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Chemotaxonomical Investigations of the Genera *Blackstonia* and *Centaurium* (*Gentianaceae*)*

By

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Key Words: Angiosperms, *Gentianaceae, Centaurium, Blackstonia.* — Secoiridoid glucosides, xanthones, chemotaxonomy.

Abstract: Methanolic extracts from aerial parts and capsules of plants of 5 populations of *Blackstonia perfoliata* and 99 populations of nine European and two American *Centaurium* species (*Gentianaceae*) have been screened by means of TLC for the secoiridoid glucosides: sweroside, swertiamarin, gentiopicroside and the m-hydroxybenzoyl esters of sweroside, namely centapicrin, desacetylcentapicrin, decentapicrin A and B as well as for the xanthones: 1,8-dihydroxy-3,5-dimethoxyxanthone, 1,8-dihydroxy-3,7-dimethoxyxanthone, 1,8-dihydroxy-3,5,6,7-tetramethoxyxanthone and xanthone-β-mono-glucosides. The taxonomical significance of the results is discussed. On the basis of chemotaxonomical evidence two *Centaurium* species, *C. pulchellum* and *C. tenuiflorum*, are placed in sect. *Parviflora* instead of sect. *Centaurium* subsect. *Parviflora*.

In many countries the aerial parts of *Blackstonia perfoliata* (L.) HUDSON and/or *Centaurium* species (*Gentianaceae*) are collected in nature for use in popular medicine. Some of these plant parts are described in national pharmacopoeias as crude plant drugs (IMBESI 1964). The crude drug "Centaurii herba" for instance is or has been described in the pharmacopoeias of many European countries. The various pharmacopoeias are not in agreement regarding the species of *Centaurium* from which this crude drug should come. These discrepancies are due mainly to the confusion about the nomenclature and delimitation of *C. erythraea* s.l. (see also Table 1).

* Part 8 in the series "Secoiridoids and Xanthones in the genus *Centaurium*". For part 7 see: VAN DER SLUIS & LABADIE (1985). – Parts of this study were presented at the 10th annual congress of "Farmacognosie en Natuurstofchemie" in Utrecht, Nederland, Nov. 11, 1983. For summary see: VAN DER SLUIS, W. G., & LABADIE, R. P. (1984), Pharm. Weekbl. **119**, 905–906.

Many synonyms for *C. erythraea* RAFN (MELDERIS 1972 a, b) are used to designate the plant origin of the crude drug "Centaurii herba"; these include *C. erythraea* RAFN[1] [Brit. Herbal Pharm. (1979)], *C. minus* MOENCH [DAB 8 (1978); ÖAB (1981)], *C. umbellatum* GILIBERT [Pharm. Franc. VIII (1965); Pharm. Helv. VI (1971); DAB 7, 2e Nachtrag (1975)], *Erythraea centaurium* (L.) PERSOON [DAB 6 (1936); Ned. Pharm. V (1940)] (SAKINA & AOTA 1976, TAKAGI & YAMAKI 1982). – *Centaurium* species also permissible according to some pharmacopoeias: *C. pulchellum* (SW.) DRUCE [Pharm. ROSS. IX (1961); Hung. Pharm. III (1970)]; *C. uliginosum* (W. & K.) BECK[2] [Hung. Pharm. III (1970)].

Table 1. Some differences in the nomenclature and delimitation of *Centaurium erythraea* s.l. according to ZELTNER (1970) and MELDERIS (1972)

ZELTNER (1970)	MELDERIS (1972)
C. minus GARS. subsp. *minus*	*C. erythraea* RAFN subsp. *erythraea*
C. majus (H. & L.) ZELTNER subsp. *majus* var. *majus*	subsp. *majus* (H. & L.) MELDERIS
subsp. *majus* var. *suffruticosum* (SALZMANN) ZELTNER	*C. suffruticosum* (GRISEB.) RONN.

The delimitation of *C. erythraea* is also disputed. The Pharm. Franc. VI (1965) advocates the use of the var. *suffruticosum* from Algerie because of its bright red flowers. A large percentage of the commercially available "Centaurii herba" belongs to this taxon. Its status however is very much debated. Both ZELTNER (1970) and MELDERIS (1972 a, b) described this taxon as a species and separated it from *C. erythraea* (Table 1). Whereas MELDERIS (1972 a, b) regarded it as a species, *C. suffruticosum* (GRISEB.) RONN., ZELTNER (1970), on the other hand, treated it as a variety of *C. majus* subsp. *majus* (Table 1).

On the basis of our results we agree with ZELTNER (1970) that the separation of *C. majus* from the *C. erythraea* s.l. complex is more useful than the separation of *C. suffruticosum* only. In this paper we use the nomenclature and delimitation of *C. majus* in the sense of ZELTNER (1970). The nomenclature and status of all the other *Centaurium* taxa are according to MELDERIS (1972 a, b) as described in Flora Europaea.

[1] The author's name is in fact RAFN and not RAFINESQUE-SCHMALZ.
[2] Syn.: *C. littorale* subsp. *uliginosum* (W. & K.) MELDERIS.

The dried parts of *B. perfoliata* have also been described as a possible substitute for "Centaurii herba" (FOURNIER 1947).

To investigate whether there is any chemical basis for substituting these species and plants of different habitats a semiquantitative chemical survey, using TLC, was instituted. The methanolic extracts from the aerial parts and capsules of plants of *B. perfoliata,* nine European and two American *Centaurium* species have been investigated, most of them from a number of populations. Special attention was paid to the occurrence of secoiridoid glucosides and some 1,8-dihydroxyxanthones. These compounds, especially the secoiridoid glucosides, are not only important in view of their pharmacological activity, but they can also be used as an important character for classification (HEGNAUER & KOOIMAN 1978, JENSEN & al. 1975).

Systematics of the Genera *Blackstonia* and *Centaurium*

Comprehensive reports on the systematics and distribution of the genus *Blackstonia* HUDSON and/or the genus *Centaurium* HILL (*Gentianaceae*) have been published by MELDERIS (1931, 1972 a, b), ZELTNER (1970), TUTIN (1972) and by JÄGER (1978).

The distribution of the genus *Blackstonia* is limited to the Mediterranean-Atlantic region in Europe and Africa (JÄGER 1978). Most authors (e.g. TUTIN 1972 and JÄGER 1978) consider this genus to consist of only one species, *Blackstonia perfoliata,* with four subspecies, whereas ZELTNER (1970) distinguishes four species. In this paper we use the nomenclature and divisions applied by TUTIN (1972) in Flora Europaea.

According to ZELTNER (1970) the basic chromosome number of *Blackstonia* is x = 10. Two subspecies are diploid and the other two, viz.: subsp. *perfoliata* and subsp. *serotina* (KOCH ex REICHB.) VOLLMANN are usually tetraploid.

The genus *Centaurium* is distributed chiefly in the northern hemisphere; in Europe it occurs especially around the Mediterranean with a concentration in the Iberian Peninsula; in America its main centre is in California and Mexico (JÄGER 1978). Difficulties have been encountered in taxonomical studies of the genus since the species are extremely variable. Parallel variation in several characters is common in a group of related species, and natural hybridization has also been reported. Species and infraspecific taxa are, therefore, often difficult to define and the literature abounds with confusing nomenclature (MELDERIS 1931, 1972 a, UBSDELL 1976 a). For this reason it is impossible to determine the exact number of species in this genus. Estimates vary from 30 to 50 (JÄGER 1978). ZELTNER (1970) made a valuable contribution to a better understanding of the relationship of the European *Centaurium* species by publishing the

results of his morphological, ecological and geographical investigation supplemented by extensive karyological studies.

According to ZELTNER (1970) the usual basic chromosome number of the European *Centaurium* species is x = 10; one species has x = 11. He found most of these species to be diploid, a few tetraploid, one hypotetraploid (2 n = 36); three had diploid and tetraploid cytodemes, the diploids occurring in the Mediterranean region and the tetraploids in central and northern Europe.

Table 2. Classification of the European *Centaurium* species into sections, subsections and greges according to various taxonomists

GRISEBACH (1839), MELDERIS (1931)	RONNIGER (1916)	ZELTNER (1970)
Sect. *Spicaria* GRISEB.	Sect. *Spicaria* GRISEB.	Sect. *Spicaria* GRISEB.
Xanthea REICHB.	*Xanthea* REICHB.	*Xanthea* REICHB.
Centaurium (*Eu-erythraea* GRISEB.)		
subsect. *Caespitosae* (RONN.) MELD.	*Caespitosae* RONN.	*Caespitosa* RONN.
		Centaurium subsect. *Parviflora* (RONN.) MELD.
subsect. *Parviflorae* (RONN.) MELD.	*Parviflorae* RONN.	
subsect. *Vulgares*		
Grex *Linariaefoliae*	*Linariaefoliae* WITTROCK	*Vulgaria* (MELD.) ZELTNER
Grex *Centaurium* (*Centauria*)	*Centaurium* (*Centauria* WITTROCK)	*Centaurium*

The species of *Centaurium* often are placed in a number of sections, one of which, sect. *Trichostylus* GRISEB., contains only American species (GILG 1897, MELDERIS 1931, ZELTNER 1970, JÄGER 1978).

The European *Centaurium* species were placed by GRISEBACH in three several sections. Some authors split up his sect. *Centaurium* (*Eu-erythraea*) into sections or subsections (Table 2). On the basis of biosystematic studies MELDERIS (1931) placed the European taxa in three sections and accepted three subsections in sect. *Centaurium*, whereas ZELTNER (1970) treated subsect. *Caespitosa* as a fourth section (Table 2).

Our results, however, support the separation of subsect. *Parviflora* (RONN.) MELDERIS from sect. *Centaurium* (Fig. 1). This results in the following division.

