Acridone Alkaloids from the Root Bark of *Severinia buxifolia* in Hainan

Tian-Shung WU* and Chien-Mao CHEN

Department of Chemistry, National Cheng Kung University, Tainan, Taiwan, 701, R.O.C.
Received July 26, 1999; accepted September 10, 1999

Eight new acridone alkaloids, buxifoliadines-A—H together with nine known acridone compounds, were isolated and characterized from the root bark of *Severinia buxifolia* which was collected in Hainan province, China. Their structures were determined by spectroscopic methods. The relationship between acridone alkaloids with collecting area is discussed. The ^{13}C-NMR spectra of the prenyl substituents at C-2 and/or C-4 of *N*-unsubstituted acridone alkaloids are also discussed.

Key words *Severinia buxifolia*; Rutaceae; acridone alkaloid; buxifoliadines-A—H.

Severinia buxifolia Tenore (*Atalantia buxifolia* (Poir.) Oliv.) (Rutaceae) is a Chinese folk medicine and has been used for treatment of chronic rheumatism, paralysis, snakebite and malaria.[1,2] Essential oils, coumarins, acridone alkaloids, sesquiterpenoids and tetranortriterpenoids have been isolated from this plant.[3—10] In our previous papers,[9,10] we reported isolation of several acridone alkaloids, coumarins and tetranortriterpenoids as cytotoxic principles and tetranortriterpenoids as antifeedant from the root bark of *S. buxifolia* which was collected in Taiwan. However, Gu and Qin have only isolated simple acridone alkaloids from the root bark of this plant, which was collected in Hainan province, China.[11,12] To understand the relationship between bioactive constituents and collecting area, the constituents of the plant collected in Hainan province were reinvestigated. We now report the isolation and characterization of seventeen acridone alkaloids including eight new compounds from the root bark of *S. buxifolia*.

Results and Discussion

The methanol extract of *S. buxifolia* was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was separated by silica gel column chromatography to give seventeen acridone alkaloids.

Buxifoliadine-A (**1**) was isolated as yellow needles, mp 155—157 °C. The 9-acridone skeleton in the molecule was suggested by ultraviolet (UV) spectroscopic absorptions at 425, 326, 278 nm,[13] and a carbonyl group absorption band at 1608 cm^{-1} in infrared (IR) spectrometry. Its molecular formula was determined as $C_{25}H_{29}NO_4$ by high-resolution electron impact mass spectrometry (HR-EI-MS). The presence of a chelated phenolic hydroxyl group at the C-1 was indicated by the ^{1}H-NMR signal at δ 14.38 (exchanged with D_2O). One-proton singlet at δ 9.23 (exchanged with D_2O) indicated another hydroxyl group in the molecule. Two singlet signals at δ 3.84 and δ 3.71 (each 3H) together with ^{13}C-NMR spectra at δ 62.0 and 47.9 were assigned for methoxy and *N*-methyl groups. In the aromatic region, ABX pattern signals at δ 7.78 (1H, dd, J=8.0, 1.6 Hz), 7.28 (1H, dd, J=8.0, 1.6 Hz), and 7.16 (1H, t, J=8.0 Hz) were attributed to H-8, H-6, and H-7, respectively. The lower field proton at δ 7.78 was deshielded by the 9-carbonyl group. In the aliphatic region, two sets of prenyl groups appeared at δ 5.33 (1H, br t, J=6.2 Hz), 3.64 (2H, d, J=6.2 Hz), 1.79 (3H, br s), 1.66 (3H, br s), and 5.28 (1H, br t, J=6.8 Hz), 3.39 (2H, d, J=6.8 Hz), 1.75 (3H, br s), 1.65 (3H, br s). To confirm the location of the hydroxyl, methoxy and two prenyl groups, a nuclear Overhauser effect (NOE) experiment (Fig.1) was conducted. The result showed the methoxy group (δ 3.84) to be within NOE distance of H-1′ (δ 3.39) and H-1″ (δ 3.64), and the hydroxyl proton (δ 9.23) to be within NOE distance of δ 7.28 (H-6), which indicated the methoxy, hydroxyl group and two prenyl groups were located at C-3, C-5; C-2 and C-4, respectively. Those were also supported by a ^{1}H-detected heteronuclear multiple bond coherence spectroscopy (HMBC) experiment (Fig. 2). On the basis of the above results, the structure of buxifoliadine-A was assigned as **1**.

Buxifoliadine-B (**2**) was obtained as yellow needles, mp 238—240 °C. Its UV and IR spectra are characteristic of an 9-acridone.[13] The molecular formula was established as $C_{24}H_{27}NO_4$ by HR-EI-MS, which was one CH_2 unit less than that of **1**. Comparing the spectral data including two dimensional (2D) NMR data with those of **1**, a *N*-methyl signal (δ_H 3.71, δ_C 47.9) in **1** was replaced by a NH group (δ_H 9.11, exchangeable with D_2O) in **2**. Based on these data, the structure of buxifoliadine-B was assigned as **2**.

Buxifoliadine-C (**3**) was isolated as yellow needles, mp 275—278 °C (dec.), and exhibited the molecular formula $C_{19}H_{19}NO_4$ by HR-EI-MS. It consisted of an acridone skeleton by comparison of spectral data with those of **2**. However, the difference between **2** and **3** in the ^{1}H-NMR spectrum, only a prenyl group signal in **2** was instead of a singlet aromatic proton at δ 6.88 in **3**. To confirm the relative location of hydroxyl, methoxy and prenyl groups, a NOESY experiment (Fig. 1) was carried out. The result showed the signal at δ 6.88 to be within NOE distance of the signal at δ 10.23 (NH) and δ 3.92 (methoxy). The signal at δ 3.34 (H-1′) was also found to show NOE with methoxy signal at δ 3.92. Therefore, the prenyl group, methoxy group and a lone aromatic proton were substituted at C-2, C-3 and C-4, respectively. This result was also supported by a HMBC experiment (Fig. 2). Based on these data, the structure of buxifoliadine-C was represented as **3**.

