

Notes

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Constituents of Cinnamomi Cortex. IV.¹⁾ Structures
of Cinncassiols C₁ Glucoside, C₂ and C₃

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The structures of three novel diterpenes, cinncassiol C₁ glucoside (1), cinncassiol C₂ (2) and cinncassiol C₃ (3), which had been isolated from the fraction exhibiting anti-complement activity of the water extractive of Cinnamomi Cortex, were characterized.

Keywords—Cinnamomi Cortex; Lauraceae; diterpene; cinncassiol C₁ glucoside; cinncassiol C₂; cinncassiol C₃

We have recently described^{1,2)} the structure elucidation of the diterpenes isolated from the fraction exhibiting anti-complement activity³⁾ of the water extractive of Cinnamomi Cortex ("Kannan Keihi," the dried bark of *Cinnamomum cassia* BLUME).

As a part of a program directed towards the elucidation of the active substance(s), we now report the characterization of three additional diterpenes named cinncassiols C₁ glucoside (1), C₂ (2) and C₃ (3).

Cinncassiol C₁ glucoside (1), an amorphous powder, $[\alpha]_D +14.5^\circ$, C₂₆H₃₈O₁₂ (field desorption mass spectrum (FD-MS): 542 (M⁺)), showed the absorptions of two ketone functions (1750 and 1645 cm⁻¹) as well as hydroxyl groups (3400 cm⁻¹; strong) in the infrared (IR) spectrum; the former resembled those of cinncassiol C₁ (4)^{2b)} and the latter functions were supposed to be glycosidic hydroxyl groups. Enzymatic hydrolysis of 1 with crude hesperidinase (Tanabe Pharm. Co., Ltd.) liberated an aglycone, mp 204.5–207°C, $[\alpha]_D +20.4^\circ$, which was identified as cinncassiol C₁ (4), and D-glucose, $[\alpha]_D +48.7^\circ$. Consequently, 1 consists of one mole each of cinncassiol C₁ (4) and D-glucose. On acetylation⁴⁾ at room temperature for 30 min with Ac₂O-pyridine, 1 yielded an acetate (5), mp 229–231°C, $[\alpha]_D +5.1^\circ$, C₃₄H₄₆O₁₆. Its electron impact mass spectrum (MS) showed peaks due to the molecular ion (*m/z* 710) and the terminal peracetylated glucosyl cation (*m/z* 331, 271, 169 and 109). The above mass spectrum of 5 indicated that four acetyl functions were introduced at the hydroxyl groups of the glucosyl moiety of 1 and that the glucosyl residue should be bound at the 19-hydroxyl of cinncassiol C₁ (4). The proton nuclear magnetic resonance (¹H NMR) spectrum (CDCl₃) of 5 showed a doublet signal (1H, d, *J*=9 Hz) at δ 4.52 ascribable to the glucosyl anomeric proton. Therefore, the structure of 1 can be represented as cinncassiol C₁ 19-O- β -D-glucopyranoside.

Cinncassiol C₂ (2), colorless needles, mp 219–221°C, $[\alpha]_D +30.5^\circ$, showed the absorptions of a five-membered ring ketone (1750 cm⁻¹) and α,β -unsaturated ketone (1650 cm⁻¹) and its molecular formula was defined as C₂₀H₂₈O₆ by FD-MS, MS (*m/z* 364 (M⁺)) and elementary analysis. The carbon-13 nuclear magnetic resonance (¹³C NMR) spectrum (CD₃OD) of 2 revealed the presence of 3 $\times$ -C-O- (δ 72.9, 79.1 and 91.9 ppm), 1 $\times$ -O-C-O- (104.8) and 1 $\times$ >C=C<_H (125.8 and 163.5). Furthermore, the signals in the ¹H NMR spectrum (CD₃OD) of 2 resembled those of cinncassiol C₁ (4) and were assigned by comparison, as follows; δ 0.99 (3H, d, *J*=6 Hz, 2-CH₃), 1.00 (3H, s, 9-CH₃), 1.11, 1.13 (each 3H, d, *J*=7 Hz, 18-(CH₃)₂),

1.30 (3H, s, 12-CH₃), 2.14, 2.41 (each 1H, d, $J=14$ Hz, 10-H₂), 2.64 (1H, m, 18-H), 3.67 (1H, d, $J=10$ Hz, 1-H) and 5.79 (1H, s, 14-H). All the above spectral data indicate that the structure of cinncassiol C₂ (2) corresponds to 19-desoxycinncassiol C₁ and is represented as shown in the formula 2.

