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Alkaloids from *Heimia salicifolia*

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Abstract

Two alkaloids, 9β,2′-dihydroxy-4″,5″-dimethoxy-lythran-12-one or 9β-hydroxyvertine (**1**) and (2*S*,4*S*,10*R*)-4-(3-hydroxy-4-methoxyphenyl)-quinolizidin-2-acetate (**2**), as well as seven known alkaloids, lythrine (**3**), dehydrodecodine (**4**), lythridine (**5**), vertine (**6**), heimidine (**7**), lyfoline (**8**) and epi-lyfoline (**9**), were isolated from *Heimia salicifolia*. The structures of these compounds were elucidated by extensive spectroscopic techniques. Furthermore, the structures of **2**, **3**, and **6** were confirmed by X-ray crystallography, including absolute configuration determination of **2** and **6**. Compounds **6** and **9** showed moderate antimalarial activity.

Keywords: *Heimia salicifolia*; Lythraceae; Alkaloids; X-ray crystallography

1. Introduction

Heimia salicifolia (H.B & K.) Link & Otto (Lythraceae) is a wild flowering shrub distributed over Mexico, Western Texas, El Salvador, Jamaica, and South America (Uruguay to Argentina) (Malone and Rother, 1994; Guzman et al., 2006). The plant has different folk names viz. hauchinal, sinicuichi, etc. in different places (Malone and Rother, 1994). It has been in use in Central and South America as antisyphilitic, antipyretic, emetic, hemostatic, general tonic, laxative, diuretic, anti-inflammatory and for its wound healing activity. It is employed to treat myalgia in the elderly and as a post partum bath. It also serves as repellant for insects and flies (Malone and Rother, 1994). The most interesting and important claim for the plant is its psychotomimetic activity relating to its folklore use as additive with intoxicants. Based on this information it was selected for investigation under our ongoing project natural products in neuroscience. The folkloric reports state that after the administration of a decoction with alcohol or plant juice, the person is said to experience variety of physiological actions like objects appearing yellow and auditory distortions where the sound of bells or voices sound as if they are from further distances (Malone and Rother, 1994). The initial phytochemical evaluation of this purported psychoactive plant lead to the isolation of five alkaloids (Blomster et al., 1964). Since then nearly 20 alkaloids have been reported from *H. salicifolia* and its related species (Malone and Rother, 1994; Xie et al., 1995). Amongst these, cryogenine was found to induce mild CNS depression in unanesthetized animals which was later supported by its passive response in hippocratic screening. It along with nesodine also caused a fall in blood pressure in experimental animals (Kaplan and Malone, 1966). The inhibition of prostaglandin synthetase by cryogenine and nesodine may explain the traditional anti-inflammatory usage of *H. salicifolia* (Lema et al., 1986). Malone and Rother in 1994 reported absence of any psychodysleptic effects in one person "double blind" screening of vertine

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and lythrine. However, these alkaloids are not yet screened for their antifungal, antimicrobial, and antimalarial potential. These alkaloids were therefore, subjected to these screens in the present investigation. These alkaloids mainly belong to biphenylquinolizidine lactone class and have been reported exclusively from the Lythraceae family (Malone and Rother, 1994). Since the structures were determined mainly by X-ray crystallography, no extensive NMR data for these alkaloids is available. In the present communication, we are reporting the isolation and structure elucidation of two new alkaloids (**1**–**2**) in addition to seven (**3**–**9**) other known alkaloids from the methanolic extract of *H. salicifolia*. The complete ^{1}H and ^{13}C NMR assignments for known alkaloids are also being reported for the first time.