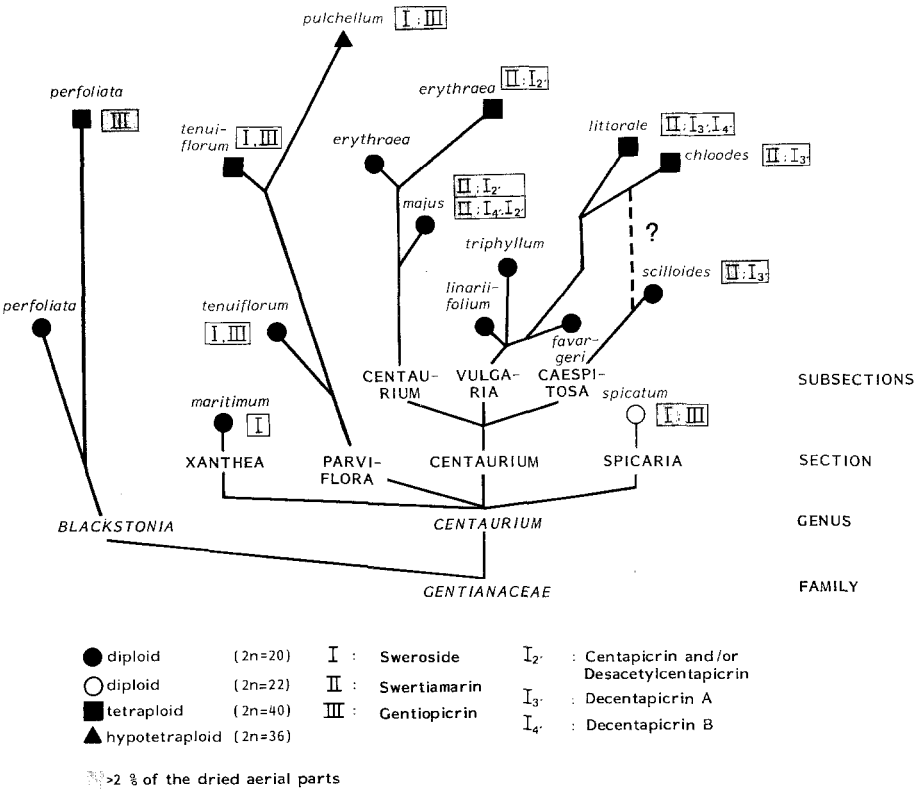


Fig. 1. Phylogenetic relationship between the European *Blackstonia* and *Centaurium* species according to ZELTNER (1970), slightly modified and supplemented by the main secoiridoid accumulation findings

I: sect. *Xanthea* RONN., containing only *C. maritimum* (L.) FRITSCH (2 n = 20).

II: sect. *Spicaria* (GRISEB.) RONN., containing *C. spicatum* (L.) FRITSCH (2 n = 22) and some related non-European species.

III: sect. *Parviflora* RONN., containing the annual European species, *C. pulchellum* (SW.) DRUCE (2 n = 38), *C. tenuiflorum* (HOFFMANNS. et LINK) FRITSCH (2 n = 20 or 40). – MELDERIS (1931) also included the American species *C. chilense* and *C. quitense* in this section. Our results, however, do not indicate a close relationship.

IV: sect. *Centaurium*, the largest section, containing about ten species, is split up into the following subsections:

A.: subsect. *Caespitosa* (RONN.) MELDERIS, containing the perennial *C. scilloides* (L. fil.) DRUCE (2 n = 20).

B.: subsect. *Vulgaria* (MELDERIS) ZELTNER, containing the narrow-leaved biennial species, of which *C. favargeri* ZELTNER, *C. triphyllum* (W. L. E. SCHMIDT) MELD. and *C. linariifolium* (LAM.) G. BECK are diploid 2 n = 20) and *C. littorale* (D. TURNER) GILMOUR and *C. chloodes* (BROT.) SAMP. are tetraploid (2 n = 40). Hexaploid plants (2 n = 60), closely resembling *C. littorale* in their morphology, proved to be hybrids of *C. littorale* and *C. erythraea* (UBSDELL 1976 b, 1979).

C.: subsect. *Centaurium,* containing the broad-leaved biennial species *C. erythraea* RAFN (2 n = 20 or 40) and *C. majus* (HOFFMANNS. et LINK) ZELTNER (2 n = 20 or 40).

Chemical Constituents

Secoiridoid Glucosides. Secoiridoid glucosides, which are secondary iridoids derived biosynthetically from an iridoid glucoside by cyclopentane ring cleavage (INOUYE & al. 1976) (Fig. 2), are known to accumulate in many species of the *Gentianaceae* and some related families (JENSEN & al. 1975). In *Blackstonia perfoliata* and some *Centaurium* species secoiridoid glucosides of the sweroside-type, bearing a vinyl group at C-9, have been reported as the main constituents in the aerial parts; gentiopicroside (gentiopicrin) (III) is the main secoiridoid glucoside found in *B. perfoliata* (FOURNIER 1947, VAN DER SLUIS & al. 1983), whereas in *C. spicatum* and in *C. pulchellum* it is sweroside (I) (VAN DER SLUIS & LABADIE 1978, 1981 b). In addition to these glucosides some m-hydroxybenzoyl esters of sweroside (I) accumulate in the ripe capsules of some *Centaurium* species; centapicrin ($I_{2'a}$) and desacetylcentapicrin ($I_{2'}$), both extremely bitter, occur in *C. erythraea* (SAKINA & AOTA 1976, VAN DER SLUIS & LABADIE 1978), decentapicrin A ($I_{3'}$) and decentapicrin B ($I_{4'}$) occur in *C. littorale* (VAN DER SLUIS & LABADIE 1981 a) (Fig. 3) and decentapicrin A in *C. linariifolium* (SEOANE, personal communication).

Xanthones. Rather simple polyoxygenated xanthones, oxygenated at least at C-1, C-3, and C-5 or C-7 with hydroxyl, methoxyl or O-glycosyl groups, are known to accumulate in many species of the *Gentianaceae* (CARPENTER & al. 1969, HOSTETTMANN & WAGNER 1977). These xanthones are very probably biosynthetized via the shikimate-malonate pathway with a benzophenone as intermediate (INOUYE & NAKAMURA 1971) (Fig. 4). The oxygenation pattern of the xanthones accumulating in plant taxa seems to be of taxonomical importance (CARPENTER & al. 1969, DA MATA REZENDA & GOTTLIEB 1973, HOSTETTMANN & WAGNER 1977).

From *Blackstonia perfoliata* we have isolated 1-hydroxy-3,7,8-trimethoxyxanthone and 1,8-dihydroxy-3,7-dimethoxyxanthone (L_3)

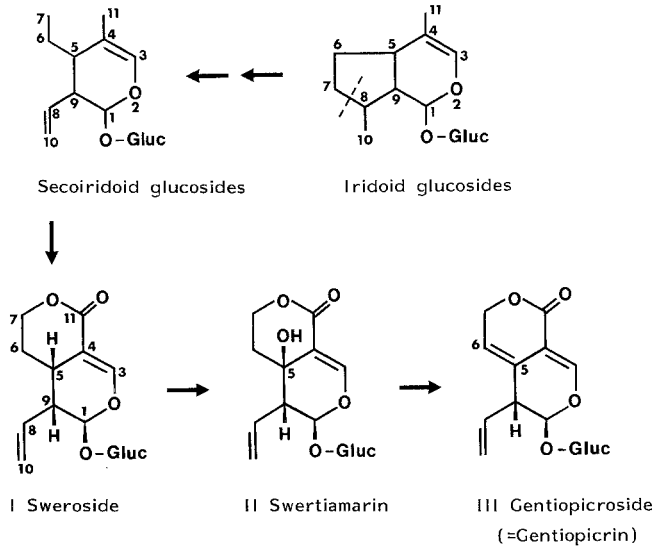
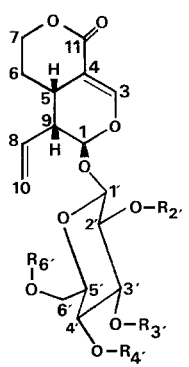


Fig. 2. Possible biosynthetic pathway of *Gentianaceae* secoiridoid glucosides according to INOUYE & al. (1976) and structural formulas of the iridoid glucosides isolated from *Gentianaceae* species



		$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{6'}$
I	Sweroside	H	H	H	H
I 2'a	Centapicrin	mOHB	Ac	H	H
I 2'	Desacetylcentapicrin	mOHB	H	H	H
I 3'	Decentapicrin A	H	mOHB	H	H
I 4'	Decentapicrin B	H	H	mOHB	H
I 6'	Decentapicrin C	H	H	H	mOHB

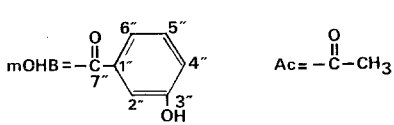


Fig. 3. Structural formulas of m-hydroxybenzoyl esters of sweroside, isolated from *Centaurium* species

(VAN DER SLUIS, to be published). To our knowledge no other xanthones have been reported for this species.

In *Centaurium* species the xanthone spectrum is rather complex. Xanthones with one or more of the oxygenation patterns: 1-2-3-5, 1-3-5-6, 1-3-5-8, 1-3-7-8, 1-3-6-7-8, and 1-3-5-6-7-8, have been isolated from *C.*

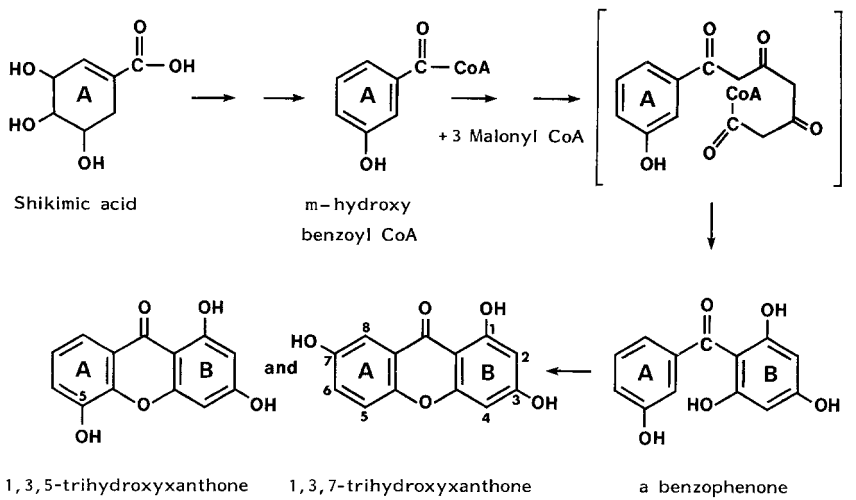


Fig. 4. Possible biosynthetic pathway of *Gentianaceae* xanthones according to INOUYE & NAKAMURA (1971)

Table 3. 1,8-dihydroxy-x-methoxyxanthones, isolated from *Blackstonia* and *Centaurium* species

Species References	Plant part	Oxygenation pattern			
		1, 3, 5, 8	1, 3, 7, 8	1, 3, 5, 6, 8	1, 3, 5, 6, 7, 8
B. perfoliata VAN DER SLUIS, to be published	root		+		
C. canchanlahuen VERSLUYS & al. 1982	whole plant	+	+		+
C. erythraea TAKAGI & YAMAKI 1982	whole plant	+	+	+	+
C. linariifolium PARRA & al. 1984	whole plant		+	+	+
C. littorale VAN DER SLUIS 1976, VAN DER SLUIS & LABADIE 1985	root	+			+
C. pulchellum MIANA & AL-HAZIMI, 1984	whole plant	+	+		

littorale (VAN DER SLUIS 1976, VAN DER SLUIS & LABADIE 1985), *C. canchalahuen* (VERSLUYS & al. 1982), *C. erythraea* (TAKAGI & YAMAKI 1982, NESSHTA & al. 1982, 1983 a, b), *C. linariifolium* (PARRA & al. 1984, Seoane, personal communication) and *C. pulchellum* (MIANA & AL-HAZIMI 1984). The 1,8-dihydroxyxanthones isolated from the above-mentioned species are listed in Table 3 and the structural formulas are given in Fig. 5. In addition to these xanthones we have detected with TLC the presence of numerous more polar xanthone aglycones and glycosides.

