NPC Natural Product Communications

2017
Vol. 12
No. 4
495 - 498

A New Ajmaline-type Alkaloid from the Roots of *Rauvolfia serpentina*

Thitima Rukachaisirikul[a,*], Suwadee Chokchaisiri[a], Parichat Suebsakwong[a], Apichart Suksamrarn[a] and Chainarong Tocharus[b]

[a]*Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand*

[b]*Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand*

thitima@ru.ac.th

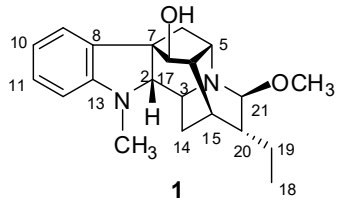
Received: October 5th, 2016; Accepted: February 22nd, 2016

A new ajmaline-type alkaloid, 21-*O*-methylisoajmaline (**1**), together with twenty-one known compounds, a mixture of *β*-sitosterol (**2**) and stigmasterol (**3**), reserpinine (**4**), tetrahydroalstonine (**5**), reserpine (**6**), venoterpine (**7**), yohimbine (**8**), 6'-*O*-(3,4,5-trimethoxybenzoyl)glomeratose A (**9**), isoajmaline (**10**), 3-*epi*-*α*-yohimbine (**11**), methyl 3,4,5-trimethoxy-*trans*-cinnamate (**12**), a mixture of *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (**13**) and stigmasterol 3-*O*-*β*-D-glucopyranoside (**14**), rescidine (**15**), 7-deoxyloganic acid (**16**), ajmaline (**17**), suaveoline (**18**), (+)-tetraphyllicine (**19**), loganic acid (**20**), 3-hydroxysarpagine (**21**), and sarpagine (**22**), were isolated from the roots of *Rauvolfia serpentina*. Their structures were elucidated by spectroscopic data analysis and comparison with literature data. Compounds **11**, **12** and **15** were for the first time identified from the genus *Rauvolfia* and **5**, **7**, **11**, **12**, **15**, **18** and **22** were found from *R. serpentina* for the first time. Compound **11** showed moderate anticholinesterase activity with IC_{50} value of 15.58 μM, whereas **6** exhibited strong vasorelaxant activity with the EC_{50} value of 0.05 μM.

Keywords: *Rauvolfia serpentina*, Apocynaceae, Alkaloids, Anticholinesterase activity, Vasorelaxant activity.

The genus *Rauvolfia* (Apocynaceae family) have attracted considerable attention being a rich source of monoterpene indole alkaloids, which have diverse structures and bioactivities [1,2]. *Rauvolfia serpentina* (L.) Benth. ex Kurz (known in Thai as Rayom) is an important medicinal plant used to cure many illnesses such as hypertension, mental agitation, gastrointestinal disorders, epilepsy, traumas, anxiety, excitement, schizophrenia, sedative insomnia and insanity [3,4]. Previous studies on this plant revealed the presence of various types of indole alkaloids, including yohimbine, heteroyohimbine, ajmaline, and sarpagine type indole alkaloids [5,6]. As part of our continuing search for biologically active compounds from Thai medicinal plants, we herein describe the isolation and structure elucidation of one new ajmaline-type alkaloid (**1**) and twenty-one known compounds (**2**-**22**) from the roots of *R. serpentina*. The anticholinesterase and vasorelaxant activities of some isolated compounds were also evaluated. In addition, the NMR data of rescidine (**15**) and sarpagine (**22**) are reported here for the first time.

Compound **1** was obtained as a pale yellow amorphous solid, $[\alpha]_D^{29}$ +59.2° (c 0.84, MeOH). The HRESIMS showed a pseudomolecular ion $[M+H]^+$ at *m/z* 341.2248 in accordance with the molecular formula $C_{21}H_{28}N_2O_2$. The UV spectrum showed maximal absorptions at 223, 244 and 282 nm. The IR spectrum indicated the presence of OH (3294 cm^{-1}) and an aromatic functionality (1625, 1607 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) showed signals for an ethyl group at δ_H 1.05 (3H, t, *J* = 7.0 Hz, H-18), 1.56 (1H, m, H-19a) and 1.69 (1H, overlapped, H-19b), a singlet for an *N*-methyl group at δ_H 2.79, and signals for four aromatic protons at δ_H 7.52 (1H, d, *J* = 7.3 Hz, H-9), 6.81 (1H, t, *J* = 7.3 Hz, H-10), 7.16 (1H, t, *J* = 7.3 Hz, H-11), and 6.76 (1H, d, *J* = 7.3 Hz, H-12). Its ^{1}H NMR spectroscopic features were closely comparable to those of isoajmaline [7], except for the presence of an additional methoxy singlet at δ_H 3.07. The position of the methoxy group at C-21 was determined from a HMBC correlation between the methoxy signal (δ_H 3.07, δ_C 43.8) and C-21 (δ_C 97.8). The *β*-orientation of the



