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C-GLYCOSYLXANTHONE AND FLAVONOID VARIATION WITHIN THE FILMY-FERNS (HYMENOPHYLLACEAE)*

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Key Word Index—*Hymenophyllum; Trichomanes;* Hymenophyllaceae; Filicales; flavonoid *C*- and *O*-glycosides; mangiferin—acetylated and benzoylated; new *C*-glycosylxanthones; dilatatin; tricetin 8-*C*-glucoside.

Abstract—The identification and distribution of flavone and flavonol-*O*-glycosides, *C*-glycosylflavones and *C*-glycosylxanthones are fully documented for *Hymenophyllum tunbridgense, H.* (*Mecodium*) *dilatatum, H.* (*M.*) *recurvatum, Trichomanes* (*Didymoglossum*) *petersii, T.* (*Cardiomanes*) *reniforme,* and *T.* (*Polyphlebium*) *venosum.* A number of novel acetylated and benzoylated *C*-glycosylxanthones, orientin and isoorientin arabinofuranosides, and tricetin 8-*C*-glucoside are reported for the first time. This preliminary study suggests that a wide variety of chemotypes is encompassed within this family, and that a thorough chemotaxonomic study should be of considerable value in resolving the much disputed subfamilial taxonomic relationships. The available data do not support the division of the family into the two classic genera *Hymenophyllum* and *Trichomanes.*

INTRODUCTION

The Hymenophyllaceae, or filmy ferns, constitute a large group of chiefly tropical or sub-tropical ferns of approximately 650 species [1]. The family may be considered to consist of essentially two genera, *Hymenophyllum* and *Trichomanes* (see [2]); however, at one time *Trichomanes* was placed in a separate family, the Trichomanaceae [3]. In contrast Copeland [4, 5] and Pichi-Sermolli [3, 6] consider the family to consist of approximately 33 genera. There is still however, divergence of opinion [1, 2, 7] and the number of genera (and other subfamilial taxa) is disputed.

Very little chemical data have been published to date for the species of the Hymenophyllaceae. In a survey for fern flavonoid aglycones Harada and Saiki [8] reported the presence of apigenin in *Hymenophyllum barbatum* and kaempferol in *H.* (*Mecodium*) *polyanthose* and *Trichomanes* (*Nesopteris*) *thysanostoma.* Voirin [9] identified kaempferol in *Trichomanes javanicum,* kaempferol and quercetin in *Hymenophyllum* sp. and 5-*O*-methylquercetin (azaleatin) in other unidentified *Trichomanes* species. Voirin also reported leucocyanidins in the seven species he studied. In a survey for flavonoid glycosides in 'primitive' leptosporangiate ferns, Wallace and Markham [10] identified kaempferol and quercetin-3-*O*-glucosides in *T.* (*Cardiomanes*) *reniforme* and their 3-*O*-rhamnoglucosides in *H.* (*Mecodium*) *demissum.* Other monoflavonoids and biflavones were not detected in these two species.

The present communication presents a preliminary investigation of the polyphenolic variation within the Hymenophyllaceae. Six selected species have been studied in depth and all flavonoids and xanthones detected were studied.

RESULTS

A summary of the results obtained in the present study is presented in Table 1 along with data derived from previous, less exhaustive, studies reported in the literature. For the species *Hymenophyllum tunbridgense, H.* (*Mecodium*) *dilatatum, H.* (*M.*) *demissum, H.* (*M.*) *recurvum, Trichomanes* (*Didymoglossum*) *petersii, T.* (*Cardiomanes*) *reniforme* and *T.* (*Polyphlebium*) *venosum* a complete evaluation of the xanthones and non-leucocyanidin type flavonoids was carried out and the absence of certain chemical characters is noted only for these species.

I. *Flavonoids*

Flavonoid-*O*-glycoside structure analyses were based on absorption spectroscopy, NMR spectroscopy, acid and enzyme hydrolyses, and subsequent product analysis by PC and/or TLC (for aglycones and *C*-glycosides), MS (for some), and GLC (for sugars). Where possible, confirmation of structure was obtained by cochromatography with authentic samples. The detection of a kaempferol *O*-glycoside in the xanthone-rich *H. recurvum* requires special comment since flavonoids could not be detected even on heavily loaded 2D-PCs of this species. The detection was accomplished by acid-hydrolysing the combined extracts from non-xanthone containing areas of 20 2D-PCs. PC of the product gave traces of a flavonol which

* Part I in the series "The Chemotaxonomy of the Hymenophyllaceae".

