Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(12):1394-1397

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Rare cyano glucosides from *Coldenia procumbens* Linn.

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ABSTRACT

The objective of the work was to isolate active phytoconstituents present in the herb Coldenia procumbens. Phytochemical investigation of the aerial parts of the plant yielded rare Nitrile glucosides, Ehretioside A1 ***1*** *and Lithospermoside (Griflonin)* ***2****. The structures of the compounds were established on the basis of various NMR and Mass spectral data. The cyano-containing compounds* ***1*** *and* ***2*** *were isolated for the first time from C. procumbens.*

Key words: *Coldenia procumbens*, Ehretioside A1, Lithospermoside, Ehretioside A1 hexa acetate, Boraginaceae.

INTRODUCTION

Coldenia procumbens Linn. (Family: Boraginaceae) is a small, annual, prostrate herb often found in seasonally flooded locations and it is a common weed [1]. It is found throughout in India, Srilanka and in other tropical countries. In Asia, it has been reported from India, Srilanka, Myanmar, China, Taiwan etc. The plant is used in Indian system of medicine namely Ayurveda and Sidha. It is widely used as an anti-diabetic [2], anti-inflammatory [3], antimicrobial [4], anthelmintic [5], analgesic [6] and CNS depressant [7]. The antihepatotoxic Wedelolactone [8] was isolated from this plant. In continuation to our phytochemical exploration of medicinal plants of Deccan region [9], we report the isolation and identification of rare cyanoglucosides **1** and **2** from the aerial part of the *C. procumbens* for the first time.

EXPERIMENTAL SECTION

2.1: General experimental procedures

IR spectra were recorded on a Perkine Elmer FT-IR 240-C spectrometer using KBr optics. NMR spectra were recorded on Burker Avance 300 MHz in $CDCl_3$ and Pyridine-*d5* using TMS as internal standard. Electron impact (EI) and chemical ionization mass spectra were recorded on a VG Micromass model 7070H instrument. All the fractions were monitored on silica gel precoated TLC plates of Merck and spots were visualization observed under UV (254 nm) and also by Anisaldehyde-sulphuric acid spray reagent heating at 110^0C. Silica gel (100-200 mesh) used for column chromatography was procured from Merck.

2.2: Plant material

The aerial part of the plant was collected from Mylaram village, Nalgonda district of Andhra Pradesh, India in November-2011 and was identified by taxonomist, Prof. V.S. Raju of Kakatiya University, Warangal, Andhra Pradesh, India. A voucher specimen was deposited at the CIMAP-Research Centre, Hyderabad, India under the accession number CP-AP-1/2011.

2.3: Extraction and Isolation

The aerial parts of *C. procumbens* freed from earthy material, shade dried and powdered. The powdered plant material (1.2 kg) was extracted with Hexane and Methanol using hot percolation method. The extracts were evaporated at reduced pressures. The resulting dry methanol extract (70 gm) was defatted with hexane and then subjected to column chromatography over silica gel (100-200 mesh). The column was eluted successively with hexane, chloroform, ethyl acetate and acetone solvents. Identical ethyl acetate washings were combined on the basis of TLC (silica gel) and evaporated *in vacuo* to afford a brownish residue (5 gm). The residue was further column chromatographed over silica gel (100-200 mesh) and eluted with solvents chloroform and increasing amounts of methanol in chloroform. The fractions collected in chloroform: methanol (80:20) gave a pure compound (150 mg) **1** as a colorless amorphous powder. Likewise, the acetone washings were also clubbed and concentrated to get colorless crude (3 g) which was column chromatographed over silica gel (100-200 mesh) and eluted with solvents acetone and increasing amounts of methanol in acetone. The fractions collected in acetone: methanol (80:20) gave a pure compound (100 mg) **2** as a colorless amorphous powder. The structure of the compound **1** and **2** were elucidated on the basis of IR, UV, $^{1}H/^{13}C$/2D-NMR, and Mass spectra.

2.4: Acetylation of Compund-1

Ehretioside A1 (10 mg) was dissolved in pyridine and to this acetic anhydride was added slowly while stirring at room temperature under nitrogen atmosphere for 12 h. Later, the solvent was removed under reduced pressure and the residue was diluted with distilled water and extracted thrice with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to get the final product. The crude was purified by column chromatography with ethyl acetate in hexane to give a pure compound (5 mg) **3** as a colorless amorphous powder.

