[Chem. Pharm. Bull. 29(9)2451—2459(1981)]

Constituents of Cinnamomi Cortex. V.[1] Structures of Five Novel Diterpenes, Cinncassiols D_1, D_1 Glucoside, D_2, D_2 Glucoside and D_3

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(Received February 12, 1981)

The structures of cinncassiol D_1 (**1**), cinncassiol D_1 glucoside (**2**), cinncassiol D_2 (**3**), cinncassiol D_2 glucoside (**4**) and cinncassiol D_3 (**5**), isolated from the fraction exhibiting anti-complement activity of the water extractive of Cinnamomi Cortex ("Tōkō 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 D_1; cinncassiol D_1 glucoside; cinncassiol D_2; cinncassiol D_2 glucoside; cinncassiol D_3; X-ray analysis

The isolation of a series of diterpenes (compounds I—XIII) from the fraction exhiviting anti-complement activity[2] of the water extractive of Cinnamomi Cortex ("Kannan Keihi" and "Tōkō 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 D_1 (**1**), D_1 glucoside (**2**),[5] D_2 (**3**), D_2 glucoside (**4**) and D_3 (**5**), which had been isolated[6] from the water extractive of "Tōkō Keihi." They are closely related to each other structurally and belong to a new type different from the above three groups.

Cinncassiol D_1 (**1**), a white powder, $[\alpha]_D$ $-11.6°$, $C_{20}H_{32}O_5$ (field desorption mass (FD-MS) spectrum (*m/z*) 352 (M^+)), showed signals due to three *tert.* CH_3 (δ 0.90 and 2×1.69), a *sec.*CH_3 (δ 1.37, d, $J=6$ Hz), a $-CH_2-O-$ (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, $C_{22}H_{34}O_6$, $[\alpha]_D$ $-10.9°$, derived from **1** by acetylation with Ac_2O–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 ^{13}C nuclear magnetic resonance (^{13}C NMR) spectrum (Table II) exhibited peaks due to $CH_3\times4$, $CH_2\times4$, $>CH\times5$, $>C<\times2$, $-CH_2-O-\times1$, $>CH-O-\times1$, $>C-O-\times2$ and $>C<\begin{matrix}O-\\O-\end{matrix}\times1$. On the basis of a comparisn of the ^1H NMR and ^{13}C 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 Ac_2O–pyridine at room temperature overnight to yield the monoacetyl monobrosylate (brosylate=*p*-bromobenzenesulfonate) (**7**) of **1**, colorless plates, mp 104—105°C, $[\alpha]_D$ $\simeq0°$, ^1H NMR ($CDCl_3$) δ: 2.03 (3H, s, -OAc), 7.65, 7.78 (each 2H, d, $J=10$ Hz, $4\times$arom.

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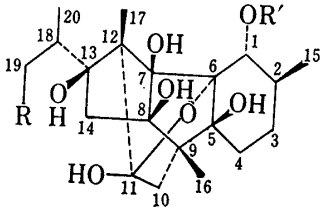
Table I. Diterpenes obtained from Cinnamomi Cortex

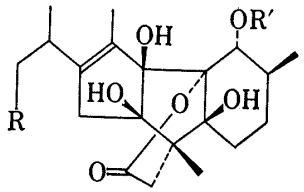
Ketal-type



I R=H, R′=Ac
cinnzeylanine[3,4a]
II R=R′=H
cinnzeylanol[3,4a]

III R=OH, R′=H
cinncassiol B[4c]
IV R=-O-β-D-glc·pyr, R′=H
cinncassiol B[4c] glucoside

Lactone-type

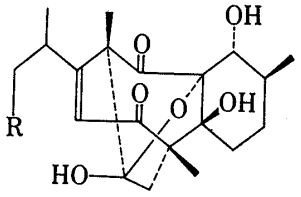


V R=H, R′=Ac
anhydrocinnzeylanine[3,4a]
VI R=R′=H
anhydrocinnzeylanol[3,4a]

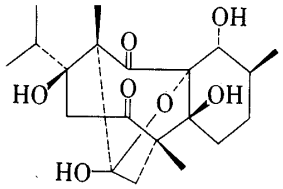
VII R=OH, R′=H
cinncassiol A[4a]
VIII R=OAc, R′=H
cinncassiol A monoacetate

IX R=-O-β-D-glc·pyr, R′=H
cinncassiol A glucoside[4a]

Diketone-type



X R=OH
cinncassiol C_1[4b]
XI R=-O-β-D-glc·pyr
cinncassiol C_1 glucoside[1]
XII R=H
cinncassiol C_2[1]



XIII cinncassiol C_3[1,3]

Table II. ^{13}C 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_5-pyridine for **1**, **2**, **3**, **12** and in $CDCl_3$ for **11**.