2. Results and discussion

Compound (**1**) was obtained as amorphous solid and gave the positive Dragendroff's test. Its molecular formula was determined as $C_{26}H_{30}NO_6$, on the basis of pseudomolecular $(M+H)^+$ ion at m/z at 452.2091 in the HRESIMS, which corroborated with its NMR spectroscopic data. The ^{1}H NMR (see Table 2) spectrum of **1** showed seven olefinic resonances, two methoxyl along with aliphatic region. The 26 carbons seen in the ^{13}C NMR spectrum (see Table 1), were accounted for two methyl, five methylene, eleven methine, and seven quaternary carbons by using DEPT experiment. The aliphatic carbons gave 1D and 2D NMR correlation which on constructing formed of substituted quinolizidine nucleus (Hedges et al., 1983). The downfield aliphatic carbons at δ 50.69, 50.85 and 64.48 indicated nitrogen neighborhood. Another signal at δ 65.86 correlated with a proton at δ 3.52 in HMQC spectrum indicated oxygenation. This proton showed double triplet ($J = 12.6$, 6.0 Hz), due to neighboring methylene protons at δ 1.61, 1.00 and methine proton at δ 2.63. The HMQC experiment revealed five unsubstituted olefinic carbons of the two phenyl rings. The proton appearing as singlet at δ 7.02 (H-3″) showed HMBC correlations with 4″–OCH_3 at δ 56.66. The proton appearing at δ 4.57 (H-4) showed HMBC (see Fig. 1) correlations with δ 40.32 (C-3), 73.06 (C-2), 110.84 (C-3″) and 132.61 (C-2″). The downfield resonance of H-4 indicated the point of attachment for phenyl ring. The two methoxyl protons at δ 3.82 and 3.78 showed HMBC correlations with 148.71 (C-4″) and 150.76 (C-5″), respectively. While the proton at δ 6.74 ($J = 13.0$ Hz) showed correlations with 126.36 (C-1″). Rest of the phenyl ring assignments were done using 1D and 2D NMR experiments. The doublet appearing at δ 5.76 (H-13) showed COSY correlation with δ 6.74 (H-14) and HMBC correlations with a carbonyl carbon at δ 170.26 (C-12), δ 130.92 (C-5′). The index of hydrogen deficiency (13) indicated presence of five rings of which two belonged to quinolizidine and two to phenyls. The broad singlet at δ 5.30 (H-2) which was correlated with the δ 73.06 (C-2), indicated oxygenation, suggesting forma-

Table 1
^{13}C NMR data for **1** (125 MHz) and **3**–**9** compounds (100 MHz), [in δ ppm, solvent CD_3OD and $CDCl_3$ (compound **3**)]

Carbon	1	3	4	5	6	7	8	9
1	29.07	37.01	36.79	37.89	35.31	35.38	37.51	34.85
2	73.06	71.19	71.05	71.94	72.65	72.55	72.07	72.06
3	40.32	39.68	38.80	38.95	40.52	39.78	39.62	39.85
4	50.85	61.55	61.82	61.66	49.30	49.36	61.25	49.77
6	50.69	52.99	53.49	54.12	51.25	51.42	53.88	50.99
7	21.34	25.98	25.51	26.65	20.50	27.38	25.27	27.17
8	35.75	24.52	24.58	25.63	26.14	26.74	26.31	25.48
9	65.86	33.03	32.66	33.82	27.21	27.38	33.43	31.69
10	64.48	60.39	63.97	61.37	58.78	58.59	63.21	59.25
12	170.26	168.47	169.99	172.82	170.00	172.69	170.20	169.76
13	119.37	119.45	118.10	48.46	119.23	48.94	119.23	119.18
14	137.15	135.68	137.98	72.69	137.20	72.66	136.98	137.37
1′	126.42	126.31	126.09	127.67	126.48	127.34	126.98	126.53
2′	157.46	153.78	159.85	155.17	157.35	155.72	156.92	157.49
3′	117.51	116.04	118.82	117.82	117.36	118.09	117.27	117.47
4′	131.73	130.65	131.98	125.08	131.66	125.03	131.38	131.66
5′	130.92	131.23	131.17	136.00	130.89	135.58	129.61	130.90
6′	132.48	131.20	132.71	129.76	132.47	129.98	132.67	132.52
1″	126.36	125.06	125.14	131.74	126.38	131.01	126.64	126.45
2″	132.61	134.79	128.23	134.37	132.57	133.46	133.14	129.76
3″	110.84	110.60	112.48	110.90	110.88	111.38	115.12	115.39
4″	148.71	147.98	118.82	148.61	148.64	148.77	147.39	147.92
5″	150.76	150.03	149.77	150.63	150.75	149.90	147.91	148.38
6″	115.75	111.30	147.61	114.76	115.64	115.27	114.05	114.32
4″–OCH_3	56.66	56.24	–	56.56	56.53	56.57	–	–
5″–OCH_3	56.62	56.53	56.54	56.64	56.56	56.67	56.48	56.56

Table 2
^{1}H NMR data for compounds **1**, **3–9** in CD_3OD[a] (400 MHz, in δ ppm)[b]

