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INVESTIGATIONS ON HOYA SPECIES. VI[1]. LATEX COMPOSITION AND LEAF PHENOLICS AND THEIR TAXONOMIC SIGNIFICANCE

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SUMMARY

Twenty five *Hoya* species were investigated for the possible use of the composition of latex triterpenes and leaf phenolics in their systematic identification. Both the phenolics (mainly apigenin-type flavones) and the latex triterpenes appeared rather specific for the species investigated. Because of its stability especially the latex particle composition may be worthwile as additional marker in the determination of *Hoya* species.

1. INTRODUCTION

The genus *Hoya* (Asclepiadaceae) comprises about two hundred species originating from South East Asia and Australia. A number of them are cultivated for ornamental purposes which has added to the existing confusion in nomenclature. A reliable systematic monograph of the genus does not exist and generally there is much doubt concerning the correctness of the names attached to the plants.

Important help in distinguishing *Hoya* species and cultivated forms may come from phytochemistry. Like other Asclepiadaceae *Hoya* species possess laticifers, which in most cases contain a milky latex. The milkiness of such latices is due to the presence of lipoid particles suspended in an aqueous serum. Main components of these particles are triterpenes en polyisoprenes. Especially the triterpenes have been found to be useful additional markers in the taxonomy of for instance the latex-bearing Euphorbiaceae (PONSINET & OURISSON 1968, NIELSEN et al. 1979). In an extensive treatise of the Apocynaceae latices VAN DIE (1955) concluded that "The composition of the coagulate (*i.e.* the particle fraction) from a given plant is essentially constant and independent of the plant part from which the latex is obtained". Likewise, in *Hoya australis* the latex triterpene composition was found to be independent of the age of the plant and even of changes in environmental factors (BAAS & NIEMANN 1979). This is quite in contrast to the composition of most other secondary plant products which often

[1] Part V of this series: F. Warnaar, in press.

varies greatly in relation to changes in environment. Flavonoids, for instance, have been found to be very dependent on season (STAUDE & REZNIK 1973, NIEMANN 1976). In Asclepidaceae like *Periploca graeca* (MELIN 1964) and *Hoya australis* (NIEMANN et al. 1980) the flavonoid pattern in young leaves was found to be quite different from that in older leaves.

The present study concerns a comparative investigation into the possible use of latex particle composition and of leaf phenolics in the identification of species of the genus *Hoya*.

2. MATERIALS AND METHODS

Plants – Leaves, petioles and stems of the investigated *Hoya* species were obtained from fresh material, currently cultivated in the greenhouses of the Botanical Laboratory of the University of Utrecht. Most of them are cuttings, originating from the Botanic Gardens of Paris, Gent, Heidelberg, Bogor, Tübingen, and München. The species were kept under the name by which they were obtained, while for some species, *viz.* coll. nrs. 9, 18, 21, 35, 110, 122, 142, 156, and 202 (see *table 3*), these names could be verified by comparison with specimens present in the Herbaria of Leiden and Kew. As is referred to in the introduction of this paper, we are not certain about the correctness of the species names. We do not believe, however, that this uncertainty – which we regard as unavoidable – invalidates the main purpose of the present work: to test the potential significance of chemical characters derived from laticifers in distinguishing *Hoya* species. Of all species used specimens were deposited in the Herbarium of the University of Utrecht.

Latex – Latex was tapped from petioles, extracted according to the method of BLIGH & DYER (1959) and the extracts were analysed either as such (*H. lacunosa* seedlings), or after saponification (all species), on a gas chromatograph with a 150 cm glass column, ID 4 mm, containing 3% SE 30 on Varaport 30, 80–100 mesh, temperature programmed from 200–300°C at 2°/min, or isotherm at 225°C. Retention times given are relative to 5 α-cholestane. Fatty acids, obtained after saponification were methylated and analysed on 25% DEGS or 10% Apiezon L as described previously (WARNAAR 1977). *H. carnosa* (nrs. 35 and 36, *table 3*) and *H. motoskei* (155-1) don't possess a particle containing latex and are omitted in *fig. 1*.