Buxifoliadine-D (**4**) was obtained as yellow needles, mp 236—238 °C. The 9-acridone skeleton in the molecule was suggested by UV, IR and ^{1}H-NMR spectra.[13] The molecular formula of **4** was determined as $C_{23}H_{23}NO_3$, on the basis of the $[M]^+$ at *m/z* 361.1677 in the HR-EI-MS. The presence of a chelated phenolic hydroxyl group at the C-1 was indicated by the ^{1}H-NMR signal at δ 14.80 (exchanged with D_2O). One-proton singlet at δ 9.84 (exchanged with D_2O) was assigned to an N-H proton. In the aromatic region of the ^{1}H-

* To whom correspondence should be addressed.

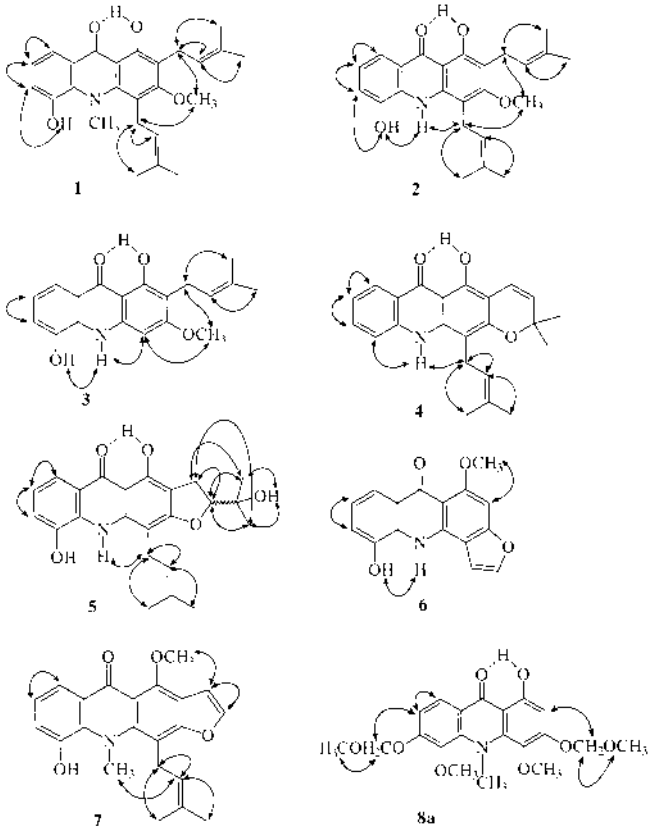


Fig. 1. The NOE Correlations of Compounds **1**—**8a**

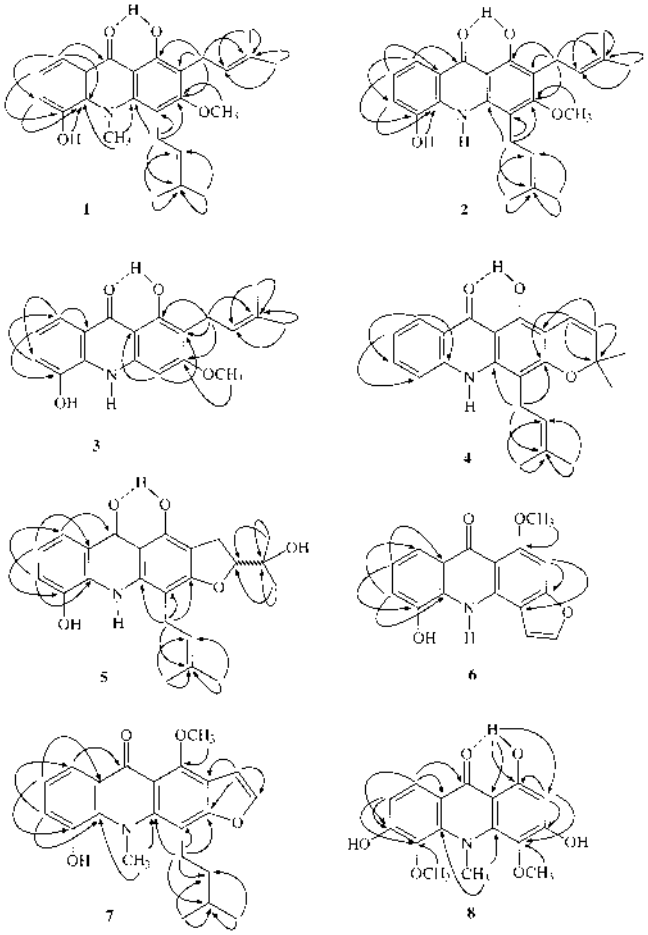


Fig. 2. ^{2}J, ^{3}J-Correlations of HMBC of Compounds **1**—**8**

NMR spectrum, four mutually coupling signals at δ 8.26 (1H, d, J=8.0 Hz), 7.70 (2H, dd, J=4.0, 1.2 Hz), and 7.28 (1H, dt, J=8.0, 4.0 Hz) were attributed to H-8, H-6 and H-5, and H-7, respectively. A prenyl group in the molecule was inferred by the signals at δ 5.15 (1H, br t, J=6.8 Hz), 3.53 (2H, d, J=6.8 Hz), 1.87 and 1.68 (each 3H, br s). The remaining signals at δ 6.74, 5.67 (each 1H, d, J=10.0 Hz), and 1.46 (6H, br s) represented the presence of a 2,2-dimethylpyrano moiety. Location of the prenyl group at C-4 and the dimethylpyran ring fused to acridone with linear orientation were proved by the presence of NOE between NH (δ 9.84) and H-1″ (δ 3.53). It was also supported by the appearance of the methylene carbon signal of the prenyl group (δ 22.2) and the C-1′ signal of pyran ring at δ 116.7.[14] On the basis of the above analysis, the structure of buxifoliadine-D was assigned as **4**.

