Conformation and Absolute Stereochemistry of Macrocalyxoforin D (= Longirabdiol)

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The conformation and the absolute stereochemistry of macrocalyxoforin D (=longirabdiol) was determined based on nuclear magnetic resonance spectroscopy, X-ray crystallographic analysis and circular dichroism spectroscopy.

Keywords macrocalyxoforin D; longirabdiol; 6,7-seco-*ent*-kaurenoid; conformation; absolute stereochemistry; nuclear magnetic resonance; X-ray analysis; circular dichroism; *Rabdosia longituba*

During the course of our systematic investigation of the diterpenoid constituents of *Rabdosia longituba* (MIQ.) (Japanese name: akichouji) (Labiateae),¹⁾ we isolated a diterpene named longirabdiol²⁾ in the early stage of the study. This compound was finally identified as macrocalyxoforin D (**1**)³⁾ by means of spectroscopic methods, and has been proposed to have a spiro-seco-kaurene structure or its enantiomer as the basic skeleton. It is known that there are two possible chair conformations for ring A in the *ent*-spiro-seco-kaurene series diterpenoids.⁴⁾ However, the conformation of ring A was not discussed in the previous report³⁾ and the absolute stereochemistry has not been determined yet. Thus, we investigated the stereochemistry of macrocalyxoforin D (**1**) by nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallographic analysis and circular dichroism (CD) spectroscopy. This paper describes the results of these experiments.

The conformation of macrocalyxoforin D (**1**) in CDCl₃ solution was examined. The assignments of the proton (¹H)- and carbon-13 (¹³C) signals were confirmed by ¹H-¹H-shift correlation spectroscopy (¹H-COSY) and (¹H-¹³C-shift) correlation spectroscopy (¹H-¹³C-COSY). The results are shown in Table I. Some of the ¹³C-signal assignments previously reported,³⁾ especially those for C-5, C-9, C-19 and C-20, were unambiguously reassigned. Based on the assignments of the ¹H-signals, we performed nuclear Overhauser enhancement (NOE) and ¹H-¹H-nuclear Overhauser enhancement correlation spectroscopy (¹H-NOESY) experiments. The results are summarized in Fig. 1. As previously mentioned, there are two possible chair conformations in ring A.⁴⁾ One has C-9 equatorial and C-20 axial orientations and the other has C-9 axial and C-20 equatorial orientations. The above mentioned results showed that macrocalyxoforin D (**1**) has a chair conformation having C-9 equatorial and C-20 axial orientations in ring A, the same as that of trichorabdal C (**3**).⁵⁾

It is also noteworthy that ring C has a boat conformation as judged from the coupling constants of H-9 (dd, *J* = 12.5 and 4.5 Hz).

A single crystal of macrocalyxoforin D 6-*O*-*p*-bromobenzoate (**2**), prepared by treatment of macrocalyxoforin D (**1**) with *p*-bromobenzoyl chloride in methylene chloride in the presence of α -pinene and grown in methanol, was then subjected to an X-ray analysis. The molecular structure of **2** is illustrated in Fig. 2, while bond

TABLE I. Proton- and Carbon-13 NMR Data for Macrocalyxoforin D (**1**)

	¹ H (CDCl ₃)	¹³ C (CDCl ₃)
1		27.6
2		18.0
3		39.4
4		39.4
5	1.43 (1H, m)	50.4
6	3.68 (1H, dd, <i>J</i> = 12.5, 3.5 Hz)	56.7
	3.88 (1H, dd, <i>J</i> = 12.5, 1 Hz)	
7		172.0
8		57.9
9	2.55 (1H, dd, <i>J</i> = 12.5, 4.5 Hz)	44.1
10		42.8
11		17.0
12		29.4
13	3.10 (1H, br dd, <i>J</i> = 9.5, 4.5 Hz)	35.1
14	2.15 (1H, d, <i>J</i> = 12 Hz)	29.5
	2.41 (1H, dd, <i>J</i> = 12.5, 4.5 Hz)	
15		203.0
16		150.2
17	5.49 (1H, br s)	118.5
	6.01 (1H, br s)	
18	1.08 (3H, s)	29.1
19	3.33 (1H, d, <i>J</i> = 11.5 Hz)	68.5
	4.02 (1H, d, <i>J</i> = 11.5 Hz)	
20	4.68 (1H, d, <i>J</i> = 12 Hz)	71.4
	4.92 (1H, d, <i>J</i> = 12 Hz)

Chart 1

Fig. 1. Summary of NOE and ^1H -NOESY Experiments
The figures in parentheses show the increments of signal intensity (%).

