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Liquid chromatographic–UV detection and liquid chromatographic–thermospray mass spectrometric analysis of *Chironia* (Gentianaceae) species

A rapid method for the screening of polyphenols in crude plant extracts

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ABSTRACT

The use of liquid chromatography–thermospray mass spectrometry (LC–TSP-MS) and liquid chromatography coupled with UV photodiode-array detection (LC–UV) in the analysis of crude plant extracts provides important structural information on metabolites directly in their biological matrices. In the LC analysis of polyphenols (such as xanthones), shift reagents can be added postcolumn and UV spectra recorded on-line. Information on the position of free hydroxyl groups can be obtained by adding reagents such as $AlCl_3$, weak and strong bases and boric acid. Thus, for certain polyphenols, the combination of LC–UV with postcolumn addition of shift reagents and LC–MS permits a full on-line structural determination involving no time-consuming isolation process. To illustrate this qualitative analytical approach, crude extracts of different Gentianaceae (*Chironia* species) were submitted to LC–TSP-MS and LC–UV measurements with postcolumn addition of shift reagents. This method permitted the identification of a large number of xanthones which are of pharmaceutical interest as potential inhibitors of monoaminooxidases.

INTRODUCTION

Species of the Gentianaceae family are known to contain secoiridoids and xanthones [1–3]. The latter class of substance is of special interest as it includes strong inhibitors of monoaminooxidase (MAO) [4,5]. Further, xanthones are useful chemotaxonomic markers [5]. In the course of an investigation of tropical and subtropical Gentianaceae [6,7], we have collected several *Chironia* species. The genus *Chironia* L. is distributed mainly in South Africa, but several species range northward into tropical Africa and Madagascar [8]. A first phytochemical study of the roots and leaves of *C. krebsii* has afforded eighteen xanthones (**1–18**) [7] (Fig. 1) and three secoiridoids, swertiamarin (**19**), gentiopicroside (**20**) and sweroside (**21**). The xanthones are currently being tested for their inhibitory activities on monoaminooxidases A and B.

For a rapid qualitative survey of the xanthones in other species and for identification of minor xanthones, different extracts of four *Chironia* species, namely *C. krebsii* Griseb., *C. palustris* Burch., *C. pupurascens* Verdoorn and *C. baccifera* L., have been screened by liquid chromatography with UV photodiode-array detection (LC–UV) with postcolumn addition of shift reagents [9,10] and by LC with thermospray mass spectrometric detection (LC–TSP-MS) [11].

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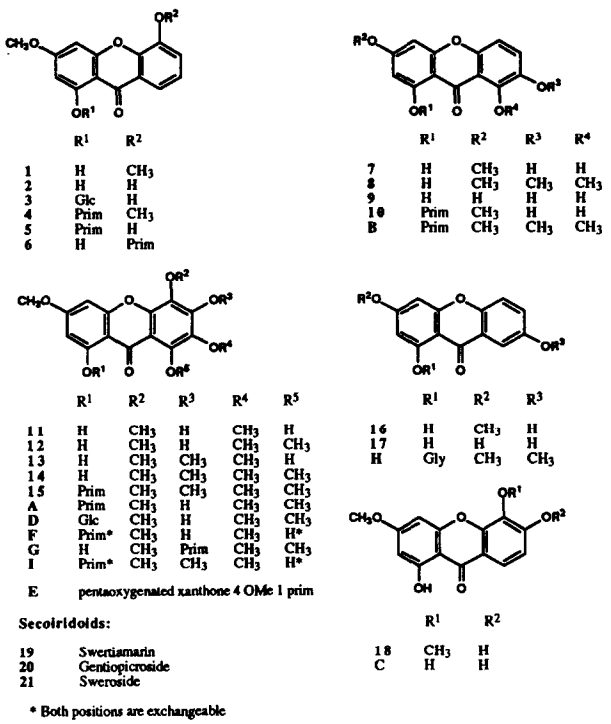

Fig. 1. Compounds found in *Chironia* species. Compounds **1–21** were isolated from *C. krebsii*. Xanthones **A–I** were identified only from MS and UV data obtained on-line. Glc = glucose; Gly = undefined glycoside; Prim = primeverose [=β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside].

The separation of the xanthones directly from a crude plant extract was achieved by reversed-phase LC using an acetonitrile–water gradient system. The same LC conditions were used for the LC–UV and LC–MS investigations.

Xanthones present characteristic UV spectra with four bands of decreasing intensity [12]. These spectra are easily recorded during the LC separation of a crude plant extract, on-line with the aid of a photodiode array detector (220–450 nm). The UV spectra depend on the substitution pattern of the polyphenols. The location of free hydroxyl groups on a xanthone nucleus can be established with the aid of classical shift reagents [13]. These reagents induce a shift of the absorption maxima and their applications to structure elucidation of flavonoids and xanthones have been extensively described [13, 14]. In this work, reagents were added to the eluate using a post-column derivatization system [9,10]. As the separations were achieved in an acidic acetonitrile–water system, the shift reagents usually used in methanol solutions required some adaptations.

LC–TSP-MS is a soft ionization technique [11] that mainly forms adduct ions such as $[M+H]^+$ with molecules. These adduct molecular ions allow a rapid determination of the molecular mass of a component directly after its elution from the LC column. The use of LC–TSP-MS has already provided on-line molecular mass information in the analysis of polyphenols and polyphenol glycosides such as flavonoids [15]. Xanthones, which are closely related to flavones, can be ionized under nearly identical conditions, with ammonium acetate as buffer and in the positive ion and filament-off modes.

This paper presents the results obtained by LC–UV with postcolumn addition of shift reagents and LC–TSP-MS in the analysis of crude extracts of Gentianaceae. The combination of structural information obtained by the two on-line detection methods is discussed.