	1	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	1.85–1.96 2.49 *d* (15.0)	1.73, 1.69	1.81	1.69	2.15–2.18 1.66–1.70	2.09–2.14 1.65–1.69	1.69 *d* (5.2)	2.21–2.29 1.66–1.72
2	5.30 *br*,*s*	5.32 *br*,*s*	5.22 *br*,*s*	5.01 *br*,*s*	5.29 *br*,*s*	4.95	5.25 *br*,*s*	5.28 *br*,*s*
3	1.97 *t* (13.0) 2.18 *d* (14.5)	2.23 9 *d* (14.8) 2.04 *t* (14.0)	2.00	2.28 *d* (13.2) 1.97	2.21–2.25 1.97–2.03	2.33 *d* (13.2) 1.74–1.77	2.18 *d* (14.8) 2.04 *d* (13.2)	2.21–2.29 2.09–2.12
4	4.57 *d* (11.5)	3.65 *d* (10.8)	4.13 *d* (9.6)	3.12 *d* (10.4)	4.73 *d* (11.2)	4.13 *d* (11.2)	3.87 *d* (11.2)	4.80 *d* (10.8)
6	2.75 *d* (13.5) 2.29 *t* (12.5)	2.66 *d* (11.2) 1.13	2.72 *d* (9.2) 1.37–1.39	2.68 *d* (12.8)	2.84 *d* (13.4) 2.41 *d* (11.2)	2.90 *d* (14.4) 2.44 *d* (10.8)	2.69 *d* (11.6) 1.21	2.91 *d* (13.6) 2.49
7	0.93 *d* (14.0) 0.78 *d* (13.5)	1.40	1.37–1.39	1.46	0.65–0.78	0.82–0.86	1.54 *d* (11.2)	1.17 1.68
8	1.61 *d* (10.0) 1.00 *d* (13.0)	1.60 1.57	1.49–1.58	1.67	1.33–1.35	1.52 *d* (10.4) 1.06	1.37	1.32–1.43
9	3.52	1.45 1.40	1.49–1.58	1.51 1.44	1.02–1.05	1.79 *d* (12.4)	1.37 1.21	1.32–1.65
10	2.63	1.95	2.29 *d* (12.1)	3.11	3.09	3.09	1.99	3.18–3.20
13	5.76 *d* (12.5)	5.83 *d* (12.2)	5.74 *d* (12.1)	2.81 2.40	5.79 *d* (12.4)	2.86 *d* (2.8) 2.38 *d* (11.2)	5.71 *d* (12.4)	5.78 *d* (12.8)
14	6.74 *d* (12.5)	6.76 *d* (12.2)	6.78 *d* (12.1)	5.01 *dd* (2.4)	6.79 *d* (12.4)	5.05 *dd* (10.8, 2.8)	6.64 *d* (12.4)	6.80 *d* (12.8)
3′	6.89 *d* (8.5)	6.97 *d* (8.4)	6.89 *d* (8.8)	6.97 *d* (8.8)	6.93 *d* (8.8)	6.98 *d* (8.4)	6.88 *d* (8.8)	6.94
4′	7.10 *d* (8.0)	7.16 *dd* (8.2, 1.8)	7.14 *d* (8.0)	7.42 *dd* (8.4, 2.0)	7.16 *dd* (8.2, 1.8)	7.42 *dd* (8.8, 2.0)	7.05 *d* (8.4)	7.15 *dd* (8.4, 2.0)
6′	6.94	7.10 *d* (2.0)	6.86	6.58 *d* (2.4)	7.02 *d* (2.4)	6.62 *d* (2.0)	6.90 *s*	7.08
3″	7.02 *s*	7.04 *s*	6.93	7.17 *s*	7.08 *s*	7.09 *s*	7.02 *s*	7.09
4″	–	–	6.89	–	–	–	–	–
6″	7.07*s*	6.92 *s*	–	6.92 *s*	7.12 *s*	6.96 *s*	6.95 *s*	6.98
4″ –OCH_3	3.78 *s*	3.85 *s*	–	3.82 *s*	3.82 *s*	3.84 *s*	–	–
5″ –OCH_3	3.82 *s*	3.89 *s*	3.82 *s*	3.87 *s*	3.86 *s*	3.87 *s*	3.78 *s*	3.84 *s*

[a] Compound **3** recorded in $CDCl_3$.
[b] Compound **1** recorded on 500 MHz. *J* values in parentheses are in Hz. Due to overlapping in the ^{1}H spectrum only detectable relative *J* (Hz) are reported.

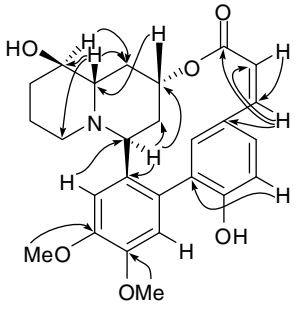


Fig. 1. Selected HMBC correlation for compound **1**.

