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CHEMICAL CONSTITUENTS OF THE BARKS OF *Litsea rubescens*

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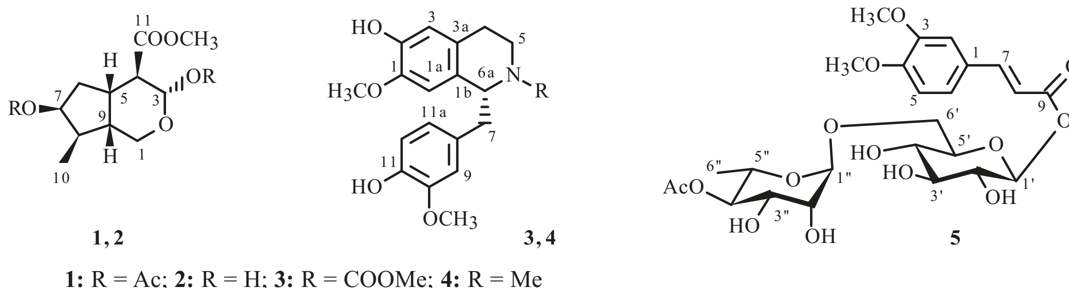
A phytochemical investigation of the ethanol extract from the barks of Litsea rubescens *resulted in the isolation of three new compounds, 3α,7β-diacetyl-8β-methyloctahydro-4β-methyl formate (**1**), (–)-*N*-methoxycarbonylorientailine (**3**), and 9-*O*-[4-*O*-acetyl-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-3,4-dimethoxycinnamic acid (**5**), together with two known compounds, 3α,7β-dihydroxy-8β-methyloctahydro-4β-methyl formate (**2**) and (–)-orientailine (**4**). The structures of the three new compounds were elucidated on the basis of 1D, 2D NMR (COSY, HMQC, HMBC, and NOESY), and mass spectral analyses.*

Keywords: *Litsea rubescens*, Lauraceae, iridoid, alkaloid, phenylpropanoid glycoside.

The genus *Litsea* (Lauraceae) is represented by 74 species in China, mostly growing in the south and southwestern parts of the country on mountains 1500 m above sea level [1]. *Litsea* plants demonstrate a wide spectrum of pharmacological properties, including antifungal, antibacterial, antidiarrheal, anti-inflammatory, anti-arthritic, anti-HIV, anti-asthma, hepatoprotective, immunomodulatory, antidiabetic, antioxidant, antitumor, and antidepressant activities [2–4]. Most *Litsea* plants contain alkaloids [5], flavonoids [6], terpenes [7, 8], lactones [9], lignans, amides, steroids, butanolides, butenolactones [2], and volatile oil constituents [10]. *Litsea rubescens*, a deciduous tree and shrub, has long been used as traditional Chinese medicine for the treatment of gastroenteralgia, edema, and rheumatic arthritis. Previous studies have shown the presence of cytotoxic chalcones in the stem barks of *L. rubescens* [11]. To find more pharmacological and structurally interesting substances, the present investigation of the ethanol extract of *L. rubescens* led to three new compounds, 3*α*,7*β*-diacetyl-8*β*-methyloctahydro-4*β*-methyl formate (**1**), (–)-*N*-methoxycarbonylorientailine (**3**), and 9-*O*-[4-*O*-acetyl-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranosyl]-3,4-dimethoxycinnamic acid (**5**), and two known compounds, 3*α*,7*β*-dihydroxy-8*β*-methyloctahydro-4*β*-methyl formate (**2**) and (–)-orientailine (**4**). Their structures were established on the basis of their chromatographic properties and by chemical and physicochemical methods.



