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Note

Alstonoside, a secoiridoid glucoside from *Alstonia scholaris*

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From the stems of *Alstonia scholaris*, a new secoiridoid glucoside, named alstonoside **1**, together with two isoflavone apioglucosides, formononetin 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside **2** and biochanin A 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside **3** are isolated and their structures are elucidated on the basis of detailed 1D and 2D-NMR and mass spectral data. Alstonoside features the presence of a ring opened monocyclic iridoid glucoside with the presence of an *O*-benzoyl moiety. This study constitutes the first report of a new secoiridoid glucoside and two isoflavone apioglucosides from this plant.

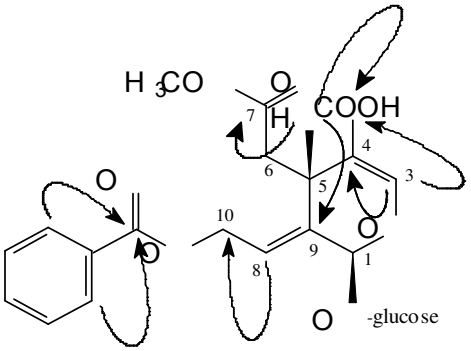
Keywords: *Alstonia scholaris,* Apocynaceae, alstonoside, secoiridoid glucoside, isoflavone apioglucosides

Alstonia scholaris (Apocynaceae Ver. name: Chattivan), is a common tree, growing upto 3.0 metre in height, distributed throughout the sub-Himalayan belt, West Bengal, Bihar, peninsular India and Southeast Asia. The bark, stem, roots and the leaves have been used traditionally as folk remedies for the treatment of many diseases including diarrhea, dysentery, malaria and snake bites[1,2]. During the last decade, *A. scholaris* has received much attention for the isolation of numerous mono and dimeric indole alkaloids, in general belonging to the picrinine class[3-6]; nevertheless not much work has been concentrated towards the isolation of non alkaloidal constituents. The chemical examination of the *n*-butanol residue of the hydroalcholic extract of the stems of *A. scholaris* is taken up. The isolation of a new secoiridoid glucoside alstonoside **1**, together with two known isoflavone apioglucosides, formononetin 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside **2** and biochanin A 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside **3** are reported (**Figure 1** and **Figure 2**). The isolation procedure and the preparation of pharmaceutical salts of alstonoside **1** has been the subject of a recent patent application[7]. The present study constitutes the first report of a new secoiridoid and two isoflavone apioglucosides from this plant.

Results and Discussion

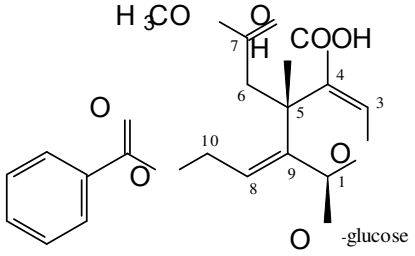
The *n*-butanol fraction of the hydroalcholic extract of the dried stems (1.5 kg) of *A. scholaris* was subjected to normal phase silica-gel chromatography to afford **1** (2.46 g) as colourless needles with $[\alpha]^{25}_{D}$ -175.98° (*c* =0.1, MeOH). The molecular formula of alstonoside **1** was determined to be $C_{24}H_{28}O_{13}$ by positive-ion –mass spectra $[M+H]^+$ *m*/*z* 525 as well as from its ^{13}C and DEPT (distortionless enhancement by polarization transfer) NMR data. The NMR spectrum of **1** (**Table I**) displayed a singlet for H-1 proton at δ 6.01 (δ_C 94.2), a characteristic H-3 signal for iridoids at 7.55 (δ_C 154.9, ref. 8,9) system with a one-proton double doublet at 4.12 (*J* = 3.7, 10.0 Hz) typical for H-5 methine exhibiting strong COSY (homonuclear correlation spectroscopy) cross peaks with a diastereopic methylene, H_2 [δ 2.56 (*J* = 10.0, 15.3 Hz) and δ 2.90 (*J* = 3.7, 15.3 Hz)] located at C-6, a triplet at δ 6.25 (*J* = 6.5 Hz) for a H-8 olefinic methine coupled to an oxymethylene, H_2-10 (δ 5.11). In addition, the NMR spectrum revealed resonances corresponding to a two-proton double doublet at δ 8.02 (2H), a one-proton triplet at 7.60 (1H) and two double doublets at 7.46 (1H) and 7.48 (1H) together with the corresponding carbon resonances, clearly indicative of an *O*-benzoyl moiety in **1.**

(**Table I,** ref 10**),** the ^{13}C NMR spectrum of **1** exhibited 24 carbon signals (**Table I**), with ten representing the aglycon, one methoxyl group (δ_C 52.2), an acid carbonyl (δ_C 169.6), seven carbons of an *O*-benzoyl moiety and six for the glucopyranose unit (δ_C 100.9, 77.9, 78.5, 71.4, 74.6 and 62.4). The *β*-configuration of the glucose moiety was determined from its large coupling constant that appeared as a doublet at δ 4.83 (*J*= 7.7 Hz) bound to δ 100.9 inferred from the HSQC (hetero nuclear single quantum coherence) experiment. The above spectral values suggested that compound **1** must be a monocyclic iridoid glucoside[11-14]. Evidence of ring opening at C-7 was observed from the COSY and HMBC (Heteronuclear multiple bond coherence) experiments. The H-5 methine showed ^3J (HMBC)