Buxifoliadine-E (**5**) was isolated as optically inactive yellow needles, mp 247—249 °C. The UV spectrum of **5** showed a typical absorption associated with a 9-acridone nucleus.[13] The molecular formula $C_{23}H_{25}NO_5$, was fixed on the basis of HR-EI-MS (*m/z* 395.1733). The ^{1}H-NMR spectrum of **5** appeared a signal at δ 14.49 (exchangeable with D_2O) due to strongly hydrogen-bonded phenolic hydroxyl proton, and three signals at δ 9.82, 9.02 and 3.82 (D_2O exchangeable) owing to a phenolic hydroxyl proton, an amino proton and an alcoholic hydroxyl group. In the aromatic region, three mutually coupling signals at δ 7.74 (1H, dd, J=8.0, 1.2 Hz), 7.20 (1H, dd, J=8.0, 1.2 Hz), and 7.08 (1H, t, J=8.0 Hz) were attributed to H-8, H-6 and H-7, respectively. Signals at δ 5.21 (1H, m), 3.55 (2H, d, J=6.8 Hz), 1.68 (3H, br s) and 1.99 (3H, br s) indicated the presence of a prenyl group in the molecule. Signals also showed from deceptively simple ABX system of three protons at δ 3.21 (1H, dd, J=15.2, 8.0 Hz), 3.16 (1H, dd, J=15.2, 9.2 Hz), and 4.79 (1H, dd, J=8.0, 9.2 Hz); two C-methyl groups at δ 1.25 and 1.28 (each 3H, br s), and a signal at δ 3.82 from a proton (OH) exchangeable with D_2O. These NMR data could be regarded as consistent with hydroxyisopropyldihydrofurano moiety in molecule. Further support of this proposition was provided by a mass fragment ion at *m/z* 336 (M^+-59), which is produced by cleavage of the hydroxy isopropyl group. To confirm the relative substitution of prenyl, hydroxyl and dihydropyran groups, a NOESY experiment (Fig. 1) was conducted. The signal at δ 3.55 (H-1″) was found to be within NOE distance of the signal at δ 9.02 (N–H), which indicated the prenyl group was located at C-4. Finally, a HMBC experiment (Fig. 2) also supported the proposed structure of buxifoliadine-E. Based on these data, the structure of buxifoliadine-E was assigned as **5**.

Buxifoliadine-F (**6**) was obtained as yellow needles, mp >280 °C. The 9-acridone skeleton in the molecule was suggested by UV and IR spectra. The molecular formula $C_{16}H_{11}NO_4$ was determined by the HR-EI-MS (*m/z* 281.0688). In the ^{1}H-NMR spectrum, ABC pattern signals at δ 8.04 (1H, dd, J=8.0, 1.6 Hz), 7.49 (1H, dd, J=7.8, 1.6 Hz) and 7.36 (1H, dd, J=8.0, 7.8 Hz) were attributed to H-8, H-6 and H-7, respectively. Signals at δ 12.43 and 10.09 (exchangeable with D_2O) were assigned as hydroxyl and amino pro-

Table 1. ^{1}H-NMR Spectral Data for Compounds **1**—**8** (Acetone-d_6, δ, Multiplicity, J, Hz)

	1	**2**	**3**	**4**	**4**[b]
1-OH/1-OCH_3	14.38[a] (1H, s)	14.47[a] (1H, s)	14.49[a] (1H, s)	14.80[a] (1H, s)	[c]
2-H					
3-OCH_3/3-OH	3.84 (3H, s)	3.66 (3H, s)	3.92 (3H, s)		
4-H/4-OCH_3			6.88 (1H, s)		
5-H/5-OCH_3/5-OH	9.23[a] (1H, s)	9.80[a] (1H, s)	9.62[a] (1H, s)	7.70 (1H, dd, 4.0, 1.2)	7.56 (1H, dd 7.2, 1.2)
6-H/6-OH	7.28 (1H, dd, 8.0, 1.6)	7.23 (1H, dd, 8.0, 1.2)	7.18 (1H, dd, 7.8, 1.2)	7.70 (1H, dd, 4.0, 1.2)	7.52 (1H, ddd, 8.0, 7.2, 1.2)
7-H	7.16 (1H, t, 8.0)	7.11 (1H, t, 8.0)	7.07 (1H, t, 7.8)	7.28 (1H, dt, 8.0, 4.0)	7.13 (1H, ddd, 8.0, 8.0, 1.2)
8-H	7.78 (1H, dd, 8.0, 1.6)	7.78 (1H, dd, 8.0, 1.2)	7.78 (1H, dd, 7.8, 1.2)	8.26 (1H, d, 8.0)	8.17 (1H, dd, 8.0, 1.2)
10-NH/10-NCH_3	3.71 (3H, s)	9.11[a] (1H, s)	10.23[a] (1H, s)	9.84[a] (1H, s)	9.73[a] (1H, s)
1′-H	3.39 (2H, d, 6.8)	3.40 (2H, d, 6.1)	3.34 (2H, d, 6.1)	6.74 (1H, d, 10.0)	6.65 (1H, d, 9.6)
2′-H	5.28 (1H, br t, 6.8)	5.22 (1H, br t, 6.1)	5.24 (1H, br t, 6.1)	5.67 (1H, d, 10.0)	5.49 (1H, d, 9.6)
3′-OH					
4′-H	1.65 (3H, br s)	1.78 (3H, br s)	1.78 (3H, br s)	1.46 (3H, br s)	1.36 (3H, br s)
5′-H	1.75 (3H, br s)	1.65 (3H, br s)	1.63 (3H, br s)	1.46 (3H, br s)	1.36 (3H, br s)
1″-H	3.64 (1H, d, 6.2)	3.66 (1H, d, 6.1)		3.53 (2H, d, 6.8)	3.45 (2H, d, 6.8)
2″-H	5.33 (1H, br t, 6.2)	5.29 (1H, br t, 6.1)		5.15 (1H, br t, 6.8)	5.07 (1H, br t, 6.8)
4″-H	1.66 (3H, br s)	2.00 (3H, br s)		1.87 (3H, br s)	1.80 (3H, br s)
5″-H	1.79 (3H, br s)	1.80 (3H, br s)		1.68 (3H, br s)	1.62 (3H, br s)