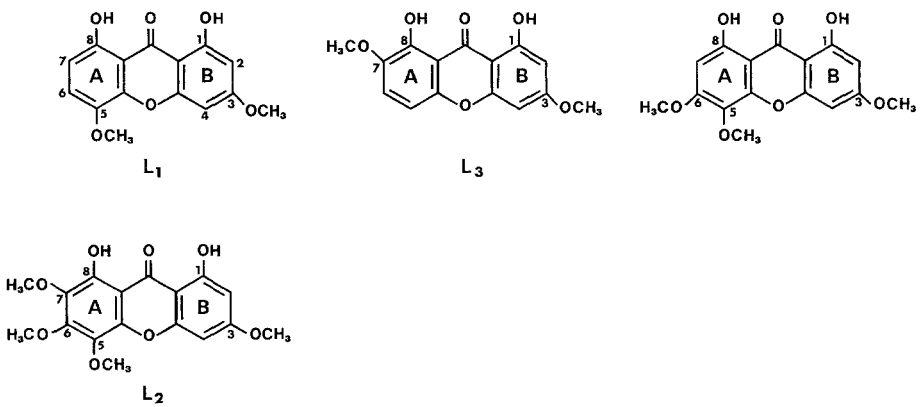


Fig. 5. Structural formulas of the 1,8-dihydroxyxanthones isolated from *Centaurium* species. Carbon numbering is according to their biosynthetic origin, ring B being of polyketide and ring A of shikimate origin

Material and Methods

Plant Material. The origins of the investigated populations of *Blackstonia* and *Centaurium* species are listed in Table 4. Photographs of specimens of a *C. erythraea* population and of two *C. majus* populations are presented in Fig. 9.

Chromosome counts on some of this plant material were made by Drs. J. C. VAN LOON of the department of "Populatie- en Evolutiebiologie", Rijksuniversiteit Utrecht, using the method described by VAN LOON (1974). The plants involved were grown in the botanical garden. The identification of the plant material was based on the treatment by MELDERIS (1972 b) in Flora Europaea, except in the case of *C. majus* which was treated according to ZELTNER (1970). Some voucher specimens have been deposited in the Herbarium of the "Farmaceutisch Laboratorium" and others in the Herbarium of the department of "Populatie- en Evolutiebiologie". All these specimens will be deposited later in the central Herbarium of the "Rijksuniversiteit Utrecht" (U). A few herbarium specimens from that central Herbarium (U) and from the "Rijksherbarium" in Leiden (L) were investigated as well.

Extraction of Plant Material. The air-dried and ground plant material was exhaustively extracted with methanol. The methanol was evaporated and the residue dissolved in methanol to give a concentration of 0.2 g plant material per ml methanol.

Authentic Samples. Sweroside (I) and gentiopicroside (III) were isolated from *C. spicatum* (F-767S3/4), (VAN DER SLUIS & LABADIE 1981 b); swertiamarin (II), decentapicrin A ($I_{3'}$) and B ($I_{4'}$) and the xanthones L_1 and L_2 from *C. littorale* (F-749V16) (VAN DER SLUIS & LABADIE 1981 a, 1985), centapicrin ($I_{2'a}$) and desacetylcentapicrin ($I_{2'}$) from *C. erythraea* (F-749C4) and the xanthone L_3 from *B. perfoliata* (F-768B2) (VAN DER SLUIS, to be published).

Chemical Methods. Identification of the chemical compounds.
Thin-layer chromatography (TLC) was used in the following ways:

a) sorbent layer: precoated silica gel 60 F-254, 10 × 10 cm (Merck),
solvent: ethyl acetate-methanol-water (77 : 15 : 8),
b) sorbent layer: as under a),
solvent: toluene-light petroleum 40–60°-ethyl formate-formic acid (42 : 42 : 14 : 2) (VAN DER SLUIS & LABADIE 1985),
c) sorbent layer: silanized precoated silica gel 60 F-254, 10 × 10 cm (Merck),
solvent: ethyl formate, saturated with water (VAN DER SLUIS & LABADIE 1981 a),
d) sorbent layer: as under c),
solvent: toluene, saturated with water (VAN DER SLUIS & LABADIE 1985).

Chromatography plates are allowed to develop in unsaturated chambers over a distance of 8 cm. Compounds are detected by observing the plates in UV_{254}, in daylight and in UV_{366} after the plates have been sprayed with 5% KOH in methanol, fast blue salt B reagent and diluted sulphuric acid, and then heated with a hair drier for about 10 minutes (VAN DER SLUIS & LABADIE 1981 a).

TLC-methods a and c were used to identify the secoiridoid glucosides as well as the unknown components X_1, X_2, X_3, and Z. Two µl of the methanolic plant extract as well as of a standard solution containing 1 mg of each sweroside (I), swertiamarin (II) and gentiopicroside (III) per ml methanol and two µl of a standard solution containing 1 mg of each centapicrin ($I_{2'a}$), desacetylcentapicrin ($I_{2'}$), decentapicrin A ($I_{3'}$) and decentapicrin B ($I_{4'}$) per ml methanol were applied to the TLC-plates. It was only with TLC-system c that sweroside (I) could be separated from swertiamarin (II) and centapicrin ($I_{2'a}$) from decentapicrin A (VAN DER SLUIS & LABADIE 1978, 1981 a). All the secoiridoid glucosides as well as the unknown compounds surveyed for quenched in UV_{254}. The m-hydroxybenzoyl esters of sweroside were detected rather specifically but not very sensitively after the plates had been sprayed with the reagent solution and heated. The spots of these compounds are red in daylight and in UV_{366} (VAN DER SLUIS & LABADIE 1981 a). The spots of the compounds X_1, X_2, and X_3 gave a blue fluorescence after the plates had been sprayed with KOH solution. When TLC-method a was used the unknown compound(s) Z appeared as a spot just above that of gentiopicroside (III) whereas with TLC-method c this compound stayed at the base. The spot was colored pinkish purple with the fast blue salt reagent.

TLC-methods b and d were used to identify the 1,8-dihydroxyanthone aglycones L_1, L_2, and L_3. Four µl of the methanolic plant solution and four µl of a standard solution containing 1 mg of each of the standard compound L_1, L_2, and L_3 per ml methanol were applied to the plates. With both TLC-systems all three xanthones were separated from each other and were colored blue to red with the reagent. The xanthone-β-mono-glucosides were identified by two-dimensional

TLC, performed on TLC-plates precoated with silica gel. Four µl of the methanolic plant extract were applied. The plate was developed in the first direction with the solvent used in TLC a. After enzymatic hydrolysis with β-glucosidase on the plate, as described by LABADIE & MORRIËN (1978) and after drying, the plate was developed in the second direction with the solvent used in TLC b (Fig. 6).

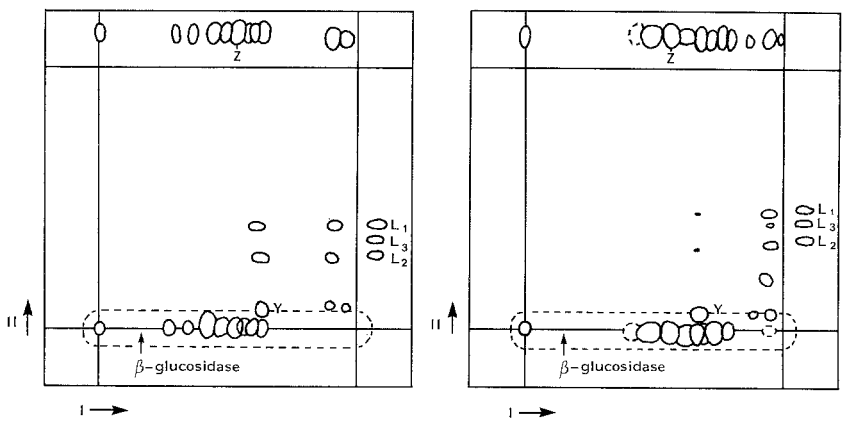


Fig. 6. Examples of two-dimensional thin-layer chromatograms of methanolic extracts from capsules of *C. tenuiflorum*, F768P13 (left) and *C. pulchellum*, F748P5 (right). The sample area of the TLC plate after the run in the first direction is incubated with β-glucosidase

Results and Discussion

Characteristic thin-layer chromatograms of secoiridoid glucosides from the aerial parts of *Blackstonia* and *Centaurium* species and of the capsules of *Centaurium* species are shown in Fig. 7 and in Fig. 8 respectively. Two-dimensional chromatograms of xanthone aglycones and xanthone-β-mono-glucosides from the capsules of *C. tenuiflorum* and *C. pulchellum* are shown in Fig. 6.

The combined results of the chemical survey of the aerial parts, roots and capsules or inflorescences of plants of five populations of *Blackstonia perfoliata* and of 99 populations of nine European and two American *Centaurium* species are presented in Table 4. In this Table populations of putative hybrids are listed within the species which they resemble most closely.

The characteristic chemical accumulation patterns found in those species of which at least two populations were investigated are shown in Table 5. The compounds listed are only those that were screened for and

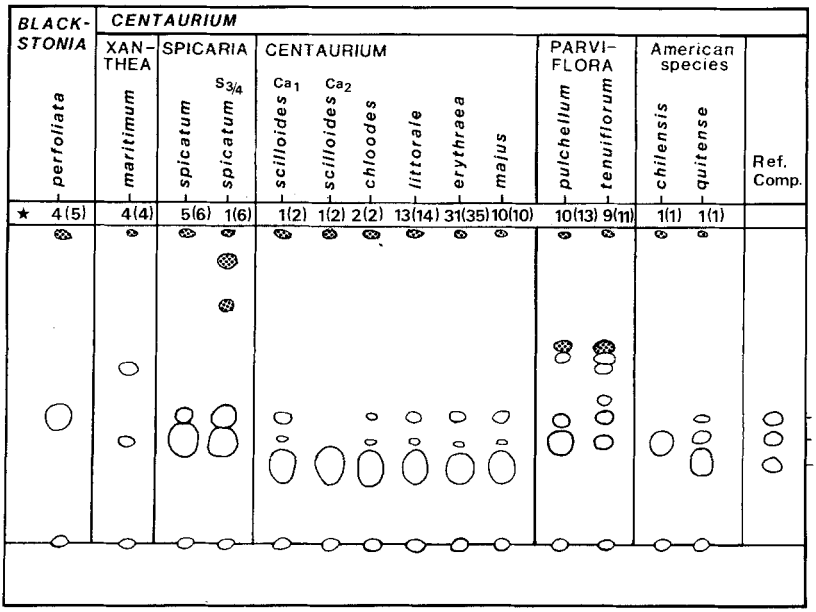


Fig. 7. Examples of thin-layer chromatograms of methanolic extracts from the aerial parts of *Blackstonia* and *Centaurium* species using TLC-system c. ★: number of populations showing this chromatogram; (): number of populations investigated; dotted areas: purple with fast blue salt reagent

that accumulate in detectable amounts in the parts of the plants investigated with the rather insensitive TLC-screening systems. Even if some compounds are not recorded, they may nevertheless be present in small quantities. We were able to isolate the xanthone L_3 from plants of a *Blackstonia perfoliata* population (B 2)[3] even though this compound could not be detected with the TLC-screening methods. Other detectable components, particularly other xanthones, are not included in Tables 4 and 5.

