Longikaurin A and B; New, Biologically Active Diterpenoids from *Rabdosia longituba*

By TETSURO FUJITA,* YOSHIO TAKEDA, and TETSURO SHINGU†

(*Faculty of Pharmaceutical Sciences, The University of Tokushima, Tokushima* 770, *Japan and* †*Faculty of Pharmaceutical Sciences, Kobe-Gakuin University, Tarumi-ku, Kobe* 673, *Japan*)

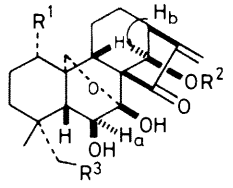
Summary Chemical investigation of the biologically active substances of *Rabdosia longituba* led to the isolation of two new diterpenoids having an *ent*-kaurene skeleton, longikaurin A (**1**) and B (**2**), and their structures were established by spectroscopic and chemical data

DURING studies on the biologically active diterpenoids of plants belonging to the genus *Rabdosia* [= *Isodon*][1] (Labiatae), we examined the constituents of the leaves of *R longituba* (Miquel) Hara [*I longitubus* (Miq) Kudo] and isolated two new diterpenoids, longikaurin A (**1**) and B (**2**), together with known kamebakaurin (**6**) [2] Longikaurin A (**1**) shows cytotoxicity‡ *in vitro* against cultured rat

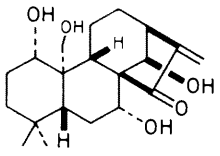
‡ 1 μg ml^{-1} of longikaurin A (**1**) shows inhibitory activity (74%) on the growth of cultured rat mammary cancer FM 3A/B cells Although longikaurin B (**2**) is likely to show a similar activity, poor solubility in Me_2SO–H_2O inhibited testing The detailed study will be published elsewhere

mammary cancer FM 3A/B cells, and both longikaurin A (**1**) and B (**2**) show antibacterial activity.§

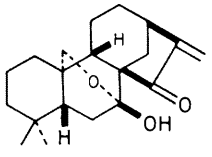
Longikaurin A (**1**), $C_{20}H_{28}O_5$, m.p. 223—225 °C, $[\alpha]_D^{25}$ −91·1° (*c* 0·21, C_5H_5N), has been shown to contain a five membered ketone conjugated with an *exo*-methylene group from the following spectral data: λ_{max} (MeOH) 235 nm (ϵ 9530); ν_{max} (Nujol) 1705 and 1640 cm^{-1}; ^1H n.m.r.¶ δ 5·52 and 6·14 (each 1H, br s); ^{13}C n.m.r.¶ δ 117·8 (t), 151·2 (s) [>C=CH_2], and 207·1 p.p.m. [ketone]. Compound (**1**) shows a strong absorption band in the i.r. spectrum (3400—3050 cm^{-1}) due to hydroxy groups and ^1H n.m.r. signals at δ 4·90 (1H, m), 6·10 (1H, m), and 6·59 (1H, d, *J* 10 Hz) which are assigned to three hydroxy groups. The ^1H [δ 3·77 (1H, d, *J* 7 Hz after D_2O treatment, H_a) and 4·76 (1H, d, *J* 2 Hz, H_b)] and ^{13}C n.m.r. signals [δ 71·7 and 72·4 p.p.m. (each doublets)] suggest that two of the three hydroxy groups are secondary and the third is tertiary. The ^{13}C n.m.r. spectrum further shows signals at δ 96·4 and 64·5 p.p.m. due to the acetalic carbon and $-CH_2O-$ group, respectively. These data show that longikaurin A (**1**) is pentacyclic. Considering the structures of diterpenoids isolated so far from *Rabdosia* species,[3] we presumed that (**1**) had the *ent*-7β,20-epoxy-kaur-16-en-15-on-7α-ol (**7**) structure as a basic skeleton. This presumption was supported by the fact that the dihydro-compound (**8**) shows a negative Cotton effect [λ_{max} (MeOH) nm (ϕ): 316 (−5141), 280 (+2981)].



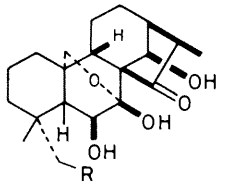
(1) $R^1 = R^2 = R^3 = H$
(2) $R^1 = R^2 = H$, $R^3 = OAc$
(3) $R^1 = R^3 = H$, $R^2 = Ac$
(4) $R^1 = OH$, $R^2 = R^3 = H$
(5) $R^1 = H$, $R^2 = Ac$, $R^3 = OAc$



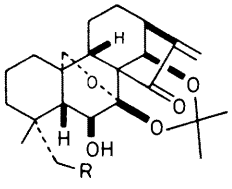
(6)



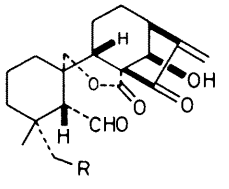
(7)



