LONGIKAURIN C, D, E AND F; NEW ANTIBACTERIAL DITERPENOIDS FROM *RABDOSIA LONGITUBA*

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Abstract — Four new diterpenoids, longikaurin C, D, E and F with antibacterial activity were isolated from the leaves of *Rabdosia longituba* and their structures were shown to be (3), (4), (5) and (6), respectively.

Recently, we isolated two new, biologically active diterpenoids, longikaurin A (1) and B (2) from the leaves of *Rabdosia longituba* (Miquel) Hara[1] and determined their structures[2]. Here, we report structural elucidations of four new other antibacterial[3] diterpenoids, longikaurin C, D, E and F, present as minor constituents in the same plant.

Longikaurin C (3), $C_{22}H_{30}O_6$, mp 248-250°C, $[\alpha]_D^{25}$ -137.5° (*c* 0.12, C_5H_5N) has the following constants: λ_{max} (MeOH) 239 nm (ε 9450); ν_{max}[4] 3450-3075, 1735, 1700, 1640, 1245 cm^{-1}; ^1H NMR[5] (C_5D_5N) δ 1.33 (s, tert. Me), 1.93 (s, OAc), 3.98 (2H, br.s), 4.30 (1H, dd, *J* 10 and 6 Hz, changed to d, *J* 6.5 Hz by D_2O treatment), 4.34, 4.62 (each 1H, each AB doublets, *J* 11 Hz). The ^{13}C NMR[5] (C_5D_5N) showed the presence of 2 $-CH_2O$ (δ 66.6), 1 >CH(OH) (δ 73.4) and 1 acetalic carbon (δ 96.0) together with an exo-methylene [δ 116.5 (t) and 153.8 (s)] and 2 carbonyl carbons (δ 170.7 and 210.2). These data, together with the fact that the dihydro-compound (8) showed a negative Cotton effect [λ_{max}(MeOH) nm (φ): 316 (-4312), 284 (+1764)] in the ORD, suggest that longikaurin C has the basic skeleton, *ent*-7β,20-epoxy-kaur-16-en-15-on-7α-ol (7). The presence of a $-CH_2OAc$ group in (3) was indicated by a signal of AB doublets (δ 4.34 and 4.62) in the ^1H NMR. This group was shown to be located at C-4α by examination of the internuclear double resonance (INDOR)[6,7] and NOE. On monitoring δ 4.62, INDOR signals due to NOE were observed on 20-H_2 (δ 3.98) and a tert. Me group. A hydroxy group is present at C-6β judging from the coupling pattern of the secondary carbinyl proton (δ 4.30) and the observation of a signal due to NOE (ca.6%) for a tert. Me group on

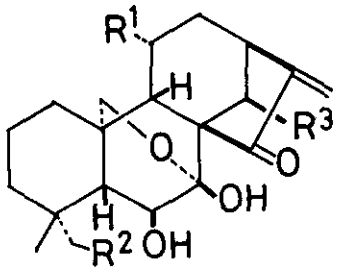
monitoring from the proton. This was also confirmed by the fact that periodate oxidation of (3) gave an aldehyde (11), mp 187-189°C [δ 4.66 (br.s, 20-H_2), 9.84 (1H, d, J 4 Hz, CHO)] showing no hydroxy group in the IR spectrum. Accordingly, longikaurin C has structure (3).

Longikaurin D (4), $C_{22}H_{30}O_7$, mp 262-264°C, $[\alpha]_D^{25}$ -109.0° (c 0.13, C_5H_5N) has the following constants: λ_{max} (MeOH) 237.5 nm (ε 8550); ν_{max} 3530, 3400-3100, 1720, 1700, 1640, 1250 cm^{-1}; ^1H NMR (C_5D_5N) δ 3.51 (d, J 11 Hz, 14α-H), 4.15 (ABdd, J 9 and 1.5 Hz, 20-H_1), 4.68 (ABd, J 11 Hz, 19-H_1), 5.18 (ABdd, J 9 and 2 Hz, 20-H_1). The ^{13}C NMR (C_5D_5N) showed signals assigned to 2 >CH(OH) (δ 65.5 and 74.2). The dihydro-compound (9) showed a negative Cotton effect [λ_{max} (MeOH) nm (φ): 316 (-6826), 283 (+1998)] in the ORD. These results suggest that longikaurin D has the same structure as longikaurin C (3), but with an additional secondary hydroxy group. This hydroxy group was deduced to be located at C-11α from the following data: 1) Longikaurin D (4) was not acetylated by the usual method with Ac_2O-C_5H_5N; 2) In the ^1H NMR, the signals assigned to 14α-H and 20-H_1 were shifted downfield, as in the case of nodosin[8]. Accordingly, the structure of longikaurin D should be represented as (4).