Fig. 2. ORTEP Drawing of Macrocallyxofornin D 6-*O*-*p*-Bromobenzoate (**2**)

TABLE II. Bond Distances (Å) of **2** Involving Non-hydrogen Atoms

Br-C(25)	1.91	O(1)-C(6)	1.53
O(1)-C(21)	1.47	O(2)-C(7)	1.25
O(3)-C(15)	1.24	O(4)-C(19)	1.40
O(5)-C(7)	1.34	O(5)-C(20)	1.45
O(6)-C(21)	1.14	C(1)-C(2)	1.57
C(1)-C(10)	1.60	C(2)-C(3)	1.47
C(3)-C(4)	1.48	C(4)-C(5)	1.61
C(4)-C(18)	1.62	C(4)-C(19)	1.64
C(5)-C(6)	1.52	C(5)-C(10)	1.54
C(7)-C(8)	1.44	C(8)-C(9)	1.60
C(8)-C(14)	1.50	C(8)-C(15)	1.57
C(9)-C(10)	1.53	C(9)-C(11)	1.53
C(10)-C(20)	1.52	C(11)-C(12)	1.61
C(12)-C(13)	1.56	C(13)-C(14)	1.57
C(13)-C(16)	1.51	C(15)-C(16)	1.49
C(16)-C(17)	1.33	C(21)-C(22)	1.48
C(22)-C(23)	1.40	C(23)-C(27)	1.37
C(23)-C(24)	1.40	C(24)-C(25)	1.27
C(25)-C(26)	1.47	C(26)-C(27)	1.44

S.D. ± 0.01 – 0.02 Å.

TABLE III. Bond Angles ($^\circ$) of **2** Involving Non-hydrogen Atoms

C(6)-O(1)-C(21)	115.6	C(20)-O(5)-C(7)	116.3
C(2)-C(1)-C(10)	116.0	C(1)-C(2)-C(3)	114.4
C(2)-C(3)-C(4)	115.2	C(3)-C(4)-C(5)	115.2
C(3)-C(4)-C(18)	109.8	C(3)-C(4)-C(19)	109.0
C(5)-C(4)-C(18)	108.5	C(5)-C(4)-C(19)	110.2
C(18)-C(4)-C(19)	103.5	C(4)-C(5)-C(10)	117.0
C(4)-C(5)-C(6)	109.4	C(10)-C(5)-C(6)	123.0
C(1)-C(10)-C(5)	110.0	C(1)-C(10)-C(9)	110.7
C(1)-C(10)-C(20)	108.4	C(5)-C(10)-C(9)	112.7
C(5)-C(10)-C(20)	108.5	C(9)-C(10)-C(20)	106.5
C(10)-C(9)-C(8)	109.7	C(10)-C(9)-C(11)	116.8
C(8)-C(9)-C(11)	107.3	C(9)-C(8)-C(14)	111.2
C(9)-C(8)-C(7)	116.0	C(9)-C(8)-C(15)	102.6
C(14)-C(8)-C(7)	116.7	C(14)-C(8)-C(15)	99.0
C(7)-C(8)-C(15)	108.9	C(8)-C(14)-C(13)	102.4
C(14)-C(13)-C(12)	111.6	C(14)-C(13)-C(16)	105.2
C(12)-C(13)-C(16)	106.9	C(13)-C(12)-C(11)	105.3
C(9)-C(11)-C(12)	115.5	O(5)-C(20)-C(10)	112.9
O(5)-C(7)-O(2)	114.1	O(5)-C(7)-C(8)	125.2
O(2)-C(7)-C(8)	120.7	O(3)-C(15)-C(8)	123.1
O(3)-C(15)-C(16)	125.4	C(8)-C(15)-C(16)	111.0
C(13)-C(16)-C(15)	103.1	C(13)-C(16)-C(17)	132.3
C(15)-C(16)-C(17)	124.3	O(4)-C(19)-C(4)	119.0
O(1)-C(6)-C(5)	107.0	O(1)-C(21)-O(6)	117.3
O(1)-C(21)-C(22)	107.0	O(6)-C(21)-C(22)	135.6
C(21)-C(22)-C(23)	111.7	C(21)-C(22)-C(27)	124.6
C(23)-C(22)-C(27)	123.4	C(22)-C(23)-C(24)	116.9
C(23)-C(24)-C(25)	122.3	Br-C(25)-C(24)	123.9
Br-C(25)-C(26)	112.3	C(24)-C(25)-C(26)	123.8
C(25)-C(26)-C(27)	114.6	C(22)-C(27)-C(26)	118.7

S.D. ± 0.9 – 1.0 °.

