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The Constituents of Cinnamomi Cortex. III.[1] Structures of Cinncassiol B and Its Glucoside

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Compounds **7**—**13** were isolated from the water extractive of Cinnamomi Cortex (the dried bark of *Cinnamomum cassia* Blume, "Tōkō Keihi" in Japanese, Lauraceae), which shows anti-complement activity. Among them, the compounds **7** and **8** were characterized by chemical and spectral means and named cinncassiol B and cinncassiol B 19-O-β-D-glucopyranoside, respectively.

Keywords——Cinnamomi Cortex; Lauraceae; diterpenes; anti-complement activity; cinncassiol B; cinncassiol B 19-O-β-D-glucopyranoside; FD-MS

In the preceding papers,[1,3] we have reported the structure elucidation of diterpenes (compounds I—VII) obtained from the water extractive of "Kannan Keihi" (Cinnamomi Cortex; the dried bark of *Cinnamomum cassia* Blume) which possesses anti-complement activity.[4] However, "Tōkō Keihi" (originated from the same plant as "Kannan Keihi") is also used as a crude drug and its water extractive also exhibits the above activity.[5] Therefore, in order to identify the active substance(s) and to compare the components, especially diterpenes, of the two crude drugs, a study of the constituents of the water extractive of "Tōkō Keihi" was undertaken.

As shown in Chart 1, the procedure of extraction and separation was similar to that used for "Kannan Keihi." In the course of this procedure, thin layer chromatographic (TLC) analyses indicated that compounds **1**—**6** were probably identical with cinnzeylanine (I), cinnzeylanol (II),[6] anhydrocinnzeylanine (III), anhydrocinnzelanol (IV), cinncassiol A (V) and cinncassiol A 19-O-β-D-glucopyranoside (VI), respectively, which had been obtained[3] from "Kannan Keihi." Seven new compounds, **7**—**13**, were isolated. This paper deals with the structure elucidations of compounds **7** and **8**.

Compound **7**, a white powder, $[\alpha]_D$ +22.7°, showed hydroxyl (3350 cm^{-1}), but no carbonyl absorption in the infrared (IR) spectrum. Based on elementary analysis and field desorption mass spectrometry (FD-MS, *m/z* 400 (M^+)), **7** was formulated as $C_{20}H_{32}O_8$, which includes one more oxygen atom than cinnzeylanol (II). Since the signal pattern in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum resembled that of II, **7** appeared to be an analogous diterpene. By comparing the ^{1}H-NMR spectrum of **7** with that of II, the signals were as-

