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XANTHONES, FLAVONES AND SECOIRIDOIDS OF AMERICAN *GENTIANA* SPECIES

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Key Word Index—*Gentiana*; Gentianaceae; xanthones: flavone *C*-glycosides; secoiridoid glycosides; chemotaxonomy.

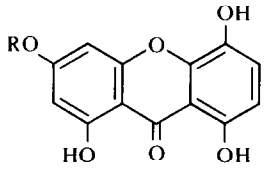
Abstract—Isoorientin, isoorientin-4′-*O*-glucoside, gentiopicrin, xanthones with 1,3,5,8 or 1,3,7,8-oxidation pattern have been isolated from five North American *Gentiana* species (*G. affinis, G. algida, G. calycosa, G. detonsa, G. strictiflora*) and one South American species (*G. cerastioides*). Separations were achieved with droplet counter-current chromatography (DCCC) or centrifugally accelerated preparative TLC. The distribution of the isolated compounds within the genus is discussed.

INTRODUCTION

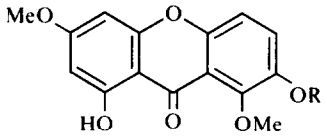
The genus *Gentiana* (Gentianaceae) contains about 400 species which are arranged, according to Kusnezov [1] in 19 sections and two subgenera, *Eugentiana* and *Gentianella*. More recently, several authors [2–6] adopted *Gentianella* as a separate genus. Among them, Smith [6] proposed a classification with 15 sections by deleting or regrouping some of the small Kusnezov sections.

Phytochemical investigation of numerous European species has led to highly interesting chemotaxonomic conclusions. Gentians are characterized by the presence of bitter principles (secoiridoid glycosides), flavone-*C*-glycosides and xanthone aglycones and glycosides. Whereas secoiridoid and flavone-*C*-glycosides occur in almost all of the species studied, xanthones were found to be the more useful markers [7]. The oxidation pattern of the xanthones is generally uniform within a particular section and is of prime importance in the chemotaxonomy of gentians. Species can be differentiated only by glycosylation characteristics.

Since no American gentian has been studied up to now and in order to confirm these earlier conclusions [7–9],



1 R = Me, bellidifolin
2 R = H, desmethylbellidifolin



4 R = Me, decussatin
3 R = H, gentiacaulein

Table 1. Distribution of the identified constituents in American *Gentiana* species

Species	Section	Xanthones	Flavone-*C*-glycosides	Secoiridoid
G. affinis	*Pneumonanthe*	—	**9, 10**	**11**
G. algida	*Frigida*	—	**9, 10**	**11**
G. calycosa	*Pneumonanthe*	—	**9, 10**	**11**
G. cerastioides	*Andicola*	**1, 2, 5, 6**	**9**	**11**
G. detonsa	*Crossopetalum*	**3, 4, 7, 8**	**9**	**11**
G. strictiflora	*Amarella*	**1, 2, 5, 6**	**9**	**11**

1 bellidifolin, **2** desmethylbellidifolin, **3** gentiacaulein, **4** decussatin, **5** bellidifolin-8-*O*-glucoside, **6** desmethylbellidifolin-8-*O*-glucoside, **7** decussatin-1-*O*-primeveroside, **8** gentiacaulein-1-*O*-primeveroside, **9** isoorientin, **10** isoorientin-4′-*O*-glucoside, **11** gentiopicrin.