Phenolics – Fresh leaves were extracted with acetone. After filtration and addition of water, lipophilic compounds were removed by extraction with ligroin. The acetone-water extract was concentrated, acidified to pH 2–3 and extracted with butanol. The butanol extract was evaporated to dryness and the residue was taken up in methanol and further purified by bandchromatography on paper with 15% acetic acid and subsequently with tert. butanol – acetic acid – water 3:1:1, sometimes followed by further purification in other solvent systems. Isolated compounds were identified by colour, R_f values, UV spectrum inclusive shifts, often by comparison with the authentic substance, and by analysis of their

acid and/or alkaline hydrolysis products. For some di-C-glycosylflavones MS of permethylated derivatives was used (BOUILLANT et al. 1975).

3. RESULTS AND DISCUSSION

To be able to evaluate interspecific variations, it is necessary to obtain insight in the variation between different individuals of the same species. For leaf flavonoids it has already been demonstrated that a large genetic variation may occur (NIEMANN & BAAS 1978). For latex the picture might be different. VAN DIE (1955) found for a number of individuals of *Plumiera acutifolia* that the polyisoprene content for a given species is subject to only slight variation.

Latex of *H. lacunosa*, the only species of which a number of seedlings were available, contains 12.2% of lipids, of which 12.3% consists of rubber and the remainder is formed by the free triterpenols β-amyrin, α-amyrin, lupeol and 24-methylenecycloartanol (12.6%), the acetates of the first three triterpenols (84.0%) and long-chain fatty acid esters of the triterpenols (3.4%) (WARNAAR, in preparation, NIEMANN et al. 1979). *Table 1* gives a comparison of the gaschromatograms of the total lipid extracts of 10 seedlings and shows that the variation found between the latices of the different individuals is negligible.

It was demonstrated before (WARNAAR, in press) that the triterpene-bound fatty acids show a specific pattern, typical for the latex involved. The pattern for *H. lacunosa* is given in *table 2*. In all seedlings investigated the same pattern was

Table 1. Variation in the total lipid extract of latex of ten seedlings of *Hoya lacunosa*. Percentages of the gas-chromatographic peaks found on a 3% SE 30 column, temperature programmed from 200–300°C at 2°/min. P = peak number, RT = retention time relative to 5 α-cholestane[1], S = seedling number, Av. = average.

P:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
RT:	0.45	0.57	0.61	0.69	0,76	0.84	1.13	1.32	1.51	1.71	1.84	1.93	1.99	2.08	2.21	2.30	2.43	2.54
S																		
1	0.03	0.2	0.2	0.4	1.8	0.4	2.6	2.6	0.4	0.8	10.0	4.0	14.2	51.7	4.0	1.6	5.0	0.4
2	–	0.2	0.2	0.4	1.6	0.4	2.4	2.2	0.4	1.1	9.7	3.5	14.3	50.6	4.7	1.7	5.5	0.8
3	0.04	0.2	0.2	0.5	1.7	0.3	2.4	2.5	0.3	0.6	8.2	5.6	13.8	53.7	3.3	1.7	4.5	0.5
4	0.04	0.3	0.2	0.5	1.6	0.6	2.9	2.5	0.5	0.8	10.0	5.2	13.5	49.4	4.0	1.7	5.8	0.4
5	–	0.2	0.1	0.5	1.7	0.2	2.6	2.6	0.2	0.6	9.7	4.9	14.6	49.8	4.1	1.8	6.0	0.4
6	0.06	0.2	0.1	0.5	1.6	0.6	2.9	2.5	0.5	0.6	9.5	4.9	14.4	49.6	4.4	1.6	5.9	0.2
7	–	0.2	0.1	0.5	1.6	0.4	2.6	2.5	0.3	0.7	10.2	4.9	14.2	50.1	4.2	1.6	5.8	+
8	–	0.2	0.1	0.6	1.9	0.4	3.1	2.9	0.6	0.7	10.3	5.4	13.0	47.0	4.7	1.7	6.5	0.2
9	–	0.2	0.1	0.5	1.4	0.4	2.4	2.2	0.4	0.9	11.1	3.6	14.1	49.6	5.0	1.5	6.3	0.3
10	–	0.2	0.2	0.4	2.0	0.1	1.9	2.2	0.4	0.9	8.8	3.3	14.4	50.3	5.1	2.1	5.9	1.3
Av.:		0.2	0.15	0.5	1.7	0.4	2.6	2.5	0.4	0.8	9.75	4.5	14.1	50.2	4.35	1.7	5.7	0.47

