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ALKALOIDS AND COUMARINS OF *SKIMMIA REEVESIANA*

TIAN SHUNG WU

Department of Applied Chemistry, Providence College of Arts and Science, Taichung, Taiwan 40211, Republic of China.

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Key Word Index—*Skimmia reevesiana*; Rutaceae; root and stem bark; quinolin-4-one alkaloids; reevesianine-A; reevesianine-B; furoquinoline alkaloids; coumarins.

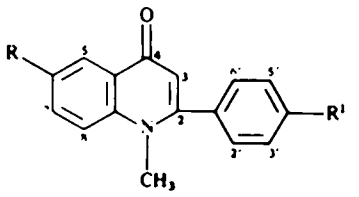
Abstract Two new quinolone alkaloids, reevesianine-A and -B, along with five known furoquinoline alkaloids, 7-isopentenyloxy-γ-fagarine, skimmianine, haplopine, evodine, evoxine, and nine known coumarins, 7-isopentenyloxy-8-isopentenylcoumarin, auraptene, osthol, isomeranzin, pranferin, *R*-(−)-columbianetin, umbelliferone, meranzin hydrate and skimmin, were isolated from the root and stem bark of *Skimmia reevesiana* collected in Taiwan. The structures of reevesianine-A and -B were established by chemical and spectroscopic methods.

INTRODUCTION

Skimmia reevesiana [1] is a shrub, distributed in Taiwan forests at medium altitudes. It is apparently unused in traditional medicine. We found that the leaf of this plant is resistant to phytophagous insects and this led us to investigate its constituents. As far as we know, no phytocemical research has been carried out on the plant. The present report describes the isolation and structure elucidation of two new quinolinone alkaloids, reevesianine-A (1) and -B (3), as well as five known furoquinoline alkaloids and nine known coumarins from the root and stem bark of *S. reevesiana* collected in Taiwan.

RESULTS AND DISCUSSION

Reevesianine-A (1) was isolated as colourless plates, mp 322–326°. The molecular formula was established as $C_{16}H_{13}NO_2$ by high resolution mass spectrometry. The UV spectrum of 1 exhibited absorption bands at 213.8, 228 (sh), 268.8, 324.2 and 336.2 nm, characteristic of a quinolin-4-one derivative [2]. This was also supported by the following evidence: (i) the IR carbonyl absorption at 1610 cm^{-1}, (ii) the chemical shift of H-5 appears at δ8.28, (iii) a carbonyl carbon at 176.8 ppm (C-4) of its acetyl derivative 2 in the ^{13}C NMR spectrum (Table 1). It also showed a deep green colour reaction with $FeCl_3$. The bathochromic shifts in UV with NaOMe, the IR band at 3400 cm^{-1} and the ^1H NMR singlet signal at δ9.72 (exchangeable with D_2O) indicated the presence of a phenolic hydroxyl group in reevesianine-A. In the ^1H NMR spectrum of 1, an *N*-methyl group occurred at δ3.66 (*s*). In the aromatic region, the farthest downfield proton appeared as a doublet at δ8.28 (H-5) which is characteristically deshielded by the adjacent carbonyl group at the *peri*-position, and the presence of the signals at δ7.40 (1H, *dd*, *J* = 3 and 8 Hz) and 7.62 7.80 (2H, *m*) due to H-8 and H-6, 7, respectively, indicated that ring A was unsubstituted. An olefinic proton also appeared as a one proton singlet at δ6.07 which is consistent with the chemical shift of the olefinic H-3 proton of the 2-substituted quinolin-4-one type. In addition, A_2B_2-type quartets at δ6.90 and 7.28 (each 2H, *J* = 9 Hz) could be



1 R = H, R¹ = OH
2 R = H, R¹ = OAc
3 R = OCH₃, R¹ = OH
4 R = OCH₃, R¹ = OAc
5 R = R¹ = H
6 R = OCH₃, R¹ = H

Table 1. ^{13}C NMR data of 2-phenylquinolin-4-one derivatives

Carbon No.	Compounds 5*†	6*†	2†	4‡
N-Me	37.26	37.36	37.62	38.09
2	154.83	153.81	154.33	153.80
3	112.58	111.80	112.38	110.80
4	177.58	176.92	176.80	174.69
4a	126.60	128.15	126.31	126.72
5	126.60	105.83	126.66	105.07
6	123.68	156.33	124.14	156.85
7	132.39	122.94	132.74	123.91
8	116.04	117.71	116.19	118.35
8a	141.94	136.02	141.87	136.49
1′	135.86	136.63	133.04	132.57
2′ and 6′	128.81§	128.80§	129.88	129.93
3′ and 5′	128.57§	128.69§	122.21	122.21
4′	129.65	129.58	151.76	151.81
6-OMe		55.86		55.81
CMe ‖ O			21.12	21.06
CMe ‖ O			169.01	168.90

