Iridoids from the Bark of *Alstonia scholaris*

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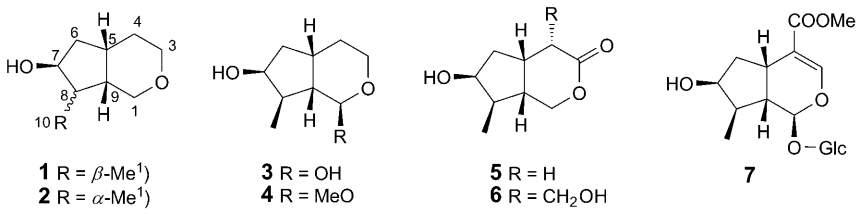
Four new 11-noriridoids named scholareins A–D (**1**–**4**), along with three known derivatives, isoboonein (**5**), alyxialactone (**6**), and loganin (**7**), were isolated from EtOH extracts of the bark of *Alstonia scholaris* by chromatographic methods. Their structures were identified by extensive mass-spectrometric and spectroscopic (especially 2D-NMR) experiments.

Introduction. – *Alstonia scholaris* (Apocynaceae) is a tree 20 to 40 m high, which is widely distributed in the tropical regions of Africa and Asia [1]. Like the other members of the genus *Alstonia*, *A. scholaris* is well known for its monoterpenoid indole alkaloids, which originate from the tryptamine–loganin/secologanin biosynthetic pathway [2–4]. Therefore, as a prototype of monoterpenoid indole alkaloids, iridoids may widely exist in *A. scholaris*. However, there was no literature reporting iridoids from this plant. This observation promoted us to investigate the nonalkaloidal part of the bark of *Alstonia scholaris*, which led to the isolation of four new iridoids, scholareins A–D[1]) (**1**–**4**), together with three known analogues, isoboonein (**5**) [5], alyxialactone (**6**) [6], and loganin (**7**) [7]. In this article, we report their isolation and structure elucidation.



Results and Discussion. – Scholarein A (**1**) possesses a molecular formula $C_9H_{16}O_2$ as evidenced by its HR-ESI-MS (*m/z* 157.1224 ($[M+H]^+$, $C_9H_{17}O_2^+$)), indicating two unsaturation degrees. The IR spectrum showed absorptions for OH (3386) and CH_2 groups (2929 cm^{-1}). The ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*), HSQC, and HMBC, ^{1}H,^{1}H-COSY, and ROESY data (*Fig. 1*) and comparison with the data of isoboonein

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

Table 1. *^{1}H-NMR Data of* **1**–**4**. δ in ppm, J in Hz.

	1[a])	**2**[b])	**3**[c])	**4**[b])
CH_2(1) or H–C(1)	3.39–3.42 (*m*)	3.61–3.63, 3.34–3.36 (2*m*)	4.89 (*d*, *J* = 4.0)	4.54 (br. *s*)
CH_2(3)	3.43–3.46, 3.30–3.33 (2*m*)	3.64–3.66, 3.57–3.59 (2*m*)	3.84–3.87, 3.36–3.39 (2*m*)	3.70–3.73, 3.49–3.52 (2*m*)
CH_2(4)	1.66–1.69, 1.12–1.15 (2*m*)	1.71–1.74, 1.51–1.53 (2*m*)	1.40–1.44, 1.28–1.31 (2*m*)	1.47–1.50, 1.34–1.36 (2*m*)
H–C(5)	2.16–2.19 (*m*)	2.32–2.35 (*m*)	2.34–2.37 (*m*)	2.38–2.41 (*m*)
CH_2(6)	1.58–1.61, 1.32–1.35 (2*m*)	1.89–1.92, 1.22–1.24 (2*m*)	1.72–1.75, 1.67–1.70 (2*m*)	1.83–1.86, 1.73–1.76 (2*m*)
H–C(7)	3.86–3.88 (*m*)	3.94–3.97 (*m*)	4.13–4.17 (*m*)	4.23–4.26 (*m*)
H–C(8)	1.45–1.47 (*m*)	2.16–2.18 (*m*)	1.91–1.93 (*m*)	2.04–2.08 (*m*)
H–C(9)	1.69–1.72 (*m*)	1.74–1.77 (*m*)	1.60–1.64 (*m*)	1.71–1.74 (*m*)
Me(10)	0.83 (*d*, *J* = 7.0)	1.02 (*d*, *J* = 7.2)	0.98 (*d*, *J* = 6.9)	1.04 (*d*, *J* = 7.0)
MeO–C(1)				3.37 (*s*)
OH–C(1)			4.92 (*s*)	
OH–C(7)			3.43 (*d*, *J* = 5.0)	

[a]) In $CDCl_3/CD_3OD$ at 400 MHz. [b]) In $CDCl_3$ at 500 MHz. [c]) In CD_3COCD_3 at 500 MHz.

