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Constituents of Cinnamomi Cortex. V.¹⁾ Structures of Five Novel Diterpenes, Cinncassiols D₁, D₁ Glucoside, D₂, D₂ Glucoside and D₃

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The structures of cinncassiol D₁ (1), cinncassiol D₁ glucoside (2), cinncassiol D₂ (3), cinncassiol D₂ glucoside (4) and cinncassiol D₃ (5), isolated from the fraction exhibiting anti-complement activity of the water extractive of Cinnamomi Cortex ("Tôkô Keihi"), have been characterized by means of chemical, spectral and X-ray analyses. They are novel pentacyclic diterpenes with a new skeleton.

Keywords—Cinnamomi Cortex; Lauraceae; diterpenes; cinncassiol D₁; cinncassiol D₁ glucoside; cinncassiol D₂; cinncassiol D₂ glucoside; cinncassiol D₃; X-ray analysis

The isolation of a series of diterpenes (compounds I—XIII) from the fraction exhibiting anti-complement activity²⁾ of the water extractive of Cinnamomi Cortex ("Kannan Keihi" and "Tôkô Keihi", the dried bark of *Cinnamomum cassia* BLUME (Lauraceae); one of the most widely used crude drugs), the identification of compounds I and II with cinnzeylanine and cinnzeylanol,³⁾ respectively, and the structure elucidation of compounds III—XIII were reported in the preceding papers.^{1,4)} These diterpenes so far obtained from Cinnamomi Cortex can be classified into three groups, namely ketal, lactone and diketone types, as shown in Table I.

As a continuation of that work, the present paper deals with the structure determination of five additional diterpenes named cinncassiol D₁ (1), D₁ glucoside (2),⁵⁾ D₂ (3), D₂ glucoside (4) and D₃ (5), which had been isolated⁶⁾ from the water extractive of "Tôkô Keihi." They are closely related to each other structurally and belong to a new type different from the above three groups.

Cinncassiol D₁ (1), a white powder, $[\alpha]_D -11.6^\circ$, C₂₀H₃₂O₅ (field desorption mass (FD-MS) spectrum (m/z) 352 (M⁺)), showed signals due to three *tert.* CH₃ (δ 0.90 and 2×1.69), a *sec.* CH₃ (δ 1.37, d, $J=6$ Hz), a -CH₂-O- (δ 3.80, d, $J=9$ Hz) and a >CH-O- (δ 4.44, br s,) in the proton nuclear magnetic resonance (¹H NMR) spectrum (*d*₅-pyridine). Since the latter two functions, the methylene and the methine bearing an oxygen atom, appeared at δ 4.01, 4.21 (each 1H, dd, $J=7, 11$ Hz) and 4.45 (1H, br s) in the ¹H NMR spectrum (*d*₅-pyridine) of the monoacetate (6), a white powder, C₂₂H₃₄O₆, $[\alpha]_D -10.9^\circ$, derived from 1 by acetylation with Ac₂O-pyridine at room temperature for 30 min, the above signals could be assigned to the hydroxymethyl group and the methine proton bearing an ether oxygen bond, respectively. Furthermore, the ¹³C nuclear magnetic resonance (¹³C NMR) spectrum (Table II) exhibited peaks due to CH₃ $\times 4$, CH₂ $\times 4$, >CH $\times 5$, >C $\times 2$, -CH₂-O- $\times 1$, >CH-O- $\times 1$, >C-O- $\times 2$ and >C<O⁻ $\times 1$. On the basis of a comparison of the ¹H NMR and ¹³C NMR spectra of 1 with those of compounds I—XIII, 1 was supposed to be a diterpene with a new skeleton. Thus, 1 was treated with *p*-bromobenzenesulfonyl chloride and pyridine at room temperature for 2 h and subsequently acetylated with Ac₂O-pyridine at room temperature overnight to yield the monoacetyl monobrosylate (brosylate=*p*-bromobenzenesulfonate) (7) of 1, colorless plates, mp 104—105°C, $[\alpha]_D \simeq 0^\circ$, ¹H NMR (CDCl₃) δ : 2.03 (3H, s, -OAc), 7.65, 7.78 (each 2H, d, $J=10$ Hz, 4 $\times$ arom.

TABLE I. Diterpenes obtained from *Cinnamomi Cortex*

Ketal-type	Lactone-type	Diketone-type
I R=H, R'=Ac cinnzeylanine ^{3,4a)}	V R=H, R'=Ac anhydrocinnzeylanine ^{3,4a)}	X R=OH cinncassiol C ₁ ^{4b)}
II R=R'=H cinnzeylanol ^{3,4a)}	VI R=R'=H anhydrocinnzeylanol ^{3,4a)}	XI R=-O-β-D-gluc·pyr cinncassiol C ₁ glucoside ¹⁾
III R=OH, R'=H cinncassiol B ^{4c)}	VII R=OH, R'=H cinncassiol A ^{4a)}	XII R=H cinncassiol C ₂ ¹⁾
IV R=-O-β-D-gluc·pyr, R'=H cinncassiol B ^{4c)} glucoside	VIII R=OAc, R'=H cinncassiol A monoacetate	
	IX R=-O-β-D-gluc·pyr, R'=H cinncassiol A glucoside ^{4a)}	XIII cinncassiol C ₃ ^{1,3)}

