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Components of Stem Barks of *Winchia calophylla* A. DC. and Their Bronchodilator Activities

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Abstract: The Dai medicinal plant *Winchia calophylla* A. DC. (Apocynaceae) has efficacy as an anti-cough and anti-asthmatic medication. In order to investigate its relative bioactive components, we studied the chemical constituents of this plant. Using repeated column chromatography, 28 compounds, including loganin, six phenolic compounds, 17 indole alkaloids, three pyridine alkaloids, and a quinoline alkaloid, were isolated from the stem barks of *W. calophylla*. Loganin, paeonol, *N* (4)-methyl akuammicine, and cantleyine exhibited a moderate relaxation effect on isolated smooth muscles of guinea-pig tracheal spirals and lung strips and may be the bioactive components responsible for the bronchodilation produced by *W. calophylla*.

Key words: alkaloid; Apocynaceae; bronchodilatation; *Winchia calophylla*.

Winchia calophylla A. DC. (Apocynaceae), distributed in Yunnan and Hainan provinces of China, India, Myanmar, and Indonesia, is a traditional Dai medicinal plant (Delectis Florae Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita 1977). It has been used as a substitute for *Alstonia scholaris* (L.) R. Br. (Apocynaceae), from which the drugs Dengtaiyekeli and Dengtaiyechongji have been made, and is used in the treatment of cough, asthma, and chronic bronchitis. However, the relative bioactive components of *W. calophylla* have not been determined. Thus, in the present study we systematically investigated the chemical constituents of this medicinal plant. Previously, we had reported on the isolation and elucidation of nine triterpenoids from the stem barks of *W. calophylla* (Zhu *et al.* 2002). In the present study, 28 known compounds, including 21 alkaloids, were isolated.

1 Results and Discussion

By comparison of their physical and EIMS, ^{1}H-, and ^{13}C-NMR, and DEPT spectral data with those already published, the 28 compounds were identified as loganin (**1**; Ikeshiro and Tomita 1984), paeonol (**2**; Patra *et al.* 1987), 4-hydroxy-3-methoxybenzoic acid (**3**; Scott 1972), 3, 4-dihydroxybenzoic acid (**4**; Scott 1972), 2, 3-dihydroxybenzoic acid (**5**; Scott 1972), sesamin (**6**; Pelter *et al.* 1978), (–)-lyoniresinol (**7**; Ohashi *et al.* 1994), echitamidine (**8**; Keawpradub *et al.* 1994), alstoqustine (**9**; Hu *et al.* 1989), *N* (4)-methyl akuammicine (**10**; Yamauchi *et al.* 1990a), *N* (4)-demethyl alstoqustine (**11**; Hu *et al.* 1989), tubotawine (**12**; Yamauchi *et al.* 1990b), rhazimanine (**13**; Rahman and Alvi 1987), echitamine (**14**; Hu *et al.* 1989; Yamauchi *et al.* 1990b; Keawpradub *et al.* 1994), 17-O-acetylechitamine (**15**; Yamauchi *et al.* 1990b),

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pseudoakuammigine (**16**; Yamauchi *et al.* 1990a), picrinine (**17**; Abe *et al.* 1989), nareline (**18**; Abe *et al.* 1989), stemmadenine (**19**; Achenbach *et al.* 1997), vallesamine (**20**; Yamauchi *et al.* 1990a), cantleyine (**21**; Ravao *et al.* 1985), isocantleyine (**22**; Zhang *et al.* 1992), venoterpine (**23**; Ravao *et al.* 1985), rutaecarpine (**24**; Tang *et al.* 1996a), evodiamine (**25**; Tang *et al.* 1996a), dehydroevodiamine hydrochloride (**26**; Arisawa *et al.* 1993), 1, 2, 3, 4-tetrahydro-1-oxo-carboline (**27**; Tang *et al.* 1996a), and 1-methyl-2-[(10*Z*)-10-pentadecanenyl]-4 (1H)-quinolone (**28**; Tang *et al.* 1996b), as shown in Fig.1.

