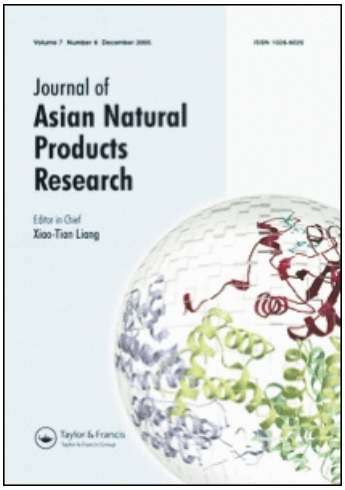

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Chemical constituents from *Alpinia tonkinensis*

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CHEMICAL CONSTITUENTS FROM *ALPINIA TONKINENSIS*

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Two new compounds, 2α-(*p*-hydroxycinnamoyl) cineole (**2**) and (*E*)-15-nor-16-oxo-8(17),12-labdadiene (**4**), along with three known compounds, *trans*-cinnamyl methyl ester (**1**), 4(15)-cadinene-6,10-diol (**3**) and isorhamnetin-3-*O*-β-D-galactosyl-(6 → 1)-α-L-rhamnoside (**5**), have been isolated from *Alpinia tonkinensis* Gangep. Their structures have been elucidated by spectral analysis.

Keywords: *Alpinia tonkinensis* Gangep.; 2α-(*p*-Hydroxy-cinnamoyl)cineole; (*E*)-15-nor-16-Oxo-8(17),12-labdadiene

INTRODUCTION

Rhizomes of *Alpinia tonkinensis* Gagnep. (Zingiberaceae) are commonly used as a spice and as an aromatic drug in Guangxi Province, China. They are also used to treat stomach-ache as a digestive [1]. So far only Qin *et al.* have analyzed the chemical constituents of their volatile oil, by GC–MS [2]. The present paper describes the isolation and structural elucidation of two new compounds, 2α-(*p*-hydroxycinnamoyl) cineole (**2**) and (*E*)-15-nor-16-oxo-8 (17),12-labdadiene (**4**) (Fig. 1), along with three known compounds, *trans*-cinnamyl methyl ester (**1**), 4(15)-cadinene-6,10-diol (**3**) and isorhamnetin-3-*O*-β-D-galactosyl-(6 → 1)-α-L-rhamnoside (**5**), from the rhizomes of *Alpinia tonkinensis* Gangep. This is the first time the three known compounds have been isolated from the genus *Alpinia*.

RESULTS AND DISCUSSION

Compound **2** was isolated as an amorphous powder. Its molecular formula was assigned as $C_{19}H_{24}O_4$ on the basis of the molecular ion peak (M^+) in the HR-EIMS at *m/z* 316.1656. The UV spectrum shows absorptions at 313, 228 and 210 nm. IR absorption bands at 3206, 1708, 1636, 1605, 1587, 1442 and 1170 cm^{-1} are attributed to hydroxy, α,β-unsaturated

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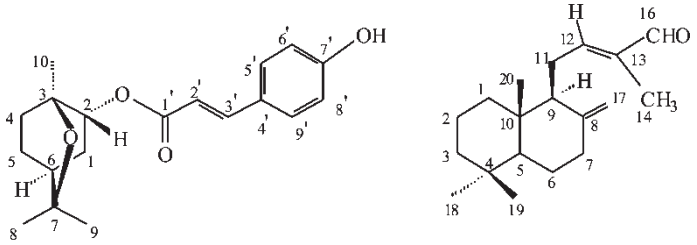
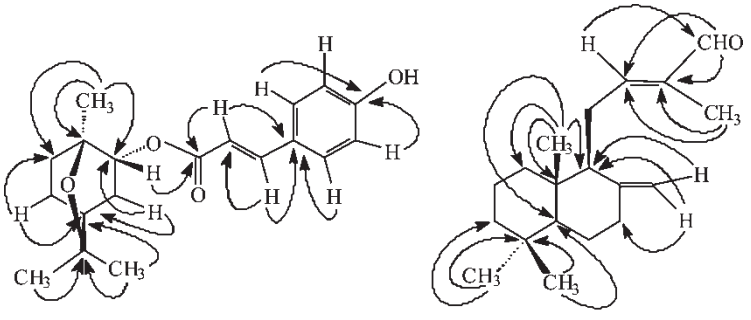


