Studies on the Chemical Constituents of *Cinnamomum camphora*

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Abstract: From *Cinnamomum camphora,* ten compounds were isolated and identified as 4-hydroxysesamin (1), 3,3′-dihydroxy-4,4′-dimethoxytetrahydrofuran lignanoid (2), 3, 4-methylenedioxyphenyl-3′,9-dihydroxy-4′-methoxy- tetrahydrofuran lignanoid (3), lyoniresinol (4) , (+)-3 -(3, 4 – methylenedioxyphenyl)- 1,2- propanediol (5), isolariciresinol (6), taiwanin C (7), chinensinaphthol l(8), borneol (9) and daucosterol(10). The structures of the isolated compounds were established by means of NMR and MS analyses. These compounds were firstly isolated from this plant. Compound 1 have inhibitory activities on the release of β-glucuronidase from rat PMNs induced by PAF.

Introduction

Cinnamomum camphora (L.) Pvesl is a plant of the *Lauraceae*. This plant is widely distributed in southern of China[1]. Its branch and stem has been used to treat spleen-stomach warming analgesic, rheumatic arthritis and hypertension etc, except for used to driving mosquitos in Chinese folk medicine. Diterpenes, alkaloids, lignanoids, flavonoids and tanins were isolated previously from *Cinnamomum* genus[2~5]. In our previous chemical investigations of *Cinnamomum camphora*, terpenes were the major chemicals. In the course of our continuing search for naturally occurring terpenoids from *Cinnamomum* genus plant, ten compounds were isolated and identified as 4-hydroxysesamin (1), 3,3 ′ -dihydroxy-4,4 ′ -dimethoxytetrahydrofuran lignanoid (2), 3,4-methylenedioxyphenyl-3 ′ , 9-dihydroxy-4 ′ -methoxy-tetrahydrofuran lignanoid (3), lyoniresinol (4), (+)-3-(3,4–methylenedioxyphenyl)- 1, 2- propanediol (5), isolariciresinol (6), taiwanin C (7), chinensinaphthol l(8), borneol (9) and daucosterol(10) from *Cinnamomum camphora* (L.) Pvesl. In this paper, we describe the isolation and structure elucidation based on modern spectroscopic data and chemical evidence.

Experimental

Apparatus and reagents. IR spectra were measured on a Nicolet IR100 infrared spectrometer, and NMR spectra were obtained with NMR AC-400 and AC-500 NMR instrument (BRUKER Company, Switzerland). EI-MS data were recorded on a VG Autospec 300. HRFABMS was performed on an Auto spec Ultima-TOF mass spectrometer, whereas ESIMS were obtained using an Agilent 1100 series LC/MSD Trap SL mass spectrometer. Melting points of the chemicals were detected on X-4 micromelting apparatus. thin layer chromatography and column chromatography was carried out on silica gel (Qingdao Marine Chemical Factory).

Plant material. *Cinnamomum camphora* (L.)Pvesl were collected from Jian County, Jiangxi Province, China in July of 2014 and authenticated by Professor Q. Lui, Jiangxi University of Traditional Chinese Medicine. A voucher specimen (2014-07-20) of the plant is deposited at the Herbarium of Jiangxi University of Traditional Chinese Medicine.

Extraction and isolation. The dried and powdered plant (5kg) was extracted(3×) with ethanol for 4h under reflux, and the combined extracts were concentrated in vacuo. The resulting extract(330g) and then suspended in water and successively extracted with light petroleum, chloroform, ethyl acetate and butanol saturated with water to give the respective extracts after solvent removal . The

chloroform-solution portion (96g) was subjected to column chromatography on silica gel (10×100cm) with PE-Acetone(100:0-1:1) to give five fractions (Fractions Ⅰ-Ⅴ), Fraction Ⅱ and Ⅲ were further subjected to silica-gel column eluted with PE-Acetone(10:1-4:1) to afford 1(8mg), 2(14mg), 3 (10mg), 4(32 mg) , 6(8mg), 7 (11mg), 8(13mg) , 9(17mg) and 10(5mg).

Anti-inflammation bioassays. The anti-inflammatory activities were assayed by measuring the inhibition of the PAF induced release of β-glucuronidase from rat PMNs in vitro as described previously5. The absorbance was read at 550nm, and then the inhibitory ratio was calculated. Ginkgolide B (sigma, 98% pure) was used as a positive control.

