Investigations on *Hoya* Species.  
III. Leaf Phenolics and Latex Lipids of *Hoya lacunosa* Bl. * 
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**Hoya lacunosa**, Asclepiadaceae, C-Glycosylflavonoids, Latex, Triterpenoids, 4-α-Methylsterols

*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 (schafoside) and 6,8-di-C-arabinosylapigenin. In the latex triterpenoids were found, often both free and in ester form, of which β-amyrin, α-amyrin, lupeol, 24-methylene cycloartenol, obtusifoliol and cyclo-eucalenol 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 triterpenoids and their esters were present. The main free alcohols are β-amyrin, α-amyrin, cycloartenol and 24-methylene cycloartenol, 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 propionate isomers and isovalerate of these triterpenoids [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. carnosae* 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 greenhouse. 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 Al₂O₃ 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
Fig. 1. HPLC analysis of Hoya lacunosa leaf phenolics. V = referent vitexin, 1, 2 and 8 are unknown, 3 = a C-hexosyl C-pentosyluteolin, 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-z-cholestan e. 1, 2 and 3 are unknown homologues, 6 = obtusifoliol, 7 = z-amyrin, 8 = a mixture of cycloeucalenol, z-amyrin and lupeol, 9 = 24-methylene cycloartanol + z-amyrin acetate, 10 = a mixture of z-amyrin acetate and lupeol acetate. Other peaks were not identified. See also Table I.

Table I. Identification of triterpenoids from Hoya lacunosa latex.

<table>
<thead>
<tr>
<th>No</th>
<th>RT [240 °]</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.71</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>1.19</td>
<td>0.7</td>
</tr>
<tr>
<td>3</td>
<td>1.62</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>1.96 tr</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.31 tr</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.67 tr</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.83 tr</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.05 17</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3.80 22</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4.15 52</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>sh. 3.3</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>4.98 tr</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>5.37 tr</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>6.12 tr</td>
<td></td>
</tr>
</tbody>
</table>

* Except for peak 14, above m/e 170, in order of decreasing intensity.
spectrum (for the C-glycosylflavones after perme- 
thylation [9]), and most of them by co-chromatogra-
phy with the original compound.

GLC conditions: a 4 mm x 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-
cholestan.

HPLC conditions: a 4.6 ID x 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 (tri-
terpenoids), 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 
the HPLC separation the flavonoids of H. lacunosa 
were mainly of the di-C-glycosylapigenin type, iden-
tified as: schaftoside (6-C-glucosyl 8-C-arabinosyla-
pigenin) [14], 6,8-di-C-arabinopyranosylapigenin 
[15], and isoschaftoside (6-C-arabinosyl 8-C-glucosa-
ylapigenin). More flavonoids were present, but final-
ly their concentration was too low for complete iden-
tification. Evidence was obtained, however, for the 
ocurrence of C-hexosyl C-pentosyl luteolin derivati-
ves.

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-glycosyl-
depsides. Of the eight phenolics indicated, 1, 2 and 8 
remained unidentified, 3 = a C-hexosyl C-pentosyllu-
tein derivative, 4 = schaftoside which co-chroma-
tographs with a phenolic depside, 5 = a glucose ester 
of ferulic acid, 6 = 6,8-di-C-arabinopyranosylapige-
nin and 7 = isoschaftoside.

Compared with H. australis and H. bella, H. lacu-
nosa shows a comparatively simple latex composi-
tion of which a gas chromatogram of the total lipid 
eXPt is given in Fig. 2. Mass spectral data, obtained 
after further separation by Al₂O₃ 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-
methylenecycloartenol were identified from the free 
triterpenol fraction (9.5%). The ester fraction contain-
ed 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 flavo-
nol glycosides. H. australis is more difficult to evalu-
ate 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 
ocurrence of C-glycoflavones also contradicts the con-
cept of Kozjek et al. [7] of a special place of the As-
clepiadaceae 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 be-
long to the common, rather ubiquitous type, al-
though generally lupeol and 2-aromatics were not found 
together in Hoya species.

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