EXPERIMENTAL

Chemicals

HPLC-grade water was prepared by distillation on a Buchi (Flawil, Switzerland) Fontavapor 210 distillation instrument and passed through a 0.50-μm filter (Millipore, Bedford, MA, USA). HPLC-grade acetonitrile from Maechler (Reinach, Basle, Switzerland) was passed through a 0.45-μm filter. Boric acid, sodium acetate, ammonium acetate and trifluoroacetic acid (TFA) were obtained from Merck (Darmstadt, Germany) and aluminium chloride, sodium hydroxide and potassium hydroxide from Fluka (Buchs, Switzerland).

LC conditions

Separations were performed on a Nova-Pak RP-18 (4 μm) column (150 × 3.9 mm I.D.) from Waters (Bedford, MA, USA), equipped with a Nova-Pak Guard precolumn. A gradient of acetonitrile–water from 5:95 to 70:30 in 50 min (1 ml/min) was used. To avoid the tailing of phenolic compounds, 0.05% of trifluoroacetic

acid was added to the solvents, leading to a pH of 3.

Shift reagents

For comparison purposes, classical shift reagents were prepared according to the standard procedure [13]. The reagents used in the post-column derivatization system were as follows (Table I): weak base, 0.5 *M* aqueous sodium acetate solution (basified with a 0.01 *M* NaOH solution to pH 8); strong base, 0.3 *M* aqueous potassium hydroxide solution; 0.3 *M* aqueous aluminium chloride solution (with this reagent, the reaction coil was heated to 90°C and the eluent was previously neutralized with 0.02 *M* NaOH solution); boric acid, a methanol–water (1:1) solution containing 0.7 *M* boric acid and 0.1 *M* sodium acetate. All the reagent solutions were filtered through a 0.50-μm filter (Millipore, Bedford, MA, USA).

LC–UV analyses

Eluent delivery was provided by an LC-9A HPLC pump (Shimadzu, Tokyo, Japan) equipped with an FCV-9AL low-pressure mixing valve and a Model 7125 injection valve with a 20-μl loop (Rheodyne, Cotati, CA, USA). Post-column addition of the bases (for neutralization of the mobile phase) and of the shift reagents was achieved with two M-6000 pumps (Waters). Neutralization of the mobile phase was effected in an Upchurch (Oak Harbor, WA, USA) mixing tree and reaction with the shifts reagent was carried out in a 10-μl Visco mixer (Lee, Westbrook, CO, USA) followed by a reaction coil. UV spectra were recorded with an HP-1040A photodiode-array detector 1040A and the data were processed on an HP-1090 Chemstation (Hewlett-Packard, Palo Alto, CA, USA) (see Fig. 4).

LC–MS analyses

A Finnigan MAT (San Jose, CA, USA) TSQ-700 triple quadrupole instrument equipped with a TSP 2 interface was used for data acquisition and processing. The temperatures of the TSP were source block 280°C, vaporizer 100°C and aerosol 280–300°C (beginning–end of gradient). The electron multiplier voltage was 1800 V, dynode 15 kV and the filament and discharge were off. Full-scan spectra from m/z 150 and 800 in the positive ion mode were obtained (scan time 1.2 s). Concerning the LC part, the eluent delivery was provided by a 600-MS pump HPLC (Waters) equipped with a gradient controller. The UV trace was recorded on-line with a Water 490-MS programmable multi-wavelength detector. Postcolumn addition of buffer (0.5 *M* ammonium acetate) was achieved with a Waters 590-MS programmable HPLC pump (0.2 ml/min) using a simple tee junction (Waters).

TABLE I

CONDITIONS FOR POSTCOLUMN ADDITION OF SHIFT REAGENTS

Shift reagent	Pump 1[a]	Flow-rate (ml/min)	pH	Pump 2[a]	Flow-rate (ml/min)	pH	Temperature (°C)
Eluent	H_2O	0.2	3	H_2O	0.4	3	Room
NaOAc	NaOAc (0.5 *M*)	0.4	7	NaOH (0.01 *M*)	0.3	8	Room
KOH	H_2O	0.2	3	KOH (0.3 *M*)	0.4	14	Room
$AlCl_3$	NaOH (0.02 *M*)	0.2	7	$AlCl_3$ (0.3 *M*)	0.4	3.5	90
$AlCl_3$[b], acid	H_2O	0.2	3	$AlCl_3$ (0.3 *M*)	0.4	2.5	90
H_3BO_3–NaOAc	NaOH	0.2	6	H_3BO_3 (0.7 *M*)–NaOAc (0.1 *M*)	0.4	7	Room

[a] For pumps 1 and 2, see Fig. 4.
[b] 0.1% TFA in the mobile phase.

Samples

The dried plant material was extracted at room temperature with solvents of increasing polarity (dichloromethane and methanol). Aerial parts and roots of the following plants of the Gentianaceae family were used: *Chironia krebsii* Griseb., collected in Malawi, *Chironia pupurascens* Verdoorn and *Chironia palustris* Burch, collected in Zimbabwe, and *Chironia baccifera* L., collected in South Africa. Solutions to be analysed were prepared by dissolving 30 mg of the root methanolic extract in 1 ml of methanol–water (1:1). The injection volume was 20 μl.

RESULTS

Reversed-phase LC on RP-18 columns with methanol–water or acetonitrile–water containing acetic acid, phosphoric acid or formic acid has been successfully applied to analyses for polar polyphenols such as xanthone glycosides and xanthone aglycones [12, 16]. A linear gradient of an acetonitrile–water system, containing trifluoroacetic acid to avoid peak tailing (pH 3), was developed to achieve the separation of the different xanthone aglycones and glycosides which were found in the crude root methanolic and dichloromethane extracts of the *Chironia* species (Fig. 2).

