Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 1659–1661

TETRAHEDRON LETTERS

Tetrapetalone A, a novel lipoxygenase inhibitor from *Streptomyces* sp.

Toshikazu Komoda,[a] Yasumasa Sugiyama,[a] Naoki Abe,[a] Misako Imachi,[b] Hiroshi Hirota[c,d] and Akira Hirota[a,*]

[a]*Laboratory of Applied Microbiology, School of Food and Nutritional Sciences, University of Shizuoka, Yada 52-1, Shizuoka 422-8526, Japan*

[b]*Bruker BioSpin K. K., 3-21-5 Ninomiya, Tsukuba 305-0051, Japan*

[c]*Protein Research Group, RIKEN Genomic Sciences Center, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan*

[d]*Science of Biological Supramolecular Systems, Yokohama City University, 1-7-29 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan*

Received 5 November 2002; revised 20 December 2002; accepted 20 December 2002

Abstract—Tetrapetalone A (**I**), $C_{26}H_{33}O_7N$, is a novel lipoxygenase inhibitor isolated from a culture filtrate of *Streptomyces* sp. USF-4727 strain. Its chemical structure was determined by its spectroscopic evidence and methylation with diazomethane. The stereochemistry was investigated by the coupling constant in ^1H NMR, NOESY data and modified Mosher's method. **I** possessed a tetracyclic skeleton and a β-rhodinosyl moiety, and this is the first report of a compound with such a tetracyclic skeleton.
© 2003 Elsevier Science Ltd. All rights reserved.

Human lipoxygenase and cyclooxygenase catalyze the first step in the arachidonic acid pathway. The resultant leukotrienes, lipoxins and prostaglandins are important classes of signaling molecules that may be involved in a variety of human diseases.[1–3] Soybean lipoxygenase (SBL) shows an activity of 15-lipoxygenase. Thus, there is a possibility that the SBL inhibitor inhibits human lipoxygenase and cyclooxygenase. Indeed, nordihydroguaiareic acid (NDGA), a natural product, inhibits the activity of SBL, human lipoxygenase and cyclooxygenase.[4]

We have studied lipoxygenase inhibitors from soil *Streptomyces* sp. strains using a well-known SBL assay.[5,6] In our previous study, we have reported a new antimycin derivative as a lipoxygenase inhibitor.[7] In this paper, we report a new lipoxygenase inhibitor, tetrapetalone A (**I**),[8] in a culture filtrate of *Streptomyces* sp. USF-4727 strain. This strain was isolated from a soil sample from Yada, Shizuoka City, Japan, and showed inhibitory activity against SBL in our screening test.

The strain USF-4727 was inoculated into 800 ml of the medium (0.4% glucose, 0.4% yeast extract, 1.0% malt extract, pH 7.3) in a 2-L Erlenmeyer flask and cultivated at 30°C for 10 days on a rotary shaker. The culture filtrate (7.2 L) was applied to Diaion HP-20 (1 kg) column. After washing with 2 L of water, the crude extract was eluted with 2 L of MeOH and 2 L of acetone. These elutants were mixed and applied onto a silica gel column eluted with an *n*-hexane–acetone system. The active elutant was charged onto a Sephadex

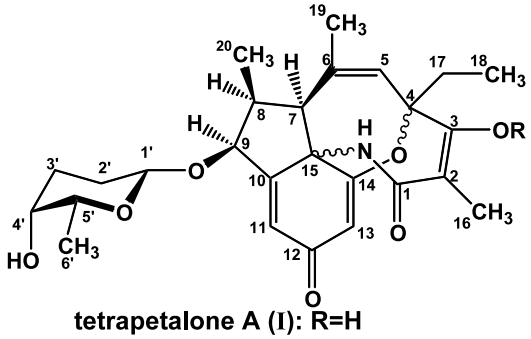

Figure 1. Structures of tetrapetalone A (**I**) and tetrapetalone A-Me (**II**).

