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REVIEW

XANTHONE GLYCOSIDES

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Key Word Index—Gentianaceae; xanthone glycosides; distribution; isolation; structure determination; synthesis; pharmacology.

Abstract—The naturally occurring xanthone glycosides are reviewed and their chemotaxonomic significance within the Gentianaceae family is discussed. The methods of isolation and structure determination, as well as synthesis are presented. The pharmacological activity of xanthone glycosides is also reported.

INTRODUCTION

Xanthones occupy an important position in the chemistry of natural products. Their structures are related to that of flavonoids and their chromatographic behaviour is also similar. Whereas flavonoids are frequently encountered in nature, xanthones have been found in a limited number of families. They always occur in Guttiferae and Gentianaceae and can be considered characteristic of these plants.

Naturally occurring xanthones were reviewed first by Roberts in 1961 [1]. Of the eleven xanthones of angiosperm origin then known, seven occurred in the Gentianaceae, three in the Guttiferae and one (mangiferin, a *C*-glucoside) in many families. Eight years later, Carpenter *et al.* [2] published a survey of the distribution of more than seventy xanthones in the Angiosperms. In the last few years, a large number of new naturally occurring xanthones, mainly glycosides, have been identified in the genera *Swertia*, *Gentiana* and *Canscora* (Gentianaceae).

The growing interest in these compounds is easily explained by their pharmacological activity (monoamine oxidase inhibition, antipsychotic action, tuberculostatic effect) as well as their importance in chemotaxonomy (as useful systematic markers [3]).

NATURALLY OCCURRING XANTHONE GLYCOSIDES

One has to make the distinction between *C*-glycosides and *O*-glycosides. The former are resistant to acidic and enzymatic hydrolysis (the sugar moiety is attached to the xanthone skeleton by a C—C bond), whereas the latter possess the classic glycosidic binding.

C-glycosides

Mangiferin **1**, the most widely distributed *C*-glucoside, was encountered for the first time in 1908 by Wiechowski [4] in *Mangifera indica* L. (Anacardiaceae). It was studied extensively by several research groups [5–8] and the conclusive structure was established as 2-*C*-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone. Mangiferin occurs widely among angiosperms and has also been identified in ferns [9, 10]. Its distribution in a large number of families has been reported [2, 11, 12]. An isomer, isomangiferin **2**, has been isolated from the aerial parts of *Anemarrhena asphodeloides* Bunge (Liliaceae) and identified as 4-*C*-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone by Aritomi and Kawasaki [13]. The relation between isomangiferin and mangiferin in structures and properties corresponds to that between flavonoid 8-*C* and 6-*C*-glucosides such as orientin and isoorientin. Another *C*-glycoside with the same substitution pattern has also been isolated from the barks of *Mangifera indica* L. On the basis of chemical and spectral data, as well as synthesis it has been formulated as 2-*C*-β-D-glucopyranosyl-3-methoxy-6,7-trihydroxyxanthone **3** or 3-*O*-methylmangiferin, usually referred to as homomangiferin [14].

In 1973, a new glycoxanthone **4** with an oxidation pattern other than that of mangiferin was found in the alcoholic extract of roots of *Canscora decussata* Schult. by Ghosal and Chaudhuri [15]. The structure of this glycoside has been established by chemical transformations and spectral (UV, IR, NMR, MS) evidence as 2-*C*-β-D-glucopyranosyl-1,3,5,6-tetrahydroxyxanthone **4**. From the rhizome of *Iris florentina* L. (Iridaceae), Arisawa *et al.* [16] have isolated in the same year another 1,3,5,6-tetraoxygenated xanthone C_2-β-D-glucoside, which they named irisxanthone. Its structure is represented by 2-*C*-β-glucopyranosyl-5-methoxy-1,3,6-trihydroxyxanthone **5**.

Two hydrolysable derivatives of xanthone-*C*-glucosides, which form a new type of glycoxanthone, have been found by Smith and Harborne [10] in the fern *Asplenium montanum* Willd. (Aspleniaceae). These two compounds are the *O*-glycosides of mangiferin and isomangiferin respectively. In both cases, the hydrolysable sugar is

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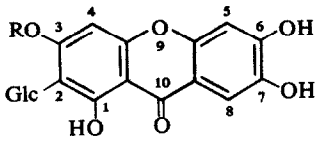
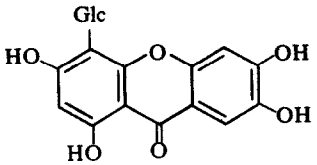
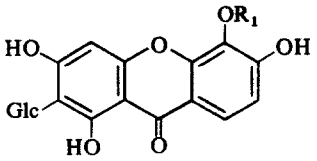


