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THE STRUCTURE OF KAMEBANIN, A NEW ANTITUMOR ENT-KAURENOID FROM ISODON KAMEBA OKUYAMA

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Chemical investigation of the bitter principles from Isodon Kameba has led to the isolation and characterization of a new ent-kaurenoid diterpene, kamebanin 1, which has in vitro cytotoxicity, in vivo tumor inhibitory, and antibacterial activities.

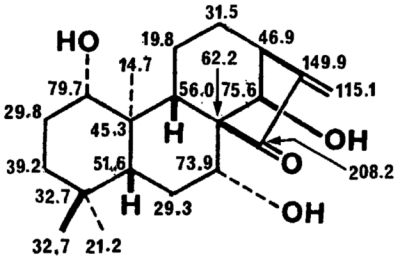
A number of ent-kaurenoids with biological activity have been isolated from Isodon species.[1,2,3] In this paper we report the structure of kamebanin isolated in 0.0012% yield from the dry leaves of Isodon Kameba (Labiatae) from which mebadonin and umbrosin A were also isolated earlier.[4,5] Kamebanin possesses significant in vitro cytotoxicity (KB) and in vivo tumor inhibitory activity against Walker intramuscular carcinosarcoma in rats,[6] and specific insecticidal activity against Lepidoptera larvae.

We propose ent-kaurene structure 1 for kamebanin, which has the following physical properties, $C_{20}H_{30}O_4$ (high resolution MS and elemental analysis), mp 266-267°C, $[\alpha]_D^{19}$ -108° (c = 1.0, dioxane), uv (EtOH) 233.5 nm (ε 7700), ir (Nujol) 3250 and 3160 (hydroxyl), and 1720 and 1640 cm^{-1} (5-membered ring ketone conjugated with exocyclic methylene). The cmr data of kamebanin, as summarized in 1, indicated the presence of three methyls, five methylenes, six methines, three tetra-substituted

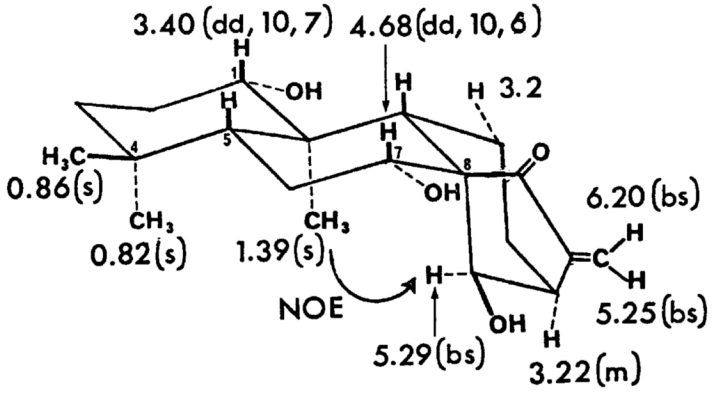
carbons, two olefinic carbons and one carbonyl carbon; the assignments are based on a combination of PND, PRFT and off-resonance decoupling techniques.[7] The pertinent pmr assignments are shown in 1a.

The above spectroscopic data and two broad singlets at 6.20 and 5.25 ppm in C_5D_5N suggest that kamebanin has a 15-oxo-16-kaurene skeleton which is typical of Isodon diterpenes. Three signals at 5.29 (bs), 4.68 (dd, 10, 6 Hz), and 3.40 ppm (dd, 10, 7 Hz) in the pmr spectrum appropriate for protons on carbons bearing hydroxyls, and three low field doublets at 79.7, 75.6, and 73.9 ppm in the cmr spectrum suggested the presence of three secondary hydroxyl groups. This was confirmed by conversion of 1 to triacetate 2. The appearance of 14-H (5.29 ppm) as a broad singlet, which collapsed to a sharp singlet upon irradiation at the frequency of 13-H (3.22 ppm, m), required the 14-hydroxyl group to be equatorial (the expected dihedral angle between 13- and 14-H $\simeq$ 90°). The abnormally low chemical shift of 7-H (4.68 ppm) is due to the deshielding effect of the 15-carbonyl group. The values of $J_{6\alpha,7}$ (10 Hz) and $J_{6\beta,7}$ (6 Hz) indicated that the 7-hydroxyl was equatorial. The stereochemistry of these two hydroxyl groups was further corroborated by conversion of 1 to acetonide 3. In addition, dihydrokamebanin 4,[8] (prepared by catalytic hydrogenation of 1) upon oxidation with Jones' reagent gave the 1,15-dione 5. The 7- and 14-hydroxyl groups were not oxidized under these conditions because of intramolecular H-bonding.