TABLE II. ¹³C NMR Spectra^{a)} of 1, 2, 3, 11 and 12

	1	2	3	11	12
C-1	88.6	88.8	89.4	88.9	44.2
C-2	40.4	40.7	36.3	35.7	78.4
C-3	28.1	28.3	41.3	40.6	47.0
C-4	51.0	51.5	87.4	87.2	89.0
C-5	54.1	54.6	62.5	61.1	53.8
C-6	77.2	77.6	76.3	75.0	76.4
C-7	82.0	82.0	81.5	82.4	83.7
C-8	48.6	48.9	49.3	48.4	48.9
C-9	37.3	37.5	42.2	41.4	42.4
C-10	25.2	26.0	26.1	25.3	26.5
C-11	107.5	107.7	107.9	106.8	107.8
C-12	57.1	57.4	57.5	56.5	57.8
C-13	40.4	41.2	40.9	40.4	40.8
C-14	41.1	41.2	44.3	42.4	44.1
C-15	25.5	25.4	24.5	23.6	13.1
C-16	24.4	24.8	22.4	21.5	22.9
C-17	13.5	14.1	13.8	12.9	13.8
C-18	36.9	34.8	37.5	33.8	37.8
C-19	67.3	75.7	67.8	78.6	67.8
C-20	10.6	10.5	10.5	8.3	10.4
C-1'		105.0		100.9	
C-2'		75.1		71.4	
C-3'		78.3		72.7	
C-4'		71.6		68.4	
C-5'		78.3		71.7	
C-6'		62.8		61.9	

^{a)} Measured in *d*₆-pyridine for 1, 2, 3, 12 and in CDCl₃ for 11.

proton). A single crystal of **7** suitable for X-ray diffraction study was obtained by recrystallization from dil. MeOH and its data were as follows; $C_{28}H_{37}SO_8Br \cdot H_2O$; monoclinic, space groups $P2_1$ ($Z=2$); lattice constants $a=10.222(6)$, $b=10.271(7)$, $c=15.155(6)$ Å, $\beta=96.91(4)^\circ$, $V=1579.6$ Å³; $D_{\text{calcd.}}=1.33$, $D_{\text{obsd.}}=1.37$ g/cm³. The cell dimensions and intensities were measured with a Syntex R_θ four-circle diffractometer with graphite-monochromated Mo($K\alpha$) radiation in an ω scan mode for 2θ less than 45° . A total of 2192 independent reflections were collected, among which 1284 reflections ($I \geq 2.0 \sigma(I)$) were stored as observed. The structure was solved by the heavy atom method using the Syntex XTL program. All the non-hydrogen atoms and all but 16 hydrogen atoms were found on a difference Fourier map. The block-diagonal least-squares method was used for refinement, the final R value being 0.105. The molecular structure with the bond lengths and angles is shown in Fig. 1.

TABLE III. Final Atomic Parameters of **7**

Atoms	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Br	11030(3)	-11151(5)	869(3)	35(1)	98(2)	102(2)	4(3)	-5(2)	11(3)
S	4742(7)	-10847(7)	256(5)	37(4)	24(4)	32(4)	-2(4)	6(3)	-2(4)
O (1)	3026(15)	-2252(18)	5505(12)	4(9)	87(13)	56(12)	-24(10)	-18(8)	-47(11)
O (6)	1351(14)	-5508(16)	3734(10)	17(7)	56(12)	31(9)	-2(8)	15(7)	-21(8)
O (7)	4268(14)	-7183(16)	4555(9)	8(8)	68(10)	29(9)	8(9)	-12(7)	-7(9)
O (11)	548(14)	-6525(18)	2426(11)	18(8)	73(13)	66(12)	-6(9)	-2(8)	-14(11)
O (19)	4312(15)	-10474(13)	1190(10)	29(9)	6(9)	53(10)	2(7)	5(8)	-4(7)
O (21)	-601(18)	-4714(23)	2257(14)	35(11)	118(18)	106(17)	8(13)	-28(11)	31(15)
OAS	4256(16)	-12170(17)	149(10)	67(11)	43(11)	36(9)	18(10)	27(9)	-8(10)
OBS	4334(14)	-9911(16)	-404(10)	28(9)	34(9)	38(10)	4(9)	7(7)	-4(9)
C (1)	2223(27)	-3140(29)	5075(18)	67(19)	72(24)	43(20)	38(19)	66(16)	-8(18)
C (2)	1246(25)	-2608(29)	4188(16)	31(15)	79(21)	26(15)	31(16)	10(12)	-5(15)
C (3)	2460(26)	-2295(31)	3548(17)	39(16)	98(22)	17(17)	12(17)	-8(13)	-32(17)
C (4)	3445(25)	-3394(25)	3681(17)	79(16)	32(17)	-33(16)	18(15)	0(13)	5(14)
C (5)	3105(25)	-4108(27)	4561(16)	43(15)	26(16)	100(17)	4(15)	-13(13)	-32(14)
C (6)	2610(22)	-5491(25)	4351(16)	25(13)	75(23)	45(15)	-10(13)	-4(12)	47(14)
C (7)	3576(19)	-6400(22)	3910(13)	35(12)	7(12)	42(14)	1(12)	17(10)	-17(12)
C (8)	4365(24)	-5519(24)	3254(17)	4(14)	21(18)	31(15)	-7(13)	23(12)	-22(12)
C (9)	3580(20)	-4341(21)	2907(13)	20(12)	26(14)	20(13)	19(11)	5(10)	29(11)
C (10)	2248(20)	-4872(24)	2384(13)	21(12)	39(14)	15(11)	-16(13)	-6(9)	13(12)
C (11)	1684(18)	-5921(24)	2892(14)	22(10)	25(13)	47(14)	-16(13)	-4(10)	-29(13)
C (12)	2611(21)	-7161(22)	3146(15)	34(13)	19(13)	48(16)	3(12)	-4(12)	1(13)
C (13)	3506(21)	-7419(21)	2415(13)	45(13)	27(13)	7(11)	-8(12)	0(10)	-22(10)
C (14)	4771(21)	-6557(23)	2657(15)	30(13)	37(16)	44(14)	-15(13)	16(11)	-21(13)
C (15)	1554(24)	-3873(26)	5757(16)	118(15)	57(15)	85(15)	-11(14)	59(13)	-26(14)
C (16)	4390(25)	-3638(23)	2303(15)	70(17)	23(16)	46(16)	-35(14)	32(14)	5(13)
C (17)	1854(23)	-8300(21)	3484(17)	35(15)	21(12)	77(20)	-14(12)	8(14)	18(13)
C (18)	3833(18)	-8869(21)	2275(12)	15(10)	25(13)	10(11)	-3(10)	-11(9)	11(10)
C (19)	4374(22)	-8986(21)	1397(13)	38(14)	21(11)	26(13)	-1(11)	-6(11)	-6(10)
C (20)	4895(22)	-9434(21)	3015(15)	56(15)	32(14)	57(16)	23(12)	10(12)	-12(12)
C (21)	-566(30)	-5936(33)	2159(19)	99(24)	50(20)	145(24)	-57(22)	4(20)	41(21)
C (22)	-1713(24)	-6709(29)	1661(17)	59(16)	96(26)	76(19)	-36(17)	5(14)	-14(18)
C (23)	6431(19)	-10996(25)	422(11)	41(12)	31(13)	17(10)	4(15)	-8(9)	-12(12)
C (24)	6975(22)	-12137(28)	792(14)	38(14)	96(20)	31(13)	6(16)	5(11)	12(15)
C (25)	8383(24)	-12149(25)	1019(17)	53(16)	44(15)	85(19)	30(15)	19(14)	35(15)
C (26)	9086(20)	-11072(32)	685(15)	37(12)	68(18)	68(16)	-12(18)	-10(11)	-24(18)
C (27)	8594(21)	-9987(20)	273(17)	27(12)	13(11)	94(20)	1(11)	22(13)	13(13)
C (28)	7165(21)	-9917(22)	142(14)	41(13)	39(14)	27(13)	4(13)	16(11)	-25(12)
HO(3)	518(14)	227(14)	402(9)						
H (31)	774(14)	304(14)	663(9)						
H (32)	829(14)	282(15)	601(9)						
H (5)	662(14)	63(15)	545(9)						
H (6)	246(13)	400(17)	473(8)						
H (8)	454(14)	-9(14)	648(10)						

