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BITTER DITERPENOIDS FROM *ISODON SHIKOKIANUS* VAR. *INTERMEDIUS*

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Key Word Index—*Isodon shikokianus* var. *intermedius*; Labiatae; diterpenoids; *ent*-kaurenoids; shikodokaurin A; shikodokaurin B.

Abstract—Four new diterpenoids were isolated from the aerial parts of *Isodon shikokianus* var. *intermedius*. The structures of two of them, shikodokaurin A and B, were established.

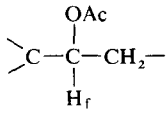
INTRODUCTION

A number of diterpenoids having *ent*-kaurene and B-seco-*ent*-kaurene skeletons have been isolated from *Isodon* species (Labiatae) [1]. From *Isodon shikokianus* var. *intermedius*, isodomedin (**1**) has been isolated [2, 3]. In the course of our chemical examination of the same plant, we have found four new, bitter diterpenoids. In this paper, we report studies on two of these substances and the establishment of their structures as **2** and **3**.

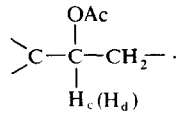
RESULTS AND DISCUSSION

The two new diterpenoids were obtained from the methanolic extract of the aerial parts of *Isodon shikokianus* var. *intermedius*. Shikodokaurin A (**2**) had a UV_{max} at 232 nm (ε = 8500, MeOH), bands at 1730, 1645 cm^{-1} in the IR spectrum, singlets at δ 5.40 and 6.14 ppm (each one proton) in the PMR spectrum, and the singnals at δ 147.0 (*s*), 118.3 (*t*) and 204.9 (*s*) ppm in the ^{13}C NMR spectrum. These data show the presence of a five-membered ring ketone conjugated with an exocyclic methylene. The PMR spectrum of **2** also shows singlets at δ 0.90, 0.94 and 1.30 ppm due to three tertiary methyl groups, singlets at δ 1.98, 2.02 and 2.12 ppm arising from three acetyl groups, a singlet at δ 3.98 ppm assignable to a hydroxy group (disappeared on adding D_2O) and the signals attributable to four protons attached to oxygenated carbons: δ 4.72 (*t*, J = 3 Hz, H_f), 4.86 (*br s*, H_e), 4.80–4.92 (H_d), 5.40 (*dd*, J = 12 and 4 Hz, H_c) ppm. All the oxygen functional groups in **2** are thus accounted for. From this, it can be assumed that **2** is a tetracyclic *ent*-kaur-16-en-15-one, and similar to other diterpenoids isolated from *Isodon* species. The absolute configuration of the C/D ring is supported by the fact that the dihydro compound (**4**) shows a negative Cotton effect in ORD.

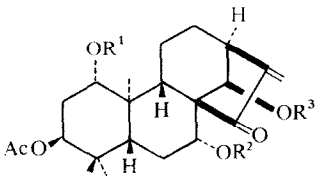
Acetylation of **2** gave the monoacetate (**5**), $C_{28}H_{38}O_9$, mp 207–208°. In the PMR spectrum, the signal of H_e suffered a downfield shift to δ 6.00 ppm (*br s*), while the other three protons [H_c, H_d δ 4.86 ppm (*dd*, J = 8 and 6 Hz), H_f] remain in the original position. Thus these three protons are attached to carbons bearing an acetoxy group. These coupling constants suggest that H_f is an equatorial proton in the partial structure,



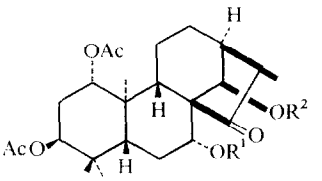
and H_c and H_d are axial protons in the partial structure,



