CHEMISTRY LETTERS, pp. 1229-1232, 1981.

STRUCTURES OF LEUKAMENINS

Yoshio TAKEDA, Tetsuro FUJITA,* and Akira UENO†

Faculty of Pharmaceutical Sciences, The University of Tokushima, Tokushima 770

†Shizuoka College of Pharmacy, Shizuoka 221

Six new diterpenoids, leukamenin A, B, C, D, E and F with 15-oxo-*ent*-kaurene skeleton were isolated from the leaves of *Rabdosia umbrosa* var. *leucantha* f. *Kameba* and their structures were deduced from chemical and spectral evidence.

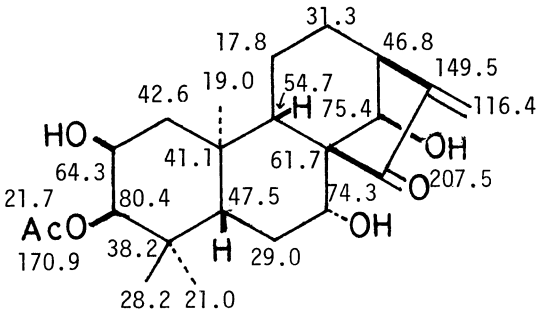
In the course of the investigations on biologically active substances of *Rabdosia*(Labiatae) plants, we have examined the constituents of the leaves of *Rabdosia umbrosa* (Maxim.) Hara var. *leucantha* (Murai) Hara f. *Kameba* (Okuyama et Ohwi) Hara[1] and isolated six new diterpenoids, leukamenin A, B, C, D, E and F. This report deals with the structure elucidation of these new compounds.

Leukamenin A (1), $C_{22}H_{32}O_6$, mp 228-230 °C, $[\alpha]_D^{21}$ -63.8° (c=1.04, MeOH), has a five membered ketone conjugated with an α-methylene group,judging from the following spectral data: λ_{max} (MeOH) 230.5 nm (ε 8664); ν_{max} (KBr)[2] 1725 and 1650 cm^{-1}; ^1H nmr (C_5D_5N)[3] δ 5.36 and 6.29 (each 1H, each br. s). The ^{13}C nmr data[3] of leukamenin A (1) are summarized in the structure (1) and showed the presence of 3 Me, 1 Ac, 4 CH_2, 7 CH, 3 tetrasubstituted carbons, 2 olefinic carbons and 2 carbonyl carbons. The ^1H nmr spectrum showed, besides the signals of 3 tert. Me groups (δ 0.94, 0.98 and 1.16) and 1 Ac group (δ 2.06), signals due to four methine protons attached to carbons having an oxygen functional group (3 OH and 1 OAc): δ 4.42 (ddd, 11, 4 and 3 Hz, H_a), 4.84 (dd, 10 and 5 Hz, H_c), 5.12 (br.s, H_d) and 5.36 (H_b, the splitting pattern was not clear because of the overlapping with one of the signals of exo-methylene). These data suggest that this compound has 15-oxo-*ent*-kaurene nucleus as a basic skeleton. In fact, the dihydro-compound (9)[4] showed a negative Cotton effect [λ_{max} (MeOH) nm (φ): 321 (-1674), 290 (+127)] in the ord.[5] The location of four oxygen functional groups were deduced as follows. In the ^1H nmr of (1), INDOR[6] signal was observed for the signal at δ 3.24 (1H, m, 13-H) on monitoring H_d. Acetylation of (1) with Ac_2O-C_5H_5N gave the triacetate (7), in the ^1H nmr spectrum of which, H_d was observed at δ 6.00 and suffered abnormal downfield shift[7] compared with the signals due to a proton attached to an acetoxy group bearing carbon, indicating that H_d was assigned