1: R = Ac; **2:** R = H; **3:** R = COOMe; **4:** R = Me

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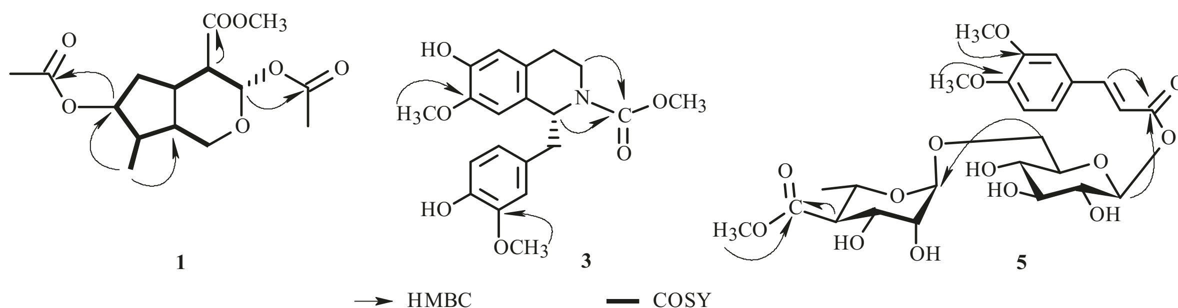

Fig. 1. Key HMBC and ^{1}H–^{1}H-COSY correlations of compounds **1**, **3**, and **5**.

Compound **1** was obtained as a colorless oil. Its molecular formula was established as $C_{15}H_{22}O_7$ deduced from the HR-ESI-MS at *m/z* 337.1259 $[M + Na]^+$ and ^{13}C NMR data analysis, indicating five degrees of unsaturation. The IR spectrum revealed the presence of C=O (1712, 1686, and 1678 cm^{-1}). Analyses of its ^{13}C NMR and DEPT spectra showed the presence of two acetyl groups (δ 170.7, 169.7, and 20.8), one quarternary carbonyl carbon (δ 173.0), six methines (two oxygenated ones), two methylenes, and two methyls (one oxygenated). The NMR spectral data of **1** were similar to those of 3*α*,7*β*-dihydroxy-8*β*-methyloctahydro-4*β*-methyl formate (**2**) [12], obtained also from the same plant. The only significant difference was the presence of two more acetyl groups in **1**. The two acetyl groups were positioned on C-3 and C-7 based on the HMBC correlaiton of H-3 (δ 5.84) and H-7 (δ 5.19) with the carbonyl carbon resonances (δ 169.7 and 170.7, respectively) (Fig. 1). The HMBC correlations of H-3 and H-5 (δ 1.88) with the carbonyl of methoxycarbonyl (δ 173.0) and of H-10 (δ_H 0.93) with C-7 (δ 78.0) and C-9 (δ 39.1) confirmed the location of methoxycarbonyl and methyl moieties at C-4 and C-8, respectively. On the basis of the observation of ROESY data similar to those of **2**, the stereochemistry of **1** was expected to be the same. The ROESY correlations of H-3/H-5/H-9 and H-4/H-6*α*/H-7 suggested that H-5, H-9, and the acetyl group on C-7 were of the *β*-configuration while the acetyl group on C-3 and H-4 were of the *α*-configuration. Therefore, **1** was inferred to be 3*α*,7*β*-diacetyl-8*β*-methyloctahydro-4*β*-methyl formate.

Compound **3**, a white amorphous powder, exhibited a molecular ion peak at *m/z* 374.1601 ($[M + H]^+$, calcd 374.1604) in the high-resolution mass spectrometry, which corresponded to the molecular formula $C_{20}H_{23}NO_6$ with 10 degrees of unsaturation. The IR spectrum of **3** indicated the existence of hydroxyl group (3430 cm^{-1}), carboxyl group (1668 cm^{-1}), and aromatic ring (1599 cm^{-1}). The ^{1}H NMR spectrum of **3** exhibited two singlets for two *para*-located aromatic protons at δ 6.55 (H-1a) and 6.39 (H-3) and an ABX system [δ 6.77 (1H, d, J =1.8 Hz, H-9), 6.74 (1H, d, J =8.0 Hz, H-12), and 6.58 (1H, dd, J = 8.0, 1.8 Hz, H-11a)]. The ^{13}C NMR spectrum showed 20 carbon signals [$OCH_3 \times 3$, CH_2 (sp^3) × 3, CH (sp^3) × 1, CH (sp^2) × 5 and C (sp^2) × 8]. The above data suggested that **3** was an isoquinoline alkaloid similar to (–)-orientailine (**4**) [13], except that a methyl group was oxygenated and esterified as an *N*-methoxycarbonyl moiety. The HMBC correlations between H-5 (δ 4.75 and 3.37), H-6a (δ 5.51) and the carbonyl carbon (δ 157.7) of the methoxycarbonyl indicated the *N*-6 location of the methoxycarbonyl group (Fig. 1). The relative configuration of **3** should be identical with that of **4** from a biogenetic perspective. Thus, the structure of **3** was established as (–)-*N*-methoxycarbonylorientailine.