Results and Discussion

Compound 1 was obtained as a colorless needles, mp 165~167℃, $[\alpha]_D$ +58.4º. EI-MS m/z(%): 371$[M+H]^+$(27), 370(M^+, 33), 352(16), 323(30), 219(8), 202(33), 201(9), 176(55), 161(34), 151(84), 149(100), 135(28), 121(45).^{1}H-NMR(Acetone-d_6, 500Hz)δ: 7.13(1H, d, J=1.5Hz, H-2′), 6.94(1H, dd, J=1.5, 8.0Hz, H-6′), 6.81(1H, d, J=8.0Hz, H-5′), 6.90(1H, d, J=1.5Hz, H-2), 6.87(1H, dd, J=2.0, 8.0Hz, H-6), 6.77(1H, d, J = 8.0Hz, H-5), 5.97(2H, s, -OCH_2O-), 5.96(2H, s, -OCH_2O-), 5.65(1H, d, J=5.0Hz, H-7′), 5.56(1H, d, J=5.0Hz, H-7), 4.88(1H, d, J=6.5Hz, 9- H or 9-OH), 4.79(1H, d, J=7.0Hz, H-9 or 9-OH), 4.21(1H, d, J=9.0Hz, H-9′), 4.00(1H, d, J=9.0Hz, H-9′), 3.10(1H, m, H-8), 2.82(1H, m, H-8′). ^{13}C-NMR(Acetone-d_6, 500Hz)δ: 148.9, 148.7, 147.9, 147.7为C-3, C-3′, C-4, C-4′, 138.7, 137.6为C-1, C-1′, 120.4, 119.9为C-6, C-6′, δ107.0~108.7(C-2, C-2′, C-5, C-5′), δ102.0~102.5(2 OCH_2O), 101.9(C-9), 87.9(C-7′), 84.1(C-7), 72.7(C-9′), 63.7(C-8), 4.8(C-8′). On the basis of relevant reference[6]and its spectral data, its structure was elucidated to be 4-hydroxysesamin.

Compound 2 was obtained as colorless needles, mp 176.0~177.0 ℃; EI-MS m/z(%): 358(M^+).^{1}H-NMR(Acetone-d_6, 500Hz): δ 7.01(1H, d, J=2Hz, H-2′), 6.85(1H, dd, J=2.0, 8.0Hz, H-6′), 6.81(1H, d, J=8.0Hz, H-5′), 7.00(1H, d, J=2Hz, H-2), 6.80(1H, dd, J=2.0, 8.0Hz, H-6), 6.71(1H, d, J = 8.0Hz, H-5), 5.62(1H, d, J=5.0Hz, H-7′), 5.54(1H, d, J=5.0Hz, H-7), 4.89(1H, d, J=6.5Hz, H-9), 4.20(1H, d, J=6.5Hz, H-9), 4.17(1H, d, J=9.0Hz, H-9′), 3.98(1H, d, J=9.0Hz, H-9′), 3.86(1H, s, -OCH_3), 3.85(3H, s, OCH_3), 3.08(1H, m, H-8), 2.86(1H, t, J=7.5Hz, H-8′).^{13}C-NMR(Acetone-d_6, 500Hz) δ: 132.8(C-1), 133.1(C-1′), 113.7(C-2), 113.3(C-2′), 151.3(C-3), 151.4(C-3′), 144.3(C-4), 144.1(C-4′), 116.8(C-5), 116.6(C-5′), 122.3(C-6), 122.5(C-6′), 84.1(C-7′), 84.3(C-7), 54.6(C-8), 54.8(C-8′), 72.9(C-9), 72.7(C-9′), 56.1, 56.2(OCH_3). Thus the structure of **2** was determined as 3, 3′-dihydroxy-4, 4′-dimethoxytetrahydrofuran lignanoid [6].