(1) R = H, mangiferin
(3) R = Me, homomangiferin



(2) isomangiferin



(4) R_1 = H
(5) R_1 = Me, irisxanthone

Table 1. Naturally occurring xanthone-*O*-glycosides

Trioxygenated xanthones Glycoside	Oxidation pattern			Natural sources	Reference
	1	3	5		
6	OMe	*O*-rut.	OH	*Canscora decussata*	[21]
	1	3	7		
7	OH	*O*-prim.	OMe	*Gentiana lutea*	[19, 22]
				G. pannonica	[23]
				G. punctata	[23]
				G. purpurea	[23]

Tetraoxygenated xanthones Glycoside	Oxidation pattern				Natural sources	Reference
	1	3	4	5		
8	*O*-glc.	OH	OMe	OMe	*Swertia bimaculata*	[24]
9	*O*-glyc.	OH	OMe	OMe	*S. bimaculata*	[25]
10	OH	*O*-glc.	OMe	OMe	*S. bimaculata*	[24]
	1	3	5	8		
11	*O*-glc.	OH	OH	OH	*S. purpurascens*	[26]
					S. racemosa	[27]
					S. randaiensis	[28]
12	*O*-glc.	OMe	OH	OH	*S. purpurascens*	[26]
					S. racemosa	[27]
					S. japonica	[1]
13	*O*-glc.	OMe	OMe	OH	*S. bimaculata*	[25]
					Frasera caroliniensis	[29]
14	OH	OH	OH	*O*-glc.	*Gentiana campestris*	[30]
					G. germanica	[31]
					G. ramosa	[31]
15	OH	OMe	OH	*O*-glc.	*G. campestris*	[30]
					G. germanica	[31]
					G. ramosa	[31]
					Swertia perennis	[32]
					S. purpurascens	[26]

Table 1—*continued*

Glycoside	Oxidation pattern				Natural sources	Reference
	1	3	7	8		
16	*O*-glc.	OH	OH	OH	*Swertia dilatata*	[27]
		—			*S. gractlescens*	[27]
					S. perennis	[32]
					Gentiana bavarica	[33]
17	*O*-prim.	OH	OH	OH	*G. bavarica*	[33]
					G. verna	[34]
18	*O*-prim.	OMe	OH	OH	*G. bavarica*	[33]
					G. verna	[34]
					G. nivalis	[35]
19	OH	OMe	*O*-rut.	OH	*G. bavarica*	[36]
20	OH	OMe	*O*-(Ac)rut.	OH	*G. bavarica*	[33, 36]
21	OH	OMe	OH	*O*-glc.	*G. verna*	[34]
22	OH	OMe	OH	*O*-prim.	*G. alpina*	[37]
					G. ciliata	[37]
					G. kochiana	[38]
23	*O*-prim.	OMe	OMe	OH	*Swertia perennis*	[32]
24	*O*-prim.	OMe	OH	OMe	*Gentiana bavarica*	[33]
					G. nivalis	[35]
					G. verna	[34]
25	OH	OMe	*O*-prim.	OMe	*G. alpina*	[37]
					G. angustifolia	[37]
					G. ciliata	[37]
					G. clusii	[37]
					G. kochiana	[38]
26	OMe	*O*-prim.	OMe	OH	*Gentiana alpina*	[37]
					G. angustifolia	[37]
					G. ciliata	[37]
					G. clusii	[37]
					G. kochiana	[38]
27	*O*-prim.	OMe	OMe	OMe	*G. alpina*	[37]
					G. bavarica	[33]
					G. ciliata	[37]
					G. clusii	[37]
					G. nivalis	[35]
					G. verna	[34, 39]
					Swertia perennis	[32]

Pentaoxygenated xanthones

Glycoside	Oxidation pattern					Natural sources	Reference
	1	2	3	4	7		
28	*O*-glyc.	OMe	OMe	OH	OMe	*Swertia bimaculata*	[25]
	1	3	4	5	8		
29	*O*-glc.	OH	OMe	OMe	OH	*Gentiana campestris*	[40]
						G. germanica	[31]
						G. ramosa	[31]