Longikaurin E (5)[9], $C_{22}H_{30}O_6$, mp 252-254°C, $[\alpha]_D^{25}$ -78.6° (c 0.21, C_5H_5N) showed the following spectral data: λ_{max} (MeOH) 237 nm (ε 8660); ν_{max} 3500-3125, 1730, 1720, 1640, 1245 cm^{-1}; ^1H NMR δ 1.12, 1.14 (2 x s, 2 x tert. Me), 2.09 (s, OAc), 3.91 (1H, dd, J 12 and 8 Hz), 4.11 (br.s, 20-H_2), 5.26 (1H, dd, J 4.5 and 4.5 Hz). The ^{13}C NMR showed the presence of 1 -CH_2O- (δ 68.9), 2 secondary carbinyl carbons (δ 68.1 and 74.5), 1 acetalic carbon (δ 95.0), an exo-methylene [δ 118.2 (t) and 151.7 (s)] and 2 carbonyl carbons (δ 169.6 and 208.4). These data suggest that the basic skeleton of this compound has structure (7). This structure is supported from the fact that the dihydro-compound (10) showed a negative Cotton effect [λ_{max} (MeOH) nm (φ): 316 (-4730), 280 (+511)]in the ORD. The location of the remaining two oxygen functional groups, -OH and -OAc, were elucidated to be located at C-6β and C-11α, respectively, by INDOR, nuclear magnetic double resonance and NOE experiments. On monitoring 6α-H (δ 3.91), an INDOR signal due to coupling was observed on the signal at δ 1.25 (d, J 8 Hz, 5-H) and a signal due to NOE was also observed for a Me group at δ 1.12. On the other hand, on irradiation at δ 1.25 the double doublet at δ 3.91 changed to a doublet (J 12 Hz) and on irradiation at δ 1.12 an NOE (13%) for the signal appeared. When monitored from 11β-H (δ 5.26), INDOR signals arising from coupling were observed on 9-H (δ 1.62, d, J 4.5 Hz) and 12β-H (δ 1.71-1.88). On irradiation at δ 5.26, the signal pattern of 12β-H was deformed and the doublet of 9-H collapsed to a singlet. The results suggest that the proton

is 11β-H which has a dihedral angle of ca 90° to 12α-H and can couple with 9-H and 12β-H. Periodic acid oxidation of (5) gave an aldehyde (12)[δ 4.45, 4.77 (each 1H, each AB doublets, J 10 Hz, 20-H_2), 9.84 (1H, d, J 4 Hz, CHO)]. Accordingly, longikaurin E was assigned structure (5).

Longikaurin F (6), $C_{24}H_{32}O_8$, mp 249-251°C, $[\alpha]_D^{25}$ -120.4° (c 0.11, C_5H_5N) showed the following spectral data: λ_{max} (MeOH) 237 nm (ε 8716); ν_{max} 3500-3100, 1730, 1720, 1645, 1250 cm^{-1}; ^1H NMR δ 1.24 (s, tert. Me), 2.04, 2.10 (2 x s, 2 x OAc), 3.76-4.18 (4H, 6-H, 20-H_2, 19-H_1), 4.46 (ABd, J 12 Hz, 19-H_1), 5.24 (dd, J 4 and 4 Hz, 11-H). The ^1H NMR spectrum is very similar to that of longikaurin E (5) except for the presence of signals due to one tert. Me and one acetoxymethyl groups instead of two tert. Me groups. In an INDOR experiment, compound (6) showed almost the same coupling pattern as that of (5), except for the above mentioned differences. A 19-acetoxy group on the skeleton of longikaurin F is suggested by analogy with congeners. Consequently, the structure of longikaurin F could be represented as (6).



(1): R^1=R^2=H; R^3=OH

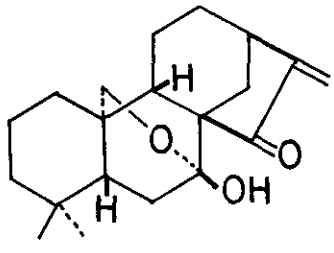
(2): R^1=H; R^2=OAc; R^3=OH

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

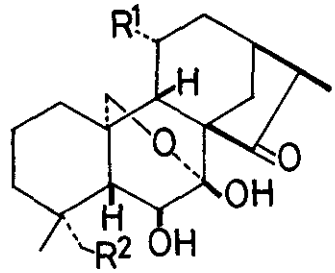
(4): R^1=OH; R^2=OAc; R^3=H

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

(6): R^1=R^2=OAc; R^3=H



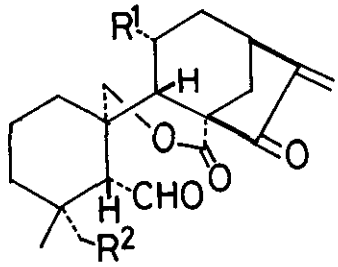
(7)



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

(9): R^1=OH; R^2=OAc

(10): R^1=OAc; R^2=H



(11): R^1=H; R^2=OAc

(12): R^1=OAc; R^2=H

NOTES AND REFERENCES

1) H. Hara, Japan J. Bot., 1972, 47, 193.

2) T. Fujita, Y. Takeda and T. Shingu, Chem. Comm., 1980, 205.

3) The minimal inhibitory concentrations(m.i.c.) of longikaurin C (3), D (4), E (5) and F (6) against *Bacillus subutilis* ATCC 6633 are 15.6, 62.5, 62.5 and 62.5 μg ml^{-1},respectively. All the diterpenoids tested showed m.i.c. of > 1000 μg ml^{-1} against *Escherichia coli* NIHJ. The detailed study will be published elsewhere.

4) IR spectra were recorded for nujol mull.

5) Unless otherwise noted, ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution, using tetramethylsilane as internal standard.

6) O. Sciacovelli, W. von Philipborn, C. Amith and D. Ginsburg, Tetrahedron, 1970, 26, 4589.

7) T. Kikuchi, M. Niwa, T. Yokoi and T. Shingu, Abstract papers of 17th Symposium on the Chemistry of Natural Products (Tokyo), 1973, p. 140.

8) E. Fujita, M. Taoka, Y. Nagao and T. Fujita, J. Chem. Soc. Perkin I, 1973, 1760.

9) This compound has recently been isolated independently from *Rabdosia shikokiana* (Makino) Hara var. *shikokiana*: M. Ochi, M. Okamura, H. Ozuki, I. Miura, I. Kubo and T. Kubota, Koen Yoshishu-Koryo, Terupen oyobi Seiyu Kagaku ni kansuru Toronkai,24th, 1980, p.229.

Received, 13th October, 1980