1

methoxy group was deduced from the observed NOE enhancement of H-21 and H-3 and the ROESY cross-peak between 21-OMe and H-5. The 17R, 21S configuration of **1** was suggested by the H-17 and H-21 signals appearing at δ 4.36 as a singlet and δ 4.63 as a doublet (*J* = 5.2 Hz), respectively, while one-proton singlet at δ 2.81 was attributed to *β*-oriented H-2 proton [8]. The *α*-oriented ethyl group at C-20 was readily verified by both the observed NOE enhancement and the ROESY correlation between H-19 and H-15. The ^{13}C NMR (DEPT) spectrum (Table 1) exhibited six sp^2 carbons (δ_C 154.2, 132.5, 129.1, 124.1, 121.5 and 111.2), one sp^3 quaternary carbon (δ_C 56.3), eight sp^3 methines (δ_C 97.8, 78.6, 76.3, 59.9, 58.3, 55.0, 47.3 and 29.2), three sp^3 methylenes (δ_C 31.9, 25.4 and 23.2) and three methyl groups (δ_C 43.8, 34.8 and 12.0). The ^{13}C NMR spectroscopic data of **1** was generally similar to those of isoajmaline [7] except for the presence of a methoxy carbon (δ_C 43.8) and the notable downfield shifts of three aminomethines C-3 (from δ_C 47.8 in isoajmaline to δ_C 58.3 in **1**), C-5 (from δ_C 48.4 in isoajmaline to δ_C 59.9 in **1**), and C-21 (from δ_C 88.1 in isoajmaline to δ_C 97.8 in **1**), which could be attributed to the presence of the methoxy group modified steric interactions in the molecular framework. Therefore, the structure of compound **1** was unambiguously elucidated as 21-*O*-methyl derivative of isoajmaline, named 21-*O*-methylisoajmaline.

The structures of the known compounds were determined through analysis of their spectroscopic data in comparison with reported values, and they were identified as a mixture of *β*-sitosterol (**2**) and

Table 1: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and HMBC correlation spectroscopic data of compound **1** in CD_3OD.

Position	δ_H (mult, J_{Hz})	δ_C	HMBC
2	2.81 (s)[a]	78.6 CH	C-3, C-6, C-7, C-8, C-14, C-17, N-CH_3
3	4.04 (br d, 9.2)	58.3 CH	
5	3.95 (br s)	59.9 CH	
6	2.24 (m)[b]	31.9 CH_2	C-2, C-7, C-17
7	-	56.3 C	
8	-	132.6 C	
9	7.52 (d, 7.3)	124.7 CH	C-7, C-10, C-11, C-12, C-13
10	6.81 (t, 7.3)	121.6 CH	C-8, C-9, C-11, C-12, C-13
11	7.16 (t, 7.3)	129.2 CH	C-8, C-9, C-10, C-12, C-13
12	6.76 (d, 7.3)	111.3 CH	C-8, C-9, C-10, C-13
13	-	154.3 C	
14a	1.69[c]	25.4 CH_2	C-2
14b	2.29[b]		
15	2.34 (m)[b]	29.3 CH	C-3, C-5, C-14, C-16, C-19, C-20, C-21
16	2.63 (br s)	55.1 CH	C-7, C-14, C-15, C-17
17	4.36 (s)	76.3 CH	C-2, C-5, C-6, C-15, C-16
18	1.05 (t, 7.0)	12.0 CH_3	C-19, C-20
19a	1.56 (m)	23.3 CH_2	C-18, C-20, C-21
19b	1.69[c]		
20	1.79 (m)	47.3 CH	C-19
21	4.63 (d, 5.2)	97.9 CH	
-OCH_3	3.07 (s)	43.8 CH_3	C-21
-NCH_3	2.79 (s)[a]	34.8 CH_3	C-2, C-13

