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Terpenoids. XLVIII.[1] New Diterpenoids from *Rabdosia shikokiana* var. *occidentalis*

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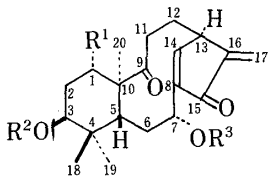
Five new kaurene-type diterpenoids, shikoccin (**1**), *O*-methylshikoccin (**2**), epoxyshikoccin (**3**), *O*-methylepoxyshikoccin (**4**) and shikoccidin (**5**) were isolated from *Rabdosia shikokiana* var. *occidentalis*. All these compounds except for **5** possess a hitherto unknown 8,9-seco-*ent*-kaurene skeleton.

Keywords——*Rabdosia shikokiana*; diterpenoid; shikoccin; shikoccidin; *ent*-kaurene; Labiatae

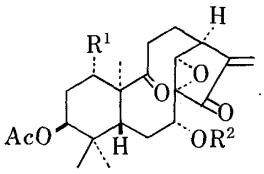
As part of our studies on biologically active constituents of *Rabdosia* plants, we isolated shikoccin (**1**), *O*-methylshikoccin (**2**), epoxyshikoccin (**3**), and *O*-methylepoxyshikoccin (**4**), all of which possess a hitherto unknown 8,9-seco-*ent*-kaurene skeleton, together with shikoccidin (**5**), a new penta-oxygenated *ent*-kaurene diterpenoid, from *Rabdosia shikokiana* (MAKINO) HARA var. *occidentalis* (MURATA) HARA (Labiatae).[2,3] Recently, 8,9-seco-*ent*-kaurene diterpenoids, shikodomedin (**6**), shikokiamedin (**7**), and rabdolatifolin (**8**) were isolated from other *Rabdosia* species.[4,5] Here, we present a full account of the work reported in preliminary communications[2,3]

Repeated column chromatography of the methanolic extracts of *R. shikokiana* var. *occidentalis* collected in Hyogo prefecture resulted in the isolation of seven crystalline compounds. Two of them were identical with the known diterpenoids, isodomedin (**9**)[6] and leukamenin E (**10**).[7] Shikoccin (**1**), $C_{22}H_{30}O_5$, mp 150—152 °C, on acetylation afforded the acetate **11**, whose crystalline structure was determined by X-ray crystallographic analysis.[8] The structure of shikoccidin (**5**), $C_{22}H_{32}O_6$, mp 178—179 °C (from MeOH), was also elucidated by the X-ray method.[2] The circular dichroism curve of dihydroshikoccidin (**12**) showed negative and positive Cotton effects at 325.5 and 293.5 nm, respectively, indicating an *ent*-kaurane skeleton.[9] Thus, the absolute structure of shikoccidin was determined to be **5**. Treatment of shikoccidin (**5**) with oxalic acid in methanol effected the retro-aldol reaction to provide shikoccin (**1**), which confirmed the position of a secondary hydroxyl group and also the absolute stereochemistry of shikoccin.

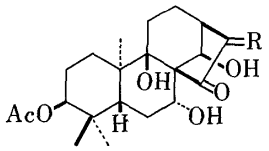
Spectral data (Table I) for *O*-methylshikoccin (**2**), $C_{23}H_{32}O_5$, mp 168—171 °C, indicated that this compound was the methyl ether of shikoccin (**1**). Treatment of shikoccin (**1**) with sulfuric acid–methanol at refluxing temperature afforded *O*-methylshikoccin (**2**) which was identical with the natural product, accompanied with deacetyl-*O*-methylshikoccin **13**, and **14**. The coupling pattern of H-7 in **2** (δ 4.18 ppm, dd, $J=13$, 6 Hz) was similar to that in **1** (δ 4.64 ppm, dd, $J=11$, 5 Hz). This indicates that the methoxyl group at C(7) in *O*-methylshikoccin (**2**) exists in the same orientation as in **1**, since major change in conformation



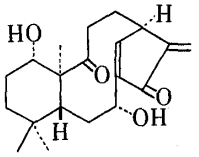
1 : $R^1=R^3=H$, $R^2=Ac$
2 : $R^1=H$, $R^2=Ac$, $R^3=Me$
6 : $R^1=OAc$, $R^2=Ac$, $R^3=H$
11 : $R^1=H$, $R^2=R^3=Ac$
13 : $R^1=R^2=H$, $R^3=Me$