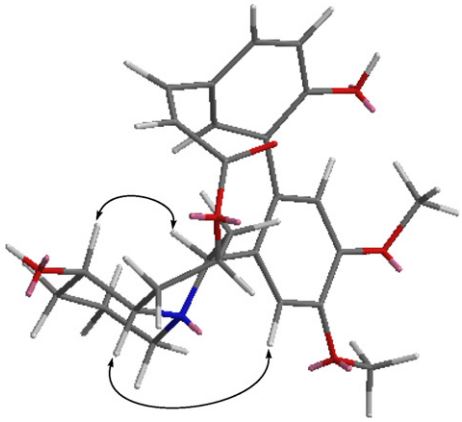


Fig. 2. Key ROESY correlations for compound **1**.

tion of another ring by attachment of lactone. Two olefinic signals at δ 5.76 (H-13) and 6.74 (H-14) appeared as doublet ($J = 12.5$ Hz), characteristic for C-13 and C-14 protons respectively. This indicated the compound to be vertine (**6**) with hydroxylation at C-9 position. The relative configuration of C-9 was assigned using its ROESY correlation. The C-2 and C-4 protons being biogenetically β- and α- oriented respectively, were used as the basis for correlations. The proton appearing at δ 4.57 (H-4) showed ROESY correlations with δ 3.52 (H-9) and δ 0.78 (H-7). The aromatic proton at δ 7.02 (H-3″) correlated with δ 2.63 (H-10) indicating its relative configuration as β. The key ROESY correlations have been shown in Fig. 2. Thus compound **1** was elucidated as 9β,2′-dihydroxy-4″,5″-dimethoxy-lythran-12-one or 9β-hydroxyvertine.

Compound (**2**) was obtained as white needles and gave positive Dragendroff's test. The molecular formula for **2** was deduced as $C_{18}H_{26}NO_4$, using pseudomolecular ion peak $(M + H)^+$ at *m*/*z* 320.1879 in the positive HRESIMS as well as from its NMR spectroscopic data. The 18 signals

Table 3
^{1}H and ^{13}C NMR data compound **2** (δ ppm, in CD_3OD)

No	^{13}C	^{1}H
1	37.29	1.53–1.69
2	69.23	5.25 (*m*)
3	37.95	1.99–2.03
4	61.37	4.09 *t* (5.2)
6	52.38	2.70 *d* (13.2) 2.25
7	25.48	1.53–1.76
8	24.48	1.29
9	31.85	1.57–1.63
10	56.03	3.07
$COOCH_3$	172.37	–
$COOCH_3$	21.41	2.03
1′	134.87	–
2′	116.82	6.87 *d* (2.0)
3′	147.78	–
4′	148.67	–
5′	112.78	6.89 *d* (8.4)
6′	121.26	6.81–6.83 *dd* (8.0,2.0)
OCH_3	56.54	3.84 *s*

^{1}H recorded at 400 MHz and ^{13}C at 100 MHz. *J* values in parentheses are in Hz.

seen in ^{13}C NMR (see Table 3) spectrum of **2** were identified as 2 methyl, 6 methylenes, 6 methines and 4 quaternary compounds using DEPT experiment. In comparison to compound **1**, the ^{1}H NMR (see Table 3) of **2** showed lack of one aromatic ring and olefinic protons of C-13 and C-14. The aliphatic region was much similar to compound **1**. Using 1D and 2D NMR data quinolizidine ring assignments were made. The downfield proton at δ 4.09 (H-4) showed HMBC correlations with 134.87 (C-1′), 112.78 (C-2′), 121.26 (C-6′), indicating the attachment of aromatic ring at C-4. The aromatic region in proton NMR showed three protons at δ 6.82 (H-6′, $J = 8.0$, 2.0 Hz) indicating *o*- and *p*-coupling, 6.89 (H-5′, $J = 8.4$ Hz), and 6.87 (H-2′, $J = 2.0$ Hz). Using COSY, HMQC and HMBC the aromatic region was assigned. The one methyl protons at δ 2.03 showed HMBC correlations with δ 172.37 indicating an acetyl group. Methoxy protons at δ 3.84 showed HMBC correlation with δ 148.67 (C-4′). The proton at δ 3.07 (H-10) was much downfield as compared to **3** in which it is α-configured (δ 1.95). In 1983, Hedges et al reported that when H-10 is β-configured, the C-4 and C-10 signals appear upfield at δ 48.0 and 57.4, respectively, as compared to α counterpart in which the signals appear at δ 60.2 and 61.4, respectively. This suggested the relative configuration of H-10 as β. This proton showed ROESY (see Fig. 3) correlations with δ 5.25, indicating acetyl group to be α configured. Moreover, the proton at δ 4.09 showed ROESY correlation with δ 2.03 of acetyl group indicating β configuration for C-4 aryl ring. Considering the biogenetic configuration of C-4 and C-2 it can be predicted that the absolute configuration of the compound must be as elucidated (Rother and Edwards, 1994). This assumption was later proved with X-ray analysis of the crystal of **2** (Fig. 4). This elucidated the compound **2** as (2*S*,4*S*,10*R*)-4-(3-hydroxy-4-methoxyphenyl)-quinolizidin-2-acetate.