2 **4**

FIGURE 1 Structure of **2** and **4**.

carbonyl, an olefinic group, and a benzene ring. The ^{1}H NMR spectrum of **2** contains a pair of *trans* doublets at δ 6.30 (1H, d, $J = 15.9$ Hz) and 7.61 (1H, d, $J = 15.9$ Hz), an AA′BB′ coupling of proton signals at δ 6.85 (2H, d, $J = 8.5$ Hz) and 7.45 (2H, d, $J = 8.5$ Hz), and one hydroxy proton signal at δ 6.20. The ^{13}C NMR spectrum of **2** contains a signal due to an α,β-unsaturated carbonyl at δ 167.1 and two olefinic carbon signals at δ 115.5 and 144.7, as well as six aromatic carbon signals at δ 126.9, 130.0, 130.0, 115.9, 115.9, 158.1. The above spectral evidences indicate that **2** contains a *p*-hydroxycinnamoyl moiety, which was further confirmed by the fragment peak at *m/z* 147 (100) in the EIMS. In addition, the ^{1}H NMR spectrum of **2** exhibits three methyl singlets at δ 1.09, 1.26, 1.31, a proton signal at δ 4.83, and the ^{13}C NMR and DEPT spectra of **2** exhibit ten carbon signals at δ 74.1 (C), 72.8 (CH), 71.3 (C), 33.9 (CH), 31.7 (CH_2), 28.9 (CH_3), 28.6 (CH_3), 26.1 (CH_2), 24.2 (CH_3), and 22.0 (CH_2), which indicate that **2** contains a bicyclic monoterpene moiety. Cross peaks in the HMBC spectrum of **2** indicate that the bicyclic monoterpene moiety is 2-hydroxycineole (Fig. 2). The HMBC spectrum of **2** shows obvious cross peak of H-2 to C-1′, indicating that the 2-hydroxycineole is esterified. Thus, the structure is elucidated as 2-(*p*-hydroxycinnamoyl)cineole. The stereochemistry of **2** was determined by the coupling constants of related protons and NOESY spectrum. A small coupling ($J = 1.6$ Hz) for H-2 in **2** (Table I) must be due to a four-bond interaction with a proton in the 4-methylene. Such long-range coupling is only favoured if the coupling protons can adopt a W-conformation. Thus, H-2 in **2** is equatorial, requiring a *trans* stereochemistry for the epoxide linkage and the cinnamate substituent. The proposed stereochemistry was confirmed by NOESY experiments, which demonstrate the correlation between H-2 and the 9-methyl [3]. Additionally, the NOESY correlations occur between H-2 and H-10, H-1e; H-1e and H-9; H-5 and H-8 (Fig. 3). Therefore, the **2** is established as 2α-(*p*-hydroxycinnamoyl)cineole (Fig. 1).



2 **4**

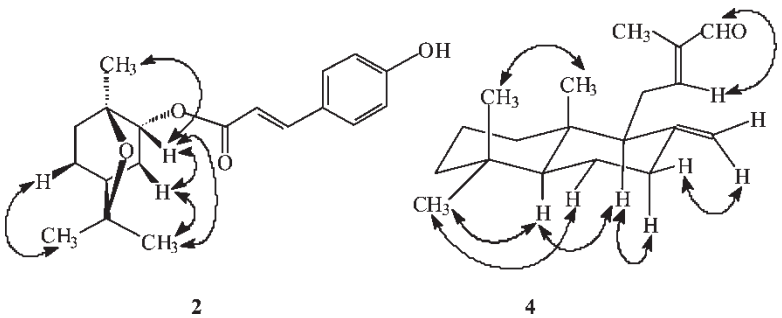
FIGURE 2 Major HMBC correlations for **2** and **4**.

