Pergamon

Tetrahedron Letters, Vol. 37, No. 22, pp. 3871-3874, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)00704-6

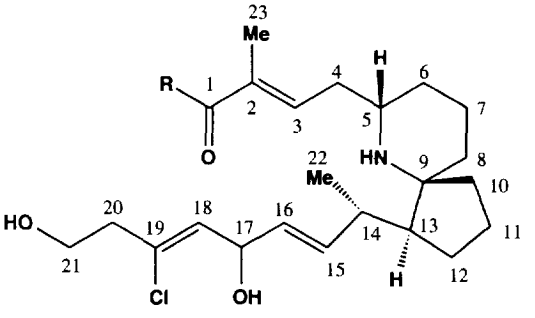
Pinnaic Acid and Tauropinnaic Acid: Two Novel Fatty Acids Composing a 6-Azaspiro[4.5]decane Unit from the Okinawan Bivalve *Pinna muricata*

Tong Chou, Makoto Kuramoto,[†] Yuko Otani, Mayumi Shikano, Kazunaga Yazawa, and Daisuke Uemura*[†]

Sagami Chemical Research Center, Nishi-Ohnuma 4-4-1, Sagamihara, Kanagawa 229, Japan
[†]Department of Chemistry, Faculty of Science, Shizuoka University, Ohya, Shizuoka 422, Japan

Abstract : Two novel fatty acids with cPLA$_2$ inhibitory activity, pinnaic acid and tauro-pinnaic acid, were isolated from the Okinawan bivalve *Pinna muricata*. Their structures, including the relative stereochemistry at C5, C9, C13 and C14, were elucidated by extensive 2D NMR spectroscopic analysis.

Specific inhibitor of phospholipase A_2 (PLA_2) has been considered to be potential drugs for the treatment of inflammation and other disease states, since PLA_2 is linked to the initial step in the cascade of enzymatic reactions which lead to the generation of inflammatory mediators.[1-3] Marine natural products, such as manoalide[4] and luffariellolide[5] have been reported to be potent PLA_2 inhibitors.[6,7] A cytosolic 85-kDa phospholipase ($cPLA_2$)[8] exhibits specificity for the release of arachidonic acid from membrane phospholipids.[9] Therefore, compounds that inhibit $cPLA_2$ activity have been targeted as anti-inflammatory agents. In our continuing search for bioactive substances from marine organisms,[10,11] two novel polyketides containing 6-azaspiro[4.5]decane unit with $cPLA_2$ inhibitory activity, pinnaic acid (**1**) and tauropinnaic acid (**2**), were isolated from the Okinawan bivalve *Pinna muricata*.[12] We report here the isolation and structural elucidation of these two compounds.



1 : R=OH
2 : R=NHCH$_2$CH$_2$SO$_3$H

The 80% EtOH extract of viscera (10kg) of *P. muricata* (3,000 individuals) was partitioned between EtOAc and water. The water layer was fractionated by column chromatography on TSK G-3000S polystyrene gel (Tosoh Co.) (50% EtOH), Sephadex LH-20 (MeOH), DEAE Sephadex A-25 (0.02 M pH 6.9 phosphate buffer), ODS-AQ (YMC, Inc.) (50% MeOH), and silica gel BW-127ZH (Fuji Silysia Co.) (30% MeOH/$CHCl_3$) to obtain two novel fatty acids, pinnaic acid (**1**) (1 mg) and tauropinnaic acid (**2**) (4 mg). The molecular formula of tauropinnaic acid (**2**) was deduced to be $C_{25}H_{41}ClN_2O_6S$ by HRFABMS (MH^+, $C_{25}H_{42}{}^{35}ClN_2O_6S$ *m/z* 533.2452, Δ -0.5 mmu). A detailed analysis of ^{1}H-NMR, ^{13}C-NMR, DEPT and ^{1}H-^{13}C COSY spectra showed that **2** contained two methyls, 11 methylenes, eight methines and four quaternary carbons (Table 1). The presence of five protons on heteroatoms was deduced from the molecular formula coupled with the ^{13}C-NMR data. Three