Table 2. Distribution of xanthones and flavone-*C*-glycosides in the genus *Gentiana*

Subgenus	Section	Species studied	Xanthones		Flavone-*C*-glycosides	Ref
			oxidation pattern	mangiferin		
Eugentiana	*Coelanthe*	6	1, 3, 7	+*	+	[14] [15]
	Pneumonanthe	*G. affinis*	—	−	+	
		G. calycosa	—	−	+	
		G. asclepiadea	—	+	+	[16]
		G. pneumonanthe	—	+	+	[17] [18]
	Othophora	none				
	Stenogyne	none				
	Frigida	*G. algida*	—	−	+	
	Aptera		—	+	+	[19]
	Isomeria	none				
	Chondrophylla	2	—	−	+	[20] [21]
	Thylacites	5	1, 3, 7, 8	−	+	[22]
	Cyclostigma	8	1, 3, 7, 8	+	+	[23]
Gentianella	*Dasystephana*	none				
	Andicola	*G. cerastioides*	1, 3, 5, 8	−	+	
	Imaicola	none				
	Stylophora	none				
	Megacodon	none				
	Amarella	*G. strictiflora*	1, 3, 5, 8	−	+	
		G. campestris	1, 3, 5, 8 and 1, 3, 4, 5, 8	+	+	[8]
		G. germanica	1, 3, 5, 8 and 1, 3, 4, 5, 8	+	+	[8]
		G. ramosa	1, 3, 5, 8 and 1, 3, 4, 5, 8	+	+	[8]
	Antarctophila	3	1, 3, 5, 8 and 1, 3, 4, 5, 8	not studied		[24] [25]
	Arctophila	none				
	Crossopetalum	*G. detonsa*	1, 3, 7, 8	−	+	
		G. ciliata	1, 3, 7 and 1, 3, 7, 8	−	+	[9] [22] [26]

Classification according to Kusnezov [1].
* Mangiferin was found only in *G. lutea*.

we undertook the investigation of several American species (Table 1). Due to the scarcity of plant material, the main purpose of the present work was to determine the presence or absence of xanthones and to isolate the main constituents with separation techniques which have only recently become available.

RESULTS AND DISCUSSION

Extraction of the dried plant material (10–15 g of aerial parts) using solvents with increasing polarity has been reported previously [10]. The $CHCl_3$ extract was examined by TLC for the presence of free xanthone aglycones and subsequently purified by droplet counter-current chromatography (DCCC) with $CHCl_3$–MeOH–H_2O (13:7:4) in the descending mode [11]. From *Gentiana strictiflora* (Rydb.) A. Nels. (= *Gentianella amarella* (L.) Börner subsp. *acuta* [Michx.] J. M. Gillett) and from *G. cerastioides* Kunth, bellidifolin (**1**) and desmethylbellidifolin (**2**) could be obtained directly in a pure form. Centrifugally accelerated prep. TLC on silica with $CHCl_3$ of the crude extract of *G. detonsa* (Rottb.) Don, furnished gentiacaulein (**3**) and decussatin (**4**) whereas no aglycone could be detected in the $CHCl_3$ extracts of *G. algida* Pall., *G. affinis* Griseb. and *G. calycosa* Griseb. The MeOH extract containing the glycosides was submitted to column chromatography on Sephadex LH20. DCCC of the main fractions with $CHCl_3$–MeOH–PrOH–H_2O (9:12:1:8) or (5:6:1:4) afforded gentiopicrin [12] and isoorientin.

From *G. strictiflora* and *G. cerastioides*, bellidifolin-1-*O*-glucoside and desmethylbellifolin-1-*O*-glucoside were also isolated by DCCC [11]. Acid hydrolysis of the MeOH extracts confirmed the presence of xanthone-*O*-glycosides. The aglycones obtained were readily separated by centrifugally accelerated TLC. In addition, isoorientin-4′-*O*-glucoside has also been isolated.

Identification was made from UV data by comparing the spectra before and after cleavage of the sugars and by comparison with authentic samples previously isolated from other *Gentiana* species. The results are summarized in Table 1. An additional secoiridoid glycoside, which is not indicated here, was isolated from *G. algida* [11]. Its structure determination is currently in progress and will be published separately [13].

As mentioned above, isoorientin **9** and gentiopicrin **11** are not useful chemotaxonomic markers since they occur in all species. However the presence of isoorientin-4′-*O*-glucoside **10** in *G. affinis*, *G. algida* and *G. calycosa* is interesting since this glycoside was identified in related European species belonging also to *Eugentiana*. The position of the investigated American gentians in the classification of Kusnezov as well as the distribution of flavone-*C*-glycosides and xanthones are given in Table 2.

