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Gas-Liquid Chromatographic Analyses of the Water-soluble compounds of Plant Latices

By

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With 1 Figure

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Summary

BAIER W. R. & HEINRICH G. 1993. Gas-liquid chromatographic analyses of the water-soluble compounds of plant latices. – *Phyton* (Horn, Austria) 33 (1): 77-85, 1 Figure. – English with German summary.

The latices of 14 plant species belonging to the families of *Euphorbiaceae*, *Asclepiadaceae*, *Moraceae*, *Nelumbonaceae*, *Papaveraceae* and *Russulaceae* were studied by means of gas-liquid chromatography. After fractioning the latices with ion-exchangers, the sugars, carbonic acids and amino acids were analyzed. The main compounds were gallic, ascorbic, glucuronic and citric acids, and also glucose. The rest was very heterogeneous.

Zusammenfassung

BAIER W. R. & HEINRICH G. 1993. Gaschromatographische Trennung der wasserlöslichen Komponenten pflanzlicher Milchsäfte. – *Phyton* (Horn, Austria) 33 (1): 77-85, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Der Milchsafte von 14 Pflanzen aus den Familien der Euphorbiaceen, Asclepiadaceen Moraceen, Nelumbonaceen, Papaveraceen und Russulaceen wurde mit Hilfe der Kapillargaschromatographie untersucht. Nach einer Fraktionierung mit Ionen-Austauschern wurden die Karbonsäuren, Zucker und Aminosäuren bestimmt. Neben zahlreichen Nebenkompontenten fanden sich meist Gallus-, Ascorbin-, Glucuron- und Zitronensäure sowie Glucose als Hauptkomponenten.

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Introduction

The milky fluid which exudes from several plants is called latex. It is contained in special vessels called laticifers. These are internal secretory structures which are either single, non-articulated cells or compound, articulated tubes. They can be branched or unbranched (MAHLBERG 1963, MAHLBERG & SABHARWAL 1968, ESAU & KOSAKAI 1975, WILSON & MAHLBERG 1978).

Latex is a mixture of various substances, occurring either in solution (hydrophilic compounds) or suspension (lipophilic compounds). Besides, it may contain a variety of cell structures (HEINRICH 1967, 1970), such as nuclei, mitochondria, ribosomes, starch-grains, rubber-grains, and luteoids. Luteoids are lysosome-like vacuols (PUJARNISCLE 1968, D'AUZAC & al. 1982).

Latex occurs in at least 12.000 plant species belonging to 900 genera. Not all genera of a family, however, are latex-bearing. This fact complicates the definition of the role of latex in plants. ULTEE (1925) distinguished latices rich in salt, rubber, protein or phytostearin. It is remarkable that nearly all latices contain varying amounts of rubber, especially *Hevea brasiliensis* is a very important source of caoutchouc (LYNEN & HENNING 1960, LYNEN 1963, BACKHAUS 1985).

The fact that latex is not a dead structure was shown by several studies (MOTHES & MEISSNER 1964, MEISSNER 1966 b, BÖHM & SCHULZE 1972).

Euphorbia is a well known latex-bearing genus (SCHULZE & al. 1967). In a previous study a number of species of this genus as well as some other genera were analyzed to determine the inorganic ions of their latices (BAIER & al. 1991). In this study we concentrate on the organic, soluble compounds of several latices previously studied by MOTHES & MEISSNER (1964) and GROENEVELD & al. (1982).

Material and Methods

The latices of 14 plants from the Botanical Garden of Graz were collected from leave-stalks of stems. *Chelidonium majus* and *Lactarius vellereus* are grown outdoors.

The samples are listed according to the anatomical features of their latex vessels and their families. Besides, the natural habitat of each plant is noted.

