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STRUCTURES OF EFFUSANINS, ANTIBACTERIAL DITERPENOIDS FROM *RABDOSIA EFFUSA*

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Five new diterpenoids, effusanin A, B, C, D and E with antibacterial activity were isolated from the stem and leaves of *Rabdosia effusa* and their structures were deduced from chemical and spectral findings.

During investigations on biologically active substances in members of the Labiatae, we examined the constituents of stem and leaves of *Rabdosia effusa* (Maxim.) Hara[1] and isolated five new antibacterial[2] diterpenoids, effusanin A (1), B (2), C (3), D (4) and E (5) together with shikokianin (6)[3], longikaurin E (7)[4] and longikaurin F (8).[4] Here we report elucidation of the structures of the new diterpenoids.

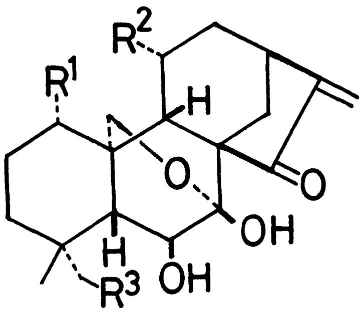
Effusanin A (1), $C_{20}H_{28}O_5$, mp 266-268 °C, $[\alpha]_D^{20}$ -79.7° (c=0.35, C_5H_5N) has a five membered ketone conjugated with an α-methylene group, judging from the following spectral data: λ_{max} (MeOH) 240 nm (ε 9712); ν_{max}[5] 1702 and 1640 cm^{-1}; ^1H nmr[6] δ 5.23 and 5.92 [each 1H, each br. s]; ^{13}C nmr[6] δ 115.8 (t), 154.5 (s)[exo-methylene] and 211.0 [ketone]. The ^1H nmr shows the presence of two tert. methyl groups (δ 1.12 and 1.17), an oxygenated methyl group [δ 4.29 and 4.70 (each 1H, each ABdd, 10 and 1 Hz)] and two protons attached to carbons having a hydroxyl group [δ 3.62 (1H, dd, 8 and 8 Hz), 4.19 (1H, dd, 11 and 7 Hz, changed to doublet, 7 Hz, after D_2O treatment)]. The ^{13}C nmr also showed a signal due to an acetalic carbon (δ 95.9), besides signals assigned to an oxygenated methyl group (δ 63.8) and two secondary carbinyl carbons (δ 73.5 and 75.0). These data suggest that effusanin A (1) has the basic skeleton, *ent*-7α-hydroxy-7β,20-epoxykaur-16-en-15-one (10).

In fact, the dihydro-compound (11) showed a negative Cotton effect [λ_{max} (MeOH) nm (ϕ): 317 (-4667), 285 (+2258)] in the ord. The locations of the two secondary hydroxyl groups were determined as follows: In an internuclear double resonance (INDOR) experiment, a signal due to NOE[7] was observed on a tert. methyl group at δ 1.12 when the double doublet at δ 4.19 was monitored. On the other hand, an NOE (10%) was observed for the double doublet at δ 4.19 on irradiation at δ 1.12. Therefore, the hydroxyl group should be located at position C-6β. Acetylation (Ac_2O-C_5H_5N) of effusanin A (1) gave the monoacetate (2)[^{1}H nmr ($CDCl_3$) δ 4.58 (1H, dd, 10 and 6 Hz, 1β-H)]. Oxidation of (1) with $NaIO_4$ gave isodocarpin (14)[8], mp 290-291 °C, $[\alpha]_D^{20}$ -141.7° (c=0.22, C_5H_5N) [ν_{max} (KBr) 1750, 1700, 1650 cm^{-1}]. Accordingly, effusanin A has the structure (1).

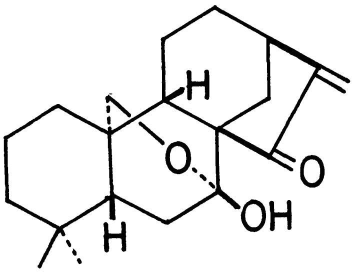
Effusanin B (2)[9], $C_{22}H_{30}O_6$, mp 258-260 °C, $[\alpha]_D^{20}$ -44.1° (c=0.28, $CHCl_3$) showed λ_{max} (MeOH) 240 nm (ε 9473) in the uv and ν_{max} 1720, 1645, 1250 cm^{-1} in the ir. This compound was identified as the monoacetate (2) of effusanin A (1).