3 : $R^1=R^2=H$
4 : $R^1=H$, $R^2=Me$
7 : $R^1=OAc$, $R^2=H$



5 : $R=CH_2$
12 : $R=\alpha$-H, β-Me



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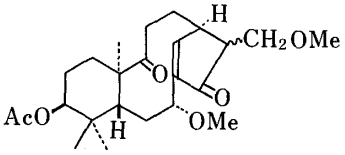

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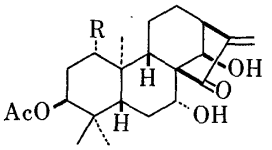

9 : R=OH
10 : R=H

TABLE I. Spectral Data for **1**–**4**, **11**, **13**, and **14**

Compd.	λ_{max}[a] nm (ε)	ν_{max}[b] cm^{-1}	^{1}H-NMR, δ ppm (Hz) H-14	H_2-17	H-7	H-3	H-13	OAc	$-CH_3$	-OMe
1	246	3450, 1725, 1695	7.23	6.13 (s)	4.64	4.73	3.63	2.10	0.98 (s)	
	(7460)	1650, 1620, 1250	(d, 3)	5.43 (s)	(dd, 11, 5)	(brt, 3)	(m)		1.01 (s)×2	
2	244	1725, 1690, 1645	7.17	6.13 (s)	4.17	4.74	3.66	2.11	0.99 (s)	3.18
	(7300)	1615, 1245, 1093	(d, 3)	5.44 (s)	(dd, 13, 6)	(brt, 3)	(m)		1.01 (s)×2	
3	232.5	3530, 1733, 1698	3.73	6.28 (s)	4.57	4.77	3.27	2.14	1.04 (s)×2	
	(6490)	1646, 1255, 1028	(s)	5.53 (d, 1)	(dd, 12, 5)	(brt, 3)	(m)		1.08 (s)	
4	233	1730, 1704, 1648	3.63	6.27 (s)	4.11	4.74	3.34	2.07	0.97 (s)	3.18
	(5110)	1255, 1108	(s)	5.48 (d, 1)	(dd, 12, 5)	(brt, 3)	(m)		1.00 (s)	
									1.08 (s)	
11	243[c]	1730, 1700, 1650	7.20	6.19 (s)	5.50	4.73	3.63	2.02	0.99 (s)	
	(6815)	1620, 1245	(d, 3)	5.45 (s)	(dd, 11, 5)	(brt, 3)	(m)	2.15	1.00 (s)	
									1.10 (s)	
13	244	3450, 1685, 1645	7.20	6.17 (s)	4.20	3.51	3.65		0.97 (s)×2	3.18
	(7740)	1616, 1098	(d, 3)	5.46 (s)	(dd, 11, 5)	(brt, 3)	(m)		1.11 (s)	
14	230	1720, 1695, 1620	7.31	3.44	4.04	4.76	3.32	2.17	0.98 (s)	3.18
	(6370)	1245, 1095	(d, 3)	(t, 10)	(dd, 11, 5)	(brt, 3)	(m)		1.01 (s)×2	3.40
				3.89						
				(dd, 10, 4)						

a) Measured in MeOH. *b*) In $CHCl_3$. *c*) In EtOH.

between **1** and **2** is unlikely.

Comparison of the spectral data of epoxyshikoccin (**3**), $C_{22}H_{30}O_6$, mp 124—126 °C, with those of shikoccin (**1**) indicated that both compounds possess the same basic skeleton. The absence of the infrared (IR) absorption at 1620 cm^{-1} and the remarkable upfield shift of the 14-H signal (from δ 7.23 ppm in **1** to δ 3.73 ppm in **3**) indicated that **3** has the structure corresponding to the 8,14-epoxy derivative of shikoccin (**1**). In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **3**, the signals at δ 148.5 ppm and δ 158.9 ppm in the spectrum of **1** were absent, but new signals at δ 64.5 ppm and δ 60.2 ppm were recognized. This supported the structure of epoxyshikoccin (**3**). α-Configuration of the epoxide ring was deduced, since no coupling between H-13 and H-14 was observed. Epoxidation of shikoccin

