Pergamon

Tetrahedron Letters, Vol. 38, No. 32, pp. 5667-5670, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01234-3

Amphimic Acids, Novel Unsaturated C28 Fatty Acids as DNA Topoisomerase I Inhibitors from an Australian Sponge *Amphimedon* sp.

Takayuki Nemoto, Makoto Ojika*, and Youji Sakagami*

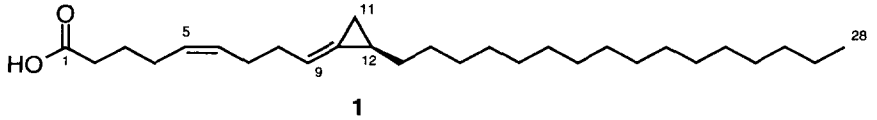
Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-01, Japan

Abstract: Amphimic acids A (**1**) and B (**2**), novel unsaturated long-chain fatty acids possessing a cyclopropylidene group and inhibiting DNA topoisomerase I, have been isolated from an Australian sponge *Amphimedon* sp. Their structures were elucidated by spectroscopic analysis and chemical degradation. The enantioselective synthesis of **1** was carried out to determine the absolute configuration.
© 1997 Elsevier Science Ltd.

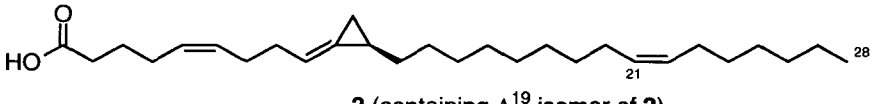
In continuation of our search for DNA topoisomerase I (topo I) inhibitors from marine invertebrates,[1] we have found two novel long-chain fatty acids termed amphimic acids A (**1**) and B (**2**) in an Australian sponge *Amphimedon* sp. In this communication we report the isolation of these new compounds and their absolute stereochemistry, which has been determined by the enantioselective synthesis of **1**.

The MeOH extract of the sponge (3.8 kg wet wt), which was collected at the Great Barrier Reef, was partitioned between EtOAc and water. The EtOAc soluble material was further partitioned between hexane and 90% MeOH. The material obtained from the hexane portion, which inhibited topo I with an IC_{50} of 30 μg/ml, was subjected to bioassay-guided fractionation using silica gel [i. benzene/EtOAc, EtOAc, and then EtOAc/MeOH, step gradient; ii. hexane/EtOAc (9:1)] to afford an active fraction (IC_{50} = 3 μg/ml). The active fraction was further separated by reversed-phase HPLC (0.01 M NH_4OAc in 95% MeOH) to give **1** containing a slight amount of impurities and **2** as colorless oils. The crude compound **1** was rechromatographed by reversed-phase HPLC [0.01 M NH_4OAc in MeCN/MeOH/H_2O (50:48:2)] to yield pure **1** (15.6 mg, 4.1 x 10^{-4}% yield based on wet weight) as colorless fine needles. The compound **2** (16.0 mg, 4.2 x 10^{-4}% yield) contained a small amount of an isomer and could not be further purified. Amphimic acids A (**1**) and B (**2**) inhibited topo I with IC_{50}s of 0.47 μM and 3.2 μM, respectively.[2]

Amphimic acid A (**1**), mp 39-39.5 °C (MeCN/Et_2O), $[\alpha]^{22}_D$ + 7.7 (*c* 0.49, MeOH), has the molecular formula of $C_{28}H_{50}O_2$ [high-resolution negative FABMS: *m/z* 417.3711 $(M-H)^-$, Δ -2.1 mmu]. IR absorption



1



2 (containing Δ^{19} isomer of **2**)

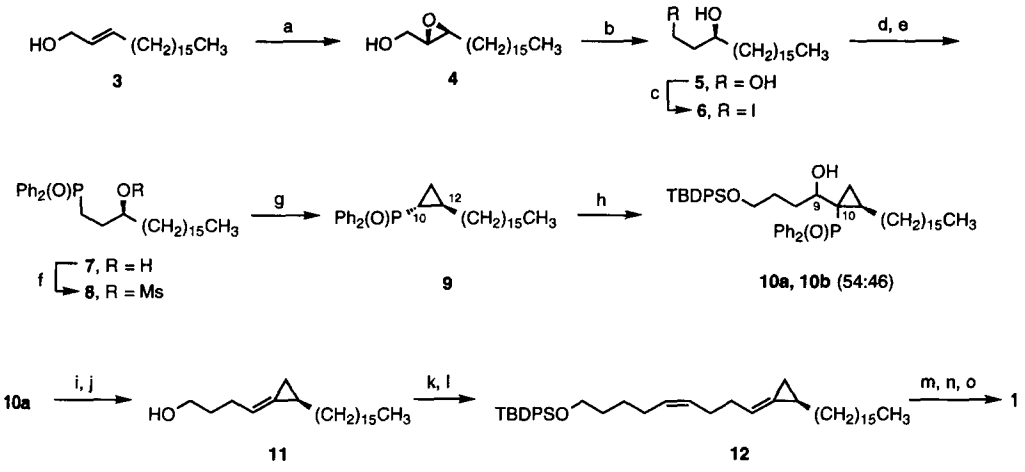
Table 1. NMR Data for **1** and **2**[a]

