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ISOQUINOLINE ALKALOIDS FROM *ACANGELISIA GUSANLUNG*

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Key Word Index—*Acangelisia gusanlung*; Menispermaceae; 8-oxyprotoberberine alkaloids; gusanlung C, D; 8-oxythalifendine; 8-oxyberberrubine.

Abstract—Studies on the stem of *Acangelisia gusanlung* yielded four new isoquinoline alkaloids, gusanlung C, D, 8-oxythalifendine and 8-oxyberberrubine together with a known alkaloid, 8-oxyberberine. Their structures were elucidated by spectroscopic analysis of the natural products and of derivatives.

INTRODUCTION

Acangelisia gusanlung H. S. Lo is a small shrub distributed in Hainan Island of southern China and the whole stem is used in folk medicine. Previous phytochemical studies have afforded two new 8-oxotetrahydroprotoberberine alkaloids, gusanlung A and B [1]. As part of our continuing researches on *A. gusanlung*, we discuss here the isolation and structural elucidation of four new compounds, gusanlung C (**1**), gusanlung D (**2**), 8-oxythalifendine (**4**), 8-oxyberberrubine (**5**) and of the known 8-oxyberberine (**3**) from the ethylacetate-soluble extract of *A. gusanlung* stem.

RESULTS AND DISCUSSION

Gusanlung C (**1**), $C_{18}H_{19}NO_4$ (HRMS), was isolated as yellow crystals, mp 104–105°. Its IR spectrum showed phenolic hydroxy (3300 cm^{-1}), amide carbonyl (1640 cm^{-1}), –NH– (3380 cm^{-1}), double bond (1610 cm^{-1}) and aromatic (1570, 1500 cm^{-1}) absorption bands. The ^1H NMR spectrum of compound **1** showed a characteristic AA′ XX′ broadened quartet (J = 8.0 Hz) at δ6.74 and 7.05 typical for the four protons in a *para*-disubstituted benzene ring, a pair of separated olefinic protons with an AX pattern (J = 15.5 Hz) at δ6.48 and 7.43, another AX quartet (J = 8.4 Hz) at δ6.81 and 7.03, the latter of which also showed a small *meta*-coupling (J = 0.5 Hz) in a benzene ring, one proton doublet (J = 0.5 Hz) at δ7.14, coupling with the doublet at δ7.03, a methoxy signal at δ3.85, two aliphatic protons as a triplet (J = 7.2 Hz) at δ2.73 and two additional aliphatic protons as a quartet (J = 7.2 Hz) at δ3.46 which exhibited coupling with signals at δ8.00 (1H, t, J = 7.2 Hz) due to NH (exchangeable), respectively (Table 1). The absorption at 1640 cm^{-1} suggested that compound **1** contained an amide or urethane carbonyl group and this was supported by the observation of a ^{13}C NMR signal at δ_C 167 (Table 2). Based on the above spectral data, **1** must contain a CH_2–CH_2–NH–CO–OMe moiety. Compound **1** was transformed into acetyl and methyl derivatives (**1a**) and (**1b**) with acetic anhydride–pyridine and diazomethane, respectively. The ^1H NMR (Table 1) spectra showed the presence of two acetyl groups at δ2.28 (3H, s), δ2.30 (3H, s) in **1a** and three methoxy signals at δ3.80 (3H, s), δ3.89 (6H, s) in **1b**, indicating that **1** contained two phenolic hydroxy groups. In order to elucidate the positions of the aromatic substituents, a 2D ^1H–^1H delayed COSY experiment for **1b** was carried out. In this experiment, the signal at δ2.80 due to H-5 was correlated with the doublet at δ6.85 indicating that this signal should be H-4. Since the methoxyl at δ3.89 and an olefinic proton at δ7.55 were correlated with the same proton doublet at δ7.00 due to H-1, the remaining two positions in this benzene ring should be substituted by a methoxy group and a double bond. On biogenetic considerations, the methoxy group and the double bond were located at C-2 and C-16a′, respectively. The 15.5 Hz J value between the olefinic protons indicated that they were in a *trans*-arrangement. The two proton doublet at δ6.88 was assigned to H-10 and H-12, because of long range coupling with the methoxyl at δ3.89. These analyses suggested the structure as **1**. The ^{13}C NMR spectral data also supported this deduction (Table 2).