Compound **5** was obtained as a colorless oil. Its molecular formula $C_{25}H_{34}O_{14}$ was determined by analyses of NMR data and verified by HR-ESI-MS (*m/z* 557.1864 $[M - H]^-$, calcd 557.1870). The IR spectrum of **5** indicated the existence of hydroxyl group (3414 and 1075 cm^{-1}), carboxyl group (1730 cm^{-1}), and aromatic ring (1610 cm^{-1}). The negative FAB-MS data at *m/z* 557 $[M - H]^-$, 395 $[M - H - 162]^-$, and 249 $[M - H - 162 - 146]^-$, combined with the ^{13}C NMR data, indicated that **5** had one hexosyl and one 6-deoxyhexosyl unit. Acid hydrolysis of **5** with 2% HCl afforded L-rhamnose and D-glucose, which was identified by GC analysis of its trimethylsilyl imidazole derivatives [14]. The rhamnopyranosyl and glucopyranosyl units were in the *α*- and *β*-configurations, respectively, by the coupling constants of their anomeric protons. In addition to the signals of sugar moieties, the ^{13}C NMR and DEPT spectra also displayed the presence of one carboxyl group [δ 165.9 (s)], two methoxy groups [δ 56.8 and 56.2 (each, q)], and eight olefinic carbons arising from a phenyl and a disubstituted double bond [δ 145.8, 115.0 (each, d)]. The ^{1}H NMR spectrum showed three aromatic protons at δ 7.16 (d, J = 1.8 Hz), 7.06 (dd, J = 8.6, 1.8 Hz), and 6.77 (d, J = 8.6 Hz) arising from a 1,3,4-trisubstituted benzene ring and two olefinic protons at δ 7.64 (d, J = 15.8 Hz) and 6.36 (d, J = 15.8 Hz) for a disubstituted *trans* double bond. These structural features indicated that **5** possessed a cinnamic acid aglycone. In the HMBC plot (Fig. 1), the correlations of glucosyl H-1′ (δ 4.82) with carboxyl carbon C-9 and of rhamnosyl H-1″ (δ 4.72) with glucosyl C-6′ (δ 67.8) indicated that the rhamnosyl (1→6) glucopyranosyl sugar chain was linked at the carboxyl group. The two methoxy groups were positioned at C-3 and C-4, and the acetyl group at C-4″, respectively, based on the HMBC correlations of the methoxy groups with C-3 and C-4, and of the proton signals (δ 4.89, H-4″) with the

carboxyl carbon of the acetyl (δ 172.5), respectively. Therefore, the structure of **5** was elucidated as 9-*O*-[4-*O*-acetyl-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-3,4-dimethoxycinnamic acid.

EXPERIMENTAL

General Procedures. Column chromatography (CC): silica gel (200–300 mesh, 10–40 μm; Qingdao Marine Chemical Factory, Qingdao, China), C_{18} reverse-phase silica gel (60 mm; Merck), Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden). Thin-layer chromatography (TLC): silica gel GF_{254} (10–40 μm; Qingdao Marine Chemical Factory, Qingdao, China). MCI Gel CHP20P (75–150 um; Mitsubishi Kasei Chemical Industries), C_{18} reversed-phase silica gel (20–45 μm; Fuji Silysia Chemical Ltd.). All solvents were distilled before use. Optical rotations: PerkinElmer 341 polarimeter. UV Spectra: Hewlett-Packard-8452A diode-array spectrophotometer. IR Spectra: Nicolet Magna FT-IR 750 spectrophotometer in cm^{-1}. ^1H and ^{13}C NMR spectra: Bruker AM-400 spectrometer, δ in ppm, J in Hz. MS: VG AutoSpec-3000 mass spectrometer, in *m/z*. EI-MS: Finnigan MAT-95 mass spectrometer. ESI-MS and HR-ESI-MS spectra: Micromass LC-MS-MS mass spectrometer.