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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, $\beta=96.91(4)°$, $V=1579.6$ Å^3; $D_{(calcd.)}=1.33$, $D_{(obsd.)}=1.37$ g/cm^3. The cell dimensions and intensities were measured with a Syntex R_3 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)						

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Atoms	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{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 10^4 for non-hydrogen atoms and by 10^3 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 Å^2)

Therefore, the chemical structure of cinncassiol D_1 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 C_5–C_6 into the C_5–C_1 bond in the ketal-type diterpene cinnzeylanol (II), for example.

Cinncassiol D_1 glucoside (**2**), a white powder, $[\alpha]_D$ −4.1°, $C_{26}H_{42}O_{10}$ (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 cinncassiol D_1 (**1**) and D-glucose. Therefore, **2** is composed with each one mole of **1** and D-glucose. A comparison of the ^{13}C 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°, derived from **2** by acetylation with Ac_2O–pyridine at room temperature for 20 min, were also consistent with the above evidence, that is, the ^1H NMR spectrum ($CDCl_3$) 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 cinncassiol D_1 19-O-β-D-glucopyranoside.

Cinncassiol D_2 (**3**), a white powder, $[\alpha]_D$ −15.4°, was formulated as $C_{20}H_{32}O_6$ 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-CH_3), 1.41 (3H, d, J=7 Hz, 18-CH_3), 1.68 (3H, s, 1-CH_3), 1.74 (3H, s, 12-CH_3), 3.85 (2H, d, J=9 Hz, 19-H_2), 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 cinncassiol D_1. The 11-O-

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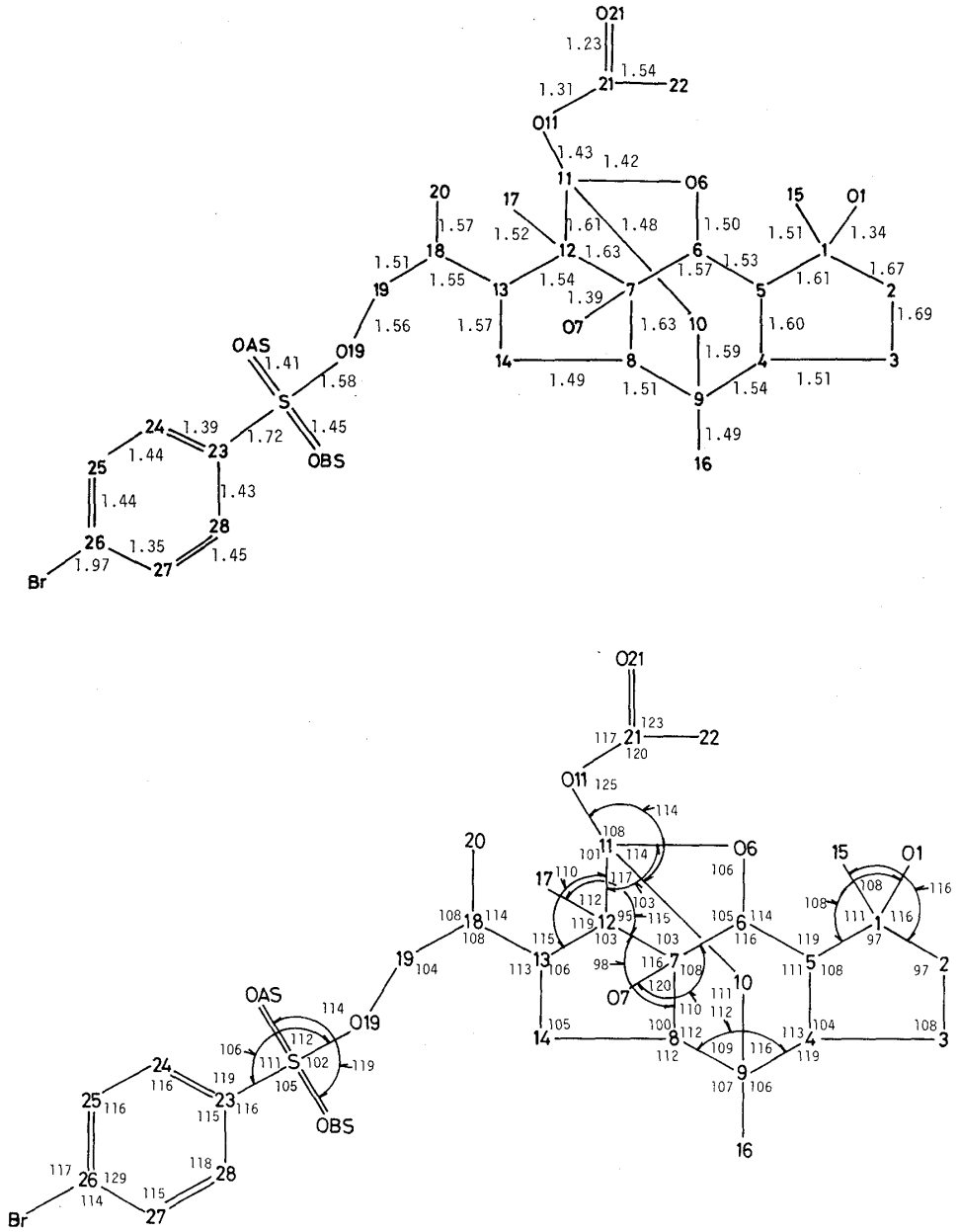