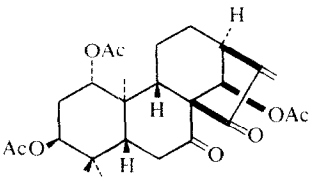
The appearance of the signal due to H_e proton, abnormally downfield in an acetate, suggests that this proton can be assigned to the C-14α-proton, which has suffered an anisotropic effect from the 15-carbonyl group and has an angle of *ca* 90° to the C-13 proton [4]. Then, the structure around the conjugated α-methylene five-membered ketone and the positions of oxygen functional groups were examined by the internuclear double resonance (INDOR) [5] spectra. On monitoring H_a or H_b, INDOR signals (probably due to coupling and NOE) were observed at H_b and H_g, or H_a and H_g, respectively. This result establishes the structure of the D-ring. Respective monitoring at H_d and H_f made it clear that both protons couple with the same methylene group (δ 1.73–1.96 ppm). When H_f was monitored, NOE's were also observed on both methyl groups at C-4 (δ 0.90 and 0.94 ppm). The results suggest that H_d is an axial proton at C-1 and H_f is an equatorial proton at C-3. On monitoring H_e, an INDOR signal was observed at H_g and a NOE was observed at the methyl group at C-10 (δ 1.30 ppm). From these results, it was established that a β-hydroxy group is at C-14 and the methyl group at C-10 is α-oriented. Finally, the signal of H_e appears downfield from those of protons [H_d and H_f] at C-1 and C-3. This acetoxy group is at C-7α position, because the proton at C-7β position suffered downfield shift due to the 15-carbonyl group. Thus, the structure of shikodokaurin A must be *ent*-kaur-16-en-15-on-1β,3α,7β,14α-tetraol 1,3,7-triacetate (**2**). Indeed, the physical data of the monoacetate (**5**) agree with those of isodomedin triacetate [2].



1 $R^1 = R^2 = R^3 = H$
3 $R^1 = R^3 = Ac$; $R^2 = H$
5 $R^1 = R^2 = R^3 = Ac$
6 $R^1 = R^3 = Ac$; $R^2 = COPh$
7 $R^1 = R^2 = Ac$; $R^3 = COPh$



4 $R^1 = Ac$; $R^2 = H$
8 $R^1 = H$; $R^2 = Ac$



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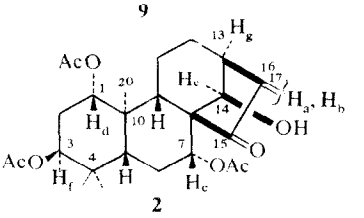


2

Shikodokaurin B (**3**) was obtained as an amorphous powder. Since this substance gradually changed to shikodokaurin A (**2**) during further purification, it was finally purified as its benzoate (**6**), which is clearly different from the benzoate (**7**) derived from **2**. Acetylation of **3** gave the monoacetate (**5**) of **2**. Therefore, **3** has the same oxygenation pattern as **2**. Further, catalytic hydrogenation of shikodokaurin A (**2**) produced dihydroshikodokaurin A (**4**) and dihydroshikodokaurin B (**8**), the latter showing a negative Cotton effect in ORD as in the case of **4**. The PMR spectrum of **3** showed two singlets at δ 1.96 and 2.08 ppm due to three acetyl groups and is very similar to that of **2** except for the signals at δ 4.12 (*dd*) and 5.98 (*br s*) ppm in **3** which correspond to the signals at δ 5.40 (H_c, *dd*, 7-H) and 4.86 (*br s*, 14α-H) ppm in **2**, respectively. Thus, the structure of shikodokaurin B must be *ent*-kaur-16-en-15-on-1β,3α,7β,14α-tetraol 1,3,14-triacetate (**3**). Treatment of **3** with Jones' reagent furnished dehydroshikodokaurin B (**9**), $C_{26}H_{34}O_8$. In the PMR spectrum of **9**, a NOE (11 %) was observed on a doublet at δ 5.74 ppm (J = 2 Hz, 14α-H) on irradiation at a methyl group (C-20) at δ 1.46 ppm. This supports the above structural assignment.

EXPERIMENTAL

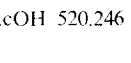
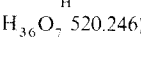
General procedures. All mps were uncorr. PMR: 100 MHz or 90 MHz, ^{13}C NMR: 22.6 MHz. All the NMR spectra were taken for $CDCl_3$ solns. Chemical shifts are given in δ(ppm) with TMS as the internal standard. All $[\alpha]_D$'s were calculated from ORD curves. MS: 40 eV unless otherwise noted. Si gel 60 F_{254} precoated plates (Merck, 0.5 mm in thickness) were used for preparative TLC unless otherwise noted.