Table 1. A summary of the flavonoid and *C*-glycosylxanthone

Classic genera	Copeland's genera [4, 5]	*C*-Glycosylxanthones									Kaempferol
		Isomangiferin	Mangiferin	6′-*O*-Acetyl-mangiferin	2′-*O*-Benzoyl-mangiferin	4′-*O*-Benzoyl-mangiferin	6′-*O*-Benzoyl-mangiferin	Di-*O*-Benzoyl-mangiferin	Dilatatin	Isodilatatin	
Hymenophyllum	*Hymenophyllum tunbridgense*	—	—	—	—	—	—	—	—	—	
	H. barbatum [8]										+ +
	H. sp. [9]										
	Mecodium demissum [10]	—	—	—	—	—	—	—	—	—	
	M. dilatatum	—	—	—	—	—	—	—	+ +	+ +	
	M. recurvum	+	+ +	—	+ +	+ +	+ +	+	—	—	
	M. polyanthose [8]										+ +
Trichomanes	*Didymoglossum petersii*	—	—	—	—	—	—	—	—	—	—
	Cephalomanes javanicum [9]										+ +
	Cardiomanes reniforme [10]	—	+ +	+ +	—	—	—	—	—	—	
	Polyphlebium venosum	—	—	—	—	—	—	—	—	—	—
	Nesopteris thysanostoma [8]										+ +

+ + = Major component, + = minor component, tr = trace component, * = details in Experimental, — = not detectable using

was spectrally (UV-vis) and chromatographically (TLC, 4 solvents) identical with kaempferol. The glycosidic nature of the natural product is inferred from the R_f values of the region from which it was extracted (0.6–0.9, TBA; 0.6–0.7, HOAc).

Major *C*-glycosylflavones in *T. petersii* and *T. (P.) venosum*, such as vitexin, isovitexin, orientin and isoorientin were identified after isomerisation by co-chromatography of each pair of isomers with standards from *Spirodela polyrhiza* [11]. The arabinosyl derivatives of vitexin and orientin, present as major constituents in *T. venosum*, were identified via acid hydrolysis and chromatographic product analysis. A ^{13}C NMR study of the orientin arabinoside, although complicated by the presence of the equivalent isoorientin arabinoside, did show two signals at 60.0 and 61.8 ppm with no other signal visible between 62 and 70 ppm. This indicates (a) that the arabinose must be present as a furanoside, and (b) that it is not attached to the 6-hydroxyl of the glucose [12, 13]. When the signals associated with an *O*-linked β-L-furanosylarabinose [14] are 'subtracted' from this spectrum, the remaining signals at 82.1 79.3, 75.8, 71 *br* and 60.0 ppm are in accord with those expected for a 2″-*O*-glycosylated *C*-glucoside (calculated from the effects of rhamnosylation [12] of vitexin [13a]) and isoorientin [13b, 25]. On this basis the orientin and isoorientin arabinosides are tentatively considered to be 2″-*O*-β-L-arabinofuranosides.

Only one other major *C*-glycosylflavone was found in *T. venosum* which had low mobility in both TBA (R_f 0.15) and HOAc (R_f 0.13), and gave UV-visible absorption data equivalent to those of a luteolin *C*-glycoside except that the 330 nm peak was not produced on addition of $AlCl_3$. This compound was defined as tricetin 8-*C*-glucoside by a variety of physical techniques. The ^{13}C NMR spectrum exhibited a two-carbon singlet at 146.6 ppm (C-3′, 5′) and a less intense signal at 138.0 ppm (C-4′) clearly distinguishing the tricetin hydroxylation pattern. In addition, the expected signals for a *C*-linked glucose [13] were also present. The ^1H NMR spectrum, with signals at 7.10 (H-2′, 6′), 6.55 (H-3) and 6.30 (H-6), defines the glycosylation site as C-8. Consistent with 8-*C*-glycosylation, the MS of the permethyl ether lacked a significant M-31 peak and exhibited the base peak at *m/e* 415 (M-175) [15]. The molecular ion was visible at *m/e* 590 (33%) (see note added in proof).