(8) R = H
(9) R = OAc



(10) R = H
(11) R = OAc



(12) R = H
(13) R = OAc

Acetylation of (**1**) (acetic anhydride–pyridine) gave the monoacetate (**3**), m.p. 233—235 °C. The ^1H n.m.r. signal for H_b of (**1**) was shifted downfield to δ 5·68 (1H, *J* 1 Hz) indicating that H_b is located at C-14α analogous to oridonin (**4**).[4] A secondary hydroxy group is located at the C-6β position. In INDOR[5,6] experiments, a signal due to nuclear Overhauser enhancement (n.O.e.) was observed for the methyl groups (δ 1·09, 6H) when monitored at the frequency of H_a, which appears as a doublet (*J* 7 Hz) after D_2O treatment. On irradiation of the methyl groups, n.O.e. (6%) was observed for H_a.

Treatment of (**1**) with 2,2-dimethoxypropane in $HCONMe_2$ in the presence of *p*-toluenesulphonic acid gave the acetonide (**10**), m.p. 194—196 °C [^1H n.m.r. δ 1·32 and 1·64 (each 3H, 2 × s, acetal Me)], confirming β-configuration of the tertiary hydroxy group at C-7. Oxidation of (**1**) with periodic acid gave the aldehyde-lactone (**12**) [^1H n.m.r. δ 9·90 (1H, d, *J* 6 Hz, aldehydic proton); i.r. ($CHCl_3$) ν_{max} 1740, 1720, and 1710 cm^{-1}], the formation of which established chemically the existence of a hydroxy group at the C-6β position. Thus it was shown that longikaurin A has the structure (**1**).

Longikaurin B (**2**), $C_{22}H_{30}O_7$, m.p. 238—239·5 °C, has the following physical properties: $[\alpha]_D^{25}$ −115·9° (*c* 0·12, C_5H_5N); λ_{max} (MeOH) 234·5 nm (ϵ 9510); ν_{max} (Nujol) 3490, 3350—3100; 1745, 1710, and 1650 cm^{-1}. The ^1H n.m.r. spectrum (in C_5D_5N) is very similar to that of longikaurin A (**1**) except for the signals due to a tertiary methyl group (δ 1·34, 3H, s), an acetyl group (δ 1·95, 3H, s), and an acetoxy methyl group [δ 4·40 and 4·68, each 1H, each AB doublets, *J* 11 Hz]. The ^{13}C n.m.r. spectrum when compared with that of (**1**) differs only in the number of methyl groups which decreases from 2 to 1 and the number of oxygenated methylene groups which increases from 1 to 2. These spectral data suggest that in the structure of longikaurin B (**2**), one of the two methyl groups at C-4 of longikaurin A (**1**) is oxidized to an acetoxy methyl group. In INDOR[5,6] experiments with (**2**), signals due to n.O.e.'s were observed for the signals at δ 3·99 and 4·11 (each 1H, AB doublets, *J* 10 Hz, 20-H_2) and δ 1·37 (tertiary methyl group) when an AB doublet at δ 4·68 was monitored. N.O.e.'s were observed for an AB doublet at δ 4·68 on irradiation of the tertiary methyl group and 20-H_2 (8 and 11%, respectively). These facts suggest that longikaurin B (**2**) corresponds to 19-acetoxylongikaurin A.

Compound (**2**) gave rise to the monoacetate (**5**), m.p. 182—183 °C [^1H n.m.r. δ 2·04 and 2·06 (each 3H, 2 × s, 2 × OAc), 5·66 (1H, br s, 14α-H)], the acetonide (**11**) [^1H n.m.r. δ 1·34 and 1·64 (each 3H, 2 × s, acetal Me)], the aldehyde-lacetone (**13**) [^1H n.m.r. δ 9·97 (1H, d, *J* 6 Hz,

§ The minimal inhibitory concentrations (m.i.c.) of (**1**) against *Staphylococcus aureus* FDA 209P and *Escherichia coli* NIHJ are 12·5 and >200 $\mu g\ ml^{-1}$, respectively. Compound (**2**) also shows m.i.c. against the same bacteria (25 and >200 $\mu g\ ml^{-1}$, respectively). The detailed study will be published elsewhere.

¶ Unless otherwise noted, ^1H n.m.r. spectra were recorded in $CDCl_3$ solution and ^{13}C n.m.r. spectra were recorded in C_5D_5N solution, using tetramethylsilane as internal standard.

aldehydic proton), ir ν_{max} ($CHCl_3$) 1740 and 1705 cm^{-1}] and the dihydro-compound (**9**) [o r d , λ_{max} (MeOH)/nm (ϕ) 317 (−4004), 280 (+3022)] These facts established that longikaurin B has the structure (**2**)

The isolation of longikaurin B (**2**), which is oxygenated at C-19, is the first example among diterpenoids having an *ent*-kaurene nucleus so far from *Rabdosia* species This substance may be a biosynthetic precursor of the 6,7-seco-*ent*-kaurenoid diterpene of the shikodonin[7] type

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