Fig. 1. Bond Lengths (Å) and Angles (°)

monoacetyl 19-O-monobrosylate derived from **3** in the same way as for **7** was treated with $COCl_2$–ether to give the 1,4-cyclic carbonate (**10**), $C_{29}H_{35}SO_{10}Br$. Since the configuration of the hydroxyl at C-1 was assumed to be β because the methyl signal at C-1 appeared at δ 1.68 (in the almost same range as that of **1**) in the ^1H NMR spectrum, the hydroxyl functions at C-4 was also supposed to be β.

Cinncassiol D_2 glucoside (**4**), a white powder, $[\alpha]_D$ −3.2°, $C_{26}H_{42}O_{11}$ (FD–MS (*m/z*): 531 (M^+ +1), 513 (M^+ −OH)), was decomposed into cinncassiol D_2 (**3**) and D-glucose on enzymatic hydrolysis, as in the case of **2**. Furthermore, the location and the mode of linkage of the glucosyl moiety were determined in the same way as for **2**. Namely, the tetraacetate (**11**), a white powder, $C_{34}H_{50}O_{15}$, $[\alpha]_D$ −17.5°, derived from **4** in the same way as for **8**, showed four acetyl signals (δ 2.03, 2.05, 2.10 and 2.17) in the ^1H NMR spectrum ($CDCl_3$) and a peak at *m/z* 331 originated from the terminal peracetylated hexosyl cation in the EI–MS spectrum, indicating that the glucosyl residue should be linked to the C-19 hydroxyl. In addition, in

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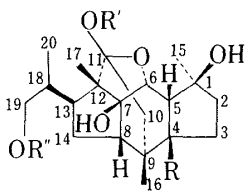

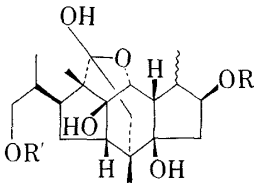

1: R=R′=R″=H
2: R=R′=H, R″=β-D-glc·pyr-
3: R=OH, R′=R″=H
4: R=OH, R′=H, R″=β-D-glc·pyr-
6: R=R′=H, R″=Ac
7: R=H, R′=Ac, R″=*p*-Br·C_6H_4·SO_2-
8: R=R′=H, R″=2′,3′,4′,6′-tetra-O-acetyl-β-D-glc·pyr-
9: R=OH, R′=H, R″=Ac
11: R=OH, R′=H, R″=2′,3′,4′,6′-tetra-O-acetyl-β-D-glc·pyr-

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.

Cinncassiol D_3 (**5**), a white powder, $C_{20}H_{32}O_6$, $[\alpha]_D$ −10.1°, has the same molecular formula as **3**, but its *Rf* 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, $C_{24}H_{36}O_8$, $[\alpha]_D$ −10.1°, 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, OAc×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, >C<u>H</u>–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-