TABLE IV. Atomic Coordinates for Non-hydrogen Atoms of **2** with Their e.s.d.'s in Parentheses

Atom	x	y	z
Br	0.8852 (3)	0.3496 (1)	0.37302 (7)
O(1)	0.913 (1)	0.1285 (6)	0.6398 (3)
O(2)	1.054 (1)	-0.3048 (6)	0.6112 (4)
O(3)	1.288 (1)	-0.1144 (7)	0.5675 (4)
O(4)	0.996 (1)	0.0352 (6)	0.8220 (4)
O(5)	1.055 (1)	-0.2067 (5)	0.6837 (3)
O(6)	0.928 (2)	0.2677 (6)	0.6774 (4)
C(1)	0.683 (2)	-0.1517 (9)	0.6824 (5)
C(2)	0.595 (2)	-0.1555 (11)	0.7468 (6)
C(3)	0.545 (2)	-0.0668 (7)	0.7717 (6)
C(4)	0.688 (2)	0.0033 (10)	0.7681 (5)
C(5)	0.790 (2)	0.0097 (8)	0.7042 (5)
C(6)	0.912 (2)	0.0917 (10)	0.7040 (6)
C(7)	1.028 (2)	-0.2245 (8)	0.6252 (6)
C(8)	0.969 (2)	-0.1602 (9)	0.5811 (5)
C(9)	0.886 (2)	-0.0684 (7)	0.6068 (4)
C(10)	0.841 (2)	-0.0799 (7)	0.6736 (5)
C(11)	0.731 (2)	-0.0411 (8)	0.5651 (6)
C(12)	0.781 (2)	-0.0340 (9)	0.4946 (6)
C(13)	0.887 (2)	-0.1226 (9)	0.4799 (5)
C(14)	0.858 (2)	-0.1962 (8)	0.5298 (5)
C(15)	1.137 (2)	-0.1263 (8)	0.5444 (6)
C(16)	1.085 (2)	-0.0995 (8)	0.4823 (5)
C(17)	1.200 (2)	-0.0704 (11)	0.4406 (6)
C(18)	0.603 (2)	0.1013 (8)	0.7835 (5)
C(19)	0.833 (2)	-0.0132 (8)	0.8225 (6)
C(20)	1.009 (2)	-0.1164 (8)	0.7036 (6)
C(21)	0.912 (2)	0.2271 (7)	0.6344 (5)
C(22)	0.902 (2)	0.2483 (8)	0.5695 (5)
C(23)	0.894 (2)	0.3411 (9)	0.5593 (5)
C(24)	0.883 (2)	0.3693 (8)	0.4995 (6)
C(25)	0.894 (2)	0.3143 (9)	0.4556 (5)
C(26)	0.910 (2)	0.2162 (8)	0.4628 (5)
C(27)	0.920 (2)	0.1859 (10)	0.5243 (5)

distances and angles involving non-hydrogen atoms are given in Tables II and III. The absolute configuration of the molecule was determined by Bijvoet's anomalous dispersion method. Dihydromacrocallyxofornin D (**4**) ob-

TABLE V. Determination of Absolute Stereochemistry

<i>h</i>	<i>k</i>	<i>l</i>	Obs. (+)	(<i>F</i> _O) (−)	Calc. (+)	(<i>F</i> _C) (−)
1	1	1	48.60	> 34.48	37.06	> 25.45
1	1	5	77.57	< 90.67	69.15	< 73.98
1	1	8	29.43	> 25.40	25.02	> 19.71
1	1	9	64.38	> 54.94	59.82	> 48.11
1	1	10	68.35	< 77.74	61.24	< 65.49
1	2	1	93.53	> 88.33	75.60	> 69.02
1	2	3	63.34	< 74.26	55.64	< 62.31
1	2	4	47.30	< 62.49	38.81	< 45.46
1	2	5	28.71	< 38.70	27.00	< 31.12
1	3	1	45.71	< 56.95	46.14	< 54.08
1	3	3	61.55	> 56.58	46.64	> 41.72
1	3	8	18.34	< 27.43	18.21	< 25.04
1	4	6	20.14	< 29.31	24.37	< 28.59
1	4	7	9.08	< 17.15	8.43	< 14.48
1	5	2	74.34	> 67.76	71.67	> 64.67
1	5	3	52.38	< 65.59	45.46	< 51.77
1	6	1	57.25	< 62.88	45.51	< 50.47
1	6	5	56.90	< 67.66	56.37	< 61.02
2	2	2	154.84	> 148.59	106.25	> 102.32
2	3	1	50.66	< 57.99	42.78	< 49.53
2	3	4	39.54	< 45.94	32.82	< 37.04
2	3	5	69.17	< 77.87	57.56	< 62.18
3	1	1	29.65	> 22.34	17.92	> 12.41
3	1	2	27.09	< 32.24	22.69	< 28.65
3	1	4	10.01	< 15.61	13.98	< 18.85
3	1	5	42.43	> 34.29	39.46	> 31.64