Table 2. *^{13}C-NMR Data* (100 MHz) *of* **1**–**4**. δ in ppm.

	1[a])	**2**[b])	**3**[c])	**4**[b])
C(1)	62.2 (*t*)	60.7 (*t*)	93.6 (*d*)	99.4 (*d*)
C(3)	61.7 (*t*)	60.6 (*t*)	58.6 (*t*)	58.1 (*t*)
C(4)	33.2 (*t*)	39.3 (*t*)	30.1 (*t*)	28.7 (*t*)
C(5)	34.4 (*d*)	34.0 (*d*)	32.1 (*d*)	31.2 (*d*)
C(6)	40.9 (*t*)	41.1 (*t*)	42.4 (*t*)	41.7 (*t*)
C(7)	74.7 (*d*)	75.5 (*d*)	73.6 (*d*)	74.0 (*d*)
C(8)	41.3 (*d*)	40.9 (*d*)	39.4 (*d*)	38.2 (*d*)
C(9)	48.4 (*d*)	47.7 (*d*)	48.8 (*d*)	46.5 (*d*)
C(10)	13.5 (*q*)	10.2 (*q*)	13.0 (*q*)	13.0 (*q*)
MeO				54.7 (*q*)

[a]) In $CDCl_3/CD_3OD$. [b]) In $CDCl_3$. [c]) In CD_3COCD_3.

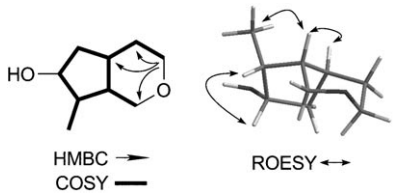


Fig. 1. *Selected HMBC, COSY, and ROESY correlations of* **1**

(**5**) [5] established the structure of scholarein A (**1**) as (5β,7β,8β,9β)-8-methyl-2-oxabicyclo[4.3.0]nonan-7-ol[1]).

The ^{13}C-NMR and DEPT spectra of **1** displayed nine signals, corresponding to one Me, four CH_2, and four CH groups. Of these, three signals were assigned to oxygenated C-atoms at δ(C) 74.7 (CH), 62.2 (CH_2), and 61.7 (CH_2). The ^{1}H-NMR spectrum displayed clear signals for a Me (δ(H) 0.83 (*d*, J = 7.0 Hz)) and a CH group (δ(H) 2.16–2.19 (*m*)); signals at δ(H) 3.30–3.33, 3.39–3.42, 3.43–3.46, and 3.86–3.88 were assigned to three oxygenated C-atoms according to the HSQC spectrum. The above data indicated the presence of a bicyclic noriridoid skeleton, which was confirmed by the ^{1}H,^{1}H-COSY cross-peaks CH_2(3)/CH_2(4), CH_2(4)/H–C(5), H–C(5)/CH_2(6), CH_2(6)/H–C(7), H–C(7)/H–C(8), H–C(8)/H–C(9), H–C(9)/CH_2(1), H–C(5)/H–C(9), and H–C(8)/Me(10). After careful analysis of the ^{1}H- and ^{13}C-NMR data of **1**, we found they were similar to those of isoboonein (**5**) [5], except for the C(3)=O group of **5** which was replaced by a CH_2 group (δ(H) 3.30–3.33 and 3.43–3.46; δ(C) 61.7) in **1**, as revealed by the HMBC C(3)/CH_2(1), CH_2(4), and H–C(5). The relative configuration of **1** was determined by a ROESY experiment. The NOE correlation H–C(5)/H–C(9) indicated the position of these H-atoms on the same side of the molecular skeleton, which were assigned to be β-oriented. The NOE correlation H–C(9)/Me(10) established the β-orientation of Me–C(8), and the NOE correlation H–C(8)/H–C(7) the β-orientation of OH–C(7).