[1] The retention times differ somewhat from those published earlier for *H. lacunosa* (NIEMANN et al. 1979) because a different column was used. The main peaks consisted of a mixture of obtusifoliol and β-amyrin (no 10), a mixture of cycloeucalenol, α-amyrin and lupeol (no 11), a mixture of 24-methylenecycloartanol and β-amyrin acetate (no 13), and the acetates of α-amyrin and lupeol (no 14).

Table 2. Long-chain fatty acids of triterpenes of *Hoya lacunosa* latex in mol%. Before the colon: number of carbon atoms in the fatty acid; after the colon; number of double bonds.

14:0	15.2%	17:0	0.7	20:0	1.6
14:1	6.4	17:1	1.0	20:1	0.4
15:0	5.5	18:0	1.7		
15:1	2.3	18:1	21.2	>20	–
16;0	24.6	18:2	8.5		
16;1	3.7	18:3	3.6		
16;2	3.7				

found, the greatest variation being less than 3%. It thus appears that also in this respect the latex triterpene composition is independent of individual variation within the species.

For twenty-two species possessing a particle containing latex the triterpene composition found after saponification is visualised in *fig. 1*. In this figure the species have been arranged according to their latex composition, starting with those with a comparatively high peak at the place of β-amyrin (RT 3.11). Most species show comparatively large peaks with the retention times of β-amyrin and that of α-amyrin and/or lupeol (RT 3.45), whereas lower ones were mainly found at RT 3.60 (cycloartenol) and 4.18 (24-methylenecycloartanol). Several minor components (white blocks in the figure) were not correlated.

In spite of the similarities found, only in one case identical chromatograms were obtained, *viz.* for the two specimens of *H. angustifolia*, of which one was obtained under the name *H. longuifolia*. Although we did not have a GC-MS available, it

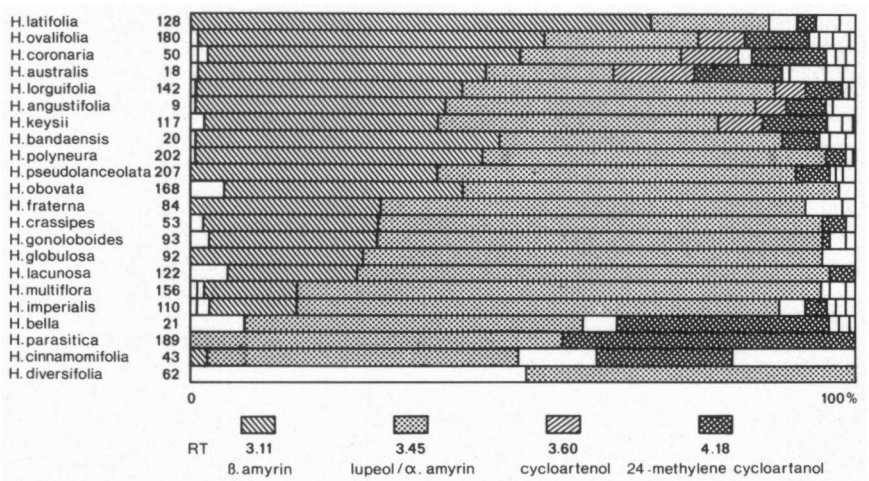

Fig. 1. Gaschromatographic "fingerprints" of triterpenes found after saponification of *Hoya* latices. The percentile contribution of the GC peaks is indicated by blocks given according to increasing retention, the retention time of the white blocs was in general not correlated with that of known triterpenoids. *H. longuifolia* and *H. angustifolia* are synonyms.