LC–UV Photodiode array detection

The use of a photodiode-array detector permits the measurement of the whole UV spectrum (220–450 nm) of each peak of the chromatogram. The attribution of the peaks to xanthones (**1–18**) and secoiridoids (**19–21**) was unambiguous, the xanthones usually exhibiting a UV spectrum consisting of four bands of decreasing intensity (200–400 nm), while the secoiridoids exhibit only one band (230–240 nm). The number of bands and the general aspects of the UV spectra of xanthones (UV spectra 1–18 in Fig. 2) allowed a first attribution of the type of oxygenation pattern encountered [12,14]. The comparison of the chromatogram of the methanolic extract of the roots with the corresponding dichloromethane extract permitted the localization of xanthone aglycones and xanthone glycosides (Fig. 2). Indeed, the extraction with dichloromethane permitted the selective acquisition of the polyphenolic aglycones only, owing to their low polarity. The peaks observed for the xanthone aglycones corresponded to the slower running peaks in the chromatogram (Fig. 2).

LC–MS thermospray detection

In order to obtain more information on the molecular masses, the crude formulae and the sugar sequence (glycosides) of the xanthones, LC–TSP-MS analysis of the extracts was carried out. The same LC conditions as for the LC–UV analysis were used (see Experimental). The ammonium acetate buffer (0.5 *M*) was added postcolumn (0.2 ml/min) to avoid modification of the chromatographic conditions. In order to obtain the optimum intensities of ions for both xanthone aglycones and glycosides, the parameters of the TSP interface were tuned with a solution of rutin (M_r 610), a flavonoid diglycoside. The vaporizer temperature range for observation of the $[M + H]^+$ ion of rutin was narrow and the optimum intensity was found for a setting of the vaporizer of 100°C with the source block at 280°C. Nevertheless, even at this temperature the $[M + H]^+$ ion was weak, and the major ion was the aglycone moiety $[A + H]^+$. The influence of the change in the composition of the eluent during in the gradient (5–70% acetonitrile) and the presence of trifluoroacetic acid in the mobile phase was found to be negligible. No improvement was observed by using the filament-on or discharge-on mode, and the analysis was finally carried out in the filament-off mode.

Under these conditions, all the peaks recorded in the UV trace (254 nm) of the different extracts gave a clearly discernible MS response in the total ion current trace (TIC) (Fig. 3). As the molar absorptivities ε of the xanthones were of the same order of magnitude [7], comparison of the UV chromatogram with the TIC trace showed a poor MS response for some compounds. Indeed, the total ion current response for highly hydroxylated xanthones with high melting point, such as **9** (1,3,7,8-tetrahydroxyxanthone, m.p. >335°C [7]), was found to be very weak compared with the UV trace of the corresponding peak. The relationship between the