Plant material. Plants were collected on Mt. Koetsu (in Tokushima Pref., Japan) in July, 1977 and identified as *Isodon shikokianus* (Makino) Hara var. *intermedius* (Kudo) Murata by Mr. G. Murata of Faculty of Science, Kyoto University. Voucher specimen (T. Fujita, No. 4) was deposited in the herbarium of Institute of Botany, Kyoto University (KYO), Kitashirakawaoiwake-cho, Sakyo-ku, Kyoto, 606, Japan.

Isolation procedures. Dried aerial parts (2.1 kg) were extracted with MeOH (58 l.) at room temp. for a month. The plant material was further extracted twice with the same quantities of MeOH at room temp. for 5 days. The MeOH extracts were combined and evapd *in vacuo*. The residue was dissolved in 90 % MeOH (1.5 l) and the soln was rinsed ×3 with *n*-hexane (total 2 l.). The *n*-hexane layer was washed with 90 % MeOH (2 × 0.5 l.). The 90 % MeOH layer was combined and concd *in vacuo* and the residue was suspended in H_2O (1 l.) and extracted with EtOAc (3 × 1 l.). After washing with H_2O (2 × 0.3 l.), the EtOAc extract was dried and evapd to give a residue (91.5 g). A portion (90 g) of the residue was chromatographed on a Si gel (2 kg) column with $CHCl_3$–Me_2CO with increasing Me_2CO content. The fractions eluted with $CHCl_3$–Me_2CO (19:1) were combined, evapd, treated with charcoal in MeOH and concd *in vacuo* to give a residue (2.88 g) which was chromatographed on a Si gel (150 g) column with Et_2O as eluent. The faster eluates gave shikokiamedin (477 mg) as an amorphous powder. The slower eluates gave a residue (522 mg) which was subjected to preparative TLC on Si gel plates (Si gel 60 PF_{254}, 0.75 mm in thickness) in Et_2O. The band with higher R_f gave somewhat crude shikodokaurin B (**3**) (143 mg). The band with lower R_f gave a residue which was recrystallized from EtOH to give shikodokaurin A (**2**) (44 mg) as needles, mp 232–234°. The eluates with $CHCl_3$–Me_2CO (9:1) gave crude shikodomedin which was recrystallized from a mixture of $CHCl_3$ and Et_2O to give pure shikodomedin (8.99 g) as needles, mp 193–194°.

The physical properties of the isolated substances are as follows:

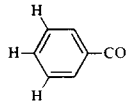
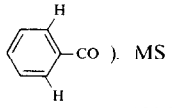
Shikodokaurin A (**2**): IR ν_{max}^{Nujol} cm^{-1}: 3600, 1730, 1645, 1250; PMR δ 0.90, 0.94, 1.30 (3 × *s*, 3 × tert. Me), 1.98, 2.02, 2.12 (3 × *s*, 3 × OAc), 3.10 (*m*, 13-H), 3.98 (*s*, OH), 4.72 (*t*, J = 3 Hz, 3-H), 4.86 (*br s*, 14-H), 4.80–4.92 (1-H), 5.40 (*dd*, J = 12 and 4 Hz, 7-H), 5.40, 6.14 (2 × *br s*, 17-H_2). MS (75 eV): *m/e* M^+ 476, M^+-AcOH 416.221 (calcd. for $C_{24}H_{32}O_6$ 416.220) (Found C, 65.36; H, 7.93. $C_{26}H_{36}O_8$ requires: C, 65.53; H, 7.61 %).

Shikodokaurin B (**3**): PMR: δ 0.88, 0.96, 1.36 (3 × *s*, 3 × tert. Me), 1.96, 2.08 (2 × *s*, 3 × OAc), 3.08 (*m*, 13-H), 4.12 (*dd*, J = 10 and 6 Hz, 7-H), 4.64–4.90 (2H, 1-H and 3-H), 5.98 (*br, s*, 14-H), 5.36, 6.12 (2 × *br, s*, 17-H_2). Attempts to purify **3** failed, because **3** gradually changed to shikodokaurin A (**2**). Accordingly, **3** was converted to a benzoate and purified. Namely, crude **3** (contained a trace of **2**, 37.4 mg) was benzoylated with PhCOCl–Py in the usual way. The product was purified on a Si gel plate (Et_2O) to give mono-benzoate (**6**) (10.5 mg). Shikodokaurin B benzoate (**6**), $[\alpha]_D^{21}$ +22.3° ($CHCl_3$, *c* 0.53); UV λ_{max}^{MeOH} nm (ε): 228 (19 220), 273 (1955); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1645, 1600; PMR: δ 0.93, 0.96, 1.48 (3 × *s*, 3 × tert. Me), 1.96, 1.99, 2.12 (3 × *s*, 3 × OAc), 3.10 (*m*, 13-H), 4.64–4.96 (2H, 1-H and 3-H), 5.25–5.44 (1H, 7-H), 5.38 (*s*, 17-H_1), 6.12 (2H, *s*, 14-H and 17-H_1), 7.32–7.64 (3H, ), 7.80–8.16 (2H, ); MS: *m/e* M^+ – AcOH 520.246 (calcd. for $C_{31}H_{36}O_7$ 520.246).