of these protons were identified by the ^{1}H-NMR spectrum measured in DMSO-d_6 [$\delta_{\text{-NHCO-}}$ 7.38 (t) ppm, $\delta_{17\text{-OH}}$ 5.06 (d) ppm and $\delta_{21\text{-OH}}$ 4.63 (t) ppm]. ^{13}C-signal at δ 169.04 (s) (C1) was assigned to an amide carbon, and signals at δ 68.80 (d) (C17) and at δ 58.06 (t) (C21) were assigned to hydroxyl-bearing carbons, respectively. Six olefinic carbons were observed at δ 136.98 (d) (C15), 134.51 (s) (C2), 132.14 (s) (C19), 130.69 (d) (C16), 128.29 (d) (C18) and 127.70 (d) (C3). The ^{1}H-^{1}H COSY spectrum of **2** gave four partial structures **a**-**d**, as shown in Fig. 1. The connectivity of C23 and C3 via C2 was implied by allylic coupling and homoallylic coupling correlations between 23-Me and H3 and between 23-Me and 4-methylene protons, while C18 and C20 were connected via C19 based on a crosspeak between H18 and 20-methylene protons.

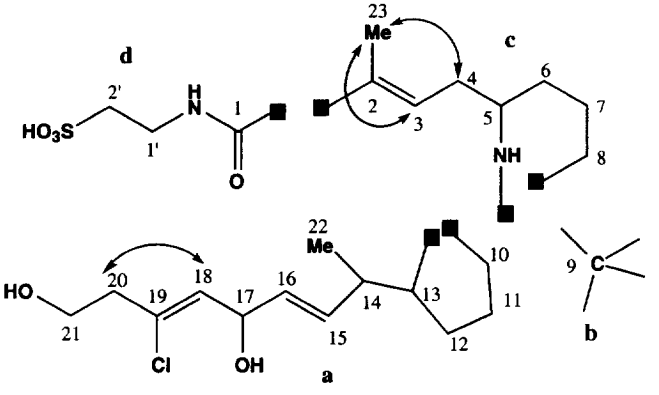


Fig. 1. Four partial structures of **2** established by the 2D-NMR (400 MHz) spectroscopy (arrows : allylic coupling correlation; ■ : quaternary carbons).

$^2J_{CH}$, $^3J_{CH}$ long-range coupling correlations in the HMBC[13] spectrum of **2** were used to assemble the four partial structural units through quaternary carbons. The nitrogen-bearing methine carbon was assigned to C5 because of its reasonable chemical shift (δ_C 53.46, δ_H 3.40). The H12/C9, H10/C9 and H8/C10 crosspeaks suggested the connectivities from C13 and C10 to C9 (δ 68.74 ppm), the last of which was considered to be a quaternary carbon bearing a nitrogen atom, and revealed that C8 of the **c** unit was connected to C10 of the **a** unit through a nitrogen-bearing quaternary carbon (C9) of the **b** unit to form a novel 6-azaspiro[4.5]decane skeleton. The presence of a 6-membered ring (C5-C9 and the secondary amine group) and a 5-membered ring (C9-C13) was supported by coupling constants typical of ring protons ($^3J_{5H\text{-}6Ha}$=12 Hz, $^3J_{5H\text{-}6Hb}$=2 Hz,[14] $^2J_{10Ha\text{-}10Hb}$=11.4 Hz,[15] $^3J_{10Ha\text{-}11Ha}$=10 Hz, $^3J_{10Ha\text{-}11Hb}$=5.6 Hz, $^3J_{10Hb\text{-}11Ha}$=7.0 Hz, $^2J_{12Ha\text{-}12Hb}$=11.7 Hz and $^3J_{12Hb\text{-}11Ha}$=6.5 Hz) and by the 6 degrees of unsaturation (four double bonds and two rings). Furthermore, HMBC correlations observed between 23-Me/C1, H3/C1 and 1'-CH_2/C1 suggested the connectivity of the **c** unit to the **d** unit through the amide carbonyl carbon C1. The chemical shifts (δ_C 49.05, δ_H 3.00) of 2'-methylene and MS fragments (*m/z* 425 [M$^+$-($CH_2CH_2SO_3H$)] in EIMS and *m/z* 426 [MH$^+$-($CH_2CH_2SO_3H$)] in CIMS) confirmed the location of the sulfonyl group (-SO_3H) adjacent to C2' as a taurine group. Since all of quaternary carbons were assigned as described above, the position of the chlorine atom was suggested to be C19 (δ 132.14 ppm).[16] Eventually, the gross structure of tauropinnaic acid (**2**) was completely elucidated. The *E* geometry of the C15-C16 double bond was derived from the large coupling constant (15.9 Hz) between H15 and H16. Additional evidence for the *E* configuration was obtained from the NOESY crosspeak between H15 and H17. The *Z* geometry of the C18-C19 double bond was established by NOESY crosspeaks between the H18 and 20-methylene protons, while the *E* geometry of the C2-C3 double bond was based on the carbon chemical shift of