The plants investigated were not all at the same stage. Most plants were investigated when they were flowering and fruiting; some, on the other hand, were studied when they were flowering, and one population of plants was investigated before anthesis.

A survey of *C. pulchellum* and *C. erythraea,* both from the Bijlmermeer near Amsterdam, at different growth stages indicated that there was not a great difference in the amounts of the compounds screened for in the aerial parts at these growth stages; however, there was a striking difference in the

[3] The collection numbers are given mostly with only the last codes (e.g. only B 2 is given instead of F-768B2). For the origins of the populations see Table 4.

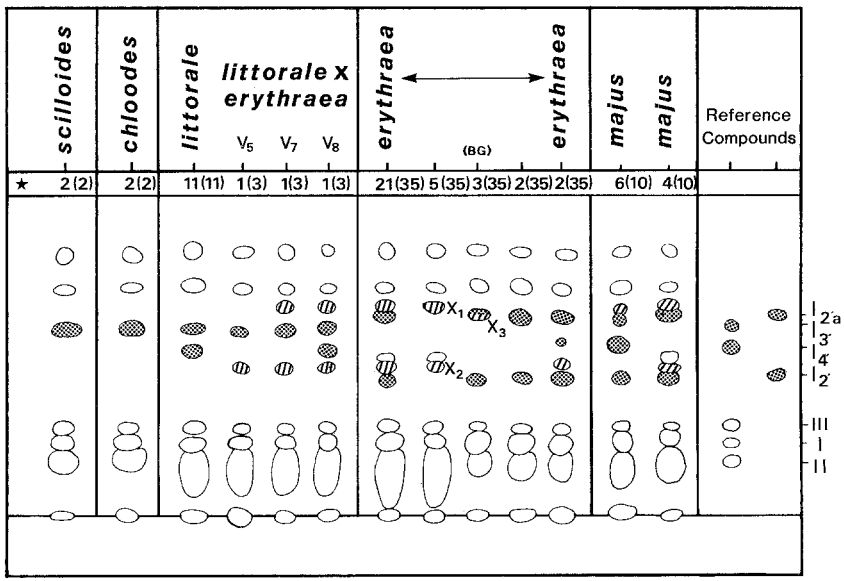


Fig. 8: Examples of thin-layer chromatograms of methanolic extracts from capsules of *C. scilloides*, *C. chloodes*, *C. littorale*, *C. erythraea*, and *C. majus* using TLC-system c. ★: number of populations showing this chromatogram; ( ): number of populations investigated; dotted areas: red with fast blue salt- H_2SO_4 reagent; hatched areas: blue fluorescence in UV_{366} with KOH reagent

compounds that accumulated in the flowers and capsules. Whereas centapicrin ($I_{2'a}$), desacetylcentapicrin ($I_{2'}$) and the unknown components X_1 and X_2 could not or could hardly be detected in the flowers of *C. erythraea* they were abundant in the capsules. This means that a chemical survey of the flowers is of only limited value as a substitute for a survey of the capsules as far as these constituents are concerned. Even if some compounds are not recorded in the flowers they may be present in the capsules.

As can be seen from Tables 4 and 5 and Figs. 7 and 8 the secoiridoids and xanthones that accumulate in the plants proved to be a very simple and reliable chemotaxonomical character. The differences in the accumulation patterns of these compounds within the species are in good agreement with the generally accepted division into genera, sections, subsections and species. Practically the only exceptions are those species that we have placed in sect. *Parviflora*.

The Genus *Blackstonia* Hudson (*B. perfoliata*)

The secoiridoids and xanthones that accumulated in the plants of the five *B. perfoliata* populations from southern Europe, one population of

subsp. *serotina* (B 1) and four populations of subsp. *perfoliata,* were almost the same. Only a few xanthones could be detected in *B. perfoliata,* L_3 being the only xanthone surveyed for that could be demonstrated. In two populations, one of which was used for the isolation of that xanthone and the less polar 1-hydroxy-3,7,8-trimethoxyxanthone (VAN DER SLUIS, to be published), L_3 was present in smaller concentrations than was detectable under the working conditions for the TLC-screening (Table 4).

In agreement with data reported in literature (FOURNIER 1947) gentiopicroside (III) is practically the only detectable secoiridoid glucoside in *B. perfoliata.* It was found to be present in large amounts in the dried aerial parts. Only in one population (B 3) was gentiopicroside (III) detected in smaller amounts, most probably due to the fact that the plants were investigated at the end of the growth period (Table 4).

In *Centaurium* the situation is quite different. In its characteristic accumulation of gentiopicroside *B. perfoliata* resembles the crude drug "Gentianae radix" (dried roots of *Gentiana lutea* L.) more than "Centaurii herba" (dried aerial parts of *C. erythraea*).

A morphological character not often mentioned is the color of the seeds. The seeds of all *B. perfoliata* plants investigated are black, like those of *C. maritimum,* whereas those of the other *Centaurium* species are brown to dark brown.

No chromosome numbers were determined for these populations. According to ZELTNER (1970) the *B. perfoliata* populations investigated are most probably tetraploid ($2n = 40$).

The Genus *Centaurium* HILL

Sect. *Xanthea* (*C. maritimum*). Four populations of *C. maritimum* from several localities in southern Europe were investigated (Table 4). Morphologically *C. maritimum* differs not only from the other *Centaurium* species in the yellow color of its flowers but also in the color of its seeds. Its seeds are black like those of *B. perfoliata,* whereas those of the other species investigated are brown to dark brown (Table 4).

The chromosome number $2n = 20$ was determined for plants from one population. This chromosome number is in accordance with the data given by ZELTNER (1970).

C. maritimum differs markedly from the other species investigated, especially in the accumulation of xanthones. None of the xanthones screened for was detected in *C. maritimum.* Of the secoiridoids screened for only sweroside (I) accumulated in detectable amounts in the aerial parts. In one population however we also detected gentiopicroside (III) in the roots and swertiamarin (II) in the capsules (Table 4). As far as the accumulation of sweroside (I), the main secoiridoid glucoside, is con-

cerned, *C. maritimum* resembles *C. pulchellum* and *C. spicatum*; but in these species this glucoside accumulates in larger amounts (Tables 4 and 5; Fig. 7). Also detected was a spot of unknown iridoid-like compounds, possibly the same as those found in *C. pulchellum* and *C. tenuiflorum*, with an Rf value of about 0.5 in TLC-system c (Fig. 7).

Sect. *Spicaria* (*C. spicatum*). Six populations of *C. spicatum*, mainly from several localities on the Atlantic coast of the Iberian peninsula, were investigated. These populations differ only slightly from each other in their morphological and chemical characters, but markedly from the other species investigated. Sweroside (I) accumulate in the aerial parts in all populations in large amounts (Table 4; Fig. 7). In this character these plants resembled *C. pulchellum*, but in this species other hitherto unknown iridoid glucosides accumulate as well (Fig. 7). One population from northern Portugal, consisting of plants of both white and pink colored flowers (S 3/4), proved to be slightly different from the others. In this population the amount of gentiopicroside (III) that accumulates in the aerial parts was almost equal to the amount of sweroside (I), whereas gentiopicroside was only a minor component in the aerial parts of the other populations. Gentiopicroside (III) is even the main component in the fruits of these plants (Fig. 7; Table 4). In addition some hitherto unknown phenoles, not detectable in the other populations, had accumulated in the aerial parts (Fig. 7).

As far as xanthones are concerned, plants of all populations except one (S 9) accumulate the compound L_1 in the aerial parts and most populations also accumulate L_2, but in low amounts. In these characters *C. spicatum* differs somewhat from the other species investigated (Tables 4 and 5).

The chromosome number $2n = 22$ was determined for one population (S 3/4). This chromosome number is in agreement with the numbers given by ZELTNER (1970) for this species.

Sect. *Centaurium*: subsect. *Caespitosa* (*C. scilloides*), **subsect. *Vulgaria*** (*C. chloodes, C. littorale*) and **subsect. *Centaurium*** (*C. erythraea, C. majus*). The secoiridoids that accumulate in the species placed in sect. *Centaurium*, viz. *C. scilloides, C. chloodes, C. littorale, C. erythraea*, and *C. majus* are very similar. All species are characterized by the accumulation of swertiamarin (II), the main secoiridoid glucoside in the aerial parts (Table 4; Fig. 7) and of one or more of the m-hydroxy-benzoyl esters of sweroside in the capsules (Table 2; Fig. 8). The amount of swertiamarin (II) in the aerial parts can be as much as eight per cent of the dried plant material.

In these characters the species within section *Centaurium* are quite distinct from all other species investigated. They differ also from *C.*

pulchellum and *C. tenuiflorum,* which are usually placed in the same section (MELDERIS 1972, ZELTNER 1970).

Subsect. *Caespitosa* (*C. scilloides*). Only two populations of *C. scilloides,* one coming from a natural habitat on the Atlantic coast of the Iberian peninsula, have been investigated (Table 4). The chromosome number 2 n = 20 was determined for one population. This chromosome number is in agreement with data given by ZELTNER (1970).

Both populations are characterized by the rich accumulation of decentapicrin A ($I_{3'}$), the only ester in the capsules. In this character this species resembles *C. chloodes.*

In the plants of both populations the xanthone L_2 accumulates in the aerial parts, whereas the two populations differ in the accumulation of xanthones L_1 and L_3 (Table 4).

Subsect. *Vulgaria* (*C. chloodes, C. littorale*). Of the five species usually included in subsect. *Vulgaria* (Fig. 1), we only investigated plant material of *C. chloodes* and of *C. littorale.*

C. chloodes. Only two populations of *C. chloodes,* one coming from a natural habitat on the Atlantic coast of the Iberian peninsula, were investigated (Table 4). The chromosome number 2 n = 40 for *C. chloodes,* was determined for one population. This chromosome number is in agreement with data given by ZELTNER (1970).

Both populations are characterized by the rich accumulation of only decentapicrin A ($I_{3'}$) in the capsules. In this character this species resembles *C. scilloides.*

In the plants of both populations the xanthone L_2 accumulates in the aerial parts, whereas the two populations differ in the accumulation of the other xanthones (Table 4).

Nevertheless plants of more populations will have to be investigated; the present results suggest that *C. scilloides* and *C. chloodes* are more closely related than is generally assumed and that *C. chloodes* should perhaps be transferred to subsect. *Caespitosa.* In this respect it is worth mentioning that in spite of the fact that ZELTNER (1970) placed *C. scilloides* in the section *Caespitosa* and *C. chloodes* in sect. *Centaurium* subsect. *Vulgaria,* he did draw a dotted line between these two species in the phylogenetic figure (Fig. 1).

