Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 8, pp. 971-974, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00139-X

RHOMBENONE: FARNESYL PROTEIN TRANSFERASE INHIBITOR FROM THE LEAVES OF *HEDERA RHOMBEA* BEAN

Byoung-Mog Kwon,* Seung-Ho Lee, Kyung-Sook Kim[1], Ihn-Rhan Lee[1], Un Chul Lee[2], Su-Hyung Hong, Song-Hae Bok

Protein Regulator RU, Korea Research Institute of Bioscience & Biotechnology, KIST, P.O Box 115 Yoosung, Taejon, Korea 305-600

[1]*College of Pharmacy, Ewha Women University, Seoul Korea*

[2]*Korea Research Institute of Ginseng and Tobacco, Taejeon, Korea*

Abstract: Rhombenone, a new dammarane analogue containing an 25-oxo, which inhibits farnesyl protein transferase (FPTase), was isolated from the leaves of *Hedera rhombea* Bean. Additional related dammaranes were also evaluated for FPTase inhibition. © 1997 Elsevier Science Ltd.

Ras farnesyl protein transferase (FPTase) is an enzyme which catalyzes the transfer of the farnesyl group from farnesyl pyrophosphate (FPP) onto cysteine 186 at the C-terminal of the Ras protein.[1,2] This is an essential step for membrane association of Ras which is critical for triggering *ras* oncogene toward tumor formation.[3,4] Recent works have demonstrated that specific inhibitors of the FPTase showed excellent efficacy *in vivo* against solid tumors in nude mouse.[5]

In the course of a screening program to discover new inhibitors of FPTase from herbal medicines, we have isolated compound **1** named rhombenone from the leaves of *Hedera rhombea* Bean (Araliaceae). The extract of the leaves of *H. rhombea* has been used as a therapeutic agent for various diseases including hemorrhage, chronic catarrh, jaundice, lithiasis and convulsion.[6,7] Herein, we describe the structure and FPTase inhibition of **1**.

Dried leaves of *H. rhombea* (3 kg) were soaked in 20 L of methanol (95%) at room temperature for 3 days. The extract was concentrated to one tenths of the volume and 2 L of water was added. The solution was extracted with ethyl acetate; after evaporation the resultant mass was triturated with methanol and filtered. The filtrate was chromatographed on Sephadex LH-20 in gel permeation mode using methanol as eluent. Active fraction was further purified by silica gel and C-18 column chromatography. Extraction and separation of *H. rhombea*-derived FPTase

inhibitory active fractions were monitored with the farnesyl transferase scintillation proximity assay (SPA) method.[8] Finally, **1** was purified by preparative TLC with 60% EtOAc/40% hexane to afford approximate 5 mg/kg as a solid.[9]

1 was analyzed for $C_{29}H_{46}O_4$ by HRCI-MS ([M+H]$^+$: 459.3501 calcd: 459.3476), ^{13}C, ^1H NMR and IR spectral data. **1** exhibited two carbonyl and a broad hydroxyl group absorption bands at 1710, 1680 and 3460 cm^{-1} in the IR spectrum, respectively. All one bond connections between ^{13}C and ^1H were elucidated by the HMQC experiment. Partial structure **A**, shown in Fig. 1, was determined from the interpretation of two and three bond HMBC correlations of C-3, C-6 and C-10 carbons, and also the strong coupling between H-6 (δ 3.92) and H-5 (δ 1.62) in the ^1H-^1H COSY spectrum. Two signals at δ 6.03 and 6.83 ppm were classified as an enone moiety. In 2D COSY spectrum of the side chain of **1**, a strong coupling between H-22 and H-23 and two and three-bond HMBC correlations to their respective neighboring protons were observed. These correlations were very important for determination of key connectivities and in verification of the partial structure **B**. Based on the above data and reported spectral data of dammarane compounds,[10] **1** was suggested to be a dammarane-type triterpene having an α,β-unsaturated side chain.

