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TWO XANTHONE GLUCOSIDES FROM *HALENIA ELLIPTICA**

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Key Word Index—*Halenia elliptica*; Gentianaceae; pentaoxygenated xanthones; tetraoxygenated xanthones, xanthone glucosides, amoebicidal activity

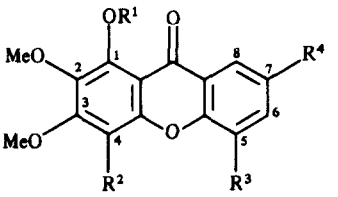
Abstract—Two new xanthone-*O*-glucosides, 2,3,7-trimethoxyxanthone-1-*O*-glucoside and 2,3,5-trimethoxyxanthone-1-*O*-glucoside were isolated from whole plant of *Halenia elliptica* and identified through their acetates. Also present are four free xanthones, 1-hydroxy-2,3,4,7-tetramethoxy, 1-hydroxy-2,3,4,5-tetramethoxy, 1-hydroxy-2,3,7-trimethoxy, and 1-hydroxy-2,3,5-trimethoxyxanthone, oleanolic acid and sitosterol glucoside. A fraction rich in xanthone glucosides showed marked antiamoebic activity.

A number of dihydroxypolymethoxyxanthones have been isolated from the leaves of *Halenia elliptica* D. Don. of Chinese origin [1], which were shown to possess marked hepatoprotective activity [2]. Besides this, three monohydroxypolymethoxyxanthones have also been isolated from other species of *Halenia* [3, 4]. Under a programme of screening Indian medicinal plants for wide range of biological activity at our Institute, the alcoholic extract of the whole plant of *H. elliptica* showed amoebicidal activity [5]. Present communication deals with the structure elucidation of two new xanthone-*O*-glucosides (**1**) and (**2**).

The antiamoebic activity was localized in greenish yellow solid matter deposited from an alcoholic plant extract as well as in chloroform-soluble fraction. Column chromatography of both the fractions, separately, yielded four known xanthones identified as 1-hydroxy-2,3,4-7-tetramethoxy- (**3**), 1-hydroxy-2,3,4,5-tetramethoxy- (**4**), 1-hydroxy-2,3,7-trimethoxy- (**5**) and 1-hydroxy-2,3,5-trimethoxyxanthone (**6**) by comparing the spectral data (UV, IR, ^{1}H NMR and MS) with literature [6–8] along with oleanolic acid and β-D-glucoside of sitosterol all isolated for the first time from *H elliptica*.

Besides the above compounds, the chloroform soluble fraction yielded an amorphous compound, which was a mixture of at least two glucosides. This was further purified through acetylation (pyridine–Ac_2O) followed by column chromatography to yield **1a** and **2a**

The UV spectrum of **1a** was similar to that of a 1,2,3,7-tetraoxygenated xanthone [6] The IR spectrum of **1a** showed ν_{max} 1750, 1280 (acetyl carbonyl), 1650 cm^{-1} (xanthone carbonyl). The ^{1}H NMR spectrum of **1a** showed the presence of four acetyl methyl protons signals between δ1.9 to 2.1, a two proton singlet at δ3.55 for C-6′ protons of acetylglucose moiety; three methoxy protons signals between δ3.80 to 3 95 and one anomeric proton of glucose moiety as doublet at δ5.4 (J, 7 Hz) indicating β-D-glucosyl linkage. The rest of the protons of the glucosyl



1 R¹ = Glc, R² = R³ = H, R⁴ = OMe
1a R¹ = Glc(Ac)₄, R² = R³ = H, R⁴ = OMe
2 R¹ = Glc, R² = R⁴ = H, R³ = OMe
2a R¹ = Glc(Ac)₄, R² = R⁴ = H, R³ = OMe
3 R¹ = R³ = H, R² = R⁴ = OMe
4 R¹ = R⁴ = H, R² = R³ = OMe
5 R¹ = R² = R³ = H, R⁴ = OMe
6 R¹ = R² = R⁴ = H, R³ = OMe

moiety appeared as multiplets centred at δ4.6. In the aromatic region, the signals at δ6.85 (1H, *s*), 7.15 (1H, *dd*, J=3 and 9 Hz; *meta* and *ortho* couplings), 7.25 (1H, *d*, J =9 Hz, *ortho* coupling) and δ7 60 ppm, (1H, *d*, J=3 Hz, *meta* coupling) were assigned to the H-4, H-6, H-5 and H-8 protons of the xanthone nucleus CIMS data of **1a** showed two peaks at *m/z* 331 (10%) and 302 (100%) which corresponded to acetylated glucose and aglycone ions respectively. On hydrolysis with 6% hydrochloric acid **1a** gave 1-hydroxy-2,3,7-trimethoxyxanthone (**5**). Thus the original glycoside **1** is 1-hydroxy-2,3,7-trimethoxyxanthone 1-*O*-β-D-glucoside (**1**).

