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# XANTHONES, SECOIRIDOIDS AND FLAVONOIDS FROM *HALENIA CORNICULATA*

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**Key Word Index** *Halenia corniculata*; Gentianaceae; xanthones; flavonoids; secoiridoids; aglycones; primeverosides; gentiobiosides; LC–UV; LC–TSP–MS.

**Abstract**—Three new xanthone glycosides and a rare flavonoid glycoside have been isolated from the whole plant methanolic extract of *Halenia corniculata* (Gentianaceae). They were identified as 2,3,7-trimethoxy-1-*O*-primeverosyloxyxanthone, 2,3,4,7-tetramethoxy-1-*O*-primeverosyloxyxanthone, 2,3,4,5,7-pentamethoxy-1-*O*-primeverosyloxyxanthone and 7-*O*-primeverosylluteolin. Combined HPLC–UV and thermospray MS analyses of both dichloromethane and methanolic crude extracts permitted the detection of six other new xanthones, tentatively identified by on-line data. Among these, one was an aglycone, 1-hydroxy-2,3,4,5,7-pentamethoxyxanthone and five were 1-*O*-glycosides: 7-hydroxy-2,3,4,5-tetramethoxy-1-*O*-primeverosyloxyxanthone, 7-hydroxy-2,3,4,5-tetramethoxy-1-*O*-gentiobiosyloxyxanthone, 2,3,7-trimethoxy-1-*O*-gentiobiosyloxyxanthone, 2,3,4,7-tetramethoxy-1-*O*-gentiobiosyloxyxanthone and 2,3,4,5,7-pentamethoxy-1-*O*-gentiobiosyloxyxanthone. Systematic use of an in-house UV database and complementary on-line MS data enabled the identification of 16 known secoiridoids, flavonoids and xanthones.

## INTRODUCTION

As a part of our ongoing phytochemical investigation of species of the Gentianaceae [1, 2], a large effort has been devoted to the search of new xanthones with potential monoamine oxidase (MAO) inhibitory activity [3]. These compounds are particularly interesting as potential new antidepressive drugs and are also useful chemotaxonomic markers. More than 50 crude extracts of Gentianaceae species have been routinely screened by HPLC, combining UV photodiode array (LC–UV) and thermospray mass spectrometry detection (LC–TSP–MS) [1]. Both UV and MS spectra obtained on-line gave important structural information, and the systematic use of an in-house UV-spectral library allowed direct identification of known compounds. An efficient targeted isolation of unidentified compounds was then performed. This type of routine analysis permitted an optimisation of the investigation and avoided unnecessary and costly isolations of trivial compounds. We report here the application of this method to a medicinal plant from Mongolia, *Halenia corniculata* L. (Cornaz), which is rich in xanthone aglycones and glycosides. The genus *Halenia* contains approximately 80 species, but only four of them have been investigated from a phytochemical viewpoint: *H. corniculata* [4–6], *H. asclepiadea* [7], *H. elliptica* [8–12] and *H. campanulata* [13].

## RESULTS AND DISCUSSION

The dried plant material was extracted at room temperature with solvents of increasing polarity (dichloromethane and methanol) and both extracts were analysed by LC–UV and LC–TSP–MS. As shown in Fig. 1, the use of a photodiode array detector allowed a rapid screening of the UV-active constituents of the crude extracts and permitted the identification of most compound classes. UV spectra with a single absorption band at 230–240 nm are characteristic for secoiridoids (**1**–**4**), while flavonoids present two maxima between 240–285 nm and 300–350 nm (**5**–**7**). UV spectra of compounds **8**–**26** exhibited four or five absorption bands of decreasing intensity, characteristic for xanthones. Figure 1 shows clearly that this latter type of compounds possesses a large diversity of UV spectra (related to the different oxidation pattern) [14]. An in-house LC-UV spectral library has been constituted with pure standard compounds previously isolated and identified in our laboratory. The same LC conditions have been used for these standard compounds and the crude extracts analyses. A computerized comparison of the UV spectrum, the retention time and the MS data allowed an unam-

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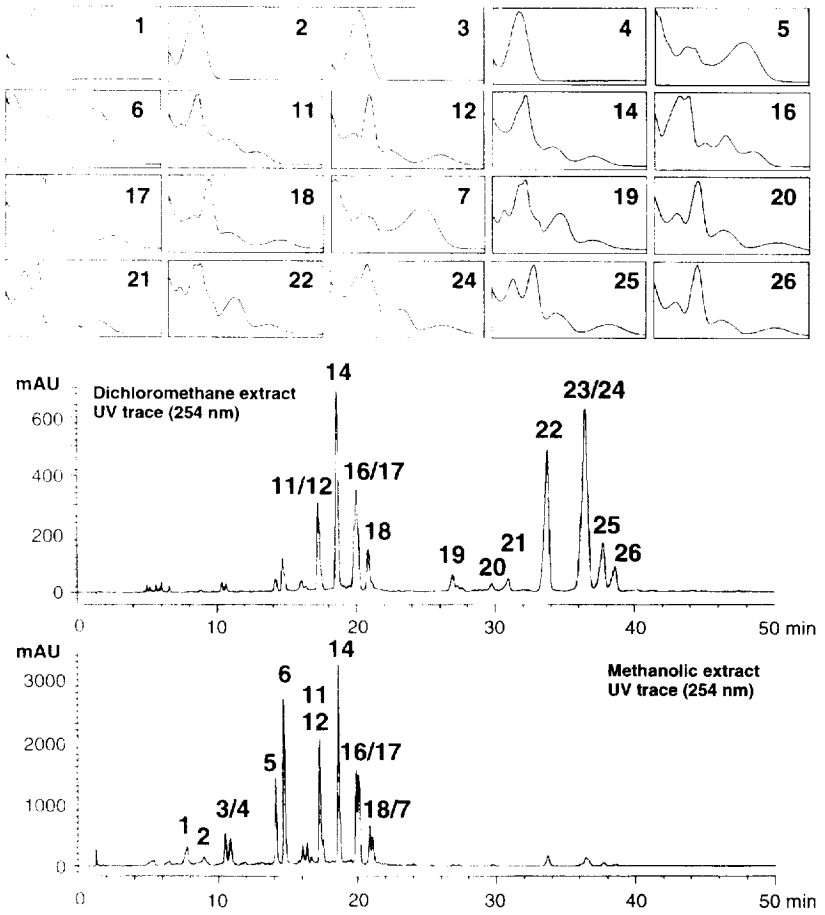