tained by catalytic hydrogenation of **1** showed a negative Cotton effect ($\Delta\epsilon_{303} - 1.06$) in the CD spectrum,⁶⁾ which is consistent with the result obtained by X-ray crystallographic analysis.

Experimental

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Ultraviolet (UV) and infrared (IR) spectra were taken with a Hitachi 330 spectrophotometer and a Hitachi 215 spectrophotometer, respectively. NMR spectra were recorded with a JEOL JNM FX-200 or a JEOL JNM GSX-400 spectrometer. Chemical shifts are given in δ values using tetramethylsilane as an internal standard. Optical rotation was determined with a Union Giken PM-201 digital polarimeter and CD spectra were recorded with a JASCO J-600 spectropolarimeter. Mass spectra (MS) were determined with a JEOL JMS D-300 spectrometer. Kieselgel 60 (0.040–0.063 mm, Merck) was used for column chromatography and Kieselgel 60 F₂₅₄ precoated plates (0.25 mm and 0.5 mm, Merck) were used for thin layer and layer chromatographies.

Isolation of Macrocalyxofornin D (=Longirabdiol) (1) Dried aerial parts (658 g) of *Rabdodia longituba* collected in Togouchi Town, Hiroshima Pref., Japan were extracted with MeOH (10 l) for 2 weeks at room temperature. The extraction was repeated again in the same manner. The MeOH extract was concentrated *in vacuo* and the residue was dissolved in 90% MeOH (440 ml). After being washed with *n*-hexane (300 ml $\times$ 3), the 90% MeOH layer was concentrated *in vacuo*. The residue was suspended in H₂O (400 ml) and the suspension was extracted with EtOAc (300 ml $\times$ 3). The EtOAc extract was washed with H₂O, dried and evaporated *in vacuo* to give a residue (16.1 g), which was chromatographed on a silica gel (600 g) column with CHCl₃–Me₂CO as the eluant, with increasing Me₂CO content. The eluate from 15% Me₂CO–CHCl₃ (2.993 g) was purified on a silica gel (120 g) column with Et₂O as the eluant and recrystallized from MeOH to give macrocalyxofornin D (=longirabdiol) (**1**) (536 mg) as colorless needles. mp 203–205°, $[\alpha]_D^{22.5} + 16.5^\circ$ ($c = 1.21$, MeOH), $[\alpha]_D^{22} - 26.4^\circ$ ($c = 4.02$, CHCl₃). UV λ_{max} (MeOH): 232 nm (ϵ 8580). IR ν_{max} (KBr): 3200,

1740, 1710, 1640, 1395, 1265, 1235, 1095, 1040, 1030, 995 cm^{−1}. ¹H- and ¹³C-NMR (see Table I). Anal. Calcd for C₂₀H₂₈O₅: C, 68.94; H, 8.10. Found: C, 68.77; H, 8.29.