II. *C-Glycosylxanthones*

The xanthones were readily recognised on a 2D-PC by their orange fluorescent appearance (in UV light) which turned to a fluorescent yellow in NH_3 vapour. None were converted to aglycones by acid treatment and all were thus considered to be *C*-glycosides.

(A) *Mangiferin and isomangiferin.* *C*-Glycosylxanthones are rare as natural products [16], the most common being mangiferin (2-*C*-β-D-glucosyl-1,3,6,7-tetrahydroxyxanthone) and its 4-*C*-glucosyl isomer, isomangiferin. Accordingly, the xanthones from *H. recurvum* and *T. reniforme* were co-chromatographically and spectrally (UV-vis) compared directly with authentic mangiferin and isomangiferin from *Asplenium* [17]. From this it was evident that *H. recurvum* contains both of these xanthones (plus others), *T. reniforme* contains mangiferin (plus one other) and *H. dilatatum* contains two xanthones very closely related, but not identical, to mangiferin and isomangiferin.

(B) 6-*O*′-*Acetylmangiferin.* The other xanthone in *T. reniforme*, is converted into mangiferin by both acid and alkaline hydrolysis. As such it was considered to be an acylated mangiferin although the liberated acid was not detectable by TLC analysis. ^1H NMR studies on both mangiferin and its acyl derivative revealed, in

variation amongst studied species of the Hymenophyllaceae

Flavonols							*C*-Glucosyl-flavones*			Flavones	
Kaempferol 3-*O*-glucoside	Kaempferol 3-*O*-rhamno-glucoside	Quercetin	Quercetin 3-*O*-glucoside	Quercetin 3-*O*-galactoside	Quercetin 3-*O*-arabinoside	Quercetin 3-*O*-rhamno-glucoside	Apigenin	Luteolin	Tricetin	Apigenin	Apigenin 7,4'-diglucoside
++	++		++	—	—	++	—	—	—	—	—
		++								+	
—	+	—	—	—	—	++	—	—	—	—	—
—	—		—	++	++	—	tr	tr	—	—	—
—	—		—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	++	++	—	—	+
++	—		++	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	++	++	++	—	—

the standard techniques of Mabry *et al.* [23].

the spectrum of the latter, only two signals not present in the spectrum of mangiferin, one due to a carbonyl carbon (171.4 ppm) and the other (21.2 ppm) due to a methyl carbon. This is interpreted as evidence of an acetyl function. The downfield position of the glucose C-6′ signal (64.9 ppm) of the acylated derivative relative to its position in the mangiferin spectrum (61.7 ppm), coupled with the upfield shift of the C-5′ signal, indicates that the glucose 6-hydroxyl is acetylated [12]. The xanthone is thus considered to be 6′-*O*-acetylmangiferin, a new natural product.

(C) *Benzoylmangiferins.* Xanthones in *H. recurvum* other than mangiferin and isomangiferin all gave identical sets of absorption spectra to mangiferin and all produced mangiferin together with benzoic acid (identified by MS and cochromatography) on acidic or alkaline treatment. These benzoylated mangiferins are more mobile than the parent compound in TBA and appear on a 2D-PC as two spots, one large and diffuse centred at R_f 0.7 (TBA), 0.3 (HOAc) and the other, smaller and more compact at R_f 0.88 (TBA), 0.46 (HOAc). Consistent with these PC mobilities, quantitative isolation of benzoic acid from each of the components revealed a benzoic acid: mangiferin ratio of 1:1 for the former and 2:1 for the latter.

