CHEMISTRY LETTERS, pp. 1613 - 1616, 1984.

STRUCTURE OF RABDOHAKUSIN

Isao KUBO,* Takeshi MATSUMOTO, Yukihiro ASAKA,† Takashi KUBOTA,† Hideo NAOKI,†† Pawel FLUDZINSKI,§ and Andrew S. KENDE§

Division of Entomology and Parasitology, College of Natural Resources, University of California, Berkeley, California 94720, U.S.A. †School of Medicine, Kinki University, Sayama-cho, Minamikawachi-gun, Osaka 589 ††Suntory Institute for Bioorganic Research, Shimamoto-cho, Mishima-gun, Osaka 618 §Department of Chemistry, University of Rochester, Rochester, New York 14627, U.S.A.

The structure of a new 8,9-seco-ent-kaurene compound, rabdohakusin, isolated from Rabdosia umbrosa var. hakusanensis (Kudo) Hara (Labiatae) has been determined by spectroscopic methods including 2D NMR.

Our continuing search for bioactive diterpenoids from Rabdosia species[1] has led us to examine the chemical components from R. umbrosa var. hakusanensis. We isolated six ent-kaurene diterpenoids from the dry leaves which had been collected in Toyama, Japan. The major diterpenoid, shikoccin (2), mp 157 °C, $[\alpha]_D^{25}$-33.0° ($CHCl_3$), was obtained in 0.1% yield. This compound had previously been isolated from R. shikokiana[2] and has antitumor activity against P 388 lymphocytic leukaemia in mice. Four other minor diterpenoids were identified[3] as kamebanin, mebadonin, umbrosin A and isodomedin, all of which have anti-tumor activity. In this communication we report the structural determination and the conformational analysis of a new minor diterpenoid (0.01% yield), rabdohakusin (1).

Rabdohakusin (1) $C_{22}H_{32}O_4$ (by CI-MS in isobutane, and elemental analysis) has the following physical properties: mp 97-98 °C; $[\alpha]_D^{25}$+78.7° (c 0.127, $CHCl_3$); UV (EtOH) 210 and 288 nm (ε 7580 and 29 resp.); IR ($CHCl_3$) 3600-3200 (hydroxyl), 1720 and 1250 (acetate), 1710 (carbonyl) and 1685 cm^{-1} (trisubstituted double bond). ^{13}C NMR data (1a) showed the presence of four methyl, six methylene, four methine, two quaternary, four olefinic, and two carbonyl carbons. The analysis of the congested ^1H NMR spectrum (360 MHz) was greatly facilitated by the use of 2D homonuclear correlation spectroscopy (COSY). The spin coupling network was shown in Fig. 1. The overlapped high-field proton resonances were analyzed by the extensive 1D double resonance experiments with the aid of $Eu(fod)_3$, and two continuous proton systems were disclosed as depicted in 1b. The coupling data of a fragment A shows a typical

trans-decalin type feature having an axial acetoxyl group, whereas the data of the fragment B suggest a considerable skewed ring system. The existence of allylic couplings, 7-H/15-H (J=2 Hz), 17-H_a/15-H (J=2 Hz), 17-H_b/15-H 17-H_a/13-H (J=2 Hz), and 17-H_b/13-H (J=1.5 Hz), established a diallyl alcohol moiety in fragment B. This was confirmed by the oxidation of 1 with MnO_2 to yield an expected enone (3), EI-MS m/z 358, UV (heptane) 266 nm. The chemical shifts of the exomethylene protons which underwent significant low field shifts (6.20 and 5.48 ppm) were closely similar to those (6.16 and 5.44 ppm) encountered in shikoccin (2), but the signal due to the other conjugated olefin proton of 3 showed a different chemical shift (6.39 ppm) from that (7.25 ppm) of 2. In the 2D NMR, the presence of cross peaks due to the non-resolved long-range couplings of H-5 and H-1ß with methyl protons at 1.20 ppm finally connected fragment A and B at C-10. These spectral and chemical data clearly suggested that rabdohakusin (1) possessed an 8,9-seco-ent-kaurene skeleton[4,5] with the 7,8-double bond.