TABLE II. ^{13}C-Chemical Shifts of **1**—**4** and **11**

Assignment	Compound 1	2	3	4	11
C-1	30.9[a]	30.8[a]	31.5[a]	31.2[a]	30.9[a]
C-2	25.8[a]	25.8[a]	25.1[a]	25.0[a]	25.9[a]
C-3	76.9	76.8	76.9	77.3	76.7
C-4	38.0	38.0	38.3	37.3	38.0
C-5	37.1	36.7	35.6	35.4	36.7
C-6	27.2[a]	27.3[a]	27.0[a]	27.1[a]	21.7
C-7	64.3	73.5	61.8	72.0	66.9
C-8	148.5	146.2	64.5	64.0	145.6[a]
C-9	214.5	213.7	213.3	213.5	214.5
C-10	53.0	52.9	52.7	52.8	52.9
C-11	35.8	33.4	32.1	31.5	32.1
C-12	22.0[a]	22.0[a]	21.9[a]	21.9[a]	21.9[a]
C-13	42.3	42.3	39.9	38.6	42.4
C-14	158.9	158.8	60.2	59.9	159.2
C-15	194.5	194.8	195.6	197.0	193.5
C-16	145.9	145.7	145.0	145.8	144.9[a]
C-17	116.3	115.8	121.9	121.9	116.7
C-18	28.2	28.3	28.0	28.3	27.9
C-19	20.9	20.8	20.8	21.0	20.9
C-20	16.8	16.8	16.6	16.6	16.8
$\underline{C}H_3CO_2$	22.0	22.0	21.6	21.7	21.0, 21.9
$CH_3\underline{C}O_2$	170.4	169.9	169.9	170.0	169.7, 170.3
CH_3O		56.4		59.1	

a) Tentatively assigned.

(**1**) with *m*-chloroperbenzoic acid (*m*-CPBA) provided the 8,14-epoxide, which was identical with **3**, confirming the structure of epoxyshikoccin (**3**).

The structure of *O*-methylepoxyshikoccin (**4**), $C_{23}H_{32}O_6$, mp 142—144 °C, was easily inferred from its spectral data (Table I) and confirmed by the conversion from *O*-methylshikoccin (**2**) with *m*-CPBA.

Though it cannot be ruled out that **2** and **4** might be artifacts, we believe them to be natural products because no trace of *O*-methylshikoccin (**2**) was obtained from the reaction of **5** with oxalic acid in methanol.

Experimental

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian T-60 NMR spectrometer or a JEOL JMN-FX100 spectrometer in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard. ^{13}C-NMR spectra were obtained with a JEOL JMN-FX100 spectrometer in $CDCl_3$ solution with TMS as an internal standard. Chemical shifts are given in δ (ppm). IR spectra were measured on a EPI-S_2 spectrophotometer. Ultraviolet (UV) spectra were measured on a Hitachi EPS-3 spectrometer. Circular dichroism (CD) spectra were obtained with a JASCO model 6 spectropolarimeter and optical rotations were taken with a JASCO DIP-181 automatic polarimeter. Preparative thin layer chromatography (PTLC) was performed on plates of silica gel (Kieselgel 60 F_{254}, Merck).

Isolation of Diterpenoids——Dried aerial parts (11 kg) of *Rabdosia shikokiana* (MAKINO) HARA var. *occidentalis* (MURATA) HARA collected in Hyogo prefecture were extracted with methanol (130 l×2) at 70—75 °C for 3 h. Methanolic extracts were concentrated under reduced pressure below 50 °C to leave a residue (568 g). A part (100 g) of the residue was treated with active charcoal in ethyl acetate and partitioned with water. The organic layer was dried over Na_2SO_4 and evaporated to leave a residue (42 g), which was chromatographed over silica gel (1.2 kg). Successive elution with $CHCl_3$. $CHCl_3$–acetone (8.5 : 1.5), $CHCl_3$–acetone (7 : 3) gave fractions a (9.3 g), b (2.6 g), c (3.4 g), d (5.2 g), e (4.2 g), f (2.1 g), and g (3.0 g).

