Notes

[Chem. Pharm. Bull. 29(9)2686—2688(1981)]

Constituents of Cinnamomi Cortex. IV.[1] Structures of Cinncassiols C_1 Glucoside, C_2 and C_3

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(Received February 2, 1981)

The structures of three novel diterpenes, cinncassiol C_1 glucoside (**1**), cinncassiol C_2 (**2**) and cinncassiol C_3 (**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 C_1 glucoside; cinncassiol C_2; cinncassiol C_3

We have recently described[1,2] the structure elucidation of the diterpenes isolated from the fraction exhibiting anti-complement activity[3] 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 C_1 glucoside (**1**), C_2 (**2**) and C_3 (**3**).

Cinncassiol C_1 glucoside (**1**), an amorphous powder, $[\alpha]_D$ +14.5°, $C_{26}H_{38}O_{12}$ (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 C_1 (**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°, which was identified as cinncassiol C_1 (**4**), and D-glucose, $[\alpha]_D$ +48.7°. Consequently, **1** consists of one mole each of cinncassiol C_1 (**4**) and D-glucose. On acetylation[4] at room temperature for 30 min with Ac_2O-pyridine, **1** yielded an acetate (**5**), mp 229—231°C, $[\alpha]_D$ +5.1°, $C_{34}H_{46}O_{16}$. 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 C_1 (**4**). The proton nuclear magnetic resonance (^1H NMR) spectrum ($CDCl_3$) 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 C_1 19-O-β-D-glucopyranoside.

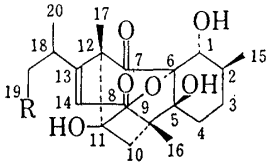
Cinncassiol C_2 (**2**), colorless needles, mp 219—221°C, $[\alpha]_D$ +30.5°, showed the absorptions of a five-membered ring ketone (1750 cm^{-1}) and α,β-unsaturated ketone (1650 cm^{-1}) and its molecular formula was defined as $C_{20}H_{28}O_6$ by FD-MS, MS (*m/z* 364 (M^+)) and elementary analysis. The carbon-13 nuclear magnetic resonance (^{13}C NMR) spectrum (CD_3OD) of **2** revealed the presence of 3×–C–O– (δ 72.9, 79.1 and 91.9 ppm), 1×–O–$\overset{|}{C}$–O– (104.8) and 1×>C=C$\langle_H$ (125.8 and 163.5). Furthermore, the signals in the ^1H NMR spectrum (CD_3OD) of **2** resembled those of cinncassiol C_1 (**4**) and were assigned by comparison, as follows; δ 0.99 (3H, d, J=6 Hz, 2-CH_3), 1.00 (3H, s, 9-CH_3), 1.11, 1.13 (each 3H, d, J=7 Hz, 18-$(CH_3)_2$),

1.30 (3H, s, 12-CH_3), 2.14, 2.41 (each 1H, d, J=14 Hz, 10-H_2), 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 C_2 (2) corresponds to 19-desoxycinncassiol C_1 and is represented as shown in the formula 2.

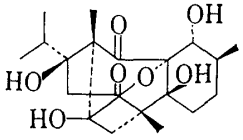
Cinncassiol C_3 (3), colorless needles, mp 221—222°C, $[\alpha]_D$ +6.1°, $C_{20}H_{30}O_7$ (FD-MS and MS (m/z) 382 (M^+)), exhibited absorptions due to ketone functions at 1730 and 1670 cm^{-1} in its IR spectrum. The ^{13}C NMR spectrum (CD_3OD) of 3 showed the presence of 4×–C–O– (δ 71.6, 77.4, 80.5 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 (CD_3OD) of 3 were assigned as follows; δ 0.95, 0.99, 1.20 (each 3H, d, J=7 Hz, 2- and 18-$(CH_3)_2$), 1.03 (3H, s, 9-CH_3), 1.25 (3H, s, 12-CH_3), 2.25 2.62 (each 1H, d, J=15 Hz, 10-H_2), 2.37, 3.66 (each 1H, d, J=13 Hz, 14-H_2), 3.69 (1H, d, J=10 Hz, 1-H). All the above signals were reminiscent of those of dioxocinnzeylanol[5] derived from cinnzeylanol (6)[2a,5,6] by HIO_4 oxidation, as reported by Tamura *et al.*, and 3 was found to be identical with that compound.

Cinncassiols C_1 glucoside (1), C_2 (2) and C_3 (3) together with cinncassiol C_1 (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.

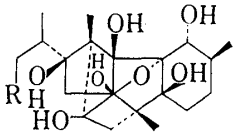
Pharmacological tests of the diterpenes, including 1, 2 and 3, so far isolated from Cinnamomi Cortex are in progress.



1 : R=-O-β-D-glc·pyr
2 : R=H
4 : R=OH
5 : R=-O-(2′, 3′, 4′, 6′-tetra-O-acetyl)-β-D-glc·pyr



3



6 : R=H
7 : R=OH

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 ^{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. 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% 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.