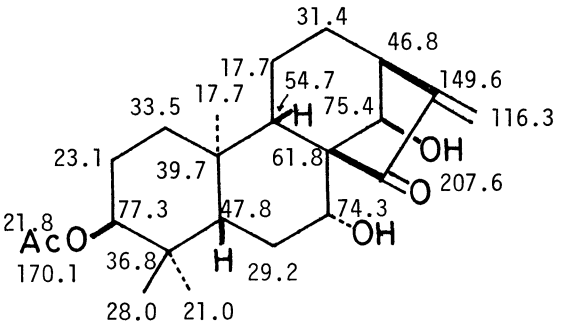
to 14α-H. *Ent*-kaurene skeleton of this substance was also supported from the fact that a NOE (19 %) was observed for H_d on irradiation at δ 1.16 (10-Me). H_a and H_b were assigned to 2α-axial and 3α-equatorial protons, respectively, from the following data. In the ^{1}H nmr of (7), respective irradiation of dimethyl groups at C-4 (δ 0.90 and 1.04) gave NOE's (13 and 12%, respectively) for H_b which was observed at δ 4.96 (d, 3 Hz) without overlapping with other signals. Accordingly, H_b was assigned to an α-equatorial proton attached to an acetoxy group bearing carbon, C-3. On monitoring from H_b, a signal due to coupling was observed for H_a and H_a changed to a double doublet (11 and 4 Hz) on irradiation at H_b in the ^{1}H nmr of (1). Above fact, together with the coupling pattern of H_a, suggests that H_a could be an axial proton at C-2. This presumption was confirmed from the fact that H_b was observed at δ 4.51 as a singlet in the ^{1}H nmr of 2,7-bisdehydro-compound[4] and a NOE (17%) was observed for H_a when irradiated at δ1.16 (10-Me) in the ^{1}H nmr of (1). Another proton, H_c, should be an axial proton which is located between a quarternary carbon and a methylene group. Treatment of (1) with 2,2-dimethoxypropane in the presence of TsOH in DMF gave an acetonide (12)[^{1}H nmr:δ 4.54 (1H, d, 2 Hz, 14α-H)]. Thus, a hydroxy group should be located at C-7α and hence H_c was assigned to the proton attached to a carbon having the hydroxy group. Accordingly, leukamenin A has structure (1).

Leukamenin B (2), C (3) and D (4) showed the following physical data: (2), $C_{24}H_{34}O_7$, mp 240-241 °C, $[\alpha]_D^{21}$ -32.5° (c=1.17, $CHCl_3$); λ_{max} (MeOH) 230.5 nm (ε 8627); ν_{max} 3600, 3550-3150, 1740, 1730 and 1650 cm^{-1}; (3), $C_{26}H_{36}O_8$, amorphous powder, $[\alpha]_D^{21}$ -21.2° (c=0.90, $CHCl_3$); λ_{max} (MeOH) 231 nm (ε 6351); ν_{max} 3600, 1730, 1650, 1265 and 1210 cm^{-1}; (4), $C_{26}H_{36}O_8$, mp 182-184 °C, $[\alpha]_D^{21}$ -27.3° (c=1.32, $CHCl_3$); λ_{max} (MeOH) 231.5 nm (ε 7360); ν_{max} 3560, 1740, 1730, 1650 and 1260-1210 cm^{-1}. Leukamenin B (2), C (3) and D (4) have the same oxidation pattern with that of leukamenin A (1) from the fact that respective acetylation of (2), (3) and (4) afforded the same acetate (7). In the ^{1}H nmr spectrum, (2) showed signals at δ 1.96 and 2.09 due to 2 Ac groups and a signal at δ 5.22(1H, ddd, 12, 4 and 3 Hz) due to H_a. Accordingly, leukamenin B has the structure (2). Leukamenin C (3) and D (4) showed signals due to 3 Ac groups, respectively, in their ^{1}H nmr. The ^{1}H nmr spectra also showed signals at δ 4.22 (dd, 9 and 7 Hz) and 5.96 (br.s) in (3), and at δ 5.43 (dd, 9 and 5 Hz) and 4.85 (br.s) in (4), which correspond to the signals at δ 4.38 (7β-H) and 4.86 (14α-H) in (2). These data indicate the structures (3) and (4) for leukamenin C and D, respectively.