Gusanlung D (**2**), $C_{18}H_{15}NO_3$ (HRMS), $[\alpha]_D^{20}$ −345° ($CHCl_3$; c 0.018), needles, mp 250–251.5°, had UV maxima at 222, 273, 294, 320 nm, similar to those of gusanlung A and B [1] indicating that compound **2** possessed the 8-oxoprotoberberine alkaloid skeleton. The IR spectrum of **2** displayed lactam carbonyl group (1650 cm^{-1}) and

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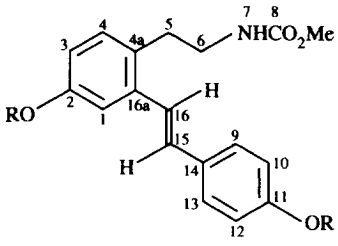

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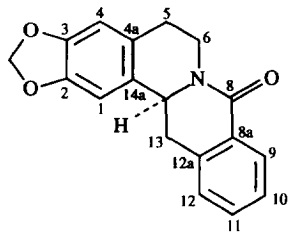
1b R = CH_3



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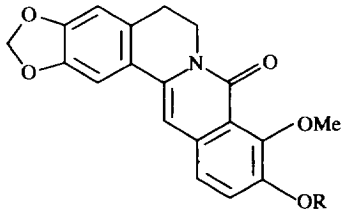

3 R = CH_3
4a R = H
4b R = $COCH_3$

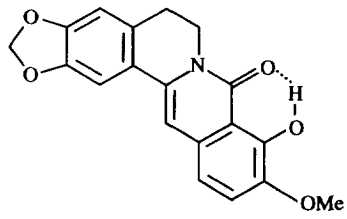

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Table 1. ^{1}H NMR data of compounds **1**, **1a** and **1b** (TMS as internal standard)

H	1*	1a†	1b‡
1	7.14 (*d*, *J* = 0.5 Hz)	7.04 (*d*, *J* = 0.5 Hz)	7.00 (*d*, *J* = 0.5 Hz)
3	7.03 (*dd*, *J* = 8.4, 0.5 Hz)	7.06 (*dd*, *J* = 8.2, 0.5 Hz)	7.07 (*dd*, *J* = 8.4, 0.5 Hz)
4	6.81 (*d*, *J* = 8.4 Hz)	6.99 (*d*, *J* = 8.2 Hz)	6.85 (*d*, *J* = 8.4 Hz)
5	2.73 (2H, *t*, *J* = 7.2 Hz)	2.85 (2H, *t*, *J* = 6.7 Hz)	2.80 (2H, *t*, *J* = 7.2 Hz)
6	3.46 (2H, *q*, *J* = 7.2 Hz)	3.62 (2H, *q*, *J* = 6.7 Hz)	3.14 (2H, *q*, *J* = 7.2 Hz)
7	8.00 (1H, *t*, *J* = 7.2 Hz)		
9	7.05 (*d*, *J* = 8.0 Hz, AA′ XX′)	7.20 (*d*, *J* = 8.2 Hz, AA′ XX′)	7.14 (*d*, *J* = 8.0 Hz, AA′ XX′)
10	6.74 (*d*, *J* = 8.0 Hz, AA′ XX′)	7.02 (*d*, *J* = 8.2 Hz, AA′ XX′)	6.88 (*d*, *J* = 8.0 Hz, AA′ XX′)
12	6.74 (*d*, *J* = 8.0 Hz, AA′ XX′)	7.02 (*d*, *J* = 8.2 Hz, AA′ XX′)	6.88 (*d*, *J* = 8.0 Hz, AA′ XX′)
13	7.05 (*d*, *J* = 8.0 Hz, AA′ XX′)	7.20 (*d*, *J* = 8.2 Hz, AA′ XX′)	7.14 (*d*, *J* = 8.0 Hz, AA′ XX′)
15	7.43 (*d*, *J* = 15.5 Hz, AX)	7.55 (*d*, *J* = 15.5 Hz, AX)	7.55 (*d*, *J* = 15.5 Hz, AX)
16	6.48 (*d*, *J* = 15.5 Hz, AX)	6.26 (*d*, *J* = 15.5 Hz, AX)	6.20 (*d*, *J* = 15.5 Hz, AX)
COMe	3.85 (*s*, 3H)	3.82 (*s*, 3H)	3.80 (*s*, 3H)
2 × OMe	—		3.89 (*s*, 6H)
2 × OCOMe	—	2.28 (*s*, 3H), 2.30 (*s*, 3H)	—

*400 MHz, CD_3COCD_3.
†400 MHz, $CDCl_3$.
‡400 MHz, $CDCl_3$.