Catalytic hydrogenation of shikodokaurin A (**2**). Shikodokaurin A (**2**) (24.5 mg) was dissolved in MeOH (3 ml) and hydrogenated over PtO_2 (2.2 mg) for 20 min. The catalyst was filtered off and the solvent removed *in vacuo* to give a residue which was

chromatographed on a Si gel plate (Et_2O). The band showing lower R_f gave dihydroshikodokaurin A (**4**) (6.3 mg), $[\alpha]_D^{21}$ +4.76° (MeOH, *c* 0.21); ORD λ_{max}^{MeOH} nm ([Φ]): 319 (−2071), 287 (+2367) (*c* 0.21): IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1735; PMR: δ 0.88, 0.94 (2 × *s*, 2 × tert. Me), 1.09 (*d*, *J* = 8 Hz, 16-Me), 128 (*s*, tert. Me), 1.98, 2.02. 2.12 (3 × *s*, 3 × OAc), 2.50 (*m*, 13-H), 2.84 (*t*, *J* = 8 Hz, 16-H), 3.88 (*m*, OH), 4.66–4.96 (3H, 1-H, 3-H and 14-H), 5.26 (*dd*, *J* = 12 and 6 Hz, 7-H); MS: *m/e* M^+ 478; $M^+ - H_2O$ 460.244 (calcd. for $C_{26}H_{36}O_7$ 460.246); M^+ −AcOH 418.239 (calcd. for $C_{24}H_{34}O_6$ 418.236). The band showing higher R_f gave dihydroshikodokaurin B (**8**) (6.6 mg), $[\alpha]_D^{21}$ + 8.7° (MeOH, *c* 0.35): ORD λ_{max}^{MeOH} nm ([Φ]): 319 (−1515), 287 (+1791) (*c* 0.35); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 1730; PMR: δ 0.86, 0.96 (2 × *s*, 2 × tert. Me), 1.12 (*d*, *J* = 8 Hz, 16-Me), 1.38 (3H, *s*, tert. Me), 1.98, 2.04, 2.08 (3 × *s*, 3 × OAc), 2.52 (*m*, 13-H), 2.72 (*t*, *J* = 8 Hz, 16-H), 4.00 (*dd*, *J* = 16 and 8 Hz, 7-H), 4.70 (*t*, *J* = 4 Hz, 3-H), 4.80 (*t*, *J* = 8 Hz, 1-H), 6.06 (*s*, 14-H); MS: *m/e* M^+ 478.253 (calcd. for $C_{26}H_{38}O_8$ 478.257); $M^+ - H_2O$ 460.244 (calcd. for $C_{26}H_{36}O_7$ 460.246): M^+ −AcOH 418.239 (calcd. for $C_{24}H_{34}O_6$ 418.236).

Acetylation of shikodokaurin A (**2**). Shikodokaurin A (**2**) (29.4 mg) was acetylated with Ac_2O–Py and the product (30 mg) was recrystallized from EtOH to give **5** as needles, mp 207–208°, $[\alpha]_D^{21}$ + 17.2° ($CHCl_3$, *c* 0.32); UV λ_{max}^{MeOH} nm (ε): 232 (7938); IR ν_{max}^{Nujol} cm^{-1}: 1735, 1640, 1250; PMR: δ 0.86, 0.96, 1.44 (3 × *s*, 3 × tert. Me), 1.92, 1.96, 2.04, 2.10 (4 × *s*, 4 × OAc), 3.02 (*m*, 13-H), 4.64 (*t*, *J* = 4 Hz, 3-H), 4.86 (*dd*, *J* = 8 and 6 Hz, 1-H), 5.22 (*dd*, *J* = 12 and 6 Hz, 7-H), 5.34 (*s*, 17-H_1), 6.00 (*s*, 14-H), 6.08 (*s*, 17-H_1); MS: *m/e* M^+ −AcOH 458.230 (calcd. for $C_{26}H_{34}O_7$).