*Data from ref. [14].
†In $CDCl_3$.
‡In $CDCl_3$ + DMSO-d_6.
Shifts marked with a § are interchangeable.

assigned to H-3',5' and H-2',6' of ring C, respectively, and coupled with the observation of a marked diamagnetic shift ($\Delta\delta - 0.33$) of H-3',5' signal by acetylation of **1**, indicated the presence of the hydroxyl group at the 4'-position in ring C. These results showed that the structure of **1** was 2-(4'-hydroxylphenyl)-*N*-methyl-quinolin-4-one which we have named reevesianine-A.

Reevesianine-B (**3**) was recrystallized from methanol as colourless plates, mp 304–306°. The mass spectrum (observed: 281.1014; calculated: 281.1050) established the molecular formula as $C_{17}H_{15}NO_3$. The UV spectrum of **3** showed a close resemblance to that of **1**, thus suggesting a 2-substituted quinolin-4-one structure for the compound. The ^{1}H NMR spectrum of **3** showed the presence of two three proton singlets at δ3.66 and 3.90 due to the *N*-methyl and methoxyl groups. A pair of A_2B_2-type doublets at δ6.90 and 7.28 (each 2H, *d*, J = 9 Hz) could be attributed to the H-3',5' and H-2',6' C-ring protons, respectively. A sharp one proton singlet at δ6.04 could be assigned to a lone aromatic proton at H-3. An ABX pattern signals at δ7.69 (1H, *d*, J = 3 Hz), 7.66 (1H, *d*, J = 10 Hz) and 7.32 (1H, *dd*, J = 3 and 10 Hz) were assigned to H-5, H-8 and H-7, respectively. The deshielding of H-5 is reasonable because it lies in the *peri*-position with respect to the 4-carbonyl moiety. The presence of a methoxyl group at the 6-position was indicated by the *meta*-coupling between H-5 and H-7 (J = 3 Hz). A hydroxyl group signal appeared at δ9.73 (exchangeable with D_2O) as a singlet and was located at the 4'-position from the observation of a diamagnetic shift ($\Delta\delta - 0.32$ ppm) of the H-3',5' signal at δ7.22 in **3** on acetylation of the hydroxyl group. All these data suggested the alkaloid reevesianine-B (**3**) to be 2-(4'-hydroxylphenyl)-6-methoxyl-*N*-methylquinolin-4-one.

The known compounds, auraptene (**14**) [3], osthol (**15**) [4], umbelliferone (**19**) [5] and skimmianine (**8**) [6], were isolated and identified by comparison with authentic specimens. Physical constants and spectroscopic data (UV, IR, NMR, mass spectrometry) of 7-isopentenyloxy-γ-fagarine (**7**) [7], haplopine (**9**) [8], evodine (**10**) [9], evoxine (**11**) [10], skimmin (**12**) [11], 7-isopentenyloxy-8-isopentenylcoumarin (**13**) [12], isomeranzin (**16**) [5], pranferin (**17**) [13], *R*-(−)-columbianetin (**18**) [4], and meranzin hydrate (**20**) [8] were in agreement with litrature data.

Among the above compounds, the insect feeding inhibitory activity of evoxine (**11**) has been reported by Yajima *et al.* [10]. The isolation of evoxine from *S. reevesiana* could represent the insect antifeeding properties of this plant.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR (100 MHz) and ^{13}C NMR (25 MHz) were recorded in $CDCl_3$ except where noted. Chemical shifts are shown in ppm (δ) with TMS as int. std. MS were recorded using a direct inlet system. UV were determined in MeOH and IR recorded in KBr except where noted.

Plant material. Stem and root bark of *S. reevesiana* Fortune was collected in August, 1980 in the mountain of Arisan, Chia-I, Taiwan and identified by Prof. C. S. Kuoh. A voucher sample is deposited in the Herbarium of Chia-Nan Junior College of Pharmacy, Tainan, Taiwan.