the 23-Me signal at δ 11.3 ppm and confirmed by the NOESY crosspeak between 23-Me and 4-methylene protons.

Table 1. NMR Data of Pinnaic Acid (**1**) and Tauropinnaic Acid (**2**) (400 MHz, in CD_3OD).

	pinnaic acid (**1**)		tauropinnaic acid (**2**)		
Position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	HMBC
1	170.14 (s)		169.04 (s)		
2	134.51 (s)		134.51 (s)		
3	127.71 (d)	6.45 (t, 7.5)	127.71 (d)	6.28 (t, 7.2)	C1, C23
4	31.53 (t)	2.56 (br.)	31.24 (t)	2.56 (br.)	C2
5	53.46 (d)	3.40 (m)	53.46 (d)	3.40 (m) [14]	
6	27.20 (t)	1.44, 1.92	27.21 (t)	1.44, 1.92 (Hb, br.dd, 12.0, 2.0) [15]	
7	19.46 (t)	1.64, 1.82	19.38 (t)	1.64, 1.82	
8	34.17 (t)	1.68, 1.82	34.11 (t)	1.68, 1.82	C10
9	68.48 (s)		68.74 (s)		
10	33.70 (t)	1.86, 2.15	33.74 (t)	1.86, 2.15 (Ha, dd, 11.4, 7.0) [15]	C9, C12
11	21.66 (t)	1.55, 1.84	21.55 (t)	1.55, 1.84	
12	28.54 (t)	1.58, 1.98	28.37 (t)	1.55, 1.98 (Ha, dd, 11.7, 6.5) [15]	C9, C11
13	54.21 (d)	1.79 (m)	54.21 (d)	1.79 (m)	
14	36.37 (d)	2.40 (m)	36.17 (d)	2.40 (m)	
15	136.97 (d)	5.82 (dd, 8.8, 15.6)	136.98 (d)	5.82 (dd, 9.7, 15.9)	C14, C16, C17
16	130.75 (d)	5.64 (dd, 6.8, 15.6)	130.69 (d)	5.64 (dd, 6.5, 15.9)	C14, C15, C18
17	68.84 (d)	5.03 (dd, 6.8, 7.9)	68.80 (d)	5.03 (dd, 6.5, 7.6)	C15
18	128.22 (d)	5.72 (d, 7.9)	128.29 (d)	5.72 (d, 7.6)	C19, C20
19	132.01 (s)		132.14 (s)		
20	41.65 (t)	2.55 (t, 6.5)	41.65 (t)	2.55 (t, 6.5)	C18, C19, C21
21	58.06 (t)	3.78 (t, 6.5)	58.06 (t)	3.78 (t, 6.5)	C19, C20
22	19.03 (q)	1.08 (d, 7.0)	18.96 (q)	1.08 (d, 7.0)	C13, C14, C15
23	11.86 (q)	1.88 (s)	11.30 (q)	1.88 (s)	C1, C2, C3
1'			35.06 (t)	3.68 (t, 6.5)	C2', C1
2'			49.05 (t)	3.00 (t, 6.5)	C1'