[a,b] Partially overlapping signals, [c] Overlapping signals

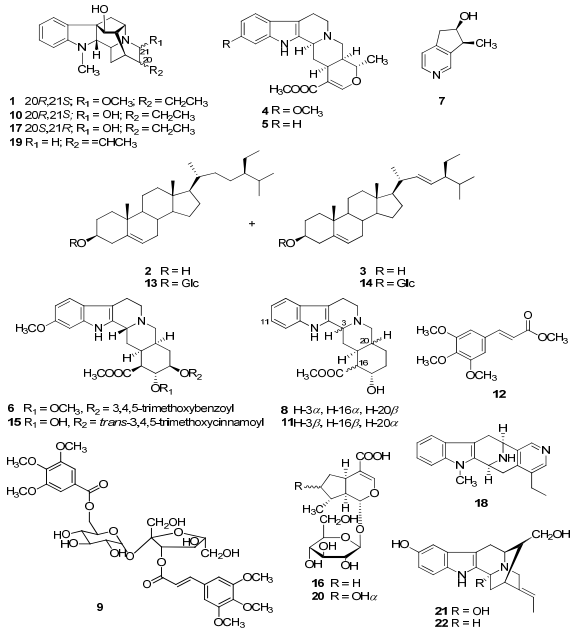


Figure 1: Structures of isolated compounds from *R. serpentina*.

stigmasterol (**3**) [9], reserpinine (**4**) [10], tetrahydroalstonine (**5**) [11,12], reserpine (**6**) [12,13], venoterpine (**7**) [14,15], yohimbine (**8**) [16], 6'-*O*-(3,4,5-trimethoxybenzoyl)glomeratose A (**9**) [6], isoajmaline (**10**) [7], 3-*epi*-α-yohimbine (**11**) [17], methyl 3,4,5-trimethoxy-*trans*-cinnamate (**12**) [18], a mixture of β-sitosterol 3-*O*-β-D-glucopyranoside (**13**) and stigmasterol 3-*O*-β-D-glucopyranoside (**14**) [19], rescidine (**15**) [12], 7-deoxyloganic acid (**16**) [20], ajmaline (**17**) [7], suaveoline (**18**) [21], (+)-tetraphyllicine (**19**) [22], loganic acid (**20**) [23], 3-hydroxysarpagine (**21**) [6] and sarpagine (**22**) [24] (Figure 1). Among the known compounds, compounds **11**, **12** and **15** were first identified from genus *Rauvolfia* and **5**, **7**, **11**, **12**, **15**, **18** and **22** were isolated from *R. serpentina* for the first time.

Some isolates were tested for anticholinesterase and vasorelaxant activities. The results are shown in Table 2. Compounds **18** and **1** showed moderate anticholinesterase activity with IC_{50} values of 15.58 and 31.21 μM, respectively. Compound **6** exhibited the most active vasorelaxant activity with the EC_{50} values of 0.05 μM or approximately 1.6 fold more active than the positive control acetylcholine whereas compounds **11**, **9** and **21** showed strong activity with the EC_{50} values of 0.09, 0.54 and 0.97 μM, respectively.

Table 2: IC_{50} and EC_{50} values of some isolated compounds for anticholinesterase and vasorelaxant activities, respectively.

Compds	IC_{50} (μM) ±S.E.M.[d]	EC_{50} (μM)
1	31.21±1.20	-
4	Inactive[b]	-
5	151.74±0.10	20.20
6	Inactive[b]	0.05
7	Inactive[b]	-
8	247.34±2.93	16.47
9	Inactive[b]	0.54
10	Inactive[b]	-
11	106.87±1.39	0.09
12	Inactive[b]	-
15	64.22±0.27	-
16	Inactive[b]	-
17	Inactive[b]	6.94
18	15.58±0.24	23.10
19	Inactive[b]	-
20	Inactive[b]	3.47
21	76.41±1.19	0.97
22	88.50±1.20	-
Galanthamine[a]	1.45±0.04	-
Acetylcholine[c]	-	0.08

[a]Reference drug, [b]Inactive at 0.1 mg/ml, [c]As acetylcholine iodide, [d]Standard error of the mean of three assays.

Experimental

General experimental procedures: Optical rotations were measured on a JASCO-1020 polarimeter. UV spectra were measured on an UV-1800 Shimadsu UV spectrophotometer. ATR-FTIR spectra were obtained using a Perkin Elmer FT-IR spectrum 400 spectrometer. 1D and 2D NMR spectra were recorded on a Bruker AVANCE 400 NMR spectrometer. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. ES-MS and HR-ESI-MS were recorded on a Finnigan LC-Q mass spectrometer and a micrOTOP-II mass spectrometer, respectively. Column chromatography (CC) was carried out using Merck silica gel 60 (<0.063 mm) and Amersham Biosciences Sephadex LH-20. For TLC, Merck precoated silica gel 60 F254 plates were used. Spots on TLC were visualized under UV light and by spraying with anisaldehyde-H_2SO_4 reagent followed by heating.