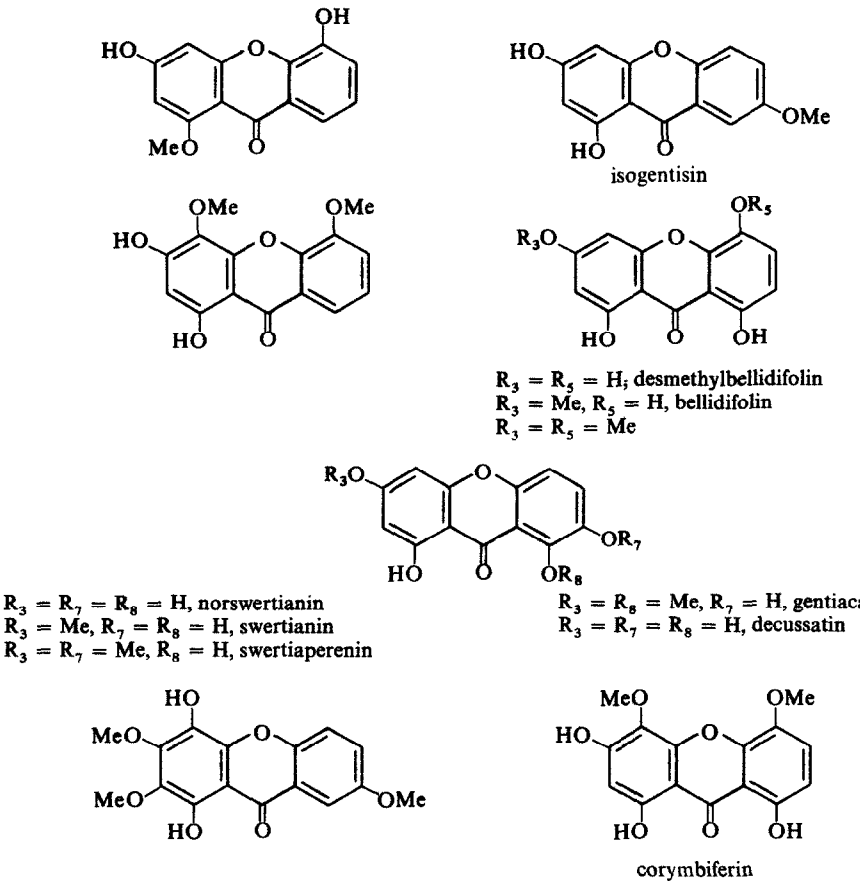
glc: glucose, prim: primverose, glyc. unidentified sugar, rut: rutinose.

attached to the *C*-glycosyl moiety of the xanthone in a yet undefined position. The characteristics of these glycosides are similar to that of the *O*-glycosyl-6- or 8-*C*-glycosides of flavones. The numerous cases of analogous flavones recently found suggests that corresponding xanthones may be soon discovered.

Xanthone-*C*-glycosides present a unique taxonomic character in plants and both in their distribution and biogenesis they are more closely related to flavonoids than to xanthones [2, 17]. Mangiferin co-occurs commonly with isoorientin, but has recently been found to co-occur also with different groups of polyoxygenated xanthones (see Table 1).

O-*glycosides*

Glycosides were not common at the time of the last review on xanthones, published in 1969. Apart from mangiferin, a *C*-glucoside, the only reported examples of *O*-glycosides were gentiacauloside **25** isolated from *Gentiana acaulis* [18], gentioside **7** from *G. lutea* [19] and



Aglycones of naturally occurring xanthone-*O*-glycosides

swertianolin **12** from *Swertia japonica* [1] (Gentianaceae). More than twenty xanthone-*O*-glycosides are now known and their natural occurrence is restricted so far to the members of the family Gentianaceae (see Table 1). However, present day isolation techniques will certainly provide the means of discovering glycosides in other families which contain polyoxygenated xanthones. Among the known compounds are *O*-monosides and *O*-biosides. β-D-glucose is the only monosaccharide to be encountered, whereas in the analogous flavones, other sugars are frequently found, such as D-galactose, L-rhamnose, L-arabinose and D-apiose [20]. Only two disaccharides have been discovered to date. These are primeverose or 6-*O*-(β-D-xylopyranosyl)-β-D-glucopyranose which occur frequently and rutinose or 6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranose which appears to be rarer and has only been identified in a trioxygenated xanthone of *Canscora decussata* Schult. [21] and in a tetraoxygenated xanthone of *Gentiana bavarica* L. [33]. In contrast, Wagner [20] has described 25 types of disaccharides in various flavonoid biosides. The first xanthone diglucoside, norswertianin-1-*O*-glucosyl-3-*O*-glucoside has been isolated recently from *Swertia perennis* [64].