Effusanin C (3), $C_{22}H_{30}O_7$, mp 243-245 °C, $[\alpha]_D^{21}$ -54.0° (c=0.46, C_5H_5N) gave the following uv and ir data: λ_{max} (MeOH) 240 nm (ε 9608); ν_{max} 1740, 1700, 1640, 1235 cm^{-1}. The ^{1}H and ^{13}C nmr are very similar to those of effusanin A (1) except that there is only one tert. methyl group and there are signals of a -CH_2OAc group: ^{1}H nmr [δ 1.94 (3H, s, OCOC$\underline{H}_3$), 4.84 (1H, ABd, 11 Hz)]; ^{13}C nmr [δ 170.1 (O$\underline{C}$OCH_3), 66.5 or 64.4 (-$\underline{C}H_2$OAc)]. These data suggest that effusanin C has a structure, in which one of the tert. methyl groups at C-4 in (1) is oxidised to an acetoxymethyl group. Acetylation (Ac_2O-C_5H_5N) of (3) gave the monoacetate (4), which was identical with effusanin D (4), $C_{24}H_{32}O_8$, mp 188-190 °C, $[\alpha]_D^{21}$ -28.2° (c=0.41, $CHCl_3$). The dihydro-compound (12) of effusanin D (4) showed a negative Cotton effect [λ_{max} (MeOH) nm (ϕ): 318 (-4802), 285 (+2115)] in the ord. Oxidation of (3) with $NaIO_4$ gave a lactone (15), mp 270-272 °C. The acetoxymethyl group was deduced to be located at C-4α from the fact that, in the ^{1}H nmr (C_5D_5N-$CDCl_3$) of (15), a signal due to NOE for the tert. methyl group was observed when a signal at δ 2.20 (1H, s, 5-H) was monitored. Accordingly, the structures of effusanin C and D should be represented as (3) and (4), respectively.

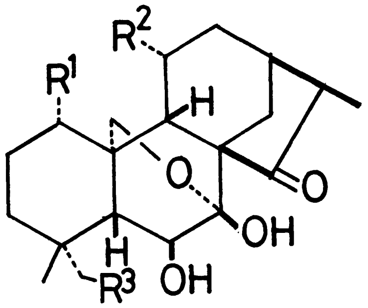
Effusanin E (5), $C_{20}H_{28}O_6$, mp 250-252 °C, $[\alpha]_D^{21}$ -81.3° (c=0.28, C_5H_5N) showed the following spectral data: λ_{max} (MeOH) 238.5 nm (ε 8335); ν_{max} 3600-3050, 1702, 1642 cm^{-1}. Besides the signals of two tert. methyl groups (δ 1.24 and 1.34), the ^{1}H nmr showed signals due to three protons on carbons bearing a hydroxyl group [δ 3.84 (1H, dd, 10 and 6 Hz), 4.28 (1H, dd, 11 and 7 Hz, changed to a doublet, 7 Hz, after D_2O treatment), 4.50 (1H, m, changed to a double doublet, 5 and 5 Hz, after D_2O treatment)] and signals assigned to a -CH_2O- group [δ 4.32 (1H, ABd, 11 Hz), 5.13 (1H, ABdd, 11 and 1 Hz)]. The ^{13}C nmr showed the presence of an acetalic carbon (δ 96.3), an oxygenated methyl carbon (δ 65.7) and three secondary carbinyl carbons (δ 75.3, 73.5 and 67.0). These data suggest that effusanin E has the same structure as effusanin A (1) but with an additional secondary hydroxyl group. In fact, the dihydro-compound (13) showed a negative Cotton effect [λ_{max} (MeOH) nm (ϕ): 317 (-4396), 284 (+1617)] in the ord. The additional hydroxyl group was concluded to be located at C-11α, because the proton signals assigned to 20-H_1 and 14α-H were shifted to δ 5.13 and 3.64 (1H, d, 12 Hz), respectively, like those of longikaurin D (9)[4]. Oxidation of (5) with $NaIO_4$ gave nodosin (16)[10], mp 303-305 °C, $[\alpha]_D^{21}$ -141.2° (c=0.24, C_5H_5N); ν_{max} (KBr) 1750, 1702, 1642 cm^{-1}. Accordingly, effusanin E has the structure (5).



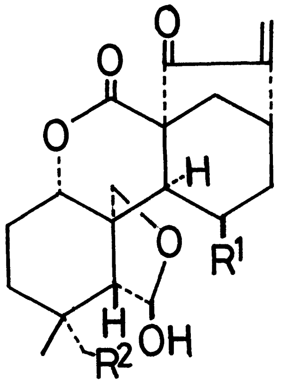
(1): R^1=OH; R^2=R^3=H
(2): R^1=OAc; R^2=R^3=H
(3): R^1=OH; R^2=H; R^3=OAc
(4): R^1=R^3=OAc; R^2=H
(5): R^1=R^2=OH; R^3=H
(6): R^1=R^2=OAc; R^3=H
(7): R^1=R^3=H; R^2=OAc
(8): R^1=H; R^2=R^3=OAc
(9): R^1=H; R^2=OH; R^3=OAc



(10)



(11): R^1=OH; R^2=R^3=H
(12): R^1=R^3=OAc; R^2=H
(13): R^1=R^2=OH; R^3=H



(14): R^1=R^2=H
(15): R^1=H; R^2=OAc
(16): R^1=OH; R^2=H

REFERENCES AND FOOTNOTES

1. H. Hara, Japan J. Bot., 47, 193 (1972).
2. The minimal inhibitory concentrations (m.i.c.) of effusanin A (1), B (2), C (3), D (4) and E (5) against *Bacillus subtilis* ATCC 6633 are 62.5, 31.2, 62.5, 31.2 and 62.5 $\mu g\ ml^{-1}$, respectively. All the diterpenoids tested show m.i.c. of > 1000 $\mu g\ ml^{-1}$ against *Escherichia coli* NIHJ. The detailed study will be published elsewhere.
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5. Unless otherwise noted, all ir spectral data were obtained for Nujol mull.
6. Unless otherwise noted, ^{1}H and ^{13}C nmr spectra were recorded in C_5D_5N solution, using tetramethylsilane as internal standard.
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