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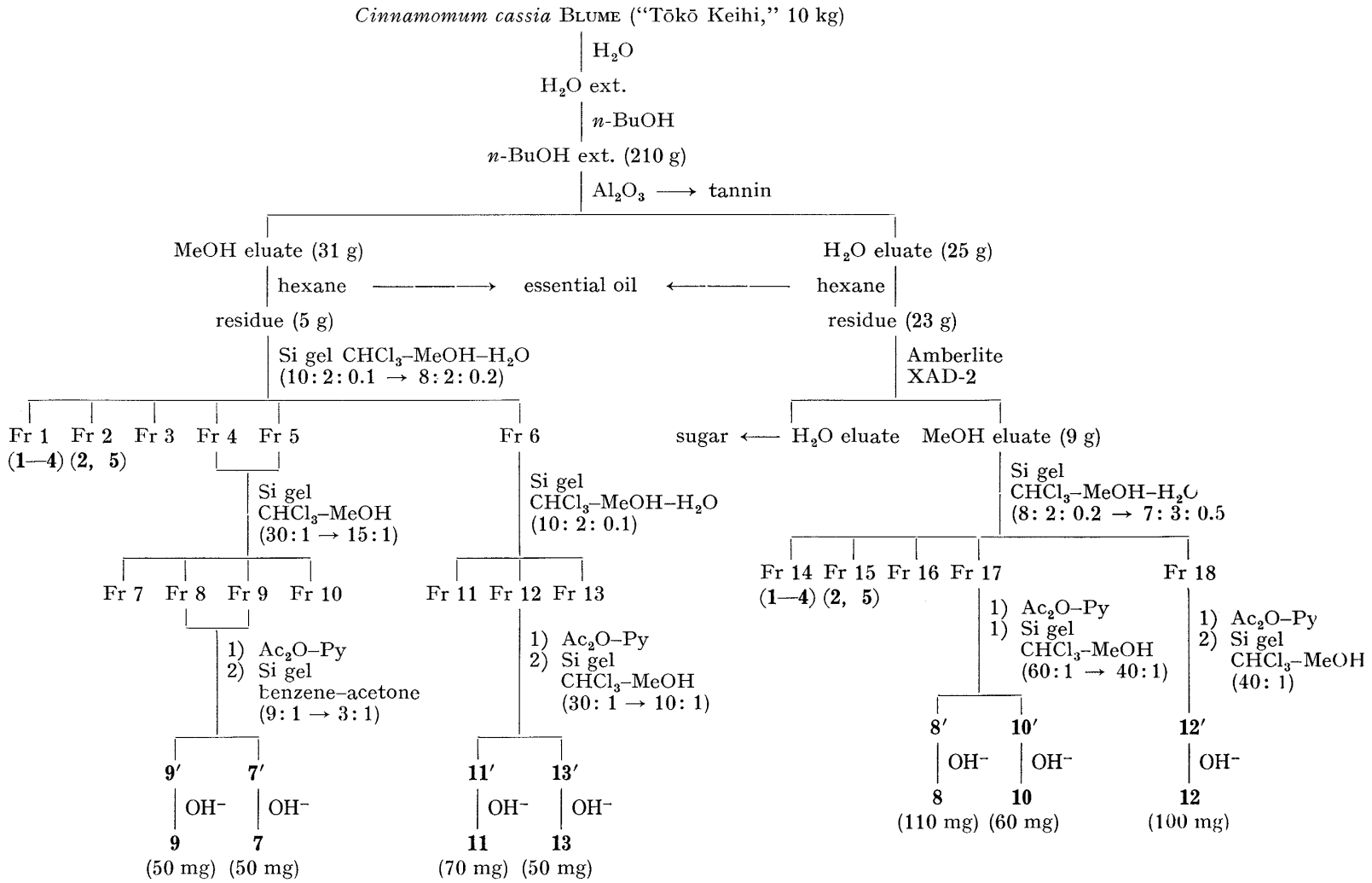

Chart 1

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signed as follows; δ 1.22 (3H, singlet (s), 9-CH_3), 1.29 (3H, doublet (d), J=6 Hz, 18-CH_3), 1.49 (3H, d, J=7 Hz, 2-CH_3), 2.21 (3H, s, 12-CH_3), 2.51, 2.90 (each 1H, d, J=15 Hz, 14-methylene protons) and 4.57 (1H, d, J=10 Hz, 1-H). In addition, the signals at δ 4.23 (1H, doublet of doublet (d d), J=4, 10 Hz) and 4.64 (1H, d d, J=5, 10 Hz) are attributable to the hydroxymethyl moiety at C-18, because irradiation of the signal around δ 1.80 changed the above two doublet-of-doublet signals into two doublets, together with a change of the doublet signal due to the 18-methyl group into a singlet. Therefore, **7** is assumed to be 19-hydroxylated cinnzeylanol with a hemiacetal structure. To confirm this tentative structure, **7** was refluxed with 5% methanolic hydrochloric acid to yield a single product identical with cinncassiol A (V). Since V had already been correlated to anhydrocinnzeylanol (IV) by lithium aluminum hydride reduction of cinncassiol A 19-O-monotosylate (V'), the configurations of the asymmetric carbons except for C-12, -13 and -18 in **7** are same as those of V. Among the unknown configurations, those at C-12 and -13 are probably as shown in formula **7** in Chart 2, since the C_{11}-C_{12} bond should be connected to C-12 from the α-side, and the elimination of the hydroxyl at C-13 by acid treatment would occur in the case of *anti*-parallel coplanarity between the C_{13}–OH and the C_{11}–C_{12} bonds, indicating β-configuration of the C_{13}–OH bond as in the case of the formation of III from I. The configuration of C-18 remains to be determined. Based on the above findings, the structure of **7** was deduced to be as shown in Chart 2, and it was named cinncassiol B.