Plant Material. The barks of *L. rubescens* were collected in Chuxiong, Yunnan Province, China, in June 2014. A specimen (LR20140601), identified by one of the authors (X. Mao), was deposited in the Herbarium of the College of Biological Resources and Environment Science, Qujing Normal University, Qujing, Yunnan, China.

Extraction and Isolation. The dried barks of *L. rubescens* (4.7 kg) were powdered and extracted with 70% ethanol three times under reflux for 15 h and then concentrated under reduced pressure to give a crude extract (108.5 g). The crude extract was partitioned between equal volumes of ethyl acetate and water to provide an EtOAc-soluble (60.3 g) and an aqueous layer. The EtOAc-soluble fraction was subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH (100:0, 5 L; 90:10, 5 L; 80:20, 5 L; 70:30, 5 L; 60:40, 5 L; 50:50, 5 L) to yield seven fractions (F1–F7). Fraction F2 (3.3 g) was further subjected to silica gel column chromatography eluting with $CHCl_3$–MeOH (90:10, 500 mL; 80:20, 500 mL; 70:30, 500 mL; 60:40, 500 mL; 50:50, 500 mL) to give three subfractions F2a (216 mg), F2b (103 mg), and F2c (156 mg). Subfraction F2a was separated by repeated column chromatography over Sephadex LH-20 ($CHCl_3$–MeOH, 1:1, 500 mL and MeOH, 500 mL), then purified on a silica gel column with *n*-hexane–EtOAc (7:3, 200 mL) as eluant to yield **1** (34.7 mg) and **2** (45.1 mg). Fraction F5 (petroleum ether–acetone, 30:1, 1.3 g) was applied to an ODS column eluting with MeOH–H_2O (30:70, 40:60, 50:50) to provide three subfractions (F5a, F5b, and F5c), F5b (MeOH–H_2O, 20:80, 226 mg) was repeatedly chromatographed on silica gel (150 g, 60 × 2.8 cm, $CHCl_3$–MeOH, 20:1→10:1) and then purified on a Sephadex LH-20 column eluting with MeOH–H_2O (50:50) to afford **4** (78 mg). Subfraction F5c was purified by preparative RP-HPLC (ODS column, 250 × 20 mm) eluting with MeOH–H_2O (20:80) to obtain **3** (77 mg) and **5** (66 mg).

Sugar Analysis of Compound 5. A solution of **5** (about 5.0 mg) in 2% HCl (dioxane–H_2O, 1:1, 1 mL) was heated at 95°C for 2 h based on a previous reference [15]. The reaction solution was extracted with chloroform three times. The aqueous layer was passed through an Amberlite IRA-401 (OH^- form) column, and the eluate was condensed until dry to yield the monosaccharide mixture. The solution of the sugar part obtained as described above in pyridine (2 mL) was added to L-cysteine methyl ester hydrochloride (1.5 mg) and kept at 60°C for 1 h each. Then trimethylsilylimidazole (1.5 mL) was added to the reaction mixture and the whole kept at 60°C for 30 min. The supernatant was subjected to GC analysis under the following conditions: column temperature: 180/280°C, programmed increase: 3°C/min, carrier gas N_2 (1 mL/min), injector and detector temperature 250°C, injection volume 4 μL, and split ratio 1:50. Configuration identification of D-glucose and L-rhamnose was carried out by comparison with their derivative retention times (Hara et al., 1987). Retention times in GC of standard D/L-glucose and L/D-rhamnose derivatives were 19.450/19.856 and 15.849/16.312 min, respectively. By comparing with the retention time of the authentic sugars in the form of derivatives under the same condition, the sugar moieties of compound **5** were determined to be D-glucose and L-rhamnose. All chemical reagents and standard sugars were purchased from Sigma-Aldrich.