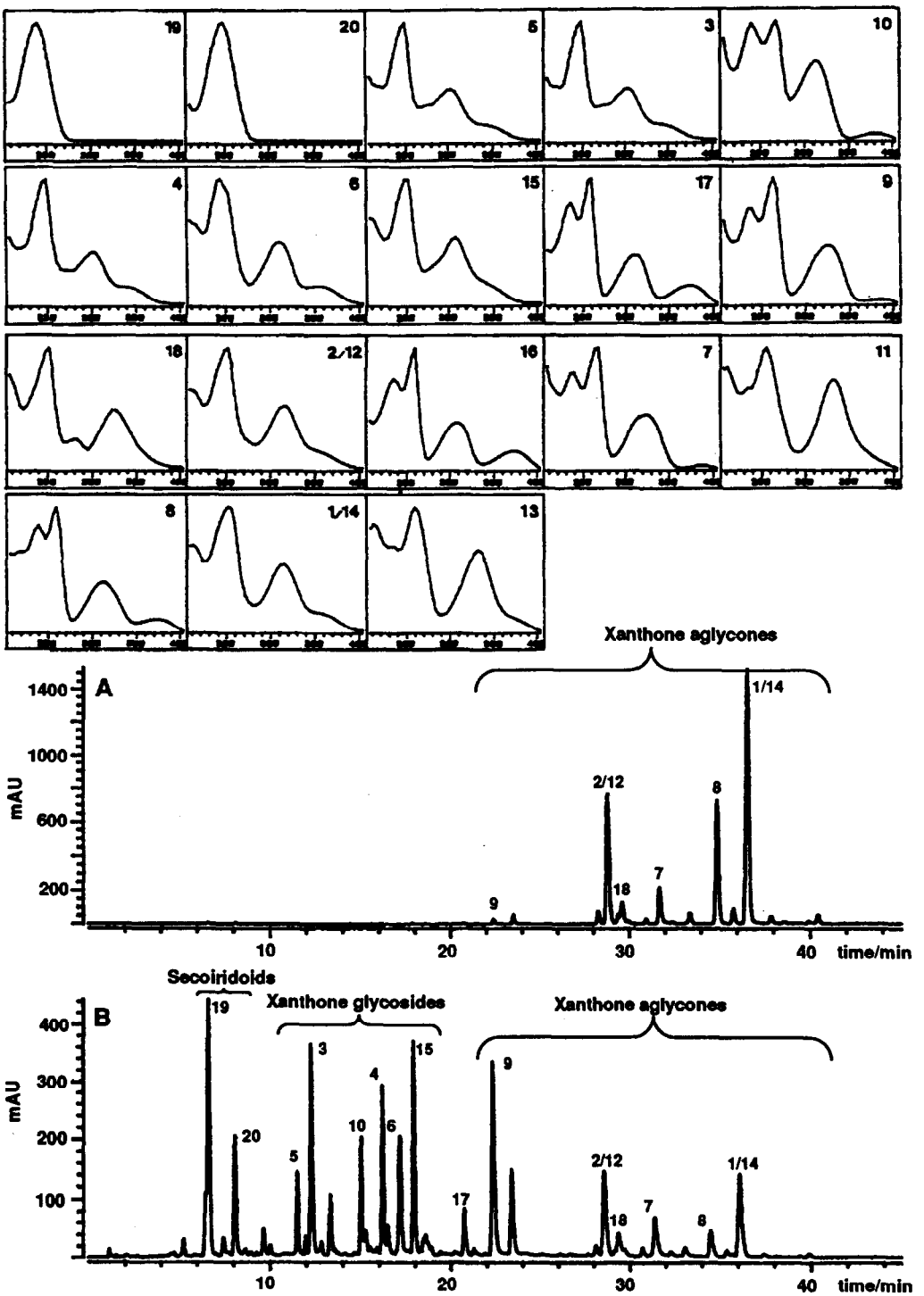

Fig. 2. Comparison of the LC–UV traces of (A) the root dichloromethane and (B) the root methanolic extracts of *C. krebsii*. The UV spectra displayed were recorded from chromatogram B. UV traces were recorded at 254 nm. UV spectra were recorded from 200 to 400 nm. For identities of the peaks, see Fig. 1.

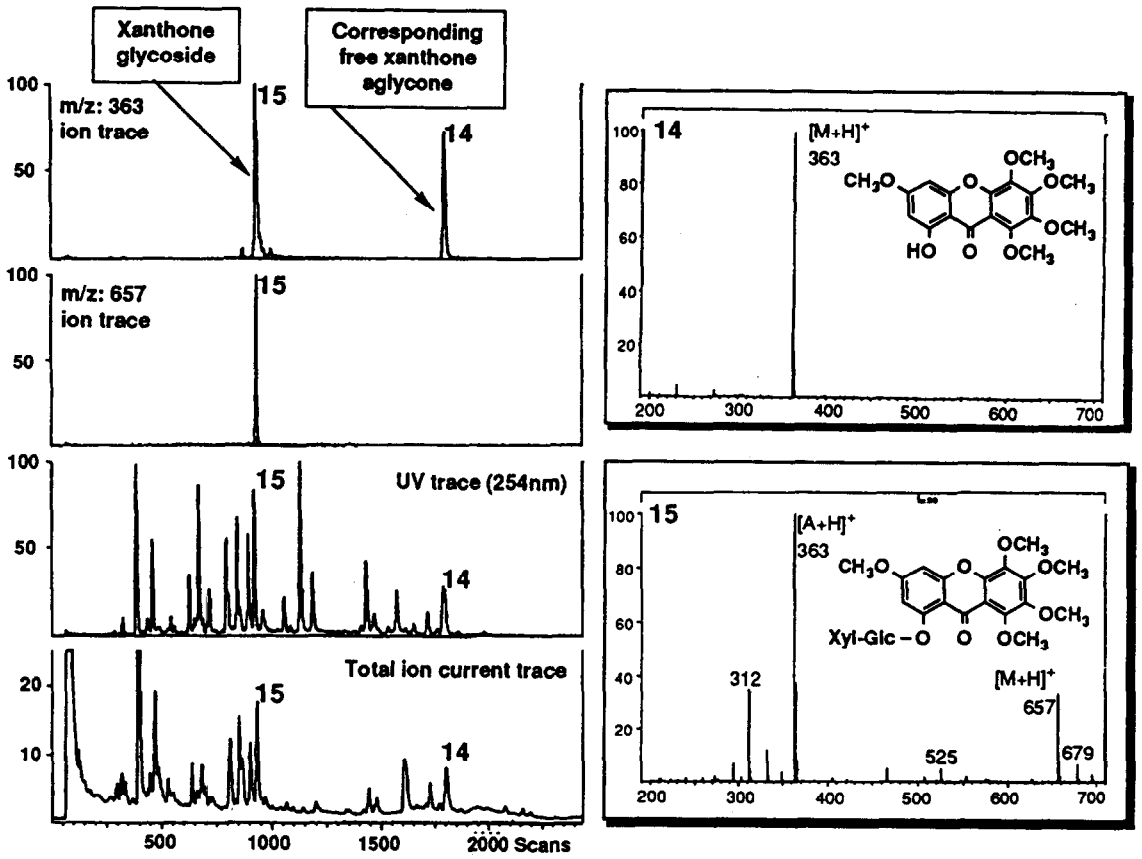

Fig. 3. LC–TSP-MS analysis of the root methanolic extract of *C. krebsii*. The ion trace for *m/z* 657 displayed corresponds to the pseudo-molecular ion $[M+H]^+$ of xanthone **15**. The ion at *m/z* 363 is the main fragment of **15** and corresponds to the pseudo-molecular ion of its corresponding free aglycone **14** also present in the extract.

TIC intensity and melting point was investigated by injecting equal amounts (2.5 μg) of xanthones with melting points varying from 158 to >335°C (**1**, 173°C; **2**, 272°C, **7**, 226°C; **8**, 158°C; **9**, >335°C). Under the conditions used for LC–TSP-MS analysis of the extracts, the TIC for a non-volatile compound such as **9** (m.p. >335°C) was about 30 000 times lower than for a more volatile xanthone such as **8** (m.p. 158°C). In the same way, the MS responses of xanthones **1**, **2** and **7** were dependent on their melting points. Hence LC–TSP-MS alone cannot be used for direct semi-quantitative determination of xanthones.

The TSP mass spectra of the xanthone aglycones recorded on-line from the liquid chromatogram exhibited only the $[M+H]^+$ ions as main peak; the molecular mass determination was thus unambiguous (see Figs. 3 and 5). For simple xanthones, the number of hydroxyl and methoxyl substituents was deduced by subtracting the molecular mass of the xanthone nucleus (M_r 196) from that of the aglycones.

