A xanthone glycoside from aerial parts of *Swertia paniculata*

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**Abstract** Column chromatography of purified butanol extract obtained after fractionation from aqueous methanolic extract of aerial parts of *Swertia paniculata* afforded one xanthone glycoside (1,5-dihydroxy-3-methoxyxanthone-8-O-β-D-glucopyranoside). Structure of this compound was elucidated using spectroscopic techniques. This is the first report of isolation of this compound from *S. paniculata*.

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1. Introduction

*Swertia paniculata* WALL (D. Don), family Gentianaceae, is widely distributed throughout the temperate region of the Western Himalayas at altitudes of 5000–8000 ft, from Kashmir to Nepal and in the Lushai hills of Mizoram at altitudes of 1500–2400 m. It is used in the Indian system of medicine (ISM) as a bitter tonic and in the treatment of certain mental disorders, such as melancholia (Chopra et al., 1956).

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2. Material and methods

2.1. General

Melting point was determined on electro thermal (UK-made) apparatus on centigrade scale and was uncorrected. Ultra-violet absorption spectrum was recorded on Perkin-Elmer Lambda Bio 20 UV spectrophotometer. Infrared spectrum was recorded on Perkin-Elmer 1710 infrared Fourier Transform spectrometer. $^1$H and $^{13}$C NMR were recorded on a Bruker AVANCE DRX-300 (300 and 75 MHz) equipped with 5 mm inverse multinuclear probe head. Tetramethyl silane (TMS) was used as an internal standard and chemical shift values, coupling constant ($J$) in Hertz. EIMS, electron impact mass spectra, were recorded at 70 eV on Micromass Quattro II triple quadrapole mass spectrometer. ESMS, electron impact mass spectra, were recorded at 70 eV on JEOL-JMS D-100 spectrometer. Column chromatography (PC) was carried out on Whatman paper (4:1:5, v/v/v), developed by spraying with silver nitrate solution.

2.2. Plant material

*S. paniculata* Wall (D. Don), family Gentianaceae, aerial parts were collected from Bharmour (Chamba) Palampur, Himachal Pradesh (9000 ft height) and identified by Dr. S. P. Jain, Scientist, Botany and Pharmacognosy Division, Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, where a voucher specimen No. 11470 has been retained.

2.3. Extraction and isolation

The air-dried and powdered aerial parts (5 Kg) of *S. paniculata* were extracted with aqueous methanol (20%) to yield aqueous methanolic extract, which was filtered and the filtrate was concentrated up to one liter and then fractionated with n-hexane, chloroform, ethyl acetate and butanol (Ahmad et al., 2004). The n-butanol extract (46.38 g) thus obtained was further fractionated to purify the extract. In the n-butanol extract (44 g), water was added (70 ml) and then extracted with chloroform (30 ml x 3) to yield chloroform extract (4.69 g). The remaining water was concentrated to half and then fractionated with acetonitrile by adding salt (NaCl) to yield acetonitrile extract (16 g) and water extract (32.73 g). Column chromatography of acetonitrile extract (16 g) was packed in chloroform and elution was carried out with increasing polarity of chloroform–methanol (94:6, 92:8, 90:10, 85:15, 80:20, v/v). Similar fractions were pooled together on the basis of TLC behavior. One major compound was isolated from fraction number 30–35, while eluting the column with chloroform–methanol (85:15) as yellow powder. Compound 1 is isolated for the first time from *S. paniculata*.

2.4. 1,5-Dihydroxy-3-methoxyxanthone-8-O-β-D-glucopyranoside (I)

Yellow powder (32.6 mg, 0.000652%, w/w), m.p. 194–196 °C, C$_{20}$H$_{20}$O$_{11}$ (M$^+$ 436), $R_f$ 0.32 (CHCl$_3$–MeOH, 88:12, v/v), IR $\nu_{\text{max}}$ (KBr): 3410 (OH), 2400 (C–H), 1740 (C=O), 1700, 1650, 1560 cm$^{-1}$. UV $\lambda_{\text{max}}$ (EtOH): 224, 253, 276, 325 nm. $^1$H NMR (300 MHz, Py-$d_5$): $\delta$ 3.63 (3H, s, OCH$_3$), 4.16–4.66 (6H, m, sugar protons), 5.47 (1H, d, $J$ 7.2 Hz, H-1'), 6.20 (1H, d, $J$ 2.4 Hz, H-2'), 6.48 (1H, d, $J$ 2.4 Hz, H-4'), 7.38 (1H, d, $J$ 8.7 Hz, H-6), 7.60 (1H, d, $J$ 8.7 Hz, H-7). $^{13}$C NMR (75 MHz, Py-$d_5$): 164.2 (C-1), 98.0 (C-2), 167.1 (C-3), 157.5 (C-4a), 146.5 (C-4b), 113.4 (C-5), 151.1 (C-6), 113.9 (C-8a), 106.0 (C-8b), 182.5 (C-9), 56.0 (OCH$_3$). Glc moiety: 104.9 (C-1'), 75.5 (C-2'), 78.0 (C-3'), 71.5 (C-4'), 79.6 (C-5'), 62.8 (C-6'). ESMS $m/z$: 459.2 (M + Na)$^+$, 437.1 (M + H)$^+$, 436 (M)$^+$, C$_{20}$H$_{20}$O$_{11}$, MS $m/z$: 274 (M–glucose unit)$^+$, C$_{14}$H$_{10}$O$_{6}$ (100), 245 (M–29)$^+$ (21), 231 (M–43)$^+$ (12), 217 (M–55)$^+$ (25), 204 (14), 153 (12), 138 (17), 123, 73, 69, 43.