Position	1			2	
	^1H[b]	^{13}C	HMBC[c]	^1H	^{13}C
1	-	178.4 s	H2, 3	-	178.9 s
2	2.37 t (7.5)	33.4 t	H3, 4	2.36 t (7.5)	33.4 t
3	1.71 tt (7.5, 7.4)	24.6 t	H2, 4, 5	1.71 tt (7.5, 7.5)	24.6 t
4	2.11 dt (7.2, 7.4)	26.5 t	H2, 3, 5, 6	2.11 dt (7.5, 7.5)	26.5 t
5	5.34 dt (10.6, 7.2)	128.4 d	H3, 4, 7	5.34 dt (10.6, 7.5)	128.4 d
6	5.44 dt (10.6, 6.7)	130.6 d	H4, 7, 8	5.44 dt (10.6, 7.5)	130.9 d
7	2.16 m	27.2 t	H5, 6, 8, 9	2.18 m	27.2 t
8	2.18 m	31.9 t	H6, 7, 9	2.18 m	31.8 t
9	5.76 m	116.7 d	H7, 8, 11	5.76 m	116.8 d
10	-	128.0 s	H8, 11	-	128.0 s
11	0.64 m	8.5 t	H9, 13	0.64 m	8.5 t
	1.14 dd (7.8, 7.8)			1.14 dd (7.8, 7.8)	
12	1.35 m	15.3 d	H9, 11, 13	1.35 m	15.3 d
13-19	1.2-1.4 m	22.7-33.1 t		1.2-1.4 m	29.0-31.8 t
20	1.2-1.4 m	22.7-33.1 t		2.02 m	27.2 t
21	1.2-1.4 m	22.7-33.1 t		5.35 m	129.9 d
22	1.2-1.4 m	22.7-33.1 t		5.35 m	129.9 d
23	1.2-1.4 m	22.7-33.1 t		2.02 m	27.2 t
24-27	1.2-1.4 m	22.7-33.1 t		1.2-1.4 m	29.0-31.8 t
28	0.88 t (6.8)	14.1 q		0.88 t (6.8)	14.1 q

[a] Spectra were recorded at 400 MHz for ^1H and at 100 MHz for ^{13}C using $CDCl_3$ as solvent. Chemical shifts are in δ values. [b] Coupling constants (Hz) are in parentheses. [c] Parameters were optimized for J_{CH} = 7.5 and 10 Hz.

bands at 3400-2500 and 1710 cm^{-1} ($CHCl_3$) and a ^{13}C NMR signal at δ 178.4 indicated the presence of a carboxyl group. The strong signal at δ 1.2-1.4 and three olefinic methine signals in the ^1H NMR spectrum suggested that **1** is an unsaturated fatty acid. ^1H and ^{13}C NMR signals were assigned as shown in Table 1 by a ^1H-^{13}C COSY experiment. COSY spectra for **1** provided partial structures C2-C13 and C27-C28. The presence of a cyclopropylidene moiety (C9-C12) was demonstrated by HMBC correlations (C9/H11, C10/H11, C11/H9, and C12/H9) and long-range ^1H-^1H couplings (H9/H11 and H9/H12). The connectivity of the carboxyl group with the C-2 methylene was also determined by HMBC data (C1/H2 and C1/H3). The remaining methylenes (C14-C26) should be located between C13 and C27. The *Z* geometry at C5 and the *E* at C9 were determined by a coupling constant ($J_{H5,H6}$ = 10.6 Hz) and NOESY correlations (H8/H11), respectively. These findings finally disclosed the gross structure of amphimic acid A (**1**).