C. littorale. Fourteen populations of subsp. *littorale,* growing along the coasts of "Sverige" (Sweden), "Danmark" (Denmark) and "Nederland" (The Netherlands) (Table 4) were investigated. The plants studied include small erect specimens from a population in "Sverige" (V 3), tall erect specimens from populations in "Danmark" (V 4) and in

"Nederland" (V 6, 10, 11, 13, 15, and 16) and tall procumbent specimens from three populations in "Nederland" (V 12, 14, and 17) (Table 5). The last mentioned taxon was treated by JONKER (1950) as forma *iberoides* and by FREYSEN (1976) as var. *iberoides*.

Cultivation experiments with seeds of some of these populations (V 3, 10, 12, and 16) showed that their characters are retained under cultivation. This is in agreement with cultivation experiments done by FREYSEN (1976) with tall erect and procumbent plants of some varieties. The chromosome number $2n = 40$ determined for three populations is in agreement with that reported by ZELTNER (1970).

The secoiridoids and xanthones that accumulate and were screened for are almost identical in the plants of the varieties investigated. All plants are characterized by the rich accumulation of decentapicrin A and B. They are present in almost equal amounts in the capsule. In the aerial parts of most plants L_2 was the only xanthone screened for that was found accumulating.

The accumulation pattern in plants of three populations is slightly different (V 5, V 7, and V 8/9). The three populations differ from the others in the color of the flowers and they look rather like a hybrid of *C. erythraea* and *C. littorale* photographed by ZELTNER (1970) (photograph 32). One of these populations from the island "Skiermûntseach" (Schiermonnikoog) in "Nederland" (V 8/9), consisting of plants with both dark and light colored flowers, accumulates in the capsules in addition to decentapicrin A and B small amounts of desacetylcentapicrin ($I_{2'}$) and the unknown compounds X_1 and X_2. The capsules of the other two populations, constisting of plants with only light colored flowers, small specimens from the island Rømø in "Danmark" (V 5) and tall ones from the island "Skiermûntseach" (V 7), accumulate decentapicrin A ($I_{3'}$) but not decentapicrin B ($I_{4'}$). Instead of this ester component very small amounts of desacetylcentapicrin ($I_{2'}$) were detected together with both of the unknown components X_1 and X_2 (V 7) or with only X_2 (V 5). Because the accumulation of desacetylcentapicrin ($I_{2'}$) and of the unknown components X_1 and X_2 in the capsules proved to be very characteristic for *C. erythraea* (Table 4; Fig. 8), these results strongly suggest that the plants of these three populations are hybrids of *C. littorale* and *C. erythraea*. The occurrence of both *C. erythraea* and *C. littorale* on the island "Skiermûntseach" is known (MENNEMA & al. 1984). Crossing experiments between *C. littorale* and *C. erythraea* and chemical investigation of the hybrids are needed to study whether these chemical characters can be inherited and, if so, to what extent.

On the basis of our results and taxonomic relations we expect that plants of the other *Centaurium* species not investigated by us and grouped

in sect. *Centaurium* subsect. *Vulgaria,* viz.: *C. favargeri, C. linariifolium* and *C. triphyllum* (Fig. 1), accumulate swertiamarin (II) in large amounts in the aerial parts and decentapicrin A ($I_{3'}$) with or without decentapicrin B ($I_{4'}$) in the capsules. This statement is supported by the fact that decentapicrin A was isolated from *C. linariifolium* (SEOANE, personal communication).

Subsect. *Centaurium* (*C. erythraea* and *C. majus*). Forty populations of *C. erythraea* from all over Europe, and ten populations of *C. majus* have been investigated.

The chromosome number $2n = 40$ was determined in fourteen populations of *C. erythraea* and $2n = 20$ in three populations of *C. majus*. These chromosome numbers are in agreement with the data reported by ZELTNER (1970). With only a few exceptions, partly due to the differences in the growth stage of the plants investigated, these species accumulate the very bitter esters centapicrin ($I_{2'a}$) and desacetylcentapicrin ($I_{2'}$) as well as the blue fluorescing unknown components X_1 and X_2 in varying amounts in the capsules and in the aerial parts the xanthone aglycone L_2. These characters, especially the accumulation of the strongly bitter esters in the capsules, are very characteristic for *C. erythraea* and *C. majus* (Tables 2 and 3; Fig. 8). The accumulation of the very bitter esters in their fruits can easily be used for the identification of these species by tasting successively stem or leaf parts and fruit parts. Only in the above-mentioned two species are the fruits much more bitter than the stem or leaf parts.

A few chemovarieties can be distinguished in *C. erythraea.* Three populations from Bulgaria (B-25077, B-25112, and B-25218), as well as a population of the subsp. *turcicum* (VELEN.) MELDERIS (C 21), accumulate another unknown blue fluorescing component X_3 (Table 5; Fig. 8) in the capsules, instead of X_1 and X_2. Morphologically these three populations from Bulgaria are no different from the other populations investigated in the same country.

A population from Athos (B-G20477; Fig. 9) and a population from "Corse" (Corsica) (C 12) accumulate in the capsules the very bitter esters, but not the unknown components X_1 and X_2, whereas another population from "Corse" (C 9) accumulates X_1 and X_2 but not the strongly bitter esters. Morphologically the Athos population (B-G20477) also differs markedly from the other *C. erythraea* populations especially in the flowers and capsules (Fig. 9). The Athos population plants can probably be regarded as a subspecies, but further research is needed before we can reach a more definite conclusion.

The investigated populations of *C. majus* showed a marked difference in the ester and blue fluorescent components that accumulate. Whereas all three populations from Portugal which we investigated accumulate the

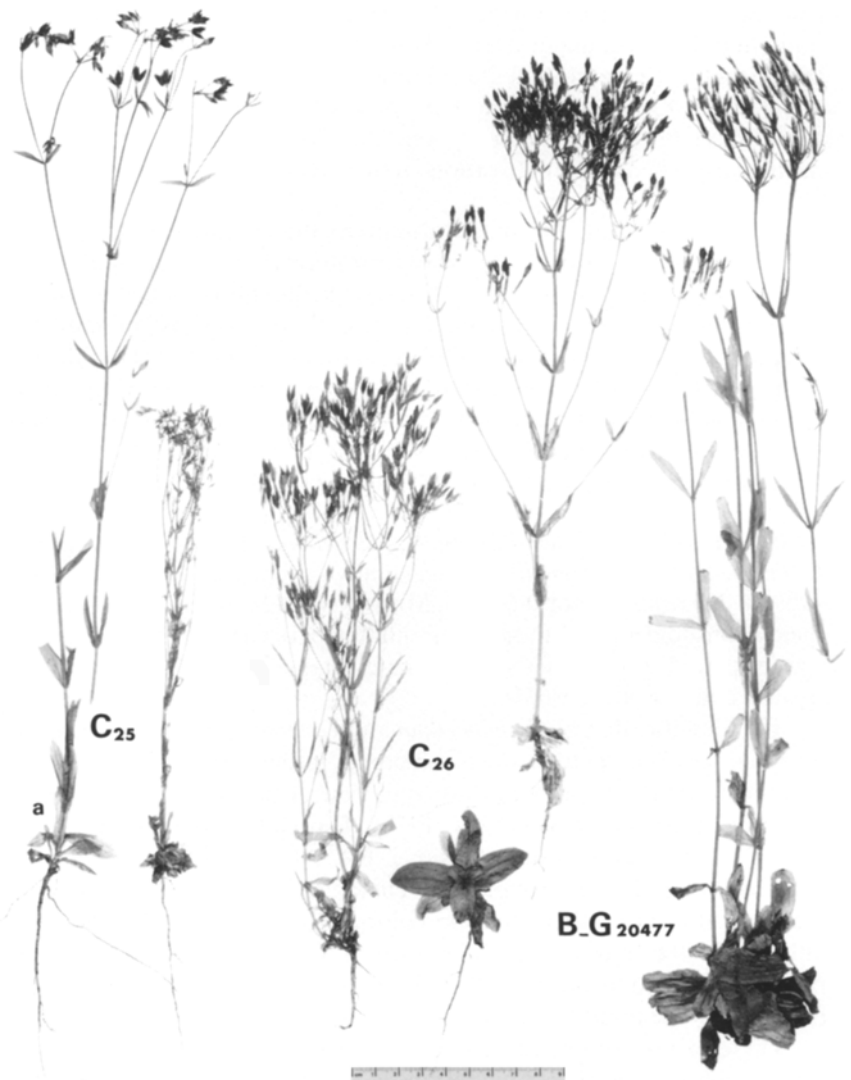


Fig. 9. Characteristic specimens of plants of the Athos populations of *Centaurium erythraea* (B-G20477) and from populations of *C. majus* subsp. majus in northern España (Spain) (C 25) and in the Algarve, Portugal (C 26). For the origins of the populations see Table 4

same components in the capsules as most *C. erythraea* populations, all four populations from northern "España" (Spain) accumulate decentapicrin B ($I_{4'}$) as the major ester and only a small amount of one or both of the strongly bitter esters and the unknown components X_1 and X_2 (Tables 4 and 5; Fig. 8). Morphologically the plants of northern "España" differ from those of southern Portugal in the shape of their flowers and, particularly when under cultivation, in their very lax inflorescence (Fig. 9, C 25 a).

In their chemical accumulation pattern the commercial samples of Atlas quality "Centaurii herba" originating from the subsp. *majus* var. *suffruticosum* partly (C 30 and C 31) resemble the subsp. *majus* var. *majus* populations in northern "España" and partly (C 29) those of southern Portugal. More investigations of plant material of this variety with ripe fruits will have to be done in order to obtain more insight into their taxonomic position. In any case these results suggest that subsp. *majus* var. *suffruticosum* should not be treated as a species separate from subsp. *majus* var. *majus* as proposed by MELDERIS (1972).

Sect. *Parviflora* (*C. tenuiflorum, C. pulchellum*). The European species *C. pulchellum* and *C. tenuiflorum,* generally grouped in sect. *Centaurium* subsect. *Parviflora* (ZELTNER 1970, MELDERIS 1972), are quite different in their secoiridoid and xanthone accumulation pattern from the *Centaurium* species placed in the other subsections of that section and are therefore treated under sect. *Parviflora.*

The identification of *C. pulchellum* and *C. tenuiflorum* on the basis of morphological characters only is sometimes rather difficult in the case of plant material from southern Europe in view of the great morphological variation encountered (ZELTNER 1970). Especially populations of *C. pulchellum* that grow in meadows (e.g. P 4, P 10, and P 11) resemble *C. tenuiflorum* in their morphological characters. Ten populations from locations all over southern Europe, consisting of plants usually branched only above the middle, were identified as *C. tenuiflorum* and thirteen populations from locations in West Europe with plants mostly branched from below the middle were identified as *C. pulchellum.*

These identifications are partly supported by cytological investigations (Table 4). One of the populations of *C. tenuiflorum* investigated (P 9) is diploid ($2n = 20$) and belongs to the subsp. *acutiflorum* (ROUY ex SCHOTT) ZELTNER. The other populations of this species are tetraploid ($2n = 40$) or very probably tetraploid (MELDERIS 1972, ZELTNER 1970, photographs 21 and 22) and belong to the subsp. *tenuiflorum.*

The accumulation of the chemical compounds screened for is rather uniform within the different populations of *C. pulchellum* and to a lesser extent within the various populations of *C. tenuiflorum.* Both species are

characterized by the accumulation of xanthone-β-mono-glucosides and the unknown compound(s) Z and in this respect are rather distinct from the other *Centaurium* species and *Blackstonia perfoliata* (Tables 4 and 5). The species also differ from the species placed in the section *Centaurium* in the accumulation of secoiridoids. Only small amounts of swertiamarin (II) are found in the aerial parts and no m-hydroxy-benzoyl esters of sweroside are found in the capsules.