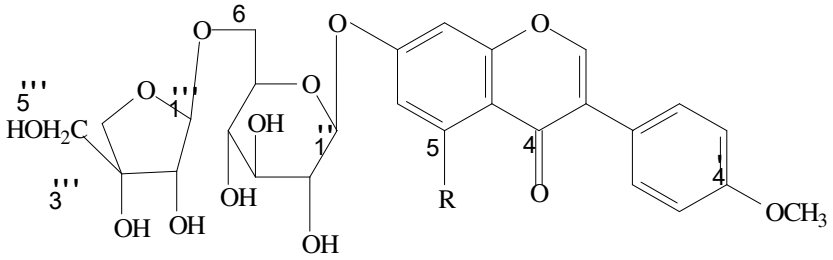


Key HMBC correlations of **1**

Figure 1



1



2 R=H
3 R=OH

Figure 2

correlation with the ester carbonyl at C-7 (δ_C 173.4) and a ^2J couplings with the methylene carbon at C-6 and the quaternary carbon at C-9 (δ_C 131.5) requiring the substitution of the methyl ester on C-5, while the H-8 methine exhibited ^2J (HMBC) correlations with the quaternary carbon at C-9 (δ_C 131.5), the oxymethylene at C-10 (δ_C 62.6) and ^3J couplings with the methine carbon at C-1 (δ_C 94.2) suggesting the placement of the *O*-benzoyl moiety at C-10. The G-1 proton of the sugar showed ^3J correlation with the methine carbon at δ_C 94.2 linking the sugar to C-1 of the pyran ring, while the carbonyl group at C-7 was esterified. The stereochemistry at C-1 and C-5 were determined from strong NOE interactions that were observed between H-1α/H-6α and H-5β/H-6β, further supported by the coupling constants between H-6α, H-6β and the H-5 methine (see **Table I**). Based on the above findings, the configuration at C-5 was considered to be *R* and on biosynthetic grounds, C-1 was also found to be *R* (with 1*α* and 5*β*-oriented H (ref. 8,12). Full assignments of the ^{1}H and ^{13}C NMR signals were accomplished using ^{1}H-^{1}H COSY, HSQC and HMBC experiments (**Table I**). Consequently, compound **1** was characterized as alstonoside, a new secoiridoid glucoside. This is the first report of an iridoid isolated from this plant.

Two isoflavone apioglucosides **2** and **3** were isolated and characterized by 1D and 2D NMR and mass

Table I — NMR Assignments for (in MeOH-d_4 , 400/100 MHz) Alstonoside **1**

H/C	^{1}H	^{13}C †	COSY	HMBC
1α	6.01 s	94.2		C-1′, C-8, C-9
3	7.55 s	154.9		C-4, COOH, C-5
4		109.4		
5β	4.12 dd (3.7, 10.0)	32.5	H-6α,β	C-4, C-9, C-6
6	α) 2.56 dd (10.0,15.3) β) 2.90 dd (3.7, 15.3)	40.7		COOR, C-4, C-9
7		173.4		
8	6.25 br t (6.5)	124.1	H-10a	COO, C-9
9		131.5		
10	5.11 dd (6.5, 13.2)4.98 [b]	62.6	H-8	COO, C-9
11-COOH		169.6		
1′	4.83 d (7.7)	100.9	G-2′	C-1
2′	3.32 [b]	74.6		
3′	3.38 [b]	78.5		
4′	3.32 [b]	71.4		
5′	3.40[b]	77.9		
6′	3.68[c] 3.88 (1.5, 10.0)	62.4		
O-benzoyl				
1″		134.6		
2″/6″	8.02 dd (7.2, 1.3)	130.5 [a]	H-3″	COO
3″	7.46 dd (7.2, 1.2)	129.6 [a]		
4″	7.60 br t (7.6)	134.3	H-5	
5″	7.48 dd (7.6, 1.2)	129.6		
CO		167.8		
$COOCH_3$	3. 64, br s	52.2		CO

†Assignments were further confirmed by HSQC (^{13}C-^{1}H) heteronuclear coupling experiment. Coupling constants (*J*) parentheses. [a]Signals within a column are interchangeable.
[b]Overlapping with MeOH. [c]Signal pattern unclear due to overlapping.

spectral data. Compound **2** was identified as formononetin 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside[13], while **3** indicated to be a hydroxylated derivative of **2** having an additional mass of 16 units. The spectral data for **3** was found to be consistent with the reported literature data for biochanin A 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside[14,15].