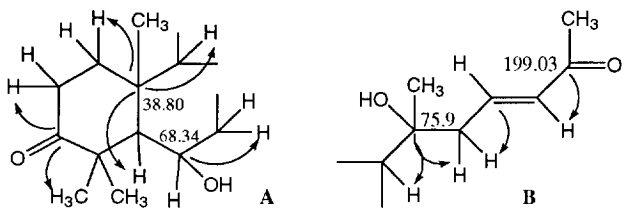
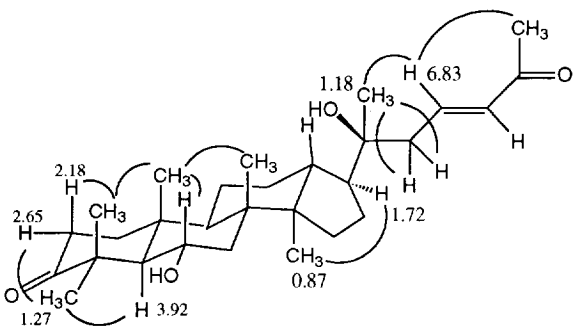


Fig. 1 Structure determination with HMBC connections



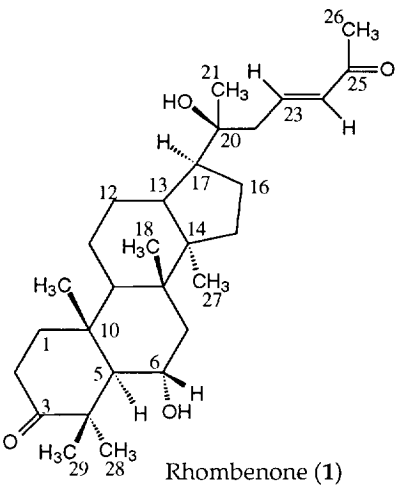
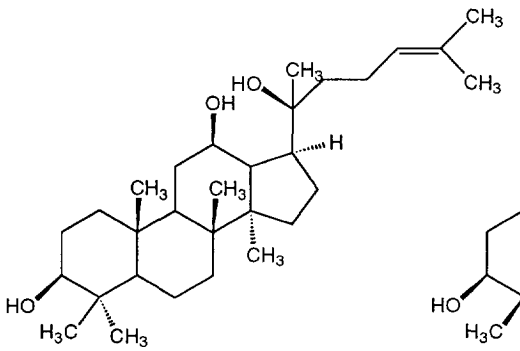


Fig. 2 Stereochemistry of compound **1** by NOESY

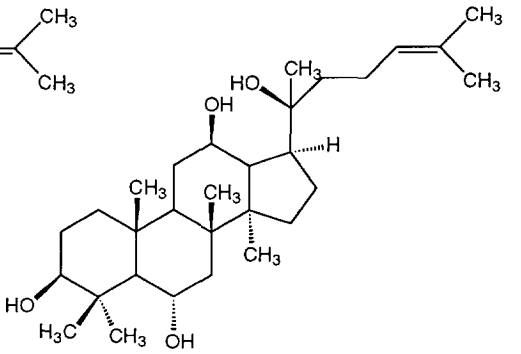
The relative stereochemistry of four-ring systems was clarified by the NOESY spectrum. The configuration of C-17 was assigned as S by the correlation between H-17 (δ 1.71) and H-27 (δ 0. 87) in NOESY spectrum. Correlations between H-21 and H-16, 17 were not detected, however, strong interactions between the H-21 signal at δ 1.18 and H-22, H-23 were observed in NOESY spectrum as shown Fig. 2, which led to the assignment of C-20 configuration as S.[10,11] The geometry of the methyl of C-26 and H-23 was determined to be *syn* on the basis of NOE experiments. Stereochemistry of the olefinic protons was assigned as *trans* by coupling constant (J=15.9 Hz). By comparison of ^{13}C-NMR spectral data of **1** and 3-oxo-20(S)-dammar-24-ene-6α, 20, 26-triol,[10] **1** is a member of a large group of triterpenoids and the first 27-demethyl dammarane analogue.

FPTase inhibitory activity was measured against partially purified FPTase enzyme prepared from rat brain[2,12] and biotin-YRASNRSCAIM acceptor peptide using a scintillation proximity assay method.[8] The isolated enzyme was confirmed by the positive control with gliotoxin (IC_{50} of 2 μM), which is a known FPTase inhibitor.[13] **1** showed an IC_{50} of 2.3 μM against above assay system.