C. pulchellum and *C. tenuiflorum* differ from each other mainly in the quantities of sweroside (I) and xanthones accumulating. Whereas sweroside (I) is the main component of *C. pulchellum,* it is only a minor component in *C. tenuiflorum* and is present in almost the same quantity as gentiopicroside (III) (Tables 4 and 5). Other hitherto unknown components, also detectable in most populations of *C. pulchellum,* are the main components in *C. tenuiflorum.*

In all populations of *C. pulchellum* the xanthones L_1, L_2, and L_3 are present in rather large amounts, whereas in the various populations of *C. tenuiflorum* these xanthones are present in more variable amounts (Table 4). The composition of the xanthone-β-mono-glucosides is also different in the two species. Whereas the β-monoglucosides of L_1 and L_2 are present in rather large amounts in the capsules of *C. tenuiflorum* these glucosides were hardly detectable at all in the capsules of *C. pulchellum* (Table 4; Fig. 6).

In the capsules of both species the β-mono-glucosides of the unknown xanthone(s) Y were detected, but not those of L_3 (Fig. 6). Also present are blue fluorescenting compounds, possibly identical with the unknown compounds X_1, X_2, or X_3 (Fig. 6; Table 5).

American *Centaurium* Species. Of the American *Centaurium* species placed by MELDERIS (1931) in sect. *Centaurium* subsect. *Parviflora* we have investigated *C. quitense* from one population on the Bahama Islands and a very old sample of "Herba Chanchalaguae" from the museum of the Farmaceutisch Laboratorium, regarded as belonging to *C. chilense.*

There is a marked difference in the chemical composition of the plants of these two species. *C. quitense* accumulates swertiamarin (II) as the main secoiridoid glucoside in the aerial parts whereas *C. chilense* accumulates sweroside. In plants of *C. chilense* the xanthone L_3 is detected and in plants of *C. quitense* the xanthone L_2 and a hitherto unknown xanthone. This unknown xanthone has an Rf value just below that of L_3 and has not been detected in any of the other *Centaurium* and *Blackstonia* species investigated. Neither *C. chilense* nor *C. quitense* contains any xanthone-β-mono-glucosides or the unknown compound(s) Z, whereas these compounds are all very characteristic of the European members of sect. *Parviflora.*

Table 4. Origin of investigated plant material and results of the TLC screening of *Blackstonia* and *Centaurium* species. – Locality: Abbreviations for countries: B = Belgium, BG = Bulgaria, BS = Bahamas, D = Federal Republic of Germany, DDR = German Democratic Republic, DK = Danmark, GR = Greece, E = Spain, F = France, I = Italy, MA = Morocco, NL = Nederlands, P = Portugal, RCH = Chile, S = Sweden, TR = Turkey, YU = Yugoslavia. – Geographical names are given in the local language. Herbarium-Coll. no.: Herbaria deposit and collection number: F: Farmaceutisch Laboratorium, Utrecht: will be deposited in the Herbarium, Utrecht (U); B: Populatie- en Evolutiebiologie, Utrecht: deposited in the Herbarium, Utrecht (U); U: Herbarium, Utrecht (U); L: Rijksherbarium, Leiden (L). – Stage: fl: flowering, fr: fruiting. – Color abbreviations: bl = black, br = brown, d = dark, p = pink, r = red, y = yellow, w = white. – Remarks: cult.: in culture. – Habit: a = annual, b = biennial, p = perennial. – Part: ae: aerial part; cp: capsule; fl: flower; rt: root. Secoiridoid glucosides: + + + = > 2%, + + = 0.5–2%; + = 0.1–0.5%; – = < 0.1%. – Xanthones: + = 0.2–0.5%; – = < 0.2%

Genus (Sub)section Species Locality Herbarium-coll. no.; stage; chromosome no. (2n)	Color of flower of seed; remarks	Part	Secoiridoid glucosides							Xanthones				Unknown components	
			I	II	III	$I_{2'a}$	$I_{2'}$	$I_{3'}$	$I_{4'}$	L_1	L_2	L_3	β-mono-glucosides L_1/L_2	X_1/X_2	X_3
Blackstonia HUDS.															
B. perfoliata															
F, Corse, Bonifacio F-786B1; fl	yellow/black	ae	–	–	+ + +	–	–	–	–	–	–	–	–	–	–
E, Navarra, Pamplona	yellow/black	ae	–	–	+ + +	–	–	–	–	–	–	–	–	–	–
F-768B2; fl and fr		cp	–	–	+ + +	–	–	–	–	–	–	–	–	–	–
		rt	–	–	+	–	–	–	–	–	–	–	–	–	–
E, Logroño, Logroño F-767B3; fl and fr	yellow/black	ae	–	–	+	–	–	–	–	–	–	+	–	–	–
E, Santander, Suances F-767B4; fl and fr	yellow/black	ae	–	–	+ + +	–	–	–	–	–	–	+	–	–	–
P, Algarve, Lagos, Odeaxere F-786B5; fl and fr	yellow/black	ae	–	–	+ + +	–	–	–	–	–	–	+	–	–	–
Centaurium HILL															
Sect. *Xanthea*															
C. maritimum															
F, Corse, Porto Vecchio, Trinité, F-786X3: fl and fr	yellow/black	ae	+	–	–	–	–	–	–	–	–	–	–	–	–
F, Corse, Ajaccio F-786X4; fl and fr	yellow/black	ae	+ +	–	–	–	–	–	–	–	–	–	–	–	–
P, H. B. Coimbra (I.S. '78) F-78X1; fl; 2n = 20	yellow/black cult.	ae	+	–	–	–	–	–	–	–	–	–	–	–	–

P, W. Algarve, Carrapateire	yellow/black	ae	+	–	–	–	–	–	–	–	–	–	–	–
F-786X2; fl and fr		cp	+	+	–	–	–	–	–	–	–	–	–	–
		rt	+	–	+	–	–	–	–	–	–	–	–	–
Sect. *Spicaria*														
C. spicatum														
E, Galicia, Pontevedra, Gondomar	pink/white (brown)	ae	+ +	–	+	–	–	–	–	+	+	–	–	–	–
F-767S1/2; fr and fl														
P, Minho, Caminha;	pink/white	ae	+ + +	–	+ + +	–	–	–	–					
F-7675S3/4; fr and fl	(brown)	cp												
2 n = 22		rt	+	–	+									
P, H. B. Coimbra (I.S. '78)	pink/(brown)	ae	+ + +	–	+	–	–	–	–	+	±	–	–	–	–
F-78S5; fl	cult.													
P, W. Algarve, Carrapateire	pink	ae	+ +	–	+	–	–	–	–	+	±	–	–	–	–
F-786S6; fl														
P, Algarve, Portimão	pink/white	ae	+ + +	±	+	–	–	–	–	+	–	–	–	–	–
F-786S7/8; fl (and fr)														
MA, Maroc (L-23418; Ch. Chauvage subno. 17451) F-64S9	pink	fl	+ + +	–	+	–	–	–	–	–	–	–	–	–	–
Sect. *Caespitosa*														
C. scilloides														
E, Galicia, Pontevedra, Gondomar	pink/brown	ae	±	+ + +	+					+	+	–	–		
F-767Ca1; fl and fr		cp	±	+ + +	+ +	–	–	+ +	–					–	–
NL, H. B. Utrecht (I.S. '75)	pink/dark brown	ae	–	+ + +	–					+	+	+	–		
F-75Ca2; fl and fr; 2 n = 20	cult.	cp	+	+ + +	+	–	–	+ +	–					–	–
Sect. *Vulgaria*														
C. chloodes														
E, Galicia, Corrubedo, Playa Ladeira; F-768V1; fr (and fl); 2 n = 40	red/brown	ae	±	+ +	+					+	+	+	–		
		cp	+	+ +	+	–	–	+ +	–					–	–
D, H. B. München (I.S. '75)	red/brown	ae	–	+ + +	+					–	+	±	–		
F-75V2; fl and fr	cult.	cp	+	+ +	+	–	–	+ +	–					–	–
C. littorale														
S, Bohuslän, Ellös	red/brown	ae	+	+ +	+					–	+	–	–		
F-748V3; fr (and fl); 2 n = 40	c. 10 cm	cp	+	+ + +	+	–	–	+ +	+ +	–	–	–		–	–
DK, N. Slesvig, Rømø, Havnesand	red/brown	ae	+	+ + +	+					–	+	–	–		
F-838V4; fr (and fl)		cp	+	+ +	+	–	–	+ +	+ +					–	–
DK, N. Slesvig; Rømø, Havnesand	pink/brown c. 10 cm	ae	+	+ + +	+					–	+	–	–		
		cp	+	+ +	+	–	±	+ +	–					–/+ +	–

Table 4 (continued)