Keywords: soybean lipoxygenase inhibitor; *Streptomyces*.
* Corresponding author. Tel.: +81-54-264-5552; fax: +81-54-264-5099; e-mail: hirotaa@fns1.u-shizuoka-ken.ac.jp

0040-4039/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
PII: S0040-4039(03)00007-8

LH-20 (MeOH) column to give a crude oil. This oil was further purified by a silica gel column eluted with *n*-hexane/acetone = 45/55. Finally, the active fraction was crystallized from the *n*-hexane–acetone system to yield tetrapetalone A (**I**)[8] (20 mg).

I was obtained as pale yellow amorphous powder, mp: 190°C. The data of HRFAB MS gave a molecular formula, $C_{26}H_{33}O_7N$, for **I** and the IR spectrum showed the existence of ketone (1670 cm^{-1}) and hydroxy groups (3400 cm^{-1}). In the structure elucidation of **I**, two fragments were derived from the results of the ^1H, ^{13}C NMR, DEPT spectra and ^1H–^1H COSY, HMQC data in CD_3OD. One was a fragment from C-5 to C-9 with two methyls (C-19 and 20). The other fragment was an ethyl group of C-17 and C-18. These two fragments and an α,β-unsaturated carbonyl group (C-1 to 3) with a branched methyl (C-16) were connected based on the HMBC spectral data, constructing a partial structure A (Fig. 2). In addition, 2D-INADEQUATE data indicated the presence of two other partial structures (B and C, Fig. 3). These three partial structures were joined into partial structure D (Fig. 4) from the results of 2D-INADEQUATE and HMBC spectra. In the HMBC spectrum of tetrapetalone A-Me (**II**, $C_{27}H_{35}O_7N$) obtained by methylation of **I** with diazomethane, a cross peak between the methoxy methyl proton (δ_H: 4.2 ppm) and C-3 was observed (Fig. 5), and, furthermore, this carbon (C-3) also had a cross peak with 5-H and 17-H even after methylation. These results indicated that an OH group was connected to C-3 in partial structure D.

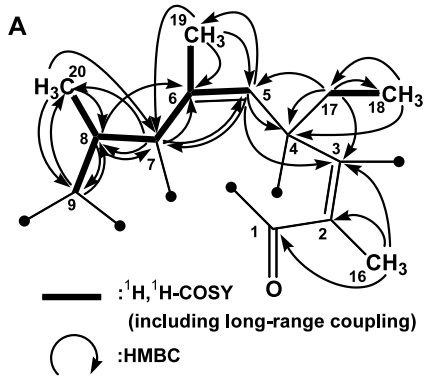

Figure 2. Partial structure A.

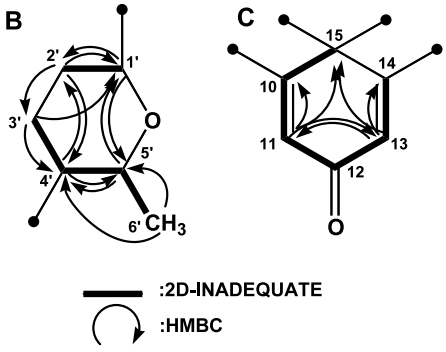

Figure 3. Partial structures B and C.

In the ^1H NMR spectrum of **I** using DMSO-d_6, two new broad signals were observed. Their chemical shifts suggested that one (δ_H: 6.3 ppm) was an amide proton signal, and the other was a hydroxy proton signal (δ_H: 4.4 ppm). In the ^1H–^1H COSY spectrum, the hydroxy proton (δ_H: 4.4 ppm) had a cross peak with 4′-H. In the HMBC spectrum, the amide proton (δ_H: 6.3 ppm) showed a cross peak with C-7 and C-15, respectively. These results suggested that C-4′ was connected to the OH group and C-15 to amide NH. Accordingly, the remaining C-1 carbonyl carbon (δ_C: 177.6 ppm) formed an amide bond with C-15-NH.

From the chemical shift in the ^{13}C NMR spectrum and the molecular formula, $C_{26}H_{33}O_7N$, C-4 (δ_C: 69.3 ppm) and C-14 (δ_C: 156.1 ppm) should be connected through one oxygen atom. Thus, the planar structure of **I** was established as shown in Figure 1.