TABLE I ^{1}H (400 MHz in $CDCl_3$) and ^{13}C NMR (100 MHz in CD_3COCD_3) spectral data of **2** and **4**

2			**4**		
Position	δ_H	δ_C	*Position*	δ_H	δ_C
1	2.67 (1H, ddd, J = 12.2, 9.7, 3.3 Hz)	31.7	1	1.07, 1.70	40.1
	1.38 (1H,ddd, J = 13.6, 3.8, 3.3 Hz)		2	1.34, 1.42	20.3
2	4.83 (1H, ddd, J = 9.7, 2.9, 1.6 Hz)	72.8	3	1.24, 1.40	43.1
3		71.3	4		34.5
4	1.95, 1.65	26.1	5	1.22 (1H, m)	56.4
5	2.05, 1.57	22.0	6	1.36, 1.76	25.5
6	1.61 (1H, m)	33.9	7	2.02, 2.45	38.9
7		74.1	8		149.5
8	1.31 (3H, s)	28.6	9	2.05 (1H, m)	57.5
9	1.26 (3H, s)	28.9	10		40.6
10	1.09 (3H, s)	24.2	11	2.10, 2.50	25.3
1′		167.1	12	6.72 (1H, m)	159.3
2′	6.30 (1H, d, J = 15.9 Hz)	115.5	13		138.2
3′	7.61 (1H, d, J = 15.9 Hz)	144.7	14	3.33 (3H, s)	29.7
4′		126.9	16	9.33 (1H, s)	194.3
5′ 9′	6.85 (2H, d, J = 8.5 Hz)	130.0	17	4.53, 4.85 (br s)	108.8
6′ 8′	7.45 (2H, d, J = 8.5 Hz)	115.9	18	0.89 (3H, s)	34.3
7′		158.1	19	0.84 (3H, s)	22.4
			20	0.78 (3H, s)	15.1
OH	6.20				

Extensive spectral analysis, including ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC, NOESY and HR-EIMS, has allowed the proton and carbon signals of **2** to be assigned as in Table I.

Compound **4** was isolated as a yellow liquid. Its molecular formula was assigned as $C_{19}H_{30}O$ on the basis of the molecular ion peak (M^+) in the HR-EIMS at *m/z* 274.2286. IR absorption bands at 1685 and 1641 cm^{-1} are attributed to α,β-unsaturated carbonyl and olefinic groups, respectively. ^{1}H NMR signals at δ 0.89 (3H, s), 0.84 (3H, s) and 0.78 (3H, s), as well as those at δ 4.53 (1H, br s) and 4.85 (1H, br s), are characteristic of a labdane-type diterpenoid and assigned to the methyl groups at C-18, C-19, and C-20 as well as to the methylene group at C-17, respectively, which is confirmed by the fragment peak at *m/z* 137 (100) in the EIMS. The ^{13}C NMR spectrum and DEPT experiment for **4** confirmed the existence of 19 carbons and four methyls, seven methylenes, three methines, five quaternary carbons, which contain an α,β-unsaturated carbonyl at δ 194.3 and four olefinic carbon signals at δ 159.3 and 138.2, 108.8 and 149.5, as well as four methyl carbon signals at δ 29.7, 34.3, 22.4, 15.1. The above ^{13}C NMR signals provide further evidence of a labdane-type diterpenoid. According to the NOESY spectra (Fig. 3) and comparison of ^{13}C NMR data with literature values [4], the relative configuration of **4** is identical with those of labdane-type



2 **4**

FIGURE 3 Major NOESY correlations for **2** and **4**.

diterpenoids isolated from the plants in the family Zingiberaceae. The structure of the side-chain (C-11 to C-16) of **4** was deduced from its HMBC spectrum. In the HMBC spectrum (Fig. 2), the olefinic signal at δ 6.72 (1H, m, H-12) and the methyl signal at δ 3.33 (3H, s, H-14) are correlated with the signal of the aldehyde group at δ 194.3 (C-16). The stereochemistry of the double bond between C-12 and C-13 of **4** was determined from the NOESY spectrum. An obvious NOE correlation between the signal of the aldehyde proton at δ 9.33 (H-16) and the olefinic signal at δ 6.72 (H-12) indicates an *E*-configuration for the double bond. Accordingly, the structure of the side-chain was established. Therefore, the structure of **4** is (*E*)-15-nor-16-oxo-8(17),12-labdadiene (Fig. 1). Extensive spectra analysis, including ^{1}H and ^{13}C NMR, DEPT, HMBC and NOESY, has allowed proton and carbon signals of **4** to be assigned as in Table I.