Genus (Sub)section Species Locality Herbarium-coll. no.; stage; chromosome no. (2 n)	Color of flower of seed; remarks	Part	Secoiridoid glucosides							Xanthones				Unknown components	
			I	II	III	$I_{2'a}$	$I_{2'}$	$I_{3'}$	$I_{4'}$	L_1	L_2	L_3	β-mono-glucosides L_1/L_2	X_1/X_2	X_3
F-838V5; fl (and fr)															
NL, "Skiermûntseach" (Fr),	red/brown	ae	±	++	+					−	±	−	−		
Balch		cp	+	++	+	−	−	++	++					−	−
F-748V6; fl and fr															
NL, "Skiermûntseach"; (Fr), J. de	pink/brown	ae	++	++	+					−	±	−	−		
Jongpad; F-748V7; fl and fr		cp	+	++	+	−	±	++	−					++	−
NL, "Skiermûntseach" (Fr),	pink/red (brown)	ae	+	++	+					−	−	−	−		
Wester polder;		cp	+	++	+	−	±	+	+	−	±	−		++	−
F-748V8/9; fl and fr; 2 n = 40															
NL, "It Amelân" (Fr), 't Oerd	red/brown	ae	±	++	+					−	+	−	−		
F-748V10; fl and fr; 2 n = 40		cp	+	++	+	−	−	++	++					−	−
NL, "It Amelân" (Fr), Nesser	red/brown	ae	±	++	+					−	+	−	−		−
Dunen; F-748V11; fl and fr		cp	+	++	+	−	−	++	++					−	−
NL, "It Amelân" (Fr), Nesser	red/brown	ae	±	+++	+					−	+	−	−		
Dunen; F-748V12; fl and fr	var. *iberoides*	cp	+	++	+	−	−	++	++					−	−
NL, "Skylge" (Fr), Noordvaarder	red/brown	ae	±	+++	+					−	−	−	−		
F-749V13; fl and fr		cp	+	++	+	−	−	++	++					−	−
NL, "Skylge" (Fr), Noordvaarder	red/brown	ae	±	+++	+					−	+	−	−		
F-748V14; fl and fr	var. *iberoides*	cp	+	++	+	−	−	++	++					−	−
NL, Texel (NH), 't Horntje	red/brown	ae	±	+++	+					−	+	−	−		
F-749V15; fr and fl		cp	+	+++	±	−	−	++	++					−	−
NL, Amsterdam (NH), Bijlmermeer	red/brown	ae	±	+++	+					−	+	−	−		
F-749V16; fr and fl		cp	+	++	+	−	−	++	++					−	−
NL, Voorne-Putten (ZH), Oost-	red/brown	ae	±	+++	+					−	++	−	−		
voorne; F-748V17; fl and fr	var. *iberoides*	cp	+	++	+	−	−	++	++					−	−
Sect. *Centaurium*															
C. erythraea															
DK, Sjaelland, Rørvig	pink	ae	±	+++	±					−	±	−	−		
F-80C1; fl		fl	+	+++	+	±	±	−	−	−	±	−		++	−
DDR, Aschersleben (H. B. Halle, S.I.	pink/brown	ae	±	+++	+					−	±	−	−		
'74, no. 962); F-74C36; fl and fr	cult.	cp	+	+++	+	±	±	−	−					++	−

DDR, Rudolstadt (H. B. Halle, I.S.	red/brown	ae	±	+ + +	+					–	±	–	–		
'74, no. 963); F-74C37 fl and fr	cult.	cp	+	+ + +	+	±	±	–	–					+ +	–
DDR, Thüringen, Triptis (H. B. Halle,	red/brown	ae	±	+ + +	+					–	±	–	–		
I.S. '74, no. 946); F-74C35; fl and fr	cult.	cp	+	+ + +	+	+	+	–	–					+ +	–
D, Emsland, Bentheim	pink/red/brown	ae	±	+ + +	+					–	+	–	–		
F-829C2 and C3: fl and fr; 2n = 40		cp	+	+ +	+	+	+	–	–	–	+	–		+ +	–
NL, Amsterdam (NH), Bijlmermeer	pink/brown	ae	±	+ + +	+					–	+	–	–		
F-749C4; fl and fr; 2n = 40		cp	+	+ + +	+	+ +	+ +	–	–					+ +	–
NL, ibid.; fl		ae	±	+ + +	+					–	+	–	–		
F-748C4a		fl	+	+ + +	+	–	–	–	–					±/–	–
NL, ibid.; fr		ae	+	+ + +	+					–	+	–	–		
F-749C4b		cp	+	+ + +	+	+ +	+ +	–	–					+ +	–
B, Voerstreek, Teuven	pink	ae	±	+ + +	+					–	±	–	–		
F-828C5; fl		fl	±	+ +	+	–	±	–	–	–	+	–		–/+	–
F, Artois, Marquise	pink/brown	ae	±	+ + +	+					–	+	–	–		
F-808C6; fr (and fl)		cp				–	±	–	–	–	+	–		–/+	–
F, Bretagne, Lac du Guelédan,	pink/brown	ae	+	+ + +	+					–	±	–	–		
St. Brigitte; F-768C7; fl and fr		cp	+	+ + +	+	+	+	–	–					+	–
F, Bretagne, Côtes-du-Nord,	pink/brown	ae	±	+ + +	+					–	+ +	–	–		
Trebeurden; F-768C8; fr (and fl); 2n = 40	var. *capitatum*	cp				±	±	–	–					+ +	–
F, Corse, Porto Vecchio,	pink/brown	ae	+	+ + +	+					–	±	–	–		
Trinité; F-786C9; fl and fr		cp				–	–	–	–					+ +	–
F, Corse, Bonifacio	pink	ae	+	+ + +	+					–	+ +	–	–		
F-768C10 and C12; fl		fl				+	+	–	–					–	–
F, Corse, Ajaccio	red	ae	±	+ + +	+					–	+ +	–	–		
F-786C13; fl		fl				–	–	–	–					±/–	–
F, Pyrénées-Orientals, Port	pink/brown	ae	±	+ + +	+					–	+	–	–		
Vendres: F-596C34; fl and fr		cp				±	+ +	–	–					+ +	–
E, Huesca, Boltaña	pink/brown	ae	+ +	+ + +	+					–	+	–	–		
F-757C14; fl and fr		cp				+ +	+ +	–	–	–	+	–		–/+	–
E, Huesca, Boltaña	pink/brown	ae	+	+ + +	+					–	+	–	–		
F-757C15; fl and fr		cp	+ +	+ + +	+	+ +	+ +	–	±	–	+	–		–/+	–
E, Navarra, Erasum	pink/brown	ae	+	+ + +	+					–	+	+	–		
B-2477; fl and fr; 2n = 40	cult.	cp	+ +	+ + +	+	+ +	+ +	–	–	–	±	–		+ +	–
E, Santander, Suances	pink/brown	ae	±	+ + +	+					–	+	–	–		
F-768C16; fl and fr		cp	+ +	+ + +	+	+	+	–	–	–	+	–		+	–
E, Santander, Santillana	pink/brown	ae	±	+ + +	±					–	+	–	–		
F-768C32; fl and fr		cp	+	+ + +	+	+	+	–	–					+	–
E, Santander, Potes	pink/brown	ae	+ +	+ + +	+					–	±	–	–		
F-768C17; fl and fr		cp				±	±	–	–					±	–

Table 4 (continued)

Genus (Sub)section Species Locality Herbarium-coll. no.; stage; chromosome no. (2n)	Color of flower of seed; remarks	Part	Secoiridoid glucosides							Xanthones				Unknown components	
			I	II	III	$I_{2'a}$	$I_{2'}$	$I_{3'}$	$I_{4'}$	L_1	L_2	L_3	β-mono-glucosides L_1/L_2	X_1/X_2	X_3
I, Lago Maggiore, Valle Cannobina, Orasso; F-787C18; fl	pink	ae	±	+ + +	+					−	+	−	−		
		fl				±	−	−	−					±	−
YU, Bosna-Hercegovina, Višegrad	pink/brown	ae	+ +	+ + +	+					−	+	−	−		
B-17354; fl and fr; 2n = 40	cult.	cp				±	±	−	−					+ +	−
YU, Bosna-Hercegovina, Višegrad	red/brown	ae	+ +	+ + +	+					−	+ +	−	−		
B-17355; fl and fr; 2n = 40	cult.	cp				−	−	−	−					+	−
GR, Athos, Karyes	pink/brown	ae	±	+ + +	+					−	+	−	−		
B-G20477; fr and fl; 2n = 40	cult.	cp				+	+	−	−					−	−
BG, Balčik	pink: c. 20 cm	ae	+	+ + +	+					−	+ +	−	−		
F-747C19; fl		fl	±	+ + +	±	−	−	−	−					±	−
BG, Varna, Zlatni Pjasaci	pink; c. 30 cm	ae	+	+ + +	+					−	+ +	−	−		
F-747C20; fl		fl	+	+ +	±	+	+ +	−	−					+	−
BG, Stara Planina, Gurkovo, Voda; B-25077; fl and fr; 2n = 40	pink/brown cult.	ae cp	+ +	+ + + + +	+ +	 + +	 + +	 −	 −	− −	+ −	− −	− 	 −	 + +
BG, Stara Planina, Trojan, Aprilci;	pink/brown	ae	±	+ + +	+					−	−	−	−		
B-25112; fl and fr	cult.	cp	+	+ +	+	±	+	−	−	−	−	−		−	+
BG, Stara Planina, Trojanski Pr.	red/brown	ae	±	+ + +	+					−	+	−	−		
B-25128; fl and fr; 2n = 40	cult.	cp	+	+ + +	+	+	+	−	−	−	−	−		+	−
BG, Sofia	red/brown	ae	±	+ + +	+					−	−	−	−		
B-25145; fl and fr; 2n = 40	cult.	cp	+	+ + +	+	±	+	−	−	−	±	−		+	−
BG, Rila Planina, Rila	red/brown	ae	±	+ + +	+					−	+	−	−		
B-25194; fl and fr; 2n = 40	cult.	cp	+	+ +	±	+	+ +	−	−	−	−	−		+	−
BG, Rila Planina, Blagoevgrad	red/brown	ae	+	+ + +	+					−	−	−			
B-25218; fl and fr; 2n = 40	cult.	cp	+ +	+ +	±	+ +	+ +	−	−	−	−	−		−	+ +
BG, Pazardžik − Peštera	red/brown	ae	+	+ + +	+				−		+	−	−		
B-25244; fl and fr; 2n = 40	cult.	cp	±	+ +	+	±	+	−	−	−	+	−		+	−
BG, Rodopen, Čudnite Mostove	pink/brown	ae	+	+ + +	+					−	−	−	−		
B-25265; fl and fr; 2n = 40	cult.	cp	+ +	+ +	+	+ +	+	−	−	−	±	−		+/−	−
TR, Istanbul, Adalar, Büyükada	pink	ae	+	+ + +	+					−	±	−	−		
F-796C21; fl	subsp. *turcicum*	fl	+	+ + +	+	−	−	−	−					−	+