Plant Material: The roots of *R. serpentina* were purchased from Chao Kom Per herbal store, Bangkok in May 2013. A voucher specimen (Thitima Rukachaisirikul, No. 009) was deposited at the Faculty of Science, Ramkhamhaeng University, Thailand.

Extraction and isolation: The air-dried, powdered roots of *R. serpentina* (2.7 kg) were extracted successively with n-hexane, EtOAc and MeOH at room temperature. The hexane, EtOAc and MeOH extracts were filtered and concentrated to dryness under reduced pressure. The hexane extract (15.6 g) was subjected to CC (silica gel; hexane-EtOAc gradient) to give 6 fractions (H1-H6). Fr. H2 (1.8 g) was purified by CC (silica gel; hexane-EtOAc, 90:10) to afford a mixture of **2** and **3** (0.42 g), whereas fr. H4 (0.38 g) was rechromatographed by CC twice in succession (silica gel; CH_2Cl_2-MeOH, 95.5:0.5 and hexane-EtOAc, 70:30) to yield **4** (82.7 mg). The EtOAc extract (38.2 g) was subjected to CC (silica gel; hexane, hexane-EtOAc, EtOAc, EtOAc-MeOH, MeOH gradient) to give 14 fractions (E1-E14). Fr. E6 (1.21 g) was separated on Sephadex LH-20, eluted with MeOH-CH_2Cl_2 (70:30) and further purified by CC (silica gel; hexane-EtOAc, 80:20) to give **5** (10.1 mg). Fr. E8 (3.80 g) was separated by CC twice in succession (silica gel; CH_2Cl_2-MeOH, 95.5:0.5 and hexane-EtOAc-MeOH, 60:40:1) to furnish **6** (304.5 mg). Fr. E9 (2.05 g) was fractionated by CC (silica gel; CH_2Cl_2-MeOH, 95.5:0.5) and further resubjected to CC (reversed

phase RP-18; MeOH-H_2O, 60:40) to obtain 7 fractions (E9.1-E9.7). Subfr. E9.1 (95.1 mg) was purified by CC (silica gel; CH_2Cl_2-MeOH, 99:1) to afford **7** (33.6 mg), whereas subfr. E9.3 (0.41 g) furnished **8** (201.1 mg). Fr. E10 (1.98 g) was separated on Sephadex LH-20, eluted with MeOH-CH_2Cl_2 (80:20) and further purified by CC (silica gel; CH_2Cl_2-MeOH, 94:6) to obtain **9** (105.5 mg). Fr. E11 (7.33 g) was rechromatographed by CC twice in succession (silica gel; CH_2Cl_2-MeOH, 94:6) to afford **10** (4.4 mg). Fr. E12 (1.17 g) was purified in a similar manner as fr. E10 to give **11** (45.6 mg). The MeOH extract (198.7 g) was subjected to CC (silica gel; hexane, hexane-EtOAc, EtOAc, EtOAc-MeOH, MeOH gradient) to give 14 fractions (M1-M14). Fr. M3 (0.38 g) was purified on Sephadex LH-20, eluted with MeOH-CH_2Cl_2 (80:20) to yield **12** (24.5 mg). Fr. M6 (4.06 g) was rechromatographed by CC (silica gel; CH_2Cl_2-MeOH, 98:2) to obtain a mixture of **13** and **14** (11.0 mg). Fr. M7 (8.40 g) was fractionated by CC twice in succession (silica gel; CH_2Cl_2-MeOH, 96:4 and hexane-EtOAc, 94:6) to obtain 7 fractions (M7.1-M7.7). Subfr. M7.1 (0.77 g) was rechromatographed by CC (silica gel; CH_2Cl_2-MeOH, 99:1) to afford **15** (4.6 mg), whereas subfr. M7.7 (0.10 g) was separated by CC twice in succession (silica gel; CH_2Cl_2-MeOH, 90:10 and hexane-EtOAc-MeOH, 60:40:2) to give **16** (20.0 mg). Fr. M8 (26.09 g) was purified by CC (silica gel; CH_2Cl_2-MeOH, 93:7) to obtain **17** (3.45 g). Fr. M9 (25.31 g) was subjected to CC (silica gel; CH_2Cl_2-MeOH gradient) to give 11 fractions (M9.1-M9.11). Subfrs. M9.7 (0.10 g) and M9.8 (0.42 g) was separately rechromatographed by CC (silica gel; CH_2Cl_2-MeOH, 98:2 and 94:6, respectively) to furnish **18** (23.3 mg) and **19** (7.3 mg), respectively. Subfr. M9.9 (2.12 g) was separated by CC twice in succession (silica gel; hexane-EtOAc-MeOH, 60:40:3 and CH_2Cl_2-MeOH, 94:6) to afford **1** (9.8 mg). Subfr. M9.11 (8.75 g) was reseparated on Sephadex LH-20, eluted with MeOH to obtain 4 fractions (M9.11.1-M9.11.4). Subfrs. M9.11.2 (2.19 g) and M9.11.3 (3.17 g) were separately subjected to CC (silica gel; CH_2Cl_2-MeOH, 90:10) to give **20** (217.5 mg) and **21** (47.8 mg), respectively. Fr. M10 (13.76 g) was further purified by CC three times in succession (silica gel; CH_2Cl_2-MeOH, 90:10) to furnish **22** (11.0 mg).