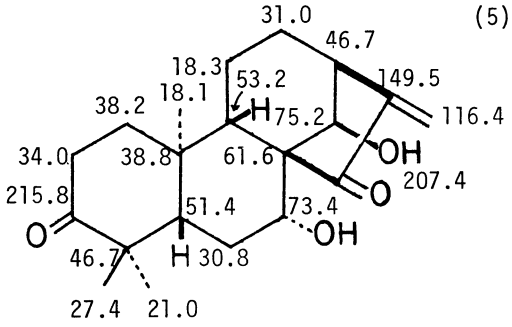
Leukamenin E (5), $C_{22}H_{32}O_5$, mp 148-149 °C, $[\alpha]_D^{22}$ -55.8° (c=0.39, MeOH) showed the following data: λ_{max} (MeOH) 230.5 nm (ε 7670); ν_{max} 3600, 3550-3150, 1720, 1645 and 1260 cm^{-1}; ^{1}H nmr δ 2.05 (3H, s, Ac), 4.40 (1H, dd, 10 and 5 Hz), 4.64 (1H, t, 3 Hz) and 4.87 (1H, br.s). The ^{13}C nmr data are summarized in the structure (5). The above mentioned data suggest that leukamenin E has a structure corresponding to 2-deoxyleukamenin A (5). This presumption was confirmed by the fact that a NOE (9%) was observed for the signal at δ 4.64 (3α-H) when irradiated at δ0.93 (one of the dimethyl groups at C-4) and by the following reaction. Leukamenin E (5) gave the diacetate (8)[^{1}H nmr δ 6.01



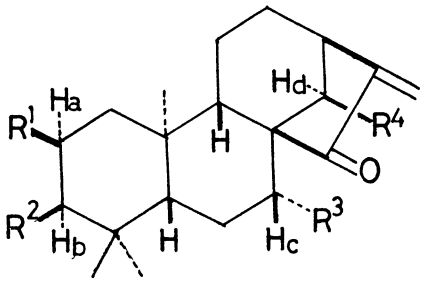
(1)

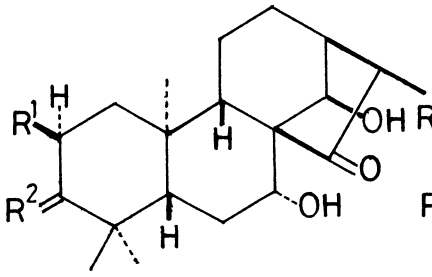


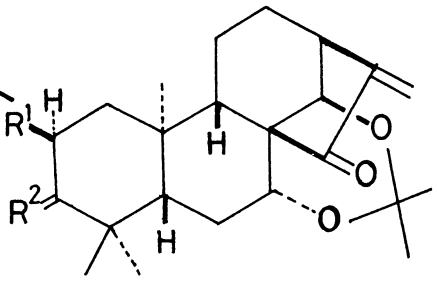
(5)



(6)







(1): $R^1=R^3=R^4=OH$; $R^2=OAc$

(2): $R^1=R^2=OAc$; $R^3=R^4=OH$

(3): $R^1=R^2=R^4=OAc$; $R^3=OH$

(4): $R^1=R^2=R^3=OAc$; $R^4=OH$

(5): $R^1=H$; $R^2=OAc$; $R^3=R^4=OH$

(7): $R^1=R^2=R^3=R^4=OAc$

(8): $R^1=H$; $R^2=R^3=R^4=OAc$

(9): $R^1=OH$; $R^2=\alpha$-H, β-OAc

(10): $R^1=H$; $R^2=\alpha$-H, β-OAc

(11): $R^1=H$; $R^2=O$

(12): $R^1=OH$; $R^2=\alpha$-H, β-OAc

(13): $R^1=H$; $R^2=\alpha$-H, β-OAc

(14): $R^1=H$; $R^2=O$

(br.s), 14α-H], an acetonide (13) [^{1}H nmr δ 4.22 (dd, 11 and 8 Hz, 7β-H) and 4.54 (d, 2 Hz, 14α-H)] and dihydro-compound (10)[ord: λ_{max} (MeOH) nm (ϕ): 319 (-2297), 287 (+981)]. Accordingly, leukamenin E has structure (5).