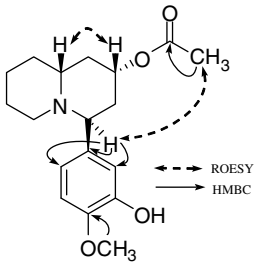


Fig. 3. Key HMBC and ROESY for **2.**

In addition to the two new alkaloids (**1**–**2**), lythrine (**3**), dehydrodecodine (**4**), lythridine (**5**), vertine (**6**), heimidine (**7**), lyfoline (**8**), and epi-lyfoline (**9**) were isolated and identified by spectroscopic techniques. Compounds **3**–**9** have been reported previously but no comprehensive NMR data is available. Hence, complete ^{1}H and ^{13}C assignments of **3**–**9** are being reported by using extensive 1D and 2D NMR spectroscopy.

Except **4**, the compound **3**–**6**, **5**–**7**, and **8**–**9** they exist as stereoisomers differing at 10-H (α/β). However, compounds with 10-Hβ can be easily distinguished by an upfield shift in ^{13}C NMR shift values (δ) of C-10 and C-4 in comparison to their counterparts (Hedges et al., 1983). This is evident from the ^{13}C shift values for lythrine (**3**) and vertine (**6**) in Table 1. The structures of **3** (Fig. 5) and **6** (Fig. 6) were further confirmed by X-ray crystallographic analysis.

Compounds **2, 3**, **5, 6**, **8**, and **9** were screened for antimicrobial and antimalarial activity according to the meth-

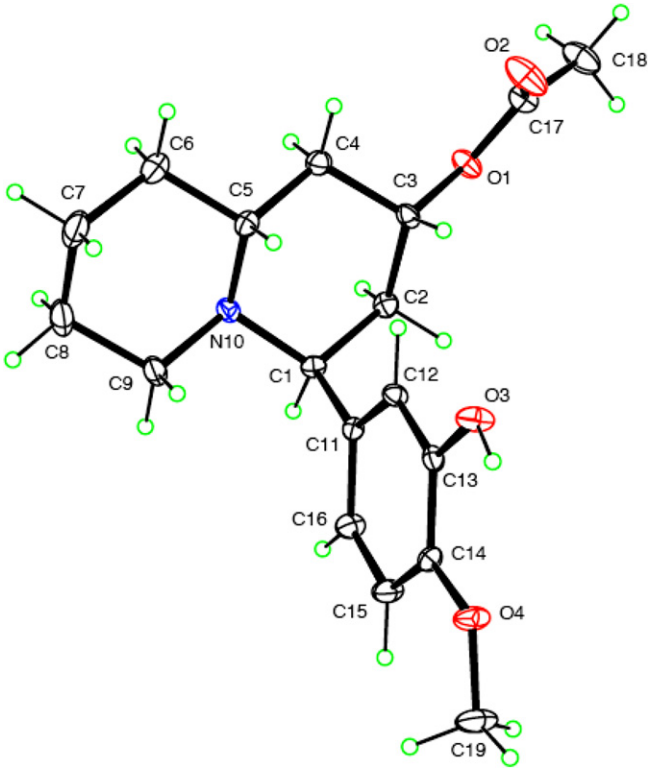


Fig. 4. ORTEP drawing for compound **2.**

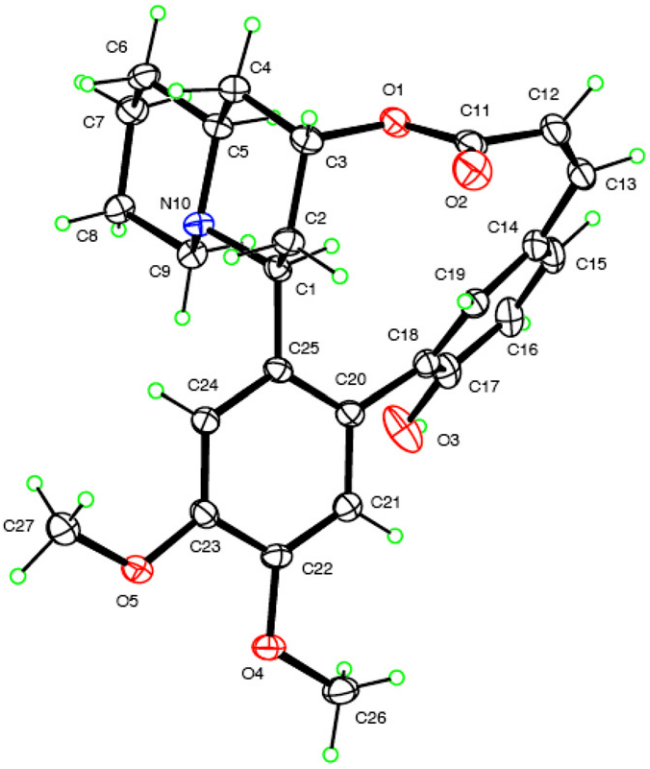


Fig. 5. ORTEP drawing for lythrine (**3**).