The UV spectrum of **2a** (see Experimental) was similar to that of 1,2,3,5-tetraoxygenated xanthones [6] The IR spectrum of **2a** showed ν_{max} 1745, 1250 (acetyl carbonyl), 1650 cm^{-1} (xanthone carbonyl). The ^{1}H NMR of (**2a**) was similar to that of **1a** except for the signals in the aromatic region which accounted for the presence of 1,2,3,5-tetra oxygenation pattern of xanthone nucleus. A one proton singlet due to H-4 appeared at δ6.9, a double doublet at δ7.10 (J, 2 and 8 Hz) of H-6, a triplet at δ7 30 (J, 8 Hz) due

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to H-7 (experiencing *ortho* coupling with H-6 and H-8) and a double doublet at δ7.75 (J, 2 and 8 Hz) could be assigned to H-8 On acid hydrolysis **2a** yielded 1-hydroxy-2,3,5-trimethoxyxanthone (**6**). CIMS (CH_4) of **2a** also supported the presence of tetraacetylglucosyl ion at m/z 331 (10%) and tetraoxygenated xanthone ion (**6**) at m/z 302 (100%) Thus **2** is the 1-*O*-β-D-glucoside of 1-hydroxy-2,3,5-trimethoxyxanthone.

Free xanthones **3–6** were devoid of anti-amoebic activity which was concentrated in the xanthone glucoside fraction. This is the first report on the occurrence of xanthone-*O*-glucosides with 1,2,3,5- and 1,2,3,7-tetraoxygenation pattern in family Gentianaceae with special reference to genus *Halenia* [9–14].

EXPERIMENTAL

Mps uncorr IR spectra were recorded as KBr pellets ^{1}H NMR spectra were recorded in $CDCl_3$ either at 90 MHz or at 400 MHz using TMS as int reference in δ(ppm)

Plant material The plant material was collected from Dugalbitta and Chopta of district Chamoli (Garhwal, Himalayas), U P, India by Botany Division of the Institute during the flowering season and a voucher specimen is preserved

Extraction and isolation The air-dried, powdered whole plant was extracted with alcohol The alcoholic extract on concentration deposited a greenish yellow solid (~20 g) which was filtered off The remaining alcoholic extract was concd and fractionated into hexane, $CHCl_3$, *n*-butanol and aq fractions. The greenish yellow solid (5 0 g) was chromatographed over silica gel (250 g) using a *n*-hexane–Me_2CO gradient, collecting fractions of 200 ml each On elution with hexane–Me_2CO (19 1) fractions 22–40 yielded **3** which was crystallized from MeOH as dark yellow needles (100 mg) and fractions 44–66 yielded **4** which was also crystallized from methanol as yellow fluffy compound (600 mg) On subsequent elution with hexane–Me_2CO (9 1) fractions 67–82 gave a mixture of two compounds which on repeated crystallization from hot MeOH and on keeping for 4–6 hr yielded **6** as a fluffy compound (400 mg) and **5** as a yellow crystalline compound (200 mg) from hot MeOH on keeping overnight Compounds **5** and **6** were further purified on a silica gel column using hexane–EtOAc (19 1) as eluent, followed by subsequent crystallization as earlier From the previous column, elution with hexane–Me_2CO (9 1), fractions 81–101 gave a triterpene (Liebermann–Burchard test positive for triterpene), as a crystalline solid (600 mg) from $CHCl_3$–MeOH (1 3), mp 250–255°, EIMS (m/z) 456 $[M]^+$ with a base peak at m/z 248 (characteristic for oleanolic acid or oleanane series) Co TLC, IR, NMR and MS confirmed its identity as oleanolic acid The subsequent fraction on elution with hexane–Me_2CO (3 1) gave a mixture of compounds which on keeping with $CHCl_3$–MeOH (1 3) afforded a greenish white compound identified as sitosterol β-D-glucoside (200 mg), mp 280–282° (dec)