21-*O*-methylisoajmaline (**1**)
Pale yellow amorphous solid.
$[\alpha]_D^{29}$: +59.2° (*c* 0.84, MeOH).
UV (MeOH) λ_{max} (log ε) 223 (2.61), 244 (1.07), 282 (0.75) nm.
IR (ATR): 3294, 2961, 2930, 2873, 1625, 1607, 1465, 1356, 1294, 1228, 1158, 1124, 1073, 1049, 1022, 933, 860, 836, 809, 759, 744 cm^{-1}.
^1H and ^{13}C NMR (CD_3OD): Table 1.
ESMS (+ve): *m/z* (%) 341.8 $[M+H]^+$ (100).
HR-ESI-MS (+ve): *m/z* 341.2248 $[M+H]^+$ (Calcd. for $C_{21}H_{28}N_2O_2$ +H, 341.2229).

Rescidine (**15**)
Pale yellow amorphous solid.
$[\alpha]_D^{28}$: -46.4° (*c* 0.56, $CHCl_3$) [lit. $[\alpha]_D^{22}$ -63.4° (*c* 1.0, $CHCl_3$)] [25].
^1H NMR (400 MHz, $CDCl_3$)**:** δ 1.74 (1H, br d, *J* = 13.6 Hz, H_a-14), 1.89 (1H, br d, *J* = 12.0 Hz, H-20), 1.97 (1H, br d, *J* = 13.2 Hz, H_a-19), 2.20 (1H, dd, *J* = 13.5, 5.0 Hz, H_b-14), 2.29 (1H, q-like, *J* = 12.2 Hz, H_b-19), 2.31 (1H, br d, *J* = 13.2 Hz, H-15), 2.46 (1H, H_a-6)[a], 2.47 (1H, H_a-21)[a], 2.64 (1H, dd, *J* = 11.0, 4.2 Hz, H-16), 2.96 (1H, m, H_b-6), 3.04 (1H, br d, *J* = 8.8 Hz, H_b-21), 3.16 (2H, d, *J* = 5.2 Hz, H-5), 3.83 (3H, s, 11-OCH_3), 3.84 (3H, s, 16-OCH_3), 3.86 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.87 (3H, s, 4′-OCH_3), 4.23 (1H, t, *J* = 10.2 Hz, H-17), 4.43 (1H, br s, H-3), 4.86 (1H, m, H-18), 6.35 (1H, d, *J* = 15.8 Hz, H-α), 6.74 (2H, s, H-2′, H-6′)[b], 6.77 (1H, H-10)[b], 6.83 (1H, br s, H-12), 7.31 (1H, d, *J* = 8.4 Hz, H-9), 7.55 (1H, br s, -NH), 7.61 (1H, d, *J* = 15.8 Hz, H-β), [a]Overlapping signals, [b] Partially overlapping signals.
^{13}C NMR (100 MHz, $CDCl_3$): δ 16.8 (C-6), 24.1 (C-14), 29.2 (C-19), 32.0 (C-15), 33.9 (C-20), 49.0 (C-21), 51.1 (C-5), 52.1 (C-16)[c], 52.2 (16-O$\underline{C}H_3$)[c], 53.8 (C-3), 55.8 (11-O$\underline{C}H_3$), 56.1 (4′-O$\underline{C}H_3$), 60.9 (3′, 5′-O$\underline{C}H_3$), 68.7 (C-17), 76.5 (C-18), 95.2 (C-12), 105.3 (C-2′, 6′), 109.1 (C-7), 109.6 (C-10), 117.1 (C-α), 118.5 (C-9), 122.2 (C-8), 129.8 (C-1′), 130.2 (C-2), 136.4 (C-13), 145.2 (C-4′,β), 153.4 (C-3′, 5′), 156.3 (C-11), 166.8 (CO), 173.2 ($\underline{C}O_2CH_3$), [c]Assignments with the same superscript may be interchanged.
ESMS (+*ve*): *m/z* (%) 621.8 $[M+H]^+$ (100).