RESULTS AND DISCUSSION

3.1: Structure elucidation of compound 1: It is identified was Ehretioside A1

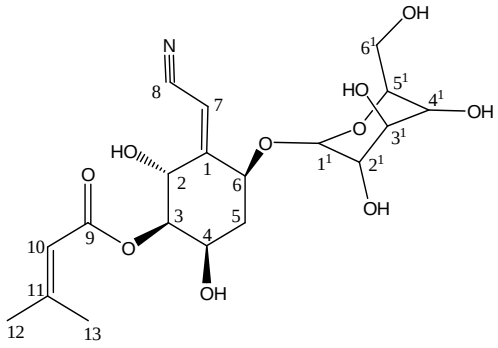
The structure of the isolated compound 1 was elucidated on the basis of IR, $^{1}H/^{13}C$/2D-NMR, and Mass spectra as Ehretioside A1. ^{1}H and ^{13}C NMR spectra indicated the presence of β-D-glucopyranose by signals at δ 5.07 (d, J-7.8 Hz, H1′) and δ104.04 (C1′). The molecular ion peak in ESI-MS spectrum at m/z 268 $(M+H\text{-}Glu)^+$ indicated the molecular formula $C_{13}H_{17}NO_5$ for the aglycon. The ^{1}H-NMR spectrum displayed an olefinic proton (H-10) at δ5.57 (t, J= 1.2 Hz) coupled with protons of two methyl groups at 1.57 and 2.08 (each 3H, d, J= 1.1 Hz) indicating a senecioic acid ester moiety. This was further confirmed by the signals at δ 117.9 and 158.3 for C-10 and C-11 respectively in the ^{13}C-NMR spectrum. Ehretioside A1 showed the methine signals of cyanomethylene group at (δ_H 6.29 (d, J = 1.8 Hz), δ_C 97.00 ppm) and a quaternary carbon (δ_C 167.33 ppm) (Table-1). The ESI-MS of Ehretioside A1 gave $(M+Na)^+$ peak at m/z 452 corresponding to the molecular formula $C_{19}H_{27}NO_{10}Na$. The IR (KBr) ν_{max} spectrum showed absorption at 3473 (OH), 3416, 3234, 2914, 2228 (CN) and 1690 (CO), 1643, 1430, 1371, 1239, 1163, 1072 cm^{-1}. The UV spectrum showed absorption at λ max: 232 nm.

3.2: Structure elucidation of compound 2: It is identified as Lithospermoside (Griflonin)

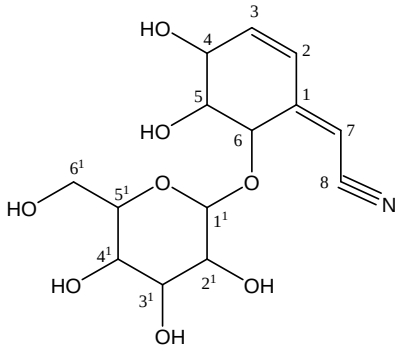
The structure of the isolated compound 2 was elucidated on the basis of IR, $^{1}H/^{13}C$/2D-NMR, and Mass spectra as Lithospermoside (2). The presence of a peak at 105.68 is typical for anomeric carbons of glucosides (C-1^1), additional peaks at 79.78 79.72, 76.33, 72.74, and 64.42 ppm support the idea that the compound contains b-glucopyranose moiety. The peak at 119.1 ppm can be assigned to a nitrile carbon. In addition to these seven carbons, there are peaks at 72.74, 77.1 and 78.7 (carbons bearing oxygen, C-4, 5, 6), and 98.49, 127.85, 140.84, and 157.52 ppm (aromatic or vinyl carbons, C-7, 2, 3, 1). A DEPT spectrum reveals that the peaks at 157.52 (C-1) and 119.1 (C-8) ppm lack protons, and all other carbons bear one hydrogen. The ^{1}H NMR spectrum confirms the presence of a glucosyl group in the compound by the presence of a doublet at 5.64 ppm (H-1^1), a series of double doublet-like signals centered at 3.97, 4.23, 4.20, 4.34, 4.55 and a ddd centered at 4.37 ppm (Table -1) (H-5^1, 3^1, 4^1, 6^1b, 6^1a, 2^1). The patterns of all other protons in the molecule are more complex. Two vinyl protans appear at 6.23 (H- 2) and 6.36 (H-3) ppm, respectively; an additional vinyl signals found at 5.62 (H-7) ppm. Three protons are on carbons bearing oxygen: at 4.69, 4.48 and 5.26 (H-4, 5, 6) ppm; one of these must be the site of attachment of the glucosyl group. The ESI-MS of Lithospermoside (2) gave $(MS)^+$ peak at m/z 330 corresponding to the molecular formula $C_{14}H_{19}NO_8$. The IR (KBr) ν_{max} spectrum showed absorption at 3449 (OH), 2916, 2223 (CN), 639, 1381, 1189, 1110 cm^{-1}.