The aglycones are tri-, tetra- or pentaoxygenated. The 1,3 oxidation pattern is found in each compound with substitution in the 4, 5, 7 or 8 positions. In the known *O*-glycosides, substitution never occurs in position 6. Until now only two trioxygenated glycosides have been identified: 1-hydroxy-7-methoxy-3-*O*-primeverosylxanthone **7** in the roots of different *Gentiana* spp. [19, 23] and 5-hydroxy-1-methoxy-3-*O*-rutinosylxanthone **6** in the aerial parts of *Canscora decussata* Schult. [21]. On the other hand, the tetraoxygenated glycosides are more common, in particular those containing the 1, 3, 7, 8 oxidation pattern. To date 12 compounds having this pattern are known: two glycosides, two rutinosides and eight primeverosides. Kaldas *et al.* [40] are the only ones to have fully characterized a pentaoxygenated xanthone. This glycoside 1-*O*-β-D-glucopyranosyl-3,8-dihydroxy-4,5-dimethoxyxanthone **29**, was isolated from the leaves of *Gentiana campestris* L. and may be identical to a corymbiferine (1,3,8-trihydroxy-4,5-dimethoxyxanthone) derivative mentioned by Ross [41] in his work on a species from New Zealand related to *Gentiana campestris* L. This author has not, however, defined the position of the glucose on the xanthone moiety.

The first acyl-*O*-glycoside was isolated by Hostettmann *et al.* [33] from the aerial parts of *Gentiana bavarica* L. This compound was shown to be 7-*O*-rutinosylswertianine **20** with an acetyl group in an undefined position of the sugar moiety. Quite recently, by use of ^{13}C-NMR spectroscopy, these authors were able to locate the acetyl group in position 4 of the rhamnose [36]. By contrast, many acylated flavone glycosides are known [20, 42].

A final *O*-glycoside should be mentioned: euxanthic acid (1-hydroxy-7-*O*-glucuronylxanthone), a degradation product of mangiferin, which occurs in the urine of cows fed on mango leaves [1].

Table 2. Distribution of xanthone glycosides (*O*- and *C*-glycosides) in Gentianaceae

Gentiana Section	Species	Glycoside	Ref.
Amarella	*G. campestris*	**14** (1, 3, 5, 8)	[30]
		15 (1, 3, 5, 8)	[30]
		29 (1, 3, 4, 5, 8)	[40]
		1 mangiferin	[30]
	G. germanica	**14** (1, 3, 5, 8)	[31]
		15 (1, 3, 5, 8)	[31]
		29 (1, 3, 4, 5, 8)	[31]
		1 mangiferin	[31]
	G. ramosa	**14** (1, 3, 5, 8)	[31]
		15 (1, 3, 5, 8)	[31]
		29 (1, 3, 4, 5, 8)	[31]
		1 mangiferin	[31]
Antarctophila	*G. corymbifera*	**29** (1, 3, 4, 5, 8)	[41]
Aptera	*G. cruciata*	**1** mangiferin	[44]
Coelanthe	*G. lutea*	**7** (1, 3, 7)	[19, 22]
		1 mangiferin	[22]
	G. pannonica	**7** (1, 3, 7)	[23]
	G. punctata	**7** (1, 3, 7)	[23]
	G. purpurea	**7** (1, 3, 7)	[23]
Crossopetalum	*G. ciliata*	**22** (1, 3, 7, 8)	[37]
		25 (1, 3, 7, 8)	[37]
		26 (1, 3, 7, 8)	[37]
Cyclostigma	*G. bavarica*	**16** (1, 3, 7, 8)	[33]
		17 (1, 3, 7, 8)	[33]
		18 (1, 3, 7, 8)	[33]
		19 (1, 3, 7, 8)	[36]
		20 (1, 3, 7, 8)	[33, 36]
		24 (1, 3, 7, 8)	[33]
		27 (1, 3, 7, 8)	[33]
	G. favrati	**1** mangiferin	[35]
	G. nivalis	**18** (1, 3, 7, 8)	[35]
		24 (1, 3, 7, 8)	[35]
		27 (1, 3, 7, 8)	[35]
		1 mangiferin	[35]
	G. utriculosa	**1** mangiferin	[35]
	G. verna	**17** (1, 3, 7, 8)	[34]
		18 (1, 3, 7, 8)	[34]
		21 (1, 3, 7, 8)	[34]
		24 (1, 3, 7, 8)	[34]
		27 (1, 3, 7, 8)	[34]
Pneumonanthe	*G. asclepiadea*	**1** mangiferin	[46]
	G. pneumonanthe	**1** mangiferin	[50]
Thylacites	*G. alpina*	**22** (1, 3, 7, 8)	[37]
		25 (1, 3, 7, 8)	[37]
		26 (1, 3, 7, 8)	[37]
		27 (1, 3, 7, 8)	[37]
	G. angustifolia	**25** (1, 3, 7, 8)	[37]
		26 (1, 3, 7, 8)	[37]
	G. clusii	**25** (1, 3, 7, 8)	[37]
		26 (1, 3, 7, 8)	[37]
		27 (1, 3, 7, 8)	[37]
	G. kochiana	**22** (1, 3, 7, 8)	[38]
		25 (1, 3, 7, 8)	[38]
		26 (1, 3, 7, 8)	[38]
	S. bimaculata	**8** (1, 3, 4, 5)	[24]
		9 (1, 3, 4, 5)	[25]
		10 (1, 3, 4, 5)	[24]
		13 (1, 3, 5, 8)	[25]
		29 (1, 2, 3, 4, 7)	[25]
	S. chirata	**1** mangiferin	[48]
	S. dilatata	**16** (1, 3, 7, 8)	[27]
		1 mangiferin	[27]
	S. gracilescens	**16** (1, 3, 7, 8)	[27]
		1 mangiferin	[27]
	S. japonica	**12** (1, 3, 5, 8)	[1]
	S. perennis	**15** (1, 3, 5, 8)	[32]
		16 (1, 3, 7, 8)	[32]
		23 (1, 3, 7, 8)	[32]
		27 (1, 3, 7, 8)	[32]
		1 mangiferin	[32]
	S. purpurescens	**11** (1, 3, 5, 8)	[26]
		12 (1, 3, 5, 8)	[26]
		15 (1, 3, 5, 8)	[26]
	S. racemosa	**11** (1, 3, 5, 8)	[27]
		12 (1, 3, 5, 8)	[27]
	S. randaiensis	**11** (1, 3, 5, 8)	[28]
	S. swertopsis	**1** mangiferin	[48]
	S. tosanensis	**12** (1, 3, 5, 8)	[1]
	C. decussata	**6** (1, 3, 5)	[21]
		1 mangiferin	[15]
		4 glycoxanthone	[15]