Atoms	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> ₁₁	<i>B</i> ₂₂	<i>B</i> ₃₃	<i>B</i> ₁₂	<i>B</i> ₁₃	<i>B</i> ₂₃
H (13)	273(14)	246(15)	180(9)						
H (14)	525(14)	432(15)	195(9)						
H (151)	886(14)	84(15)	441(9)						
H (152)	860(14)	162(14)	395(9)						
H (153)	734(15)	133(15)	375(9)						
H (161)	574(14)	129(15)	814(10)						
H (162)	538(14)	222(15)	768(9)						
H (17)	177(14)	118(15)	390(9)						
H (18)	295(14)	55(15)	228(9)						
H (191)	385(14)	28(15)	133(9)						
H (192)	361(15)	153(15)	86(9)						
H (20)	470(14)	-36(14)	310(10)						
H (221)	868(14)	313(14)	112(9)						
H (222)	799(14)	309(14)	200(9)						
H (27)	903(14)	61(15)	1015(10)						

Atomic co-ordinates, multiplied by 10⁴ for non-hydrogen atoms and by 10³ for hydrogen atoms, and thermal parameters,^{a)} multiplied by 10.

The anisotropic temperature factors are of the form

$$\exp[-(1/4)(B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)].$$

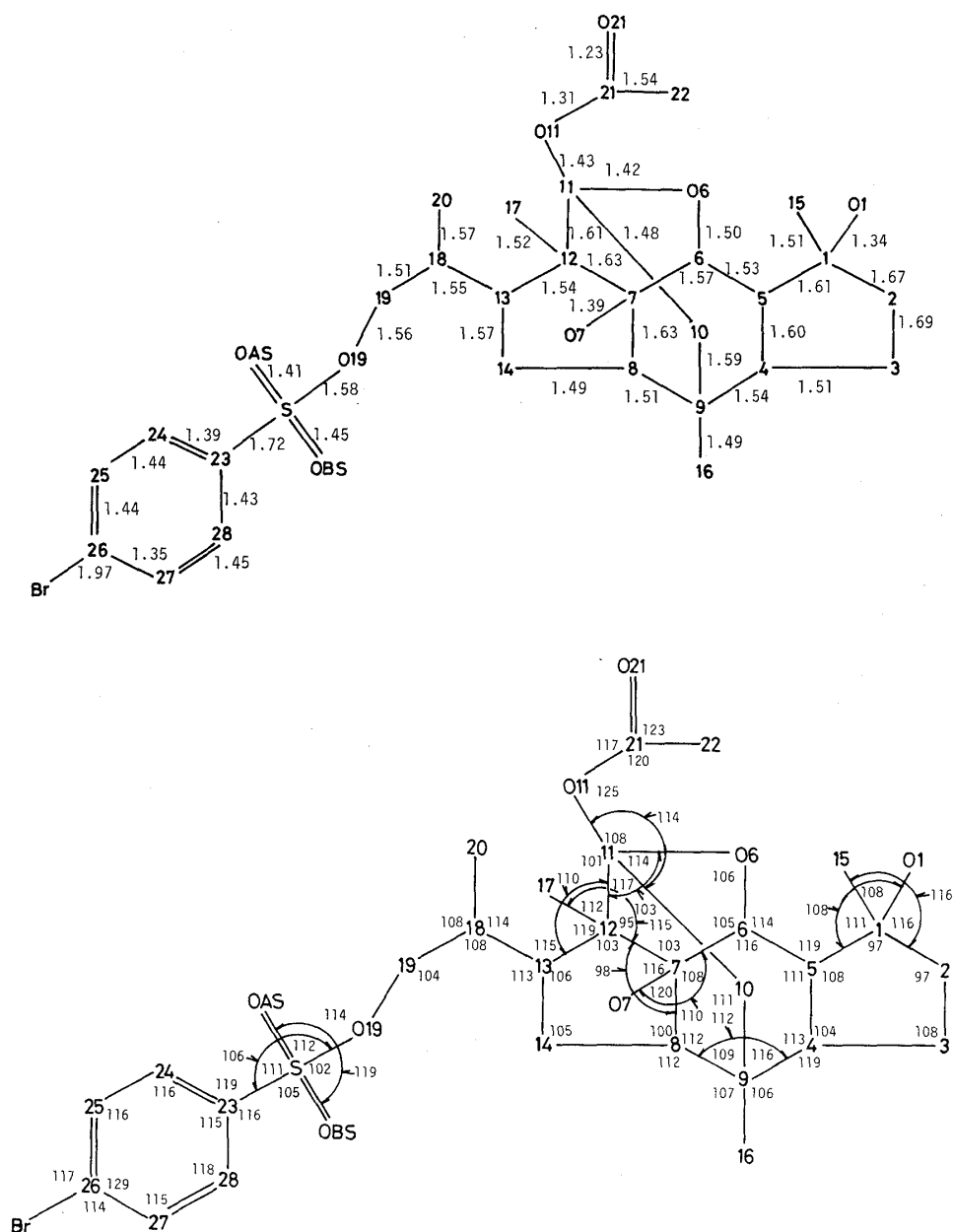
a) Thermal parameters for hydrogen atoms are fixed at the average temperature factor ($B=4.07 \text{ \AA}^2$)