3α,7β-Diacetyl-8β-methyloctahydro-4β-methyl Formate (1). Colorless oil, $[\alpha]_D^{20}$ +78.81° (*c* 0.110, CH_3Cl). IR (KBr, ν_{max}, cm^{-1}): 2940, 1712, 1686, 1678, 1275, 1033, 925. ^1H NMR (400 MHz, Me_2CO-d_6, δ, ppm, J/Hz): 5.84 (1H, d, J = 8.6, H-3), 5.19 (1H, m, H-7), 3.88 (1H, dd, J = 12.6, 5.0, H-1b), 3.73 (1H, dd, J = 12.6, 6.0, H-1a), 3.63 (3H, s, CO_2Me), 2.51 (1H, m, H-4), 2.44 (1H, m, H-6β), 2.03 (1H, m, H-9), 1.99 (6H, s, COMe × 2), 1.98 (1H, m, H-8), 1.88 (1H, m, H-5), 1.86 (1H, m, H-6α), 0.93 (3H, d, J = 6.6, H-10). ^{13}C NMR (100 MHz, Me_2CO-d_6, δ, ppm): 173.0 (C-11), 170.7 (MeCO-7), 169.7 (MeCO-3), 94.3 (C-3), 78.0 (C-7), 64.7 (C-1), 52.3 (CO_2Me), 49.5 (C-4), 43.5 (C-8), 39.1 (C-9), 37.9 (C-6 and C-5), 20.8 (COMe × 2), 12.5 (C-10). EI-MS *m/z* 314 $[M]^+$; HR-ESI-MS *m/z* 337.1259 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_7Na$, 337.1263).

(–)-*N*-Methoxycarbonylorientailine (3). White amorphous powder, $[\alpha]_D^{20}$ –18.34° (*c* 0.100, MeOH). UV (MeOH, λ_{max}, nm): 301 (3.77), 284 (3.88), 216 (4.26). IR (KBr, ν_{max}, cm^{-1}): 3430, 1668, 1599, 1520, 1465, 1209, 1020. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.77 (1H, d, J = 1.8, H-9), 6.74 (1H, d, J = 8.0, H-12), 6.58 (1H, dd, J = 8.0, 1.8, H-11a), 6.55 (1H, s, H-1a), 6.39 (1H, s, H-3), 5.51 (1H, m, H-6a), 4.75 (1H, m, H-5b), 3.86 (3H, s, 1-OMe), 3.85 (3H, s, 10-OMe), 3.71 (3H, s, CO_2Me), 3.37 (1H, m, H-5a), 2.75 (1H, m, H-7b), 2.56 (1H, m, H-4a), 2.64 (1H, m, H-7a), 2.37 (1H, m, H-4b). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 157.7 (CO_2Me), 145.4 (C-10), 145.1 (C-1), 144.9 (C-2), 143.4 (C-11), 133.3 (C-8), 130.4 (C-3a), 125.3 (C-1b), 120.9 (C-1a), 115.6 (C-12), 113.6 (C-3), 110.5 (C-11a), 110.3 (C-9), 57.8 (C-6a), 55.9 (1-OMe), 55.8 (10-OMe), 53.4 (CO_2Me), 46.8 (C-5), 40.8 (C-7), 24.9 (C-4). ESI-MS *m/z* 374 $[M + H]^+$; HR-ESI-MS *m/z* 374.1601 $[M + H]^+$ (calcd for $C_{20}H_{24}NO_6$, 374.1604).