The diffuse 'monobenzoate' spot resisted all attempts at resolution by repeated chromatography but was demonstrated to consist of a mixture of three monobenzoates by ^{13}C NMR spectroscopy. When the complex ^{13}C NMR spectrum was compared with that of mangiferin it was apparent that a number of the sugar carbon signals had been partially shifted both upfield and downfield due to benzoylation. Thus, a portion of the C-6′ signal of mangiferin appeared at 65.2 ppm instead of 61.7 ppm indicating the presence of a 6′-*O*-benzoate. As expected [12] this shift is accompanied by a 3 ppm upfield shift of a portion of the C-5′ signal to *ca* 79 ppm. In a similar manner the presence of a 2′-*O*-benzoate was indicated by the 3 ppm downfield shift of a portion of the C-2′ signal to *ca* 73.7 and 3 ppm upfield shifts of C-1′ and C-3′ to 70.7 and *ca* 76.8 ppm, respectively. The 4′-*O*-benzoate likewise was evidenced by similar shifts in the C-3′, 4′ and 5′ signals. Integration of the spectrum indicated all three benzoates to be present at about the same level. A similar ^{13}C NMR study on the dibenzoate was prevented by lack of sufficient material.

D. *Dilatatin* (C-*hexosylxanthone from* H. dilatatum). The major xanthone in *H. dilatatum* gave identical absorption spectra and elemental analysis data to mangiferin [18] but was slightly different chromatographically and had a higher optical rotation in pyridine ($[\alpha]_D^{20} = 43.1°$, mangiferin = 35.5° [19]). Differences were also evident in the ^1H and ^{13}C NMR spectra of the two. These include the chemical shift of the glycosyl H-1 signal at 5.05 ppm, $J = 9.4$ Hz (in DMSO-d_6), compared with mangiferin which was found to exhibit this signal at 4.62 ppm, and the chemical shifts of the six sugar carbons which differ markedly from those of mangiferin (see Experimental). It is evident from the above that the major xanthone in *H. dilatatum* is not mangiferin but a closely related 2-*C*-β-D-hexosyl-1,3,6,7-tetrahydroxyxanthone in which the hexose is not glucose. As such it is a new natural product and the name dilatatin is proposed for it. Acid treatment interconverts this xanthone with the other minor xanthone in the same plant which has the same absorption characteristics and is accordingly considered to be the 4-*C*-hexosyl isomer (isodilatatin). The hexose does not appear to be galactose either since the published ^{13}C NMR spectrum of *C*-linked β-D-galactose [20] does not match the spectrum obtained here. A final analysis of this data must await the availability of spectra of other authentic *C*-linked hexosides.

DISCUSSION

The potential for a chemotaxonomic study of this family is highlighted by the wide polyphenolic variation exhibited by the species so far investigated (Table 1). This range extends from species containing xanthones and lacking chromatographically detectable flavonoids (*H. recurvum*) through species containing only flavonol 3-*O*-glycosides, (*H. tunbridgense* and *H. demissum*) or only *C*-glycosylflavones, (*T. venosum*), to species containing both *C*-glycosylxanthones and flavonol 3-*O*-glycosides, (*T. reniforme*), *C*-glycosylflavones and flavone *O*-glycosides (*T. petersii*) and a combination of flavonol 3-*O*-glycosides, *C*-glycosylflavones and *C*-glycosylxanthones (*H. dilatatum*). Another, *H. barbatum*, may contain only flavone *O*-glycosides [8]; but a complete analysis of this plant has not yet been published.

Although it is premature to base biosystematic schemes on the limited data available, it is of interest to note that the historically all-inclusive genera, *Hymenophyllum* and *Trichomanes*, are not clearly delineated by their polyphenolic characteristics. Indeed there is a good deal of overlap; *C*-glycosylxanthones, flavonol 3-*O*-glycosides, flavone *O*-glycosides and *C*-glycosylflavones being found in both groups. The present data thus do not appear to support the two genera hypothesis. In fact the finding of *C*-glycosylxanthones in members of both of the classic genera, in view of the apparent rarity of these compounds in ferns [17], could well indicate a common origin for the two types of soral development (which distinguish these 'genera').

C-Glycosylxanthones are generally considered to be closely related biosynthetically to *C*-glycosylflavones with which they commonly co-occur [16, 21]. It is therefore of interest in these ferns that the two groups occur largely independent of one another, *T. reniforme* and *H. recurvum* accumulating *C*-glycosylxanthones to the exclusion of *C*-glycosylflavones and *T. venosum* and *T. petersii* accumulating *C*-glycosylflavones to the exclusion of *C*-glycosylxanthones. The two major biosynthetic pathways for polyphenolics represented in the studied members of the Hymenophyllaceae may thus be considered as those leading to the production of *C*-glycosides (xanthones *or* flavones) and flavonol *O*-glycosides. Both pathways are operative only in two of the studied species, *H. dilatatum* and *T. reniforme* and it is significant that both are considered by Copeland [4] to be primitive species within the family.