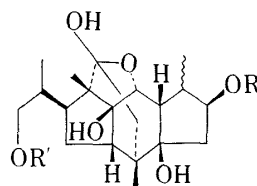
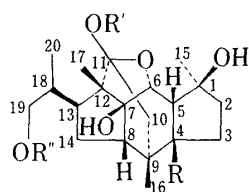
Therefore, the chemical structure of cinnassiol D₁ is represented by the formula **1** or its enantiomer; however the latter could be excluded because the configurations, at C-7, -9, -11 and -12 for example, are most likely identical with those of cinnzeylanol (II). **1** is a novel pentacyclic diterpene consisting of three five-membered rings and two six-membered ones, and its skeleton corresponds to a migrated form from the C₅-C₆ into the C₅-C₁ bond in the ketal-type diterpene cinnzeylanol (II), for example.

Cinnassiol D₁ glucoside (**2**), a white powder, $[\alpha]_D -4.1^\circ$, C₂₆H₄₂O₁₀ (FD-MS (*m/z*): 553 (M+K⁺), 537 (M+Na⁺)), showed strong absorption (3380 cm⁻¹) due to hydroxyl functions. On the assumption that it was a glycoside of a diterpene, **2** was hydrolyzed with crude hesperidinase (Tanabe Pharm. Co., Ltd.) to give an aglycone identical with cinnassiol D₁ (**1**) and D-glucose. Therefore, **2** is composed with each one mole of **1** and D-glucose. A comparison of the ¹³C NMR spectrum of **2** with that of **1** revealed that the signals due to C-19 and C-18 were shifted by +8.4 and -2.1 ppm, respectively, indicating that the glucosyl moiety is bound with the C-19 hydroxyl.⁷⁾ The spectral data of the tetraacetate (**8**), a white powder, $[\alpha]_D -18.6^\circ$, derived from **2** by acetylation with Ac₂O-pyridine at room temperature for 20 min, were also consistent with the above evidence, that is, the ¹H NMR spectrum (CDCl₃) showed the presence of four acetyl signals at δ 2.00, 2.03, 2.05 and 2.09 and the electron impact mass spectrum (EI-MS) exhibited a peak due to the terminal peracetylated hexosyl cation (*m/z* 331), indicating that the glucosyl moiety should be linked to the C-19 hydroxyl. Moreover, a doublet signal with a *J*=7 Hz at δ 4.49 indicated that the glucosyl linkage has the β -configuration. Consequently, **2** can be represented as cinnassiol D₁ 19-O- β -D-glucopyranoside.

Cinnassiol D₂ (**3**), a white powder, $[\alpha]_D -15.4^\circ$, was formulated as C₂₀H₃₂O₆ based on elementary analysis and the EI-MS spectrum (*m/z* 368 (M⁺)); it contains one more oxygen than **1**. The ¹H NMR spectrum of **3** showed a pattern similar to that of **1** and a comparison of both ¹H NMR spectrum (*d*₅-pyridine) suggested the following signal assignments in the ¹H NMR spectrum of **3**; δ 1.27 (3H, s, 9-CH₃), 1.41 (3H, d, *J*=7 Hz, 18-CH₃), 1.68 (3H, s, 1-CH₃), 1.74 (3H, s, 12-CH₃), 3.85 (2H, d, *J*=9 Hz, 19-H₂), 4.49 (1H, d, *J*=2 Hz, 6-H). Since irradiation of a doublet signal at δ 4.49 (6-H) changed a broad singlet signal at δ 2.75 to a sharp singlet, the signal at δ 2.75 was assignable to 5-H and consequently the carbons at C-1 and -4 should be quaternary ones.

Therefore, the structure of **3** was supposed to be 4-hydroxy cinnassiol D₁. The 11-O-





- 1: $R=R'=R''=H$
 2: $R=R'=H$, $R''=\beta\text{-D-gluc}\cdot\text{pyr-}$
 3: $R=OH$, $R'=R''=H$
 4: $R=OH$, $R'=H$, $R''=\beta\text{-D-gluc}\cdot\text{pyr-}$
 6: $R=R'=H$, $R''=Ac$
 7: $R=H$, $R'=Ac$, $R''=p\text{-Br}\cdot\text{C}_6\text{H}_4\cdot\text{SO}_2\text{-}$
 8: $R=R'=H$, $R''=2',3',4',6'\text{-tetra-O-acetyl-}\beta\text{-D-gluc}\cdot\text{pyr-}$
 9: $R=OH$, $R'=H$, $R''=Ac$
 11: $R=OH$, $R'=H$, $R''=2',3',4',6'\text{-tetra-O-acetyl-}\beta\text{-D-gluc}\cdot\text{pyr-}$

- 5: $R=R'=H$
 12: $R=R'=Ac$
 13: $R=H$, $R'=CH_3$

the ^{13}C NMR spectrum (CDCl_3) the signals due to the C-19 and C-18 were shifted by +10.8 and -3.7 ppm, respectively, supporting the above ^1H NMR and EI-MS evidence.