	5	**6**	**7**	**8**
1-OH/1-OCH_3	14.49[a] (1H, s)	4.13 (3H, s)	4.24 (3H, s)	14.15[a] (1H, s)
2-H		6.56 (1H, s)		6.21 (1H, s)
3-OCH_3/3-OH				9.13[a] (1H, s)
4-H/4-OCH_3				3.79 (3H, s)
5-H/5-OCH_3/5-OH	9.82[a] (1H, s)	12.43[a] (1H, s)	9.22[a] (1H, s)	3.86 (3H, s)
6-H/6-OH	7.20 (1H, dd, 8.0, 1.2)	7.49 (1H, dd, 7.8, 1.6)	7.23 (1H, dd, 8.0, 1.8)	9.13[a] (1H, s)
7-H	7.08 (1H, t, 8.0)	7.36 (1H, dd, 8.0, 7.8)	7.15 (1H, t, 8.0)	6.94 (1H, d, 9.2)
8-H	7.74 (1H, dd, 8.0, 1.2)	8.04 (1H, dd, 8.0, 1.6)	7.75 (1H, dd, 8.0, 1.8)	7.93 (1H, d, 9.2)
10-NH/10-NCH_3	9.02[a] (1H, s)	10.09[a] (1H, s)	3.63 (3H, s)	3.81 (3H, s)
1′-H	3.16 (1H, dd, 15.2, 9.2)	7.24 (1H, d, 2.0)		
	3.21 (1H, dd, 15.2, 8.0)			
2′-H	4.79 (1H, dd, 9.2, 8.0)		7.90 (1H, d, 2.0)	
3′-OH	3.82[a] (1H, s)			
4′-H	1.28 (3H, br s)			
5′-H	1.25 (3H, br s)			
1″-H	3.55 (2H, d, 6.8)	6.93 (1H, d, 3.6)	3.69 (2H, d, 6.8)	
2″-H	5.21 (1H, m)	8.70 (1H, d, 3.6)	5.39 (1H, br t, 6.8)	
4″-H	1.99 (3H, br s)		1.80 (3H, br s)	
5″-H	1.68 (3H, br s)		1.68 (3H, br s)	

a) Exchangeable with D_2O. *b*) In $CDCl_3$+$(CD_3)_2SO$. *c*) Undetected.

tons. Signals at δ 6.93 and 8.70 (each 1H, d, J=3.6 Hz) with small coupling constant were attributed to H-1″ and H-2″ of the furan ring. A NOESY experiment (Fig. 1) was conducted to confirm the relative substitution of hydroxyl and methoxy groups and orientation of the furan ring. The result showed the methoxy group (δ 4.13) to be within NOE distance of H-2 (δ 6.56), which showed the hydroxy and methoxy group to be located at C-5 and C-1, respectively, and an angular orientation for furan ring. A HMBC experiment (Fig. 2) and a C-2 at δ 94.4 in ^{13}C-NMR spectrum also supported this proposition for the structure of buxifoliadine-F. Based on these data, the structure of buxifoliadine-F was assigned as **6**.

Buxifoliadine-G (**7**) was isolated as yellow needles, mp 150—152 °C (dec.). The 9-acridone skeleton in the molecule was suggested by UV and IR spectra. Its molecular formula was determined as $C_{22}H_{21}NO_4$, from a $[M]^+$ at *m/z* 363.1470 by HR-EI-MS. In the ^{1}H-NMR spectra, ABX pattern signals at δ 7.75 (1H, dd, J=8.0, 1.8 Hz), 7.23 (1H, dd, J=8.0, 1.8 Hz), and 7.15 (1H, t, J=8.0 Hz) were attributed to H-8, H-6 and H-7, respectively. A pair of furan signals appeared at δ 7.90 and δ 7.24 (each 1H, d, J=2.0 Hz). Signals at δ 5.39 (1H, br t, J=6.8 Hz), 3.69 (2H, d, J=6.8 Hz), 1.80 (3H, br s) and 1.68 (3H, br s) indicated the presence of a prenyl group in the molecule. Two three-proton singlet signals at δ 3.63 and δ 4.24 were assigned as *N*-methyl and methoxy groups. The lower chemical shift of the *N*-methyl carbon (δ 48.0) is characteristic of the *N*-methyl-9-acridone nucleus and has a substitute at both peri-positions (C-4 and C-5).[14] The appearance of the methylene carbon signal at δ 27.5 was suggestive of the location of a prenyl moiety at C-4.[14] NOESY (Fig. 1) and HMBC (Fig. 2) experiments were carried out to determine the location of the hydroxyl and methoxy groups and orientation of the furan ring. The result showed the methoxy signal (δ 4.24) to be within NOE distance of H-1′ (δ 7.24) and the ^{1}H–^{13}C long-range correlation between the C-3 (δ 154.0) and H-1′ (δ 7.24), H-1″ (δ 3.69) which confirmed that the methoxy and hydroxyl groups were located at C-1 and C-5. Consequently, the above spectral data afforded the structure of buxifoliadine-G as **7**.