Other biochemical characters which show taxonomic potential in the Hymenophyllaceae are the glycosidic moieties of flavonol *O*-glycosides. For example *T. reniforme* accumulates 3-*O*-glycosides exclusively, *H. demissum* the 3-*O*-rhamnoglucosides and *H. tunbridgense*, both. In contrast only the 3-*O*-galactoside and 3-*O*-arabinoside were detected in *H. dilatatum*. Flavones, found so far only in *H. barbatum* [8] and *T. petersii*, should also be of taxonomic significance since the biosynthetic pathway leading to their formation is quite distinct from that of flavonols and *C*-glycosylflavones [22].

On the basis of the above it is concluded that a study of the polyphenolics of the species of the large and taxonomically cumbersome family of filmy ferns, the Hymenophyllaceae, should be a useful aid in delineating lower taxa. To this end a comprehensive survey of the family is now underway.

EXPERIMENTAL

Plant material. Trichomanes reniform Forst. f. (= *Cardiomanes reniforme* (Forst. f.) Presl.) (200 g dry wt) was collected on 31 January 1977 at the Kaitoke Waterworks Reserve, Upper Hutt, New Zealand. *Trichomanes venosum* R. Br (= *Polyphlebium venosum* Cop.) (10 g dry wt.) and *H. dilatatum* (Forst. f.) Swartz. (= *Mecodium dilatatum* (Forst. f.) Cop.) (10 g dry wt) were collected on 6 March 1977 in the Tauherenikau River Valley, Tararua Mountains, New Zealand. *H. recurvum* Gaud in Fryc. (= *M. recurvum* (Gaud in Fryc.) Cop.) (12 g dry wt) was collected on 20 January 1978 by Dr. C. W. Smith on Oahu, Hawaii and *T. petersii* Gray (10 g dry wt) and *H. tunbridgense* (L.) Smith (8 g dry wt) were collected on 24 September 1977 in the Estatoe Gorge, Pickens County, South Carolina. Voucher specimens are on file in the Herbaria of Western Carolina University and the New York Botanical Garden. Dry pinnae material was worked up for chromatography according to ref. [10].

Flavonoids. All flavonoids were isolated by 2D-PC in *t*-BuOH–HOAc–H_2O, 3:1:1 (TBA) and 15% HOAc (HOAc). Structure identification was according to ref. [23]. Confirmatory cochromatography: *O*-glycosides by PC in TBA, HOAc and H_2O; *C*-glycosides by TLC (cellulose) in TBA, HOAc, H_2O and (Si gel) EtOAc–Py–H_2O–MeOH, 16:4:2:1; aglycones by TLC in TBA, C_6H_6–HOAc–H_2O, 125:72:3 (BzAW), $CHCl_3$–HOAc–H_2O, 10:9:1 (CAW) and (Si gel) toluene–$CHCl_3$–Me_2CO, 8:5:7 (TCA). An asterisk indicates those compounds for which an authentic sample was not available. Sugars were identified by GLC (3% OV-1) as their trimethylsilylesther derivatives after liberation by acid hydrolysis. Pectinase hydrolysis and subsequent cochromatography were used to identify the *C*-glycosylflavone moiety of the X″-*O*-glycosides. Where possible R_f values were determined by 1D-PC using rutin as an internal standard; R_f (rutin): TBA 0.40–0.46; HOAc 0.52–0.58; H_2O 0.21–0.27). Flavonoids found are as follows (R_fs in TBA, HOAc and H_2O in parentheses).

