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CAMPESTROSIDE, A NEW TETRAHYDROXANTHONE GLUCOSIDE FROM *GENTIANA CAMPESTRIS*

MARYSE KALDAS*, IWAO MIURA† and KURT HOSTETTMANN†

*Institut de Chimie de l'Université, CH-2000 Neuchâtel, Switzerland; †Department of Chemistry, Columbia University, New York, NY 10027, U.S.A.

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Key Word Index—*Gentiana campestris*; Gentianaceae; xanthone; tetrahydroxanthone glucoside; chemotaxonomy.

Abstract—Campestroside has been isolated from the aerial parts of *Gentiana campestris*. From UV, MS, ^{1}H-NMR and ^{13}C-NMR data its structure has been established as 1,3,5-trihydroxy-8-β-D-glucopyranosyl-5,6,7,8-tetrahydroxanthone. Campestroside which has also been detected in *G. ramosa* and *G. germanica* is the first reported tetrahydroxanthone glycoside.

INTRODUCTION

In previous papers we reported the isolation of three *C*-glycosides, isoorientin, swertisin and mangiferin, as well as three new xanthone-*O*-glycosides from the leaves of *Gentiana campestris* L. (Gentianaceae) [1, 2]. These glycosides are accompanied by a minor congenor, campestroside, which eluded structural clarification because of the limited amount and unusual spectroscopic properties. Campestroside has also been detected in two related species, *G. germanica* Willd. and *G. ramosa* Hegetschw. The present communication deals with its structure determination as **1**.

RESULTS

Extraction of the dried plant material using solvents with increasing polarity has been reported previously [1]. Polyamide column chromatography of the methanol extract starting with MeOH–H_2O (1:1) and increasing the percentage of MeOH afforded glycoflavones, mangiferin and xanthone-*O*-glycosides [1, 2]. Compound **1** was eluted together with swertisin and subsequently purified by preparative TLC on Si gel. Filtration on Sephadex LH20 and recrystallization from MeOH afforded pale yellow needles, mp 145° (dec.).

The UV spectrum was closely related to that of xanthones [3]. The bathochromic shift observed by adding $AlCl_3$ indicated the presence of a free hydroxyl group peri to the carbonyl function. A bathochromic shift of the band at 298 nm with increasing intensity was also observed by addition of a weak base, NaOAc (see Table 1); this is typical for xanthones (or chromones)

Table 1. UV spectra of compounds **1**–**2** (max in nm, solvent = MeOH)

Compound	Pure solvent	Solvent with added		
		$AlCl_3$	NaOAc	NaOMe
1	231, 253, 260	223, 265	229, 273	228, 273
	298, 325 *sh*	312, 359	331	334
2	231, 252, 258	225, 267	229, 267	228, 267
	297, 325 *sh*	313, 360	328	332

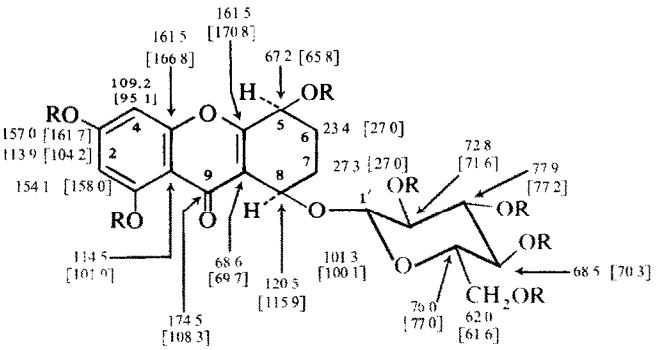
‡ To whom correspondence should be addressed.

possessing a free hydroxyl group at position 3. The high R_f value on polyamide TLC suggested that **1** must be a glycoside. When subjected to acid hydrolysis or treatment with β-D-glucosidase, it furnished, in addition to glucose, a compound (**2**) with lower R_f. Comparison of the UV spectra of **1** and **2** with and without the shift reagents (Table 1) showed no significant differences. Thus, the hydrolysable sugar was probably not attached to the chromophore. Compound **2** is rather unstable and is easily aromatized to 1,3-dihydroxyxanthone **3** [4].

