Journal of Saudi Chemical Society (2012) **16**, 419–422

King Saud University

Journal of Saudi Chemical Society

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Vincamine and 14-*epi*-vincamine indole alkaloids from *Ambelania occidentalis*

Seif-Eldin N. Ayyad [a,*], Salem A. Basaif [a], Azhar T. Al-Saggaf [b], Walied M. Alarif [c]

[a] *Chem. Dept., Faculty of Science, King Abdulaziz University, P.O. 80203, Jeddah 21589, Saudi Arabia*
[b] *Chem. Dept., Faculty of Science, Taibah University, P.O. 30100, Al-Madinah Al-Munawarah, Saudi Arabia*
[c] *Marine Chem. Dept., Faculty of Marine Sciences, King Abdulaziz University, P.O. 80207, Jeddah 21589, Saudi Arabia*

Received 25 September 2010; accepted 14 February 2011
Available online 19 February 2011

KEYWORDS

Ambelania occidentalis;
Apocynaceae;
Vincamine;
14-*epi*-vincamine

Abstract Two indole alkaloids, Vincamine **1** and 14-*epi*-vincamine **2** were isolated here for the first time from *Ambelania occidentalis*. The structures of these compounds were elucidated by one and two dimension NMR and MS spectroscopy.

1. Introduction

The genus *Ambelania* (family Apocynaceae) is distributed mainly over tropical South America. Plants of the Apocynaceae family are rich sources of structurally diversified indole alkaloids (Dewick, 2002).

A number of tropical plants were reported to elaborate alkaloids with biological activity against tropical diseases caused by protozoan parasites, including leishmaniasis, Chaga's disease, African trypanosomiasis (sleeping sickness), and malaria (Osorio et al., 2008), which encouraged us to investigate one of these plants *Ambelania occidentalis*.

A. occidentalis is routinely used in folk medicine for treating gastrointestinal disorders, even though there have been no safety trials, the genotoxicity potential of hydro-alcoholic extracts of this plant in mice was assesed; induced DNA damage was assessed in peripheral blood leucocytes and micronucleus induction was assessed in polychromatic erythrocytes from bone marrow (Castro et al., 2009). Indole alkaloids have been extensively investigated for a wide variety of pharmacological effects, such as anti-cancer (anti-tumour), anti-HIV, anti-inflammatory, anti-leshmanial activity, anti-malarial, contraceptive, bactericide, anti-hypertensive, central nervous system stimulant (CNS) and anti-addiction agents (Kam et al., 2004; Henriques et al., 1996; Carlini, 2003; Maisonneuve and Glick, 2003). In this article, we report the isolation and structure elucidation of two indole alkaloids from *A. occidentalis*.

* Corresponding author. Mobile: +966 50 500096687.
E-mail address: snayyad2@yahoo.com (S.-E. N. Ayyad).

Peer review under responsibility of King Saud University.
doi:10.1016/j.jscs.2011.02.008

2. Experimental

2.1. Apparatus and methods

1D and 2D nuclear magnetic resonance spectra were recorded on a Varian VI-500 MHz spectrometer for ^{1}H NMR and a Varian VI-300 MHz ^{1}H NMR or Bruker WM 400 MHz spectrometers. Chemical shifts are given in (ppm) relative to TMS as the internal standard. Electron impact mass spectra were determined at 70 eV on a kratos MS-25 instrument. Thin layer chromatography silica gel was used. Preparative thin layer chromatography (PTLC) was performed on silica gel (kieselgel 60, F_{254}).

2.2. Plant material

The plant *A. occidentalis* was collected from Amazon rainforests and identified by Botany group in Minnesota University, Minnesota, USA. Voucher sample is deposited at the Chemistry Department, Faculty of science, King Abdulaziz University, Jeddah, Saudi Arabia.