aromatic (1490, 1600 cm^{-1}) absorption bands. The ^{1}H NMR spectrum of **2** exhibited one methylenedioxy, three groups of seven aliphatic protons as multiplets, three aromatic proton multiplets, two aromatic protons as singlets and one aromatic proton doublet (J = 8.0 Hz) at δ8.07, characteristic of H-9 in this class of alkaloid, which is deshielded by the carbonyl group at C-8 [2]. In the 2D ^{1}H–^{1}H NMR COSY spectrum of **2**, the signal at δ8.07 (H-9) was correlated with the three aromatic proton multiplets indicating that the methylenedioxy group was in ring A and that ring D was not substituted. The ^{13}C NMR spectral data (Table 2) also supported the proposed structure **2**.

8-Oxythalidendine (**4**), light brown needles, mp 286–287°, has the molecular formula $C_{19}H_{15}NO_5$ based on the HR mass spectrum of the monoacetate (**4a**). It

gave a dark brown spot with the Dragendorff spray reagent like all 8-oxyberberine alkaloids [3]. Its IR spectrum displayed absorption of a lactam carbonyl group at 1630 cm^{-1} and a hydroxy group at 3200 cm^{-1}. The ^1H NMR spectrum of **4** (see Table 3) presented the distinctive triplets at δ2.90 and δ4.30 (H-5 and H-6) of 8-oxyberbine alkaloids, one methylenedioxy, three aromatic singlets and a two proton AB quartet. The ^1H NMR spectrum of **4** was similar to that of **3** except for one missing methoxy singlet. Acetylation of compound **4** afforded a monacetate **4a** indicating that **4** contained one phenolic hydroxyl group. Because of the facile acetylation of **4**, its structure was deduced to be 8-oxythalifendine.

Table 2. ^{13}C NMR data of compounds **1**–**3** and **5** (75 Hz, $CDCl_3$)

C	1	2	3	5
1	119.8 (*d*)	107.3 (*d*)	104.6 (*d*)	104.0 (*d*)
2	156.6 (*s*)	135.0 (*s*)	147.3 (*s*)	141.6 (*s*)
3	122.6 (*d*)	147.0 (*s*)	148.3 (*s*)	146.4 (*s*)
4	116.1 (*d*)	107.5 (*d*)	107.8 (*d*)	107.1 (*d*)
4a	131.0 (*s*)	126.5 (*s*)	119.3 (*s*)	109.6 (*s*)
5	35.7 (*t*)	29.7 (*t*)	28.6 (*t*)	28.4 (*t*)
6	41.9 (*t*)	42.0 (*t*)	39.2 (*t*)	39.1 (*t*)
8	167.0 (*s*)	162.0 (*s*)	160.0 (*s*)	164.0 (*s*)
8a	—	117.3 (*s*)	132.3 (*s*)	129.9 (*s*)
9	116.0 (*d*)	128.7[a](*d*)	151.3 (*s*)	149.0 (*s*)
10	130.4 (*d*)	127.9[a](*d*)	149.4 (*s*)	147.5 (*s*)
11	149.1 (*s*)	127.1[a](*d*)	118.8 (*d*)	114.9 (*d*)
12	130.4 (*d*)	126.8[a](*d*)	122.2 (*d*)	120.0 (*d*)
12a	—	124.6 (*s*)	129.9 (*s*)	128.9 (*s*)
13	116.0 (*d*)	33.5 (*t*)	101.1 (*d*)	103.6 (*d*)
14	148.6 (*s*)	49.4 (*d*)	135.5 (*s*)	133.6 (*s*)
14a	—	126.5 (*s*)	123.6 (*s*)	122.1 (*s*)
15	140.6 (*d*)			
16	111.6 (*d*)			
16a	128.2 (*s*)			
OMe	56.4 (*q*)		56.8 (*q*), 61.5 (*q*)	56.7 (*q*)
OCH_2O		100.9 (*t*)	101.3 (*t*)	100.6 (*t*)

[a]Interchangeable assignments.

8-Oxyberberrubine (**5**), also $C_{19}H_{15}NO_5$, was isolated as reddish brown needles, mp 240–241°. It displayed a black spot on spraying with Dragendorff reagent like compounds **3** and **4**. Its lactam carbonyl group absorption band in the IR spectrum appeared at 1640 cm^{-1} (δ_C 164). The ^1H NMR spectrum was similar to that of **4**, but the R_f values differed. Compound **4** was easily transformed into its acetate, while compound **5** resisted acylation. Hence, **5** was a positional isomer of **4** in which the phenolic hydroxyl was located at C-9. The existence of a hydrogen bond with the amide carbonyl explains the different polarities of the two compounds, while the steric hindrance of the phenol flanked by two *ortho*-substituents explains the reluctance to acetylation.