Cinnassiol D₃ (5), a white powder, $\text{C}_{20}\text{H}_{32}\text{O}_6$, $[\alpha]_D -10.1^\circ$, has the same molecular formula as 3, but its *R_f* value on thin-layer chromatography (TLC) is more smaller than that of 3. 5 was acetylated with Ac_2O -pyridine at room temperature for 30 min to yield the diacetate (12) of 5, a white powder, $\text{C}_{24}\text{H}_{36}\text{O}_8$, $[\alpha]_D -10.1^\circ$, FD-MS (m/z): 452 (M^+). A comparison of signals in the ^1H NMR spectrum (d_5 -pyridine) of 12 with those of 9 led us to make the following assignments; δ 1.25 (3H, d, $J=6$ Hz, *sec*- CH_3), 1.28 (3H, s, 9- CH_3), 1.31 (3H, d, $J=7$ Hz, 18- CH_3), 1.70 (3H, s, 12- CH_3), 2.00, 2.03 (each 3H, s, $\text{OAc}\times 2$), 4.04, 4.25 (each 1H, d d, $J=7$, 11 Hz, 19- H_2), 4.39 (1H, br s, 6-H), 5.60 (1H, d d d, $J=8$, 8, 8 Hz, $>\text{CH-OAc}$). Taking into account the ^{13}C NMR data for 12, the structure of 5 has a *sec*- CH_3 at C-1 and a new *sec*. hydroxyl at either C-2 or C-3 in place of the structure of 3. The location of the *sec*. hydroxyl was deter-

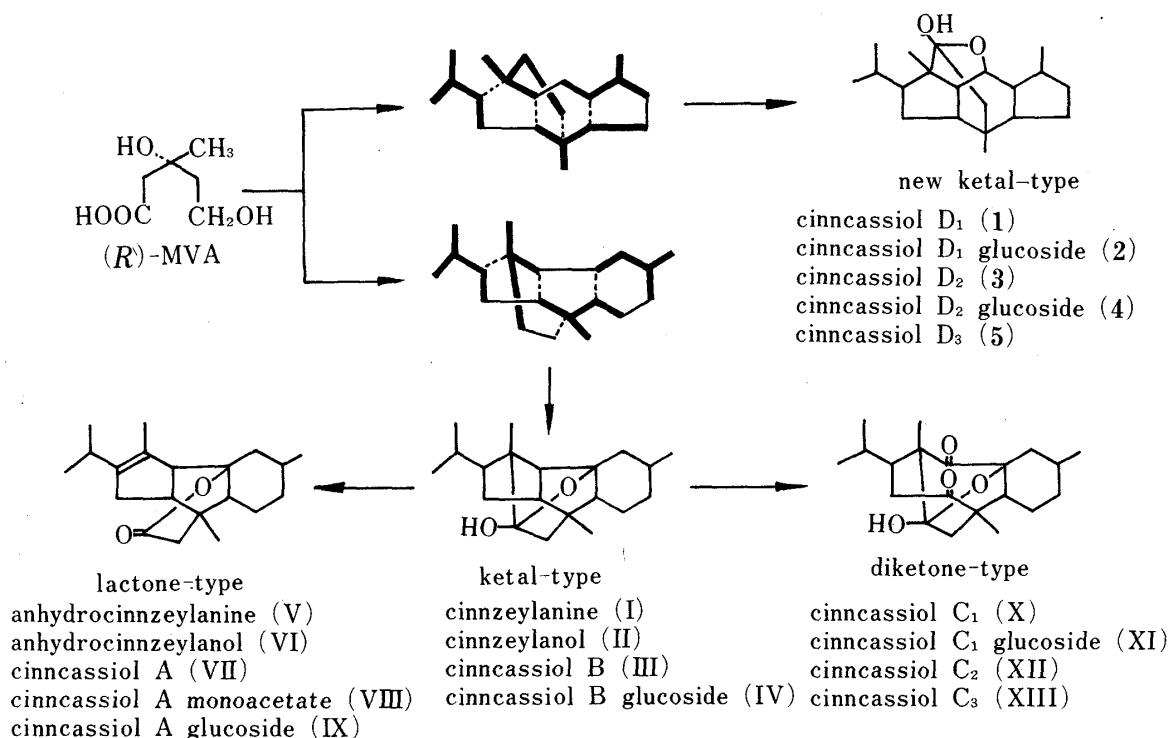


Chart 1. Possible Biogenesis of Diterpenes so far obtained from Cinnamomi Cortex

mined to be at C-2 by irradiation on 6-H→5-H→1-H→2-H→3-H in turn. The 19-O-monomethyl ether (13), $C_{21}H_{34}O_6$, derived from 5 by Kuhn's methylation, was reacted with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid (*p*-TsOH) to yield the 2,4-acetonide (14), $C_{24}H_{38}O_6$. Since the configuration of the hydroxyl at C-4 was assumed to be β because the methyl signal at C-9 appeared at δ 1.28 (in the almost same range as that of 9) in the 1H NMR spectrum, the hydroxyl function at C-2 was also supposed to be in the β -configuration.

Consequently, the structure of cinncassiol D_3 can be represented as shown in the formula 5, though the configuration at C-1 remains to be determined.

Cinncassiol D_1 (1), D_1 glucoside (2), D_2 (3), D_2 glucoside (4) and D_3 (5) are unique pentacyclic diterpenes with a new skeleton.