Fig. 1. Comparison of the LC–UV traces of dichloromethane and methanolic extracts of *Halenia corniculata*. The UV spectra of major compounds (recorded from 200 to 450 nm) enable direct determination of the compound classes. UV spectra of minor compounds (**8**–**10**, **13**, **15** and **23**) are not displayed. For LC–UV conditions, see Experimental.

biguous on-line identification of known compounds, directly from the LC analysis of the crude extracts. The identification criteria used were a matching factor greater than 95% for the UV spectrum fitting and a retention time in a window of 5% around the value of the reference compound.

With the aid of these on-line data, seven xanthones were thus identified from the analysis of the crude dichloromethane extract. These were 2,3,5-trimethoxy-1-*O*-gentiobiosyloxyxanthone (**8**), 2,3,5-trimethoxy-1-*O*-primeverosyloxyxanthone (**11**), 2,3,4,5-tetramethoxy-1-*O*-primeverosyloxyxanthone (**14**), 1-hydroxy-2,3,5-trimethoxyxanthone (**22**) and 1-hydroxy-2,3,4,5-tetramethoxyxanthone (**24**), isolated in our laboratory from *H. campanulata* [13]. 1,5-dihydroxy-2,3-dimethoxyxanthone (**19**) and 1,7-dihydroxy-2,3-dimethoxyxanthone (**21**) isolated from *Monnina sylvatica* (Polygalaceae) [15]. The aglycones **22** and **24** have previously been described in *H. corniculata* by Tankhaeva *et al.* [4]. The xanthone aglycone **22** and the corresponding 1-*O*-gentiobioside (**8**) and 1-*O*-primeveroside (**11**) constituted a particularly interesting series for the interpretation of the MS and UV data of the other xanthones detected in the extracts. As these compounds were fully characterized in the literature [13], their chromatographic behaviour as well as their spectroscopic data were used as a reference for the structure elucidation of other series of related xanthones. Under the LC–TSP–MS conditions (see experimental), which are similar to positive D/CI–MS using $NH_3$ as reactant gas [16], the xanthone aglycones (like compound **22**) showed an intense protonated molecular ion $[M + H]^+$ but almost no fragmentation. The xanthone glycosides (like **8** and **11**) gave weak sodium adduct ions $[M + Na]^+$ and an important fragment corresponding to the aglycone moiety $[A + H]^+$. The difference of 316 amu between those two peaks in the TSP–MS spectrum of the glycosyl xanthone was characteristic of the loss of a primeverosyl moiety ( – pentosyl) (132) – hexosyl (162) – Na (23) + H (1)). Likewise, a difference of 346 amu ( – hexosyl (162) – hexosyl (162) – Na (23) + H (1)) indicated the presence of a gentiobiosyl derivative. Two glycosides with the same aglycone (as **8** and **11**) were also distinguished by their retention times. Indeed, the substitution of a primerosyl

by a gentiobiosyl group results in a diminution of the retention time of more than one minute. Thus, the LC–MS data allowed an unambiguous differentiation of these two types of glycosyl derivatives. Moreover, primeverose and gentiobiose are the only disaccharides corresponding to these masses, which have been found to date in the Gentianaceae. Xanthones **8**, **11** and **22** exhibited very similar UV spectra. Indeed, the UV spectra of the two glycosyl derivatives were identical. Thus, substitution by a primeverosyl or a gentiobiosyl residue did not modify the chromophore. Nevertheless, the corresponding aglycone (**22**) gave a bathochromic shift for its second absorption band (250–270 nm) of 5–10 nm. These different considerations showed that LC–UV and LC–MS data can be used efficiently to detect other series of xanthone glycosides having a common aglycone moiety. The approach constituted the basis for a systematic search of xanthone aglycones and glycosides in the extracts.

Figure 2 illustrates the potentialities of these techniques for targeting new molecules. As shown in the chromatograms, the three xanthones **15**, **18** and **26** were localized directly in the single ion traces of the LC–MS analysis of the crude dichloromethane extract. Thus, xanthones **15**, **18** and **26** appeared in the single ion trace 363 amu, which indicated the presence of a common pentasubstituted aglycone (one hydroxyl and five methoxyl groups) in each case (Fig. 2). Compounds **15** and **18** were detected separately at 709 and 679 amu, respectively. According to the considerations discussed above, **18** was assumed to be a primeverosyl xanthone. The peak at 679 amu corresponded to $[M + Na]^+$ or $[A + 132 + 162 + Na]^+$. Compound **15** appeared to be a gentiobiosyl xanthone and was present in very small amounts. Nevertheless, it could be easily detected at 709 amu, corresponding to $[A + 162 + 162 + Na]^+$. The UV spectra of the three compounds were very similar but did not correspond to any of the available data. Thus, a rare oxidation pattern was indicated by these on-line data and isolation was subsequently undertaken.