EXPERIMENTAL

General. Mps: uncorr. UV: MeOH. IR: KBr. ^1H NMR, 300 MHz and ^{13}C NMR, 75 MHz in $CDCl_3$ with TMS as int. standard. MS: 70 eV direct inlet. Spray reagent for TLC: Dragendorff reagent.

Plant material. Acangelisia gusanlung was collected in the Hainan Province of China in November 1987, and authenticated by Professor Y. Zhong. A voucher specimen is deposted in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and isolation. The dried and ground stems of *A. gusanlung* (20 kg) were refluxed with 95% EtOH. The extract was concd to dryness under red. pres. below 60°. The residue was partitioned between EtOAc and H_2O (1:1). The EtOAc soln was evapd to yield 30 g of residue. The EtOAc extract was chromatographed on a silica gel (200–300 mesh, 1.5 kg) column (4.5 × 110 cm) eluted with hexane–EtOAc (1:1, 5 l, frs 1–25), followed by EtOAc–EtOH (9:1, 3.2 l, frs 26–42), and finally EtOAc–EtOH (7:3, 3.4 l, frs 43–60). Fractions of 200 ml were collected to afford 60 frs. Fractions 48–58 were purified by PTLC on silica gel GF_{254} using $CHCl_3$–Me_2CO (82:4) to give **1** (70 mg). Fractions 30–40 (2.09 g) were rechromatographed on a column of silica gel (0.063–0.200 mm, 100 g) eluted with $CHCl_3$–Me_2CO (41:3, 750 ml) to yield **3** (80 mg) [4] from frs 2–3, and **4** (40 mg) from frs 9–14, respectively.

Table 3. ^1H NMR data of compounds **3**, **4**, **4a** and **5** (300 MHz, $CDCl_3$)

H	3	4	4a	5
1	6.73 (*s*)	6.74 (*s*)	6.78 (*s*)	6.83 (*s*)
$-OCH_2O-$	6.00 (*s*, 2H)	6.00 (*s*, 2H)	6.10 (*s*, 2H)	6.02 (*s*, 2H)
4	6.70 (*s*)	6.72 (*s*)	6.74 (*s*)	6.72 (*s*)
5	2.90 (*t*, 2H, J = 7.2 Hz)	2.90 (*t*, 2H, J = 7.2 Hz)	2.90 (*t*, 2H, J = 7.2 Hz)	2.91 (*t*, 2H, J = 7.2 Hz)
6	4.30 (*t*, 2H, J = 7.2 Hz)	4.30 (*t*, 2H, J = 7.2 Hz)	4.30 (*t*, 2H, J = 7.2 Hz)	4.27 (*t*, 2H, J = 7.2 Hz)
C_9-OMe	4.00 (*s*, 3H)	4.04 (*s*, 3H)	3.96 (*s*, 3H)	—
C_{10}-OMe	3.94 (*s*, 3H)	—	—	3.96 (*s*, 3H)
11	7.33 (J = 7.4 Hz, AB*q*)	7.33 (J = 7.0 Hz, AB*q*)	7.32 (J = 7.0 Hz, AB*q*)	7.30 (J = 8.0 Hz, AB*q*)
12	7.28 (J = 7.4 Hz, AB*q*)	7.24 (J = 7.0 Hz, AB*q*)	7.26 (J = 7.0 Hz, AB*q*)	7.00 (J = 8.0 Hz, AB*q*)
13	7.22 (*s*)	7.20 (*s*)	7.27 (*s*)	7.21 (*s*)
OCOMe	—	—	2.37 (*s*, 3H)	—

Fractions 7–18 (2.1 g) were rechromatographed on a column of silica gel (0.063–0.200 mm, 100 g) eluted with a mixture of $CHCl_3$–cyclohexane (8:2, 900 ml) to give a green residue which was subjected to PTLC on silica gel GF_{254} using $CHCl_3$–Me_2CO (84:4) to yield **5** (R_f 0.79, 35 mg). Fractions 19–27 were purified by PTLC on silica gel GF_{254} using $CHCl_3$–MeOH (85:1) to yield **2** (R_f 0.67, 32 mg).