( ): oxidation pattern.

The various positions in which sugars are attached to the xanthone nucleus are noteworthy: of the twenty four known cases, thirteen xanthones carry their glycosidic portion in position 1, four in position 3, three in position 7 and four in position 8. The preference for position 1 is surprising in view of the vicinity of the carbonyl function; the corresponding position in the flavone nucleus (position 5) is rarely glycosylated. A study of the biosynthetic pathway leading to xanthone glycosides would be useful in understanding the frequent blocking of the position α to the carbonyl. It would be tempting to suggest that glycosylation takes place prior to the closure of the xanthone ring. The position of the glycoside linkage is not only biogenetically significant, but has a chemotaxonomic importance in the Gentianaceae [35].

CHEMOTAXONOMIC SIGNIFICANCE

Since xanthone glycosides are known so far only in the Gentianaceae, their chemotaxonomic importance is limited to this family and in particular to the genus *Gentiana*, where they mostly occur. According to Kusnezow [43] this genus is divided into two subgenera, *Eugentiana* and *Gentianella*, which are divided into sections. The genus comprises 19 sections in all. The glycoside distribution is given in table 2.

It is noteworthy that the 1,3,7,8-tetraoxygenated xanthone glycosides occur only in species of the *Cyclostigma* and *Thylacites* sections and in *Gentiana ciliata* L. (*Crossopetalum*). The remarkable similarity in the distribution of the glycosides in the *Cyclostigma* and *Thylacites* sections might confirm their phylogenetic relationship put forth by Scharfetter [44]. The uniform distribution of 1,3,5,8- and 1,3,4,5,8-oxidized xanthones in species of the *Amarella* section (subgenus *Gentianella*) was reported by Kaldas *et al.* [31]. These oxidation patterns were also found in *Gentiana corymbifera* Kirk. and *Gentiana bellidifolia* Hook. [45] which belong to the same subgenus (section *Antarctophila*).

The only trisubstituted xanthone glycoside, gentioside

7, has been encountered only in species of the *Coelanthe* section, in the roots and leaves of *Gentiana lutea* L. and in the roots of *Gentiana purpurea* L., *G. punctata* L. and *G. pannonica* Scop. It does not occur, however, in the aerial parts of these species nor in *G. villarsi* Ronn. and *G. burseri* Lapeyr., two other species of this section. *O*-glycosides are not found in species of the other sections studied (*Aptera* [46], *Pneumonanthe* [46], *Chondrophylla* [47]). The *C*-glucoside mangiferin **1** is encountered in a variety of species (see table 2) but further studies are necessary to draw any conclusion about its chemotaxonomic signficance.

In summary, the oxidation pattern of xanthone is generally uniform within a particular section and is of prime importance in the chemotaxonomy of gentians. Species are differentiated only by glycosylation characteristics. A complete investigation of *G. bavarica* L., *G. nivalis* L. and *G. verna* L. (section *Cyclostigma*) [35] showed that apart from two common glycosides (**24** and **27**), each species has its specific glycosides. Whereas gentiabavarutinoside **20** and desacetylgentiabavarutinoside **19** are found in high concentration in *G. bavarica* L., they do not occur in the other two species. On the other hand, *G. verna* L. is characterized by swertianin-8-*O*-glucoside **21**, the only 8-*O*-glycoside in the section. Finally, the several glycosides found in both *Gentiana* and *Swertia* confirm the relationships between the two genera [48].