A modified method first applied by LISS (1961) was used. The freshly - tapped latex was suspended in twice distilled water before being evaporated by freeze-drying. The pellet of each vial was resuspended in 10 ml distilled water. After filtering the samples. DOWEX 50W2 and DOWEX 1 × 4 ion-exchangers were used to obtain three fractions (KINZEL 1962). The resulting fractions (carbonic acids, amino acids, and sugars) were freeze-dried again and diluted in 0.5 ml pyridine. After silylation with REGISIL (BSTFA with 1% TMCS), 5 µl of the sample were injected into a quartz silica capillary column 25 m × 0.2 mm (DURABOND 5) at a temperature of 100°C. After five minutes the temperature was programmed to ascend to 300°C at a rate of

<i>Euphorbia enopla</i> Boiss. (<i>Euphorbiaceae</i>) non-articulated branched laticifers	S-Africa
<i>Euphorbia fimbriata</i> Scop. (<i>Euphorbiaceae</i>) non-articulated branched laticifers	S-Africa
<i>Euphorbia lathyris</i> L. (<i>Euphorbiaceae</i>) non-articulated branched laticifers	W-India
<i>Euphorbia myrsinites</i> L. (<i>Euphorbiaceae</i>) non-articulated branched laticifers	mediterranean
<i>Euphorbia ornithopus</i> Jacq. (<i>Euphorbiaceae</i>) non-articulated branched laticifers	S-Africa
<i>Euphorbia pulcherrima</i> Willd. (<i>Euphorbiaceae</i>) non-articulated branched laticifers	C-America
<i>Euphorbia schimperi</i> Presl. (<i>Euphorbiaceae</i>) non-articulated branched laticifers	S-Arabia
<i>Euphorbia stenoclada</i> H. Baill. (<i>Euphorbiaceae</i>) non-articulated branched laticifers	SW-Madagascar
<i>Asclepias syriaca</i> L. (<i>Asclepiadaceae</i>) non-articulated branched laticifers	N-America
<i>Ficus elastica</i> Roxb. (<i>Moraceae</i>) non-articulated branched laticifers	E-India
<i>Nelumbo nucifera</i> Gaertn. (<i>Nelumbonaceae</i>) articulated unbranched laticifers	Asia
<i>Chelidonium majus</i> L. (<i>Papaveraceae</i>) articulated unbranched laticifers	N-hemisphere
<i>Papaver somniferum</i> L. (<i>Papaveraceae</i>) articulated unbranched laticifers	Asia
<i>Lactarius vellereus</i> Fr. (<i>Russulaceae</i>) milky hyphes	Europe

8°C per minute. The carrier gas was hydrogen (0.95 ml/min), the make-up gas was nitrogen. GLC was carried out using a DANI 8400 gas chromatograph with FID.

Sometimes the latex was taken from several plants of the same species, growing on the same place, because the latex content of one single plant was not enough to carry out an analysis. In order to determine whether individual differences in the composition of latex exist, the latices of different *Ficus elastica* plants were analysed separately.

The composition of latices might also be dependend on the stage of plant development. To find out more about this, the latices of leaves and stems of *Ficus elastica* plants of different age were examined. No conspicuous differences in the water soluble main components of the latex could be found. Of course, the influence of the condition of the soil and the way of growing the plants also must not be neglected when regarding the composition of the latices. This is to be important to find out if a latex fingerprint is characteristic for a plant species. The repeated analysis of a sample of a derivated latex shows identical main peaks.

Results and Discussion

The weights of the three fractions (N free carbonic acids, aminoacids, and sugars) for 1g of fresh latex are given in Table 1. In eight cases the carbonic acids are the heaviest fraction, in four cases the amino acids, only in three latices the sugars override the other fractions. All latices except *Nelumbo* have less sugars than carbonic and amino acids together. All latices of succulent plants have a water content of 60–70 percent (Table 1).

Table 1

Carbonic acids, amino acids and sugars in mg per g of fresh latex and the water contents in percent of fresh weight.

	carbonic acids	amino acids	sugars	water %
<i>E. enopla</i>	12	<u>6</u>	12	64
<i>E. fimbriata</i>	36	<u>87</u>	43	64
<i>E. lathyris</i>	41	<u>49</u>	12	80
<i>E. myrsinites</i>	<u>111</u>	72	37	77
<i>E. ornithopus</i>	37	<u>61</u>	21	38
<i>E. pulcherrima</i>	<u>48</u>	12	10	84
<i>E. schimperi</i>	<u>40</u>	12	7	63
<i>E. stenoclada</i>	<u>125</u>	9	11	70
<i>Asclepias syriaca</i>	<u>29</u>	10	23	91
<i>Ficus elastica</i>	<u>49</u>	6	6	83
<i>Nelumbo nucifera</i>	6	3	<u>12</u>	91
<i>Chelidonium majus</i>	<u>52</u>	41	49	73
<i>Papaver somniferum</i>	23	<u>46</u>	23	70
<i>Lactarius vellereus</i>	5	1	<u>16</u>	74

The dominating fraction of each sample is underlined!