Scholarein B (**2**) was found to possess a molecular formula $C_9H_{16}O_2$ as evidenced by HR-ESI-MS (*m/z* 157.1218 ($[M+H]^+$, $C_9H_{17}O_2^+$)). The ^{1}H-, ^{13}C-NMR, and DEPT data of **2** were very similar to those of **1** (*Tables 1* and *2*). Their EI-MS also displayed almost the same spectrometric fragmentations (see *Exper. Part*). The difference was established by comparing their ROESY plots. In the case of **2**, the NOE correlation H–C(5)/H–C(9) indicated a *cis* relationship of these protons, which were assigned to be β-oriented. The absence of an NOE correlation H–C(9)/Me(10) and the presence of an NOE correlation H–C(8)/H–C(9) established the α-orientation of Me–C(8), and the NOE correlation Me(10)/H–C(7) the β-orientation of OH–C(7). Thus, scholarein B (**2**) was an isomer of **1** and was determined as (5β,7β,8α,9β)-8-methyl-2-oxabicyclo[4.3.0]nonan-7-ol[1]).

Scholarein C (**3**) was obtained as colorless crystals. The HR-ESI-MS afforded a molecular formula $C_9H_{16}O_3$ (*m/z* 195.1002 ($[M+Na]^+$, $C_9H_{16}NaO_3^+$)). The IR spectrum showed absorptions for OH (3360) and CH_2 groups (2944 cm^{-1}). The ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*), HSQC, and HMBC, ^{1}H,^{1}H-COSY, and ROESY data (*Fig. 2*) and comparison with the data of boonein [8] established the structure of **3** as (1β,5β,7β,8β,9β)-8-methyl-2-oxabicyclo[4.3.0]nonane-1,7-diol[1]).

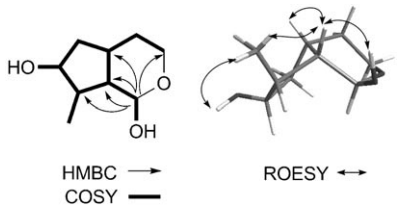


Fig. 2. *Selected HMBC, COSY, and ROESY correlations of* **3**

The ^{13}C-NMR and DEPT spectra of **3** displayed nine signals, corresponding to one Me, three CH_2, and five CH groups. Of these, three signals were assigned to oxygenated C-atoms, *i.e.*, δ(C) 93.6 (CH), 73.6 (CH), and 58.6 (CH_2). The ^{1}H-NMR spectrum showed the clear signal of a Me group (δ(H) 0.98 (*d*, J = 6.9 Hz)); four signals at δ(H) 3.36–3.39, 3.84–3.87, 4.13–4.17, and 4.89 were assigned to three oxygenated C-atoms, and the signals at δ(H) 3.43 and 4.92 were assigned to OH–C(7) and OH–C(1),

respectively, based on the HSQC and HMBC spectra. The COSY data afforded the connectivities $CH_2(3)-CH_2(4)-CH(5)-CH_2(6)-CH(7)-CH_2(8)-CH(9)-CH(1)-OH$, $CH(5)-CH(9)$, $CH(8)-Me(10)$, and $CH(7)-OH$. The above data indicated the presence of a bicyclic noriridoid skeleton, and its structure was very similar to that of boonein [8], except for the C(1)=O group of boonein which was replaced by a hemiacetal group (δ(H) 4.89; δ(C) 93.6) in **3**, as revealed by the HMBC cross-peaks C(1)/OH–C(1), H–C(9), $CH_2(3)$, H–C(5), and H–C(8). The NOE correlation H–C(5)/H–C(9) indicated a β *cis* relationship of these protons. The NOE correlation H–C(9)/OH–C(1) and a J(9,1) value of 4.0 Hz established the β-orientation of OH–C(1) [9], the NOE correlation H–C(9)/H–C(10) the β-orientation of Me–C(8), and the NOE correlation Me(10)/OH–C(7) the β-orientation of OH–C(7).

Scholarein D (**4**) has a molecular formula $C_{10}H_{18}O_3$ as determined on the basis of its HR-ESI-MS (m/z 209.1160 ($[M+Na]^+$, $C_{10}H_{18}NaO_3^+$)). The 1D- and 2D-NMR spectra (*Tables 1* and *2*) displayed similarities to those of **3**, except for one MeO group present in **4** (δ(H) 3.37; δ(C) 54.7). The key HMBC cross-peak δ(H) 3.37/δ(C) 99.4 indicated that the MeO group should be placed at C(1). Thus, scholarein D (**4**) was determined as (1β,5β,7β,8β,9β)-1-methoxy-8-methyl-2-oxabicyclo[4.3.0]nonan-7-ol[1]).

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical, Inc.*, China) and silica gel *RP-18* (20–40 µm; *Merck*). TLC: visualization by spraying with 10% H_2SO_4 in EtOH, followed by heating. Optical rotations: *Horiba-SEAP-300* spectropolarimeter. IR Spectra: *Tenor-27* spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-AM-400* and *-DRX-500* spectrometer; Me_4Si as internal standard; δ in ppm, J in Hz. MS: *VG-Autospec-3000* spectrometer (EI) and *API-Qstar-Pulsar 1* spectrometer (HR-ESI); in m/z.