EXPERIMENTAL

General Experimental Procedures

Mps were determined on an *X*4 micromelting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. UV spectra were recorded on an UV-2051 spectrophotometer in MeOH. IR spectra were recorded on an Impact-410 (Nicolet) spectrograph. 1D and 2D NMR spectra were recorded on Bruker ACF 300, 400 MHz spectrometers, using TMS as internal standard. EIMS were measured on a HP5989 mass spectrometer. HR-EIMS were measured on Jeol D-300 mass spectrometer. Silica gel H (10–40 μm) was used for column chromatography.

Plant Material

Rhizomes of *Alpinia tonkinensis* Gagnep. were collected in Nanning, Guangxi Province, China, in September, 1997, and identified by Dr Min-jian Qing, Department of Medicinal Botany, China Pharmaceutical University. A voucher specimen (No. 970914) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation

Powdered rhizomes (1.80 kg) were boiled under reflux in MeOH, and the resultant extract suspended in MeOH–water (10:90) and then further extracted with light petroleum (60–90°C). MeOH was removed from the water fraction and successively extracted with chloroform and *n*-butanol. Solvent was removed from the light petroleum fraction and the residue was chromatographed over silica gel, eluted with mixtures of light petroleum–acetone of gradually increasing polarity, to give compounds **1** (25 mg), **2** (8 mg), and **3** (14 mg). The solvent was removed from the chloroform fraction and the residue was chromatographed over silica gel, eluted with mixtures of chloroform–acetone of gradually increasing polarity, to give compound **4** (7 mg). The solvent was removed from the *n*-butanol fraction *in vacuo* and the residue was chromatographed over silica gel, eluted with mixtures of chloroform–methanol of gradually increasing polarity to give compound **5** (7 mg).

*trans-Cinnamyl methyl ester (**1**)*

$C_{10}H_{10}O_2$, yellow solid, mp 36.0–37.0°C. UV (MeOH) λ_{max} (nm): 274, 221, 215; IR (KBr) ν (cm^{-1}): 1717, 1636, 1575, 1440, 1320, 1276, 1172, 768; ^{1}H NMR (300 MHz, $CDCl_3$)

δ (ppm): 6.43 (1H, d, $J = 16.2$ Hz), 7.70 (1H, d, $J = 16.2$ Hz), 7.37 (3H, m), 7.52 (2H, m), 3.81 (3H, s, OCH_3).

2α-(p-Hydroxycinnamoyl) cineole (**2**)

$C_{19}H_{24}O_4$, amorphous powder, mp 195.0–197.0°C, $[\alpha]_D^{20}$ −10.6 (*c* 0.10, $CHCl_3$). UV (MeOH)λ_{max} (nm): 313, 228, 210; IR (KBr) ν (cm^{-1}): 3206, 2987, 2952, 1708, 1636, 1605, 1587, 1442, 1278, 1170; ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) see Table I; NOESY correlations were observed from H-2 to H-9, H-10, H-1e; H-1e to H-9; H-5 to H-8′; HR-EIMS (M^+) *m/z*: 316.1656 (calcd. for $C_{19}H_{24}O_4$, 316.1675); EIMS *m/z* (%): 316 (M^+, 9.7), 147 (100.0), 119 (12.5), 109 (13.7), 108 (14.8), 91 (19.0), 65 (12.8), 43 (44.7), 41 (17.3).

4 (15)-Cadinene-6, 10-diol (**3**)