Isolation of six xanthone glycosides (**8**, **11**, **14**, **16**, **17** and **18**) was performed from the methanolic extract by a combination of gel filtration on Sephadex LH-20 (MeOH) and medium-pressure liquid chromatography (MPLC) on RP-18. Structure determination was then achieved by $^1H$ NMR and $^{13}C$ NMR, including NOE and selective INEPT experiments. Among these six isolated compounds, three have already been described in *Halenia campanulata* (**8**, **11**, and **14**) and their spectroscopic data were in good agreement with those found in the literature [13]. The three others (**16**, **17**, and **18**) appeared to be primeverosyl xanthones because of the characteristic loss of 316 amu exhibited in their TSP–MS spectra. These latter spectra gave intense fragments corresponding to the aglycone moieties $[A + H]^+$ at 303, 333, 363 amu for compounds **16**, **17**, and **18**, respectively. The $[A + H]^+$ ions were indicative of the presence on the aglycone moieties of one OH and three OMe groups for **16**, one OH and four OMe for **17** and one OH and five OMe for **18**. An acid hydrolysis was performed on compounds **16**, **17**, and **18**, giving aglycones **23**, **25**, and **26**, respectively, and glucose and xylose residues in each case. Indications about the position of the free hydroxyl group in aglycones **23**, **25**, and **26** were obtained from their UV spectra recorded off-line with classical shift reagents. A strong bathochromic shift observed upon addition of $AlCl_3$ + HCl was characteristic of a OH group *peri* to the carbonyl function. As these aglycones were monohydroxylated, the *O*-glycosidic linkage of **16**, **17**, and **18** was thus also at position C-1 or C-8. The oxidation

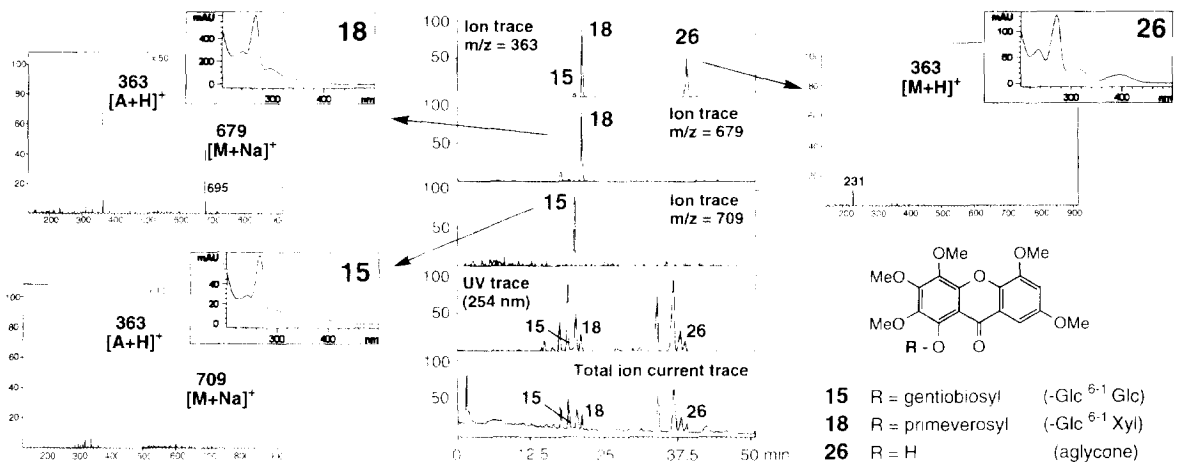


Fig. 2. LC–UV and LC–TSP–MS analysis of the crude dichloromethane extract of *Halenia corniculata*. The UV and mass spectra of three related xanthones (aglycone **20** and its two glycoside derivatives **15** and **18**) are displayed. The ion *m/z* 363 is the main fragment and corresponds to the aglycone moiety $[A + H]^+$. The ion traces displayed at *m/z* 679 and 709 correspond to the pseudo-molecular ion $[M + Na]^+$ of gentiobioside **15** and primeveroside **18**, respectively.

pattern of these glycosides and the nature of the disaccharide moieties were determined by $^1$H NMR and $^{13}$C NMR spectroscopy.