Plant Material. The bark of *Alstonia scholaris* was collected in Simao, Yunnan Province, P. R. China, and identified by Dr. *Chun-Xia Zeng*. The voucher specimen (No. LUO20060407) has been deposited with the Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The dried bark (15 kg) of *Alstonia scholaris* was extracted three times with EtOH at r.t. After evaporation of EtOH, the viscous concentrate was partitioned with AcOEt (4 × 5 l) to afford an AcOEt and H_2O extract. The AcOEt fraction (190 g) was subjected to CC prepacked silica gel (2.1 kg), $CHCl_3/Me_2CO$ 1:0 → 1:1): *Fractions 1–5*. *Fr. 2* (19.3 g) was subjected to CC (silica gel (600 g), petroleum ether/Me_2CO 3:1): **5** (800 mg), **2** (28 mg), and **4** (7 mg). *Fr. 3* (23.3 g) was subjected to CC (silica gel, $CHCl_3/Me_2CO$ 4:1): *Fr. 3a–3c*. *Fr. 3b* (3.1 g) was purified by CC (*RP-18* (50%)): **1** (25 mg). *Fr. 3c* (4.3 g) was subjected to CC (silica gel, $CHCl_3/Me_2CO$ 2:1; then *RP-18* (40%)): **3** (60 mg). *Fr. 4* (16 g) was subjected to CC (silica gel, $CHCl_3$/MeOH 7:1): **6** (18 mg). The H_2O extract (170 g) was subjected to CC (silica gel (1.8 kg), $CHCl_3$/MeOH 9:1 → 1:1): *Fractions 6–9*. *Fr. 7* (17.7 g) was purified by CC (*RP-18* (300 g)): *Fr. 7a–7d*. Compound **7** (1.3 g) was crystallized from *Fr. 7b* (7.1 g).

Scholarein A (= rel-*(4a*R*,6*R*,7*S*,7a*R*)-Octahydro-7-methylcyclopenta[*c*]pyran-6-ol*; **1**): Colorless oil. $[\alpha]_D^{22} = +60.4$ ($c = 0.19$, MeOH). IR (KBr): 3386, 2929, 1630, 1454, 1052, 976. ^{1}H- and ^{13}C-NMR ($CDCl_3/CD_3OD$): *Tables 1* and *2*. EI-MS: 156, 141, 138, 125, 111, 97, 83, 67, 55. HR-ESI-MS: 157.1224 ($[M+H]^+$, $C_9H_{17}O_2^+$; calc. 157.1229).

Scholarein B (= rel-*(4a*R*,6*R*,7*R*,7a*R*)-Octahydro-7-methylcyclopenta[*c*]pyran-6-ol*; **2**): Colorless oil. $[\alpha]_D^{22} = +30.1$ ($c = 0.15$, MeOH). IR (KBr): 3375, 2924, 1637, 1458, 1057, 1029. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 1* and *2*. EI-MS: 156, 141, 138, 125, 111, 97, 83, 67, 55. HR-ESI-MS: 157.1218 ($[M+H]^+$, $C_9H_{17}O_2^+$; calc. 157.1229).

Scholarein C (= rel-*(1*R*,4a*S*,6*S*,7*R*,7a*S*)-Octahydro-7-methylcyclopenta[*c*]pyran-1,6-diol*; **3**): Colorless crystals. M.p. 103°. $[\alpha]^{22}_{D} = +10$ ($c = 0.51$, MeOH). IR (KBr): 3360, 2944, 1629, 1457, 1134, 984. ^{1}H- and ^{13}C-NMR (CD_3COCD_3): *Tables 1* and *2*. HR-ESI-MS: 195.1002 ($[M + Na]^+$, $C_9H_{16}NaO_3^+$; calc. 195.0997).

Scholarein D (= rel-*(1*R*,4a*S*,6*S*,7*R*,7a*S*)-Octahydro-1-methoxy-1-methylcyclopenta[*c*]pyran-6-ol*; **4**): Colorless oil. $[\alpha]^{22}_{D} = +12.1$ ($c = 0.45$, MeOH). IR (KBr): 3371, 2932, 1632, 1443, 1049, 983. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 1* and *2*. HR-ESI-MS: 209.1160 ($[M + Na]^+$, $C_{10}H_{18}NaO_3^+$; calc. 209.1154).

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