harbour, J. Gilmore and K. L. Rinehart, Jr., J. Am. Chem. Soc., **106**, 1526 (1984).
20. K. L. Rinehart, Jr., J. Kobayashi, G. C. Harbour, R. G. Hughes, Jr., S. A. Mizsak and T. A. Scahill, J. Am. Chem. Soc., **106**, 1524 (1984).

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