9-*O*-[4-*O*-Acetyl-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranosyl]-3,4-dimethoxycinnamic Acid (5). Colorless oil, $[\alpha]_D^{20}$ –213.03° (*c* 0.004, MeOH). UV (MeOH, λ_{max}, nm): 204 (3.15). IR (KBr, ν_{max}, cm^{-1}): 3414, 1730, 1610, 1461, 1389, 1075. ^1H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 7.64 (d, J = 15.8, H-7), 7.16 (d, J = 1.8, H-2), 7.06 (dd, J = 8.6, 1.8, H-6), 6.77 (d, J = 8.6, H-5), 6.36 (d, J = 15.8, H-8), 4.89 (1H, m, H-4″), 4.82 (1H, d, J = 7.3, H-1′), 4.72 (1H, m, H-1″), 3.98, 3.63 (each 1H, m, H-6′), 3.96 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 3.83 (1H, m, H-4′), 3.82 (1H, m, H-3″), 3.81 (1H, m, H-5″), 3.49 (1H, m, H-3′), 3.48 (1H, m, H-2″), 3.46 (1H, m, H-2′), 3.37 (1H, m, H-5′), 2.07 (3H, s, $COCH_3$), 1.04 (3H, d, J = 8.2, H-6″). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 172.5 ($COCH_3$), 165.9 (C-9), 149.4 (C-3), 145.8 (C-7), 126.6 (C-1), 124.6 (C-6), 111.7 (C-2), 116.7 (C-5), 115.9 (C-4), 115.0 (C-8), 103.1 (C-1′), 102.0 (C-1″), 77.9 (C-2′), 76.8 (C-3′), 75.6 (C-4″), 75.0 (C-2″), 72.3 (C-4′), 71.4 (C-5′), 70.4 (C-3″), 67.8 (C-6′), 67.6 (C-5″), 56.8 (OCH_3), 56.2 (OCH_3), 40.8 (C-7), 21.1 ($COCH_3$), 17.8 (C-6″). FAB-MS (neg.) 557 $[M – H]^–$; HR-ESI-MS *m/z* 557.1864 $[M – H]^–$ (calcd for $C_{25}H_{33}O_{14}$, 557.1870).

REFERENCES

1. X. H. Yan, F. X. Zhang, H. H. Xie, and X. Y. J. Wei, *Trop. Subtrop. Bot.*, **2**, 171 (2000).
2. K. A. B. S. da Silva, L. C. Klein-Junior, S. M. Cruz, A. Caceres, N. L. M. Quintao, F. D. Monache, and V. Cechinel-Filho, *Food Chem.*, **132**, 1980 (2012).
3. D. G. Kong, Y. Zhao, G. H. Li, B. J. Chen, X. N. Wang, H. L. Zhou, H. X. Lou, D. M. Ren, and T. Shen, *J. Ethnopharmacol.*, **164**, 256 (2015).
4. W. J. Tang, Y. L. Zhang, Q. P. Xiao, C. Huang, Y. Jin, and J. Li, *Chem. Biodiv.*, **10**, 1128 (2013).
5. R. C. Rastogi and N. Borthakur, *Phytochemistry*, **19**, 998 (1980).
6. J. A. Lopez, W. Barillas, and G. L. Jorge, *Planta Med.*, **61**, 198 (1995).
7. E. H. Hakim, S. A. Achmad, M. Effendy, E. L. Ghisalberti, D. C. R. Hockless, and A. H. White, *Aust. J. Chem.*, **46**, 1355 (1993).
8. S. A. Achmad, E. L. Ghisalberti, E. H. Hakim, L. Makmur, and M. Mamurung, *Phytochemistry*, **31**, 2153 (1992).
9. H. Tanaka, T. Nakamura, K. Ichino, K. Ito, and T. Tanaka, *Phytochemistry*, **29**, 857 (1990).
10. K. P. Padmakumari and C. S. J. Narayannan, *Essent. Oil Res.*, **4**, 87 (1992).
11. L. Li, X. T. Zhao, Y. P. Luo, J. F. Zhao, X. D. Yang, and H. B. Zhang, *Bioorg. Med. Chem. Lett.*, **21**, 7431 (2011).
12. H. Corrode and E. Hardegger, *Helv. Chim. Acta*, **39**, 889 (1956).
13. J. Kunitomo, *Yakugaku Zasshi*, **81**, 1253 (1972).
14. S. Hara, H. Okabe, and K. Mihashi, *Chem. Pharm. Bull.*, **35**, 501 (1987).
15. Y. Zhang, Y. J. Zhang, M. R. Jacob, X. C. Li, and C. R. Yang, *Phytochemistry*, **69**, 264 (2008).