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Terpenoids. XX.[1] The Structure and Absolute Configuration of Lasiokaurin and Lasiodonin, New Diterpenoids from *Isodon lasiocarpus* (Hayata) Kudo

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On the basis of chemical and spectroscopic evidence, the structure and absolute configuration of lasiokaurin and lasiodonin, which were isolated from *Isodon lasiocarpus* (Hayata) Kudo, were established as *ent*-7β,20-epoxy-1β-acetoxy-15-oxo-16-kaurene-6α,7α,14α-triol(II) and *ent*-7β,20-epoxy-15-oxo-16-kaurene-1β,6α,7α,11α-tetraol(VIII), respectively.

We have isolated several kaurene-type[3] and B-secokaurene type[4] diterpenoids from *Isodon trichocarpus* Kudo and *I. japonicus* Hara, and elucidated their structures.

This paper deals with the investigation of the diterpenoid constituents in the ethereal extract from *Isodon lasiocarpus* (Hayata) Kudo collected in Taiwan.

We isolated three neutral diterpenoids, one of which was proved to be identical with the known oridonin (I).[3b] The remaining two were new compounds. The one was the major constituent and named lasiokaurin, while the other minor constituent was named lasiodonin.

Lasiokaurin, $C_{22}H_{30}O_7$, mp 228—229°, $[\alpha]_D^{17}$ −94°, was shown to be a monoacetate and its nuclear magnetic resonance (NMR) spectrum was very similar to that of oridonin. The diterpenoid on acetylation with acetic anhydride and pyridine gave a diacetate (III), which was identical with the known oridonin-1,14-diacetate.[3b] Thus, lasiokaurin was shown to be oridonin-1-acetate[3b] or oridonin-14-acetate.[3b] Finally, lasiokaurin was proved to be identical with oridonin-1-acetate (II) by its comparison with an authentic sample which was prepared by a partial hydrolysis of oridonin diacetate (III) by oxalic acid solution.

Lasiodonin, mp 252—254.5° (decomp.), $[\alpha]_D^{17}$ −100°, was proved to have the molecular formula, $C_{20}H_{28}O_6$. It was shown to have a ketone conjugated with an exomethylene group from the ultraviolet (UV) [λ_{max}^{MeOH} 241 mμ (ε=5600)], the infrared (IR) (ν_{max}^{KBr} 1705, 1640 cm^{-1}), and the NMR spectra [δ 5.93, 5.42 ppm (each 1H, br. s)]. The presence of three secondary hydroxy groups was also suggested by its IR (ν_{max}^{KBr} 3330 cm^{-1}) and NMR spectra [δ 4.19 (1H, t, J=6.5, Ha), 4.25 (1H, d, J=6, Hb), and 4.66 ppm (1H, m, Hc)]. Moreover, an AB type signal at δ 4.61 and 4.40 ppm with the coupling constant of 10 Hz in the NMR spectrum suggested the presence of an ether-type methylene. Thus, the five oxygens were characterized. In addition to these functional groups, the presence of two tertiary methyl groups was supported by the NMR spectrum [δ 1.16, 1.06 ppm (each 3H, s)].

The foregoing spectral data led to an assumption that lasiodonin has a B-secokaurene or a kaurene type frame. Since it contains neither a δ-lactone nor a five-membered ring hemiacetal, which are usually found in the B-secokaurene-type diterpenoids, it possibly

1) Part XIX: E. Fujita, T. Fujita, and Y. Nagao, *Tetrahedron*, **28**, 555 (1972).
2) Location: *Uji, Kyoto-Fu 611, Japan.*
3) *a*) E. Fujita, T. Fujita, M. Shibuya, and, T. Shingu, *Tetrahedron*, **25**, 2517 (1969); *b*) E. Fujita, T. Fujita, H. Katayama, M. Shibuya, and T. Shingu, *J. Chem. Soc.* (*C*), **1970**, 1674; *c*) E. Fujita, T. Fujita, M. Taoka, H. Katayama, and M. Shibuya, *Tetrahedron Letters*, **1970**, 421.
4) *a*) E. Fujita, T. Fujita, and M. Shibuya, *Yakugaku Zasshi*, **87**, 1076 (1967); *b*) *idem*, *Chem. Pharm. Bull.* (Tokyo), **16**, 509, 1573 (1968); *c*) See also 3*c*.

belongs to the kaurene group. Thus, the remaining unsolved oxygen is most probably present as a 7-hemiketal tertiary hydroxy group, just as in the known kaurene-type diterpenoids isolated from *Isodon* species. Subsequently, on the assumed frame IV, the foregoing functional groups were reasonably settled as follows.