Gusanlung C (**1**). Yellow needles, mp 104–105°. UV λ_{max}^{MeOH} nm: 221, 293, 319. IR ν_{max}^{KBr} cm^{-1}: 3380, 3300, 1640, 1630, 1610, 1580, 1570, 1500, 1440, 1420, 1355, 1260, 1200, 1150, 1115, 1020, 950, 810. ^1H NMR: see Table 1. ^{13}C NMR: see Table 2. EIMS *m/z*: 313 $[M]^+$, 193, 177, 149, 145, 120; HRMS *m/z*: 313.1301 $[M]^+$ (calcd for $C_{18}H_{19}NO_4$ 313. 1313).

Acetylation of compound **1**. Compound **1** (15 mg) was treated with acetic anhydride (1 ml) and pyridine (6 drops) at room temp. overnight. The reaction mixt. was treated in the usual manner to yield acetyl gusanlung C (**1a**) (8 mg), mp 155–156° EIMS *m/z*: 397 $[M]^+$, 355, 235, 219, 218, 214, 193, 192, 177, 162, 147, 145, 120. ^1H NMR: see Table 1.

Methylation of compound **1**. To 40 mg of compound **1** was added a soln of CH_2N_2 in Et_2O at 0° overnight. The solvent was removed *in vacuo* to yield an oily residue. The residue was subjected to PTLC on silica gel GF_{254} with $CHCl_3$–Me_2CO (90:5) to afford **1b** (12 mg). Recrystallization of the crude **1b** from MeOH led to pure trimethyl gusalung C (**1b**), fine needles, mp 126–127°. ^1H NMR: see Table 1. EIMS *m/z*: 341 $[M]^+$, 222, 207, 206, 191, 163, 134, 121, 97, 83, 69, 57.

Gusanlung D (**2**). Needles, mp 250–251°. $[\alpha]_D^{20}$ − 345°, ($CHCl_3$; *c* 0.018); UV λ_{max}^{MeOH} nm: 222, 273, 294, 320; IR ν_{max}^{KBr} cm^{-1}: 1650, 1620, 1600, 1485, 1450, 1425, 1405, 1390, 1280, 1254, 1205, 1145, 1075, 1040, 975, 935, 910, 850, 775. ^1H NMR: ($CDCl_3$): δ 2.70–3.40 (5H, *m*), 3.95 (1H, *m*, H-14), 4.80 (1H, *m*, H-6), 6.80 (1H, *s*, H-4), 7.35 (1H, *s*, H-1), 7.29–7.41 (3H, *m*), 8.07 (1H, *d*, J = 8.0 Hz, H-9), 6.20, 6.06 (2H, 2 *s*, OCH_2O). ^{13}C NMR: see Table 2. EIMS *m/z*: 293 $[M]^+$, 248, 235, 204, 178, 165, 152. HRMS *m/z*: 293.1017 $[M]^+$ (calcd for $C_{18}H_{15}NO_3$ 293.1047).

8-Oxythalifendine (**4**). Brown needles, mp 286–287°. UV λ_{max}^{MeOH} nm: 224, 340, 369, 191. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200, 1630, 1600, 1590, 1510, 1490, 1440, 1310, 1270, 1240, 1040, 940, 860. ^1H NMR: see Table 3. EIMS *m/z*: 337 $[M]^+$ (base peak), 322, 319, 294, 192, 164, 162, 134, 107.

Acetylation of **4**. Compound **4** (30 mg) was treated with acetic anhydride (1.5 ml) and pyridine (12 drops) at room temp. for 2 days. The reaction mixt. was treated in the usual manner to afford acetyl 8-oxythalifendine (**4a**) (11 mg), mp 199–200°. ^1H NMR: see Table 3. EIMS *m/z*: 379 $[M]^+$, 351 $[M - CO]^+$, 337 $[M - Ac]^+$, 322, 319, 294, 292, 191, 190, 178. HRMS *m/z*: 379.1027 $[M]^+$ (calcd for $C_{21}H_{17}NO_6$ 379.1050).

8-Oxyberberrubine (**5**). Reddish brown rods, mp 240–241°. UV λ_{max}^{MeOH} nm: 224, 343, 371, 391; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1640, 1600, 1515, 1490, 1455, 1390, 1320, 1270, 1230, 1180, 1090, 1035, 930, 830. ^1H NMR: see Table 3; ^{13}C NMR: see Table 2; EIMS *m/z*: 337 $[M]^+$ 322, 294, 291, 263, 262, 202, 176; HRMS *m/z*: 337.0947 $[M]^+$ (calcd for $C_{19}H_{15}NO_5$ 337. 0945).

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