ISOLATION AND STRUCTURAL DETERMINATION

Extraction and separation

Extraction of xanthone glycoside is usually carried out on dried plant material. The classical method using increasingly polar solvents (light petrol ether, chloroform, methanol) has proved to be very effective [33]. The MeOH extract is chromatographed over polyamide columns with 50% aqueous MeOH with increasing proportions of MeOH [34]. Pure compounds are obtained after purification over Sephadex LH 20 columns. In contrast to analogous flavones, xanthone glycosides are easily recrystallized from MeOH. Column chromatography on silica gel (varying proportions of ether and methanol) is used to separate glycosides containing only a few phenolic hydroxyls [21]. Preparative TLC on silica gel (AcOEt–MeOH–H_2O 21:4:3) has been employed in instances of difficult separation (compounds **19** and **20**).

Frequently used solvents in TLC are: on polyamide: MeOH–H_2O (9:1), MeOH–H_2O–AcOH (90:5:5); on cellulose: HOAc (5–30%); on silica gel: Py–H_2O–EtOAc–MeOH (12:10:80:5) and EtOAc–MeOH–H_2O (21:4:3). Chromatoplates are viewed in UV light. In certain cases, spraying with 5% KOH in MeOH has been advantageous [51].

The hydrolysis of glycosides is carried out by refluxing MeOH solutions with HCl (see standard procedure for flavonoids [52]). Enzymatic hydrolysis is also of interest in the case of glucosides. Aglycones are separated by polyamide TLC (MeOH–HOAc–H_2O, 90:5:5 or C_6H_6–MeOH–HOAc, 45:32:16) on silica gel TLC (C_6H_6–EtOAc, 3:1 or C_6H_6–$CHCl_3$, 1:1). Ample details can be found in the systematic works of Arends [53] and Saleh [54].

Recently, Hostettmann and McNair [55] separated naturally occurring xanthones by HPLC using microporous chemically bonded silica gel (Micopak CN or Micropak NH_2, solvents: hexane–$CHCl_3$ (13:7) *iso*-octane–$CHCl_3$ (3:17) or dioxane–CH_2Cl_2 (1:9), detection by UV at 254 nm). Very polar aglycones as well as glycosides are resolved on reversed-phase columns (NH_2, C_8 or C_{18} bonded silica gel) using acetonitrile–H_2O as solvent [56].

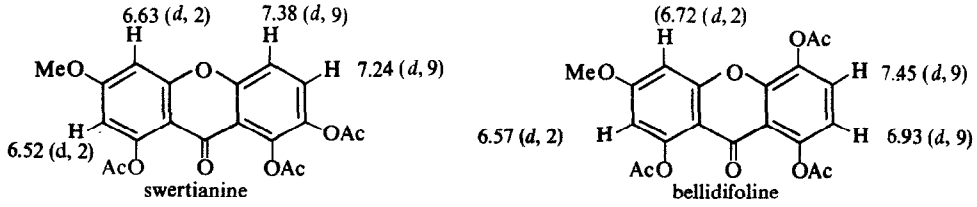
ULTRAVIOLET SPECTROSCOPY

Several papers dealing with the application of UV spectroscopy to the structure determination of xanthones have been published [57, 58]. This technique is basically useful for locating free hydroxyl groups on the xanthone skeleton. In particular, a free hydroxyl group at position 3 is easily detected by addition of NaOAc which results in a bathochromic shift of the 300–330 nm band with increasing intensity. When position 3 is blocked (e.g. methoxyl or glycosyl), the UV curve is not modified. Hydroxyl groups situated *peri* to the carbonyl function (position 1 or 8) are evidenced by the complex formed by addition of $AlCl_3$ which is stable to HCl. *Ortho*-dihydroxyl groups similarly give this complex, but can be distinguished from the former by the instability of the complexes in HCl (see also procedure used for flavonoids [59]).

Comparison of the spectra of glycosides with those of their aglycones occasionally gives directly the position of the glycosidic linkage; this is the case for position 3. However, it is practical to methylate the phenolic hydroxyls before cleavage of the sugar moiety since the sole remaining hydroxyl group is easier to determine. UV spectroscopy can also give useful information about the nature of the oxidation pattern of xanthones. However, since no systematic investigation has yet been carried out on this aspect, conclusions can only be drawn from NMR data.

