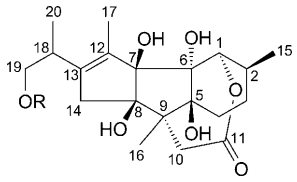
Two New Diterpenes from the Twigs of *Cinnamomum cassia*

by **Tran Minh Ngoc**[a][b], **Do Thi Ha**[a], **Ik-Soo Lee**[a], **Byung-Sun Min**[c], **Min-Kyun Na**[d], **Hyun-Ju Jung**[e], **Sang-Myung Lee**[f], and **Ki-Hwan Bae***[a]

[a]) College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea (phone: +82-42-821-5925; fax: +82-42-823-6566; e-mail: baekh@cnu.ac.kr)
[b]) National Institute of Drug Quality Control, 48 Hai Ba Trung, Ha Noi, Vietnam
[c]) College of Pharmacy, Catholic University of Daegu, Gyeongsan 712-702, Korea
[d]) College of Pharmacy, Yeungnam University, Gyeongbuk 712-749, Korea
[e]) Department of Oriental Pharmacy, Wonkwang University of Iksan, Jeonbuk 570-749, Korea
[f]) KT&G Central Research Institute, Daejoen 305-805, Korea

Two new diterpene derivatives named cinnacasol (**1**) and cinnacaside (**2**) were isolated from the MeOH extract of the twigs of *Cinnamomum cassia* BLUME. Their structures were identified based on physicochemical properties and spectroscopic-data analyses such as IR, HR-MS, and 1D- and 2D-NMR, and by comparison with published values.

Introduction. – *Cinnamomum cassia* BLUME (Lauraceae) is distributed in the southern part of mainland China, Vietnam, Myanmar, and Laos. As the bark, the twig has also been used as traditional Chinese medicine for treating dyspepsia, gastritis, blood circulation disturbances, and inflammatory diseases [1]. Chemical and pharmacological investigation on *C. cassia* has resulted in the isolation of several kinds of bioactive compounds such as cinnamaldehyde, cinnamic acid, as well as coumarins, diterpenes, and polyphenols [2][3] which exhibited antifungal, cytotoxic, antipyretic, antioxidant, and antimicrobial activities [4–9]. In the course of our phytochemical investigation program to standardize the quality of herbal medicines, two new diterpene derivatives, **1** and **2**, were isolated from the MeOH extract of the twigs of *C. cassia*.



1[1]) R = H
2[1]) R = Glc

Results and Discussion. – The MeOH extract of the twigs of *C. cassia* was partitioned into hexane-, AcOEt-, and BuOH-soluble fractions. Repeated column

[1]) Trivial atom numbering. For systematic names, see *Exper. Part.*

chromatography of the AcOEt- and BuOH-soluble fractions resulted in the purification of the two new diterpene compounds **1** and **2**.

Compound **1** was obtained as a colorless powder with $[\alpha]_D^{25} = +46.5$, and the IR spectrum showed absorption bands for a OH group and an ester CO group at 3354 and 1725 cm^{-1}. The molecular formula was established as $C_{20}H_{30}O_7$ from a molecular ion peak at *m/z* 405.2147 ($[M+Na]^+$) in the HR-EI-MS spectrum. The ^{1}H-NMR data (*Table*) showed signals for four Me groups (δ(H) 0.95, 1.03, 1.08, and 1.77), one O-bearing CH group (δ(H) 4.03), one O-bearing CH_2 group (δ(H) 3.47), and one *doublet* for a CH_2 group (δ(H) 2.42). The ^{13}C- and DEPT-NMR spectra (*Table*) indicated that **1** is a diterpene with signals for 20 C-atoms, which were classified as seven quaternary C-atoms comprising two olefinic C-atoms (δ(C) 138.2 and 142.9), four O-bearing C-atoms (δ(C) 84.8, 93.1, 97.1, and 89.2), and one sp^3 C-atom (δ(C) 48.3), three CH resonances, including one O-bearing CH group (δ(C) 72.9), and two CH groups (δ(C) 35.2 and 36.7), five CH_2 signals, containing one O-bearing CH_2 group (δ(C) 66.3), and four CH_2 groups (δ(C) 26.9, 29.1, 37.5, and 40.9), four Me groups (δ(C) 12.3, 13.7, 14.9, and 18.9), and one CO C-atom signal (δ(C) 171.8). In the comparison, ^{1}H- and ^{13}C-NMR data of **1** resemble those of cinnacassiol A [10], a diterpenoid isolated from cinnamomi cortex. The position of the OH groups at C(1), C(5), C(6), C(7), C(8), and C(19), as well as of the C=C bond between C(12) and C(13) in **1**, were determined due to the HMBC correlations from Me(16) to C(5) and C(8), from Me(17) to C(7), C(12), and C(13), and from H–C(1) to C(5), C(6), and C(7). Especially, the HMBC spectrum showed a ^3J(C,H) correlation between H–C(1) and CO group CO(11), indicating an ester linkage between O–C(1) and CO(11) (*Fig. 1*). The configuration of **1** was deduced based on a ROESY experiment, where H–C(1) was correlated with Me(15), Me(16) with H_β–C(14) (δ(H) 2.66), and H_β–C(14) with Me(20) (*Fig. 1*). Consequently, the structure of **1** was elucidated as cinnacasol.