The TSP mass spectra of xanthone glycosides usually show two weak ions corresponding to the $[M+H]^+$ and $[M+Na]^+$ adducts, and a main peak corresponding to the protonated aglycone moiety $[A+H]^+$ (see Figs. 3 and 6). In the case of diglycosides, a weak intermediate fragment due to the loss of a first sugar unit was observed (see Fig. 6). These results were in good agreement with those obtained for flavonoid glycosides [15]. The mass spectra of the xanthone diglycosides found in the *Chironia* species presented a first loss of 132 u corresponding to a pentose residue, followed by a a loss of 162 u (hexose residue) leading to the aglycone ion $[A+H]^+$. These two losses were attributable to

a primeverose moiety, a β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside disaccharide unit often encountered in the Gentianaceae family [2,16]. The monoglycosidic xanthones presented $[M+H]^+$ ions more intense than the diglycoside ions and exhibited only a loss of 162 u, attributed to be a glucosyl moiety. The structures were confirmed by a full determination of the main isolated glycosides [7].

These first LC–UV and LC–TSP-MS results afforded the molecular mass, the number of methoxyl and hydroxyl groups, the number of sugars and an idea about the substitution pattern of the xanthones found in the extracts of the *Chironia* species. In order to obtain more precise structural information on the position of the free hydroxyl groups on the xanthone nucleus, LC–UV with postcolumn addition of shift reagents for polyphenols was performed.

LC–UV photodiode-array detection with postcolumn addition of shift reagents

The use of these shift reagents played an important role in the characterization of phenolic compounds, and application of such reagents to flavonoids [13] and xanthones [12] have been extensively described. A weak base (sodium acetate) deprotonates only the more acidic phenolic groups, whereas a strong base (sodium methanolate) reacts with all phenolic groups. Aluminium chloride in neutral solution forms complexes with *ortho*-dihydroxyl groups and/or with keto functions having a hydroxyl group in an α- or *peri*-position. The former complexes are unstable when HCl is added. *ortho*-Dihydroxyl groups also form a chelate complexe with boric acid. For flavonoids and xanthones, all these reactions are in general carried out in methanol. They provide useful information about the type of flavonoid or xanthone and the oxidation pattern and the location of free hydroxyl groups.

The method employed for the postcolumn addition of shift reagents is based on the work of Hostettmann *et al.* [9] and the improvement described by Mueller-Harvey and Blackwell [10]. The experimental set-up is shown in Fig. 4. The method presented here was adapted for the use of an acidic acetonitrile–water eluent system. The shift reagents, KOH, $AlCl_3$ and H_3BO_3, were prepared as described by Hostettmann *et al.* [9]. However, the use of Na_2HPO_4 as a weak base was found to be incompatible with the acetonitrile–water system for reasons of solubility. Even at lower concentrations than those proposed by Hostettmann *et al.* [9], Na_2HPO_4 precipitated in the eluent, producing pressure instability and plugging. Na_2HPO_4 was therefore replaced with sodium acetate (0.5 *M*, 0.4 ml/min). As this weak base is not strong enough to deprotonate acidic phenolic groups in an aqueous solvent system, NaOH was added in a second HPLC pump (0.01 *M*, 0.3 ml/min) to obtain a pH of 8 (Table I, Fig. 4).

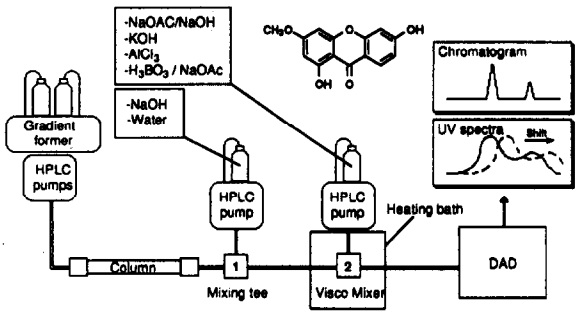

Fig. 4. Experimental set-up used for postcolumn addition of the shift reagents.

The location of *ortho*-dihydroxyl groups is not always possible in an acetonitrile–water system with the use of NaOAc–H_3BO_3 reagent [9]. However, the use of $AlCl_3$ in the acidic mobile phase (pH 3, 0.1% TFA) showed similar shifts to those observed for pure compounds having an *ortho*-dihyroxyl group with the classical $AlCl_3$–HCl [13] reagent in methanol. Hence the comparison of on-line $AlCl_3$ UV spectra at pH 7 and 3 allows the detection of the labile *ortho*-dihydroxyl complexes.

All the shift reagents were tested with an artificial mixture of previously isolated xanthones (**1**, **2**, **5**, **7**, **9**, **11** and **12**) [7], eluted under the same chromatographic conditions as those used for the extracts. The optimum concentration, flow rate and temperature are presented in Table I.

Examples of structural information obtained on-line

The structural information obtained on-line by the combination of LC–UV, LC–TSP-MS and

LC–UV with postcolumn addition of shift reagents is illustrated by two examples (Figs. 5 and 6).