NMR SPECTROSCOPY

NMR spectroscopy is the most useful method for the structural determination of xanthones. It gives information about the substituents on each ring and also about the nature of the oxidation pattern. Several authors [33, 38] have employed the acetylated derivatives in the structure determination of glycosides. The number and relative positions of acetyl and methoxyl groups can be determined by observing the shift of aromatic protons which occurs upon replacing a methoxyl group by an acetyl group. Singlets between δ 2.40 and 2.50 are indicative of acetylation *peri* to the carbonyl function (position



Isomeric tetraoxygenated xanthones: comparison of the chemical shifts of the aromatic protons.

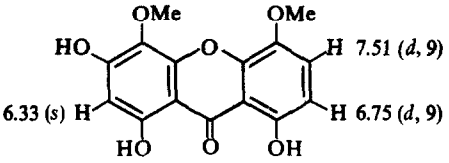
1 or 8) since for other positions on the ring the acetyl singlets fall between δ 2.30 and 2.35. The presence of a chelated OH at δ 12–13 in the spectra of the non acylated compound also confirms hydroxyl substitution at position 1 or 8. When these positions are unsubstituted, aromatic protons appear between δ 7.70 and 8.05 [60, 61].

Isomeric xanthones, tetraoxygenated in position 1,3,7,8 and 1,3,5,8, show in their ^{1}H-NMR spectra two *meta* and two *ortho* coupled protons. They can be distinguished by the fact that the *ortho* coupled protons in the 1,3,7,8-system appear at a lower field than those of the 1,3,5,8-system (e.g. swertianine [33] and bellidifoline [30]).

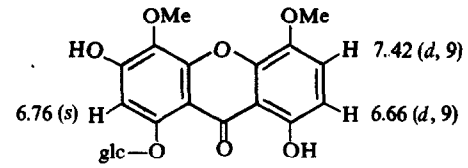
The NMR signals of the acetates of some naturally occurring *C*-glucosyl compounds in $CDCl_3$ have been examined by Gentili and Horowitz [62]. They noted that the signals of the 2″-*O*-acetyl methyl protons of 8-*C*-glucosylflavone acetates were found at higher fields than those of the corresponding 6-*C*-glucosyl compounds. In a similar manner 2-*C* and 4-*C*-isomeric glucosylxanthones can be differentiated [63].

For example, the 2″-*O*-acetyl singlet of mangiferin octaacetate appears at δ 1.79 whereas the corresponding signal is shifted to higher fields (δ 1.73) in isomangiferin octaacetate.

When only one of the hydroxyl groups *peri* to the carbonyl function is free and chelated, then protons on the aromatic ring containing the chelated hydroxyl group absorb at higher fields than the corresponding protons on the nonchelated ring. This is ascribable to the higher electron density of the chelated ring imposed by the chelation. When dealing with glycosides, this effect is best observed in DMSO-d_6 [64]. Hence comparison of the spectra of glycosides with those of their aglycones directly gives the position of attachment of the sugar moiety on the xanthone nucleus, as seen for example in corymbiferin and its glucoside **29** [40].



corymbiferin



29

Application of the nuclear Overhauser effect (NOE) to the aromatic system may be used to determine the position of substituent groups [65, 66]. The only reported example is in the structure determination of irisxanthone **5** where irradiation of the 5-OMe exerted an NOE on 4-H [16]. These results have been confirmed [67].

^{13}C-NMR spectroscopy is also useful in structure determination of xanthone glycosides and was used by Hostettmann *et al.* [36] for locating the acetyl group on the sugar moiety of gentiabavarutinoside. It is particularly diagnostic for determining the sugar linkage in disaccharides (or polysaccharides). Whereas the signal of the carbon carrying the primary alcohol appears at δ 62 in glucose, this signal is shifted to δ 67 in disaccharides possessing a 1 → 6 linkage (rutinose [36] or primeverose [68]).

A systematic ^{13}C-NMR study of naturally occurring xanthones is now in progress and will be published shortly [68]. The chemical shift of the carbonyl carbon is approximately δ 184.5 when positions 1 and 8 are substituted by hydroxyl groups. When one of these positions is occupied either by a methoxyl group or a sugar moiety, the carbonyl signal is shifted upfield by about 4 ppm; if both positions bear methoxyl groups or sugar moieties, the upfield shift is about 10 ppm. When methoxyl groups are located in positions 1 or 8, they appear at approximately δ 60–61, whereas they appear about δ 56 when located in other positions on the xanthone nucleus.