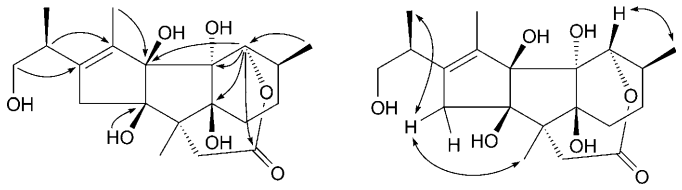


Fig. 1. *Important HMBC and ROESY correlations of* **1**

Compound **2** was obtained as a colorless powder with $[\alpha]_D^{25} = +16.5$. The molecular formula $C_{26}H_{40}O_{12}$ was established from a HR-EI-MS ion peak at *m/z* 567.6451 ($[M+Na]^+$). The IR spectrum was very similar with the one of **1**, also exhibiting absorption bands for OH groups and an ester CO group at 3354 and 1725 cm^{-1}. The ^{1}H- and ^{13}C-NMR, HMQC, and COSY data of **2** (*Table*) closely resembled those of **1**, but the signals δ(H) 4.24 and δ(C) 104.5 corresponding to an anomeric center indicated the presence of a β-glucopyranosyl unit, not present in **1**. The C-atom resonances assignable to the aglycone moiety of **2** were almost superimposable with those of **1**, except for a signal showing a downfield glycosylation shift CH_2(19) (δ(C) 73.3),

Table. *^{1}H- and ^{13}C-NMR Data* (CD_3OD, 300 and 75 MHZ) *of Compounds* **1** *and* **2**[1])[a]). δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	4.03 (d, J = 10.2)	72.9	4.04 (d, J = 10.2)	72.9
H–C(2)	1.78–1.82 (m)	35.2	1.77–1.80 (m)	35.1
CH_2(3)	1.55–1.62 (m)	29.1	1.54–1.60 (m)	29.0
CH_2(4)	1.51–1.54 (m)	26.9	1.51–1.55 (m)	26.5
C(5)	–	84.8	–	84.6
C(6)	–	93.1	–	93.0
C(7)	–	97.1	–	96.9
C(8)	–	89.2	–	89.3
C(9)	–	48.3	–	48.3
CH_2(10)	2.42 (d, J = 3.3)	40.9	2.45 (d, J = 3.3)	40.8
CO(11)	–	171.8	–	171.7
C(12)	–	138.2	–	137.3
C(13)	–	142.9	–	143.1
CH_2(14)	2.66 (br. d, J = 12.7), 2.18 (br. d, J = 12.7)	37.5	2.66 (br. d, J = 12.7), 2.18 (br. d, J = 12.7)	37.2
Me(15)	1.08 (d, J = 6.3)	18.9	1.09 (d, J = 6.3)	18.9
Me(16)	1.03 (s)	13.7	1.04 (s)	13.7
Me(17)	1.77 (s)	12.3	1.78 (s)	12.3
H–C(18)	2.71–2.75 (m)	36.7	2.88–2.92 (m)	34.4
CH_2(19)	3.47 (br. d, J = 7.5)	66.3	3.86 (overlap), 3.47 (t, J = 9.0)	73.7
Me(20)	0.95 (d, J = 6.9)	14.9	0.95 (d, J = 6.9)	14.9
H–C(1′)			4.24 (d, J = 7.8)	104.5
H–C(2′)			3.14–3.19 (m)	75.2
H–C(3′)			3.24–3.28 (m)	78.0
H–C(4′)			3.27–3.31 (m)	71.7
H–C(5′)			3.34–3.38 (m)	78.1
H–C(6′)			3.67 (dd, J = 3.6, 11.7), 3.85–3.88 (m)	62.8

[a]) Assignments were made by a combination of 1D- and 2D-NMR (COSY, HMQC, and HMBC) experiments.

revealing that the glucosyl unit is linked to the OH group at CH_2(19). This was further supported by the long-range correlations from the O-bearing CH_2(19) group (δ(H) 3.86, and 3.47) to the anomeric C-atom C(1′), and from the anomeric H-atom H–C(1′) to C(19) in the HMBC spectrum (*Fig. 2*). Accordingly, the structure of **2** was determined as cinnacaside.