Separation of Cinncassiols C_1 Glucoside (1), C_2 (2) and C_3 (3)——Further separation of fr. 3 and fr. 10 (Chart 1) previously reported[2a] by silica gel column chromatography using $CHCl_3$–MeOH (30: 1) as a solvent afforded cinncassiol C_2 (2) (30 mg) and cinncassiol C_3 (3) (25 mg) from fr. 3 and cinncassiol C_1 glucoside (1) (35 mg) from fr. 10.

Cinncassiol C_1 Glucoside (1)——A white powder, $[\alpha]_D^{20}$ +14.5° (c=0.97, MeOH). *Anal.* Calcd for $C_{26}H_{38}O_{12}$: C, 57.55; H, 7.06. Found: C, 57.88; H, 6.98. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1750 (ketone), 1645 (enone). UV λ_{max}^{EtOH} nm: 238 (ε=8600), 279 (ε=3800). ORD (c=0.064, EtOH) $[\alpha]^{21}$ (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° (c=0.57, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1740 (C=O), 1650 (enone), identical with cinncassiol C_1 (**4**), and a sugar, *Rf* 0.39 (on TLC, $CHCl_3$–MeOH–acetone–water=3:3:3:1), *Rf* 0.46 (on PPC), $[\alpha]_D^{19}$ +48.7° (c=0.37, water), identical with D-glucose.

Cinncassiol 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° (c=0.39, MeOH–$CHCl_3$=1:1). *Anal.* Calcd for $C_{34}H_{46}O_{16}$: C, 57.46; H, 6.52. Found: C, 57.58; H, 6.48. IR $\nu_{max}^{CHCl_3}$ 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×$OCOCH_3$), 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×$OCOCH_3$), 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).

Cinncassiol C_2 (2)——Colorless needles from dil.MeOH, mp 219—221°C, $[\alpha]_D^{20}$ +30.5° (c=0.82, MeOH). *Anal.* Calcd for $C_{20}H_{28}O_6 \cdot H_2O$: C, 62.81; H, 7.91. Found: C, 62.56; H, 7.88. IR ν_{max}^{KBr} cm^{-1}: 3430, 3300 (OH), 1750 (C=O) and 1650 (enone). UV λ_{max}^{EtOH} nm: 238 (ε=14900), 277 (ε=10400). ORD (c=0.055, EtOH) $[\alpha]^{20}$ (nm): −1410° (368, trough), 0° (350), +7820° (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–$\underset{|}{C}$–O–), 125.8, 163.5 ($>C=C<_H$). The signals due to the ketone functions could not be distinguished owing to the small amount of specimen available.

Cinncassiol C_3 (3)——Colorless needles from MeOH, mp 221—222°C, $[\alpha]_D^{21}$ +6.1° (c=0.33, MeOH). *Anal.* Calcd for $C_{20}H_{30}O_7 \cdot H_2O$: C, 59.98; H, 8.06. Found: C, 59.78; H, 8.04. IR ν_{max}^{KBr} cm^{-1}: 3370, 3280 (OH), 1730, 1670 (C=O). ORD (c=0.052, EtOH) $[\alpha]^{20}$ (nm): +1460° (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 (×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–$\underset{|}{C}$–O–), 222.1 (–C=O), 222.9 (–C=O).

Acknowledgement We wish to express our thanks to Mr. H. Fujiwara and Mr. K. Goto (Otsuka Pharm. Factory, Inc.) for the measurement of ^{13}C NMR spectra and to the staff of the Analytical Laboratory (Tokushima University) for elemental analysis and for the measurement of mass 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.

References and Notes

1) Part III: T. Nohara, N. Tokubuchi, M. Kuroiwa, and I. Nishioka, *Chem. Pharm. Bull.*, **28**, 2682 (1980).
2) *a*) A. Yagi, N. Tokubuchi, T. Nohara, G. Nonaka, I. Nishioka, and A. Koda, *Chem. Pharm. Bull.*, **28**, 1432 (1980); *b*) T. Nohara, I. Nishioka, N. Tokubuchi, K. Miyahara, and T. Kawasaki, *ibid.*, **28**, 1969 (1980); *c*) T. Nohara, Y. Kashiwada, T. Tomimatsu, M. Kido, N. Tokubuchi, and I. Nishioka, *Tetrahedron Lett.*, **1980**, 2647.
3) A. Koda and H. Nagai, *Proc. Symp. Wakan-Yaku*, **18**, 13 (1974); H, Nagai, M. Ichikawa, S. Watanabe, and A. Koda, *ibid.*, **11**, 51 (1978).
4) On acetylation in the same way, **4** gave 19-O-monoacetyl cinncassiol C_1.
5) A. Isogai, S. Murakoshi, A. Suzuki, and S. Tamura, *Agric. Biol. Chem.*, **41**, 1779 (1977).
6) A. Isogai, A. Suzuki, S. Tamura, S. Murakoshi, Y. Ohashi, and Y. Sasada, *Agric. Biol. Chem.*, **40**, 2305 (1976).