The wheeze-relieving activities of total alkaloids from the stem barks of *W. calophylla* and the compounds isolated were tested by determining their relaxation effect on isolated smooth muscles of guinea-pig tracheal spirals and lung strips (Xie *et al.* 1999). Loganin (**1**), cantleyine (**21**), and *N* (4)-methyl akuammicine (**10**) were able to relax contraction induced by histamine phosphate (final concentration 0.001 mg/mL), with EC_{50} values of 0.630, 0.133, and 0.750 mg/mL, respectively. In particular, the relaxation effect of compound **21** was close to that of the aminophylline control (EC_{50} 0.0240 mg/mL; Miles *et al.* 1996). Paeonol (**2**), cantleyine (**21**), and *N* (4)-methyl akuammicine (**10**) exhibited diastolic effects on the contraction of the lung strips induced by histamine phosphate (final concentration 0.001 mg/mL), with EC_{50} values of 0.480 0, 0.097 5, and 0.022 1 mg/mL, respectively. The relaxation produced by *N* (4)-methyl akuammicine was close to that produced by the aminophylline control (EC_{50} 0.015 5 mg/mL; Table 1). The other compounds tested, namely alstogustine (**9**), rhazimanine (**13**), echitamine (**14**), 17-O-acetyl echitamine (**15**), stemmadenine (**19**), cantleyine (**21**), rutaecarpine (**24**), evodiamine (**25**), and 1-methyl-2-[(10*Z*)-10-pentadecanenyl]-4 (1H)-quinolone (**28**), did not exhibit any wheeze-relieving activity under the present test conditions. Thus, it stands to reason that loganin (**1**), paeonol (**2**), cantleyine (**21**), and *N* (4)-methyl akuammicine (**10**) are the main bioactive components of *W. calophylla* in the treatment of chronic tracheitis.

2 Experimental

2.1 General experimental procedures

Optical rotations were determined on a JASCO-20C digital polarimeter (JASCO International Co., Ltd., Tokyo, Japan). IR spectra were obtained on a Bio-Rad FTS-135 IR spectrometer (Bio-Rad Laboratories Inc., USA). The ^{1}H- and ^{13}C-NMR spectra were recorded on a DRX-500 spectrometer (Bruker BioSpin Corporation, Switzerland) with TMS as the internal standard. The EI-MS measurements were performed on a VG AutoSpec-3000 mass spectrometer (VG, Manchester, UK). TLC and column chromatography were performed on plates precoated with silica gel F_{254} and over silica gel H (Qingdao Marine Chemical Plant, Qingdao, China), respectively. Solvents were distilled prior to use.

2.2 Plant materials

The stem barks of *Winchia calophylla* A. DC. were collected in Xishuangbanna, Yunnan Province of China, and identified by Professor Hong-Mao LIU and Mr Jing-Yun CUI (Xishuangbanna Tropical Botanical Garden, the Chinese Academy of Sciences, Menglun, Yunnan, China). A voucher specimen was deposited in the Xishuangbanna Tropical Botanical Garden, the Chinese Academy of Sciences.

2.3 Extraction and isolation

The dried stem barks (10.5 kg) of *W. calophylla* were extracted with 95% ethanol (EtOH; 4 ×15 L) under reflux. The syrup obtained (600 g) was partitioned between petroleum ether (PE) and water. The PE fraction was dissolved in 2% HCl (4×500 mL). The acidic solution was adjusted to pH 9–10 with concentrated ammonia and the basic solution obtained was then extracted with PE (3×600 mL), $CHCl_3$ (3×600 mL), and *n*-butanol (3×500 mL) in order. The PE fraction (17.2 g) was separated by chromatography over silica gel H, eluting with PE-ethyl acetate (2 ∶ 1) and $CHCl_3$-MeOH (200 ∶ 1) to afford compound **28** (25 mg). The $CHCl_3$ fraction (60 g) gave compounds **6** (70 mg), **8** (16 mg), **9** (12 mg), **11** (450 mg), **12** (40 mg), **14** (4.26 g), **15** (1.23 g), **16** (72 mg), **17** (23 mg), **18** (42

