Investigations on *Hoya* Species. III. Leaf Phenolics and Latex Lipids of *Hoya lacunosa* Bl. *

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Hoya lacunosa leaves contain some main C-glycosylflavonoids, which were identified as: 6-C-arabinosyl 8-C-glucosylapigenin (isoschaftoside), 6-C-glucosyl 8-C-arabinosylapigenin (schaftoside) and 6,8-di-C-arabinosylapigenin. In the latex triterpenols were found, often both free and in ester form, of which β-amyrin, α-amyrin, lupeol, 24-methylenecycloartanol, obtusifoliol and cycloeucalenol were identified in the free alcohol fraction. The esters, forming the major part of the total lipid fraction, were solely acetates of some of the above mentioned alcohols.

Introduction

A number of Asclepiadaceae have been investigated for their chemical constituents, but comparatively little is known of the genus *Hoya* [1, 2]. The only species investigated in some detail are *H. australis* and *H. bella. H. australis* has been analysed for latex lipids and wax components [3 – 5]. In the latex both free triterpenols and their esters were present. The main free alcohols are β-amyrin, α-amyrin, cycloartenol and 24-methylenecycloartanol, whereas esterification occurs mainly with cinnamic acid and to a lesser extent with acetic acid. *H. bella* latex differs in its composition, not only in the presence of the free alcohols lupeol and isobauerenol, found in addition to β-amyrin and cycloartenol, but also by the occurrence of propionates and isovalerates of these triterpenols [2]. Less is known of the leaf phenolics of the two species; *H. australis* contains large amounts of chlorogenic acid [3], some other phenolic depsides and apigenin and luteolin derivatives (O-glycosides) [16]. *H. bella* is rich in acylated flavonol glycosides esterified with ferulic acid, the latter also was found in free form in young leaves [2].

Chlorogenic acid has previously been found in leaves of *H. bandanensis* [6]. In a general screening for flavonoids in Asclepiadaceae Kozjek *et al.* [7] indicate the presence of leucocyanidin and the possible occurrence of quercetin and kaempferol in *H. carnosa* leaves. In their discussion they emphasize the absence of C-glycoflavones and xanthones, in which both the Asclepiadaceae and the Apocynaceae would differ from other families of the Contortales.

The present study describes the analysis of some leaf phenolics and latex terpenoids of *H. lacunosa.*

Materials and Methods

Plant material was obtained from *Hoya lacunosa* Bl. seedlings grown in a green-house. A voucher specimen has been deposited at the Institute for Systematic Botany of the University of Utrecht. For the investigation of the leaf phenolics, leaves were prewashed with chloroform and subsequently extracted with acetone. Lipids were removed by extraction with ligroin, the residue was concentrated and extracted with butanol. The butanol extract was used as such for chromatographic fingerprints and afterwards further separated by repeated paperchromatography (PC).

Latex was tapped from leaf stalks and a total lipid extract in chloroform was obtained according to the method of Bligh and Dyer [8]. This extract was analysed as such and after further fractionation on an Al_2O_3 column with increasing concentrations of diethyl ether in petroleum ether. In some cases further separation by thin-layer chromatography (TLC) appeared necessary.

Fractions and isolated compounds were analysed by PC and/or TLC and by gas-liquid chromatography (GLC) and/or high-performance liquid chromatography (HPLC) with adequate referent compounds. Apart from the phenolic depside (5), all compounds were finally identified by their mass

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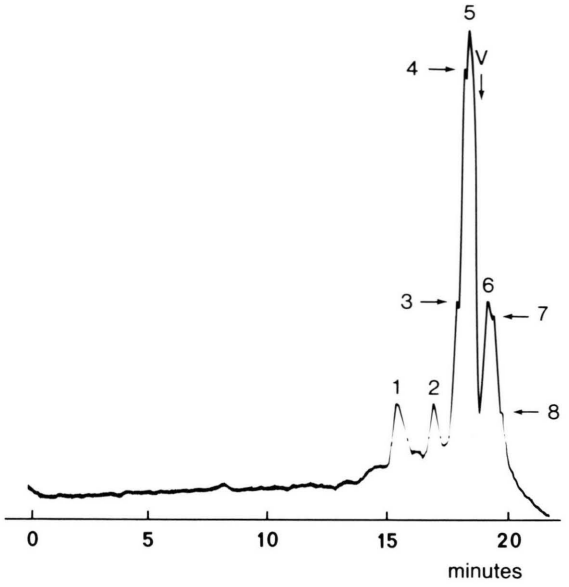