The non-succulent higher plants (*Euphorbia lathyris*, *E. pulcherrima*, *Ficus elastica*, *Chelidonium majus*, and *Papaver somniferum*) show a water content of 80 percent. The latices of *Asclepias syriaca* and *Nelumbo nucifera* contain even more water (90%). The latex of *Euphorbia ornithopus* is atypical with only 40% of water. The water content of the latices differs widely and seems to depend on environmental conditions. Table 2 lists the data of the carbonic acid fractions obtained from GLC-analysis. Many latices show a characteristic peak-triplet caused by gallic, ascorbic and glucuronic acids, often in combination with quinic and citric acids. In addition there are other acids from the Krebs-cycle.

Typical for the sugar fraction of the latices (Table 3) are glucose and an unidentified substance close to the glucose peak. Remarkable is that the

Table 2

Carbonic acids in mg per g of fresh latex of different plants sorted according to retention times.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
oxalic	0.8	—	—	—	—	—	—	0.5	0.2	—	0.1	—	0.2	0.1
glycolic	—	1.4	—	—	5.1	—	0.2	—	—	—	—	2.2	—	—
nicotinic	—	—	1.8	1.9	—	—	0.1	0.3	—	—	—	—	—	—
maleinic	—	—	—	—	—	—	—	—	—	—	0.1	2.4	—	—
malonic	—	—	2.3	—	2.5	—	—	1.1	—	—	—	—	—	—
succinic	—	—	—	—	—	—	—	—	—	—	—	—	0.7	1.0
fumaric	—	—	—	—	—	—	—	—	0.3	—	1.0	20.4	1.2	0.2
malic	—	—	6.6	—	—	—	—	0.2	0.5	0.7	0.8	10.0	4.0	1.7
tartaric	—	—	—	—	—	3.2	—	0.2	0.5	—	—	—	—	0.9
ketoglutaric	—	—	—	—	—	9.3	—	—	—	—	—	—	—	—
shikimic	—	—	—	0.5	—	1.3	0.1	—	—	—	—	2.0	—	—
citric	—	—	4.0	0.6	—	1.1	0.2	4.4	0.7	1.5	0.2	1.9	1.0	—
quinic	0.2	—	—	—	—	1.3	1.2	1.0	0.6	0.5	0.1	—	—	—
gallic	1.6	8.6	15.4	1.1	—	0.9	—	—	5.0	14.6	0.6	—	—	—
ascorbic	1.6	7.3	—	1.0	—	1.0	—	—	0.2	12.9	0.6	—	—	0.1
glucuronic	0.9	6.2	—	1.1	—	1.8	—	100	—	14.1	0.3	—	—	—
ferulic	2.2	—	—	—	18.7	2.5	36.0	—	2.5	—	—	—	—	—
coffeic	—	—	1.5	—	—	—	—	—	—	—	—	—	—	—

1 *Euphorbia enopla*, 2 *Euphorbia fimbriata*, 3 *Euphorbia lathyris*, 4 *Euphorbia myrsinites*, 5 *Euphorbia ornithopus*, 6 *Euphorbia pulcherrima*, 7 *Euphorbia schimperii*, 8 *Euphorbia stenoclada*, 9 *Asclepias syriaca*, 10 *Ficus elastica*, 11 *Nelumbo nucifera*, 12 *Chelidonium majus*, 13 *Papaver somniferum*, 14 *Lactarius vellereus*

latex of *Lactarius* seems not to contain regular sugars. Perhaps, it has lactones or alcohols instead of them.

Table 4 shows the identified amino acids of the latices. This fraction is less uniform than the others. Many amino acids are "latex-specific" (MOTHES & MEISSNER 1964). Many latices also contain tryptophan and citrulline. Besides, this fraction also contains the alkaloids of the latex. Fig. 1. shows the GLC-separation of the various fractions of the latex of *Nelumbo*.

It is not astonishing that many of these carbonic acids are intermediates from the Krebs-cycle or from the path of shikimic acid syntheses. But acids like ascorbic and glucuronic acids in connection with glucose and fructose show a third pathway of synthesis of which intermediates are found: it is the way of vitamin C synthesis. Perhaps latices are able to produce ascorbic acid themselves. In agreement with

MEISSNER (1966a) one can see that the pH of the latices relates to the quantities of carbonic acids found.