Buxifoliadine-H (**8**) was obtained as yellow needles, mp 215—217 °C. The UV and IR spectral data of **8** was also suggested the presence of a 1-hydroxy-9-acridone skeleton in the molecule.[13] The HR-EI-MS indicated the molecular formula as $C_{16}H_{15}NO_6$. The ^{1}H-NMR spectrum of **8** showed the

Table 2. ^{13}C-NMR Spectral Data for Compounds **1**—**9** (Acetone-d_6, δ)

	1	**2**	**3**	**4**	**4**[a)]	**5**	**6**	**7**	**8**	**9**
1	160.8	160.9	160.7	158.0	156.4	158.1	162.1	157.5	159.9	162.4
1-OCH_3							56.8	60.4		
2	116.1	114.2	109.2	103.0	103.0	108.2	94.4	114.9	96.6	109.7
3	165.6	163.3	164.4	157.7	156.3	165.5	164.0	154.0	157.4	158.6
3-OCH_3	62.0	62.4	56.0							
4	115.2	109.1	88.3	104.1	101.7	97.3	108.6	118.1	128.5	98.8
4-OCH_3									59.6	
4a	149.2	139.6	145.6	141.8	139.9	141.5	[b)]	150.3	104.9	141.8
5	149.5	145.5	142.1	118.1	117.3	145.3	148.1	149.9	136.0	118.0
5-OCH_3									59.6	
6	120.5	121.0	121.6	134.6	132.9	116.5	121.0	105.5	155.5	134.4
7	124.0	122.8	116.6	122.4	121.0	121.9	124.9	123.9	112.1	122.3
8	117.2	116.7	116.9	126.1	126.2	116.8	118.9	117.4	121.9	126.2
8a	125.8	127.5	132.0	120.3	118.9	121.1	126.2	129.0	117.0	120.9
9	184.2	182.8	181.7	182.3	180.9	181.9	[b)]	177.5	180.7	182.0
9a	105.3	107.4	105.3	105.0	104.0	106.0	109.7	110.6	141.3	104.9
10-NCH_3	47.9							48.0	44.7	
10a	138.2	135.8	142.5	141.1	140.6	131.7	128.1	139.0	141.9	137.0
1′	23.2	23.1	21.9	116.7	115.8	27.7		119.6		21.8
2′	123.7	122.0	122.0	127.5	126.2	92.0		145.2		126.6
3′	131.4	131.2	130.9	78.4	77.0	71.6				131.0
4′	17.9	17.9	17.8	28.4	27.8	25.9				18.0
5′	25.7	25.8	25.9	28.4	27.8	29.5				25.9
1″	27.1	23.9		22.2	21.3	23.4	105.5	27.5		116.7
2″	124.4	124.3		132.4	122.0	122.5	126.3	124.8		123.7
3″	132.1	132.0		122.9	131.0	135.2		132.0		77.8
4″	18.1	18.2		18.1	17.7	18.1		18.1		27.9
5″	25.8	25.9		25.8	25.4	25.4		25.8		27.9

a) In $CDCl_3$+$(CD_3)_2SO$. *b*) Signals not detected due to small amount of sample.

presence of three phenolic hydroxyl protons at δ 14.15 and 9.13 (all exchangeable with D_2O). The lower signal at δ 14.15 indicated that one hydroxyl group was intramolecular hydrogen bonded and located at C-1 of the 9-acridone nucleus. In the aromatic region, two mutually coupling signals at δ 7.93 and 6.94 (each 1H, d, J=9.2 Hz) were attributed to H-8 and H-7. Three methyl signals bearing heteroatom at δ 3.81, 3.86 and 3.79 were assigned as *N*-methyl and two methoxy groups. In the HMBC experiment (Fig. 2) of **8** showed the ^{1}H–^{13}C long-range coupling between the lone aromatic proton signal at δ 6.21 (H-2) and δ 159.9 (C-1), 157.4 (C-3), 128.5 (C-4), 104.9 (C-4a); between δ 6.94 (H-7) and δ 136.0 (C-5). This result indicated two methoxy groups were located at C-4 and C-5. To confirm the relative substitution of methoxy and hydroxyl groups, a NOESY experiment (Fig. 1) was carried out for the *O*-methoxymethyl derivative (**8a**), which was prepared by treatment of **8** with chloromethyl methyl ether in dried acetone and anhydrous K_2CO_3. The methylene protons of the methoxymethyl ether moiety of **8a** at δ 5.37 (2H, s, H-1′) and 5.41 (H-1″) were found to be within NOE distance of δ 6.49 (H-2) and δ 7.21 (H-7), respectively. All these data are in agreement with the structure **8** for buxifoliadine-H.

The known compounds, severifoline (**9**),[8] atalaphyllinine (**10**),[15] atalaphyllidine (**11**),[16] citrusinine-I (**12**), -II (**13**),[15] *N*-methyl atalaphylline (**14**),[17] 1,2,3-trihydroxy acridone (**15**),[18] 5-hydroxy-*N*-methyl severifoline (**16**),[19,20] and glycocitrine-I (**17**)[21] were also isolated from the root bark of *Severinia buxifolia* collected in Hainan province. Their structures were characterized by comparison of their spectroscopic data (UV, IR, NMR, MS) with literature values.