Compound 3 was obtained as a colorless needles, mp226~227℃. EI-MS m/z(%): 373$[M+H]^+$, (5), 372(M^+, 31), 354(5), 325(8), 202(5), 192(12), 176(19), 161(59), 151(100), 149(30), 135(56), 121(8).^{1}H-NMR(Acetone-d_6, 500Hz): δ 7.13(1H, d, J=1.5Hz, H-2′), 6.94(1H, dd, J=2.0, 8.0Hz, H-6′), 6.80(1H, d, J=8.0Hz, H-5′), 7.00(1H, d, J=1.5Hz, H-2), 6.84(1H, dd, J=2.0, 8.0Hz, H-6), 6.77(1H, d, J = 8.0Hz, H-5), 5.96(2H, s, -OCH_2O-), 5.62(1H, d, J=5.0Hz, H-7′), 5.54(1H, d, J=5.0Hz, H-7), 4.89(1H, d, J=6.5Hz, H-9), 4.77(1H, d, J=6.5Hz, H-9), 4.21(1H, d, J=9.0Hz, H-9′), 3.98(1H, d, J=9.0Hz, H-9′), 3.85(3H, s, OCH_3), 3.08(1H, m, H-8), 2.86(1H, t, J=7.5Hz, H-8′).^{13}C-NMR(Acetone-d_6, 500Hz) δ: 138.7(C-1), 134.8(C-1′), 107.7(C-2), 110.3(C-2′), 148.7(C-3), 148.4(C-3′), 147.7(C-4), 146.9(C-4′), 108.4(C-5), 115.6(C-5′), 120.3(C-6), 119.5(C-6′), 84.3(C-7), 88.0(C-7′), 63.5(C-8), 54.8(C-8′), 101.9(C-9), 72.7(C-9′), 102.5(OCH_2O), 56.2(OCH_3). On the basis of relevant reference[6] and its spectral data, its structure was elucidated to be 3, 4-methylenedioxyphenyl-3′, 9-dihydroxy-4′-methoxy- tetrahydrofuran lignanoid.

Compound 4 was obtained as a colorless needles, mp189-191℃. ESI-MS m/z(%): 443$[M+Na]^+$, 459$[M+K]^+$, 419$[M-H]^+$.^{1}H-NMR(DMSO-d_6, 500Hz): δ 1.40~1.46(1H, m, 8-H), 1.81~1.86(1H, m, 8′-H), 2.42(1H, dd, J=15.0Hz, 7-H), 2.61(1H, dd, J=15.0Hz, 7-H), 3.21~3.48(4H, m, H-9, 9′), 3.62(6H, s, 3′, 5′-OCH_3), 3.76(3H, s, 3-OCH_3), 4.23(1H, d, J=5.5Hz, 7′-H), 4.49(1H, br.s, OH),

4.64(1H, br.s, OH), 6.28(2H, s, 2′, 6′- H), 6.54(1H, s, 2-H). ^{13}C-NMR(DMSO-d_6, 125Hz) δ: 128.6(C-1), 106.6(C-2), 146.8(C-3), 137.2(C-4), 146.4(C-5), 125.0(C-6), 32.2(C-7), 40.1(C-8), 64.6(C-9), 137.7(C-1′), 105.9(C- 2′, 6′), 147.5(C-3′, 5′), 133.4(C-4′), 46.6(C- 7′), 40.3(C-8′), 62.2(C-9′), 55.7(3-OCH_3), 58.9(5-OCH_3), 56.1(3′, 5′-OCH_3). On the basis of relevant reference[7] and its spectral data, its structure was elucidated to be lyoniresinol.

Compound 5 was obtained as a colorless needles from chloroform. EI-MS m/z(%): 197[M＋H]$^+$(10), 196(M^+, 100), 165(17), 135(97), 121(4). ^{1}H-NMR($CDCl_3$, 500Hz) δ: 2.02(br, OH), 2.65(2H, dd J=14, 8Hz, H-7), 3.52(1H, dd, J=11, 7.0Hz, H-9), 3.70(1H, dd, J=11, 3.0Hz, H-9), 3.90(1H, m, H-8), 5.94(2H, s, OCH_2O), 6.67(1H, d, J=8.0Hz, H-5), 6.72(1H, s, H-2), 6.76(1H, d, J=8Hz, C-6). ^{13}C-NMR($CDCl_3$, 125Hz) δ: 131.3(C-1), 109.6(C-2), 147.8(C-3), 146.3(C-4), 108.4(C-5), 122.2(C-6), 39.5(C-7), 73.0(C-8), 66.0(C-9), 100.9(OCH_2O). On the basis of relevant reference[8,9] and its spectral data, its structure was elucidated to be (+)-3-(3,4–methylenedioxyphenyl)- 1, 2- propanediol (Phenylpropanoid A).