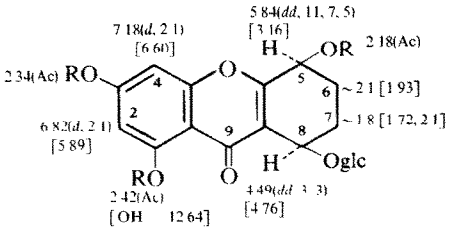
The proton-noise decoupled and off-resonance decoupled ^{13}C-NMR of campestroside **1** and its heptaacetate **1a**, mp 135°, $(M+1)^+$ peak at 721 (chemical ionization MS, methane carrier gas), showed the presence of the following groups for the aglycone moiety: two methylenes, two aliphatic methines, two aromatic methines, six quaternary carbons, and one carbonyl carbon (Scheme 1). Since only 2–3 mg each of campestroside and its heptaacetate were available, a microtube and micro-probe were employed for these measurements. Assignment was made by comparing data from literature for flavonoids [5] or xanthones [6].

The ^{1}H-NMR data for the aglycone portion are summarized in Scheme 2. Except for the 6-H and 7-H signals, all other proton chemical shifts and coupling constants, including those for the glucose moiety, were fully clarified by measurements of **1** and **1a** (at 270, 220 and 100 MHz) in different solvents, mixed solvents, and extensive decouplings. The 220 MHz spectrum of acetate **1a** taken in $CDCl_3$ containing 10% deuteriobenzene clearly shows the coupling pattern of most protons.

A free hydroxyl group peri to the carbonyl is present because of a chelated 12.64 δ signal in **1** (in DMSO-d_6). The 2.1 Hz J values of the two aromatic protons indicate that they are meta-coupled. These data, in conjunction with the presence of two aromatic acetates at 2.34 and 2.42 δ (characteristic of 1,3-dihydroxyxanthones [7]) complete derivation of the substitution pattern for C(1) through C(4). Presence of hydroxyl groups at C(1) and C(3) are corroborated by the UV shift data described above (Table 1). The spectrum of **1a** also showed the presence of five aliphatic acetyl groups: four of them, between 1.94 and 2.10 δ belonging to the sugar moiety and another one at 2.18 δ.



Scheme 1. ^{13}C-NMR (25 MHz) data of **1a** in $CDCl_3$.
Numerals in [ ] are ppm of **1** in DMSO-d



Scheme 2. ^{1}H-NMR (220 MHz) data of **1a** in $CDCl_3$
Numerals in[ ]are ppm of **1** in DMSO-d_6.

The contiguous proton system comprising C(5) through C(8) was clarified except for full analyses of the methylene protons attached to C(6) and C(7), irradiation at 2.1 δ (H-C(6)) caused the *dd* at 5.66 δ (H-C(5)) to collapse to a singlet. Similarly, irradiation at 1.5 δ (H-C(7)) changed the doublet of doublets at 4.81 (H-C(8)) to a singlet. These double resonance experiments were carried out in $CDCl_3$ containing 10% deuteriobenzene.

As far as the planar structure is concerned, there are only two alternative structures left for consideration, namely that with the glucose attached to C(5) and the other to C(8). Of the two carbinyl protons in **1** at 3.16 (clear *dd* shape) and 4.76 δ (triplet-shaped *dd*) the latter is clearly assignable to H-C(8) because of the carbonyl anisotropic effect. Upon acetylation the 3.16 ppm signal undergoes a large 2.68 ppm down-field shift (although in a different solvent) whereas the 4.76 δ signal is hardly affected. The glucose unit is hence attached to C(8).

The H-C(8) signal lacks large coupling constants (*dd*, $J_{1/2}$ = 3.3 Hz) and is therefore equatorial, i.e. the glucose moiety is quasi-axial. In contrast, the H-C(5) signal appears as a clear doublet of doublets (*dd*, $J_{1/2}$ = 11, 7.5 Hz). The H-C(5) thus is quasi-axial. In order to account for such coupling behaviors, the tetrahydro ring must adopt a quasi-chair form with axial 8-glucose and equatorial 5-OH, i.e. the two-oxygen substituents are *cis*. Molecular models show that if the shapes of the H-C(5) and H-C(8) signals were to be rationalized by a twist boat form having transoid 5- and 8-substituents, the methylenes at C(6) and C(7) would have to be almost totally eclipsed. This arrangement can therefore be ruled out. The glucose linkage at C(1') is β as evidenced by the value of $J_{1\,2}$ = 8 Hz.