2.3. Extraction and isolation

Extraction of the dried aerial parts (200 g) by methanol (400–800 ml) was carried out three times at room temperature. The extracts were combined and concentrated under reduced pressure to obtain a dark brown residue (~20.1 g, 10% of the dry weight of the plant). The crude extract was acidified using 0.5 N HCl solution and extracted with diethyl ether to remove the non-basic compounds. The aqueous phase was then made alkaline with $NaHCO_3$ and extracted with chloroform to remove the basic compounds, and the chloroform layer dried over anhydrous sodium sulphate and evaporated under reduced pressure to yield the crude alkaloid fraction (~1.5 g, 0.75% dry wt.). This crude fraction was chromatographed on a silica gel column (60 g, 40 × 1.5 cm) and eluted successively with chloroform, chloroform–ethyl acetate, and chloroform–methanol. Fractions of ~50 ml were collected, followed by TLC on silica-gel plates, with Dragendorff's reagent spray used for visualisation. Promising fractions were further purified by preparative thin layer silica gel chromatography (PTLC), using the appropriate solvent system. Fractions eluted by chloroform/ethylacetate (9.5:0.5) afforded substance **1** at R_f 0.67. Increasing the polarity to chloroform/methanol (9.2:0.8), afforded substance **2** at R_f 0.43.

Vincamine (**1**): white solid (20 mg, 0.01% dry weight): EIMS (probe) 70 eV, *m/z* (rel. int.): 354 (47) [M, $C_{21}H_{26}N_2O_3]^+$, 307 (88) $[M-H_2O-C_2H_5]^+$, 266 (100), 224 (32), 168 (20); HREIMS Obsd. *m/z* = 354.1954 [M, $C_{21}H_{26}N_2O_3]^+$ requires *m/z* = 354.1943; ^{1}H NMR ($CDCl_3$): δ 7.49 [1H, dd, J = 5.7, 3.0 Hz, H-9], 7.15 [1H, ddd, J = 6.9, 5.9, 3.8 Hz, H-10], 7.14 [1H, ddd, J = 6.9, 5.0, 3.0 Hz, H-11], 7.10 [1H, dd, J = 5.6, 3.6 Hz, H-12], 4.62 [1H, br s, OH], 3.83 [3H, s, OMe], 3.64 [1H, m, H-3], 3.39 [1H, ddd, J = 13.7, 5.6, 2.8 Hz, H-5], 3.32 [1H, ddd, J = 13.6, 8.0, 3.5 Hz, H-5′], 3.00 [1H, ddd, J = 16.4, 7.8, 2.8 Hz, H-6], 2.69 [1H, m, H-19], 2.58 [1H, ddd, J = 16.4, 4.7, 3.2 Hz, H-6′], 2.34 [1H, m, H-19′], 2.32 [1H, dq, J = 14.2, 7.3 Hz, H-20], 2.24 [1H, d, J = 14.5 Hz, Ha-15], 2.15 [1H, d, J = 14.4 Hz, Hb-15], 1.83 [1H, m, H-18], 1.67 [1H, m, H-17], 1.49 [1H, ddq, J = 14.1, 6.8 Hz, H-20′], 1.49 [1H, m, H-17′], 1.43 [1H, m, H-18′], 0.92 [3H, t, J = 7.5 Hz, H-21]; ^{13}C NMR ($CDCl_3$) and H–H COSY are shown in Table 1.

14-epi-vincamine (**2**): Yellowish white solid (8 mg, 0.004% dry weight): EIMS (probe) 70 eV, *m/z* (rel. int.): 354 (10) [M, $C_{21}H_{26}N_2O_3]^+$, 336 (30) $[M^+-H_2O]$, 307 (69) $[M-H_2O-C_2H_5]^+$, 266 (100); HREIMS Obsd. *m/z* = 354.1962 [M, $C_{21}H_{26}N_2O_3]^+$ requires *m/z* = 354.1943; ^{1}H NMR ($CDCl_3$): δ 7.44 [1H, dd, J = 5.8, 3.1 Hz, H-9], 7.32 [1H, dd, J = 5.3, 2.9 Hz, H-12], 7.13 [1H, ddd, J = 8.1, 5.8, 2.7 Hz, H-11], 7.12 [1H, ddd, J = 7.2, 5.8, 2.7 Hz, H-10], 3.92 [1H, m, H-3], 3.73 [3H, s, OMe], 3.64 [1H, s, OH], 3.28 [1H, ddd, J = 13.4, 6.4, 4.6 Hz, H-5], 3.16 [1H, ddd, J = 13.6, 7.9, 2.5 Hz, H-5′], 2.93 [1H, ddd, J = 11.6, 6.2, 2.5 Hz, H-6], 2.60 [1H, d, J = 14.8 Hz, Hb-15], 2.51 [1H, m, H-19], 2.48 [1H, ddd, J = 11.3, 7.6, 4.0 Hz, H-6′], 2.19 [1H, dq, J = 14.3, 7.5 Hz, H-20], 2.08 [1H, d, J = 14.9 Hz, Ha-15], 2.04 [1H, m, H-19′], 1.72 [1H, m, H-18], 1.46 [1H, ddq, J = 14.2, 7.2 Hz, H-20′], 1.32 [1H, m, H-17], 1.25 [2H, m, H-17′, H-18′]; ^{13}C NMR ($CDCl_3$) and H–H COSY are shown in Table 1.