The absolute stereochemistry of **1** was elucidated by the enantioselective synthesis outlined in Scheme 1. The Sharpless asymmetric epoxidation of allyl alcohol **3**[3] gave epoxide **4** with 95% ee,[4] and subsequent regioselective reduction provided 1,3-diol **5** exclusively.[5] Monoiodination of **5** gave primary iodide **6**, which was converted to phosphine oxide **7** via a phosphonium salt. Mesylation of the secondary hydroxyl group of **7** followed by cyclization of the resulting mesylate **8** provided cyclopropane compound **9**. The *trans* stereochemistry of **9** was determined by NOESY experiments (NOEs at H10/H13 and H12/Ar-H). The reaction of carbanion of **9** with an aldehyde provided a diastereomeric mixture of β hydroxy phosphine oxides in high yield.[6] The mixture was chromatographed on silica gel to afford two mixtures **10a** and **10b** in the ratio of 54:46, which were presumed to be 9,10-*threo* and 9,10-*erythro* isomers, respectively, judging from the product from the following elimination reaction of **10a**. After deprotection of the major isomers **10a**, the elimination reaction with a base provided cyclopropylidene compound **11** with the *E* geometry exclusively. After oxidation of the hydroxyl group of **11**, the resulting aldehyde was coupled with a phosphonium salt by the Wittig reaction

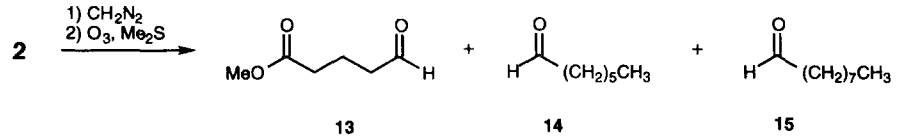


(a) *t*-BuOOH, (+)-DET, Ti(O-*i*-Pr)$_4$, CH_2Cl_2, -10 °C, 76%. (b) Red-Al, THF, 0 °C, 94%. (c) I_2, PPh_3, HMPA, toluene, rt, 72%. (d) PPh_3, $CaCO_3$, CH_3CN, 80 °C. (e) NaOH, THF/H_2O, 50 °C, 72% (2 steps). (f) MsCl, Et_3N, CH_2Cl_2, 0 °C, 100%. (g) NaN(TMS)$_2$, THF, 0 °C, 87%. (h) TBDPSO$(CH_2)_3$CHO, LDA, THF, -78 °C then 0 °C, 100%. (i) Bu_4NF, THF, rt, 71%. (j) NaH, DMF, 70 °C, 80%. (k) DMSO, $(COCl)_2$, Et_3N, -78 °C then 0 °C, 91%. (l) TBDPSO$(CH_2)_5PPh_3I$, NaN(TMS)$_2$, toluene, rt, 100%. (m) Bu_4NF, THF, rt, 100%. (n) DMSO, $(COCl)_2$, Et_3N, -78 °C then 0 °C, 79%. (o) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, rt, 97%.

Scheme 1

to provide olefin **12**. Deprotection of the silyl group of **12** followed by two-step oxidation[7] gave amphimic acid A (**1**) [mp. 39.5-40 °C, $[\alpha]^{23}_D$ +8.8 (*c* 0.40, MeOH)], which was identical with the natural compound in all respects (IR, NMR, MS, TLC) including topo I inhibitory activity (IC_{50} = 0.65 μM). Thus, the absolute configuration of **1** was determined to be 12*R* unambiguously.

Amphimic acid B (**2**), $[\alpha]^{27}_D$ +6.2 (*c* 0.98, MeOH), has the molecular formula of $C_{28}H_{48}O_2$ [high resolution negative FABMS: *m/z* 415.3551 $(M-H)^-$, Δ -2.5 mmu] and is presumed to be a dehydro analogue of **1** by comparison of the NMR data (Table 1) and molecular formula between **1** and **2**. Although, among the three double bonds in the molecule, two (C5 and C9) are located at the same positions as those in **1**, the position of the remaining one could not be determined by NMR analysis. We first examined negative-mode FAB-MS/MS experiments, which is known to be useful for determining the double-bond position in unsaturated fatty acids.[8] Thus, collisionally activated dissociation spectra obtained by linked scan MS/MS using the precursor ion $(M-H)^-$ of **2** showed two large product ion peaks at *m/z* 289 and 343, which were due to selective cleavage of two C-C bonds allylic to the double bond, indicating that the location of the third double bond was at C21. This characteristic fragment pattern was, however, made less clear-cut than those of typical monounsaturated fatty acids such as oleic acid by overlapping of another doublet of product ions. This observation led us to suspect the presence of a regioisomer in the sample of **2**. To confirm this, **2** was subjected to a degradation reaction (Scheme 2). GC-MS analysis of the reaction product showed the presence of C7 aldehyde **14** and C9 aldehyde **15** in the ratio of ca. 3:1 together with **13**,[9] establishing that the position of the remaining double bond in **2** was at C21 and that the Δ^{19} isomer existed in the sample as a minor component. The chemical shifts of the carbons (C20 and C23 in **2**) allylic to the double bond showed the *Z* geometry of C21.[10] Therefore, **2** is the 21-dehydro derivative of **1** that exists together with the Δ^{19} isomer as an inseparable 3:1 mixture, though the NMR spectra