Compound 6 was obtained as white powder. ^{1}H-NMR(DMSO-d_6, 400 MHz)δ: 6.46 (1H, dd, J = 1.7, 8.1 Hz, H-6), 6.66 (1H, d, J = 8 Hz, H-5), 6.70 (1H, d, J = 1.7 Hz, H-2), 6.36 (1H, s, H-5‘), 6.71 (1H, s, H-2’); ^{13}C-NMR (DMSO-d_6, 100MHz)δ: 136.61(C-1), 115.3 (C-2), 148.6 (C-3), 145.5(C-4), 115.7(C-5), 124.6(C-6), 46.2(C-7), 44.8(C-8), 638(C-9), 128.3(C-1’), 112.2(C-2’), 147.4(C-3’), 145.6(C-4’), 117.3 (C-5’), 133.2(C-6’), 33.2(C-7’), 35.9(C-8’), 65.6(C-9’), 56.4(OCH_3), 56.3(OCH_3). On the basis of relevant reference [10] and its spectral data, its structure was elucidated to be isolariciresinol.

Compound 7 was obtained as white powder. ^{1}H-NMR($CDCl_3$, 500MHz) δ: 7.59(1H, s), 7.08(1H, s), 6.97(1H, d, J =7.5 Hz), 6.77(1H, dd, J=5, 8Hz), 6.80(2H, d, J=5Hz), 6.11(2H, d, -OCH2O-), 6.07(2H, d, -OCH2O-), 5.54(2H, s), 4.11(3H, s, -OCH3). ^{13}C-NMR($CDCl_3$, 125MHz) δ: 14.7(C-1), 130.8(C-2), 103.1(C-3), 128.0(C-4), 102.4(C-5), 149.2(C-6), 148.8(C-7), 98.5(C-8), 119.6(C-9), 131.6(C-10), 67.0(C-11), 169(C-12), 102.5(C-13), 101.0(C-14), 125.1(C-1′), 114.7(C-2′), 148.7(C-3′), 148.6(C-4′), 111.7(C-5′), 123.1(C-6′). On the basis of relevant reference [11] and its spectral data, its structure was elucidated to be taiwanin C.

Compound 8 was obtained as white powder. ^{1}H-NMR(DMSO-d_6, 500MHz) δ:10.44(1H, s, -OH), 7.62(1H, s, H-8), 7.06(1H, d, H-5′), 6.86(2H, d, H-2′, 6′), 6.78(1H, t, H-5), 6.17(2H, s, -OCH_2O-), 5.36(2H, s, H-11), 3.84(3H, s, 3′-OCH_3), 3.71(3H, s, 4′-OCH_3). ^{13}C-NMR (DMSO-d_6, 125MHz) δ: 14.7(C-1), 130.8(C-2), 103.1(C-3), 128.0(C-4), 102.4(C-5), 149.2(C-6), 148.8(C-7), 98.5(C-8), 119.6(C-9), 131.6(C-10), 67.0(C-11), 169.0(C-12), 102.5(C-13), 125.1(C-1′), 114.7(C-2′), 148.7(C-3′), 148.6(C-4′), 111.7(C-5′), 123.1(C-6′), 55.99(-OCH_3), 55.92(-OCH_3). On the basis of relevant reference [11] and its spectral data, its structure was elucidated to be chinensinaphthol.

Compound 9 was obtained as salmon needles, mp204~208℃. ^{1}H-NMR($CDCl_3$, 400Hz) δ: 0.92(3H, s, CH_3), 0.93(3H, s, CH_3), 1.33(3H, s, CH_3), 3.53(1H, m), 1.41~1.48(2H, m), 1.57~1.62(1H, m), 1.73~1.76(1H, d), 1.79 ~ 1.86 (1H, m), 1.94~2.05(H, m). ^{13}C-NMR($CDCl_3$, 100Hz) δ: 13.3, 19.8, 23.6, 30.3, 35.4, 45.6, 76.2, 50.5, 52.6. On the basis of spectral data, its structure was elucidated to be borneol.