A possible biogenetic route to the diterpenes so far isolated from Cinnamomi Cortex is shown in Chart 1.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus (a hot-stage type) and are uncorrected. The specific rotations were measured with a Union Giken PM-201 automatic digital polarimeter. The IR spectra were obtained with a JASCO DS-701 spectrometer. The 1H NMR and ^{13}C NMR spectra were recorded with JEOL JNM-PS-100 (100 MHz) and JEOL JNM-FX-90Q (22.5 MHz) spectrometers, respectively, with tetramethylsilane as an internal standard. Mass spectra (FD and EI) were recorded on a JEOL JMS-D-300 mass spectrometer. Silica gel (Kieselgel 60; Merck) was used for column chromatography. TLC was carried out on Merck plates precoated with Kieselgel 60 using a, $CHCl_3$ -MeOH-water (7:3:0.2); b, $CHCl_3$ -MeOH-water (8:2:0.2); c, $CHCl_3$ -MeOH (10:1); d, *n*-hexane-acetone (1:1), as solvent systems. Detection was done by spraying 10% H_2SO_4 followed by heating and UV irradiation ($\lambda=366$ nm). Paper-partition chromatography (PPC) for sugar was conducted on Toyo Roshi No. 50 paper using the upper layer of *n*-BuOH-pyridine-water (6:2:3)+pyridine (1) as a solvent and aniline hydrogen phthalate as a staining agent.

Cinncassiol D_1 (1)—A white powder, *Rf* 0.67 (solv. a), $[\alpha]_D^{25} -11.6^\circ$ ($c=0.86$, MeOH). IR ν_{max}^{KBr} cm^{-1} : 3400 (OH). *Anal.* Calcd for $C_{20}H_{32}O_5$: C, 68.15; H, 9.15. Found: C, 68.22; H, 9.18. FD-MS (m/z): 352 (M^+). EI-MS (m/z): 352 (M^+), 334, 319, 316, 303, 275, 257, 216, 197, 179, 167, 157, 149, 121, 108. 1H NMR (δ , d_5 -pyridine) (ppm): 0.90 (3H, s, 9- CH_3), 1.37 (3H, d, $J=6$ Hz, 18- CH_3), 1.69 (6H, s, 1- and 12- CH_3), 3.80 (2H, d, $J=9$ Hz, 19- H_2), 4.44 (1H, br s, 6-H).

Cinncassiol D_1 19-O-Monoacetate (6)—1 (22 mg) was treated with Ac_2O (2 ml) and pyridine (2 ml) at room temperature for 25 min to give the monoacetate (6), a white powder (16 mg), $[\alpha]_D^{25} -10.9^\circ$ ($c=0.79$, MeOH). *Anal.* Calcd for $C_{22}H_{34}O_6$: C, 66.98; H, 8.69. Found: C, 66.72; H, 8.66. 1H NMR ($CDCl_3$) δ (ppm): 0.93 (3H, s, 9- CH_3), 1.00 (3H, d, $J=7$ Hz, 18- CH_3), 1.12 (3H, s, 12- CH_3), 1.38 (3H, s, 1- CH_3), 2.08 (3H, s, 19-OAc), 3.78—3.96 (3H, m, 6-H and 19- H_2); (d_5 -pyridine): 0.94 (3H, s, 9- CH_3), 1.29 (3H, d, $J=7$ Hz, 18- CH_3), 1.65, 1.70 (each 3H, s, 1- and 12- CH_3), 2.01 (3H, s, OAc), 4.01, 4.21 (each 1H, d d, $J=7, 11$ Hz, 19- H_2), 4.45 (1H, br s, 6-H).

11-Monoacetyl Cinncassiol D_1 19-O-Monobrosylate (7)—A mixture of 1 (63 mg) and *p*-bromobenzene-sulfonyl chloride (150 mg) in pyridine (5 ml) was kept standing overnight at room temperature. Then, water was added to the reaction mixture, the product was extracted with *n*-BuOH and the solvent was evaporated off *in vacuo* to leave a residue which was chromatographed on silica gel with *n*-hexane-AcOEt (2:3→1:2) as the solvent to give the monobrosylate of 1 (*Rf* 0.30, solv. d). The monobrosylate was acetylated with Ac_2O -pyridine (3 ml each) at room temperature overnight. After usual work-up, the product was purified by silica gel column chromatography with $CHCl_3$ -MeOH (60:1) as the solvent to yield the monoacetyl monobrosylate (7) of 1 (25 mg). A single crystal suitable for an X-ray diffraction study was obtained by recrystallization from dil.MeOH. Colorless plates, mp 104—105°C, $[\alpha]_D^{18} \simeq 0^\circ$ ($c=0.59$, MeOH), *Rf* 0.40 (solv. d). *Anal.* Calcd for $C_{28}H_{37}SO_8 \cdot H_2O$: C, 53.25; H, 6.22. Found: C, 53.57; H, 6.38. 1H NMR ($CDCl_3$) δ (ppm): 0.90, 1.07, 1.38 (each 3H, s, 3 $\times$ *tert*. CH_3), 0.95 (3H, d, $J=7$ Hz, *sec*. CH_3), 2.03 (3H, s, OAc), 3.87 (2H, d, $J=6$ Hz, $-CH_2-O-$), 3.90 (1H, d, $J=2$ Hz, $>CH-O-$), 7.65, 7.78 (each 2H, d, $J=10$ Hz, 4 $\times$ arom. proton).

Cinncassiol D_1 Glucoside (2)—A white powder, *Rf* 0.33 (solv. a), $[\alpha]_D^{18} -4.1^\circ$ ($c=0.29$, MeOH), IR ν_{max}^{KBr} cm^{-1} : 3380 (OH). *Anal.* Calcd for $C_{26}H_{42}O_{10}$: C, 60.68; H, 8.23. Found: C, 60.37; H, 8.37. FD-MS (m/z): 553 ($M+K^+$), 537 ($M+Na^+$).