Table 3. Acridone Alkaloids Isolated from *S. buxifolia* in Taiwan and Hainan Province

Type	Compound	Taiwan (3.4 kg)[10)]	Hainan province (980 g)
Prenyl	Atalaphyllinine	50 mg	0.8 mg
	N-Methyl atalaphylline	40 mg	3 mg
	Atalaphylline	2.4 g	—
	Glycocitrine-I	—	32 mg
	Buxifoliadine-A	—	450 mg
	Buxifoliadine-B	—	238 mg
	Buxifoliadine-C	—	5 mg
Prenyl and Pyran	Severifoline	50 mg	23 mg
	N-Methyl severifoline	80 mg	—
	5-Hydroxy-*N*-methyl severifoline	2.1 g	1 mg
	Buxifoliadine-D	—	15 mg
	Atalaphyllidine	—	0.5 mg
Furan and/or Prenyl	Buxifoliadine-E	—	2 mg
	Buxifoliadine-F	—	2 mg
	Buxifoliadine-G	—	15 mg
Simple	Buxifoliadine-H	—	232 mg
	Citrusinine-I	—	32 mg
	Citrusinine-II	—	40 mg
	1,2,3-Trihydroxyacridone	—	3 mg

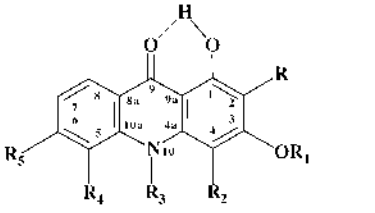
This work and our previous results show that the constitutions of the root bark of *S. buxifolia* vary according to area. (Table 3) Prenyl and pyrano type acridone alkaloids occur in both Taiwan and Hainan; simple and furano type acridone alkaloids were found only in Hainan. This is the reason why the pharmacological activities of traditional Chinese medicines are related strictly to the area in which collected. In this study, we found the methylene carbon of the prenyl groups at C-2 and/or C-4 of the *N*-unsubstituted acridone alkaloids appeared in the region of 21.8—23.9 ppm, which was different from the *N*-methyl acridone alkaloids in the ^{13}C-NMR spectra.[14] Therefore, this methylene carbon was not used to distinguish the substitution of the prenyl group at C-2 or C-4 in *N*-unsubstituted acridone alkaloids.

Experimental

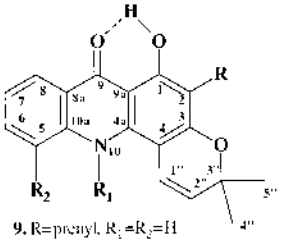
General Experiment Procedures Melting points were determined on a Yanaco micro melting points apparatus. IR spectra (KBr), UV spectra (MeOH) and mass spectra were recorded with a Shimadzu FT-IR-8501 instrument, Hitachi U-3210 spectrophotometer, VG 70-250S instrument. ^{1}H- and ^{13}C-NMR spectra were recorded with Varian unity plus 400 MHz and AMX-400 spectrophotometer ($CDCl_3$, $(CD_3)_2CO$, $(CD_3)_2SO$, D_2O). Chemical shift values are showed in ppm (δ) with tetramethylsilane (TMS) as an internal standard.

Plant Material The plant material (980 g) used for this study (root bark of *Severinia buxifolia*) was collected in February 1997 in Hainan province, China, and identified by Prof. C. S. Kuoh. A voucher specimen is deposited in the Herbarium of the National Cheng Kung University, Tainan, Taiwan, R.O.C.

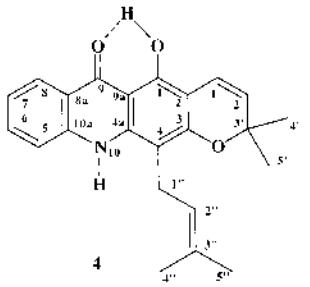
Extraction and Separation The dried root bark of *S. buxifolia* (980 g) was extracted with hot methanol and concentrated under reduced pressure to give a deep brown syrup (180 g). The syrup was partitioned successively between H_2O and $CHCl_3$. The $CHCl_3$ layer (120 g) was concentrated under reduced pressure to give brown syrup, which was directly subjected to silica gel column chromatography with successive elution with *n*-hexane, C_6H_6, EtOAc, and MeOH, to afford twenty fractions. Fraction 3 (eluate *n*-hexane) was rechromatographed on silica gel and eluted with a gradient of *n*-hexane and EtOAc (1 : 0—0 : 1) to give buxifoliadine-D (**4**) (15 mg). Fraction 9 (eluate benzene) was rechromatographed over silica gel column with *n*-



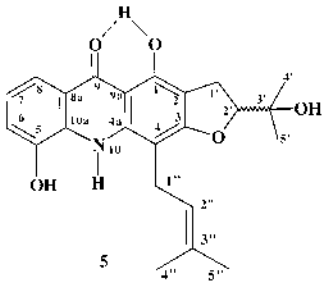
1. R=R_5=prenyl, R_1=R_2=CH_3, R_4=OH, R_3=H
2. R=R_2=prenyl, R_1=CH_3, R_3=R_5=H, R_4=OH
3. R=prenyl, R_1=CH_3, R_2=R_4=R_5=H, R_4=OH
8. R=R_1=H, R_2=R_4=OCH_3, R_3=CH_3, R_5=CH_3,
8a. R=H, R_1=CH_2OCH_3, R_2=R_4=OCH_3, R_3=CH_3, R_5=OCH_2OCH_3
12. R=R_5=H, R_1=R_2=CH_3, R_3=OCH_3, R_4=OH
13. R=R_2=R_5=H, R_1=CH_3, R_3=OCH_3, R_4=OH
14. R=R_2=prenyl, R_1=R_5=H, R_3=CH_3, R_4=OH
15. R=OH, R_1=R_2=R_3=R_4=R_5=H
17. R=R_5=H, R_1=R_3=CH_3, R_2=prenyl, R_4=OH



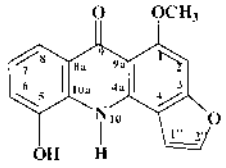
9. R=prenyl, R_2=R_1=H
10. R=prenyl, R_2=H, R_1=OH
16. R=prenyl, R_1=CH_3, R_2=OH
11. R=H, R_1=CH_3, R_2=OH