Leukamenin F (6),[8] $C_{20}H_{28}O_4$, mp 223-224 °C, $[\alpha]_D^{22}$ -170° (c=0.34, MeOH), showed the following data: λ_{max} (MeOH) 230.5 nm (ε 8371); ν_{max} 3600, 3550-3150, 1730, 1705 and 1650 cm^{-1}; ^{1}H nmr δ 1.09 (6H, s, 2 tert. Me), 1.16 (3H, s, tert. Me), 4.32 (m, dd after D_2O treatment, 10 and 5 Hz, 7β-H),

4.83 (br.s, 14α-H). The ^{13}C nmr data are summarized in the structure (6). These data suggest that leukamenin F has a structure, *ent*-7β,14α-dihydroxy-kaur-16-en-15-one as skeleton. This was supported by MS (EI), in which strong ion peaks were observed at m/e 194 and 176 arising from B, C and D ring as in the case of umbrosin A.[9] The ir (1705 cm^{-1}) and ^{13}C nmr (δ 215.8) suggest the presence of an additional six-membered ketone in (6), which should be located on A ring, namely, at C-1, 2 or 3. Leukamenin F (6) showed different spectral data with those of 1-dehydrokamebanin and 2-dehydro-umbrosin A. Thus, the carbonyl group should be located at C-3. Leukamenin F (6) gave the 7,14-di-acetate [^1H nmr δ 5.96 (br.s, 14α-H)], an acetonide (14)[^1H nmr δ 4.18 (dd, 11 and 7 Hz, 7β-H) and 4.56 (d, 2 Hz, 14α-H)] and dihydro-compound (11)[ord: λ_{max} (MeOH) nm (φ): 317 (-3477), 279 (-393)]. Accordingly, leukamenin F has structure (6).

Only three speciemen of 15-oxo-*ent*-kaurenoids oxidised at C-2, umbrosin A and B[9] and mebadonin,[10] have been isolated so far. Four of the six new diterpenoids reported here are oxidised both at C-2 and C-3 and the first example of this oxidation pattern. The examination of the biological activities are now in progress.

References

1. H. Hara, Japan J. Bot., 47, 193 (1972).
2. Unless otherwise noted, all ir spectra were recorded for $CHCl_3$ solutions.
3. Unless otherwise noted, ^1H nmr were recorded for $CDCl_3$ solutions. All ^{13}C nmr spectra were taken for C_5D_5N solutions and the assignements are based on the related compounds; I. Kubo, I. Miura, K. Nakanishi, T. Kamikawa, T. Isobe, and T. Kubota, J. Chem. Soc. Chem. Comm., 555 (1977); J.R. Hanson, M. Siverns, F. Piozzi, and G. Savona, J. Chem. Soc. Perkin I, 114 (1976).
4. Compound (9) and 2,7-bisdehydro-compound were obtained by hydrogenation (H_2-PtO_2) and Jones oxidation of (1), respectively.
5. T. Kubota, T. Matsuura, T. Tsutsui, S. Uyeo, H. Irie, A. Numata, T. Fujita, and T. Suzuki, Tetrahedron, 22, 1659 (1966).
6. O. Sciacovelli, W.von Philipborn, C. Amith, and D. Ginsburg, Tetrahedron, 26, 4589 (1970).
7. T. Fujita, Y. Takeda, and T. Shingu, J. Chem. Soc. Chem. Comm., 205 (1980).
8. This compound has recently been isolated independently from *Rabdosia japonica* (burm. f.) Hara var. *glaucocalyx* (Maxim.) Hara: personal communication from Mr. Sun Han-dong, Kunming Institute of Botany, Academia Sinica, China.
9. I. Kubo, T. Kamikawa, T. Isobe, and T. Kubota, Bull. Chem. Soc. Japan, 47, 1277 (1974).
10. K. Hirotsu, T. Kamikawa, T. Kubota, and A. Shimada, Chemistry Letters, 1973, 255.

(Received June 19, 1981)