Conclusion

Monomeric and dimeric indole alkaloids are characteristic of *Alstonia* and are also known to be widely distributed throughout the Apocynaceae members that include *Rauwolfia, Hunteria* and *Vinca*[3-5,16-19]. Although, iridoids and flavonoids are rarely encountered in *Alstonia*, limited reports of iridoids and flavonoids within the Apocynaceae family are known and include, *Rauwolfia, Plumeria* and *Cerbera*[20-24]. Alstonoside **1** belongs to the secoiridoid class that is commonly encountered in the Oleaceae[8]. It is interesting to note that previous work on the roots afforded a flavanone glycoside, 8,3′,4′-trihydroxy flavanone-7-*O*-α-L-rhamnopyranoside[25], but the present study constitutes the first report of an iridoid and two isoflavone apioglucosides from the stems of *A. scholaris*.

Experimental Section

Silica-gel 100-200 mesh (Merck) was used for column chromatography. Thin Layer Chromatography was carried out in silica-gel 60 F_{254} pre-coated

aluminum plates (0.2 mm, Merck).: UV detection was carried at 254 and 366 nm using Camag UV chamber and spraying with 5% MeOH-H_2SO_4, followed by heating at 105ºC for 1–2 min. Analytical HPLC-UV analysis were performed on WATERS 2496 (separations module) HPLC system with a WATERS 2996 photodiode array detector (PDA) and a RPC-18 column (150 mm × 4.6 mm), 5µ (Phenomenex). Mass spectra were obtained using a WATERS 2496 separations module- LC system with a MS-MSD Quattro micro in ESI mode (Multi mode ionization). NMR: ^{1}H NMR, ^{13}C NMR, DEPT-135, ^{1}H–^{1}H COSY, ^{13}C–^{1}H HSQC and ^{13}C–^{1}H HMBC spectra were taken in Avance Bruker DRX 400 spectrometer. Chemical shifts δ were given in ppm and coupling constants *J* in Hz. The spectra were measured in methanol-d_4 for the iridoid glycoside 1 and DMSO-d_6 for the isoflavone glycosides **2** and **3**.

Plant Material: The stems of *A. scholaris* were collected at Madhya pradesh, India, in June 2006. A voucher specimen (ALS/06/332) has been deposited at the Herbarium, Herbal drug Research, Ranbaxy Research Labs, Research and Development-II, Gurgaon, Haryana, India.

Extraction and Isolation: Air-dried stems (1.5 Kg) were extracted twice at room temperature with MeOH: H_2O (1:1; 2 × 1500 mL) for 24 hr. The combined extracts were concentrated in *vacuo* to yield a brown residue (67.2 g), which was partitioned with chloroform and subsequently with *n*-butanol. The chloroform residue obtained after concentration under *vacuo* was left aside. Successive extractions with *n*-butanol (2 × 500 mL) afforded after evaporation in *vacuo*, a brown residue (44 g). The *n*-butanol residue (30 g) was divided into twenty four fractions (A-X) by normal phase silica-gel chromatography, eluting with pure chloroform and gradually increasing polarity by addition of methanol. Based on their respective TLC profiles, fractions were pooled and concentrated. Fraction Q-S were found to be similar and hence the crude mixture after crystallization with aqueous methanol yielded colourless crystals of **1** (2.46 g). Similarly, fraction U constituted an amorphous solid (2 g) containing atleast two compounds on TLC, which was further purified by column chromatography using isocratic elution with $CHCl_3$: MeOH: H_2O (7:3:0.5) to give pure **2** and **3** in appreciable yields (0.032 g and 0.018 g). All three isolated compounds **1-3** were analyzed for their purity in RP-HPLC (RP C-18 column, 250 mm × 4.60 mm, 5µ (Phenomenex). The elution profile consisted of a linear gradient, 15-90 % v/v acetonitrile in 0.05% v/v aqueous formic acid over 35 min (flow rate: 1.0 mL/min; sample injection volume was 10 µL, UV detection at 232 nm). Compound **1** had a retention time: 13.18 min, Compound **2,** retention time: 12.81 min and Compound **3,** retention time: 13.74 min.

Alstonoside 1: colourless needles from MeOH; m.p. 125-26 °C; $[\alpha]^{25}_D$ -175.98° (*c* =0.1, MeOH), R_f :0.45 in $CHCl_3$: MeOH: H_2O (6.5:3:0.5); UV λ_{max} nm (log ε): 201 (2.45), 232 (3.88); LC-ESIMS $[M+H]^+$ *m/z* 525; ^{1}H and ^{13}C NMR (methanol-d_4) spectral data: see **Table I**.

Formononetin 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside 2: Colourless crystalline powder from MeOH; R_f :0.55 in $CHCl_3$: MeOH: H_2O (6.5:3:0.5); UV λ_{max} nm (log ε): 256 (3.42), 381 (sh); LC-ESIMS $[M+H]^+$ *m/z* 563; ^{1}H and ^{13}C NMR spectral data in agreement with literature values[13].

Biochanin A 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside 3: Colourless crystalline powder from MeOH; R_f :0.58 in $CHCl_3$: MeOH: H_2O (6.5:3:0.5); UV λ_{max} nm (log ε): 261 (3.42), 381 (sh); LC-ESIMS $[M+H]^+$ *m/z* 579; ^{1}H and ^{13}C NMR spectral data in agreement with literature values[14,15].

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