The first example shows the structure determi-

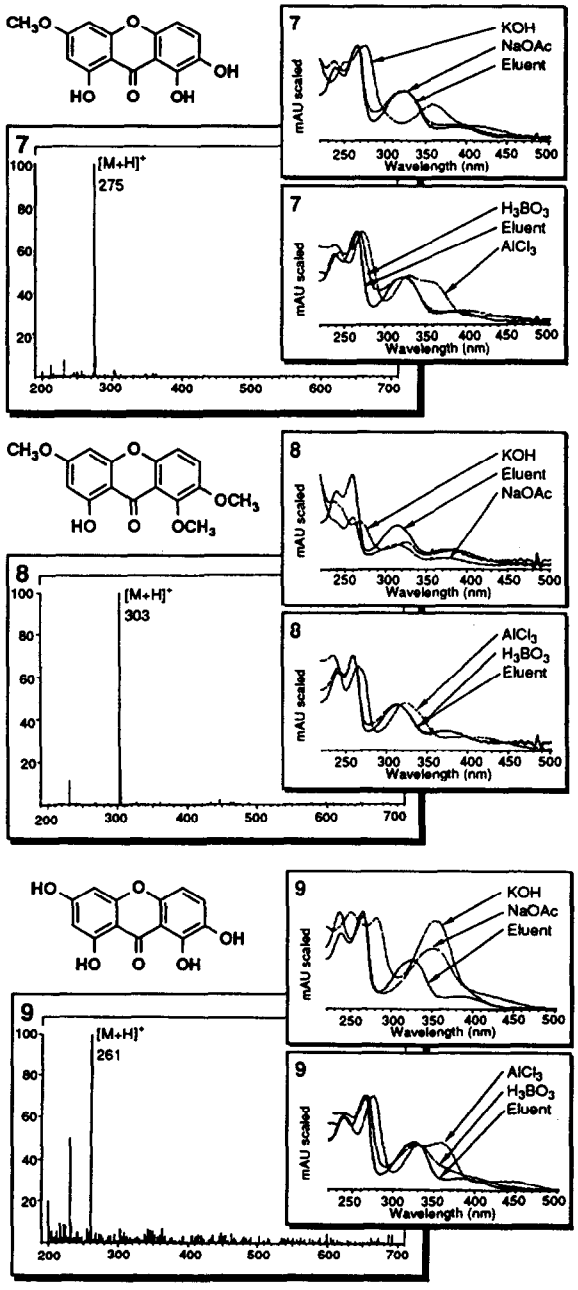

Fig. 5. Summary of all structural information obtained online for three 1,3,7,8-tetrasubstituted xanthone aglycones. Mass and UV spectra of **7**, **8** and **9** were recorded from the liquid chromatogram of *C. krebsii*.

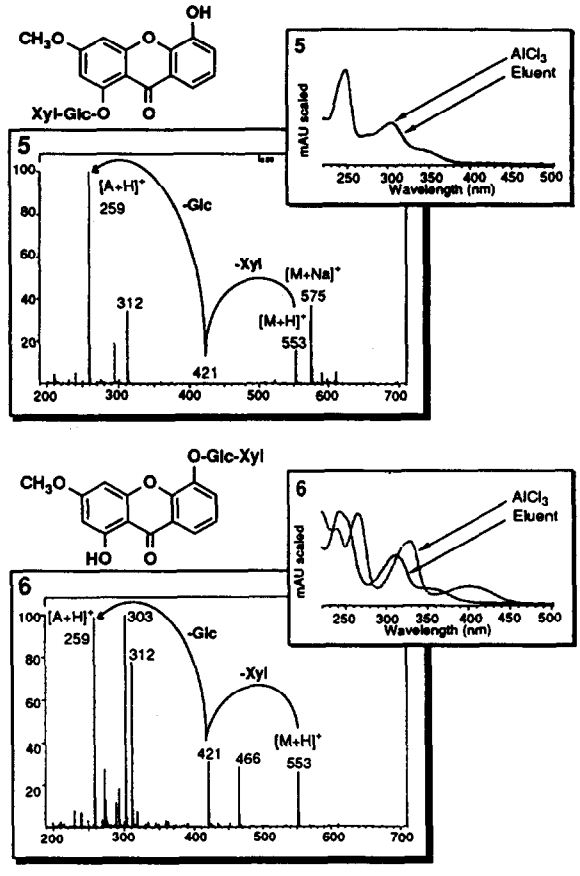

Fig. 6. Differentiation of two isomeric xanthone diglycosides. The TSP mass spectra of **5** and **6** are comparable, and both xanthones have a molecular mass of 552. The only difference between them is shown by the $AlCl_3$ spectrum, proving the difference in the position of attachment of the dissacharide unit in **5** and **6**. Mass and UV spectra of **5** and **6** were recorded from the liquid chromatogram of *C. krebsii*.

nation of three xanthone aglycones (**7–9**) in the LC–UV analysis of the root methanolic extract of *C. krebsii*. These xanthones presented nearly the same UV spectra (Fig. 2). According to Kaldas [16], the UV spectra of **7–9** with four absorption maxima and a higher intensity of band II (Table II) is characteristic of tetraoxygenated 1,3,7,8-xanthones with a free hydroxyl in position 1. The TSP mass spectra recorded online permit the molecular mass determination and the assignment of the number and the type of substituents of **7** (M_r 274: 3 OH, 1 OMe), **8** (M_r 302: 1 OH, 3 OMe) and **9** (M_r 260: 4 OH)

TABLE II

ON-LINE STRUCTURAL INFORMATION FOR COMPOUNDS **1–18**

Abbreviations: Al = $AlCl_3$ in neutralized mobile phase; KO = KOH; Na = NaOAc; Bo = H_3BO_3–NaOAc; AlH = $AlCl_3$ in acidic mobile phase; I–IV = UV absorption maxima bands with the wavelength in nm and in parentheses the relative intensity in %, according to ref. 17; ckrm = *C. krebsii* root methanolic extract; cbrm = *C. baccifera* root methanolic extract; ckad = *C. krebsii* aerial part dichloromethane extract; the TSP-MS values are the mass (in a.m.u.) of the ions $[M + H]^+$ and $[A + H]^+$; nd = not detected.