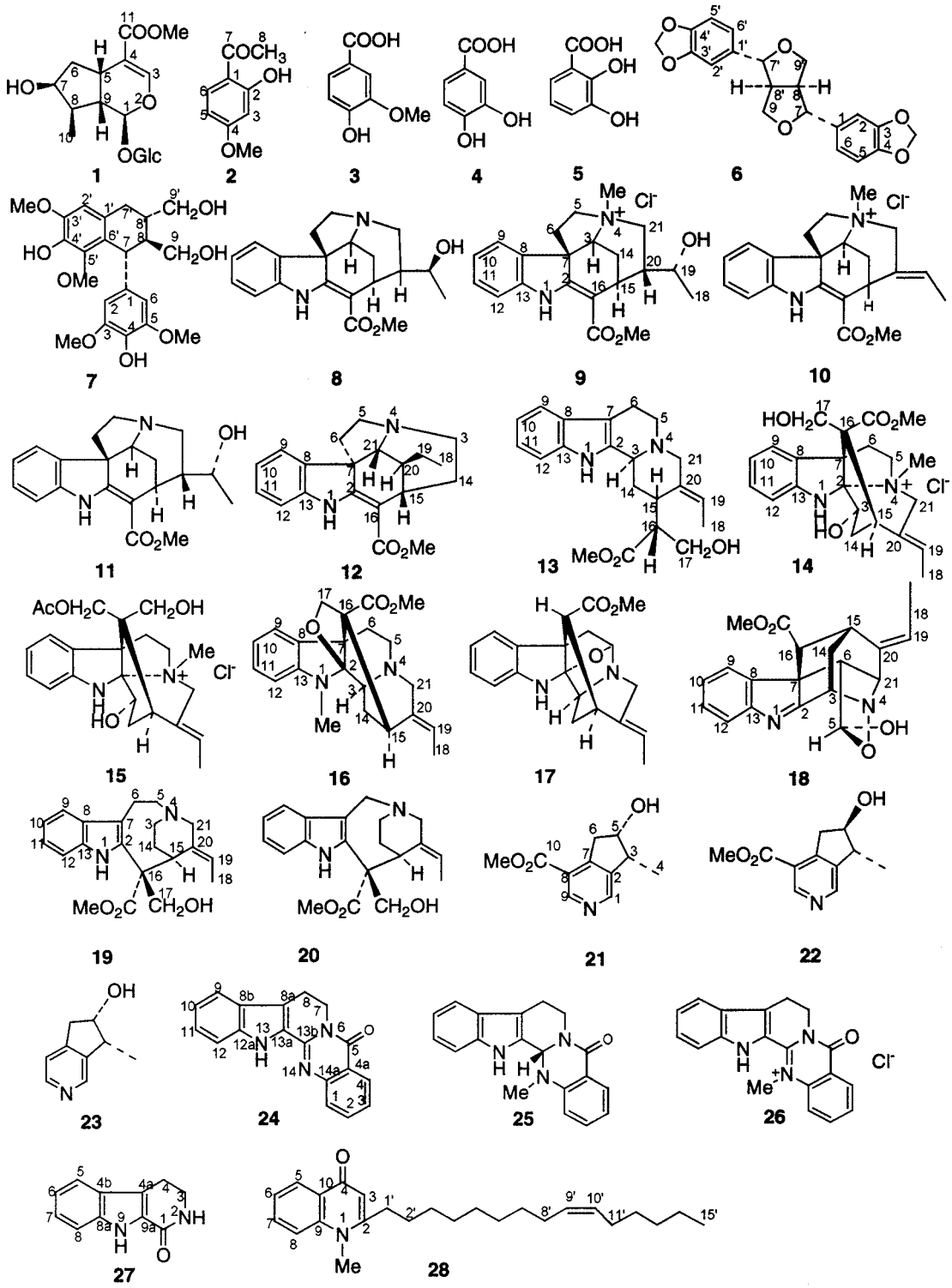

Fig. 1. The structures of compounds **1**–**28**.

mg) **19** (60 mg) **20** (13 mg), **21** (37 mg), **22** (28 mg), **23** (19 mg), **24** (approximately 100 mg), **25** (30 mg), and **26** (40 mg). The butanol fraction (180 g) was subjected to column chromatography over silica gel H

Table 1 Relaxations produced by various compounds on the histamine-induced contractile response in guinea-pig tracheal spirals and lung strips (n = 5)*

Samples	Trachea (EC_{50}; mg/mL)	Lung strips (EC_{50}; mg/mL)
N (4)-methyl akuammicine (**10**)	0.750 0	0.022 1
Cantleyine (**21**)	0.133 0	0.097 5
Loganin (**1**)	0.630 0	–
Paeonol (**2**)	–	0.480 0
P-Bu †	1.000 0	0.877 0
P-H_2O‡	1.180 0	–
P-Alk §	0.269 0	–
Aminophylline	0.024 0	0.015 5

*, the concentration of histamine used was 0.001 g/mL and, under these conditions, compounds **9**, **13** – **15** 19, **24**, **25** and **28** were inactive. †, the *n*-butanol fraction. ‡, the H_2O fraction. §, total alkaloids from ethanol extracts of the stem barks of *Winchia calophylla*.

to afford compounds **1** (1.25 g), **2** (1.372 g), **3** (28 mg), **4** (32 mg), **5** (41 mg), **7** (24 mg), **8** (36 mg), **10** (450 mg), **11** (49 mg), **13** (6 mg), and **27** (57 mg).