For compound **16**, the $^1$H NMR spectrum exhibited signals for four aromatic protons. Two of them were typical of *meta* coupled protons ($J$ = 2.9 Hz) while a third one indicated an *ortho* coupling ($J$ = 9.0 Hz). These three signals, forming a ABM system, were characteristic of a B-ring substituted at position C-6 or C-7. The carbon chemical shifts of the methoxyl groups (usually $\delta$55–56, but $\delta$60–62 when *ortho*-disubstituted [17]) were $\delta$55.6, 56.5 and 60.7. The spectroscopic data obtained for the aglycone moiety (**23**) were in good agreement with those found in the literature for 1-hydroxy-2,3,7-trimethoxyxanthone [7, 18]. For compound **17**, only three aromatic proton signals of a ABC system were recorded. In the $^{13}$C NMR spectrum, full substitution in the A-ring was confirmed by three *ortho*-disubstituted methoxyl signals. An additional signal at $\delta$55.7 was indicative of the presence of a fourth methoxyl group in the B-ring. As for **16**, comparison with the literature permitted the identification of the aglycone moiety (**25**) as 1-hydroxy-2,3,4,7-tetramethoxyxanthone. The substitution at position C-7 for xanthones **16** and **17** was confirmed by the methylation of 1,7-dihydroxy-2,3,4-trimethoxyxanthone, isolated by Bashir *et al.* [15]. In this compound, the lack of shift with a weak base excluded the possibility of an attachment of the hydroxyl group at position C-6. HPLC-UV (DAD) analysis and UV fitting of the peak spectra showed that the partial methylation of this aglycone gave compound **25**. Thus, the substitution at position C-7 of **17** was confirmed. As the B-ring $^{13}$C NMR signals of **16** and **17** were very similar, the C-7 substitution of **17** was also demonstrated. For **16** and **17**, glycosidation at C-1 was confirmed by the upfield shift of C-1 and C-3, together with a downfield shift for C-2 and C-4, in comparison to the data reported for aglycones having the same A-ring substitution [12, 15]. Both $^{13}$C NMR spectra of **16** and **17** showed 11 carbon signals due to the sugar residue. Signals for anomeric carbon atoms were observed at $\delta$104.2 and $\delta$103.4, confirming the presence of glucosyl and xylosyl moieties [17]. In each case, the C-6' carbon of the glucosyl moiety was shifted downfield to $\delta$68.1–68.2, indicating the presence of primeverosyl units. The two doublets ($J$ = 7.6 Hz each) attributable to the anomeric protons of the disaccharide moiety appeared at $\delta$4.0 and $\delta$4.9. Thus, **16** was identified as 2,3,7-trimethoxy-1-*O*-primeverosyloxyxanthone and **17** as 2,3,4,7-tetramethoxy-1-*O*-primeverosyloxyxanthone. These are, to our knowledge, new natural products. HPLC analyses showed that the two aglycones resulting from acid hydrolysis of **16** and **17** were also present in the dichloromethane extract.

The $^1$H NMR spectrum of xanthone **18** exhibited two doublets ($J$ = 2.8 Hz each) characteristic of a pair of *meta* coupled aromatic protons overlapping at $\delta$7.04. Intense singlets between $\delta$3.8 and $\delta$4.1 were indicative of the presence of five methoxyl groups. The $^{13}$C NMR spectra showed 29 carbon signals, 11 of which were due to the sugar residue, five to the methoxyl groups and 13 to the xanthone nucleus. $^1$H NMR and $^{13}$C NMR signals attributable to the disaccharide moiety were identical to those observed for glycosides **16** and **17**, proving the presence of a primeverosyl residue. $^{13}$C NMR signals for methoxyl groups appeared at $\delta$55.6, 56.7, 61.2, 61.3 and 61.4. In order to accommodate three *ortho*-disubstituted methoxyl groups and two *meta*-aromatic protons, the A-ring had to be completely substituted by one primeverosyl and three methoxyl groups. The substitution on the B-ring was determined by a selective INEPT experiment. By imposing selective pulses on the signals of the two overlapping aromatic protons H-6 and H-8 (at $\delta$7.04), it was possible to show the connectivity with the C-5 and C-7 carbon atoms and that of the carbonyl function. Substitution by methoxyl groups at positions C-5 and C-7 was thus demonstrated and compound **18** was identified as 2,3,4,5,7-pentamethoxy-1-*O*-primeverosyloxyxanthone. Thus, as indicated by the LC–UV and LC–MS data, **18** is a new natural compound. HPLC analysis showed that the corresponding aglycone (**26**), 1-hydroxy-2,3,4,5,7-pentamethoxyxanthone, obtained by acid hydrolysis of **18** was also present in the dichloromethane extract. Aglycone **26** is thus also a genuine constituent of the plant and is, to our knowledge, a new natural product.

The three gentiobiosides detected on-line (**13**, **10** and **15**) have been tentatively identified as 2,3,7-trimethoxy-1-*O*-gentiobiosyloxyxanthone (**13**), 2,3,4,7-tetramethoxy-1-*O*-gentiobiosyloxyxanthone (**10**) and 2,3,4,5,7-pentamethoxy-1-*O*-gentiobiosyloxyxanthone (**15**). As shown in Fig. 2, xanthone **15** was assumed to be a gentiobioside because of the characteristic loss of 346 amu in the TSP-mass spectrum. The presence of a gentiobiosyl moiety in xanthones **10** and **13** was also indicated by their TSP–mass spectra. The three compounds **13**, **10** and **15** were present in very small amounts in the extracts and were not isolated. However, they were easily located in the LC–MS chromatograms by displaying the specific single ion traces corresponding to the aglycone moiety $[A + H]^+$. The aglycones of **13**, **10** and **15** appeared at 303, 333, 363 amu, respectively, as found for the glycosides **16** (2,3,7-trimethoxy-1-*O*-primeverosyloxyxanthone), **17** (2,3,4,7-tetramethoxy-1-*O*-primeverosyloxyxanthone) and **18** (and 2,3,4,5,7-pentamethoxy-1-*O*-primeverosyloxyxanthone) identified above (see Fig. 3). The LC–UV data clearly showed the similarity of the UV spectra of **13**, **10** and **15** with the corresponding primeverosides **16**, **17** and **18**. Moreover, the respective difference in the retention time was in each case between 1.3 and 1.7 minutes. These on-line data implied that the oxidation patterns of **13**, **10** and **15** were the same as those of **16**, **17** and **18**, respectively. Acid hydrolysis of fractions containing small amounts of glycosides **13**, **10** and **15** gave aglycones **23**, **25**, and **26**, respectively, and glucose residues, confirming the structures obtained on-line. These three gentiobiosyl xanthones (**13**, **10** and **15**) have not yet been reported in the literature.