The relative stereochemistry of 2-methyltetrahydropyran was consistent with that of β-rhodinose on the basis of the chemical shift and coupling constant in the ^1H NMR spectrum.[9–11] NOESY correlation for 1′-H and 5′-H supported this stereochemistry. The absolute stereochemistry of this ring was determined by modified Mosher's method.[12] **II** was treated with (*R*)-(−)- and (*S*)-(+)-2-methoxy-2-trifluoromethyl-2-phenylacetyl chlorides (MTPACls) to afford the C-4′-(*S*) and (*R*) MTPA esters of **II**. The positive $\Delta\delta$ values (δ_S–δ_R) in the ^1H NMR spectrum were observed for 2′ and 3′-H, while the negative $\Delta\delta$ values were located at 5′ and 6′-H. This result revealed the absolute stereochem-

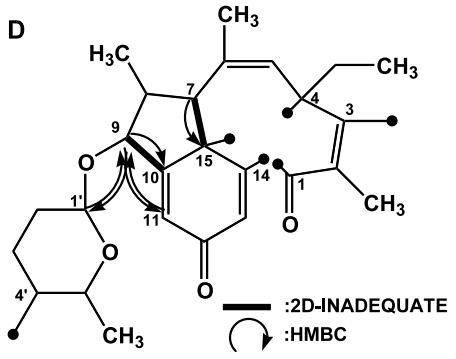

Figure 4. Partial structure D.

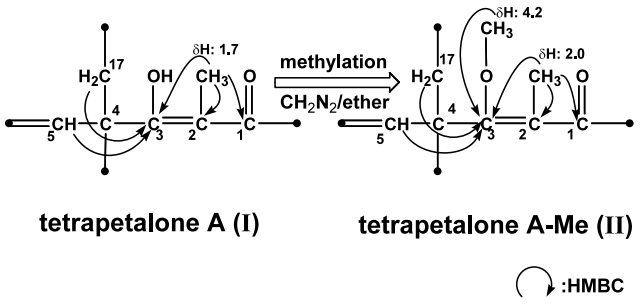

Figure 5. Partial structure of tetrapetalone A-Me (**II**).

istry of C-4′ to be *R* configuration, and then all the stereochemistry of this ring was assigned as Figure 1.

The coupling constant, 7-H/8-H (9.6 Hz) and 8-H/9-H (10.0 Hz) in the ^{1}H NMR spectrum, indicated these three protons to be *syn*. In addition, cross peaks for 7-H/8-H, 8-H/9-H and 7-H/9-H in the NOESY spectrum supported this relative stereochemistry. The absolute stereochemistry of C-9 was determined by modified Mosher's method.[12] After acid hydrolysis of **II**, we obtained an aglycon of **II** (**III**). Treating **III** with MTPACls, C-9-(*S*) and (*R*)-MTPA esters of **III** were yielded. In these esters, the $\Delta\delta$ values (δ_S–δ_R) of 11 and 13-H in the ^{1}H NMR spectrum were positive. Meanwhile, 5, 7, 8, 9, 19 and 20-H showed negative values. These results indicated 9*S* configuration. Then the stereochemistry of C-7, 8 and 9 was determined as Figure 1. The stereochemistry of the two remaining carbons (C-4 and 15) is under investigation.

Tetrapetalone A (**I**) possessed a novel tetracyclic skeleton and a β-rhodinosyl moiety, and this is the first report of a compound with this skeleton. Though the stereochemistry of the tetracyclic skeleton has not yet been determined completely, the characteristic conformation of the four rings was interesting. Further investigation is required to clarify the stereochemistry of this skeleton.

I inhibited SBL (IC_{50}: 190 μM) as well as two well-known lipoxygenase inhibitors, NDGA (IC_{50}: 290 μM) and kojic acid (IC_{50}: 110 μM). On the other hand, **II** showed little inhibitory activity against SBL even at the concentration of 1mM. Consequently, we considered that the hydroxy group at C-3 might contribute to this activity.