3. Results and discussion

Chromatographic separation of the extract of *A. occidentalis* afforded two indole alkaloids (**1** and **2**).

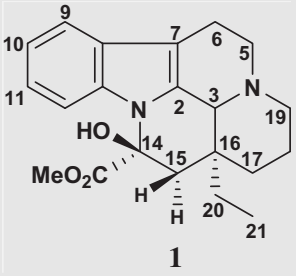
Compound **1** gave M^+ at *m/z* 354, in accordance with $C_{21}H_{26}N_2O_3$. The ^{13}C NMR spectrum (Table 1) gave a total of 21 separate signal carbon resonances and DEPT spectrum showed two methyl, seven methylene, five methine and seven quaternary carbons in agreement with the suggested molecular formula, in addition, the presence of methyl ester was confirmed by signals at δ 173.9 and 53.9 for carbonyl and methoxy groups, respectively. Also the C-14 showed a signal at δ 81.3 due to carbinol carbon.

The ^{1}H NMR spectrum showed significant signals characteristic for four aromatic methine protons of the indole system at δ 7.49 (1H, dd, J = 5.7, 3.0 Hz, H-9), 7.15 (1H, ddd, J = 6.9, 5.9, 3.8 Hz, H-10), 7.14 (1H, ddd, J = 6.9, 5.0, 3.0 Hz, H-11), 7.10 (1H, dd, J = 5.6, 3.6 Hz, H-12), the ^{1}H NMR also showed a methyl ester group at δ 3.83 (3H, s). The H–H COSY and HSQC spectral data revealed in addition to the aromatic protons, three fragments CH_2CH_2N, $NCH_2CH_2CH_2$ and an isolated aminomethine, corresponding to the C(6)–C(5), C(19)–C(18)–C(17) and C(3), respectively. The CH_2CH_2N fragment is branched from the aromatic C-7 as indicated by the observed three-bond correlation from C-2 to H-6 in the HMBC spectrum. The $NCH_2CH_2CH_2$ fragment was deduced by H–H COSY and correlation between C-16 with H-17 and 18. The aminomethine C-3 was expected from the correlation between C-2 and H-3 by HMBC spectrum.

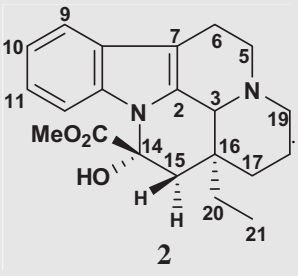
The location of a methyl ester and hydroxy group at C-14 was expected from ^{13}C NMR, DEPT spectrum and correlation from methylene hydrogen H-15 to C-14 in HMBC. The substituent, ethyl side chain C(20)–C(21) at δ 2.32, 1.49 and 0.92 attachment to C-16 was deduced by the long range coupling between H-20 to H-3 from H–H COSY.

As the compound contains four double bonds of indole and one carbonyl group, also the compound has ten degrees of unsaturation from molecular formula $C_{21}H_{26}N_2O_3$, so the

Table 1 ^{13}C NMR data of compounds **1** and **2**, including results of H–H COSY.