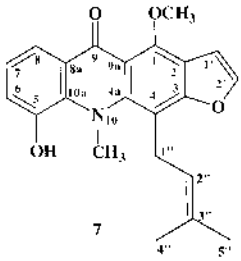
4



5



6



7

hexane–iso-Pr_2O (3 : 1) as eluent to afford buxifoliadine-A (**1**) (450 mg) and severifoline (**9**) (23 mg). Fraction 10 (eluate EtOAc) was rechromatographed over silica gel column and eluted with *n*-hexane–iso-Pr_2O (4 : 1) to obtain *N*-methyl atalaphylline (**14**) (3 mg) and 1,2,3-trihydroxy acridone (**15**) (3 mg). Fraction 11 (eluate EtOAc) was separated on silica gel column chromatography and eluted with a gradient of *n*-hexane–EtOAc (3 : 1) to give buxifoliadine-B (**2**) (238 mg), glycocitrine-I (**17**) (32 mg) and 5-hydroxy-*N*-methyl severifoline (**16**) (1 mg). Fraction 14 (eluate methanol) was rechromatographed over silica gel column and eluted with $CHCl_3$–Me_2CO (14 : 1) to afford buxifoliadine-C (**3**) (5 mg), buxifoliadine-E (**5**) (2 mg), buxifoliadine-F (**6**) (6 mg), buxifoliadine-G (**7**) (15 mg), buxifoliadine-H (**8**) (232 mg), atalaphyllinine (**10**) (0.8 mg), atalaphyllidine (**11**) (0.5 mg), citrusinine-I (**12**) (32 mg) and citrusinine-II (**13**) (40 mg), successively.

Buxifoliadine-A (**1**): Yellow needles (Me_2CO), mp: 155—157 °C. IR (KBr) cm^{-1}: 3140, 1608, 1564. 1504. UV λ_{max} nm (log ε): 425 (3.65), 326 (3.90), 278 (4.54). EI-MS *m/z* (rel. int. %): 407 (M^+, 10), 364 (5), 352 (10), 269 (13), 243 (5), 220 (17), 131 (37), 119 (21), 69 (100). HR-EI-MS *m/z*: 407.2097 (Calcd for $C_{25}H_{29}NO_4$: 407.2098).

Buxifoliadine-B (**2**): Yellow needles (Me_2CO), mp: 238—240 °C. IR (KBr) cm^{-1}: 3352, 3150, 2918, 1637, 1542. UV λ_{max} nm, (log ε): 408 (3.82), 322 (4.13), 309 (4.17), 259 (4.71). EI-MS *m/z* (rel. int. %): 393 (M^+, 100), 377 (21), 362 (26), 350 (79), 338 (94), 322 (28), 306 (17), 294 (36), 280 (17), 268 (20). HR-EI-MS *m/z*: 393.1941 (Calcd for $C_{24}H_{27}NO_4$: 393.1940).

Buxifoliadine-C (**3**): Yellow needles (Me_2CO), mp: 275—278 °C (dec.), IR (KBr) cm^{-1}: 3401, 3100, 1651, 1583. UV λ_{max} nm (log ε): 394 (2.74), 283 (3.63), 275 (sh) (3.53), 273 (3.55), 262 (3.66), 237 (sh) (3.19), 231 (3.23). EI-MS *m/z* (rel. int. %): 325 (M^+,62), 310 (36), 282 (100), 270 (89), 269 (53), 257 (14). HR-EI-MS *m/z*: 325.1316 (Calcd for $C_{19}H_{19}NO_4$: 325.1314).

Buxifoliadine-D (**4**): Yellow needles (Me_2CO), mp: 236—238 °C. IR (KBr) cm^{-1}: 3854, 3740, 1697, 1528. UV λ_{max} nm (log ε): 403 (1.84), 380 (1.72), 337 (sh) (2.02), 309 (sh) (3.00), 277 (3.32). EI-MS *m/z* (rel. int. %): 361 (M^+, 21), 346 (24), 269 (19), 220 (20), 131 (40), 69 (100). HR-EI-MS *m/z*: 361.1677 (Calcd for $C_{23}H_{23}NO_3$: 361.1678).

Buxifoliadine-E (**5**): Yellow needles (Me_2CO), mp: 247—249 °C. $[\alpha]_D$ ±0° (*c*=0.1, MeOH). IR (KBr) cm^{-1}: 3448, 1655, 1541, 1508. UV λ_{max} nm (log ε): 393 (3.86), 330 (sh) (3.85), 284 (4.68), 277 (4.59), 273 (4.60), 270 (4.59), 259 (4.72). EI-MS *m/z* (rel. int. %): 395 (M^+, 100), 380 (9), 377 (6), 362 (10), 340 (15), 336 (19), 334 (11), 327 (33), 322 (26), 308 (15), 306 (12), 294 (27), 292 (14), 280 (57), 268 (54), 252 (14), 240 (9). HR-EI-MS *m/z*: 395.1733 (Calcd for $C_{23}H_{25}NO_5$: 395.1734).

Buxifoliadine-F (**6**): Yellow needles (Me_2CO), mp>280 °C. IR (KBr) cm^{-1}: 3450, 2926, 2853, 1686, 1655, 1638, 1560, 1508. UV λ_{max} nm (log ε): 384 (3.55), 379 (3.53), 259 (4.23). EI-MS *m/z* (rel. int. %): 281 (M^+, 100), 252 (15), 238 (32), 208 (32), 194 (11). HR-EI-MS *m/z*: 281.0688 (Calcd for $C_{16}H_{11}NO_4$: 281.0688).