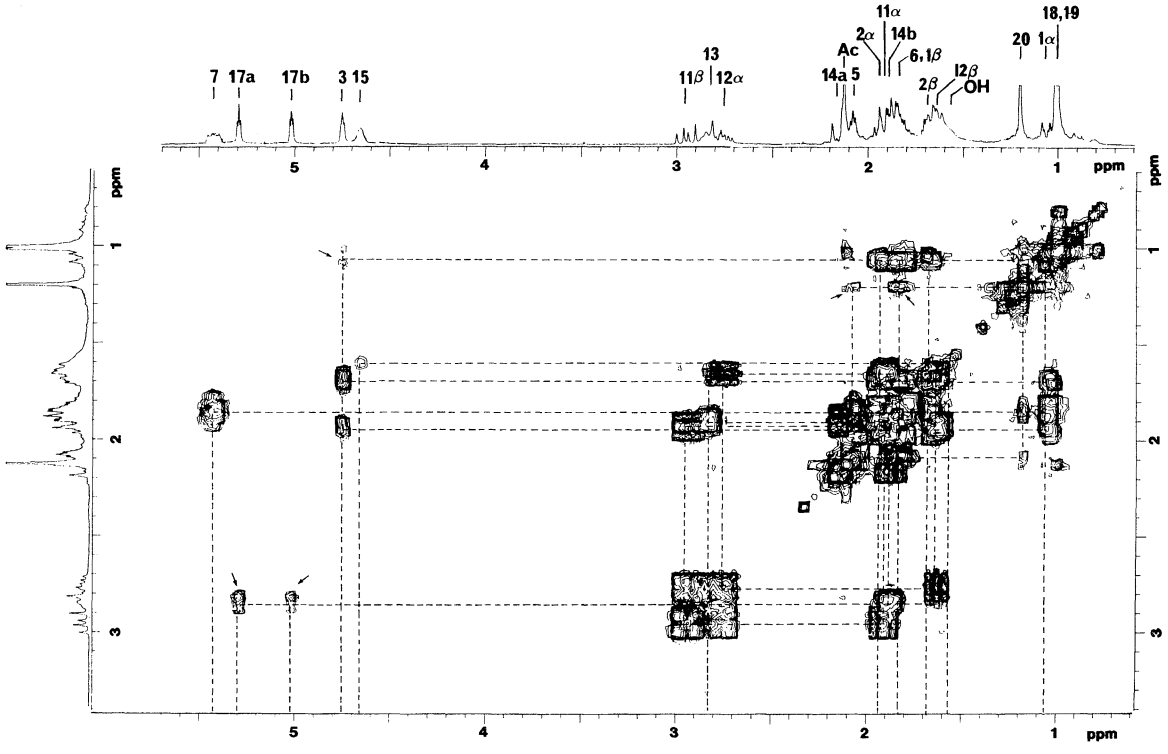
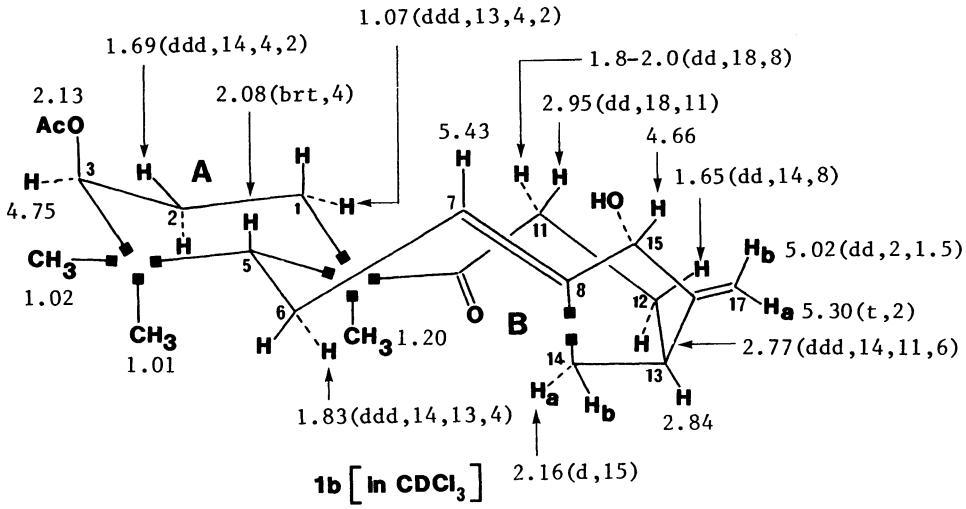


Fig. 1. The 2D NMR spectrum of rabdohakusin.