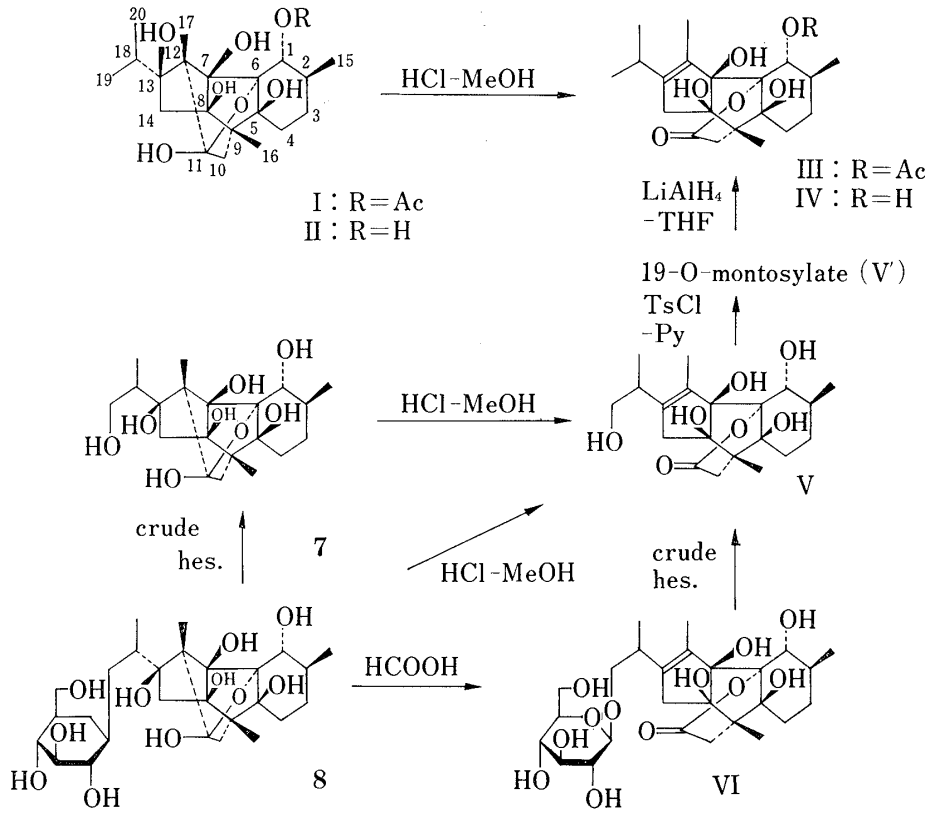

Chart 2

Compound **8**, a white powder, $[\alpha]_D$ −20.0°, showed a strong hydroxyl absorption (3440 cm^{-1}) in its IR spectrum, which is reminiscent of a glycosidic one. On enzymatic hydrolysis using crude hesperidinase (Tanabe Seiyaku Co., Ltd.) **8** liberated an aglycone, which was identical with cinncassiol B (**7**), and glucose. On the other hand, **8** on treatment with 5% hydrochloric acid afforded V and glucose. Since the FD-MS spectrum exhibits two peaks due to m/z 1147 $[2M+Na]^+$ and 585 $[M+Na]^+$, **8** is cinncassiol B glucoside, consisting of one

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mole of each. Furthermore, **8** on treatment with 44% formic acid afforded a product identical with cinncassiol A 19-O-β-D-glucopyranoside (VI). The structure of **8** was therefore decided to be cinncassiol B 19-O-β-D-glucopyranoside.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus (a hot stage type) and are uncorrected. The IR spectra were obtained with a JASCO DS-701 G spectrometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded with JEOL PS-100 and XL-100 spectrometers, respectively, with tetramethylsilane as an internal standard. The electron-impact mass (EI-MS) and FD-MS spectra were recorded on a JEOL JMS-01SG-2 mass spectrometer. The specific rotations were measured with a JASCO DIP-SL automatic polarimeter. Silica gel (Kiesel gel 60, Merck) and alumina (Aluminiumoxid 90 aktiv, Merck) were used for column chromatography. TLC was carried out on Merck plates precoated with Kiesel gel 60. Detection was done by spraying 10% H_2SO_4 and heating. Paper partition chromatography (PPC) of sugar was conducted on Toyo Roshi No. 50 paper using a mixture of pyridine (1) and the upper phase of *n*-BuOH–pyridine–water (6: 2: 3). Detection was carried out with aniline hydrogene phthalate as a spray reagent.