Sarpagine (**22**)
Pale yellow amorphous solid.
$[\alpha]_D^{29}$: +5.1° (*c* 1.11, pyr) [lit. $[\alpha]_D^{21}$: +48.4° (*c* 0.95, pyr)] [26].
^1H NMR (400 MHz, $CDCl_3$ + CD_3OD (1:1)): δ 1.67 (3H, d, *J* = 5.6 Hz, H-18), 1.95 (1H, br d, *J* = 14.8 Hz, H_a-14), 2.03 (1H, d, *J* = 7.2 Hz, H-16), 2.30 (1H, t, *J* = 11.6 Hz, H_b-14), 2.83 (1H, d, *J* = 16.1 Hz, H_a-6), 3.00 (1H, br s, H-15), 3.14 (1H, dd, *J* = 16.1, 4.6 Hz, H_b-6), 3.28 (1H, H-5)[a], 3.50 (2H, d, *J* = 7.2 Hz, H-17), 3.86 (1H, d, *J* = 15.8 Hz, H_a-21), 3.95 (1H, d, *J* = 15.8 Hz, H_b-21), 4.63 (1H, d, *J* = 9.6 Hz, H-3), 5.59 (1H, br d , *J* = 6.4 Hz, H-19), 6.68 (1H, d, *J* = 8.6 Hz, H-11), 6.81 (1H, br s, H-9), 7.15 (1H, d, *J* = 8.6 Hz, H-12), 7.82 (1H, br s, NH), [a] Overlapping signals.
^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD (1:1)): δ 13.1 (C-18), 26.7 (C-6), 27.7 (C-15), 33.0 (C-14), 44.2 (C-16), 55.5 (C-21), 52.7 (C-3), 57.8 (C-5), 64.1 (C-17), 102.9 (C-7), 103.3 (C-9), 112.7 (C-11)[b], 112.9 (C-12)[b], 121.7 (C-19), 128.5 (C-8), 130.0 (C-20), 133.1 (C-13), 135.4 (C-2), 151.6 (C-10), [b]Assignments with the same superscript may be interchanged.
ESMS (+*ve*): *m/z* (%) 311.8 $[M+H]^+$ (100).

Anticholinesterase activity testing: Acetylcholinesterase (AChE) inhibitions were determined spectrophotometrically using acetylthiocholine as substrate, by modifying the method of Ellman [27,28]. Briefly, in the 96-well plates, 140 µl of 10 mM sodium phosphate buffer (pH 8.0), 20 µl of a solution of AChE (0.2 units/mL in 10 mM sodium phosphate buffer, pH 8.0) and 20 µl of test compound solution dissolved in 80% methanol (a final concentration of 0.1 mg/mL) were mixed and incubated at room temperature for 15 min. The reaction was started by adding 20 µl of mixture solution of 5 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) in 10 mM sodium phosphate buffer (pH 8.0), containing 0.1% bovine serum albumin (BSA) and 5 mM acetylcholine iodine (ATCI) in 10 mM sodium phosphate buffer, pH 8.0 (5:1). The hydrolysis of acetylthiocholine was determined by monitoring the formation of the yellow 5-thio-2-nitrobenzoate anion as result of reaction with DTNB and thiocholines, catalyzed by enzymes at a wavelength of 405 nm and the absorbance was measured after 2 minutes of incubation at room temperature. Percentage of inhibition was calculated by comparing the rate of enzymatic hydrolysis of ATCI for the sample to that of the blank (80% methanol in buffer). Galanthamine was used as a reference standard. Every experiment was done in triplicate.