Cinncassiol C₃ (3), colorless needles, mp 221–222°C, $[\alpha]_D^{25} +6.1^\circ$, C₂₀H₃₀O₇ (FD-MS and MS (m/z) 382 (M⁺)), exhibited absorptions due to ketone functions at 1730 and 1670 cm⁻¹ in its IR spectrum. The ¹³C NMR spectrum (CD₃OD) of 3 showed the presence of 4 × -C-O- (δ 71.6, 77.4, 80.5 and 90.5), 1 × -O-C-O- (103.7) and 2 × -C=O (222.1 and 222.9). The signals in the ¹H NMR spectrum (CD₃OD) of 3 were assigned as follows; δ 0.95, 0.99, 1.20 (each 3H, d, $J=7$ Hz, 2- and 18-(CH₃)₂), 1.03 (3H, s, 9-CH₃), 1.25 (3H, s, 12-CH₃), 2.25–2.62 (each 1H, d, $J=15$ Hz, 10-H₂), 2.37, 3.66 (each 1H, d, $J=13$ Hz, 14-H₂), 3.69 (1H, d, $J=10$ Hz, 1-H). All the above signals were reminiscent of those of dioxocinnzeylanol⁵⁾ derived from cinnzeylanol (6)^{2a,5,6)} by HIO₄ oxidation, as reported by Tamura *et al.*, and 3 was found to be identical with that compound.

Cinncassios C₁ glucoside (1), C₂ (2) and C₃ (3) together with cinncassiol C₁ (4) previously reported are novel and highly oxygenated tetracyclic diterpenes possessing an eight-membered ring carrying two ketone functions in their molecules. These diketone-type diterpenes were assumed to be biogenetically derived through an oxidative cleavage of the glycol at C-7 and C-8 in ketal-type diterpenes, cinncassiol B (7)^{2c)} for example.

Pharmacological tests of the diterpenes, including 1, 2 and 3, so far isolated from Cinnamomi Cortex are in progress.

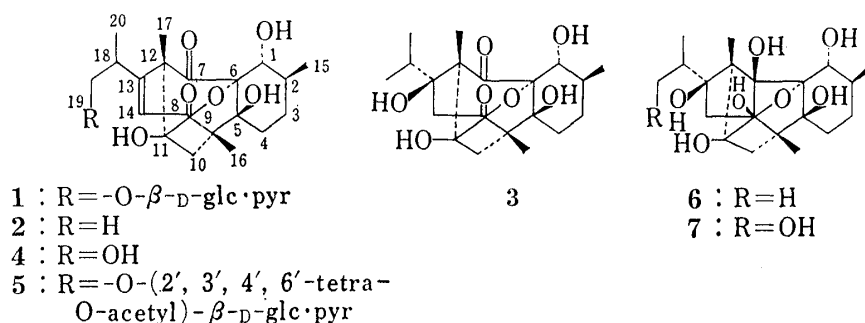


Fig. 1

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, the ultraviolet (UV) and the optical rotatory dispersion (ORD) spectra were obtained with a Hitachi EPI-G2 spectrometer, a Shimadzu UV-200 double beam spectrophotometer and a JASCO ORD/UV-5 recording spectropolarimeter, respectively. The ¹H NMR and ¹³C 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. The MS and FD-MS spectra were recorded on a JEOL JMS-D-300 mass spectrometer. Silica gel (Kieselgel 60; Merck) was used for column chromatography. Thin layer chromatography (TLC) was carried out on Merck plates precoated with Kieselgel 60. Detection was done by spraying 10% H₂SO₄ 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.

Separation of Cinncassios C₁ Glucoside (1), C₂ (2) and C₃ (3)—Further separation of fr. 3 and fr. 10 (Chart 1) previously reported^{2a)} by silica gel column chromatography using CHCl₃-MeOH (30:1) as a solvent afforded cinncassiol C₂ (2) (30 mg) and cinncassiol C₃ (3) (25 mg) from fr. 3 and cinncassiol C₁ glucoside (1) (35 mg) from fr. 10.

Cinncassiol C₁ Glucoside (1)—A white powder, $[\alpha]_D^{20} +14.5^\circ$ ($c=0.97$, MeOH). *Anal.* Calcd for C₂₆H₃₈O₁₂: C, 57.55; H, 7.06. Found: C, 57.88; H, 6.98. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3400 (OH), 1750 (ketone), 1645 (enone). UV $\lambda_{\max}^{\text{EtOH}}$ nm: 238 ($\epsilon=8600$), 279 ($\epsilon=3800$). ORD ($c=0.064$, EtOH) $[\alpha]_D^{21}$ (nm): -1330° (372, trough), 0° (350), +6640° (296, peak).

Enzymatic Hydrolysis of 1—A mixture of 1 (22 mg) and crude hesperidinase (10 mg) in dist. water (4 ml) was incubated at 37°C for 2 h, then the reaction mixture was evaporated to dryness under reduced pressure to give a residue. MeOH (15 ml) was added and the soluble portion was taken up. The solution was evaporated to dryness to give a residue, which was chromatographed over silica gel eluting with CHCl_3 -MeOH-water (7:3:0.2) to afford an aglycone, mp 204–207°C, $[\alpha]_D^{20} + 20.4^\circ$ ($c=0.57$, MeOH), IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3460 (OH), 1740 (C=O), 1650 (enone), identical with cinnassiol C_1 (4), and a sugar, R_f 0.39 (on TLC, CHCl_3 -MeOH-acetone-water=3:3:3:1), R_f 0.46 (on PPC), $[\alpha]_D^{19} + 48.7^\circ$ ($c=0.37$, water), identical with D-glucose.