The relative stereochemistry at C5, C9, C13 and C14 around the 6-azaspiro[4.5]decane moiety (Fig. 2) was based on an analysis of $^3J_{H\text{-}H}$ coupling constants and NOEs correlations from NOESY data[17] and the NOE difference spectrum of **2**. The large coupling constant between H5 and H6a (12 Hz[14]) suggested that H5 was axial. The stereochemistry at C5 and C9 were then assigned based on the NOEs correlations between H5 and H10a and between H7b and H10a. The stereochemistry at C13 was determined by the NOESY crosspeak 22-Me/H10b, and the weak NOEs correlation 22-Me/H5, whereas the stereochemistry at C14 was derived from the NOESY crosspeak H14/H12b.

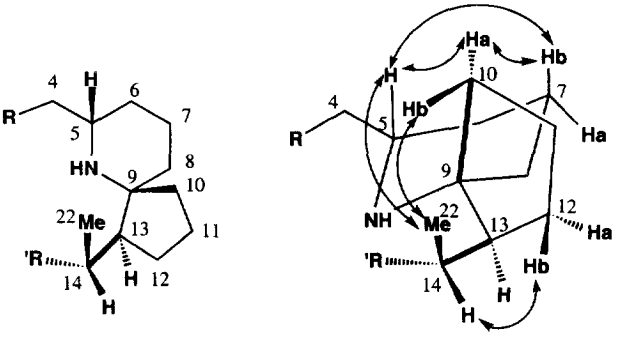


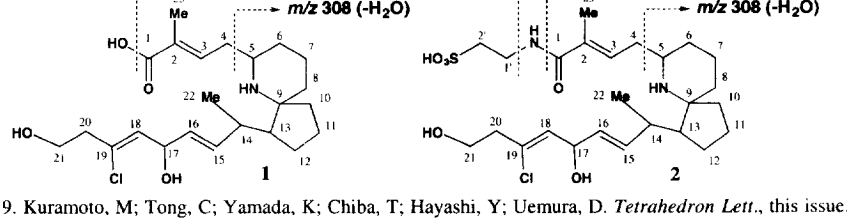
Fig.2. The relative configurations at C5, C9, C13 and C14 of the 6-azaspiro[4.5]decane moiety of **2** (arrows : NOEs correlations observed in the NOESY spectra and in the NOE difference spectrum).

The molecular formula of pinnaic acid (**1**) was determined to be $C_{23}H_{36}ClNO_4$ by HRFABMS (MH^+, $C_{23}H_{37}{}^{35}ClNO_4$ *m/z* 426.2412, Δ +0.1 mmu). The ^1H and ^{13}C data of **1** (Table 1) resembled those for **2** except for the absence of the two methylene units C1'-C2' and the adjacent sulfonyl group. The common carbon framework from C5 to C21 in **1** and **2** was also confirmed by the strong MS fragment at *m/z* 308/310 observed in each EIMS spectra.[18] C1 of **1** was assigned to a carboxyl carbon based on the MS fragment peak at *m/z* 408.[17] Thus, the structure of pinnaic acid was clarified as **1**.

Pinnaic acid (**1**) and tauropinnaic acid (**2**) inhibited $cPLA_2$ activity in vitro with IC_{50} values of 0.2 mM and 0.09 mM, respectively. These two compounds are the first members of a new group of marine alkaloids. Compound **1** has the same carbon skeleton as halichlorine[19] except for the stereochemistry at C14. Halichlorine was isolated from the black marine sponge *Halichondria okadai*.