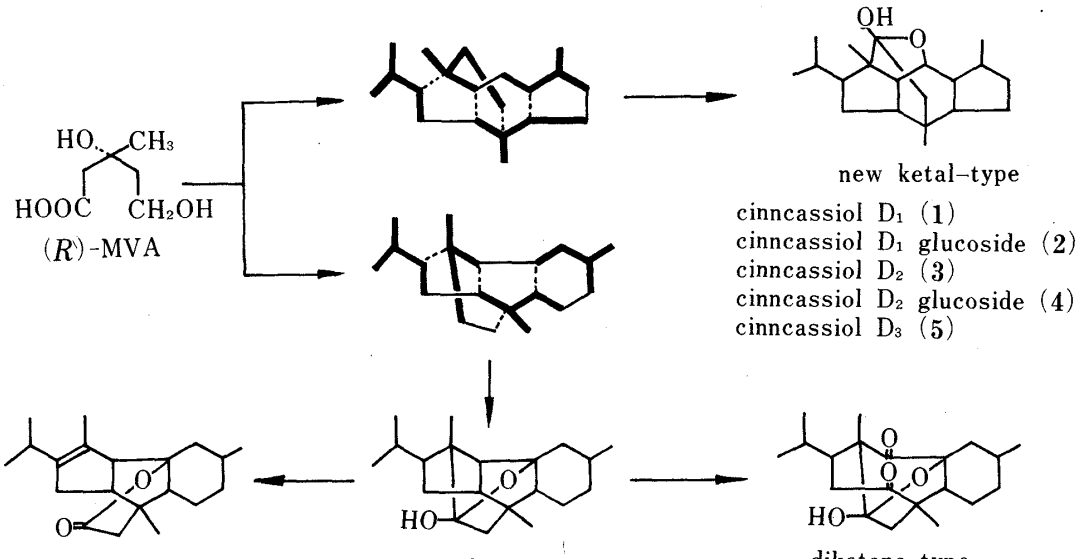

Chart 1. Possible Biogenesis of Diterpenes so far obtained from Cinnamomi Cortex

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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 ^{1}H 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 ^{1}H 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 (λ=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), $[\alpha]_D^{26}$ −11.6° (*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. ^{1}H NMR (d_5-pyridine) δ (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° (*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. ^{1}H 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*-bromobenzenesulfonyl 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, $[\alpha]_D^{18} \simeq 0°$ (*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. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.90, 1.07, 1.38 (each 3H, s, 3×*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×arom. proton).

Cinncassiol D_1 Glucoside (**2**)——A white powder, *Rf* 0.33 (solv. a), $[\alpha]_D^{12}$ −4.1° (*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° (*c*=0.32, MeOH), EI-MS (*m/z*): 352 (M^+),

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identical with cinncassiol D_1 (**1**) and the sugar, a syrup, *Rf* 0.39 (on TLC, solv. $CHCl_3$–MeOH–acetone–water=3:3:3:1), *Rf* 0.44 (on PPC), $[\alpha]_D^{17}$ +42.6° (*c*=0.28, water), identical with D-glucose.

2′,3′,4′,6′-Tetra-O-Acetyl Cinncassiol D_1 Glucoside (8)——**2** (12 mg) was acetylated with Ac_2O (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), $[\alpha]_D^{22}$ −18.6° (*c*=1.08, MeOH). *Anal.* Calcd for $C_{34}H_{50}O_{14}$: C, 59.81; H, 7.38. Found: C, 59.66; H, 7.36. EI-MS (*m/z*): 664 (M^+-H_2O), 646, 628, 584, 331.102 ($C_{14}H_{19}O_9^+$=331.101, terminal peracetylated hexosyl cation), 169, 109. ^1H NMR ($CDCl_3$) δ (ppm): 0.92 (3H, s, 9-CH_3), 0.93 (3H, d, *J*=7 Hz, 18-CH_3), 1.09 (3H, s, 12-CH_3), 1.38 (3H, s, 1-CH_3), 2.00, 2.03, 2.05, 2.09 (each 3H, s, OAc×4), 3.60—3.84 (3H, m, 5′-H and 19-H_2), 4.49 (1H, d, *J*=7 Hz, 1′-H). ^{13}C NMR ($CDCl_3$) 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 D_2 (3)——A white powder, *Rf* 0.56 (solv. a), $[\alpha]_D^{16}$ −15.4° (*c*=0.94, MeOH). *Anal.* Calcd for $C_{20}H_{32}O_6$: C, 65.19; H, 8.75. Found: C, 65.44; H, 8.62. IR ν_{max}^{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-CH_3), 1.41 (3H, d, *J*=7 Hz, 18-CH_3), 1.68 (3H, s, 1-CH_3), 1.74 (3H, s, 12-CH_3), 2.75 (1H, br s, 5-H), 3.85 (2H, d, *J*=9 Hz, 19-H_2), 4.49 (1H, d, *J*=2 Hz, 6-H).

Cinncassiol D_2 19-O-Monoacetate (9)——**3** (16 mg) was treated with Ac_2O (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° (*c*=1.10, MeOH). *Anal.* Calcd for $C_{22}H_{34}O_7$: 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-CH_3), 1.31 (3H, d, *J*=7 Hz, 18-CH_3), 1.65 (1H, s, 12-CH_3), 1.68 (3H, s, 1-CH_3), 2.04 (3H, s, OAc×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). ^{13}C 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 D_2 19-O-Monobrosylate——11-O-Monoacetyl cinncassiol D_2 19-O-monobrosylate was prepared from **3** in the manner described for **7**. The resulting monoacetyl monobrosylate (15 mg) was treated with $COCl_2$–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 $C_{29}H_{35}SO_{10}Br$: C, 53.13; H, 5.38. Found: C, 53.44; H, 5.44. EI-MS (*m/z*): 612 ($M^+-C_2H_2O$), 594 (M^+—AcOH).