References

1. Samuelsson, B. *Science* **1983**, *220*, 568–575.
2. Ghosh, J.; Myers, C. E. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 13182–13187.
3. Bell, R. L.; Young, P. R.; Albert, D.; Lanni, C.; Summers, J. B.; Brooks, D. W.; Rubin, P.; Carter, G. W. I. *Int. J. Immunopharmacol.* **1992**, *14*, 505–510.
4. Safayhi, H.; Mack, T.; Sabieraj, J.; Anozodo, M. I.; Subramanian, L. R.; Ammon, E. P. T. *J. Pharmacol. Exp. Ther.* **1992**, *261*, 1143–1146.
5. Axelrod, B.; Cheesbrough, T. M.; Laakso, S. *Methods Enzymol.* **1981**, *71*, 441–451.
6. Tappel, A. L.; Lumdberg, W. O.; Boyer, P. D. *Arch. Biochem. Biophys.* **1953**, *42*, 293–304.
7. Komoda, T.; Morimitsu, Y.; Hirota, H.; Hirota, A. *Biosci. Biotechnol. Biochem.* **1995**, *59*, 924–926.
8. *Tetrapetalone A (**I**)*: pale yellow amorphous powder, mp: 190°C. HRFAB MS $[M+H]^+$, m/z 472.2354 (472.2335 calcd for $C_{26}H_{34}O_7N$). UV–vis λ_{max} (MeOH): 385 nm (ε 10 200), 240 nm (ε 13 800); IR ν_{max} (KBr) cm^{-1}: 3400, 1670, 1380, 1300, 1250, 1170, 1060, 1020. ^{1}H NMR (CD_3OD, 400 MHz) δ_H: 0.7 (t, 3H, 18-H), 1.25 (d, 3H, 6′-H), 1.35 (d, 3H, 20-H), 1.70 (s, 3H, 16-H), 1.75, 1.87 (m, each 1H, 2′-H), 1.78, 1.95 (m, each 1H, 3′-H), 1.80 (s, 3H, 19-H), 1.85, 3.14 (m, each 1H, 17-H), 2.0 (m, 1H, 8-H), 3.3 (br. d, 1H, 7-H, overlapped with solvent peak), 3.5 (br. s, 1H, 4′-H,), 3.65 (q, 1H, 5′-H), 4.6 (dd, 1H, 1′-H), 4.8 (dd, 1H, 9-H), 5.7 (s, 1H, 5-H), 5.95 (s, 1H, 11-H), 6.75 (s, 1H, 13-H), ^{13}C NMR (CD_3OD, 100 MHz) δ_C: 5.6 (q, C-16), 7.3 (q, C-18), 17.5 (q, C-6'), 20.2 (q, C-20), 22.1 (q, C-19), 24.8 (t, C-17), 26.7 (t, C-2′), 30.9 (t, C-3′), 41.8 (d, C-8,), 56.0 (d, C-7), 67.2 (d, C-4′), 69.3 (s, C-4), 73.9 (s, C-15), 75.4 (d, C-5′), 82.8 (d, C-9), 103.0 (s, C-2), 103.3 (d, C-1′), 114.9 (d, C-13), 116.3 (d, C-11), 125.6 (d, C-5), 141.2 (s, C-6), 156.1 (s, C-14), 167.2 (s, C-10), 176.0 (s, C-3), 177.6 (s, C-1), 189.6 (s, C-12).
9. Hayakawa, Y.; Furihata, K.; Seto, H.; Otake, H. *Tetrahedron Lett.* **1985**, *26*, 3475–3478.
10. Kawamura, N.; Sawa, R.; Takahashi, Y.; Sawa, T.; Kinoshita, N.; Naganawa, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1995**, *48*, 1521–1524.
11. Canedo, L. M.; Puentes, J. L. F.; Baz, J. P.; Huang, X.-H.; Rinehart, K. L. *J. Antibiot.* **2000**, *53*, 479–483.
12. Otani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.