Fraction b was chromatographed over silica gel (80 g) with $CHCl_3$ to give crude *O*-methylepoxyshikoccin (**4**),

which was purified by PTLC with $CHCl_3$–acetone (10 : 1) to afford pure **4** (3.5 mg), mp 142—144 °C (from hexane–isopropyl ether), $[\alpha]_D^{25}$ +24.2° (c=0.60, MeOH). *Anal.* Calcd for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 67.98; H, 8.07. UV, IR, and ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. Further elution with $CHCl_3$ afforded *O*-methylshikoccin (**2**) (49 mg), mp 168—171 °C (from hexane–isopropyl ether), $[\alpha]_D^{25}$ −4.5° (c=0.40, MeOH). *Anal.* Calcd for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.39; H, 8.54. UV, IR, and ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Fraction c was chromatographed over silica gel (100 g) with $CHCl_3$ followed by PTLC with $CHCl_3$–acetone (8 : 2) to give epoxyshikoccin (**3**) (91 mg), mp 124—126 °C (from ethyl acetate–isopropyl ether). $[\alpha]_D^{25}$ −6.3° (c=0.35, MeOH). *Anal.* Calcd for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 67.38; H, 7.58. UV, IR, and ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Column chromatography of fraction d over silica gel (150 g) with $CHCl_3$ and subsequent PTLC with $CHCl_3$–acetone (9 : 1) afforded leukamenin E (**10**)[7] (38 mg), mp 200—204 °C (from ethyl acetate), $[\alpha]_D^{25}$ −53° (c=0.155, MeOH).

Crude shikoccin (**1**) (3.46 g) was obtained from fraction e after column chromatography over silica gel (150 g) with $CHCl_3$. Fraction f was chromatographed over silica gel (75 g). Elution with $CHCl_3$ afforded crude shikoccin (**1**) (1.18 g). The combined crude material was recrystallized from ethyl acetate–isopropyl ether to yield pure shikoccin (**1**) (3.74 g), mp 150—152 °C, $[\alpha]_D^{25}$ −37 °C (c=0.24, $CHCl_3$). *Anal.* Calcd for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.32; H, 8.12. UV, IR, and ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. Further elution with $CHCl_3$–acetone (9 : 1) afforded shikoccidin (**5**) (282 mg), mp 178—179 °C (from MeOH), $[\alpha]_D^{27}$ −3.2° (c=0.20, MeOH). *Anal.* Calcd for $C_{22}H_{32}O_6$: C, 67.32; H, 8.22. Found: C, 67.12; H, 8.46. UV λ_{max}^{MeOH} nm (ε): 231 (6700). IR ν_{max}^{KBr} cm^{-1}: 3450, 3400, 1730, 1720, 1650, 1260. ^{1}H-NMR: 0.94 (3H, s, –CH_3), 0.96 (3H, s, –CH_3), 1.19 (3H, s, –CH_3), 2.04 (3H, s, –$OCOCH_3$), 3.10 (1H, m, H-13), 4.65 (1H, br s, H-3), 4.76 (1H, dd, J=11, 5 Hz, H-7), 5.01 (1H, d, J=1 Hz, H-14), 5.45, 6. 21 (each 1H, br s, H_2-17). ^{13}C-NMR: 18.7 (q, C-20), 21.3 (q, C-19), 21.9 (q, –$OCOCH_3$), 22.7 (t, C-1), 26.0 (t, C-12), 27.8 (t, C-2), 28.3 (q, C-18), 30.6 (t, C-6), 31.2 (t, C-11), 36.8 (s, C-4), 40.5 (d, C-13), 44.4 (s, C-10), 45.6 (d, C-5), 66.2 (s, C-8), 71.6 (d, C-7), 75.0 (d, C-14), 77.5 (d, C-3), 81.1 (s, C-9), 118.8 (t, C-17), 147.4 (s, C-16), 170.7 (s, –$OCOCH_3$), 207.5 (s, C-15).

Column chromatography of fraction g over silica gel (65 g) with $CHCl_3$–acetone (9 : 1) afforded a fraction (423 mg) containing isodomedin (**9**) as a main component. This fraction was further separated by column chromatography over silica gel (8.5 g) with $CHCl_3$–acetone (8.5 : 1.5) followed by PTLC to afford isodomedin (**9**)[6] (54 mg), mp 209—212 °C (from aqueous EtOH), $[\alpha]_D^{25}$ −44.3° (c=0.1, MeOH).

Shikoccin Acetate (11)——A solution of shikoccin (**1**) (335 mg) in 6 ml of a 1 : 1 mixture of acetic anhydride and pyridine was kept overnight. Usual work-up followed by column chromatography over silica gel with $CHCl_3$ afforded shikoccin acetate (**11**) (337 mg), mp 183—185 °C (from MeOH). *Anal.* Calcd for $C_{24}H_{32}O_6$: C, 69.21; H, 7.74. Found: C, 69.04; H, 7.92. UV, IR, and ^{1}H-NMR: Table I.