The α,β-unsaturated ketone was put on C-15. A secondary hydroxy group was settled on C-6 with the β-configuration, because the chemical shift, the coupling pattern, and the coupling constant of Hb reasonably explained the α-hydrogen on C-6. The second secondary hydroxy group was supposed to be present at C-1 or C-3 with α-configuration, considering from the NMR data of Ha. All of the kaurene- and B-secokaurene-type diterpenoids isolated so far, however, have been shown to have the O-function at C-1. The third secondary hydroxy group was assumed to be present at C-11 or C-12, because the NMR data only allowed the assignment of Hc to C-11-H or C-12-H. But the C-11 prefers to C-12, because no diterpenoids having the O-function at C-12 have been isolated from *Isodon* species, while some diterpenoids oxygenated at C-11 have been found. These assumption was proved to be correct by the following experiments.

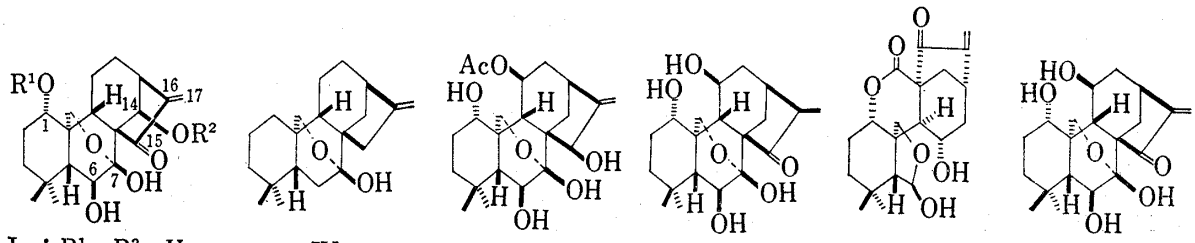

Chart 1

Dihydro-derivative of lasiodonin, prepared by catalytic hydrogenation, was found to be identical with a product VI formed by a treatment of sodoponin (V)[3c] with 15% hydrochloric acid. Moreover, lasiodonin was treated with periodate to yield epinodosin (VII)[3c,5] expectedly.

The foregoing facts established that lasiokaurin was *ent*-7β,20-epoxy-1β-acetoxy-15-oxo-16-kaurene-6α,7α,14α-triol (II) and lasiodonin was *ent*-7β,20-epoxy-15-oxo-16-kaurene-1β,6α,7α,11α-tetraol (VIII).

Experimental

Melting points were determined on a Yanagimoto micro m.p. apparatus and uncorrected. The UV spectra were taken on a Hitachi model EPS-3 recording Spectrophotometer, IR spectra on a Hitachi model EPI-S2 Spectrophotometer, NMR spectra on a Varian A-60 Spectrometer, using TMS as internal standard, and mass spectra on a JEOL model JMS-OISG Mass Spectrometer. Specific rotations were measured by Jasco DIP-SL-type Automatic Polarimeter. Mallinckrodt Silicic acid was used for column chromatography, and Nakarai Silica Layer G for thin-layer chromatography.

Extraction of the Plant Material and Isolation of the Diterpenoids——The dried leaves and stems of *Isodon lasiocarpus* (HAYATA) KUDO (3 kg) were soaked in ether (10 liter) and allowed to stand for 2 months at room temperature. The ethereal extract was concentrated to a syrup, to which MeOH (30 liter) was added. The methanolic solution was refluxed for 2 hr under the addition of charcoal (100 g) and filtered. Concentration of the solution to 0.5 liter led to precipitation of the crude crystalline lasiokaurin (7.6 g). Further concentration additionally yielded the crude lasiokaurin (4.4 g). The mother liquor was completely evaporated to remove the solvent. The residue was extracted with EtOAc. The extract was washed with 10% Na_2CO_3[6] and then water and dried (Na_2SO_4). Evaporation of the solvent left a syrupy residue (26 g), which was chromatographed on silica gel (1 kg) column with elution by CH_2Cl_2–acetone (8:2) to separate lasiokaurin (1.5 g), lasiodonin (81 mg), and oridonin (402 mg).

5) T. Kubota and I. Kubo, *Chem. Comm.*, **1968**, 763.
6) The investigation of the acidic diterpenoids will be discussed in the future.

Oridonin(I)——Recrystallization of the crude material from MeOH gave colorless needles, mp 248—250°, $[\alpha]_D^{17}$ −46° (c=1, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3400, 3200, 1705, 1645. NMR (D_5-pyridine) δ: 1.13 (3H, s), 1.20 (3H, s), 3.65 (1H, t, J=8, C-1-H), 4.29 (1H, q, J=7, 10, C-6-H), 4.42, 4.78 (each 1H, AB type, J=10 Hz, C-20 H_2), 5.35 (1H, s, C-14-H), 5.86 (1H, br s, OH), 5.53, 6.31 (each 1H, s, C-17 H_2). The substance was proved to be identical with an authentic sample of oridonin by mixture mp determination and comparison of their IR and NMR spectra.