Fig. 1. HPLC analysis of *Hoya lacunosa* leaf phenolics. V = referent vitexin, 1, 2 and 8 are unknown, 3 = a C-hexosyl C-pentosylluteolin, 4 = schaftoside mixed with a phenolic depside, 5 = the glucose ester of ferulic acid, 6 = 6,8-di-C-arabinopyranosylapigenin and 7 = isoschaftoside.

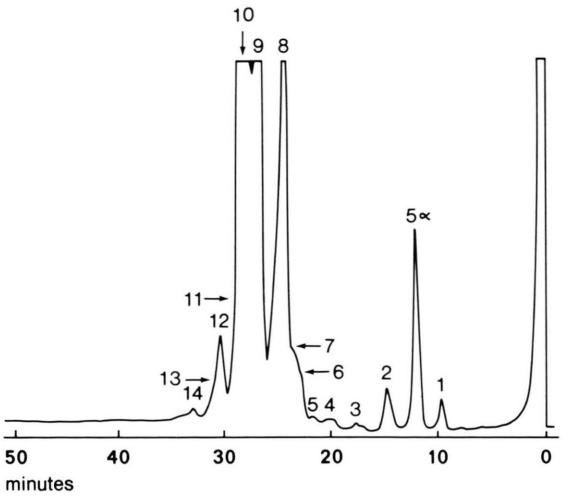

Fig. 2. Gas-chromatogram of the unsaponified total lipid fraction of *Hoya lacunosa* latex on a 3% SE 30 column, temperature-programmed at 2 ° min from 200 – 300 °C. Internal standard 5-α-cholestane. 1, 2 and 3 are unknown homologues, 6 = obtusifoliol, 7 = β-amyrin, 8 = a mixture of cycloeucalenol, α-amyrin and lupeol, 9 = 24-methylenecycloartanol + β-amyrin acetate, 10 = a mixture of α-amyrin acetate and lupeol acetate. Other peaks were not identified. See also Table I.

Table I. Identification of triterpenoids from *Hoya lacunosa* latex.

Total lipid peak			M^+	Fractions obtained after Al_2O_3 and TLC separation main fragments *	base peak	identity
No	RT [240 °]	%				
1	0.71	0.4	378	378 350 281	57	homologues, no triterpenoid
2	1.19	0.7	406	406 378 308	57	homologues, no triterpenoid
3	1.62	0.1	434	434 280 354 221 210 250	57	homologues, no triterpenoid
4	1.96	tr	386	231 232 386 217 371 246 387 233 357	231	unknown
5	2.31	tr	426	218 203 424 189 190 426 217 219 205	218	unknown triterpenol
6	2.67	tr	426	411 426 245 410 201 233 327 227 393	411	obtusifoliol
			522	522 411 217 245 523 426 412	57	unknown
7	2.83	tr	426	218 203 219 189 426 190	218	β-amyrin
8	3.05	17	426	408 393 175 411 189 300 426 409 301	109	cycloeucalenol
			426	218 426 189 203 (207) 408 411	218	α-amyrin
			426	189 218 203 207 393 411	53	lupeol
			424	218 203 424 217 406 391 189 191	109	unknown triterpenol
9	3.80	22	440	422 300 407 440 175 203 216 379	69	24-methylenecycloartanol
			468	218 203 189 468 453 408	218	β-amyrin acetate
10	4.15	52	468	218 189 203 468 249 453 408	218	α-amyrin acetate
			468	189 218 203 468 207 393	53	lupeol acetate
11	sh.	tr	472	218 189 203 209 472 408 458 364	218	unknown ester
12	4.98	3.3	482	273 175 408 285 393 409 203 365	273	unknown
			468	189 190 468 191 249 203 408 393	189	unknown ester
13	5.37	tr	482	207 273 232 482 466 422 407 175	207	unknown ester
14	6.12	tr	494	494 74 87 75 143 495 199	494	no triterpenoid

* Except for peak 14, above *m/e* 170, in order of decreasing intensity.

spectrum (for the C-glycosylflavones after permethylation [9]), and most of them by co-chromatography with the original compound.