Table 3. Leaf phenolics of *Hoya species*[1].

Species	No:	Fe	PC	Chl	Fls	AcFls	Ap	Lu	Vit	Iv	di-C
H. angustifolia Elmer	9			−					+		+
H. australis R. Br. ex Traill.	18			+			+	+			
H. bandaensis Schlechter	20			+	+		+				
H. bella Hook.	21	+		−	+	+					
H. carnosa R. Br.	35			+							+
H. carnosa R. Br. var. variegata	36			+						+	+
H. cinnamomifolia Hook.	43					no sufficient material					
H. coronaria Blume	50			+			+				
H. crassipes Turcz.	53			+							+?
H. diversifolia Blume	62-1		+	−						+	+?
H. fraterna Blume	84										+
H. globulosa Hook.	92		+	+			+				
H. gonoloboides Regel	93			+							+
H. imperialis Lindl.	110			−	+						
H. keysii F. M. Bailey	117			+			+	+			
H. lacunosa Blume	122	+		−							+
H. latifolia Don.	128			+						+	
H. macrophylla Wallich	146-1			−				no main flavonoids			
H. minima Cost	153	+	tr								+
H. motoskei Teysm.	155-1			+						+	+
H. multiflora Blume	156	+		−		tr		no main flavonoids			
H. obovata Decne	168-1			+						+	
H. ovalifolia Wight & Arn.	180			+			+	+			
H. parasitica Wall. ex Traill.	189-2		+	+					+	+	+
H. polyneura Hook.	202	+					+	+	+		+
H. pseudolanceolata Cost.	207			+							+

[1] The numbers refer to the collection number of the plants cultivated in the greenhouses of the Botanical Laboratory, University of Utrecht, Chl = chlorogenic acid, Fe = ferulic acid and/or derivatives, PC = *p*-coumaric acid and/or derivatives, Fls = flavonols, AcFls = acylated flavonol glycoside, Ap = apigenin O-glycoside(s), Lu = luteolin O-glycoside(s) and/or derivatives (a.o. chrysoeriol), Vit = vitexin and/or its O-glycosides, Iv = isovitexin and/or its O-glycosides, di-C = apigenin-di-C-glycoside(s), tr = trace.

appears that even these partly unidentified triterpene "fingerprints" are sufficiently specific for the identification of the species.

Data on leaf phenolics are given in *table 3*. With a few exceptions all species investigated contain apigenin-type flavones as main flavonoids. Differences between the species were mainly found in the presence or absence of mono- or di-C-glycosylation. The main phenolic depside, found in most species, is chlorogenic acid.

H. bella and *H. imperialis* stand out by having flavonols instead of flavones in their leaves, *H. bandaensis* contains both types of flavonoids and *H. macrophylla* and *H. multiflora* differ by the near absence of flavonoids. The flavonoids found in low concentration in *H. multiflora* are acylated flavonol glycosides. *H. bella* is the only one of the 22 species investigated with a rather different triterpene composition. *H. bella*, *H. imperialis* and *H. multiflora*, however, are clearly

distinguished from the other species by their latex long-chain fatty acid composition (WARNAAR, in preparation). For the five species mentioned the flavonoid composition may be of taxonomic value; whether mono- or di-C-glycosylation of the flavones has any taxonomic potential remains doubtful, even more so in view of possible individual and/or age-dependent variability. It is remarkable, however, that almost all species with apigenin-O-glycosides appear in the upper half of the tabulation of species according to their latex triterpene composition (*fig. 1*).

Summarizing, leaf phenolics appear less convenient as additional marker for *Hoya* taxonomy. The stable and species-specific latex particle composition, expressed as percentile distribution of not necessarily known components, render latex triterpenoid "fingerprints" a very useful means of identification. The only drawback is that living plants are needed and that there are a few species without particle containing latices.

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