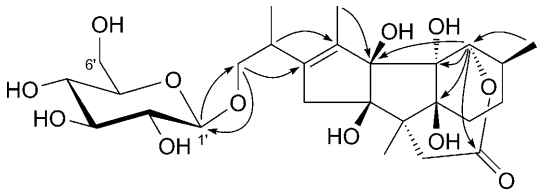


Fig. 2. *Important HMBC correlations of* **2**

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; *Kieselgel 60*, 70–230 mesh and 230–400 mesh, *Merck*). TLC: *Merck* pre-coated silica gel *60* F_{254} and/or *RP-18* F_{254s} plates (0.25 mm), and compounds were observed under UV light of 254 and 365 nm, or visualized by spraying the dried plates with 10% H_2SO_4, followed by heating at 180°. Optical rotations: *JASCO DIP-370* polarimeter. FT-NMR Spectra: *Bruker DRX-300* spectrometer (^{1}H-NMR, 300 MHz; ^{13}C-NMR, 75 MHz) using CD_3OD as the solvent and TMS as an internal standard; chemical shifts (δ) were expressed in ppm with reference to the TMS signals. 2D-NMR (HMQC, HMBC, and ROESY) Experiments: *Bruker Avance 500* spectrometer. HR-MS: *JMS-700 Mstation* mass spectrometer.

Plant Material. The sliced twig of *C. cassia* was purchased from the pharmacy store in Daejeon, Korea in November 2006, and the twig was identified by Prof. *Ki-Hwan Bae*. A voucher specimen CN 95125 was deposited at the herbarium in the College of Pharmacy, Chungnam National University.

Extraction and Isolation. The twig slices of *C. cassia* (30.0 kg) were extracted with hot MeOH (60 l × 3 times) for 2 d. The MeOH extracts were filtered, combined, and concentrated *in vacuo*, resulting in a residue (1650 g). The residue was suspended in H_2O and then fractionated successively with hexane, AcOEt, and BuOH, producing a hexane-soluble fraction (410.0 g), an AcOEt-soluble fraction (230.5 g), and a BuOH-soluble fraction (135.0 g), resp. The AcOEt-soluble fraction (230.5) was chromatographed on a SiO_2 column with a stepwise gradient of $CHCl_3$ and MeOH 60 : 1 to 0 : 1 to yield eight fractions, *E1–E8*. *E6* was rechromatographed on a SiO_2 column with hexane/AcOEt 10 : 1 to 2 : 1 as the eluting solvent system, and prep. HPLC (*YMC-ODS*, 10 × 250 mm, 5 μm) eluted with MeOH/H_2O 40 : 60 to afford **1** (5.6 mg). The BuOH-soluble fraction was suspended in H_2O and then applied to a *Diaion HP 20* column with a H_2O/MeOH gradient to give the fractions *B1–B14*. *B5* was subjected to SiO_2 CC, using CH_3Cl/MeOH/H_2O 15 : 1 : 0.1 to 1 : 1 : 0.1 as eluent to yield nine subfractions, *B5.1–B5.9*. *B5.5* was applied to a *Sephadex LH-20* column, eluted with MeOH/H_2O 1 : 5, and then to *YMC-ODS* CC, with a mobile phase system MeOH/H_2O 1 : 4 to give **2** (16.5 mg).

Cinnacasol (=*(3a*R,*3b*R,*4*R,*5*S,*7a*S,*8*R,*8a*R*)-1,3a,3b,4,5,6,7,7a,8,8a-Decahydro-3a,3b,7a,8a-tetrahydroxy-2-[(2*S*)-1-hydroxypropan-2-yl]-3,5,8-trimethyl-4,8-(epoxyethano)cyclopenta[*a*]inden-10-one*; **1**). Colorless amorphous powder. $[\alpha]_D^{25} = +46.5$ ($c = 0.4$, MeOH). UV (MeOH): 213 (2.93). IR (KBr): 3354 (OH), 2900, 1725 (C=O), 1240, 1087 (−CH=CH−), 954, 751. ^{1}H-NMR (300 MHz, CD_3OD) and ^{13}C-NMR (75 MHz, CD_3OD): *Table.* HR-EI-MS: 405.2147 ($[M+Na]^+$, $C_{20}H_{30}NaO_7^+$; calc. 405.2157).