The shikimic acid derivatives lead to the amino acid tryptophan and the alkaloids resulting from the latter. Citrulline occurs frequently in latices. Perhaps, it supplies the latex with nitrogen for synthesis of alkaloids.

Table 3

Sugars in mg per g of fresh latex of different plants sorted according to retention times.

	mannose	fructose	galactose ?	glucose	inositol	sucrose	maltose	lactose
1	—	—	—	1.7	1.2	—	—	—
2	—	—	—	5.3	2.2	—	1.1	—
3	0.3	0.4	0.7	2.2	2.0	1.5	—	—
4	—	1.1	3.4	8.0	11.9	—	1.7	—
5	—	—	—	1.2	2.6	—	0.5	—
6	0.3	0.7	—	5.6	1.6	0.1	—	0.5
7	—	0.2	0.2	0.4	0.4	—	—	—
8	0.3	—	—	0.2	0.2	8.5	—	—
9	0.4	0.4	—	—	1.6	—	0.4	0.2
10	—	—	—	—	0.5	0.2	0.6	0.4
11	0.3	2.8	—	—	3.0	0.2	—	—
12	—	—	—	1.6	4.1	2.9	—	2.4
13	0.1	0.3	0.2	0.2	2.2	1.2	6.4	2.5
14	—	—	—	—	—	—	—	—

1 *Euphorbia enopla*, 2 *Euphorbia fimbriata*, 3 *Euphorbia lathyris*, 4 *Euphorbia myrsinites*, 5 *Euphorbia ornithopus*, 6 *Euphorbia pulcherrima*, 7 *Euphorbia schimperi*, 8 *Euphorbia stenoclada*, 9 *Asclepias syriaca*, 10 *Ficus elastica*, 11 *Nelumbo nucifera*, 12 *Chelidonium majus*, 13 *Papaver somniferum*, 14 *Lactarius vellereus*

Some amino acids are possibly retained by the ion exchanger. This could be the reason that some common amino acids were missing in this fraction.

Similarities are found in *Euphorbia enopla*, *Euphorbia fimbriata*, and *Euphorbia ornithopus* on one hand and in the leafy species like *Euphorbia lathyris*, *Euphorbia pulcherrima*, and *Euphorbia schimperi* on the other hand. *Euphorbia myrsinites* and *Euphorbia stenoclada* are atypical. The latter belongs to an endemic group of *Euphorbiae* of South-Madagascar. There they are associated with the characteristic *Didiereaceae* of this area.