Buxifoliadine-G (**7**): Yellow needles (Me_2CO), mp: 150—152 °C (dec.). IR (KBr) cm^{-1}: 3200, 1679, 1599. UV λ_{max} nm (log ε): 399 (3.05), 287 (sh) (3.68), 277 (3.71), 248 (3.44). EI-MS *m/z* (rel. int. %): 363 (M^+, 84), 348 (100), 318 (39), 308 (29), 306 (34). HR-EI-MS *m/z*: 363.1470 (Calcd for $C_{22}H_{21}NO_4$: 363.1471).

Buxifoliadine-H (**8**): Yellow needles (Me_2CO), mp: 215—217 °C. IR (KBr) cm^{-1}: 3400, 1636, 1599, 1556. UVλ_{max} nm (log ε): 390 (2.53), 332 (2.93), 313 (2.67), 269 (sh) (3.41), 260 (3.45), 234 (2.60), 221 (2.94). EI-MS *m/z* (rel. int. %): 317 (M^+, 47), 302 (100), 287 (27), 78 (34), 69 (27). HR-EI-MS *m/z*: 317.0899 (Calcd for $C_{16}H_{15}NO_6$: 317.0901).

Methoxymethylation of Buxifoliadine-H (8) A mixture of **8** (50 mg), anhydrous K_2CO_3 (1.0 g) and dried Me_2CO (20 ml) was stirred at room temperature for 10 min, and then chloromethyl methyl ether (0.5 ml) was added. The reaction mixture was refluxed at 70 °C. After 1 h, the reaction mixture was evaporated. The residue was chromatographed on silica gel column and eluted with *n*-hexane–EtOAc (14 : 1) to afford **8a** as yellow needles (8 mg, 13%). $C_{20}H_{23}NO_8$, mp: 132—134 °C. IR (KBr) cm^{-1}: 3600—3400, 1630, 1589. UV λ_{max} nm (log ε): 332 (2.76), 306 (2.70), 270 (3.44), 260 (sh) (3.40), 220 (2.85). ^{1}H-NMR (400 MHz, acetone-d_6): δ 3.52 (3H, s, OCH_3-2), 3.53 (3H, s, OCH_3-2″), 3.79 (3H, s, NCH_3), 3.81 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 5.37 (2H, s, OCH_2O-1′), 5.41 (2H, s, OCH_2O-1″), 6.49 (1H, s, H-2), 7.21 (1H, d, J=9.2 Hz, H-7), 7.97 (1H, d, J=9.2 Hz, H-8), 13.97 (1H, s, 1-OH). ^{13}C-NMR (100 MHz, acetone-d_6): δ 47.4 (NCH_3), 56.6 (OCH_3), 56.7 (OCH_3), 60.8 (OCH_3), 60.9 (OCH_3), 95.6 (OCH_2O), 95.8 (OCH_2O), 97.5 (C-2), 107.2 (C-9a), 112.4 (C-7), 119.8 (C-8a), 122.5 (C-8), 132.0 (C-4), 140.2 (C-5), 143.2 (C-4a), 143.7 (C-10a), 156.6 (C-6), 158.4 (C-3), 160.6 (C-1), 182.6 (C-9).

Acknowledgements The authors are grateful for financial support from the National Science Council of the Republic of China (Grant NSC-88-2314-B-006-041).

Reference

1) Liu T. S., Lai M. C., "Flora of Taiwan," Vol. **2** , Epoch. Publishing Co., Ltd., Taiwan, 1977, p. 525.
2) Sasaki S., "Khoyo Taiwan Minkan Yakuyo Shokubutsu Shi," Khobunkan, Taipei, 1924, p. 36.
3) Scora R. W., *Phytochemistry*, **5**, 823—825 (1966).
4) Tin-Wa M., Scora R. W., Kumanoto J., *Lloydia*, **35**, 183—185 (1972).
5) Tin-Wa M., Bonomo S., Scora R. W., *Planta Med*., **37**, 379—380 (1979).
6) Dreyer D. L., *Tetrahedron*, **23**, 4613 (1967).
7) Wu T. S., Niwa M., Furukawa H., *Phytochemistry*, **23**, 595—597 (1984).
8) Wu T. S., Kuoh C. S., Furukawa H., *Phytochemistry*, **21**, 1771—1773 (1982).
9) Wu T. S., Leu Y. L., Chan Y. Y., Wu P. L., Kuoh C. S., Wu S. J., Wang Y., *Phytochemistry*, **45**, 1393—1398 (1997).
10) Wu T. S., Leu Y. L., Chan Y. Y., Lin F. W., Li C. Y., Shi L. S., Kuo S. C., Chen C. F., Wu Y. C., *Phytochemistry*, **49**, 1467—1470 (1998).
11) Gu G. M., *Yaoxue Xuebao*, **22**, 886—888 (1987).
12) Qin D. K, *Yaoxue Xuebao*, **21**, 683—685 (1986).
13) Brown R. D., Lahey F. N., *Aust. J. Sci. Res., Ser. A*., **3**, 593—595 (1972).
14) Furukawa H., Yogo M., Wu T. S., *Chem. Pharm. Bull*., **31**, 3084—3090 (1983).
15) Basa S. C., *Phytochemistry*, **14**, 835—836 (1975).
16) Wu T. S., Kuoh C. S., Furukawa. H., *Phytochemistry*, **22**, 1493—1497 (1983).
17) Govindachari T. R., Viswanathan N., *Tetrahedron*, **26**, 2905—2908 (1970).
18) Chankravanti D., *J. Chem. Soc*., 3337—3340 (1953).
19) Fraser A. W., Lewis R. J., *J. Chem. Soc., Perkin Trans. 1*, 1173—1175 (1973).
20) Rastogi K., Kapil R. S., Popli S. P., *Phytochemistry*, **19**, 945—948 (1980).
21) Wu T. S., Kuoh C. S., Furukawa H., *J. Chem. Soc., Perkin Trans. 1*, 1681—1688 (1983).