Compound	Mixture	TSP-MS		Shifted UV spectra					UV spectra					Extract
		M+H	A+H	Al	KO	Na	Bo	AlH	I	II	II′	III	IV	
1	**1+14**		273	+	+	−	−	−	251(100)			315(52)	359(13)	ckrm
2	**2+12**		259	+	+	−	−	−	251(100)			315(49)	359(11)	ckrm
3	**3+?**	421	259	+	+	+	+	−	239(100)	265(87)		315(63)	379(12)	ckrm
4		567	263	−	−	−	−	−	247(100)	269(32)		303(43)	339(14)sh	ckrm
5		553	259	−	+	−	−	−	247(100)	275(28)		303(41)	341(12)sh	ckrm
6		553	259	+	+	−	−	−	241(100)	260(90)sh		311(51)	349(16)	ckrm
7			275	+	+	−	−	+	238(75)	265(100)		323(44)	387(10)	ckrm
8			303	+	+	−	−	−	239(80)	259(100)		314(40)	373(10)	ckrm
9			261	+	+	+	+	+	237(79)	263(100)		325(51)	385(11)	ckrm
10		569	275	+	+	−	+	−	239(100)	267(100)		313(68)	379(13)	ckrm
11			335	+	+	+	−	−	254(100)			329(61)		ckrm
12	**12+2**		349	+	+	−	−	−	251(100)			315(49)	359(11)	ckrm
13			349	nd	nd	nd	nd	nd	235(63)	261(100)		331(71)	373(13)sh	ckad
14	**14+1**		363	+	+	−	−	−	251(100)			315(52)	359(13)	ckrm
15		657	363	−	−	−	−	−	250(100)			308(15)	347(15)sh	ckrm
16	sh		259	+	+	nd	nd	−	247(100)		277(23)	319(50)		ckrm
17			245	+	+	+	+	−	235(81)	258(100)		312(47)	372(17)	ckrm
18			289	+	+	+	+	−	246(100)		277(25)	319(50)		ckrm

(Fig. 5). The shifted UV spectra recorded on-line for **9** confirmed this compound to be a 1,3,7,8-tetrahydroxyxanthone. Indeed, the shift observed with the NaOAc spectrum indicated an acidic phenol in position 3. The presence of a free hydroxyl in positions 1 and 8 was characterized by the substantial shift recorded with $AlCl_3$ and finally the presence of an *ortho*-dihydroxyl group was confirmed by the shift due to the complexation of boric acid (Fig. 5). Xanthone **7** exhibited the same shifts as those recorded for **9**, except that the NaOAc spectra remained unchanged in this instance, indicating the presence of a methoxyl instead of a hydroxyl group in position 3. The structure of **7** was thus attributable to a 1,7,8-trihydroxy-3-methoxyxanthone. The KOH spectra of **8** show a large decrease in the band intensity and only a very small shift, indicating no free hydroxyl group, with the exception of a chelated one. This was confirmed by the shift measured with $AlCl_3$. The NaOAc and H_3BO_3 spectra remained unchanged, confirming the structure of **8** as a 1-hydroxy-3,7,8-trimethoxyxanthone (Fig. 5).

The second example shows the differentiation and structural determination of two isomeric xanthone glycosides. Compounds **5** and **6** (Fig. 2) exhibited nearly the same UV spectra, indicating the same oxygenation pattern (probably 1,3,5-) [17]. The TSP mass spectra of both **5** and **6** were comparable: they both exhibited an $[M + H]^+$ ion at m/z 553 and presented a consecutive loss of 132 and 162 u, leading to their respective aglycone ions $[A + H]^+$ at m/z 259 (Fig. 6). The mass of the aglycone ions was characteristic of a xanthone with one methoxyl and two hydroxyl groups and the presence of a pentose and hexose moiety was due to a primeverosyl residue. No shift was observed for either compound with the weak base (Table II), indicating no free hydroxyl

TABLE III

ON-LINE STRUCTURAL INFORMATION OF COMPOUNDS A–I

Abbreviations as in Table II; cparm = *C. palustris* root methanolic extract; cpurm = *C. pupurascens* root methanolic extract.

Compound	TSP-MS		Shifted UV spectra					UV spectra					Extract
	M+H	A+H	Al	KO	Na	Bo	AlH	I	II	II′	III	IV	
A	643	349	−	+	+	−	−	248(100)		283(27)sh	311(52)		cpurm
B	597	303	−	−	−	−	−	243(100)	251(49)sh		305(51)	355(18)sh	cpurm
C		275	+	+	+	+	+	251(100)		281(25)	326(49)		ckrm
D	511	349	−	+	+	−	−	247(100)		283(29)sh	311(51)		cpurm
E	627	333	−	−	−	−	−	246(100)		277(31)	313(58)		cpurm
F	629	335	+	+	+	+	−	247(100)	261(77)sh		325(73)		cparm
G	643	349	+	+	−	−	−	249(100)	263(86)sh		321(73)	365(15)sh	cparm
H	nd	275	−	−	−	−	−	239(87)	253(100)		309(41)	353(21)sh	cparm
I	597	303	−	−	−	−	−	247(100)			319(71)		cbrm

in position 3. The only difference between these two isomers is shown by the spectra recorded using the $AlCl_3$ reagent. In the case of **6**, an important shift was observed which is characteristic of a chelated hydroxyl group in position 1, whereas for **5** no shift was recorded (Fig. 6). The primeverosyl moiety is thus attached in position 1 in **5** and in position 5 in **6** and the structures can be established as 5-hydroxy-3-methoxy-1-O-primeverosylxanthone (**5**) and 1-hydroxy-3-methoxy-5-O-primeverosylxanthone (**6**).

The structures of all the compounds were deduced following the same procedure (Tables II and III). Xanthones **1–18** have all been isolated from *C. krebsii* for further testing on monoaminooxidases A and B [7] and their structures, established by classical spectroscopic methods, were in good agreement with the on-line spectroscopic information obtained by both LC–MS and LC–UV methods (Table II). The structure of compounds **A–E** was established only on the basis of data obtained on-line (Table III). The structure assignment was not only based on UV and MS data but also on chemotaxonomic considerations.