To survey the inhibitory activities of dammaranes, protopannaxydiol (**2**) and triol (**3**) were tested under the same condition as did for **1**. **2** and **3** isolated from panax ginseng[14] exhibited only about 20% inhibition at 100 μM.



Protopannaxydiol (**2**)



Protopannaxytriol (**3**)

Acknowledgements: B. M. Kwon thanks Drs. N. Kohl and J. B. Gibbs (Merck Research Laboratories) for helpful discussion of the FPTase project and Dr. H. M. Kim for the measurements of cytotoxicity. This research was supported by the MOST (Ministry of Science and Technology) and MAFF-SGRP (Ministry of Agriculture, Forestry and Fisheries-Special Grants Research Program) in Korea.

REFERENCES AND NOTES

1. Gibbs, J. B. *Cell*, **1991**, *65*, 1-4.
2. Reiss, Y.; Goldstein, J. L.; Seabra, M. C.; Casey, P. J.; Brown, M. S. *Cell*, **1990**, *62*, 1-4.
3. Kohl, N. E.; Mosser, S. D.; deSolms, J. S.; Giuliani, E. A.; Pompliano, D. L.; Graham, S. L.; Smith, R. L.; Scolnick, E. M.; Oliff, A.; Gibbs, J. B. *Science*, **1993**, *260*, 1934-1937.
4. James, G.T.; Goldstein, J. L.; Brown, M. S.; Rawson, T. E.; Somers, T. C.; McDowell, R. S. Crowley, C. W.; Lucas, B. K.; Levinson, A. D.; Marsters, J. C. *Science*, **1993**, *260*, 1937-1942.
5. Kohl, N. E.; Omer, C. A.; Conner M. W.; Anthony, N. J.; Davide, J. P.; Mosser, S. D.; deSolms, J. S.; Giuliani, E. A.; Gomez, R. P.; Graham, S. L.; Hamilton, K.; Handt, L. K.; Hartman, G. D.; Koblan, K. S.; Kral, A. M.; Miller, P. J.; O'Neill, T. J.; Rands, E.; Shaber, M. D.; Oliff, A.; Gibbs, J. B. *Nature Med.* **1995**, *1*, 792-797.
6. Chung, B.S.; Shin, M.K., Ed., *Hyangyak Daesajun*, **1990**; pp. 436-437, Young Rim Sa, Seoul, Korea.
7. Lee, I.R.; Kim, J.S.; Lee, S.H. *Arch. Pharm. Res.* **1993**, *16*, 331-335.
8. Streptavidin scintillation proximity assay beads were purchased from Amersham, Arlington Heights, IL, USA.
9. **1**: $[\alpha]_D^{20} = +126.08$ (MeOH, c. 0.03) and the detail NMR data of **1** will be published in *Arch. Pharm. Res.* **1997**, *20*, 000. Compound **1** exhibited very mild cytotoxicities with GI_{50} values ranging from 40 to 100 μM against human tumor cell lines, which were NCI-H23 (lung), HCT15 (colon), HT29 (colon), LOX-IMVI (melanoma) and M14 (melanoma).
10. Kizu, H.; Koshijima, M.; Hayashi, M.; Tomimori, T. *Chem. Pharm. Bull.* **1985**, *33*, 1400-1406.
11. The C-20 cofiguration in other dammares, which were isolated from natural sources, was also assigned as S. Hisham, A.; Ajitha Bai, M. D.; Fujimoto, Y.; Hara, N.; Shimada, H. *Mag. Res. Chem.* **1996**, *34*, 146-150.
12. Harwood, Jr., H. J. *Anal. Biochem.* **1995**, *226*, 268-278.
13. der Pyl, D. V.; Inokoshi, J.; Shiomi, K.; Yang, H.; Takeshima, H.; Omura, S. *J. Antibiotics*, **1992**,*45*, 1802-1805.
14. Shibata, S.; Fujuta, M.; Itokawa, H.; Tanaka, O.; Ishii, T. *Chem. Pharm. Bull.* **1963**, *11*, 759-761.

(*Received in Japan* 23 *January* 1997; *accepted* 8 *March* 1997)