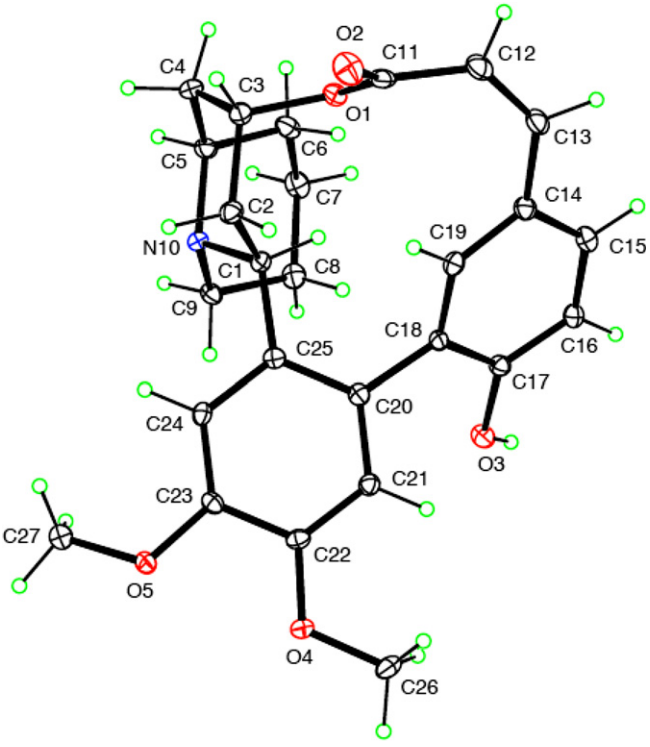


Fig. 6. ORTEP drawing for vertine (**6**).

ods described earlier (Jain et al., 2005). None of the compounds exhibited antimicrobial activity. Compound **9** exhibited moderate antimalarial activity against *Plasmodium falciparum* [Chloroquine sensitive (D6) and chloroquine resistant (W2) clones] with an IC_{50} value of 2.8 μg/ml, compared to the positive control chloroquine that has IC_{50} values of 13 and 115 ng/ml and artemisinin that has IC_{50} values of 12 and 6 ng/ml for D6 and W2 clones respectively. Compound **8** with 10-H_α was found to be inactive when compared to its counterpart of compound **9** that has 10-H_β. Compound **6** exhibited weak antimalarial activity against *P. falciparum* [IC_{50} 4.76 μg/ml for both D6 and W2 clones]. Compound **6** differs from compound **9** in having methoxy at C-4″. The decrease in potency of **6** can attribute to methoxy pattern in quinolizidine alkaloids. All the compounds did not show any cytotoxicity against vero cells.

3. Concluding remarks

This is the first comprehensive report on alkaloidal constitution of *H. salicifolia*. In the present communication two new compounds are being reported. In addition, complete ^1H and ^{13}C NMR data for the known compounds along with the help of X-ray crystallography clear differences between the 10-H_α and 10-H_β compounds.

4. Experimental

4.1. General experimental procedures

Melting point was recorded on Thomas Hoover Unimelt melting point apparatus. UV spectra were recorded on Varian Cary 50Bio UV–VIS spectrophotometer. NMR spectra were recorded on Varian AS 400 NMR Spectrometer using CD_3OD ($CDCl_3$ for **3**) and Bruker Avance 500 spectrometer (for **1**). Chemical shifts were reported in δ units (ppm) and coupling constants (J) in Hz. Optical rotation were measured on a Rudolph Research Auto Pol IV Polarimeter. The IR spectra were recorded using NaCl pellet on a Bruker, Tensor 27 FT-IR Spectrometer. The HRESIMS data was obtained on a Agilent Series 1100 SL mass spectrometer. Column chromatography was performed by using silica gel (JT Baker, 40 μm for flash chromatography). TLC analyses were carried out on silica gel 60 F_{254} plates (Merck, Germany).

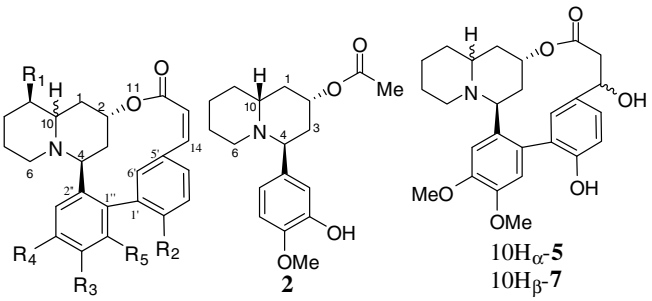
4.2. Plant material

The leaves of *H. salicifolia* (H.B & K.) Link & Otto were purchased from Bouncing Bear Botanicals and identified by Dr. Vaishali Joshi of National Center for Natural Products Research (NCNPR), University of Mississippi, USA. The voucher specimen (voucher no 2765) has been deposited at the NCNPR, University of Mississippi.