CONCLUSIONS

LC–MS and LC–UV comparisons of the root methanolic extracts of four *Chironia* species permitted a precise assignment of the peaks encountered in all species (Fig. 7). From a chemotaxonomic viewpoint, this analysis shows that the 1,3,5-, 1,3,7,8- and 1,3,5,6,7,8-substituted xanthones occur in all species and that several compounds with each substitution pattern are present. The 1,3,7- and 1,3,5,6-substituted xanthones appear to be rare and are not detected in all species (Table IV). Whereas the type of aglycone is almost the same in all four species, the type of glycosidation of the xanthones differs widely from one species to another.

According to this example, the combination of LC–TSP-MS, LC–UV and LC–UV with post-column addition of shift reagents is a powerful tool for the analysis of polyphenols in crude plant extracts. These coupled techniques give a very precise idea of the plant constituents. They allow a rapid screening of the extract and reliable identification with a minute amount of material. MS and UV data provide useful structural information. The detection of minor components is possible using single ion monitoring. MS and UV information for each peak permits a reliable comparison of chromatograms of different plant species. Full structural assignment of unknowns is not possible in all instances, but the information obtained gives a good idea of the type of compounds screeened and permits a targetted isolation of the metabolites of interest. Other plant extracts containing different types of

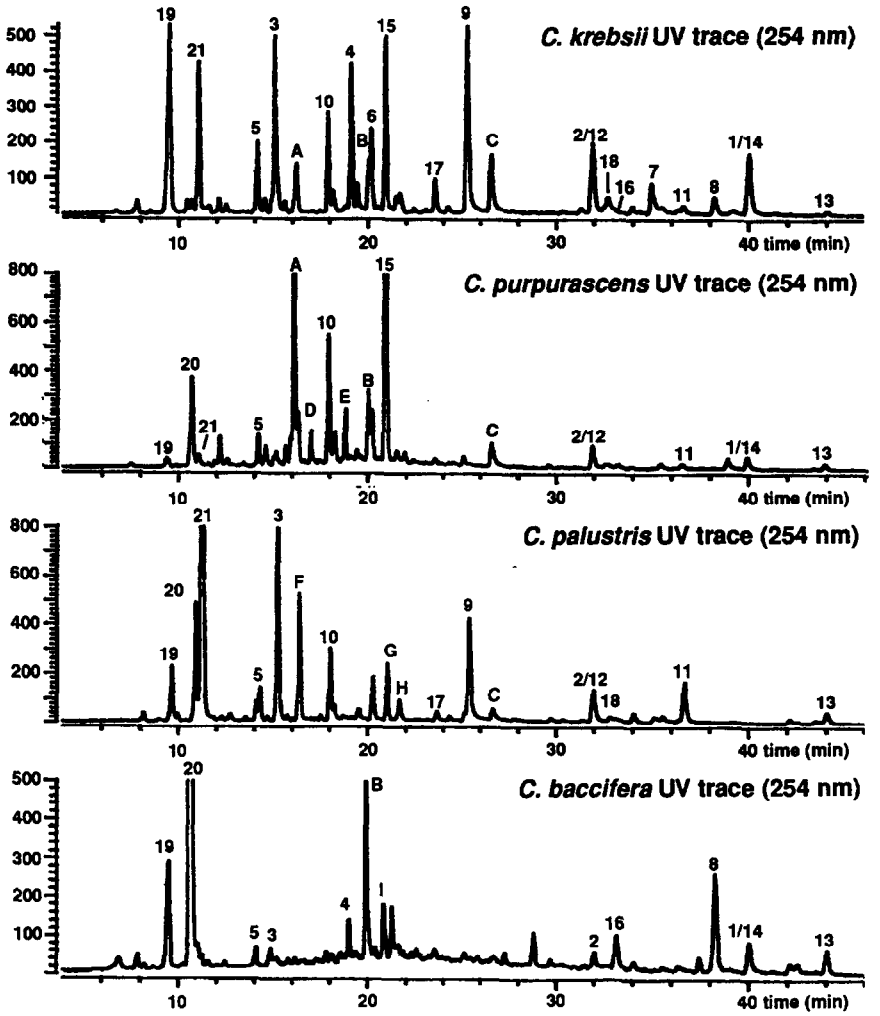

Fig. 7. Comparison of the LC–UV traces (254 nm) of the root methanolic extract of four *Chironia* species. The aglycone peaks are comparable in all chromatograms. Greater variability is observed for the xanthone glycosides between the species.

TABLE IV

OCCURRENCE OF THE IDENTIFIED XANTHONES IN THE FOUR *CHIRONIA* SPECIES

Plus signs in bold type indicate that the corresponding compounds appear as major peak in the LC–UV traces. The ε values being of the same order of magnitude for all xanthones [7], their relative abundances can be estimated in a semi-quantitative manner.

Oxidation	1,3,5-						1,3,7-			1,3,5,6-		1,3,7,8-					1,3,5,6,7,8-									
	Agl		Gly				Agl		Gly	Agl		Agl			Gly		Agl				Gly					
Compounds	**1**	**2**	**3**	**4**	**5**	**6**	**16**	**17**	**H**	**18**	**C**	**7**	**8**	**9**	**10**	**B**	**11**	**12**	**13**	**14**	**15**	**A**	**D**	**F**	**G**	**I**
C. krebsii	+	+	**+**	**+**	+	+	+	+		+	+	+	+	**+**	+	+	+	+	+	+	**+**	+				
C. purpurascens	+	+			**+**						**+**				**+**	+	+	+	+	+	**+**	**+**	+			
C. palustris		+	**+**		**+**			+	+	+	+			**+**	**+**		+	+	+					**+**	+	
C. baccifera	+	+	+		+		+						**+**			**+**			+	+						+

polyphenols are currently being screened by LC using a combination of these on-line detection methods.

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