Isolation of Diterpenes——As shown in Chart 1, extraction and isolation were carried out as in the case of "Kannan Keihi." During this procedure, seven kinds of compounds (**7**—**13**) were isolated as their acetyl derivatives (**7′**—**13′**, derived by acetylation of the appropriate fractions with Ac_2O–pyridine (1: 1, 6—8 ml in each case) at room temperature for 20 min) in order to achieve more facile separation. Each acetate thus obtained was regenerated by reaction with 3% KOH–MeOH (3—5 ml in each case) at room temperature for 30 min to afford compounds **7**—**13**. In addition, it was recognized that compounds **1**—**6** were thin-layer-chromatographically identical with compounds I—VI, respectively, in this procedure (*Rf* values, **1**, 0.53 (=I); **2**, 0.26 (=II); **3**, 0.79 (=III); **4**, 0.60 (=IV); **5**, 0.22 (=V); solv. $CHCl_3$–MeOH=10: 1, **6**, 0.18 (=IV); solv. $CHCl_3$–MeOH–H_2O=8: 2: 0.2).

Cinncassiol B (**7**)——A white powder, $[\alpha]_D^{17}$ +22.7° (c=0.44, MeOH), $[M]_D$ +90.8°, *Anal.* Calcd for $C_{20}H_{32}O_8$: C, 59.98; H, 8.05. Found: C, 59.72; H, 8.01. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH). EI–MS (m/z): 400 (M^+), 316, 305, 195, 178, 177, 170, 163, 149, 139. FD–MS (m/z): 400 (M^+). ^{1}H-NMR (d_5-pyridine) δ: 1.22 (3H, s, 9-CH_3), 1.29 (3H, d, J=6 Hz, 18-CH_3), 1.49 (3H, d, J=7 Hz, 2-CH_3), 2.21 (3H, s, 12-CH_3), 2.51, 2.90 (each 1H, d, J=15 Hz, 14-H_2), 4.23 (1H, d d, J=4, 10 Hz, 19-H), 4.57 (1H, J=10 Hz, 1-H), 4.64 (1H, d d, J=5, 10 Hz, 19-H′). ^{13}C-NMR (d_5-pyridine) δ (multiplicities by off-resonance): 10.1 (quartet (q)), 11.5 (q), 14.6 (q), 19.3 (q), 27.5 (triplet (t)), 29.3 (t), 35.0 (d), 39.6 (d), 44.1 (t), 48.5 (s), 50.4 (t), 65.2 (t), 72.4 (d), 83.6 (s), 84.9 (s), 89.8 (s), 98.1 (s), 102.0 (s): total 18 carbons; the other two carbons could not be distinguished due to the small amount of **7** available.

Cinncassiol B 19-O-Monoacetate——**7** (15 mg) was acetylated with Ac_2O–pyridine (1: 1, 2 ml) at room temperature for 1 hr. After usual work-up, purification by silica gel column chromatography (solv. $CHCl_3$–MeOH=20: 1) provided the acetate. A white powder, $[\alpha]_D^{21}$ +9.7° (c=1.24, MeOH), EI-MS (m/z): 442 (M^+), 347, 323, 287, 259, 221, 212, 195, 189, 177, 163, 155, 152, 137. ^{1}H-NMR (d_5-pyridine) δ: 1.21 (3H, s, 9-CH_3), 1.27, 1.38 (each 3H, d, J=6 Hz, 2- and 18-CH_3), 1.99 (3H, s, –OCOCH_3), 2.08 (3H, s, 12-CH_3), 2.50, 2.95 (each 1H, d, J=15 Hz, 14-H_2), 4.52 (1H, d, J=10 Hz, 1-H), 4.62 (1H, d d, J=9, 10 Hz, 19-H), 5.17 (1H, d d, J=5, 10 Hz, 19-H′).