Compounds **9**, **12** and **20** were present in too small amounts in the extracts to allow NMR experiments. However, acid hydrolysis of fractions containing **9** and **12**

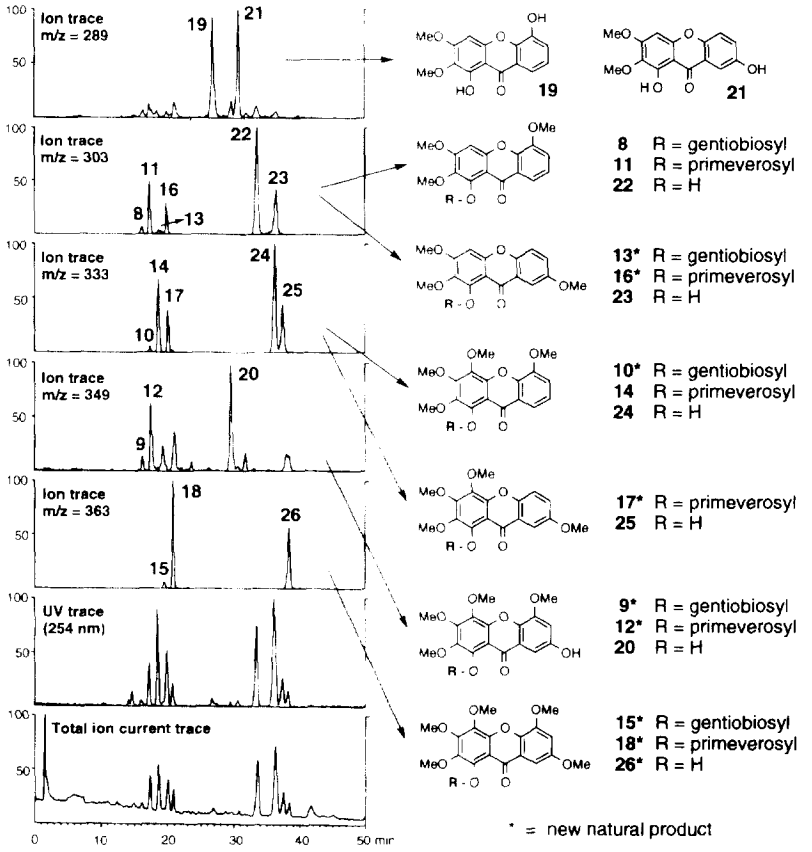


Fig. 3. LC–UV and LC–TSP–MS analysis of the crude dichloromethane extract and structures of all xanthones identified in *Halenia corniculata*. For each oxidation pattern, the aglycone moiety ion trace is displayed. Thus, series of xanthones with the same aglycone can be observed. For LC–MS conditions, see Experimental.

showed that these two xanthone glycosides had the same aglycone (**20**), also found in dichloromethane extract (see Fig. 3) TSP–MS data indicated that **9** was a gentiobioside, while **12** was a primeveroside. TSP–MS spectra of these three related xanthones showed a large fragment corresponding to the aglycone moiety at 349 amu, indicating the presence of two hydroxyl and four methoxyl groups. The UV spectra, exhibiting an intense absorption band at 260–280 nm, were very similar to those obtained for the series of xanthones **15**, **18** and **26**. Thus, MS data and the comparison of UV spectra indicated that the oxidation pattern of **9**, **12** and **20** was the same as that of xanthones **15**, **18** and **26** (1,2,3,4,5,7-*O*-hexasubstituted xanthones). Aglycone **20** was identified as 1,7-dihydroxy-2,3,4,5-tetramethoxyxanthone by comparison with a pure standard compound isolated by one of us (Odontuya) [6]. The spectroscopic data obtained for **20** were in accord with those reported in the literature [11]. The position of the *O*-glycosidic linkage of **12** was determined by an LC–UV analysis of the crude extract with post-column addition of $AlCl_3$ [1]. The absence of bathochromic shift was indicative of a glycosidation at C-1. Because of the poor resolution, the same observation was not possible for **9**. However, as the UV spectra of **9** and **12** were identical, the glycosidation at C-1 was also confirmed for **9**. Thus, the two glycosides **9** and **12** were tentatively identified on-line as 7-hydroxy-2,3,4,5-tetramethoxy-1-*O*-gentiobiosyloxyxanthone (**9**) and 7-hydroxy-2,3,4,5-tetramethoxy-1-*O*-primeverosyloxyxanthone (**12**). No previous references to these compounds have, to our knowledge, been reported.

The LC–UV and LC–TSP–MS analysis of the crude dichloromethane extract, as well as the complete structures of the 19 xanthones identified in *Halenia corniculata* are given in Fig. 3. Among the nine new natural products (**9**, **10**, **12**, **13**, **15**, **16**, **17**, **18** and **26**), three have been isolated and fully characterized, while six have been tentatively identified from on-line data. In this figure, in addition to the UV trace and the total ion current trace, xanthone aglycone ion traces are displayed. For each oxidation pattern, the aglycone and both gentiobioside and primeveroside derivatives appeared clearly in the chromatograms, except for the aglycone **25**, which corresponding gentiobioside could not be detected. It was also observed that gentiobiosides were present in much smaller amounts than primeveroside derivatives. Consequently, MS detection was essential for such minor compounds. For reason of convenience, all the xanthones were classified in six series of related aglycone and glycosides (see Fig. 3).

According to the literature on *Halenia* species, the oxidation patterns 1,2,3,5-, 1,2,3,4,5- and 1,2,3,4,5,7- with so many methoxyl groups are characteristic of this genus [4 13]. This chemotaxonomic aspect was corroborated in this study and is all the more interesting because such a constancy is not encountered in most other genera of the Gentianaceae. For five different aglycones, the presence in the extract of both corresponding primeverosides and gentiobiosides has been shown. This is also, to our knowledge, a feature of the genus *Halenia* which does not appear in other genera of the family.