Cinnassiol C_1 19-O-(2',3',4',6'-Tetra-O-acetyl)- β -D-glucopyranoside (5)—1 (10 mg) was acetylated with Ac_2O (1 ml) and pyridine (2 ml) at room temperature for 30 min to give a tetraacetate (5) of 1. Colorless needles from dil. MeOH, mp 229–231°C, $[\alpha]_D^{20} + 5.1^\circ$ ($c=0.39$, MeOH- $\text{CHCl}_3=1:1$). Anal. Calcd for $\text{C}_{34}\text{H}_{46}\text{O}_{16}$: C, 57.46; H, 6.52. Found: C, 57.58; H, 6.48. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1750 (C=O), 1645 (enone). MS (m/z): 710 (M^+), 500, 331, 169, 109. FD-MS (m/z): 710 (M^+). ^1H NMR (CDCl_3) δ (ppm): 1.02 (3H, s, 9- CH_3), 1.03 (3H, d, $J=6$ Hz, 18- CH_3), 1.13 (3H, d, $J=7$ Hz, 2- CH_3), 1.32 (3H, s, 12- CH_3), 1.98, 2.01, 2.03, 2.08 (each 3H, s, 4 $\times$ OCOCH₃), 3.50–3.90 (4H, m, 1-H, 5'-H and 19- CH_2), 4.18 (2H, br.s, 6'- CH_2), 4.52 (1H, d, $J=9$ Hz, 1'-H), 4.84–5.25 (3H, m, 2',3' and 4'-H), 5.80 (1H, s, 14-H); (pyridine- d_5): 1.24 (3H, d, $J=8$ Hz, 18- CH_3), 1.31 (3H, s, 9- CH_3), 1.36 (3H, d, $J=7$ Hz, 2- CH_3), 2.01, 2.05 (4 $\times$ OCOCH₃), 2.16 (3H, s, 12- CH_3), 2.58, 2.88 (each 1H, d, $J=15$ Hz, 10- CH_2), 4.01 (2H, d, $J=8$ Hz, 19- CH_2), 4.38 (1H, d, $J=12$ Hz, 1-H), 4.50–4.58 (2H, m, 6'- CH_2), 5.05 (1H, d, $J=10$ Hz, 1'-H), 5.35–5.93 (3H, m, 2',3',4'-H), 6.15 (1H, s, 14-H).

Cinnassiol C_2 (2)—Colorless needles from dil. MeOH, mp 219–221°C, $[\alpha]_D^{20} + 30.5^\circ$ ($c=0.82$, MeOH). Anal. Calcd for $\text{C}_{20}\text{H}_{28}\text{O}_6 \cdot \text{H}_2\text{O}$: C, 62.81; H, 7.91. Found: C, 62.56; H, 7.88. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3430, 3300 (OH), 1750 (C=O) and 1650 (enone). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 238 ($\epsilon=14900$), 277 ($\epsilon=10400$). ORD ($c=0.055$, EtOH) $[\alpha]^{20}$ (nm): -1410° (368, trough), 0° (350), $+7820^\circ$ (298, peak). MS (m/z): 364 (M^+), 336, 221, 194, 193, 179, 151, 123. FD-MS (m/z): 364 (M^+). ^{13}C NMR (CD_3OD) δ (ppm): 13.3, 18.8, 21.6, 22.4, 22.9, 28.4, 29.2, 31.5, 43.5, 54.2, 60.6, 72.9 (–C–O–), 79.1 (–C–O–), 91.9 (–C–O–), 104.8 (–O– $\dot{\text{C}}$ –O–), 125.8, 163.5 ($>\text{C}=\text{C}<\text{H}$). The signals due to the ketone functions could not be distinguished owing to the small amount of specimen available.

Cinnassiol C_3 (3)—Colorless needles from MeOH, mp 221–222°C, $[\alpha]_D^{21} + 6.1^\circ$ ($c=0.33$, MeOH). Anal. Calcd for $\text{C}_{20}\text{H}_{30}\text{O}_7 \cdot \text{H}_2\text{O}$: C, 59.98; H, 8.06. Found: C, 59.78; H, 8.04. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3370, 3280 (OH), 1730, 1670 (C=O). ORD ($c=0.052$, EtOH) $[\alpha]^{20}$ (nm): $+1460^\circ$ (345, peak), 0° (326), -3610° (276, trough). MS (m/z): 382 (M^+), 311, 293, 178, 169, 163. FD-MS (m/z): 382 (M^+). ^{13}C NMR (CD_3OD) δ (ppm): 11.9, 17.8 ($\times 2$), 18.7, 20.9, 28.3, 28.6, 34.8, 36.7, 44.3, 49.4, 52.4, 60.5, 71.6 (–C–O–), 77.4 (–C–O–), 80.5 (–C–O–), 90.5 (–C–O–), 103.7 (–O– $\dot{\text{C}}$ –O–), 222.1 (–C=O), 222.9 (–C=O).

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