1



2

Carbon No.	Compd. No.					
	1			**2**		
	δ_C	DEPT	H–H COSY	δ_C	DEPT	H–H COSY
2	131.4	s	–	130.9	s	–
3	58.7	d	–	58.3	d	–
5	50.3	t	5′, 6, 6′	50.5	t	5′, 6, 6′
5′	–	–	5, 6, 6′	–	–	5, 6, 6′
6	16.2	t	5, 5′, 6′	16.1	t	5, 5′, 6′
6′	–	–	5, 5′, 6	–	–	5, 5′, 6
7	105.3	s	–	105.7	s	–
8	128.3	s	–	128.0	s	–
9	118.1	d	10, 11	117.7	d	10, 11
10	119.9	d	9, 11, 12	119.8	d	9, 11, 12
11	121.5	d	9, 10, 12	121.2	d	9, 10, 12
12	109.9	d	10, 11	111.5	d	10, 11
13	133.8	s	–	134.9	s	–
14	81.3	s	–	82.1	s	–
15′	43.8	t	15′	44.1	t	15′
15	–	–	15	–	–	15
16	34.7	s	–	35.9	s	–
17	24.3	t	17′, 18, 18′	23.8	t	17′, 18, 18′
17′	–	–	17, 18, 18′	–	–	17, 18, 18′
18	19.9	t	17, 17′, 18′, 19, 19′	20.2	t	17, 17′, 18′, 19, 19′
18′	–	–	17, 17′, 18, 19, 19′	–	–	17, 17′, 18, 19, 19′
19	44.0	t	18, 18′, 19′	46.6	t	18, 18′, 19′
19′	–	–	18, 18′, 19	–	–	18, 18′, 19
20	28.3	t	20′, 21	28.5	t	20′, 21
20′	–	–	20, 21	–	–	20, 21
21	7.1	q	20, 20′	7.1	q	20, 20′
C=O	173.9	s	–	172.3	s	–
OMe	53.9	q	–	53.0	q	–
OH	–	–	–	–	–	–

structure should be pentacyclic. From the comparison between the above results and the literature data (Schlittler and Furlenmeier, 1953; Cordell et al., 2001) the compound **1** was assigned as Vincamine.

Compound **2** spectral data (^1H and ^{13}C NMR) are closely similar to those of compound **1**, only a significant difference between both molecules could be rationalized in terms of **2** being the 14-epimar of compound **1**. Specifically, the difference between compound **1** and **2** was deduced from the deshielding of carboxy ester group on the ^1H NMR of H-12 at δ 7.10 of **1** to 7.32 of **2** and ^{13}C NMR of C-12, C-13, C-14 at δ 109.9, 133.8, 81.3 of **1** to 111.5, 134.9, 82.1 of **2**, respectively. So the two structures are Vincamine (**1**) and 14-*epi*-vincamine (**2**) (Atta-ur-Rahman and Sultana, 1984). The two metabolites are known products from other plants and isolated here for the first time.

4. Conclusion

The tropical South America plant *A. occidentalis* is a prolific source of indole alkaloids of high medicinal importance. The two indole alkaloids Vincamine (**1**) and 14-Epivincamine (**2**) were identified here for the first time together from this plant, although they were identified separately from plants of the family Apocynaceae.

Acknowledgments

We thank Botany group in Minnesota University, Minnesota, USA for identification and collection of *A. occidentalis*. We also are thankful to the researcher's assistants of chemistry

department King Abdulaziz University, for their help in the laboratory work.

References

Atta-ur-Rahman, Sultana, M., 1984. Synthetic approaches to the huntaria alkaloids. Heterocycles 22 (4), 841.

Carlini, E.A., 2003. Plants and the central nervous system. Pharmacol. Biochem. Behav. 75, 501.

Castro, L.S., Perazzo, F.F., Maistro, E.L., 2009. Genotoxicity testing of *A. occidentalis*. Genetics Mol. Res. 8, 440.

Cordell, G.A., Quinn-Beattie, M.L., Farnsworth, N.R., 2001. The potential of alkaloids in drug discovery. Phytother. Res. 15, 183.

Dewick, P.M., 2002. Medicinal Natural Products: A Biosynthetic Approach. John Wiley & Sons, Chichester, 350.

Henriques, A.T., Melo, A.A., Moreno, P.R.H., Ene, L.L., Henriques, J.A.P., Schapoval, E.E.S., 1996. *Ervatamia coronaria*: chemical constituents and some pharmacological activities. J. Ethnopharmacol. 50, 19.

Kam, T., Sim, K., Pang, H., Koyano, T., Hayashi, M., Komiyama, K., 2004. Cytotoxic effects and reversal of multidrug resistance by ibogan and related indole alkaloids. Bioorg. Med. Chem. Lett. 14, 4487.

Maisonneuve, I.M., Glick, S.D., 2003. Anti-addictive actions of an iboga alkaloid congener: a novel mechanism for a novel treatment. Pharmacol. Biochem. Behav. 75, 607.

Osorio, E.J., Robledo, S.M., Bastida, J., 2008. Alkaloids with antiprotozoal activity. Alkaloids 66, 113.

Schlittler, E., Furlenmeier, A., 1953. Vincamine, ein alkaloid aus *Vinca minor* L. Apocynaceae. Helv. Chim. Acta 36, 2017–2020.