Cinnacaside (=*2-[(3a*R,*3b*R,*4*R,*5*S,*7a*S,*8*R,*8a*R*)-1,3a,3b,4,5,6,7,7a,8,8a-Decahydro-3a,3b,7a,8a-tetrahydroxy-3,5,8-trimethyl-10-oxo-4,8-(epoxyethano)cyclopenta[*a*]inden-2-yl]propyl β-*D*-Glucopyranoside*; **2**). Colorless amorphous powder. $[\alpha]_D^{25} = +16.5$ ($c = 0.4$, MeOH). UV (MeOH): 213 (2.93). IR (KBr): 3354 (OH), 2900, 1725 (C=O), 1240, 1087 (−CH=CH−), 954, 751. ^{1}H-NMR (300 MHz, CD_3OD) and ^{13}C-NMR (75 MHz, CD_3OD): *Table.* HR-EI-MS: 567.6451 ($[M+Na]^+$, $C_{26}H_{40}NaO_{12}^+$; calc. 567.6402).

Acid Hydrolysis. The soln. of **2** (5.2 mg) in 0.5N H_2SO_4 (dioxane/H_2O 1 : 1, 2 ml) was heated at 100° for 2 h. The mixture was diluted with H_2O (3 ml) and extracted with $CHCl_3$ (3 × 5 ml). The H_2O layer was dried *in vacuo* after neutralization with 1N NaOH and passed through a *Sep-Pak* C_{18} cartridge (*Waters*, Milford, MA). The sugar was identified as glucose (R_f 0.26), by TLC in $CHCl_3$/MeOH/H_2O 10 : 5 : 1 with an authentic sample of D-glucose. The remaining eluate was concentrated to dryness, and the residue was stirred with D-cysteine methyl ester hydrochloride, hexamethyldisilazane and Me_3SiCl in pyridine using the same procedures as described in [11]. After the reactions, the supernatant was analyzed by GC (column: cap. column *BD-5*, 0.25 mm × 30 m, detector: FID, detector temp.: 300°, injector temp.: 270°, carrier gas, N_2; column temp. 210°). The peak corresponding to the D-glucosyl derivative appeared at t_R of 8.55 min.

This work was supported by a grant from the *Korea Food and Drug Administration* (08182 Crude Drugs 257) for Studies on the Identification of the Biologically Active Components from Oriental Herbal Medicines (2007–2008).

REFERENCES

[1] D. T. Loi, 'Vietnamese Medicinal Plants and Ingredients', Medical Publish House, Hanoi, Vienam, 2004, p. 862.
[2] T. Nohara, Y. Kashiwada, K. Murakami, T. Tomimatsu, M. Kido, A. Yagi, I. Nishioka, *Chem. Pharm. Bull.* **1981**, *29*, 2451.
[3] K. Yazaki, T. Okudu, *Phytochemistry* **1990**, *29*, 1559.
[4] H. B. Singh, M. Srivastava, A. B. Singh, A. K. Srivastava, *Allergy* **1995**, *50*, 995.
[5] B. M. Kwon, S. H. Lee, S. U. Choi, S. H. Park, C. O. Lee, Y. K. Cho, N. D. Sung, S. H. Bok, *Arch. Pharmacol Res.* **1998**, *21*, 147.
[6] M. Kurokawa, C. A. Kumeda, J. Yamamura, T. Kamiyama, K. Shiraki, *Eur. J. Pharmacol.* **1998**, *348*, 45.
[7] C.-C. Lin, S.-J. Wu, C.-H. Chang, L.-T. Ng, *Phytother. Res.* **2003**, *17*, 726.
[8] H.-O. Kim, S.-W. Park, H.-D. Park, *Food Microbiol.* **2004**, *21*, 105.
[9] S.-S. Cheng, J.-Y. Liu, K.-H. Tsai, W.-J. Chen, S.-T. Chang, *J. Agric. Food Chem.* **2004**, *52*, 4395.
[10] A. Yagi, N. Tokubuchi, T. Nohara, G. Nonaka, I. Nishioka, A. Koda, *Chem. Pharm. Bull* **1980**, *28*, 1432.
[11] S. Hara, H. Okabe, K. Mihashi, *Chem. Pharm. Bull.* **1987**, *35*, 501.

Received April 20, 2009