MASS SPECTROMETRY

This technique has had few applications in the structure determination of xanthone glycosides. The first was due to Prox [69] who established the fragmentation pattern of mangiferin and related *C*-glycosides. Aritomi *et al.* [70] obtained satisfactory results by working on peracetylated derivatives of the same and analogous compounds. In mass spectra of *O*-glycosides, no discernable molecular ion peak can be observed, but an important fragment ion peak due to the aglycone moiety appears, followed by further fragmentations. Significant fragment ions from the loss of OH, H_2O and CHO are typical for xanthones and related compounds with a methoxy substituent *peri* to a carbonyl function [21,71]. Recently, Hostettmann *et al.* [72] undertook a systematic study of permethylated xanthone-*O*-glycosides. A weak molecular peak could be obtained and it was possible to differentiate between several oxidation patterns.

SYNTHESIS

Since mangiferin was the first xanthone glycoside which has been found in plants and structurally elucidated, the first synthetic approach was concentrated on this glycoside. The synthesis was performed independently by Nott and Roberts [7] and Bhatia and Seshadri [8], both using Chopin's [42] method for the synthesis of 6-*C*- and 8-*C*-glycosides of 5,7-dihydroxyflavonoids. In this method the corresponding xanthone aglycones were condensed with a large excess of α-acetobromo sugars in anhydrous methanol in the presence of sodium iodide and sodium methoxide. After hydrolysis of the *O*-glycosides and purification, the synthetic *C*-glycoside was obtained in small amounts. It was identical in every respect with natural mangiferin, thereby confirming the 2-position of the sugar and its β-configuration.

The first synthesis of an *O*-glycoside has been performed in our laboratory recently [73] following a procedure used before in the flavonoid field [20]. With 1,3-dihydroxy-7-methoxyxanthone (isogentisin) as starting material, we obtain the 3-*O*-glucoside by condensing it with α-acetobromglucose in the presence of silver carbonate and pyridine as solvent. The reaction product was saponified and the glycoside obtained after column and preparative chromatography in *ca* 20% yield. Besides the glucoside, the synthesis of the naturally occurring 3-*O*-primvero-

side of isogentisin (= gentiosid) and some other xanthone-mono- and diglycosides have been already achieved or will be completed shortly. The glycosidation of polyoxygenated xanthones will require partially benzylated or methylated xanthones before condensation and perhaps also modified Koenigs–Knorr procedures.

PHARMACOLOGY

Mangiferin was the first xanthone glycoside to be investigated pharmacologically. The initial results reported by Finegan *et al.* [74] on the diuretic and cardiotonic action of mangiferin in animals could not be confirmed fully by Bhattacharya *et al.* [75]. However, by investigating extracts, fractions and pure xanthones of *Canscora decussata*, they found a remarkable CNS stimulating effect of mangiferin in doses of 50–100 mg/kg which could be blocked by pretreatment of chlorpromazine. This effect could be evidenced by hyperactivity, fine tremors, piloerection, increased spontaneous motility, sedation, potention of the analgesia and potentiation of amphetamine toxicity in aggregated mice. This effect is not fully exhibited or only to much smaller extent by the corresponding free xanthones. According to *in vitro* experiments it could be shown that this action was mediated via a monoaminooxidase inhibition [76]. The mangiferin-induced potentiation of the antinociceptive effect of morphine, like that of nialamide, was 5-hydroxytryptamine mediated [77].

In contrast to mangiferin and other polyoxygenated xanthones, Ghosal *et al.* [78] found for xanthone *O*-glycosides and related compounds produced a CNS depressant or antipsychotic action in mice and rats. This opposite effect of *O*-glycosides raises the question of structure–activity relationships. Investigations with model compounds have been started recently by an Italian research group [79] to clarify this problem. The question as to which xanthones are metabolized in the body is also unknown. There is only one report on mangiferin by Wiechowski [80]. After feeding mangiferin (mangin) to rabbits, the author isolated euxanthic acid from the urine which has been identified as the *C*-glucuronoside of a dihydroxy-xanthone. It is likely that in addition to the oxidation of the sugar moiety, two hydroxyl groups are lost.

In summary, these results may give a reasonable explanation for the therapeutic use of *Canscora decussta* (Gentianaceaea) in some mental disorders e.g. melancholia. The use of the same plant against liver diseases in indigeneous medicine can also be correlated with mangiferin, since only this *C*-glycoside and not the total xanthones causes a significant choleretic effect [75].

In the tuberculostatic activity reported for xanthones, the aglycones showed a higher activity (10 μg/ml) in comparison with mangiferin and 3,5,8-trihydroxy-xanthone-1-*O*-glucoside (norswertianolin) [81]. The minimum inhibitory concentration (MIC) for mangiferin was in the order of 200 μg/ml.

Addendum—After completion of this review article a publication on the isolation of mangiferin-*O*-glucoside and isomangiferin-*O*-glucoside from *Hedysarum flavescens* [82] came to our knowledge. As in the case of those from *Asplenium montanum* [10], the position of the *O*-sugar could not be determined.

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