Compound 10 was obtained as white powder. mp 294~296℃. ^{1}H-NMR(DMSO-d_6, 400 MHz)δ: 3.93~3.96(1H, m, H-3), 2.43~2.47(1H, m, H-4α), 2.7~2.72(1H, m, H-3β), 5.03(1H, d, *J*=7.7Hz, H-6), 0.64(3H, s, H-18), 0.92(3H, s, H-19), 0.99(3H, d, J=6.0Hz, H-21), 0.88(3H, d, *J*=7.5Hz, H-26), 0.83(3H, d, *J*=7, 0Hz, H-27), 0.85(3H, t, *J*=7.2 Hz, H-29), 5.38(1H, d, *J*=4.5 Hz, H-1′), 4.00~4.56(5H, m). ^{13}C-NMR (DMSO-d_6, 100MHz) δ: 37.31(C-1), 29.74(C-2), 77.66(C-3), 39.35(C-4), 140.75(C-5), 121.64(C-6), 32.0(C-7), 31.86(C-8), 50.08(C-9), 36.67(C-10), 21.07(C-11), 39.57(C-12), 42.26(C-13), 56.65 (C-14), 24.33(C-15), 28.27(C-16), 55.91(C-17), 12.18(C-18), 19.43(C-19), 36.16(C-20), 19.43 (C-21), 33.82(C-22), 25.92(C-23), 45.62(C-24), 29.18(C-25), 19.08(C-26), 19.01(C-27), 23.08 (C-28), 11.97(C-29), 101.30(C-1′), 75, 1(C-2′),

78.42(C-3′), 70.54(C-4′), 78.30(C-5′), 61.56(C-6′). On the basis of spectral data, its structure was elucidated to be daucosterol.

Summary

All above compounds were first isolated from this plant. For compouds **1** and **4,** the inhibitory ratios were 38.8%($P<0.05$) and 29.4%($P>0.05$) at concentration of 10^{-5} mol L^{-1}. This suggested that 1 have inhibitory activities on the release of β-glucuronidase from rat PMNs induced by PAF.

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References

[1] X. W. Li: In Chinese Flora; Science Press: Beijing, 1982; 31:1 82.

[2] Lee HJ, Hyun EA, Yoon WJ, Kim BH, Rhee MH, Kang HK, Cho JY, Yoo ES. In vitro anti-inflammatory and anti-oxidative effects of Cinnamomum camphora extracts. J Ethnopharmacol. 2006; 103(2): 208-216.

[3] Yin-Mei Chiung，Hideo Hayashi，Hiroshi Matsumoto et al. New metabolites， tetrahydrofuran lignans，produced by Streptomyces. The Journal of Antibiotics，1994，47(4):487-491.

[4] Satoshi Yamauchi, Tomoko Ina, Takuya Kirikihira et al. Synthesis and Antioxidant Activity of Oxygenated Furofuran Lignans. Biosci. Biotechnol. Biochem. , 2004 , 68(1):183-192.

[5] Jing Xing Pan, Otto D. Hensens, Deborah L. Zink et al. Lignans with Platelet Activating Factor Antagonist Acitvity from Magnolia Biondii. Phytochemistry. 1987, 26(5):1377-1379.

[6] A.S.R. Anjaneyulu; A. Madhusudhana Rao; V. Kameswara Rao et al. Novel hydroxy lignans from the heartwood of Gmelina arborea. Tetrahedron. 1977, 133: 133-143.

[7] N. Kaneda; A. D.Kinghorn; N.R.Farnsworth et al. Two diarylheptanoids and lignan from Casuarina Junghuhniana. Phytochemistry. 1990, 29(10): 3366-3368.

[8] Rukachaisirikul, Thitima; Intaraudom, Jukkapong; Chawanasak, Suppachai et al. Phenylpropanoids from Cinnamomum parthenoxylon. National Science and Technology Development Agency. 2000, 26(3): 159-161.

[9] Hoeke, M.; Haensel, R. New investigation of Sassafras root. Archiv der Pharmazie und Berichte der Deutschen Pharmazeutischen Gesellschaft 1972, 305(1): 33-9.

[10] Zhi-Zhen Zhang, Dean Guo, Chang-Ling Li, et al. Gaultherins A and B, two ligans from Gaultheria yunnanensis. Phytochemistry, 1999, 51:469-472.

[11] Guorui Liu, Jun Wu, Meihua Yang. Complete assignments of (1)H and (13)C NMR data for three new arylnaphthalide lignans from Justicia procumbens. Magn Reson Chem. 2007, 46:283-286.