4.3. Extraction and isolation of alkaloids

Powdered leaves (1 kg) of *H. salicifolia* were extracted with methanol (2 l × 1 h ×3). The extracts were combined

and concentrated under red. pres. to give an extract (136 g). This was dissolved in 700 ml of 2% hydrochloric acid and filtered after 6 h. The filtrate was extracted with $CHCl_3$ thrice and ammonia was added to adjust pH 9. This was subsequently extracted with $CHCl_3$ several times until the $CHCl_3$ layer showed negative to the Dragendroff's reagent. The chloroform extract was concentrated under red. pres. to give alkaloidal fraction (12.6 g). This fraction was subjected to column chromatography on silica gel (600 g – mesh 40 μm) with a gradient of $CHCl_3$, $CHCl_3$:MeOH (95:5), $CHCl_3$:MeOH (90:10) and $CHCl_3$:MeOH (80:20). Fractions, 30 ml each were pooled on the basis of TLC with Dragendroff's reagent. This afforded five fractions A (429 mg), B (445 mg), C (1.185 g), D (1.095 g) and E (1.883 g). Fr. A subjected to CC and eluted with $CHCl_3$:MeOH: NH_4OH (95:5:0.1 v/v) afforded **3** (60 mg) and **2** (200 mg), whereas fr. B afforded **9** (92 mg), **4** (60 mg) and **5** (445 mg). Fractions C, D, and E on CC under same conditions gave compounds **6** (329 mg), **1** (25 mg), **7** (30 mg), **8** (92 mg) and **9** (120 mg).



	10-H	R_1	R_2	R_3	R_4	R_5
1	β	OH	OH	OMe	OMe	H
3	α	H	OH	OMe	OMe	H
4	α	H	OH	OMe	H	OH
6	β	H	OH	OMe	OMe	H
8	α	H	OH	OMe	OH	H
9	β	H	OH	OMe	OH	H

4.3.1. 9β-Hydroxyvertine (*1*)

White powder; UV (λ_{max}) 283.0; $[\alpha]_D^{20}$ +12.0 (*c* 0.15%, MeOH); IR (NaCl) ν_{max} 3392, 2924, 1699, 1117 cm^{-1}; ^{13}C and ^{1}H NMR see Tables 1 and 2; positive HRESIMS *m/z*: 452.2091 $(M+H)^+$ (calc. for $C_{26}H_{30}NO_6$, 452.2073).

4.3.2. (2S,4S,10R)-4-(3-hydroxy-4-methoxyphenyl)-quinolizidin-2-acetate (*2*)

White needle; m.p. 155–156 °C; UV (λ_{max}) 281.0; $[\alpha]_D^{20}$ +10.7 (*c* 0.15%, MeOH); IR (NaCl) ν_{max} 3740, 2928, 2362, 1729, 1248 cm^{-1}; ^{13}C and ^{1}H NMR see Table 3; positive HRESIMS *m/z*: 320.1879 $(M+H)^+$ (calc. for $C_{18}H_{26}NO_4$, 320.1862).

4.3.3. Lythrine (*3*)

White needle; UV (λ_{max}) 280.0; $[\alpha]_D^{20}$ +33.9 (*c* 0.10%, $CHCl_3$), $[[\alpha]_D^{25}$ +32.5 (*c* 0.305% $CHCl_3$) Blomster et al., 1964]; ^{13}C and ^{1}H NMR see Tables 1 and 2; positive HRESIMS *m/z*: 436.2044 $(M+H)^+$ (calc. for $C_{26}H_{30}NO_5$, 436.2124).

4.3.4. Dehydrodecodine (*4*)

Light yellow powder; UV (λ_{max}) 285.0; $[\alpha]_D^{20}$ +149.3 (*c* 0.15%, MeOH); ^{13}C and ^{1}H NMR see Tables 1 and 2; positive HRESIMS *m/z*: 422.1952 $(M+H)^+$ (calc. for $C_{25}H_{27}NO_5$,422.1967).

4.3.5. Lythridine (*5*)

Yellow powder; UV (λ_{max}) 280.0; $[\alpha]_D^{20}$ +49.3 (*c* 0.15%, MeOH), $[[\alpha]_D^{25}$ −153.4 (*c* 0.3 $CHCl_3$) Douglas et al., 1964]; ^{13}C and ^{1}H NMR see Tables 1 and 2; positive HRESIMS *m/z*: 454.2257 $(M+H)^+$ (calc. for $C_{26}H_{32}NO_6$,454.2230).