Acknowledgments. We are grateful to Dr. H. Naoki, Suntory Institute for Bioorganic Research, for performing HRFABMS measurements. This work was financially supported by a grant from Ono Pharmaceutical, a grant from Banyu Chemical, and by the Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture, Japan.

References and Notes :

1. Dennis, E. A. In : The Enzymes. Boyer, P. D. (ed.), *Academic Press*, New York **1983**, 307-353.
2. van den Bosch, H. *Biochim. Biophys. Acta*, **1980**, 604, 191-246.
3. Arita, H.; Nakano, T.; Hanasaki, K. *Prog. Lipid Res.*, **1989**, 28, 273-301.
4. Scheuer, P. J.; de Silva, E. D. *Tetrahedron Lett.* **1980**, 21, 1611-1614.
5. Albizati, K. F.; Holman, T.; Faulkner, D. J.; Glaser, K. B.; Jacobs, R. S. *Experientia* **1987**, 43, 949-950.
6. Potts, B. C. M.; Faulkner, D. J.; de Carvalho, M. S.; Jacobs, R. S. *J. Am. Chem. Soc.* **1992**, 114, 5093-5100.
7. Potts, B. C. M.; Faulkner, D. J.; Jacobs, R. S. *J. Nat. Prod.* **1992**, 55, 1701-1717.
8. Kramer, R. M. et al. *Exp. Med. Biol.* **1990**, 275, 35-54.
9. Kim, D. K.; Kudo, I.; Fujimori, Y.; Mizushima, H.; Masuda, M.; Kikuchi, R.; Ikizawa, K; Inoue, K. *J. Biochem.*, **1990**, 108, 903-906.
10. Uemura, D.; Hirata, Y. In : Atta-ur-Rahman (ed.); *Studies in Natural Products Chemistry.*, Vol. 5, Part B, Elsevier, Amsterdam, **1989**, 377-398.
11. Uemura, D. In : Bioactive Polyethers. Scheuer, P. J. (ed.); *Bioorganic Marine Chemistry*, Vol. 4, Springer, Berlin. New York, **1991**, 1-31.
12. Uemura, D.; Chou, T.; Haino, T.; Nagatsu, A.; Fukuzawa, S.; Zheng, S. Z.; Chen, H. S. *J. Am. Chem. Soc.* **1995**, 117, 1155-1156.
13. Bax, A; Summers, M. F. *J. Am. Chem. Soc.* **1986**, 108, 2093-2094.
14. The *J* value of H5 (and $^3J_{H14\text{-}H13}$, 9.0 Hz of H14) was assigned using a homodecoupling technique.
15. H10a (δ 1.45 ppm, dddd, $^2J_{gem}$=14 Hz, $^3J_{H10b\text{-}H11}$=5.6 Hz, $^3J_{H10b\text{-}H11'}$=10 Hz), H10b (δ 1.78 ppm, br.dd, $^2J_{gem}$=13.5 Hz, $^3J_{H10a\text{-}H11}$=6.6 Hz), and H12a (δ 1.58 ppm, dddd, $^2J_{gem}$=14 Hz, $^3J_{H12a\text{-}H11}$=3.2 Hz, $^3J_{H12a\text{-}H11'}$=10 Hz) were also observed in the ^1H-NMR and ^1H-^1H COSY spectra measured DMSO-d_6.
16. Breitmaier, E.; Volter, W. *Carbon-13 NMR Spectroscopy*; VCH: Weinheim, New York, 1990; pp. 198-206.
17. Phase-sensitive NOESY spectra of **2** were acquired in CD_3OD with mixing times of 250 ms (400 MHz, Bruker-AM400).
18. 

19. Kuramoto, M; Tong, C; Yamada, K; Chiba, T; Hayashi, Y; Uemura, D. *Tetrahedron Lett.*, this issue.

(*Received in Japan* 4 *March* 1996; *revised* 11 *April* 1996; *accepted* 12 *April* 1996)