Enzymatic Hydrolysis of Cinncassiol D_1 Glucoside (2)—A mixture of 2 (22 mg) and crude hesperidinase (8 mg) (Tanabe Pharm. Co., Ltd.) in dist. water (5 ml) was incubated at 39°C for 4 h. The reaction mixture was evaporated to dryness under reduced pressure to give a residue, to which MeOH was added.

The solution was filtered and the filtrate was subjected to Sephadex LH-20 chromatography. Elution with MeOH afforded the aglycone, a white powder, $[\alpha]_D^{17} -10.9^\circ$ ($c=0.32$, MeOH), EI-MS (m/z): 352 (M^+),

identical with cinncassiol D₁ (1) and the sugar, a syrup, *Rf* 0.39 (on TLC, solv. CHCl₃-MeOH-acetone-water=3:3:3:1), *Rf* 0.44 (on PPC), $[\alpha]_D^{25} + 42.6^\circ$ ($c=0.28$, water), identical with D-glucose.

2',3',4',6'-Tetra-O-Acetyl Cinncassiol D₁ Glucoside (8)—2 (12 mg) was acetylated with Ac₂O (3 ml) and pyridine (4 ml) at room temperature for 20 min to yield the tetraacetate (8) of 2. A white powder (10 mg), *Rf* 0.33 (solv. c), $[\alpha]_D^{25} - 18.6^\circ$ ($c=1.08$, MeOH). *Anal.* Calcd for C₃₄H₅₀O₁₄: C, 59.81; H, 7.38. Found: C, 59.66; H, 7.36. EI-MS (*m/z*): 664 (M⁺-H₂O), 646, 628, 584, 331.102 (C₁₄H₁₉O₉⁺=331.101, terminal peracetylated hexosyl cation), 169, 109. ¹H NMR (CDCl₃) δ (ppm): 0.92 (3H, s, 9-CH₃), 0.93 (3H, d, *J*=7 Hz, 18-CH₃), 1.09 (3H, s, 12-CH₃), 1.38 (3H, s, 1-CH₃), 2.00, 2.03, 2.05, 2.09 (each 3H, s, OAc $\times$ 4), 3.60—3.84 (3H, m, 5'-H and 19-H₂), 4.49 (1H, d, *J*=7 Hz, 1'-H). ¹³C NMR (CDCl₃) δ (ppm): 8.7, 12.9, 24.1, 24.6, 25.2, 27.8, 33.8, 37.2, 39.8, 40.0, 40.2, 47.7, 50.4, 53.6, 56.5, 62.0 (C-6'), 68.5 (C-4'), 71.5 (C-2'), 71.7 (C-5'), 72.8 (C-3'), 75.1, 76.7, 83.1, 88.8, 101.0 (C-1'), 106.9. The acetyl signals are not given.

Cinncassiol D₂ (3)—A white powder, *Rf* 0.56 (solv. a), $[\alpha]_D^{25} - 15.4^\circ$ ($c=0.94$, MeOH). *Anal.* Calcd for C₂₀H₃₂O₆: C, 65.19; H, 8.75. Found: C, 65.44; H, 8.62. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3390 (OH). EI-MS (*m/z*): 368 (M⁺), 273, 255, 215, 185, 175, 166, 157, 149. ¹H NMR (*d*₅-pyridine) δ (ppm): 1.27 (3H, s, 9-CH₃), 1.41 (3H, d, *J*=7 Hz, 18-CH₃), 1.68 (3H, s, 1-CH₃), 1.74 (3H, s, 12-CH₃), 2.75 (1H, br s, 5-H), 3.85 (2H, d, *J*=9 Hz, 19-H₂), 4.49 (1H, d, *J*=2 Hz, 6-H).

Cinncassiol D₂ 19-O-Monoacetate (9)—3 (16 mg) was treated with Ac₂O (2 ml) and pyridine (4 ml) at room temperature for 30 min to yield the 19-O-monoacetate (9) of 3. A white powder (13 mg), *Rf* 0.15 (solv. c), $[\alpha]_D^{25} - 12.7^\circ$ ($c=1.10$, MeOH). *Anal.* Calcd for C₂₂H₃₄O₇: C, 64.37; H, 8.35. Found: C, 64.26; H, 8.33. EI-MS (*m/z*): 410 (M⁺), 334, 315, 274, 255, 215, 213, 197, 139, 137, 121. ¹H NMR (*d*₅-pyridine) δ (ppm): 1.29 (3H, s, 9-CH₃), 1.31 (3H, d, *J*=7 Hz, 18-CH₃), 1.65 (1H, s, 12-CH₃), 1.68 (3H, s, 1-CH₃), 2.04 (3H, s, OAc $\times$ 1), 2.73 (1H, d, *J*=2 Hz, 5-H), 4.06 (1H, d d, *J*=7, 11 Hz, 19-H), 4.25 (1H, d d, *J*=7, 11 Hz, 19-H'), 4.46 (1H, d, *J*=2 Hz, 6-H). ¹³C NMR (*d*₅-pyridine) δ (ppm): 10.3 (q), 13.6 (q), 20.8 (d), 22.4 (q), 24.5 (q), 26.4 (t), 33.8 (d), 36.2 (t), 41.2 (d), 41.6 (t), 42.1 (s), 44.2 (t), 49.2 (d), 57.4 (s), 62.3 (d), 69.8 (t), 76.1 (d), 81.3 (s), 87.1 (s), 89.0 (s), 107.3 (s), 170.5 (s).

1,4-Cyclic Carbonate (10) of 11-O-Acetyl Cinncassiol D₂ 19-O-Monobrosylate—11-O-Monoacetyl cinncassiol D₂ 19-O-monobrosylate was prepared from 3 in the manner described for 7. The resulting monoacetyl monobrosylate (15 mg) was treated with COCl₂-ether (0.3 ml) at room temperature for 30 min, then poured into ice-water. The resulting precipitates were collected and purified by silica gel column chromatography. Elution with *n*-hexane-acetone (2:1) gave the carbonate (10). A white powder (8 mg), *Rf* 0.51 (solv. d), *Anal.* Calcd for C₂₉H₃₅SO₁₀Br: C, 53.13; H, 5.38. Found: C, 53.44; H, 5.44. EI-MS (*m/z*): 612 (M⁺-C₂H₂O), 594 (M⁺-AcOH).