H. tunbridgense: Kaempferol 3-*O*-glucoside (0.72, 0.51, 0.18), kaempferol 3-*O*-rhamnoglucoside (0.60, 0.61, 0.32), quercetin 3-*O*-glucoside (0.58, 0.47, 0.13), quercetin 3-*O*-rhamnoglucoside (0.47, 0.58, 0.28). *H. dilatatum*: Quercetin 3-*O*-galactoside (0.57, 0.37), quercetin 3-*O*-arabinoside (0.57, 0.26), vicenin-2, lucenin-2. *T. petersii*: Orientin (0.26, 0.18, 0.03), isoorientin (0.43, 0.40, 0.08), orientin X″-*O*-glucoside* (0.31, 0.66, 0.38), vitexin (0.50, 0.28, 0.05), isovitexin (0.65, 0.54, 0.18), isovitexin X″-*O*-glucoside* (0.42, 0.76, 0.86), apigenin 7, 4′-di-*O*-glucoside* (0.19, 0.67, 0.17). The aglycone from the last compound was chromatographically identical with apigenin and had MS peaks at *m/e* 270 (100%) 153 (35%), 152 (25%), 121 (27%). The 7,4′-di-*O*-substitution was evidenced by NaOMe induced band I decrease in intensity. *T. venosum*: Orientin, isoorientin, isoorientin 2″-*O*-arabinofuranoside* (0.50, 0.66, 0.85) the major component, vitexin X″-*O*-arabinoside* (0.59, 0.70, 0.67), tricetin 8-*C*-glucoside (0.15, 0.13, 0.04) and an X″-*O*-glycoside of its 6-*C*-glucoside isomer (0.24, 0.56, 0.29). The 6-*C*-glucoside isomer (0.20, 0.22, 0.05) was present in trace amounts as also was a 7-*O*-substituted lucenin-2-type compound (0.13, 0.46, 0.61). UV-visible absorption data for tricetin 8-*C*-glucoside and its X″-*O*-glycoside: λ_{max} 251, 269, 354 (MeOH); 268, 340sh, 410 dec. (NaOMe); 272, 309sh,

420 ($AlCl_3$); 276, 300sh, 360sh, 390 ($AlCl_3$/HCl); ^{13}C NMR (DMSO-d_6): 182, 164.6, 163.0, 160.6, 146.6, 139, 106.6, 104.2, 102.8, 98.5, 82.3, 79.1, 73.4, 71.1, 70.1, 62.3 ppm (weak spectrum, C-9 and C-1′ quaternaries not visible). NMR data for orientin/isoorientin *O*-arabinosides are essentially as previously reported [13a, 23]; solvent used, DMSO-d_6.

Xanthone C-glycosides from T. reniforme *and* H. recurvum. All 8 compounds (2 from *T. reniforme* and 6 from *H. recurvum* were isolated and purified by 2D-PC (TBA, HOAc) and gave essentially the same absorption spectra: (MeOH) 240, 255, 270sh, 312, 365; (NaOMe) 240, 248sh, 270, 302, 390; ($AlCl_3$) 237, 268, 286sh, 352, 415; ($AlCl_3$/HCl) 232, 265, 280sh, 336, 403 nm. One xanthone from each source cochromatographed with authentic mangiferin from *Asplenium* [17], R_f (TBA) 0.39, (HOAc) 0.41 on PC and on TLC: (cellulose) R_f (H_2O) 0.14 and (polyamide, S and S) R_f (MeOH–HOAc–H_2O, 18:1:1) 0.47, and a minor xanthone from *H. recurvum* R_f (TBA) 0.31, (HOAc) 0.24 cochromatographed with isomangiferin [17].

The only other xanthone from *T. reniforme* R_f (TBA) 0.60, (HOAc) 0.56 was converted to mangiferin by acid (2 N HCl, 1 hr, 100°) and alkaline (2 N NaOH, 20°, 2 hr) treatment. ^{13}C NMR (17 mg in DMSO-d_6): 179.6, 171.4, 164.5, 162.4, 157.0, 155.5, 151.7, 144.5, 111.8, 108.4, 107.5, 103.0, 101.8, 93.7, 79.2 (C-3′ or 5′), 78.6 (C-3′ or 5′), 73.7 (C-1′), 70.9 (C-2′, 4′), 64.9 (C-6′), 21.2 ppm. ^{13}C NMR (3.4 mg mangiferin): 179.3, 164.1 162.0, 156.5, 154.5, 151.1, 144.0, 111.9, 108.3, 107.7, 102.8, 101.5, 93.6, 81.7 (C-5′), 79.2 (C-3′) 73.4 (C-1′), 70.8 (C-2′ or 4′), 70.6 (C-2′ or 4′), 61.7 (C-6′). Sugar carbon assignments are based on the revised assignments for C-linked glucoses recently published by Chari *et al.* [25]. ^1H NMR (DMSO-d_6): 13.73*s* (OH), 7.38*s* (H-8), 6.85*s* (H-5) 6.37*s* (H-4), 5.05*d* (J = 9.4 Hz, H-1″), 4.25–3.2*m*, ppm. ^1H NMR mangiferin: 13.77*s*, 7.39*s*, 6.87*s*, 6.39*s*, 4.62*d* (J = 9.4 Hz), 4.25–3.0*m* ppm.