DISCUSSION

The structure of campestroside, the first tetrahydroxanthone to be found in nature, can thus be expressed as in **1** except for its absolute configuration. Its occurrence was unexpected and is of great biogenetic and chemotaxonomic interest, especially because the corresponding xanthone glucoside possessing the same oxidation pattern and carrying the hydrolyzable sugar at the same position is present in relatively high concentrations in the same plant [1]. Campestroside, which has also been detected in *Gentiana germanica* Willd. and *Gentiana ramosa* Hegetschw., confirms the close similarity of the species of the *Amarella* section (subgenus *Gentianella*) [8].

EXPERIMENTAL

Gentiana campestris L. and *Gentiana germanica* Willd. were collected near Sainte-Croix, Vaud (Switzerland) and *Gentiana ramosa* Hegetschw. in the Simplon region (Switzerland). 90 g of dried leaves and stems of *G. campestris* were extracted by refluxing with solvents of increasing polarity [1]. The MeOH extract was chromatographed over a polyamide column (Macherey-Nagel SC_6) column (6.5 cm × 100 cm) starting with MeOH–H_2O (1:1) and increasing the percentage of MeOH. Fractions containing **1** were chromatographed a second time over a polyamide column (4 cm × 45 cm). A gradient elution starting with MeOH–H_2O (3:7) and switching to MeOH–H_2O (1:1) afforded a mixture of **1** and swertisin [2]. This mixture was subsequently separated by preparative-TLC on Si gel (type 60 F_{254} from Merck; 2 mm thickness), two developments using AcOEt–MeOH–H_2O (100:16.5:7) followed by AcOEt–MeOH (100:16.5) as solvents. **1** was eluted from the plate with MeOH (Soxlhet extraction) and purified by filtration on Sephadex LH20 (solvent MeOH). Recrystallization from MeOH afforded 10 mg pale yellow needles: mp 145° (dec.); R_f = 0.8 on polyamide Macherey–Nagel DC_{11}, MeOH–AcOH–H_2O (90:5:5) (solvent a); R_f = 0.33 on Si gel 60 F_{254} Merck AcOEt–MeOH–H_2O (100:16.5:7) (solvent b). Acid or enzymatic hydrolysis, as well as sugar identification, were carried out as described previously [1, 2]. Compound **2** (1,3,5,8-tetrahydroxy-5,6,7,8-tetrahydroxanthone) could only be obtained in a very small amount. R_f = 0.6 (solvent a); R_f = 0.75 (solvent b). For UV data, see Table 1. Acetylation of **1** (Ac_2O and Py) afforded a hexaacetate (**1a**): mp 135°, recrystallized from EtOH. Compound **1** was isolated in a similar manner from *G. germanica* Willd. and *G. ramosa* Hegetschw. Identification was carried out by comparison of the chromatographic behavior before and after hydrolysis as well as the UV spectra. The ^{1}H-NMR spectra were recorded at 100 MHz on a Varian HA-100 or at

220 MHz on a Varian HR-220 instrument. The ^{13}C-NMR spectra were recorded on a JEOL PS-100 Fourier transform spectrometer operating at 25.0 MHz. Chemical shifts are expressed in ppm with tetramethylsilane as int. stand.

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REFERENCES

1. Kaldas, M., Hostettmann, K. and Jacot-Guillarmod, A. (1974) *Helv. Chim. Acta* **57**, 2557.
2. Kaldas, M., Hostettmann, K. and Jacot-Guillarmod, A. (1975) *Helv. Chim. Acta* **58**, 2188.
3. De Barros Correa, D., Fonseca, L. B., Silva, E., Gottlieb, O. R. and Gonçalves, S. J. (1970) *Phytochemistry* **9**, 447.
4. Guyot, M. and Mentzer, C. (1965) *Bull. Soc. Chim. France* 2558.
5. Wagner, H., Chari, V. M. and Sonnenbichler, J. (1976) *Tetrahedron Letters* 1799.
6. Hostettmann, K., Jacot-Guillarmod, A. and Chari, V. M. (1976) *Helv. Chim. Acta* **59**, 2592.
7. Rivaille, P., Massicot, J., Guyot, M. and Plouvier, V. (1969) *Phytochemistry* **8**, 1533.
8. Kaldas, M (1977) PhD Thesis, University of Neuchâtel.