C. majus															
E, Huesca, Boltaña	red/brown	ae	+	+ + +	+					−	+ +	−	−		
F-757C22; fl and fr		cp	+	+ +	±	±	±	−	+ +					+	−
E, Huesca, Barbastro	red/brown	ae	+	+ + +	+					−	+ +	−	−		
F-757C23; fl and fr		cp	+ +	+ + +	+	+	+	−	+ +					+/−	−
E, Soria, Calatanazor	red/brown	ae	+	+ + +	+					−	±	−	−		
F-768C24; fl and fr; 2 n = 20		cp	+	+ + +	±	±	±	±	+ +					±	−
E, Cataluña, Figueras, Llado	red/brown	ae	±	+ + +	±					−	+ +	−	−		
F-757C25; fl and fr; 2 n = 20		cp				−	+	−	+ +					+	−
P, Algarve, Lagos	red	ae	±	+ + +	+					−	±	−	−		
F-786C26; fl and fr		cp				+ +	+ +	−	−					+ +	−
P, Algarve, Lagos, Odeaxere	red/brown	ae	±	+ + +	±					−	−	−	−		
F-786C27; fl and fr		cp				+ +	+ +	−	−					+ +	−
P, Algarve, Carrapateire	red/pink (brown)	ae	±	+ + +	±					−	±	−	−		
F-786C28; fl and fr; 2 n = 20		cp				+ +	+ +	−	−					+ +	−
MA, Atlas Mountains (imported	red/brown	ae	±	+ + +	±					−	±	−	−		
by Caesar & Loretz, Hamburg, D)	var. *suffruticosum*	cp				+ +	+ +	−	−					+ +	−
F-78C29; fl and fr															
MA, ibid.; F-78C30; fl	red	ae	±	+ + +	±					−	±	−	−		
	var. *suffruticosum*	fl				−	−	−	+					−	−
MA, ibid.; (imported by Lehner	red	ae	±	+ + +	±					−	±	−	−		
AG, Muttenz, CH) F-76C31; fl	var. *suffruticosum*	fl				+	?	?	+					−	−
Sect. *Parviflora*															
C. pulchellum															
DK, N. Slesvig, Rømø, Havnesand	red/(dark) brown	ae	+ +	−	+	−	−	−	−	+	+	+			
F-838P1; fl and fr															
NL, "Skiermûntseach" (Fr), Kobbe-	red/dark brown	ae	+ +	−	+					+	+	+			
dunen; F-748P2; fl and fr;		cp	+ +	−	+	−	−	−	−	+	+	±	±	?	?
2 n = 36															
NL, "Skiermûntseach" (Fr), Balch	red/brown	ae	+ +	−	+					+	+	+			
F-748P3; fl and fr; 2 n = 36		cp	+ +	−	+	−	−	−	−	+	+	+	±	?	?
NL, "Skylge" (Fr), Boschplaat	red/dark brown	ae	+ +	−	+					+	+	+			
F-748P4; fl and fr; 2 n = 36		cp	+ +	−	+	−	−	−	−	+	+	−	±	?	?
NL, "Skylge" (Fr), Noordvaarder	red/brown	ae	+ +	−	+					+	+	+			
F-748P5; fl and fr; 2 n = 36		cp	+ +	−	+	−	−	−	−	+	+	±	±	?	?
NL, "Flylân" (Fr), Kroonpolders	red/brown	ae	+ +	−	+					+	+	+			
F-779P6; fl and fr		cp	+ +	−	+	−	−	−	−	+	+	±	−	?	?
NL, Texel (NH), Slufter	red/brown	ae	+ +	−	+					+	+	+			
F-749P7; fl and fr; 2 n = 36		cp	+ + +	−	+	−	−	−	−	+	+	+	±	?	?
NL, Amsterdam (NH), Bijlmermeer	red/brown	ae	+ +	−	+					+	+	+			
F-749P8; fl and fr; 2 n = 36		cp	+ +	−	+	−	−	−	−	+	+	+	±	?	?

Table 4 (continued)

Genus (Sub)section Species Locality Herbarium-coll. no.; stage; chromosome no. (2 n)	Color of flower of seed; remarks	Part	Secoiridoid glucosides							Xanthones				Unknown components	
			I	II	III	$I_{2'a}$	$I_{2'}$	$I_{3'}$	$I_{4'}$	L_1	L_2	L_3	β-mono-glucosides L_1/L_2	X_1/X_2	X_3
NL, ibid.; fl		ae	+ +	−	+					+	+	+			
		fl	+ +	−	+	−	−	−	−	+	+	+	±	?	?
NL, ibid.; fl		ae	+ +	−	+					+	+	+			
		fr	+ +	−	+	−	−	−	−	+	+	+	±	?	?
E, Cataluña, Figueras, Llado F-726P10; fl	red; 20–30 cm	ae	+ +	−	+	−	−	−	−	+	+	+ +			
E, Cataluña, Figueras, Llado	red/dark brown	ae	+ + +	−	+					+	+	+			
F-747P24; fl and fr; 2 n = 36		cp	+ + +	−	+	−	−	−	−	+ +	+ +	+ +	−	?	?
		rt	+	−	+										
E, Huesca, Boltaña	red/brown	ae	+ + +	−	+ +					+ +	+ +	+ +			
F-757P11; fl and fr		cp	+ + +	−	+	−	−	−	−	+	+	+	±	?	?
E, Santander, Suances	red/brown	ae	+ +	+ +	+					+	+	+			
F-767P12; fl and fr		cp	+	+ +	+	−	−	−	−	+ +	+ +	+ +	+/−	?	?
		rt	−	±	+										
P, Algarve, Lagos, Odeaxere	red/brown	ae	+ + +	−	+					+	+	+ +			
railway: F-786P15; fl and fr		cp	+ + +	±	+	−	−	−	−	+	+	+ +	−	?	?
		rt	+ + +	−	+										
C. tenuiflorum															
F, Gironde, Saint Estèphe	pink/brown;	ae	+	−	+					±	−	−			
F-777P9; fl and fr; 2 n = 20	c. 30 cm, thick stem	cp	±	−	+	−	−	−	−	+	+	−	+	?	?
	subsp. *acutiflorum*	rt	−	−	+					+	+	+			
E, Cataluña, Figueras, Llado	red/dark brown	ae	+	+	+					−	+	−			
F-737P23; fl and fr		cp	+	+	+	−	−	−	−	+	+	−	+ +	?	?
		rt	+	−	+										

E, Santander, Suances	red/brown	ae	+	−	+					+	+	−			
F-767P22; fl and fr		cp	+	+	+	−	−	−	−	+ +	+ +	+	+	?	?
		rt	±	±	+										
P, Minho, Caminha	red/brown	ae	+	−	+					+	−	+			
F-768P13; fl and fr		cp	+	±	+	−	−	−	−	+ +	+ +	−	+ +	?	?
P, Algarve, Lagos, Odeaxere	red/brown	ae	+	−	+					±	−	−			
beach; F-786P14; fl and fr;		cp	+	±	+	−	−	−	−	±	+ +	±	+	?	?
2 n = 40															
P, Algarve, Lagos, Odeaxere	pale lilac (dark	ae	+	−	+					+ +	+ +	+ +			
field; F-786P16; fl (and fr)	brown); thick	cp	+ +	+	+	−	−	−	−	+ +	+ +	+ +	+ +	?	?
	stem	rt	−	−	+										
ibid.; F-786P17; fl and fr	red (dark brown); thick stem	ae	+	−	+					+ +	+ +	+ +			
P, Algarve, Portimão	red/dark brown	ae	+	−	+					+ +	+ +	−			
F-786P18; fl and fr; 2 n = 40		cp	+ +	+	+	−	−	−	−	+ +	+ +	+ +	+	?	?
P, Algarve, Carrapateire	red/brown	ae	+	−	+					±	−	−	+ +		
F-786P19; fl and fr	15–30 cm	cp	+ +	+	+	−	−	−	−	+ +	+ +	−	+ +	?	?
I. Sicilia, Catania	red	ae	+	−	+	−	−	−	−	+	+	+			
F-806P20; prefl															
TR, Istanbul, Adalar, Büyükada	red	ae	+	−	+	−	−	−	−	+	+	+			
F-796P21; fl															
American species															
C. quitense															
(*C. brittonii*)															
Bahamas, Inagua	pink/white	ae	+	+ + +	+	−	−	−	−	−	+	−	−	−	−
U-174195 B (H. Danbar 364)	(brown)														
fl and fr.															
C. chilense															
„Chili", "Canchalaguae herba"		ae	+ +	−	−	−	−	−	−	−	−	±	−	−	−
F-Ch1		fl	+ + +	−	−	−	−	−	−	−	−	±	−	−	−
		rt	+	−	−	−	−	−	−	−	−	±	−	−	−

Table 5. Summary of the TLC screening results for *Blackstonia* and *Centaurium* species investigated from at least two populations. For abbreviations and notes see Table 4. Chromosome numbers (2 n) according to ZELTNER (1970)* and own counts; var. = variable

Genus (Sub)section species	2 n	Color of flower	Habit	Part	Secoiridoid glucosides							Xanthones					Unknown components	
					I	II	III	$I_{2'a}$	$I_{2'}$	$I_{3'}$	$I_{4'}$	L_1	L_2	L_3	β-mono glucosides		$X_1/X_2/X_3$	Z
															L_1/L_2	Y		
Blackstonia																		
B. perfoliata	40*	y	a	ae	–	–	+ + +	–	–	–	–	–	–	±	–	–	–	–
Centaurum																		
Sect. *Xanthea*																		
C. maritimum	20	y	a	ae	+	–	–					–	–	–	–	–	–	–
				cp				–	–	–	–							
Spicaria																		
Sect. *Spicaria*																		
C. spicatum	22	p/w	a	ae	+ + +	–	+					+	±	–	–	–	–	–
				cp				–	–	–	–							

Sect. *Parviflora*																		
C. pulchellum		36	p	a	ae	++	−	+					+	+	+				+
					cp	++	−	+	−	−	−	−	+	+	+	±	+	?	++
C. tenuiflorum		40/20	p	a	ae	+	−	+					+	var.	var.				+
					cp	+	+	+	−	−	−	−	+	+	var.	+	+	?	++
Sect. *Caespitosa*																		
C. scilloides		20	r	p	ae	±	+++	±					var.	+	var.				−
					cp				−	−	++	−				−	−	−	
Sect. *Vulgaria*																		
C. chloodes		40	r	b	ae	±	+++	+					var.	+	var.				−
					cp				−	−	++	−				−	−	−	
C. littorale		40	r\	b	ae	+	+++	+					−	+	−				−
					cp				−	−	++	++				−	−	−	
Sect. *Centaurium*																		
C. erythraea		40	p/r	b	ae	±	+++	+					−	±	−				−
					cp				+	+	−	−				−	−	+	
C. majus	N. Spain	20	r	b	ae	±	+++	±											−
					cp				+	+	−	++	−	+	−	−	−	±	
	Algarve	20	r	b	ae	±	+++	±											−
					cp				++	++	−	−	−	±	−	−	−	++	

Although chemical investigations of these species from more localities are clearly needed, our results do not indicate a close relationship between these two species, or between these two species and the European *Centaurium* species placed in sect. *Parviflora*.

Conclusions

The accumulation of xanthones and particularly secoiridoid glucosides in *Blackstonia perfoliata* and in *Centaurium* species is a very valuable character at the level of species and (sub)sections.

Because *B. perfoliata, C. maritimum, C. spicatum, C. pulchellum,* and *C. tenuiflorum* differ markedly from *C. erythraea* in the amounts of the main components they are not good replacement species for *C. erythraea* as a source of "Centaurii herba". In their main constituents *C. scilloides, C. chloodes, C. littorale,* and *C. majus* closely resemble *C. erythraea*. Therefore they might be substituted for *C. erythraea*. Concerning the bitterness of the ripe fruits, only the fruits of certain populations of *C. majus* are very bitter (as are those of *C. erythraea*) because they contain rather large amounts of the intensely bitter m-hydrobenzoyl esters centapicrin and/or desacetyl centapicrin. "Centaurii herba" however is harvested at the flowering stage and not at the fruiting stage.

More chemical investigations are needed in order to get a better understanding of the systematic position of the *C. majus* subspecies and varieties as well as of the various non-European *Centaurium* species.

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