It is noteworthy that 1,3,7,8-tetraoxygenated and 1,3,7-trioxygenated xanthones occur only in some species of *Eugentiana* and in *G. detonsa* and *G. ciliata* (*Gentianella*, section *Crossopetalum*). On the other hand, the species of *Gentianella* are characterized by the presence of 1,3,5,8-tetraoxygenated and 1,3,4,5,8-pentaoxygenated xanthones. This might justify the exclusion of section *Crossopetalum* from *Gentianella*. Indeed, on the basis of morphological and cytological criteria, Ma [27] proposed a new genus for *Crossopetalum*. Whereas 1,3,7,8-oxidized xanthones occur in *Eugentiana* (*Thylacites* and *Cyclostigma*) and 1,3,5,8-oxidized xanthones in all the sections of *Gentianella* studied, these two oxidation patterns never occur simultaneously in *Gentiana* but co-occur in *Swertia*, a closely related genus [8, 28].

The present study shows that the oxidation pattern of xanthones is a chemotaxonomic character of prime importance. It confirms the anatomical, morphological, cytological, palynological and caryological findings which led recently to the separation of *Gentianella* from *Gentiana* [2–6]. At the present time numerous authors recognize this distinction and thus, subgenus *Eugentiana* becomes genus *Gentiana*, subgenus *Gentianella* becomes genus *Gentianella*. For section *Crossopetalum* the genus *Gentianopsis* has been proposed [27].

The xanthone-*C*-glucoside mangiferin **11** is encountered in several species, but further studies are necessary to draw any conclusion about its chemotaxonomic significance. It would be of great interest to investigate at least one or two species of the sections which have not been studied at this date in order to complete the chemotaxonomy of *Gentiana* and *Gentianella*.

EXPERIMENTAL

Origin of plant material. *G. affinis* Griseb.: Albany, Wyoming; *G. algida* Pall.: Mount Evans, Colorado; *G. calycosa* Griseb.: Red Mountain Pass, Colorado; *G. detonsa* (Rottb.) Don: south of Yellowstone National Park, Wyoming; *G. strictiflora* (Rydb.) A. Nels.: Albany, Wyoming; *G. cerastioides* Kunth: Cotopaxi Mountain, Ecuador.

Isolation. The aerial parts of the plants were extracted successively with petrol, $CHCl_3$ and MeOH at room temp. All the DCCC separations were carried out on a Model A apparatus (Tokyo Rikakikai, Tokyo, Japan). The apparatus consists of a number of glass tubes (length 400 mm, i.d. 2 mm) interconnected in series by capillary Teflon tubes (i.d. 0.5 mm). In the present studies, 300 tubes were used. The flow-rate was 10–15 ml/hr, depending on the solvent system, and the eluates were collected in 1–3 ml fractions. The fractions were monitored by UV spectroscopy at 300 nm or checked by TLC on pre-coated silica gel aluminium sheets (Merck). The solvent systems were the lower layer of $CHCl_3$–MeOH–H_2O (13:7:4), $CHCl_3$–MeOH–PrOH–H_2O (9:12:1:8) or $CHCl_3$–MeOH–PrOH–H_2O (5:6:1:4). Centrifugally accelerated prep. TLC was achieved with a Chromatotrom Model 7924 (Harrison Research, Palo Alto, USA). The rotors were coated with silica gel (layer thickness 2 mm) and $CHCl_3$ or $CHCl_3$ with increasing amounts of MeOH were used as solvent (flow rate 5 ml/min). Up to 200 mg of sample could be separated in about 30 min. MeOH was the solvent used for Sephadex LH20 column chromatography.

Hydrolysis and recording of the UV spectra with the usual shift reagents were made according to standard procedures [29]. R_f values and spectral data of all the isolated compounds were in accordance with authentic samples.

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