Two glycosyl flavonoids, present in large amounts in the methanolic extract, were isolated by gel filtration on Sephadex LH-20 (MeOH). Structure determination was then achieved by $^1$H NMR and $^{13}$C NMR. These compounds were identified as 7-*O*-primeverosylluteolin or cesioside (**5**) and 7-*O*-glucosylluteolin or glucoluteolin (**6**). Acid hydrolysis of these two flavonoids gave luteolin (compound **7**), an aglycone also present in the methanolic extract and identified by comparison with a pure standard compound. While luteolin and glucoluteolin are widespread in Gentianaceae species, cesioside is a rare primeverosyl flavonoid. It was previously isolated from *Salix repens* (Salicaceae) [21], but was fully characterized by Markham *et al.* [22] from *Dacrydium* species (Podocarpaceae). Cesioside has not yet been found in the Gentianaceae and this is, to our knowledge, the first flavonoid primeveroside to be isolated from the family.

Secoiridoids are very common in numerous genera of the Gentianaceae, but are rather rare in *Halenia* species. LC–UV and LC–TSP–MS analysis allowed the on-line detection and identification of four secoiridoids (swertiamarin (**1**), sweroside (**2**), *epi*-vogeloside (**3**) and vogeloside (**4**)) in *H. corniculata* by comparison with pure reference compounds. Compounds **3** and **4**, originally isolated from *Anthocleista vogelii* (Loganiaceae) [19] and *Lonicera japonica* (Caprifoliaceae) [20], were described in *H. campanulata* [13], but swertiamarin and sweroside have never been reported in the genus *Halenia*.

In conclusion, LC–UV and LC–MS analyses do not replace other techniques of structure elucidation, but have shown their potentiality to detect compounds present in very small amounts directly in crude extracts. Moreover, they appear to be particularly efficient for indicating the presence of new xanthone or flavonoid glycosides corresponding to known aglycones. It has also been shown that this method of on-line identification can be applied to common flavonoids and secoiridoids.

## EXPERIMENTAL

*Plant material.* Whole plants of *Halenia corniculata* L. (Cornaz) were collected in 1992 by one of us (Purev) in the vicinity of Ulaanbaatar and identified by Dr Sanchir (Herbarium of Botanical Institute, Ulaanbaatar, Republic of Mongolia). A voucher specimen (no 92012) is deposited at the Institute of Pharmacognosy and Phytochemistry (University of Lausanne, Switzerland).

*Extraction.* 205 g of dry powdered whole plant were extracted at room temperature successively with $CH_2Cl_2$ (3 × 1000 ml) and MeOH (3 × 1000 ml) and afforded 9 g and 34 g of extracts, respectively.

*LC–UV analysis.* Reversed-phase HPLC of the crude extracts was carried out with a Waters 600 MS solvent delivery system, an on-line UV Hewlett-Packard 1050 series photodiode array detector and a Waters 590 MS pump for post-column addition of the buffer. Separation was achieved by using a Waters NovaPak RP-18 column (4 μm, 150 × 3.9 mm) with a MeCN–$H_2O$ gradient (5:95 to 65:35) (containing 0.05% TFA) over 50 min. The flow-rate was 1 ml min$^{-1}$, the UV trace was observed at 254 nm and UV spectra were recorded between 190 and 600 nm.

*LC–TSP–MS analysis.* An aqueous buffer of 0.5 M of $NH_4OAc$ was added post-column (0.2 ml min$^{-1}$) to help ionization. A thermospray (Finnigan MAT) interface was used with the following conditions: source temperature 280°C, vaporizer 100°C, aerosol 300°C, filament off and positive ion mode. MS detection was achieved on a Finnigan MAT TSQ 700 triple quadrupole instrument. Spectra (150–900 amu) were recorded every 3 sec.

*Isolation of the xanthone and flavonoid glycosides.* A portion (10 g) of the methanolic extract was fractionated by gel filtration on Sephadex LH-20 with MeOH as eluent. Twelve fractions were collected (1–12). The fraction 2, enriched in xanthone glycoside (3.3 g) was subjected to medium-pressure liquid chromatography (MPLC) in two batches on a LiChroprep RP-18 column (15–25 μm, 12 × 460 mm, Merck) with MeOH–$H_2O$ (40:60) at a flow rate of 3 ml min$^{-1}$ and yielded 4 mg of **8**, 12 mg of **11**, 25 mg of **14**, 8 mg of **16**, 20 mg of **17** and 15 mg of **18**. Pure compounds **5** (390 mg) and **6** (570 mg) were obtained directly from fractions 5 and 7, respectively.

*Hydrolysis of xanthone and flavonoid glycosides.* Aglycones **20**, **26** and **7** were obtained after acid hydrolysis of the corresponding glycosides (1–2 mg) according to the standard procedure. Sugars were analysed by TLC on silica with EtOAc–$H_2O$–MeOH–HOAc (13:3:3:4) and visualized by spraying with *p*-anisidine phthalate [23].

*Methylation.* 1,7-Dihydroxy-2,3,4-trimethoxyxanthone (1.6 mg), $K_2CO_3$ (25 mg), and MeI (0.1 ml) were added to 2 ml of HPLC-grade acetone. The mixture was refluxed and gave 1-hydroxy-2,3,4,7-tetramethoxyxanthone after 2 hr and 1,2,3,4,7-pentamethoxyxanthone after 10 hr.