Scheme 2

look like those of a single compound. Both **1** and **2** show similar specific rotations, indicating that the absolute stereochemistry of **2** is identical with that of **1**.

Although a number of long-chain fatty acids were isolated from marine sponges,[11] cyclopropylidene-containing fatty acids such as **1** have not been reported as natural compounds.[12] Amphimic acids A (**1**) is 7-fold more active than **2** and approximately 100-fold more active than typical C18 fatty acids such as oleic acid, suggesting that not only the linearity of the molecule but also carbon-chain length, rather than the presence of the cyclopropylidene moiety, play important roles in topo I inhibitory activity. Since **1** shows also cytotoxicity against P388 leukemia cells with an IC_{50} value (1.8 μM) similar to that in topo I inhibitory activity, the respective activities may be correlated to each other. These structure-activity relationships would be clarified by future studies on the isolation and characterization of other related long-chain fatty acids that we found in the same sponge.

Acknowledgement. This work was supported by Grant-in-Aid for Scientific Research on Priority Areas (Natural Supramolecules No. 08219218) from the Ministry of Education, Science, Sports, and Culture, Japan, by Research for the Future from Japan Society for the Promotion of Science, and by Daiko Foundation. We are grateful to Professor Toshiwo Andoh (Soka University) for providing us with topo I and Dr. Yoshikazu Shizuri (Marine Biotechnology Institute) for helpful discussions.

REFERENCES AND NOTES

1. Ojika, M.; Yoshino, G.; Sakagami, Y. *Tetrahedron Lett.* **1997**, *38*, 4235-4238.
2. See ref. 1 for details of topo I inhibition assay.
3. Smolic, S.; Kvita, V.; Weichet, J.; Trcka, V. *Collection Czechoslov. Chem. Communs.* **1960**, *25*, 259-264.
4. The enantioselectivity was determined by ^1H NMR analysis of an MTPA ester.
5. Finan, J. M.; Kishi, Y. *Tetrahedron Lett.* **1982**, *23*, 2719-2722.
6. (a) Buss, A. D.; Warren, S. *J. Chem. Soc. Perkin Trans. I*, **1985**, 2307-2325. (b) Buss, A. D; Warren S. *J. Chem. Soc., Chem. Commun.* **1981**, 100-101.
7. Kraus, G. A; Taschner, M. J. *J. Org. Chem.* **1980**, *45*, 1175-1176.
8. Tomer, K. B.; Crow, F. W.; Gross, M. L. *J. Am. Chem. Soc.* **1983**, *105*, 5487-5488.
9. These compounds were analyzed under the following conditions: capillary column, DB-17 (J&W) 15 m x 0.25 mm I.D.; time program, initial temp 50 °C, rate 40 °C/min. Retention times of **13-15** were 2'45", 2'16", and 3'16", respectively.
10. Wenkert, E.; Buckwalter, B. L.; Burfitt, I. R.; Gasic, M. J.; Gottlieb, H. E.; Hagaman, E. W.; Schell, F. M.; Wovkulich, P. M. in "*Topics in Carbon-13 NMR Spectroscopy*," ed by Levy, G. C., Wiley-Interscience, New York, **1976**, Vol. 2, Chap. 2, pp 81-121.
11. For example, (a) Barnathan, G.; Kornprobst, J.-M.; Doumenq, P.; Miralles, J. *Lipids* **1996**, *31*, 193-200. (b) Carballeira, N. M.; Shalabi, F. *J. Nat. Prod.* **1994**, *57*, 1152-1159. (c) Christie, W. W.; Brechany, E. Y.; Marekov, I. N.; Stefanov, K. L.; Andreev, S. N. *Comp. Biochem. Physiol.* **1994**, *109B*, 245-252.
12. A synthetic study on a cyclopropylidene-containing fatty acid is reported: Misra, R. N. *Tetrahedron Lett.* **1985**, *26*, 1973-1976.

(*Received in Japan* 12 *May* 1997; *revised* 12 *June* 1997; *accepted* 18 *June* 1997)