4.3.6. Vertine (*6*)

White needle; UV (λ_{max}) 285.0; $[\alpha]_D^{20}$ +69.9 (*c* 0.10%, MeOH), $[[\alpha]_D^{25}$ +61.0 (*c* 0.99% $CHCl_3$) Blomster et al., 1964]; ^{13}C and ^{1}H NMR see Tables 1 and 2; positive HRESIMS *m/z*: 436.2298 $(M+H)^+$ (calc. for $C_{26}H_{30}NO_5$,436.2124).

4.3.7. Heimidine (*7*)

White powder; UV (λ_{max}) 285.0; $[\alpha]_D^{20}$ −101.3 (*c* 0.15%, MeOH); ^{13}C and ^{1}H NMR see Tables 1 and 2; positive HRESIMS *m/z*: 454.2171 $(M+H)^+$ (calc. for $C_{26}H_{32}NO_6$, 454.2230).

4.3.8. Lyfoline (*8*)

Yellow powder; UV (λ_{max}) 281.0; $[\alpha]_D^{20}$ +70.6 (*c* 0.15%, MeOH); ^{13}C and ^{1}H NMR see Tables 1 and 2; positive HRESIMS *m/z*: 422.2238 $(M+H)^+$ (calc. for $C_{25}H_{28}NO_5$, 422.1967).

4.3.9. Epi-lyfoline (*9*)

Yellow powder; UV (λ_{max}) 285.0; $[\alpha]_D^{20}$ −75.9 (*c* 0.15%, MeOH); ^{13}C and ^{1}H NMR see Tables 1 and 2; positive HRESIMS *m/z*: 422.1975 $(M+H)^+$ (calc. for $C_{25}H_{28}NO_5$, 422.1967).

4.4. X-ray crystallography

The crystal structures of solvates of **2**, **3**, and **6** were determined, using data collected at $T = 90$ K with Mo Kα ($\lambda = 0.71073$ Å) radiation on a Nonius Kappa CCD diffractometer equipped with an Oxford Cryostream cooler. Crystal data: (2*S*,4*S*,10*R*)-4-(3-hydroxy-4-methoxyphenyl)-quinolizidin-2-acetate (**2**) chloroform solvate, $C_{18}H_{25}NO_4 \cdot CHCl_3$, monoclinic, C2, $a = 22.400(4)$, $b = 8.1642(12)$, $c = 15.783(3)$ Å, $\beta = 133.486(6)°$, $V = 2094.2(7)$ $Å^3$, $Z = 4$, $D_x = 1.392$ mg m^{-3}, $\theta_{max} = 32.4°$, $R = 0.049$ for 5958 data and 250 refined parameters, CCDC 661139. The absolute configuration was determined by refinement of the Flack parameter (Flack, 1983) to a value of −0.09(5). Fig. 4 shows the structure of the molecule.

Crystal data: lythrine (**3**) dihydrate, $C_{26}H_{29}NO_5 \cdot 2H_2O$, orthorhombic, $P2_12_12_1$, $a = 10.4543(12)$, $b = 11.5659(14)$, $c = 20.349(3)$ Å, $V = 2460.5(5)$ Å^3, $Z = 4$, $D_x = 1.273$ mg m^{-3}, $R = 0.054$ for 4178 data and 314 refined parameters, CCDC 661140. Fig. 5 shows the structure of the molecule.

Crystal data: vertine (**6**) 3:1 chloroform solvate, $C_{26}H_{29}NO_5 \cdot 3CHCl_3$, orthorhombic, $P2_12_12_1$, $a = 11.7368(12)$, $b = 13.6399(10)$, $c = 21.206(2)$ Å, $V = 3394.8(5)$ Å^3, $Z = 4$, $D_x = 1.553$ mg m^{-3}, $\theta_{max} = 33.7°$, $R = 0.041$ for 13,319 data and 422 refined parameters, CCDC 661141. The absolute configuration was determined by refinement of the Flack parameter to a value of −0.04(3). Fig. 6 shows the structure of the molecule. Details of all three structure determinations can be found in supplementary material. CCDC 661139–661141 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

4.5. Biological study

4.5.1. Assay for in vitro antimalarial activity

The assay based on the determination of plasmodial LDH activity as described earlier (Jain et al., 2005).

4.5.2. Assay for in vitro antimicrobial activity

The assay was performed using a modified version of the NCCLS methods as described previously (Jain et al., 2005). The samples were tested up to highest concentrations of 20 μg/ml.

4.5.3. Assay for in vitro cytotoxicity

Cytotoxicity of the compounds was determined by Neutral red Assay as described earlier (Mustafa et al., 2004).

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