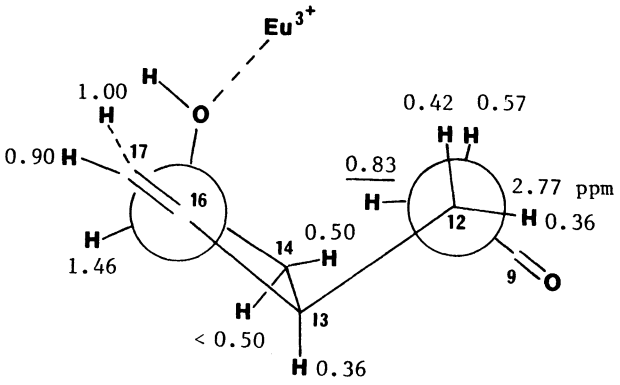
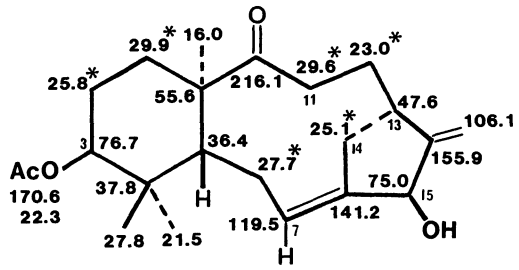
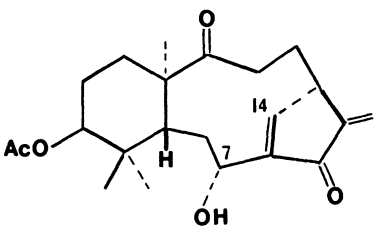


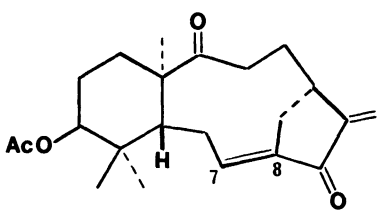
Fig. 2. The local conformation of rabdohakusin and Eu(fod)$_3$ (o.35 eq)-induced shifts. $\Delta\delta$(7-H)=1.09



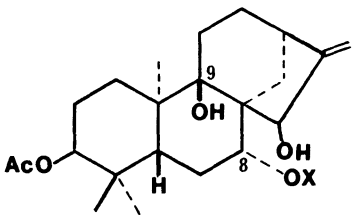
* Methylene carbon signals were tentatively assigned.



2



3



4

The configuration of the 7,8-double bond was assigned as 6,14-cis based on biogenetic consideration that rabdohakusin (1) could be derived from a corresponding ent-kaurene precursor (4) by 1,4-elimination. The acetoxyl group was positioned at C-3ß[6] because of the cross peak arising from the W-type coupling between 3-H and 1-H_{eq} observed in the COSY spectrum. The $Eu(fod)_3$-induced shift data shown in Fig. 2 indicated the predominant coordination of the LSR with the 15-OH. The large downfield shift of the 11ß-H made it clear that the orientation of the hydroxyl group was ß, and this also proposed the most plausible ten-membered ring conformation as illustrated in Fig. 2. The preference for this conformer even though it has a considerable torsion to minimize trans-annular nonbonded repulsion[7] between ketone carbonyl and 7,8-double bond was supported by the following reasons. (i) In the 2D NMR spectrum, the lack of the cross peaks due to the couplings of 11α-H/12α-H and 11ß-H/12ß-H indicates the dihedral angles of $H_{11\alpha}$-C(11)-C(12)-$H_{12\alpha}$ and $H_{11ß}$-C(11)-C(12)-$H_{12ß}$ to be approximately 90°. (ii) The small coupling of H-5 (J=4 Hz) with H-6 is consistent with the eclipsed conformation forced by the torsional arrangement shown in Fig. 2. (iii) Unusual downfield shift (2.77 ppm) of 12α-H is probably due to a carbonyl anisotropy caused by syn orientation.

In view of the fact that the absolute configuration of all Rabdosia kaurene diterpenoids known to date is the same as that of an ent-kaurane, it seems reasonable to assume that the structure of rabdohakusin is ent-8,9-seco-3ß-acetoxy-15ß-hydroxy-kaur-7,16-dien-9-one.

The authentic sample of shikoccin was kindly provided by Professor E. Fujita, Kyoto University.

References

1) I. Kubo, T. Kamikawa, T. Isobe, and T. Kubota, J. Chem. Soc., Chem. Commun., 1980, 1206 and references therein.
2) E. Fujita, N. Ito, I. Uchida, K. Fuji, T. Taga, and K. Osaki, J. Chem. Soc., Chem. Commun., 1979, 806.
3) I. Kubo, I. Ganjian, and T. Kubota, Phytochemistry, 21, 81 (1982).
4) M. Node, N. Ito, K. Fuji, and E. Fujita, Chem. Pharm. Bull., 30, 2639 (1982).
5) M. Pinar, M. Rico, C. Pascual, and B. Fernandez, Phytochemistry, 22, 2775 (1983).
6) The comparison of the ^{13}C NMR data with those of 2[4] also supported the correctness of this assignment.
7) This interaction becomes serious for the other possible conformation arising from rotation of the C-10-C-12 bond.

(Received June 23, 1984)