3.2: Structure elucidation of compound 3: It is identified as Ehretioside A1 hexa acetate

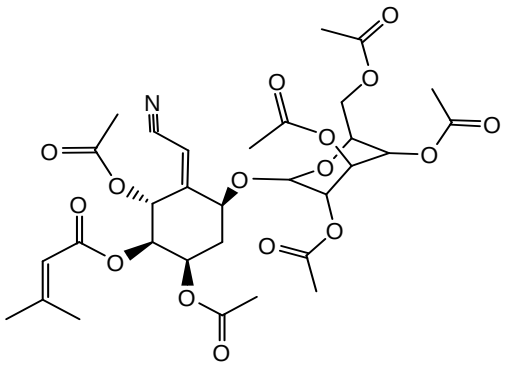
The structure of the acetate derivative **3** was elucidated on the basis of ^{1}H-NMR as Ehretioside A1 hexa acetate. The spectrum displayed a bunch of six methyl signals resonating between δ1.92 to δ2.15 ppm, integrating for 18 protons, indicates the presence of six hydroxyls in the parent compound **1**.



1 Eheretioside A1



2 Lithospermoside (Griflonin)



3 Ehretioside A1 hexa acetate

Table-1: ^{1}H (300MHz) and ^{13}C (75MHz) NMR data of Ehretioside A1 and Lithospermoside in pyridine-d_5

Position	Ehretioside A1				Lithospermoside			
	^{1}H ppm	^{13}C NMR	Nature of carbon	HMBC	^{1}H ppm	^{13}C NMR	Nature of carbon	HMBC
1	---	166.33	Q	---	---	157.52	--Q--	6.23, 6.36, 4.48, 5.26, 5.62
2	5.72	69.20	CH	4.69	6.23	127.85	CH	5.26, 5.62
3	5.22	80.29	CH	5.72	6.36	140.84	CH	6.23
4	4.69	69.04	CH	6.29, 5.57, 5.22	4.69	72.74	CH	6.23
5	1.90, 2.72	35.65	CH_2		4.48	77.11	CH	5.26
6	5.56	77.20	CH	5.07, 6.29	5.26	78.70	CH	5.64, 6.23, 5.62
7	6.29	97.00	CH	5.72, 5.57	5.62	98.49	CH	6.23, 5.26
8	---	118.31	Q	6.29, 5.72	---	117.80	Q	5.26, 5.62
9	---	167.33	Q	5.23, 2.08, 1.57	---	---	---	---
10	5.57	117.91	CH	2.08, 1.57	---	---	---	---
11	---	158.35	Q	2.08, 1.57	---	---	---	---
12	1.57	28.22	CH_3	2.08, 5.57	---	---	---	---
13	2.08	21.23	CH_3	1.57, 5.57	---	---	---	---
1′	5.07	104.04	CH	5.57, 4.01	5.64	105.68	CH	---
2′	4.01	76.40	CH	4.48, 4.23	4.37	76.33	CH	---
3′	4.23	79.70	CH	4.23, 5.07	4.23	79.78	CH	---
4′	4.23	72.70	CH	4.48, 4.23	4.20	72.79	CH	---
5′	3.95	79.90	CH	4.01	3.97	79.72	CH	---
6′	4.48, 4.33	64.05	CH_2	4.23	4.55, 4.34	64.42	CH_2	---

CONCLUSION

Present phytochemical investigation of *C. procumbens* led to the isolation of Ehretioside A1 and Lithospermoside. Ehretioside A1 was earlier reported from *Ehretia dentate* [10] and *Ehretia philippinensis* [11]. Lithospermoside was reported from *Cercis chinensis* [12] and *Semiaquilegia adoxoides* [13]. Thus, occurrence of Ehretioside A1 and Lithospermoside in the genus *Coldenia* of Boraginaceae implicates its usefulness as a chemotaxonomic marker within this family.

Acknowledgment
Authors thank the Director, CIMAP, Lucknow, India for his constant encouragement and support.

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