Cinncassiol D_2 Glucoside (4)——A white powder, *Rf* 0.25 (solv. a), $[\alpha]_D^{17}$ −3.2° (*c*=0.44, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH). FD-MS (*m/z*): 531 (M^++1), 513 (M^+–OH). *Anal.* Calcd for $C_{26}H_{42}O_{11}$: 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 D_2 (**3**), together with D-glucose, a syrup, *Rf* 0.39 (on TLC, solv. $CHCl_3$–MeOH–acetone–water=3:3:3:1), *Rf* 0.45 (on PPC), $[\alpha]_D^{18}$ +47.6° (*c*=0.42, water).

2′,3′,4′,6′-Tetra-O-acetyl Cinncassiol D_2 Glucoside (11)——**4** (12 mg) was acetylated with Ac_2O (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), $[\alpha]_D^{26}$ −17.5° (*c*=0.57, MeOH). *Anal.* Calcd for $C_{34}H_{50}O_{15}$: C, 58.44; H, 7.21. Found: C, 58.61; H, 7.27. EI-MS (*m/z*): 662 (M^+-2H_2O), 331 (terminal peracetylated hexosyl cation), 271, 255, 237, 223, 169, 109. ^1H NMR ($CDCl_3$) δ (ppm): 0.94 (3H, d, *J*=7 Hz, 18-CH_3), 0.98 (3H, s, 9-CH_3), 1.09 (3H, s, 12-CH_3), 1.37 (3H, s, 1-CH_3), 2.03, 2.05, 2.10, 2.17 (each 3H, s, OAc×4), 3.62—4.85 (3H, m, 5′-H and 19-H_2), 4.50 (1H, d, *J*=7 Hz, 1′-H).

Cinncassiol D_3 (5)——A white powder, *Rf* 0.45 (solv. a), $[\alpha]_D^{27}$ −10.1° (*c*=0.79, MeOH). *Anal.* Calcd for $C_{20}H_{32}O_6$: 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-CH_3), 1.40 (3H, d, *J*=7 Hz, 1-CH_3), 1.49 (3H, d, *J*=6 Hz, 18-CH_3), 1.74 (3H, s, 12-CH_3), 3.84 (2H, d, *J*=8 Hz, 19-H_2).

Cinncassiol D_3 2,19-Diacetate (12)——**5** (25 mg) was acetylated with Ac_2O (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° (*c*=0.79, MeOH). *Anal.* Calcd for $C_{24}H_{36}O_8$: 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-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, OAc×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-H_2), 4.39 (1H, br s, 6-H), 5.60 (1H, d d d, *J*=8, 8, 8 Hz, 2-H).

Cinncassiol D_3 19-O-Monomethylether (13)——A mixture of **5** (18 mg), Ag_2O (30 mg), CH_3I (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 $CHCl_3$–MeOH (20:1) gave the monomethylether (**13**) of **5**. A syrup (12 mg), *Rf* 0.38 (solv. b). *Anal.* Calcd for $C_{21}H_{34}O_6$: C, 65.94; H, 8.96. Found: C, 65.77; H, 9.08.

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19-O-Monomethyl Cinncassiol D_3 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. $NaHCO_3$ solution, the reaction mixture was concentrated under reduced pressure to give a residue which was chromatographed on a silica gel column. Elution with $CHCl_3$–MeOH (25: 1) gave 19-O-monomethyl cinncassiol D_3 2,4-acetonide (**14**). A white powder (5 mg), *Rf* 0.55 (solv. b). *Anal.* Calcd for $C_{24}H_{38}O_6$: C, 60.22; H, 9.07. Found: C, 59.98; H, 9.00.

Acknowledgement We wish to express our thanks to Mr. H. Fujiwara and Mr. K. Goto (Otsuka Pharm. Factory, Inc.) for measurement of the ^{13}C NMR spectra and to the staff of the Analytical Laboratory (Tokushima University) for elemental analysis and for measurement of the EI-MS and ^1H NMR spectra. We are also grateful to Dr. Y. Egawa (Tanabe Pharm. Co., Ltd.) for supplying crude hesperidinase and to Mr. I. Maetani (Kyushu University) for the measurement of FD-MS.

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