Cinncassiol D₂ Glucoside (4)—A white powder, *Rf* 0.25 (solv. a), $[\alpha]_D^{17} - 3.2^\circ$ ($c=0.44$, MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3400 (OH). FD-MS (*m/z*): 531 (M⁺+1), 513 (M⁺-OH). *Anal.* Calcd for C₂₆H₄₂O₁₁: C, 58.85; H, 7.98. Found: C, 58.61; H, 7.99.

Enzymatic Hydrolysis of 4—A mixture of 4 (18 mg) and crude hesperidinase (10 mg) in dist. water (3 ml) was incubated at 38°C for 3 h. After treatment in the same manner as for 2, the aglycone was obtained as a white powder (8 mg), *Rf* 0.56 (solv. a), identical with cinncassiol D₂ (3), together with D-glucose, a syrup, *Rf* 0.39 (on TLC, solv. CHCl₃-MeOH-acetone-water=3:3:3:1), *Rf* 0.45 (on PPC), $[\alpha]_D^{19} + 47.6^\circ$ ($c=0.42$, water).

2',3',4',6'-Tetra-O-acetyl Cinncassiol D₂ Glucoside (11)—4 (12 mg) was acetylated with Ac₂O (3 ml) and pyridine (4 ml) at room temperature for 30 min to give the tetraacetate (11) of 4. A white powder (10 mg), *Rf* 0.23 (solv. c), $[\alpha]_D^{25} - 17.5^\circ$ ($c=0.57$, MeOH). *Anal.* Calcd for C₃₄H₅₀O₁₅: C, 58.44; H, 7.21. Found: C, 58.61; H, 7.27. EI-MS (*m/z*): 662 (M⁺-2H₂O), 331 (terminal peracetylated hexosyl cation), 271, 255, 237, 223, 169, 109. ¹H NMR (CDCl₃) δ (ppm): 0.94 (3H, d, *J*=7 Hz, 18-CH₃), 0.98 (3H, s, 9-CH₃), 1.09 (3H, s, 12-CH₃), 1.37 (3H, s, 1-CH₃), 2.03, 2.05, 2.10, 2.17 (each 3H, s, OAc $\times$ 4), 3.62—4.85 (3H, m, 5'-H and 19-H₂), 4.50 (1H, d, *J*=7 Hz, 1'-H).

Cinncassiol D₃ (5)—A white powder, *Rf* 0.45 (solv. a), $[\alpha]_D^{27} - 10.1^\circ$ ($c=0.79$, MeOH). *Anal.* Calcd for C₂₀H₃₂O₆: C, 65.19; H, 8.75. Found: C, 65.37; H, 8.72. ¹H NMR (*d*₅-pyridine) δ (ppm): 1.27 (3H, s, 9-CH₃), 1.40 (3H, d, *J*=7 Hz, 1-CH₃), 1.49 (3H, d, *J*=6 Hz, 18-CH₃), 1.74 (3H, s, 12-CH₃), 3.84 (2H, d, *J*=8 Hz, 19-H₂).

Cinncassiol D₃ 2,19-Diacetate (12)—5 (25 mg) was acetylated with Ac₂O (3 ml) and pyridine (5 ml) at room temperature for 30 min to give the diacetate (12) of 5. A white powder (23 mg), *Rf* 0.42 (solv. c), $[\alpha]_D^{27} - 10.1^\circ$ ($c=0.79$, MeOH). *Anal.* Calcd for C₂₄H₃₆O₈: C, 63.70; H, 8.02. Found: C, 63.91; H, 7.98. FD-MS (*m/z*): 452 (M⁺). ¹H NMR (*d*₅-pyridine) δ (ppm): 1.25 (3H, d, *J*=6 Hz, 1-CH₃), 1.28 (3H, s, 9-CH₃), 1.31 (3H, d, *J*=7 Hz, 18-CH₃), 1.70 (3H, s, 12-CH₃), 2.00, 2.03 (each 3H, s, OAc $\times$ 2), 2.80—2.96 (2H, m, 1- and 5-H), 3.20 (1H, d d, *J*=8, 14 Hz, 3-H), 4.04, 4.25 (each 1H, d d, *J*=7, 11 Hz, 19-H₂), 4.39 (1H, br s, 6-H), 5.60 (1H, d d d, *J*=8, 8, 8 Hz, 2-H).

Cinncassiol D₃ 19-O-Monomethylether (13)—A mixture of 5 (18 mg), Ag₂O (30 mg), CH₃I (2 ml) and dimethylformamide (1 ml) was stirred at room temperature for 3 h. After usual work-up, the product was purified by silica gel column chromatography. Elution with CHCl₃-MeOH (20:1) gave the monomethylether (13) of 5. A syrup (12 mg), *Rf* 0.38 (solv. b). *Anal.* Calcd for C₂₁H₃₄O₆: C, 65.94; H, 8.96. Found: C, 65.77; H, 9.08.

19-O-Monomethyl Cinnassiol D₃ 2,4-Acetonide (14)—A solution of **13** (10 mg), 2,2-dimethoxypropane (2 ml) and *p*-TsOH (1 mg) was stirred for 2.5 h at room temperature. After neutralization with aq. NaHCO₃ solution, the reaction mixture was concentrated under reduced pressure to give a residue which was chromatographed on a silica gel column. Elution with CHCl₃–MeOH (25: 1) gave 19-O-monomethyl cinnassiol D₃ 2,4-acetonide (**14**). A white powder (5 mg), *R_f* 0.55 (solv. b). *Anal.* Calcd for C₂₄H₃₈O₆: C, 60.22; H, 9.07. Found: C, 59.98; H, 9.00.

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