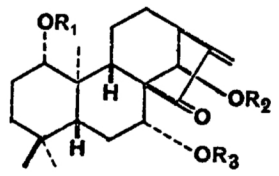
High resolution EI/MS peaks of 1 at m/e 194 and 176, which were formed by cleavage of the B-ring, showed the existence of the third hydroxyl group in the A-ring.[1] The splitting pattern dd of the C<u>H</u>-OH signal at 3.40 ppm showed that a methylene group was adjacent to this proton, and thus that the hydroxyl group should be attached either to C-1 or C-3. Analysis of the J values of this dd signal (10 and 7 Hz) showed the hydroxyl group to be equatorial. This hydroxyl group was determined to be at position 1 from the following pmr data: In compound 5, the methyl signal at 1.56 ppm (know to be 10α from NOE studies) is shifted more than the other two methyls by $Eu(fod)_3$ shift reagent which can only be rationalysed by the carbonyl at C-1, and not C-3 position. In addition, the unusually low field resonance of 11α-H in kamebanin 1 can be explained by the deshielding effect of a hydroxyl group at C-1 position. On the other hand, dihydrokamebanin 4 showed a negative CD curve ($\Delta\varepsilon_{300}$ -0.56) in MeOH and hence the D-ring should be β-oriented.[9,10] This was further supported by the negative CD curve ($\Delta\varepsilon_{300}$ -1.98) of dione 5 in MeOH.



(1) Kamebanin cmr data (δ/ppm) for d_5-pyridine solution

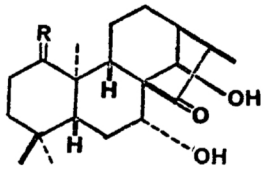


(1a) Kamebanin pmr data in d_5-pyridine solution; δ values; multiplicity and J values (in Hz) in parentheses



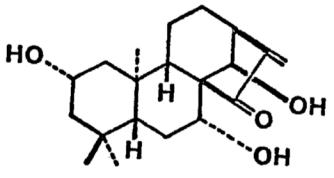
(2) $R_1=R_2=R_3=Ac$

(3) $R_1=H$, R_2, R_3 = $>C(CH_3)_2$

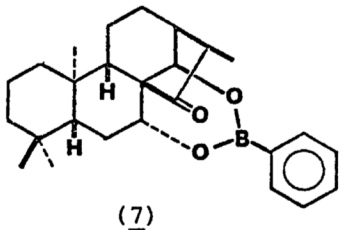


(4) R= 

(5) R=O



(6)



(7)

The structure was confirmed by correlation with mebadonin 6 whose structure had been determined by X-ray crystallography.[4] The hydroxyl groups of 5 were protected with phenylboronic acid, followed by removal of the carbonyl group (formation of the dithioketal and treatment with Raney Ni) to give compound 7, which was identical in all respects with the compound derived from 6 in the same manner.[11]

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References and Footnotes

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6. The cytotoxicity (KB) effect (LD_{50}) was 5.1 μg/ml. We thank Professor Francis J. Schmitz, Department of Chemistry, University of Oklahoma, for taking care of the bioassay.
7. P.R. Zanno, I. Miura, K. Nakanishi, and D.L. Elder, J. Am. Chem. Soc., 97, 1975 (1975) and I. Kubo and I. Miura, to be published.
8. Since the reagent is known to attack from the least hindered side, the newly formed 16-CH_3 of 4 is presumably assigned the β(R) configuration. Satisfactory spectra and/or elemental analyses were obtained for all the new compounds here reported.
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11. This work was partly supported by NIH grant CA11572.

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