2.4 Bioassay tests

Hartley guinea pigs of either sex, weighing 350 – 500 g, were obtained from the Experimental Animal Center, Kunming Medical College. Guinea pigs were killed by a blow to the head. The trachea and lungs were isolated quickly and cleaned of surrounding connective tissue. Tracheal spirals and lung strips were prepared according to methods described previously (Xie *et al.* 1999) and suspended in a 20 mL organ bath in oxygenized Krebs' solution of the following composition (in mmol/L): NaCl 128 ; KCl 4.7; $CaCl_2$ 2.2; $MgCl_2$ 0.3; NaH_2PO_4 0.8; $NaHCO_3$ 12; glucose 5.6 mmol/L, pH 7.2. The organ baths were maintained at 37 °C and the Krebs' solution was bubbled continuously with oxygen to maintain a pH value of 7.4 ± 0.5. Tension was measured isometrically with force transducers and responses were recorded on a multichannel polygraph recorder. The spirals and strips were loaded with an initial tension of 2 and 0.5 g, respectively. After an equilibration period of 2 h, both strips were precontracted by histamine at a concentration of 0.001 mg/mL. Relaxation responses to drugs were measured in a cumulative manner and are expressed as a percentage of the maximal contraction to histamine.

2.5 Identification

Loganin (1) White amorphous powder. $[\alpha]_D^{24}$ –37.5° (*c* 1.10, MeOH). EI-MS *m/z* (%): 228 (73), 210 (76), 182 (86), 179 (100), 139 (87). ^{13}C-NMR (100 MHz, MeOH-d_4) δ: 97.8 (d, C-1), 152.1 (d, C-3), 114.1 (s, C-4), 32.1 (d, C-5), 42.7 (t, C-6), 75.0 (d, C-7), 42.1 (d, C-8), 46.6 (d, C-9), 13.4 (q, C-10), 169.6 (s, C-11), 100.1 (d, C-1'), 74.8 (d, C-2'), 78.3 (d, C-3'), 71.6 (d, C-4'), 78.1 (d, C-5'), 62.8 (t, C-6'), 51.6 (q, OCH_3).

Paeonol (2) Amorphous powder. EI-MS *m/z* (%): 166 (M^+, 28), 151 (100), 84 (35), 55 (19). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 113.8 (s, C-1), 165.1 (s, C-2), 100.7 (d, C-3), 166.0 (s, C-4), 107.5 (d, C-5), 132.2 (d, C-6), 202.5 (s, C-7), 26.1 (q, C-8), 55.4 (q, OCH_3).

***N* (4)-methyl akuammicine (10)** Yellowish amorphous powder. $[\alpha]_D^{24}$–146.5° (*c* 0.22, MeOH). EI-MS *m/z* (%): 336 (M^+ – HCl, 36), 322 (M^+ – MeCl, 42), 278 (59), 264 (85), 180 (55), 158 (55), 122 (100). ^{13}C-NMR (100 MHz, MeOH-d_4) δ: 164.9 (s, C-2), 74.4 (d, C-3), 65.5 (t, C-5), 43.5 (t, C-6), 57.1 (s, C-7), 132.1 (s, C-8), 122.0 (d, C-9), 122.7 (d, C-10), 130.3 (d, C-11), 111.6 (d, C-12), 144.9 (s, C-13), 28.8 (t, C-14), 29.6 (d, C-15), 102.7 (s, C-16), 13.7 (q, C-18), 129.9 (d, C-19), 134.5 (s, C-20), 66.8 (t, C-21), 51.9 (q, –OCH_3), 168.1 (s, –CO_2R), 52.5 (q, NCH_3).

Cantleyine (21) Yellowish amorphous powder. $[\alpha]_D^{24}$ –51.0° (*c* 0.68, $CHCl_3$). EI-MS *m/z* (%): 207 (M^+, 78), 189 (16), 179 (56), 175 (77), 147 (100). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 148.0 (d, C-1), 142.6 (s, C-2), 42.5 (d, C-3), 11.8 (q, C-4), 74.7 (d, C-5), 42.2 (t, C-6), 153.2 (s, C-7), 123.1 (s, C-8), 149.2 (d, C-9), 166.0 (s, C-10), 52.0 (q, –OCH_3).

Rutaecarpine (24) Yellowish amorphous powder. EI-MS *m/z* (%): 287 (M^+, 100), 258 (16), 155 (12), 129 (13), 102 (14). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 127.2 (d, C-1), 134.5 (d, C-2), 126.2 (d, C-3), 127.5 (d, C-4), 121.9 (s, C-4a), 161.6 (s, C-5), 41.4 (t, C-7) 19.8 (t, C-8), 118.4 (s, C-8a), 126.2 (s, C-8b), 120.7 (d, C-9), 120.5 (d, C-10), 125.5 (d, C-11), 113.0 (d,

C-12), 139.8 (s, C-12a), 128.5 (s, C-13a), 146.0 (s, C-13b), 148.5 (s, C-14a).

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