The $CHCl_3$ fraction was extracted with C_6H_6 to remove the free xanthones leaving a blackish gummy matter The C_6H_6 fraction on chromatography over silica gel yielded compounds **3–6** The C_6H_6-insoluble black gummy matter was dissolved in MeOH and pptd with EtOAc. The pptd glycoside was filtered off and dried *in vacuo* to get an amorphous powder (8 0 g). The amorphous glycosidic powder (5.0 g) showing antiamoebic activity was CC over silica gel (250 g) using EtOAc–MeOH gradient. On elution with EtOAc–MeOH (9 1) fractions 27–44 of 200 ml each deposited compound A, as a yellowish white solid on concn, which was filtered and recrystallized from hot MeOH as white amorphous powder (800 mg)

Hydrolysis of compound **A**. 20 mg of (**A**) was taken in MeOH and refluxed for 30 min with 6% HCl (2 ml) on a water bath After neutralization, MeOH was distilled off under red pressure and the reaction mixture extracted with $CHCl_3$ The $CHCl_3$ layer on TLC examination showed the presence of two aglycones, **3** and **4** Co-paper chromatography with authentic sample of glucose showed the presence of glucose as the only sugar (spray reagent aniline phthalate, solvent *n*-butanol saturated with H_2O· 20 hr run, descending)

Acetylation of compound **A** 600 mg of **A** in pyridine (0 5 ml) and Ac_2O (1 5 ml) was left overnight and processed The acetylated product (500 mg) on chromatography over silica gel (20 g) yielded **1a** and **2a** as amorphous powders (150 and 250 mg) on elution with hexane–Me_2CO (4 1) and (3 1) respectively

Compound **1a** Amorphous powder, mp 110–112°, R_f 0 40 (hexane–Me_2CO 3 2), UV λ_{max}^{MeOH} nm (log ε) 227sh (4 37), 240 (4 46) 254 (4 44) 285sh (3 98), 309 (3 99) 358 (3 80) Hydrolysis in MeOH and 6% HCl at 100°for 30 min gave the aglycone, 1-hydroxy-2,3,7-trimethoxyxanthone (**5**) (co TLC, mmp, UV, mass and ^{1}H NMR data)

Compound **2a** Amorphous powder, mp 105–106°, R_f 0 36 (hexane–Me_2CO 3 2) UV λ_{max}^{MeOH} nm (log ε) 223sh (3 88), 248 (4 05), 268sh (3 82) 285sh (3 62) 308sh (3 52) 350 (3 40) No change with $AlCl_3$, NaOMe and NaOAc CIMS (CH_4) m/z 331 (25% acetylated glucose), 302 ($[M]^+$, 100%, aglycone)

On hydrolysis, **2a** yielded the aglycone, 1-hydroxy-2,3,5-trimethoxyxanthone (**6**) (co-TLC, mmp, UV, mass and ^{1}H NMR data)

Compound **3** Yellow crystalline compound from methanol, mp 116–117° Analysed for $C_{17}H_{16}O_7$ (M^+ 332 m/z) R_f 0 44 (hexane–Me_2CO, 9 1), green colour with $FeCl_3$ UV, IR, NMR was identical to that reported previously [6, 8]

Compound **4** Yellow fluffy compound from MeOH, mp 147–150°, $C_{17}H_{16}O_7$ ($[M]^+$ 332 m/z) Green colour with $FeCl_3$ IR, UV and NMR was similar to that reported [6, 8] R_f 0 28 (hexane–Me_2CO 9 1)

Compound **5** Yellow crystalline compound from MeOH, mp 168–170°, $C_{16}H_{14}O_6$ (M^+ 302 m/z) Green colour with $FeCl_3$, R_f 0 48 (hexane–Me_2CO 3 1), UV, IR and NMR was identical to that reported previously [6]

Compound **6** Yellowish fluffy compound from MeOH, mp 184–186°, $C_{16}H_{14}O_6$ (M^+ 302 m/z) Green colour with $FeCl_3$ R_f 0 51 (hexane–Me_2CO 3 1) UV, IR and NMR was identical to that reported [6]

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