Dihydroshikoccidin (12)——Shikoccidin (**5**) (50 mg) was hydrogenated over 5% Pd–C (60 mg) in MeOH. Purification by column chromatography over silica gel (5 g) with $CHCl_3$ gave dihydroshikoccidin (**12**) (23 mg), mp 172—174 °C (from $CHCl_3$–hexane). *Anal.* Calcd for $C_{22}H_{34}O_6$: C, 66.98; H, 8.69. Found: C, 67.05; H, 8.39. CD (c= 0.275, MeOH) $[\theta]^{25}$ (nm): +880 (293.5) (positive max), 0 (312), −610 (325.5) (negative max). IR ν_{max}^{KBr} cm^{-1}: 3550, 3420, 1725, 1255. ^{1}H-NMR: 0.95 (6H, s, –CH_3 × 2), 1.20 (3H, s, –CH_3), 1.13 (3H, d, J=7.5 Hz, –CH_3), 2.03 (3H, s, $OCOCH_3$), 2.59 (1H, s, –OH), 3.02 (1H, m, H-13), 3.30 (1H, s, –OH), 4.64 (2H, m, 3-H, 7-H), 5.17 (1H, s, H-14), 5.46 (1H, s, –OH).

Shikoccin (1) from Shikoccidin (5)——A solution of oxalic acid (220 mg) in MeOH–water (2 : 1, 6 ml) was added to a solution of shikoccidin (**5**) (71 mg) in MeOH (2 ml). After being refluxed for 34 h, the mixture was concentrated under reduced pressure to leave a residue, to which water was added. Extractive work-up with $CHCl_3$ followed by PTLC with $CHCl_3$–acetone (10 : 1) gave shikoccin (**1**) (51 mg) along with recovered shikoccidin (**5**) (12 mg).

***O*-Methylshikoccin (2), Deacetyl-*O*-methylshikoccin (13), and 14 from Shikoccin (1)**——A solution of shikoccin (**1**) (500 mg) in MeOH (15 ml) and conc. H_2SO_4 (0.5 ml) was refluxed for 45 h and neutralized with 5% Na_2CO_3 solution. After being concentrated, the solution was diluted with water and extracted with CH_2Cl_2. The organic layer was washed, dried over Na_2SO_4, and evaporated to give an oily residue (510 mg). Column chromatography over silica gel (20 g) with $CHCl_3$ afforded *O*-methylshikoccin (**2**) (202 mg) and deacetyl-*O*-methylshikoccin (**13**) (193 mg), mp 171—174 °C (from ethyl acetate–isopropyl ether). *Anal.* Calcd for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.78; H, 8.79. UV, IR, and ^{1}H-NMR: Table I. Further elution with $CHCl_3$ yielded **14** (49 mg), mp 131—133 °C (from hexane–isopropyl ether). *Anal.* Calcd for $C_{24}H_{36}O_6$: C, 68.54; H, 8.63. Found: C, 68.60; H, 8.78. UV, IR, and ^{1}H-NMR: Table I.

Epoxyshikoccin (3) from Shikoccin (1)——Shikoccin (**1**) (132 mg) was treated with *m*-CPBA (135 mg) in $CHCl_3$ (5 ml) in the dark for 8 d. After addition of 5% aq. Na_2CO_3, the mixture was extracted with $CHCl_3$. The organic layer was washed with water, dried over Na_2SO_4, and evaporated to afford crude epoxyshikoccin (**3**) (138 mg). Purification by PTLC with $CHCl_3$–acetone (10 : 1) followed by recrystallization from ethyl acetate–isopropyl ether yielded pure epoxyshikoccin (**3**) (78 mg).

***O*-Methylepoxyshikoccin (4) from *O*-Methylshikoccin (2)**——*O*-Methylshikoccin (**2**) (55 mg) was oxidized with *m*-CPBA (75 mg) in $CHCl_3$ (2 ml) in the dark for 2 weeks. Usual work-up as described for **3** followed by PTLC with benzene–ethyl acetate (4 : 1) and subsequent recrystallization from hexane–isopropyl ether afforded *O*-

methylepoxyshikoccin (**4**) (20 mg).

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