2.5. Acidic hydrolysis of I

Isolated xanthone glycoside (6.0 mg) was refluxed with 15% methanolic HCl (5 mL) for 4 h, on completion of the reaction, reaction mixture was diluted with water and extracted with chloroform (15 mL x 4) and dried over anhydrous sodium sulphate. Solvent was evaporated and recrystallized in chloroform to furnish the aglycon, m.p. 265–267 °C. UV $\lambda_{\text{max}}$ (EtOH): 227, 239, 253, 274, 294, 334 nm; EIMS: $m/z$ 274 [M]$^+$ correspond to molecular formula C$_{14}$H$_{10}$O$_{6}$. Aqueous mother liquor was neutralized with BaCO$_3$ and filtered. The filtrate was concentrated and checked for sugar on paper chromatography (PC) with the authentic sample and was identified as α-glucose.

3. Results and discussion

Compound 1 was obtained as a yellow powder from chloroform–methanol (85:15) fractions, m.p. 194–196 °C. The IR spectrum showed the presence of hydroxyl group (3410 cm$^{-1}$) and a carbonyl group (1740 cm$^{-1}$). The mass peak at $m/z$ 436 in ESMS corresponds to the molecular formula C$_{20}$H$_{20}$O$_{11}$.

A singlet at $\delta$ 3.63 in $^1$H NMR was assigned for the methoxy protons. The insolubility of compound in 5% aqueous
sodium carbonate located the methoxy group at C-3 position (Ghosal et al., 1973). A multiplet at $\delta$ 4.16-4.66 was assigned for six sugar protons. Two doublets at $\delta$ 6.20 ($J_{2,4}$ 2.4 Hz) and $\delta$ 6.48 ($J_{2,4}$ 2.4 Hz) were assigned for meta-coupled protons present at H-2 and H-4, respectively. The other two doublets were assigned for ortho-coupled protons present at $\delta$ 7.38 ($J_{7,8}$ 8.7 Hz) and $\delta$ 7.60 ($J_{7,8}$ 8.7 Hz). The $\beta$-linkage of glucose was confirmed by the coupling constant of the anomeric proton appearing at $\delta$ 5.47 ($J_{2,3}$ 7.2 Hz).

The $^{13}$C NMR showed a set of signals ($\delta$ 62.8, 71.5, 75.5, 78.0, 79.6 and 104.9 ppm) indicating for the presence of glucose unit. The presence of glucose moiety was confirmed by the acidic hydrolysis of the compound, which yielded $\alpha$-glucose identified by co-paper chromatography with the authentic sample. The aglycon part, m.p. 265-267 °C. MS: $m/z$ 274 [M$^+$,C$_{14}$H$_{10}$O$_6$] was identified as 1,5,8-trihydroxy-3-methoxy xanthone (I), which was confirmed by comparison with the authentic sample (Chhakravarty et al., 1994; Ghosal et al., 1973).

Thus, on the basis of above evidences and on comparison with reported data (Ishimaru et al., 1990; Sakamoto et al., 1982; Kaldas et al., 1974) compound I was established as 1,5-dihydroxy-3-methoxy xanthone-8-O-$\beta$-D-glucopyranoside (also known as bellidifolin-8-O-$\beta$-D-glucopyranoside), which is commonly known as swertianolin (trivial name) and was isolated from different sources (Wang et al., 2010; Menkovic et al., 2002) but this is the first report of isolation and characterization of the same from *Swertia* paniculata. Studies reported that some xanthenes isolated from *Gentiana compestris* and *Gentiana amurelle* ssp. acuta possessed Acetylcholinesterase (AChE) and monoamine oxidase (MAO) inhibitory activities, in which, swertianolin, the 8-O-glucopyranoside form of bellidifolin, gave 93.6% inhibition of MAO-B activity at 10$^{-5}$ M (Urbain et al., 2004, 2008). Monoamines oxidases (MAO) are a family of enzymes that catalyze the oxidation of monoamines. They are found bound to the outer membrane of mitochondria in most cell types in the body. Monoaminergic pathways are highly responsive to aversive stimuli and play a crucial role in the control of affect, cognition, endocrine secretion, chronobiotic rhythms, appetite, and motor function, all of which are profoundly disrupted in depressive states. Monoamine oxidase inhibitors (MAOIs) are a class of antidepressant drugs prescribed for the treatment of depression. They are particularly effective in treating atypical depression. MAOIs act by inhibiting the activity of monoamine oxidase, thus preventing the breakdown of monoamine neurotransmitters and thereby increasing their availability. There are two isoforms of monoamine oxidase, monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B). MAO-A preferentially deaminates serotonin, melatonin, epinephrine and norepinephrine. MAO-B preferentially deaminates phenylethylamine and trace amines. Dopamine is equally deaminated by both types and MAO-B acts in the brain to degrade dopamine. Thus, inhibiting MAO-B is a therapeutic strategy for the treatment of parkinson’s disease. As from the reports of Urbain and coworkers (Urbain et al., 2004, 2008) swertianolin (1,5-dihydroxy-3-methoxy xanthone-8-O-$\beta$-D-glucopyranoside) can be utilized in the preparation of drugs for the treatment of depression. We have got only 0.000652% of swertianolin during this study while Zhang and Yang (1994) isolated 0.77% from Tibetan medicinal plant *Gentiana azuro* and Inayat-ur-Rahman et al. (2000) isolated 0.125% (all w/w) of the same from *Swertia ciliata*. It suggests that for utilization of swertianolin in the treatment of depression at commercial scale, *G. azuro* may be the choice as pharmaceutical crop.

### 4. Conclusion

Finally, based on the above discussion, structure of compound I was assigned as 1,5-dihydroxy-3-methoxy xanthone-8-O-$\beta$-D-glucopyranoside (swertianolin) and this is the first isolation report of the same from *S. paniculata*.

### Conflict of Interest

None.

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### References


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