Vasorelaxant activity testing

1. Animals: Male Sprague-Dawley rats (age 8 weeks) were obtained from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand. The animals were housed under a 12:12-h light-dark cycle and maintained at 24±1 ºC. Animal feed and water was supplied *ad libitum*.

2. Smooth muscle tone determination: Aortic rings 4 mm in length were cut and immediately placed in 100% oxygen-saturated

HEPES-buffer physiological salt solution (HPSS: 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, 11 mM glucose, pH 7.4). The ring was equilibrated for 2 h with several changes of HPSS. The changes in isometric force were recorded on a computer system using the Chart version 7 PowerLab ADInstruments (2009) program. The resting tension was precontracted with phenylephrine (PE). The % relaxation of the aortic rings against log concentration of the compounds at 10^{-12} to 10^{-4} M is presented in Figure S14 (in Supplementary data). The EC_{50} of tested compounds is presented in Table 2.

3. Statistical analysis: Statistical analysis was performed with one way analysis of variance (ANOVA). The differences were considered statistically significant when compared to normal control at $P < 0.05$.

Supplementary data: UV, IR, NMR and HR-ESI-MS spectra of compound **1,** NMR data of compounds **15** and **22**, and the vasorelaxant profile of the tested compounds were included.

Acknowledgments - This work was supported by a fund from Ramkhamhaeng University. Partial support from the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education is gratefully acknowledged. Suwadee thanks PERCH-CIC for a scholarship.