Macrocalyxofornin D 6-*O*-*p*-Bromobenzoate (2) α -Pinene (0.2 ml) and *p*-bromobenzoyl chloride (270 mg) were added to a solution of macrocalyxofornin D (=longirabdiol) (**1**) (10.5 mg) in CH₂Cl₂ (5 ml), and the mixture was refluxed for 4 h. After cooling down, the mixture was poured into ice water and the resulting precipitates were extracted with CHCl₃ (20 ml $\times$ 3). The extracts were combined, washed with saturated NaCl aqueous solution, dried over MgSO₄, and evaporated *in vacuo*. The residue was purified by column chromatography (SiO₂, 45 g, solvent: CHCl₃–Me₂CO) and layer chromatography (solvent: CHCl₃–Me₂CO (19:1) developed three times), successively, to give 6-*O*-*p*-bromobenzoyl macrocalyxofornin D (**2**) (8.6 mg). Recrystallization from MeOH gave colorless needles, mp 189–191°C. IR ν_{max} (CHCl₃): 3550–3400, 1740, 1710, 1640, 1590, 1480, 1400, 1270, 1110, 1100, 1070, 1010 cm^{−1}. ¹H-NMR (CDCl₃) δ : 1.21 (3H, s, 18-H₃), 1.96 (1H, dd, $J = 7, 2.5$ Hz, 5-H), 2.11 (1H, d, $J = 12.5$ Hz, 14 α -H), 2.43 (2H, m, 9-H, 14 β -H), 3.02 (1H, dd, $J = 9.5, 4.5$ Hz, 13-H), 3.66 (2H, ABd, $J = 11$ Hz, 19-H₂), 4.32 (1H, dd, $J = 13, 7$ Hz, 6-H₁), 4.45 (1H, d, $J = 11.5$ Hz, 20-H₁), 4.71 (1H, d, $J = 11.5$ Hz, 20-H₁), 4.80 (1H, dd, $J = 13, 2.5$ Hz, 6-H₁), 5.42, 5.98 (each 1H, br s, 17-H₂), 7.56, 7.77 (each 2H, d, $J = 8.5$ Hz, *p*-bromobenzoyl). MS m/z : 330.1803 (M–C₇H₅O₂Br)⁺. Calcd for C₂₀H₂₆O₄: 330.1832. FAB-MS m/z : 553 and 555 (M + Na)⁺ (+ NaI), 569 and 571 (M + K)⁺ (+ KI). Anal. Calcd for C₂₇H₃₁BrO₆: C, 61.02; H, 5.88. Found: C, 60.53; H, 5.95.

X-Ray Analysis of Macrocalyxofornin D *p*-Bromobenzoate (2) C₂₇H₃₁BrO₆, $M = 531.4$. Orthorhombic, $a = 7.425$ (3), $b = 14.824$ (8), $c = 20.216$ (22) Å, $Z = 4$, $D_x = 1.57$ g·cm^{−3}, space group $P2_12_12_1$ (MoK α) = 20.0 cm^{−1}. The cell dimensions and intensities were measured on a Syntex R-3 four-circle diffractometer with graphite-monochromated MoK α radiation in the ω -scan mode for $2\theta < 45^\circ$, and 1238 independent reflections [$I > 1.96 \lambda(I)$] were used for the structure analysis. The structure was solved by the direct method using MULTAN on a Syntex XTL computer. Refinement by the block-diagonal least-squares method led to a final R value of 0.095. Atomic coordinates for non-hydrogen atoms are given in Table IV. The absolute configuration of the molecule was determined by Bijvoet's anomalous-dispersion method based on the observed and calculated structure factors of 26 Friedel pairs (see Table V).

Dihydromacrocalyxofornin D (4) Pd–C (5%) (10 mg) was added to a solution of macrocalyxofornin D (**1**) (10.6 mg) in MeOH (5 ml). The mixture was stirred for 1 h at room temperature under an atmosphere of hydrogen. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. The residue was purified by layer chromatography [solvent: CHCl₃–Me₂CO (4:1) developed twice] to give the dihydro compound (**4**)^{5b)} (5.9 mg), which crystallized on addition of MeOH. mp 195–197°C. IR ν_{max} (CHCl₃): 3370, 1740, 1710, 1260–1190, 1090, 1015 cm^{−1}. ¹H-NMR (CDCl₃) δ : 1.08 (3H, s, 18-H₃), 1.14 (1H, d, $J = 6.5$ Hz, 16-Me), 2.21 (1H, dd, $J = 12.5, 1$ Hz, 14 α -H), 3.31 (1H, d, $J = 11.5$ Hz, 19-H₁), 3.69 (1H, dd, $J = 13, 3.5$ Hz, 6-H₁), 3.88 (1H, dd, $J = 13, 1.5$ Hz, 6-H₁), 4.00 (1H, d, $J = 11.5$ Hz, 19-H₁), 4.66 (1H, d, $J = 12$ Hz, 20-H₁), 4.99 (1H, d, $J = 12$ Hz, 20-H₁). MS m/z : 350.2121 (M)⁺. Calcd for C₂₀H₃₀O₅: 350.2094. CD (MeOH): $\Delta\epsilon_{303} - 1.06$.

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