Benzoylation of shikodokaurin A (**2**). Shikodokaurin A (**2**) (17.2 mg)was benzoylated with PhCOCl–Py and the product was purified on a Si gel plate (Et_2O) to give monobenzoate (**7**) (10.3 mg). $[\alpha]_D^{21} \simeq 0°$ ($CHCl_3$, *c* 0.65); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1640, 1600; PMR: δ 0.86, 0.98 (2 × *s*, 2 × tert. Me), 1.22 (*s*, 1 × OAc), 1.52 (3H, *s*, tert. Me), 2.00, 2.10 (2 × *s*, 2 × OAc), 3.20 (*m*, 13-H), 4.72 (*t*, *J* = 4 Hz, 3-H), 4.82 (*t*, *J* = 8 Hz, 1-H), 5.22 (*dd*, *J* = 10 and 4 Hz, 7-H), 5.44 (*s*, 17-H_1), 6.20, 6.28 (2 × *s*, 14-H and/or 17-H_1), 7.36–7.72 (3H, ), 7.92–8.24 (2H, ). MS (75 eV): *m/e* M^+ − AcOH 520.246 (calcd, for $C_{31}H_{36}O_7$ 520.246).

Acetylation of shikodokaurin B (**3**). Shikodokaurin B (**3**) (30 mg) was acetylated with Ac_2O–Py and the product was purified on a Si gel plate (Et_2O) to give monoacetate (**5**) (25 mg) which recrystallized from EtOH as needles, mp 209–210°, $[\alpha]_D^{21}$ + 13.3° ($CHCl_3$, *c* 0.30). UV λ_{max}^{MeOH} nm (ε): 232 (9284); IR ν_{max}^{Nujol} cm^{-1}: 1735, 1640, 1250; PMR: δ 0.86, 0.96, 1.44 (3 × *s*, 3 × tert. Me), 1.92, 1.96, 2.04, 2.10 (4 × *s*, 4 × OAc), 3.02 (*m*, 13-H), 4.64 (*t*, *J* = 4 Hz, 3-H), 4.86 (*dd*, *J* = 8 and 6 Hz, 1-H), 5.22 (*dd*, *J* = 12 and 6 Hz, 7-H), 5.34 (*s*, 17-H_1), 6.00 (*s*, 14-H), 6.08 (*s*, 17-H_1); MS: *m/e* M^+ −AcOH 458.234 (calcd. for $C_{26}H_{34}O_7$ 458.230). This substance was identified with acetate (**5**) derived from shikodokaurin A (**2**) mmp and comparisons of IR (Nujol) and PMR ($CDCl_3$).

Jones' oxidation of shikodokaurin B (**3**). A soln of **3** (71 mg) in Me_2CO (2 ml) was stirred with Jones' reagent (0.1 ml) under ice cooling for 1.5 hr. The reaction mixture was diluted with H_2O (15 ml), extracted with $CHCl_3$ (15 ml × 2), dried (dry $MgSO_4$) and evapd *in vacuo*. The residue was purified on a Si gel plate (CH_2Cl_2–Me_2CO 9:1) to give dehydroshikodokaurin B (**9**) (49 mg), $[\alpha]_D^{21}$ −17.4° ($CHCl_3$, *c* 0.96); UV λ_{max}^{MeOH} nm (ε): 229 (4876); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1645; PMR: δ 0.84, 0.98. 1.46 (3 × *s*, 3 × tert. Me), 1.98, 2.02, 2.10 (3 × *s*, 3 × OAc), 3.12 (*m*, 13-H), 4.74 (*t*, *J* = 4 Hz, 3-H), 4.90 (*t*, *J* = 8 Hz, 1-H), 5.74 (*d*, *J* = 2 Hz, 14-H), 5.42, 6.16 (2 × *s*, 17-H_2); MS: *m/e* M^+ 474.225 (calcd. for $C_{26}H_{34}O_8$ 474.225); $M^+ - CH_2{=}C{=}O$ 432.212 (calcd. for $C_{24}H_{32}O_7$ 432.215).

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