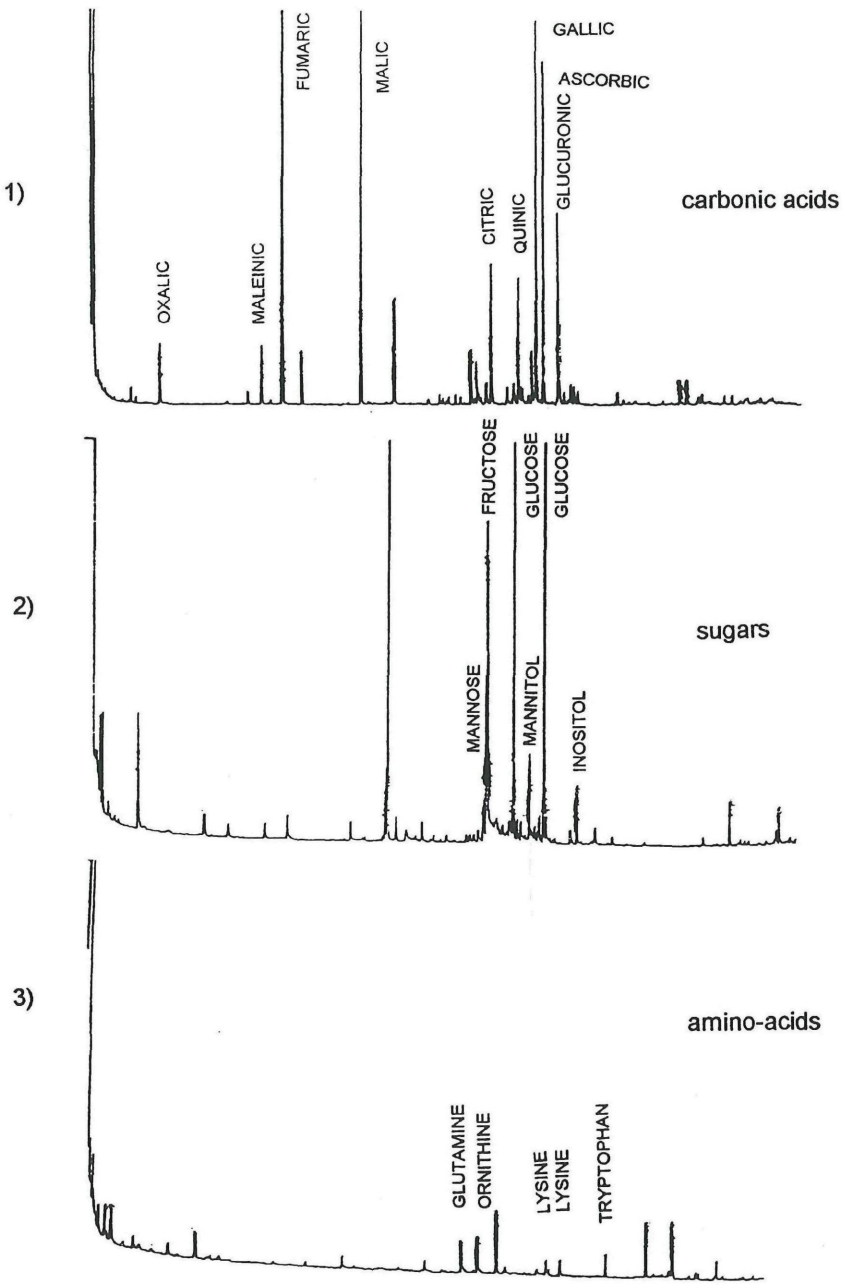


Fig. 1. GLC-Separation of the latex of *Nelumbo nucifera*.

To sum up, latex primarily seems to be a special medium for metabolic waste (e. g. caoutchouc). Besides, it also functions as storage (starch!), as a defense against being eaten and as wound-closer (MAHLBERG 1975).

A connection between the anatomy of the laticifers and their contents cannot be seen.

Table 4

Amino acids in mg per g of fresh latex of different plants sorted according to retention times.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Alanine	–	18.9	–	–	16.7	–	1.6	–	–	–	–	–	–	–
Valine	0.4	2.9	–	–	9.2	–	1.3	–	–	–	–	–	1.6	–
Proline	–	–	1.9	20.7	–	–	–	–	–	–	–	–	–	–
Methionine	–	–	–	–	–	–	–	–	–	–	–	1.2	1.5	–
Asparagine	–	–	–	–	–	–	–	–	–	–	–	1.0	2.4	–
Pheylalanine	–	–	–	–	–	–	–	–	–	–	–	1.1	0.8	0.4
Glutamine	–	–	–	–	–	–	–	–	–	0.5	0.3	0.9	0.9	–
Ornithine	0.4	–	1.2	–	–	–	–	0.3	–	–	0.2	–	–	0.2
?	0.4	2.3	–	2.1	8.4	–	1.2	–	0.7	0.5	0.4	–	–	–
Tryptophan	0.4	–	1.7	5.5	–	–	–	–	4.8	0.3	0.1	–	–	–
Cysteine	–	–	–	1.9	–	–	0.2	–	–	–	–	–	–	–
Citrulline	1.3	–	4.7	–	–	–	–	–	0.9	2.5	0.2	–	–	–
Lysine	0.8	–	2.6	–	–	–	–	–	1.6	–	–	–	–	0.1

1 *Euphorbia enopla*, 2 *Euphorbia fimbriata*, 3 *Euphorbia lathyris*, 4 *Euphorbia myrsinites*, 5 *Euphorbia ornithopus*, 6 *Euphorbia pulcherrima*, 7 *Euphorbia schimperii*, 8 *Euphorbia stenoclada*, 9 *Asclepias syriaca*, 10 *Ficus elastica*, 11 *Nelumbo nucifera*, 12 *Chelidonium majus*, 13 *Papaver somniferum*, 14 *Lactarius vellereus*

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