$C_{23}H_{24}O_{11}$, Colorless needles, mp 148.0–150.0°C, $[\alpha]_D^{20}$ + 15.7 (*c* 0.20, $CHCl_3$). IR (KBr) ν (cm^{-1}): 3370, 2959, 2951,1654, 1413, 1384, 1178, 886; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.23 (1H, dd, $J = 3.8$, 12.7 Hz, H-1), 1.60 (1H, m, H-2), 1.86 (1H, m, H-2), 1.98 (1H, m, H-3), 2.40 (1H, m, H-3), 1.92 (1H, m, H-5), 2.54 (1H, dd, $J = 1.9$, 13.3 Hz, H-5), 1.15 (1H, dt, $J = 2.5$, 10.4 Hz, H-7), 1.45 (1H, m, H-8), 1.78 (1H, m, H-8), 1.82 (1H, m, H-9), 1.42 (1H, m, H-9), 2.14 (1H, m, H-11), 0.93 (3H, s, C-12-CH_3), 0.94 (3H, s, C-13-CH_3) 1.13 (3H, s, C-14-CH_3), 4.76 (1H, dd, $J = 2.2$, 5.2 Hz, H-15), 4.87 (1H, dd, $J = 2.2$, 5.2 Hz, H-15), 3.60 (6-OH), 2.19 (10-OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 50.2 (C-1), 22.8 (C-2), 34.2 (C-3), 145.9 (C-4), 45.5 (C-5), 76.4 (C-6), 51.5 (C-7), 16.5 (C-8), 41.2 (C-9), 71.9 (C-10), 25.5 (C-11), 23.6 (C-12-CH_3), 18.4 (C-13-CH_3), 28.1 (C-14-CH_3), 111.4 (C-15); EIMS *m/z* (%): 238 (M^+, 4.6), 223 (22.0), 111 (31.1), 109 (23.1), 71 (30.2), 69 (37.0), 55 (55.7), 43 (100.0), 41 (58.1).

(E) 15-nor-16-Oxo-8 (17),12-labdadiene (**4**)

$C_{19}H_{30}O$, yellow liquid, $[\alpha]_D^{20}$ +18.6 (*c* 0.05 $CHCl_3$). IR (KBr) ν (cm^{-1}): 3075, 2935, 2861,1685, 1641, 1385, 1268, 1119, 1082, 985; ^1H (400 MHz, CD_3COCD_3) and ^{13}C NMR (100 MHz, CD_3COCD_3) see Table I; HR-EIMS $(M)^+$ *m/z*: 274.2286 (calcd. for $C_{19}H_{24}O_4$ 274.2295); EIMS *m/z* (%): 274 (M^+, 5.0), 137 (100.0), 123 (72.7), 95 (93.7), 91 (65.9), 81 (99.0), 69 (82.1), 55 (81.3), 41 (92.7).

Iisorhamnetin-3-O-β-D-galactosyl-(6 → 1)-α-L-rhamnoside (**5**)

$C_{28}H_{32}O_{16}$, yellow powder, mp >300.0°C, HCl–Mg reaction (+), Molish reaction (+). UV (MeOH) λ_{max} (nm): 255, 355; 265, 398 ($AlCl_3$); 265, 398 ($AlCl_3$/HCl); 265, 415 (NaOMe); IR (KBr) ν (cm^{-1}): 3429, 1659, 1263, 1052, 1027, 1008, 824, 762; ^1H NMR (300 MHz, DMSO) δ (ppm): 12.60 (1H, s, 5-OH), 8.00 (1H, brs, H-2′), 7.49 (1H, d, $J = 8.8$ Hz, H-6′), 6.90 (1H, d, $J = 8.4$ Hz, H-5′), 6.44 (1H, brs, H-8), 6.20 (1H, d, $J = 7.9$ Hz, gal-H-1), 5.22 (1H, brs, rha-H-1), 3.85 (3H, s, OCH_3), 1.05 (3H, d, $J = 5.9$ Hz, rha-CH_3); ^{13}C NMR (75 MHz, DMSO–d_6) δ (ppm): 156.8 (C-2), 133.5 (C-3), 177.7 (C-4), 161.6 (C-5), 99.1 (C-6), 164.6 (C-7), 94.1 (C-8), 156.8 (C-9), 104.4 (C-10), 121.4 (C-1′), 113.8 (C-2′), 149.8 (C-3′), 147.4 (C-4′), 115.5 (C-5′), 122.4 (C-6′), 102.2 (gal-C-1), 71.5 (gal-C-2), 73.3 (gal-C-3), 68.3 (gal-C-4), 74.0 (gal-C-5), 65.7 (gal-C-6), 100.5 (rha-C-1), 70.8 (rha-C-2), 71.0 (rha-C-3), 72.2 (rha-C-4), 68.6 (rha-C-5), 18.2 (rha-CH_3).

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