Lasiokaurin(II)——Recrystallization of the crude material from MeOH afforded colorless prisms, mp 228—229°, $[\alpha]_D^{17}$ −94° (c=1, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3350, 1725, 1710, 1640. UV λ_{max}^{MeOH} mμ: 238.5 (ε=8000). NMR ($CDCl_3$) δ: 1.14 (6H, s), 1.98 (3H, s, OCOCH$_3$), 3.81 (1H, q, J=7, 11, C-6-H), 4.22 (2H s, C-20 H_2), 4.62 (1H, m, C-1-H), 4.86 (1H, d, J=1, C-14-H), 5.55, 6.18 (each 1H, s, C-17 H_2), 6.45 (1H, d, J=11 Hz, C-6-OH). Mass Spectrum m/e: 406 (M^+), 388 (M^+-H_2O), 346 (M^+—AcOH). Calcd. for $C_{22}H_{30}O_7$: M 406. The substance was proved to be identical with an authentic sample of oridonin-1-acetate, $[\alpha]_D^{27}$ −97° (c=0.02, pyridine), by mixture mp determination and comparison of their spectrosopic data.

Acetylation of Lasiokaurin——A mixture of lasiokaurin (64 mg) and Ac_2O–pyridine (1:1) (2 ml) was allowed to stand at room temperature overnight. After addition of EtOH for decomposing the excess Ac_2O, the solvent was distilled off under the reduced pressure to give a residue (80 mg), which was column-chromatographed on silica gel with elution by CH_2Cl_2 to afford an oily diacetate (62 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3370, 1740, 1721, 1645. NMR ($CDCl_3$) δ: 1.13 (6H, s), 2.02, 2.08 (each 3H, s, 2×CH$_3$COO), 3.82 [1H, q, J=6, 10, it changes to a doublet (J=6) on addition of D_2O, C-6-H], 4.28 (2H, s, C-20 H_2), 4.63 (1H, t, J=5, C-1-H), 5.55, 6.19 (each 1H, s, C-17 CH$_2$), 5.85 (1H, s, C-14-H), 6.19 (1H, d, J=10 Hz, OH). The substance was proved to be identical with oridonin-1,14-diacetate by the comparison of their IR and NMR spectra.

Lasiodonin(VIII)——Recrystallization of the crude diterpenoid from MeOH gave colorless triangular crystals, mp 252—254.5° (decomp.), $[\alpha]_D^{17}$ −100° (c=1, pyridine). UV λ_{max}^{MeOH} 241 mμ (ε=5600). IR ν_{max}^{KBr} cm^{-1}: 3330, 1705, 1640. NMR (D_5-pyridine-D_2O) δ: 1.16, 1.06 (each 3H, s), 4.19 (1H, t, J=6.5, C-1-H), 4.25 (1H, d, J=6, C-6-H), 4.66 (1H, m, C-11-H), 4.40, 4.61 (each 1H, AB type, J=10 Hz, C-20 H_2), 5.42, 5.93 (each 1H, br s, C-17 H_2). Mass Spectrum m/e: 364. 189 (M^+) ($C_{20}H_{28}O_6$ requires: 364.188). *Anal.* Calcd. for $C_{20}H_{28}O_6 \cdot CH_3OH$: 63.61; H, 8.14. Found: C, 64.01; H, 8.14. This compound is photo-sensitive and easily colored yellow on standing.

Sodium Periodate Oxidation of Lasiodonin——To a solution of lasiodonin (10 mg) in MeOH (5 ml), $NaIO_4$ (40 mg) in water (0.5 ml) was added, and the mixture was stirred for 5 days at room temperature. Methanol was distilled off and the residue was dissolved in EtOAc. The solution, after washing with water and drying (Na_2SO_4), was evaporated *in vacuo* to give a crystalline residue (6 mg), which was purified by passing through a silica gel column by elution with CH_2Cl_2–acetone (9:1) to yield a purified compound (3 mg), mp 246—249°. IR ν_{max}^{KBr} cm^{-1}: 3250, 1750, 1715, 1640. This compound was proved to be identical with an authentic sample of epinodosin by mixture mp determination and the comparison of their IR spectra.

Catalytic Hydrogenation of Lasiodonin——Lasiodonin (7 mg) in MeOH (3 ml) was subjected to hydrogenation on PtO_2 (5 mg) overnight. After confirmation of no absorption at 241 mμ in UV spectrum the catalyst was filtered off, and the filtrate was evaporated to dryness. The residue (10 mg) was eluted with CH_2Cl_2: acetone (8:2) on a silica gel column to give dihydrolasiodonin (5 mg), whose IR spectrum was compeltely identical with that of VI derived from sodoponin. IR ν_{max}^{KBr} cm^{-1}: 3320, 1715.

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