Isolation. Fresh root and stem bark (1.2 kg) was extracted × 5 with EtOH. The EtOH extract was concd to 500 ml, cooled and filtered from **12** (13.2 g). The filtrate was finally concd to a brown syrup which was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was removed, the alkaloid extracted with 5% HCl solution to afford 106 g of a dark syrup. This syrup (12 g) was chromatographed on silica gel and the column eluted with C_6H_6–Me_2CO (9:1) to give 9 fractions. Fraction 2 was re-chromatographed on silica gel and eluted with *n*-hexane EtOAc (4:1) to afford **13** (3 mg), **14** (6 mg) and **15** (10 mg), successively. Fraction 3 was also subjected to CC on silica gel and eluted with same solvent system to give **16** (12 mg) and **17** (210 mg), respectively. Fraction 4 was repeatedly chromatographed over silica gel and elution with C_6H_6–Me_2CO (9:1) afforded **18** (35 mg) and **19** (21 mg). Fraction 9 was purified by silica gel CC and $CHCl_3$ Me_2CO (9:1) elution gave **20** (2.4 g). The 5% HCl fraction was made alkaline with conc. NH_3 and further extracted with $CHCl_3$. Evaporation of the $CHCl_3$ soln gave the total alkaloid fraction (1.1 g) which was chromatographed over silica gel and elution with $CHCl_3$ Me_2CO (9:1) afforded **7** (37 mg), **8** (3 mg), **9** (7 mg), **10** (53 mg), **11** (350 mg), **1** (6 mg) and **3** (4 mg), successively.

Reevesianine-A (**1**). Colourless plates from MeOH, mp 322–326° (found: $[M]^+$ 251.0912; $C_{16}H_{13}NO_2$ requires 251.0945). A dark green colour in reaction with $FeCl_3$. UV λ_{max} nm (log ε): 213.8 (4.44), 228 (sh, 4.31), 268.8 (4.16), 324.2 (4.15), 336.2 (4.16). λ_{max}^{+NaOMe} nm (log ε): 212.2 (4.46), 242.8 (4.39), 273.4 (4.01), 287 (3.94), 325 (4.18), 335.6 (4.24). IR ν_{max} cm^{-1}: 3400, 1610, 1595, 1580, 1550, 1535. MS *m/z*: 251 ([M]$^+$, 100%), 223, 181, 111, 104, 102, 89, 77.

Acetylation of **1**. Treatment of **1** with Ac_2O and NaOAc at 100° for 3 hr gave acetate **2** as colourless plates from Me_2CO, mp 124–126°, $C_{18}H_{15}NO_3$. UV λ_{max} nm: 217, 252, 328 and 338. IR ν_{max} cm^{-1}: 1755, 1620, 1600, 1565. MS *m/z*: 293 [M]$^+$, 251 (100%), 223. ^{1}H NMR: δ2.34 (3H, *s*, OAc), 3.67 (3H, *s*, *N*-Me), 6.42 (1H, *s*, H-3), 7.23 (2H, *d*, J = 9 Hz, H-3',5'), 7.44 (2H, *d*, J = 9 Hz, H-2',6'), 7.34–7.82 (3H, *m*, H-6,7,8), 8.45 (1H, *dd*, J = 1.5 and 8 Hz, H-5).

Reevesianine-B (**3**). Colourless plates from MeOH, mp 304–306° (found: $[M]^+$ 281.1014; $C_{17}H_{15}NO_3$ requires 281.1050). It showed a positive reaction with $FeCl_3$. UV λ_{max} nm (log ε): 213.8 (3.96), 238.2 (3.95), 269.4 (3.86), 320.2 (3.57), 336.4 (3.63) and 350.6 (3.60). λ_{max}^{+NaOMe} nm (log ε): 247.8 (4.03), 279 (3.65), 338.4 (3.82) and 348.4 (3.82). IR ν_{max} cm^{-1}: 3400, 1605, 1590, 1550. MS *m/z*: 281 ([M]$^+$, 100%), 280, 266, 252, 251, 238, 213, 210, 148, 84, 66.