Acid Treatment of 7 giving Cinncassiol A (**V**)——**7** (30 mg) was refluxed with 5% KOH–MeOH for 1 hr. After neutralization with 3% KOH–MeOH and filtration to remove the salts deposited, the filtrate was subjected to Sephadex LH-20 column chromatography, eluting with MeOH, to give the product (10 mg), mp 170—173°, $[\alpha]_D^{14}$ +33.2° (c=0.42, MeOH), which was identical with cinncassiol A (V) as regards mp, $[\alpha]_D$, IR, EI-MS and ^{1}H-NMR.

Cinncassiol B 19-O-β-D-Glucopyranoside (**8**)——A white powder, $[\alpha]_D^{16}$ −20.0° (c=0.15, MeOH); $[M]_D$ −112.4°. *Anal.* Calcd for $C_{26}H_{42}O_{13}$: C, 55.50; H, 7.53. Found: C, 55.81; H, 7.41. IR ν_{max}^{KBr} cm^{-1}: 3440 (OH). FD–MS (m/z): 1147 $(2M+Na)^+$, 585 $(M+Na)^+$.

Cinncassiol B 19-O-2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranoside (**8′**)——**8** (15 mg) was acetylated with Ac_2O–pyridine (1: 1, 3 ml) at room temperature for 20 min. After usual work-up, the product was purified by silica gel column chromatography (solv. $CHCl_3$–MeOH=50: 1) to afford the tetraacetate (**8′**) of **8**. A white powder, $[\alpha]_D^{16}$ −4.6° (c=1.29, MeOH). *Anal.* Calcd for $C_{34}H_{50}O_{17}$: C, 55.88; H, 6.90. Found: C, 55.49; H, 6.92. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH). EI–MS (m/z): 694 (M^+-2H_2O), 500, 331, 242, 203, 195, 177, 169, 152. ^{1}H-NMR (d_5-pyridine) δ: 1.20 (3H, s, 9-CH_3), 1.27, 1.43 (each 3H, d, J=7 Hz, 2- and 18-CH_3), 1.96, 2.00, 2.02, 2.07 (each 3H, s, 4×–OCOCH_3), 2.11 (3H, s, 12-CH_3), 2.46, 2.94 (each 1H, d, J=15 Hz, 14-H_2).

Enzymatic Hydrolysis of 8——A mixture of **8** (30 mg) in AcOH-AcONa buffer solution (4 ml, pH 4.4) was incubated with crude hesperidinase (10 mg) at 40°. After 40 hr, the reaction mixture was concentrated *in vacuo* to give the residue. MeOH was added and the mixture was filtered. The filtrate was passed through Sephadex LH-20, eluting with MeOH. The first eluate contained the aglycone (7 mg), $[\alpha]_D^{15}$ +25.2° (c=0.32, MeOH), which was identical with cinncassiol B (**7**) as regards $[\alpha]_D$, IR and EI-MS. The next fraction was identical with glucose on PPC (Rf=0.36).

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Treatment of 8 with 5% HCl-MeOH——A solution of **8** (15 mg) in 5% HCl–MeOH (1 ml) was refluxed for 55 min, then diluted with water (1 ml), and the reaction mixture was passed through an Amberlite IRA-400 column. The eluate was concentrated to give a syrup, which was then subjected to Sephadex G-15 column chromatography to afford glucose (methyl glucoside) and cinncassiol A (V) (mp 167—170°, $[\alpha]_D^{16}$ +29.8° (c=0.23, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1725 (lactone), EI-MS (m/z): 364 (M^+-H_2O, $C_{20}H_{28}O_6^+$), 195, 177, 170).

Treatment of 8 with 44% Formic Acid——**8** (30 mg) was dissolved in 44% HCOOH (3 ml) and left to stand overnight at room temperature. The mixture was neutralized with aq. $NaHCO_3$ solution and concentrated *in vacuo* to give the residue, which was passed through Sephadex LH-20 with MeOH to afford a syrup, which in turn was purified by silica gel column chromatography (solv. $CHCl_3$–MeOH–H_2O=8: 2: 0.2) to give the product (VI), 15 mg (a white powder), $[\alpha]_D^{16}$ +6.2° (c=0.52, MeOH), identical with cinncassiol A 19-O-β-D-glucopyranoside (VI) as regards $[\alpha]_D$, IR and FD–MS.

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