References

[1] Mittal B, Meenakshi S, Sharma A, Gothecha VK. (**2012**) Phytochemical & pharmacological activity of *Rauvolfia serpentina* - a review. *International Journal of Ayurvedic and Herbal Medicine*, ***2***, 427-434.
[2] Kumari R, Rathi B, Rani A, Bhatnagar S. (**2013**) *Rauvolfia serpentina* L. Benth. ex Kurz.: Phytochemical, pharmacological and therapeutic aspects. *International Journal of Pharmaceutical Sciences Review and Research, **23***, 348-355.
[3] Poonam, Agrawal S, Mishra S. (**2013**) Physiological, biochemical and modern biotechnological approach to improvement of *Rauvolfia serpentina*. *IOSR Journal of Pharmacy and Biological Sciences*, ***6***, 73-78.
[4] Meena AK, Bansal P, Kumar S. (**2009**) Plants-herbal wealth as a potential source of ayurvedic drugs. *Asian Journal of Traditional Medicines*, ***4***, 152-170.
[5] Wachsmuth O, Matusch R. (**2002**) Anhydronium bases from *Rauvolfia serpentina*. *Phytochemistry*, ***61***, 705-709.
[6] Itoh A, Kumashiro T, Yamaguchi M, Nagakura N, Mizushina Y, Nishi T, Tanahashi T. (**2005**) Indole alkaloids and other constituents of *Rauwolfia serpentina*. *Journal of Natural Products*, ***68***, 848-852.
[7] Jokela R, Lounasmaa M. (**1996**) A ^{1}H- and ^{13}C-NMR study of seven ajmaline-type alkaloids. *Planta Medica*, ***62***, 577-579.
[8] Lounasmaa M, Jokela R. (**1985**) A 400 MHz ^{1}H NMR study of twelve ajmaline-type alkaloids. *Heterocycles*, ***23***, 1503-1508.
[9] Chaturvedula VSP, Prakash I. (**2012**) Isolation of stigmasterol and β-sitosterol from the dichloromethane extract of *Rubus suavissimus*. *International Current Pharmaceutical Journal*, ***1***, 239-242.
[10] Mukhopadhyay G, Mukherjee B, Patra A, Chatterjee A, Ghosh R, Roychowdhury P, Kawazura H. (**1991**) 11-Methoxytetrahydroalstonine, a heteroyohimbinoid alkaloid from *Vinca major*. *Phytochemistry*, ***30***, 2447-2449.
[11] Hofle G, Heinstein P, Stockigt J, Zenk MH. (**1980**) ^{1}H-NMR Analysis of ajmaline-type alkaloids of the 3α series. *Planta Medica*, ***40***, 120-126.
[12] Wenkert E, Chang CJ, Chawla HPS, Cochran DW, Hagaman EW, King JC, Orito K. (**1976**) General methods of synthesis of indole alkaloids. 14. Short routes of construction of yohimboid and ajmalicinoid alkaloid systems and their ^{13}C nuclear magnetic resonance spectral analysis. *Journal of the American Chemical Society*, ***98***, 3645-3655.
[13] Lounasmaa M, Tolvanen A. (**1985**) A 400 MHz ^{1}H NMR study of four basic reserpine alkaloids. *Heterocycles*, ***23***, 371-375.
[14] Arthur HR, Johns SR, Lamberton JA, Loo SN. (**1967**) A new monoterpenoid alkaloid (RW47) from *Rauwolfia verticillata* (Lour.) Bail. of Hong Kong. *Australian Journal of Chemistry*, ***20***, 2505-2508.
[15] Ravao T, Richard B, Zeches M, Massiot G, Le Men-Olivier L. (**1985**) The confriguration of venoterpine. *Tetrahedron Letters*, ***26***, 837-838.
[16] dos Santos Torres ZE, Silveira ER, Rocha e Silva LF, Lima ES, de Vasconcellos MC, de Andrade Uchoa DE, Filho RB, Pohlit AM. (**2013**) Chemical composition of *Aspidosperma ulei* Markgr. and antiplasmodial activity of selected indole alkaloids. *Molecules*, ***18***, 6281-6297.
[17] Falkenhagen H, Stockigt J, Kuzovkina IN, Alterman IE, Kolshorn H. (**1993**) Indole alkaloids from hairy roots of *Rauwolfia serpentina*. *Canadian Journal of Chemistry*, ***71***, 2201-2203.
[18] Ralph S, Ralph J. (**2009**) NMR database of lignin and cell wall model compounds. Available at URL www.glbrc.org/databases_and_software/nmrdatabase/
[19] Rai NP, Adhikari BB, Paudel A, Masuda K, Mckelvey RD, Manandhar MD. (**2006**) Phytochemical constituents of the flowers of *Sarcococca coriacea* of Nepalese origin. *Journal of Nepal Chemical Society*, ***21***, 1-7.
[20] Muhammad I, Dunbar DC, Khan RA, Ganzera M, Khan IA. (**2001**) Investigation of una de gato I. 7-deoxyloganic acid and ^{15}N NMR spectroscopic studies on pentacyclic oxindole alkaloids from *Uncaria tomentosa*. *Phytochemistry*, ***57***, 781-785.
[21] Endreb S, Takayama H, Suda S, Kitajima M, Aimi N, Sakai SI, Stockigt J. (**1993**) Alkaloids from *Rauwolfia serpentina* cell cultures treated with ajmaline. *Phytochemistry*, ***32***, 725-730.
[22] Djerassi C, Fishman J, Gorman M, Kutney JP, Pakrashi SC. (**1957**) Alkaloid studies. XVI. Alkaloids of *Rauwolfia tetraphylla* L. The structures of tetraphylline and tetraphyllicine. *Journal of the American Chemical Society*, ***79***, 1217-1222.
[23] Nakamoto K, Otsuka H, Yamasaki K. (**1988**) 7-*O*-Acetyl loganic acid from *Alangium platanifolium* var. *trilobum*. *Phytochemistry*, ***27***, 1856-1858.
[24] Zhao S, Liao X, Wang T, Flippen-Anderson J, Cook JM. (**2003**) The enantiospecific, stereospecific total synthesis of the ring-A oxygenated sarpagine indole alkaloids (+)-majvinine, (+)-10-methoxyaffinisine, and (+)-N_a-methylsarpagine, as well as the total synthesis of the *Alstonia* bisindole alkaloid macralstonidine. *Journal of Organic Chemistry*, ***68***, 6279-6295.
[25] Popelak A, Haack E, Lettenbauer G, Spingler H. (**1961**) Rescidin, ein alkaloid aus *Rauwolfia volmitoria* Afz. *Die Naturwissenschaften*, ***48***, 73-74.
[26] Weiming C, Yaping Y, Xiaotian L. (**1983**) Alkaloids from roots of *Alstonia yunnanensis*. *Planta Medica*, ***49***, 62.
[27] Ellman GL, Courtney KD, Andres VJr, Feather-Stone RM. (**1961**) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, ***7***, 88-95.
[28] Bolognesi ML, Banzi R, Bartolini M, Cavalli A, Tarozzi A, Andrisano V, Minarini A, Rosini M, Tumiatti V, Bergamini C, Fato R, Lenaz G, Hrelia P, Cattaneo A, Recanatini M, Melchiorre C. (**2007**) Novel class of quinone-bearing polyamines as multi-target-directed ligands to combat Alzheimer's disease. *Journal of Medicinal Chemistry*, ***50***, 4882-4897.