Acetylation of **3**. **3** was treated in the same manner as **1** to afford **4** as colourless plates, mp 200–202°, $C_{19}H_{17}NO_4$. UV λ_{max} nm: 218, 259, 340 and 353. IR ν_{max} cm^{-1}: 1750, 1625, 1600, 1580, 1570 and 1510. MS *m/z*: 323 ([M]$^+$, 100%), 293, 281, 280, 266, 252 and 251. ^{1}H NMR ($CDCl_3$ + Me_2CO-d_6): δ2.35 (3H, *s*, OAc), 3.66 (3H, *s*, *N*-Me), 3.96 (3H, *s*, OMe), 6.24 (1H, *s*, H-3), 7.22 (2H, *d*, J = 9 Hz, H-3',5'), 7.54 (2H, *d*, J = 9 Hz, H-2',6'), 7.20–7.40 (2H, *m*, H-7,8) and 7.84 (1H, *d*, J = 3 Hz, H-5).

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AJMALICIDINE, AN ALKALOID FROM *RAUWOLFIA SERPENTINA*

SALIMUZZAMAN SIDDIQUI,* S. SALMAN AHMAD, S. IMTIAZ HAIDER and BINA SHAHEEN SIDDIQUI

H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-32, Pakistan

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Key Word Index *Rauwolfia serpentina*; Apocynaceae; root; alkaloid; ajmalicidine.

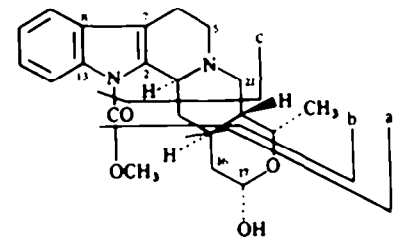
Abstract—A new heteroyohimban alkaloid, ajmalicidine, has been isolated from the roots of *Rauwolfia serpentina* of Thai origin. Its structure has been elucidated as 1-carbomethoxy-17α-hydroxy-16-decarbomethoxy 16,17-dihydro ajmalicine through chemical and spectral studies.

INTRODUCTION

Considering the importance of *Rauwolfia* alkaloids in the treatment of cardiovascular diseases and the variations recorded in the literature [1–3] with respect to the alkaloidal constituents of the roots due to varying soil and climatic conditions, studies of the alkaloids of roots collected from Nepal and Thailand were undertaken and two new alkaloids from roots originating in Nepal have been communicated earlier [4, 5]. The present paper deals with the isolation and structural elucidation of a new indole alkaloid, ajmalicidine (**1**), obtained from root material collected in Thailand.

RESULTS AND DISCUSSION

Ajmalicidine was obtained as a light yellow crystalline solid which on recrystallization from methanol–ethyl acetate formed irregular plates, mp 235–236°, $[\alpha]_D^{20°} = +190°$ ($CHCl_3$) with molecular formula $C_{21}H_{26}N_2O_4$ (elemental analysis and high resolution mass, $[M]^+$ 370.1874). The IR spectrum in chloroform showed OH stretching at 3330 cm^{-1}, in addition to a prominent band at 1720 cm^{-1} (C=O). The UV spectrum in methanol showed maxima at 208, 225 and 285 nm characteristic of an indole nucleus [6, 7]. Apart from the $[M]^+$, the mass spectrum showed peaks at *m/z* 339.1620 $[M - OCH_3]^+$, 311.1752 $[M - COOCH_3]^+$ and 170.0847, 156.0809 and 144.0813 related to ion fragments $[C_{11}H_{10}N_2]^+$, $[C_{11}H_{10}N]^+$ and $[C_{10}H_{10}N]^+$, respectively, which are characteristic of β-carbolines [8]. The spectral data of **1** showed that it belonged to the heteroyohimban series of alkaloids [9, 10]. The ^1H NMR of ajmalicidine showed a four-proton multiplet extending from δ7.38 to 6.93 for the aromatic region. The appearance of a sharp three-proton singlet at δ3.80 in the ^1H NMR and the signals at δ174.57 (C=O) and 52.36 (OCH_3) in the ^{13}C NMR suggested the presence of a carbomethoxy function in the molecule. The remaining two oxygen functions were accounted for as follows. A one-proton doublet of quartets at δ4.18 exhibited a CH_3 CH(CH) O– system, while a one-proton doublet of doublets at δ5.07 along with a signal at δ91.79 in the ^{13}C NMR suggested a hemiacetal function. These observations indicated the partial structure $-CH_2-CH(OH)-O-CH(CH)-CH_3$ which was confirmed



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