2,3,7-*Trimethoxy*-1-O-*gentiobiosyloxyxanthone* (**13**). HPLC: (RP-18, system described above) $R_t$ = 18.3 min. UV $\lambda_{max}^{on\text{-}line}$ nm: 241, 258, 283, 317, 357. TSP–MS: *m/z* 619 [M + Na]$^+$, 303 [aglycone + H]$^+$.

2,3,7-*Trimethoxy*-1-O-*primeverosyloxyxanthone* (**16**). Pale yellow amorphous powder, mp 165–167°. $[\alpha]_D^{25}$ − 36° ($H_2O$; *c* 1.0). HPLC: (RP-18, system described above) $R_t$ = 19.8 min. UV $\lambda_{max}^{MeOH}$ nm: 249, 256, 283, 316, 357. Unchanged upon addition of $AlCl_3$ or NaOMe. TSP–MS: *m/z* 619 [M + Na]$^+$, 303 [aglycone + H]$^+$. $^1$H NMR (200 MHz, DMSO-$d_6$): δ 2.7–4.1 (12H, *m*, sugar residue), 3.80, 3.88, 3.98, (3H each, *s*, 3 × MeO), 4.97 (1H, *d*, *J* = 7.6 Hz, H-1′), 7.04 (1H, *s*, H-4), 7.41 (1H, *dd*,

$J$ = 9.0, 2.9 Hz, H-6), 7.54 (1H, *d*, $J$ = 2.9 Hz, H-8), 7.54 (1H, *d*, $J$ = 9.0 Hz, H-5). $^{13}$C NMR (50 MHz, DMSO-$d_6$): $\delta$159.0 (C-1), 138.9 (C-2), 153.9 (C-3), 96.9 (C-4), 148.4 (C-4a), 149.1 (C-4b), 118.7 (C-5), 123.8 (C-6), 155.6 (C-7), 106.0 (C-8), 121.9 (C-8a), 108.6 (C-8b), 174.7 (C=O), 104.2 (C-1'), 73.9 (C-2'),, 76.3[a] (C-3'), 69.8 (C-4'), 76.5[a] (C-5'), 67.8 (C-6'), 103.3 (C-1''), 73.1 (C-2''), 76.3[a] (C-3''), 69.4 (C-4''), 65.3 (C-5''), 55.6, 56.5 (2 × MeO), 60.7 (MeO-2). ([a]Assignments interchangeable.)

2,3,4,5-*Tetramethoxy*-1-O-*gentiobiosyloxyxanthone* (**10**). HPLC: (RP-18, system described above) $R_t$ = 16.8 min. UV $\lambda_{max}^{on\text{-}line}$ nm: 242 (sh), 256, 299, 364. Unchanged upon addition of $AlCl_3$. TSP–MS: $m/z$ 679 $[M + Na]^+$, 333 [aglycone + H]$^+$.

2,3,4,5-*Tetramethoxy*-1-O-primeverosyloxyxanthone (**14**). Pale yellow amorphous powder, mp 152–155°. $[\alpha]_D^{25}$ −80° ($H_2O$: *c* 1.0). HPLC: (RP-18, system described above) $R_t$ = 18.6 min. UV $\lambda_{max}^{MeOH}$ nm: 240 (sh), 257, 298, 362. Unchanged upon addition of $AlCl_3$ or NaOMe. TSP–MS: $m/z$ 649 $[M + Na]^+$, 333 [aglycone + H]$^+$. $^1$H NMR (200 MHz, DMSO-$d_6$): $\delta$2.7–4.1 (12H, *m*, sugar residue), 3.84, 4.00, 4.01, 4.05 (3H each, *s*, 4 × MeO), 4.91 (1H, *d*, $J$ = 7.6 Hz, H-1'), 7.35 (1H, *t*, $J$ = 8.0 Hz, H-7), 7.46 (1H, *dd*, $J$ = 8.0, 1.6 Hz, H-6), 7.67 (1H, *dd*, $J$ = 8.0, 1.6 Hz, H-8). $^{13}$C NMR (50 MHz, DMSO-$d_6$): $\delta$147.0 (C-1), 137.4 (C-2), 152.3 (C-3), 142.5 (C-4), 144.7[a] (C-4a), 144.3[a] (C-4b), 148.1 (C-5), 116.5[c] (C-6), 123.8 (C-7), 116.2[c] (C-8), 122.2 (C-8a), 111.2 (C-8b), 175.4 (C=O), 104.2 (C-1'), 73.9 (C-2'), 76.3[b] (C-3'), 69.8 (C-4'), 76.3[b] (C-5'), 68.2 (C-6'), 103.5 (C-1''), 73.1 (C-2''), 76.3[b] (C-3''), 69.4 (C-4''), 65.3 (C-5''), 56.6 (MeO-5), 61.2, 61.3, 61.4 (3 × MeO). ([a–c]Assignments interchangeable.)