All other xanthones from *H. recurvum* yielded mangiferin and an acid (M^+ 122, 105 ($PhCO^+$), 77 (Ph^+), 51, *m/e*) which cochromatographed with benzoic acid on cellulose TLC in 5% NH_4OH in EtOH and BuOH–HCO_2H–H_2O (10:1:5); $KMnO_4$ spray reagent [24]. Quantification of the amount of benzoic acid produced was carried out by spectrophotometry (log ξ_{364}mangiferin = 4.1, 148 mg/l. benzoic acid had OD 0.915 at 275 nm). The ^{13}C NMR spectrum of the extract (12 mg) from the monobenzoate spot exhibited signals (in DMSO-d_6) at 81.5 (0.25 carbons), multiplet centred at 79 (1.3C), 76.8 (0.5C), multiplet centred at 73.7 (1.4C) multiplet centred at 70.7 (1.9C), 65.2 (0.3C) and 61.6 (0.7C) ppm. Assignments derived from this are as follows: 6′-O-*benzoate*; *ca* 79 (C-3′), *ca* 79 (C-5′), *ca* 73.7 (C-1′), *ca* 70.7 (C-2′), *ca* 70.7 (C-4′), 65.2 (C-6′) ppm; 2′-O-*benzoate*; 76.8 (C-3′), 81.5 (C-5′), *ca* 70.7 (C-1′), *ca* 73.7 (C-2′), *ca* 70.7 (C-4′), 61.6 (C-6′) ppm; 4′-O-*benzoate*; 76.8 (C-3′), *ca* 79 (C-5′), *ca* 70.7 (C-2′), *ca* 73.7 (C-1′, C-4′), 61.6 (C-6′) ppm.

Xanthone C-glycosides from H. dilatatum. Dilatatin the major xanthone from *H. dilatatum* was obtained as a pale yellow powder when the Me_2CO–H_2O extract was partly evapd and permitted to stand for several days (yield: 0.6 g from 11 g dry wt plant material). This compound was recrystallised × 2 from MeOH–H_2O (9:1) in a Bolton extractor to give pale yellow crystals, mp 272° with decomp., elemental analysis; Found: C, 52.25; H, 4.55, Calc. for $C_{19}H_{18}O_{11} \cdot \frac{1}{2}H_2O$: C, 52.24; H, 4.51%; $[\alpha]_D^{20}$ Py + 43.1° (589 nm), +44.9° (578 nm), +51.8° (546 nm), +81.6° (436) nm). ^1H NMR data (DMSO-d_6, ppm from TMS): 13.73 (OH), 7.38 (H-8), 6.85 (H-5), 6.37 (H-4), 5.05*d* (H-1′ J = 9.4 Hz), multiple signals 4.26–3.16 (sugar protons). ^{13}C NMR data (100 mg in DMSO-d_6): 179.4, 164.3, 162.1, 156.6, 154.3, 151.1, 143.9, 112.1, 108.5, 107.8, 103.0, 101.7, 94.0, 76.7, 71.9, 68.9, 68.2, 68.1, 61.7 ppm. Acid treatment of this xanthone (R_f TBA 0.44, HOAc 0.38) isomerized it reversibility to isodilatatin, the minor xanthone constituent of this plant (R_f TBA 0.37, HOAc 0.16).

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NOTE ADDED IN PROOF

The tricetin 8-C-glucoside isolated from *T. venosum* may now be designated 'affinetin' since a ^{13}C NMR study of isoaffinetin (cf. Krause, J. (1976) *Z. Pflanzenphysiol.* **79,** 465), kindly supplied by Dr. J. Krause, has proven isoaffinetin to be tricetin 6-C-β-D-glucoside.