GLC conditions: a 4 mm × 1.20 m glass column with 3% SE 30 on Varaport-30 eluted either at 240 °C or temperature-programmed from 200 to 300 °C, at 2 ° min, general internal standard 5-α-cholestane.

HPLC conditions: a 4.6 ID × 250 mm Zorbax ODS column at 50 °C and 1100 psi (7500 kPa, flow around 0.5 ml/min) eluted with either methanol with 0.1% of phosphoric acid (triterpenes [10, 11]) or with a gradient (45 – 100, concave 2, 3%/min on a Dupont 830 chromatograph) of methanol-water again with 0.1% of phosphoric acid (flavonoids [12, 13] slightly altered). The compounds were detected with both a fixed wavelength UV detector at 254 nm and a Dupont 837 spectrophotometer at 215 nm (triterpenoids), 335 or 360 nm (flavonoids). As internal standard uvaol and vitexin were used.

Results and Discussion

From a first PC screening of its butanol extract *H. lacunosa* appeared quite different from the previously investigated species *H. bella* and *H. australis*, but rather similar to *H. multiflora* Bl. With most solvents the two species (H. l. and H. m.) showed two distinct flavonoid spots and one major phenolic depside. On further analysis the flavonoids of *H. lacunosa* appeared mainly of the di-C-glycosylapigenin type, identified as: schaftoside (6-C-glucosyl 8-C-arabinosylapigenin) [14], 6,8-di-C-arabinopyranosylapigenin [15], and isoschaftoside (6-C-arabinosyl 8-C-glucosylapigenin). More flavonoids were present, but finally their concentration was too low for complete identification. Evidence was obtained, however, for the occurrence of C-hexosyl C-pentosyl luteolin derivatives.

A good HPLC separation of the mixture was not obtained (see Fig. 1), partly because the phenolic depside(s) elute in the same area as the C-glycosides. Of the eight phenolics indicated, 1, 2 and 8 remained unidentified, 3 = a C-hexosyl C-pentosylluteolin derivative, 4 = schaftoside which co-chromatographs with a phenolic depside, 5 = a glucose ester of ferulic acid, 6 = 6,8-di-C-arabinopyranosylapigenin and 7 = isoschaftoside.

Compared with *H. australis* and *H. bella*, *H. lacunosa* shows a comparatively simple latex composition of which a gas chromatogram of the total lipid extract is given in Fig. 2. Mass spectral data, obtained after further separation by Al_2O_3 and TLC, are summarized in Table I. A surprising lack of esters other than acetates, exists, although, like in other *Hoya* species, esters form the major part (about 80%) of the total latex lipid (compare: *H. australis* 74% esters, of which, however, 57.7% cinnamates). On further analysis β-amyrin, α-amyrin, lupeol and 24-methylenecycloartanol were identified from the free triterpenol fraction (9.5%). The ester fraction contained the acetates of the first three triterpenols. Sterols could not be detected, but 4-α-methylsterols (7.5%) were present, two of which were identified as obtusifoliol and cycloeucalenol.

It thus seems that both in its latex composition and in the phenolic leaf composition *H. lacunosa* again is fundamentally different from the previously investigated *Hoya* species. In the phenolic region it is quite distinct from *H. bella* with its acylated flavonol glycosides. *H. australis* is more difficult to evaluate because high concentrations of chlorogenic acid tend to mask the low concentrations of flavonoids present. Most of its flavonoids are O-glycosides [16] contrary to the C-glycosides of *H. lacunosa*. The occurrence of C-glycoflavones also contradicts the concept of Kozjek *et al.* [7] of a special place of the Asclepiadaceae within the Contortales based on among others the absence of those compounds. For its latex a marked distinction of *H. lacunosa* is found in the simple esters composition, the triterpenols found belong to the common, rather ubiquiteous type, although generally lupeol and α-amyrin were not found together in *Hoya* species.

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