2,3,4,7-*Tetramethoxy*-1-O-*primeverosyloxyxanthone* (**17**). Pale yellow amorphous powder, mp 142–145°. $[\alpha]_D^{25}$ −42° ($H_2O$: *c* 1.0). HPLC: (RP-18, system described above) $R_t$ = 20.1 min. UV $\lambda_{max}^{MeOH}$ nm: 242, 263, 292, 314 (sh), 374. Unchanged upon addition of $AlCl_3$ or NaOMe. TSP–MS: $m/z$ 649 $[M + Na]^+$, 333 [aglycone + H]$^+$. $^1$H NMR (200 MHz, DMSO-$d_6$): $\delta$2.7–4.1 (12H, *m*, sugar residue), 3.85, 3.88, 3.97, 4.06, (3H each, *s*, 4 × MeO), 4.92 (1H, *d*, $J$ = 7.6 Hz, H-1'), 7.43 (1H, *dd*, $J$ = 9.1, 3.0 Hz, H-6), 7.53 (1H, *d*, $J$ = 3.0 Hz, H-8), 7.62 (1H, *d*, $J$ = 9.1 Hz, H-5). $^{13}$C NMR (50 MHz, DMSO-$d_6$): $\delta$147.2 (C-1), 137.2 (C-2), 152.2 (C-3), 142.3 (C-4), 144.3 (C-4a), 149.0 (C-4b), 119.2 (C-5), 124.1 (C-6), 155.7 (C-7), 105.9 (C-8), 121.7 (C-8a), 110.9 (C-8b), 175.1 (C=O), 104.2 (C-1'), 73.9 (C-2'), 76.2[a] (C-3'), 69.8 (C-4'), 76.2[a] (C-5'), 68.1 (C-6'), 103.4 (C-1''), 73.1 (C-2''), 76.2[a] (C-3''), 69.4 (C-4''), 65.3 (C-5''), 55.7 (MeO-7), 61.2, 61.3, 61.5 (3 × MeO). ([a]Assignments interchangeable).

2,3,4,5,7-*Pentamethoxy*-1-O-*gentiobiosyloxyxanthone* (**15**). HPLC: (RP-18, system described above) $R_t$ = 19.5 min. UV $\lambda_{max}^{on\text{-}line}$ nm: 243, 266, 295, 376. Unchanged upon addition of $AlCl_3$. TSP–MS: $m/z$ 709 $[M + Na]^+$, 363 [aglycone + H]$^+$.

2,3,4,5,7-*Pentamethoxy*-1-O-*primeverosyloxyxanthone* (**18**). Pale yellow amorphous powder, mp 140–143°. $[\alpha]_D^{25}$ −32° ($H_2O$: *c* 1.0). HPLC: (RP-18, system described above) $R_t$ = 20.9 min. UV $\lambda_{max}^{MeOH}$ nm: 242, 266, 295, 379. Unchanged upon addition of $AlCl_3$ or NaOMe. TSP–MS: $m/z$ 679 $[M + Na]^+$, 363 [aglycone + H]$^+$. $^1$H NMR (200 MHz, DMSO-$d_6$): $\delta$2.7–4.1 (12H, *m*, sugar residue), 3.83, 3.87, 3.98, 4.00, 4.05 (3H, each *s*, 5 × MeO), 4.91 (1H, *d*, $J$ = 7.4 Hz, H-1'), 7.04 (1H, *d*, $J$ = 2.8 Hz, H-5), 7.04 (1H, *d*, $J$ = 2.8 Hz, H-7). $^{13}$C NMR (50 MHz, DMSO-$d_6$): $\delta$147.0 (C-1), 137.4 (C-2), 152.0 (C-3), 142.5 (C-4), 144.1 (C-4a), 140.1 (C-4b), 149.3 (C-5), 106.3 (C-6), 155.7 (C-7), 96.0 (C-8), 122.1 (C-8a), 110.8 (C-8b), 175.0 (C=O), 104.2 (C-1'), 73.9 (C-2'), 76.2[a] (C-3'), 69.8 (C-4'), 76.3[a] (C-5'), 68.2 (C-6'), 103.5 (C-1''), 73.1 (C-2''), 76.3[a] (C-3''), 69.4 (C-4''), 65.3 (C-5''), 55.6, 56.7 (2 × MeO), 61.2, 61.3, 61.4 (3 × MeO). ([a]Assignments interchangeable.)

1-*Hydroxy*-2,3,4,5,7-*pentamethoxyxanthone* (**26**). HPLC: (RP-18, system described above) $R_t$ = 38.6 min. UV $\lambda_{max}^{MeOH}$ nm: 235, 269, 307, 396. $\lambda_{max}^{AlCl_3+HCl}$ nm: 235, 286, 328, 456. $\lambda_{max}^{NaOMe}$ nm: 239, 278, 315, 420. Unchanged upon addition of NaOAc. TSP–MS: $m/z$ 363 $[M + H]^+$.

7-*Hydroxy*-2,3,4,5-*tetramethoxy*-1-O-*gentiobiosyloxyxanthone* (**9**). HPLC: (RP-18, system described above) $R_t$ = 16.1 min. UV $\lambda_{max}^{on\text{-}line}$ nm: 241, 265, 297, 381. TSP–MS: $m/z$ 695 $[M + Na]^+$, 349 [aglycone + H]$^+$.

7-*Hydroxy*-2,3,4,5-*tetramethoxy*-1-O-*primeverosyloxyxanthone* (**12**). HPLC: (RP-18, system described above) $R_t$ = 17.2 min. UV $\lambda_{max}^{on\text{-}line}$ nm: 241, 265, 296, 383. Unchanged upon addition of $AlCl_3$. TSP–MS: $m/z$ 665 $[M + Na]^+$, 349 [aglycone + H]$^+$.

1,7-*Dihydroxy*-2,3,4,5-*tetramethoxyxanthone* (**20**). Yellow amorphous powder, mp 225–227°. HPLC: (RP-18, system described above) $R_t$ = 30.0 min. UV and $^1$H NMR as [11] and [6]. $^{13}$C NMR (50 MHz, DMSO-$d_6$): $\delta$149.2 (C-1), 134.5 (C-2), 153.4 (C-3), 132.2 (C-4), 145.2 (C-4a), 139.8 (C-4b), 149.3 (C-5), 107.2 (C-6), 154.0 (C-7), 98.1 (C-8), 120.1 (C-8a), 104.1 (C-8b), 180.8 (C=O), 55.4, (MeO-5), 60.4, 61.2, 61.3 (3 × MeO).

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