On the Chemical Nature of Epicuticular Waxes in Some Succulent *Kalanchoe* and *Senecio* Species

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Leaves and other aerial parts of several succulent species each of *Kalanchoe* and *Senecio* exhibit more or less obvious wax coatings. The major components of these waxes were identified to be pentacyclic triterpenes. Some of these are rare natural products, while others are rather widespread.

Introduction

Most species of *Kalanchoe* (Crassulaceae) are more or less succulent xerophytes. The large Compositae genus *Senecio* also houses many species with succulent appearance. In both genera the presence of waxy material on their leaf surfaces and also on stem surfaces is rather widespread. In the course of our search for plants producing exudate flavonoids (c.f. Wollenweber, 1990) we have studied several species each of both genera. They all were found to be devoid of epicuticular flavonoid aglycones, but we happened to isolate some of the triterpenoid constituents forming the "leaf wax" (the term wax being used here in the botanical sense, regardless of the chemical definition; c.f. Barthlott and Wollenweber, 1981). Since to our knowledge externally accumulated triterpenoids have not been reported so far from neither *Kalanchoe* nor *Senecio* (Mahato *et al.*, 1991), we studied the major constituents of their leaf waxes. As it turned out the wax does not only appear very similar in both genera, but also its composition is very similar, which suggests joint publication of these results.

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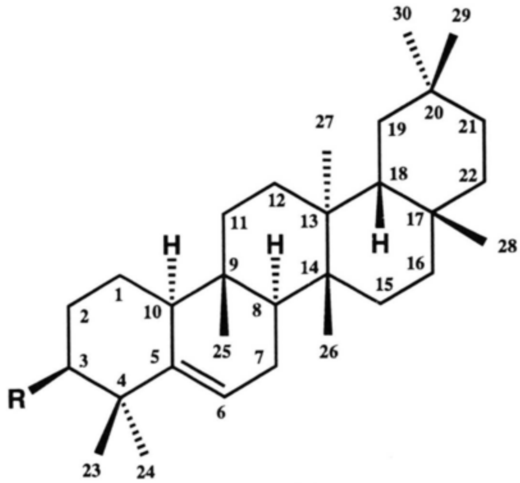
Material and Methods

All plant material used in the present study was collected from plants cultivated in greenhouses of the Botanical Garden at Darmstadt. In each case fresh plant material was briefly rinsed with chloroform to dissolve the waxy epicuticular material. After evaporation of the solvent, the normally colourless residue was dissolved in toluene and applied to column chromatography on silica. Elution was done with toluene and increasing quantities of methylethyl ketone and methanol. Fractions were monitored by TLC on silica plates with solvents A (toluene–methylethyl ketone 9:1) and B (toluene–dioxane–glacial acetic acid 18:5:1). Terpenoids were visualized by spraying silica plates with $MnCl_2$ reagent, followed by heating (Jork *et al.*, 1989). Mass spectra were recorded on a Varian MAT 311 at 70eV by direct inlet. ^{1}H-NMR, NOE, ^{13}C-NMR and HMQC-spectra were recorded on a Bruker AC 400 (400 MHz respectively 100.6 MHz). All triterpenoids were identified by comparison of the ^{1}H-NMR data with those of authentic compounds or with literature data. MS-fragmentation patterns, especially Retro-Diels-Alder reactions, were also helpful for structure elucidation (cf. Budzikiewicz *et al.*, 1963).

Results and Discussion

In most of the *Kalanchoe* and *Senecio* species analyzed here an epicuticular layer of waxy material is more or less apparent on leaves, on stems, and sometimes also on the bracts of the involucra. In *Senecio kleinia* Less. the wax is conspicuous on the succulent stems. In *Kalanchoe thyrsiflora* Harv. and particularly in *K. pumila* Baker the wax production is so rich that it forms a fragile chalky layer on the leaf surfaces. Long-chain saturated hydrocarbons were long known as components of this lipophilic material (Hegnauer, 1964), but the major wax constituents are obviously triterpenes. Those that we have identified are presented in Table IV and the results are briefly discussed in the following.



	R
1	=O
2	OH
3	OAc

Fig. 1. Structural formulae of glutinone (**1**), glutin (**2**) and glutin acetate (**3**).

The leaf wax of *Kalanchoe fedtschenkoi* Hamet et Perr. de la Bâthie exhibits several terpenoid spots on TLC plates, with one dominating. The relevant product was isolated as colourless crystals. NMR studies showed it to be a mixture of ca 80% glut-5-en-3-β-ol (D:B-friedolean-5-en-3β-ol) with 20% friedelin. PMR signals of the former product are identical with those previously reported for this compound (Matsugana *et al.*, 1988). Friedelin is identified by comparison of its CNMR spectrum with that of authentic friedelin. Both triterpenes have been found earlier, along with taraxerol, in flowers of *Kalanchoe spathulata* (Gaind *et al.*, 1976), but their presence in the leaf wax had not been considered so far.

The wax of *Kalanchoe gastonis-bonnieri* Hamet et Perr. de la Bâthie exhibits one major terpenoid spot on TLC which is due to β-amyrenone, as the ^{1}H-NMR spectrum readily confirms. The same is true for *Kalanchoe thyrsiflora* Harv.

From the leaf wax of *Kalanchoe marnieriana* Jacobs. we isolated a mixture of two triterpenes, namely 90% of glutin (**2**) and 10% of friedelin.

Kalanchoe miniata. Hilsenb. et Boj. also exhibits a series of terpenoids, two of which have been crystallized. They were identified as β-amyrine acetate and glutinone (**1**).

From *Kalanchoe pumila* again a nonpolar triterpene was obtained in crystalline form. This was shown to be β-amyrenone. α-Amyrin, β-amyrin and sitosterol have been found as constituents of *Kalanchoe pinnata* long ago (Gaind and Gupta, 1972), but they were isolated from "pulverized leaves", so also in this case their external localization had not been noticed.

Table I. ^{1}H-NMR-data of glutinone (**1**), glutine (**2**) and glutinacetate (**3**) ($CDCl_3$, δ in ppm).

Proton	1	*J* [Hz]	2	*J* [Hz]	3	*J* [Hz]
1	1.84	dddd (14; 4; 4; 4)	*		*	
1′	1.60	m	*		*	
2	2.38	ddd (15; 4; 4)	*		*	
2′	2.46	ddd (15; 10; 5)	*		*	
3	–		3.46	dd (3; 3)	4.69	dd (3; 3)
6	5.69	m	5.64	m	5.56	m
7	1.97	m (2H)	*		*	
8	1.66	dd (12; 6)	*		*	
10	2.23	m	*		*	
18	1.59	m	*		*	
19	0.92	dd (13; 2)	*		*	
23	1.21	s	1.14	s	1.07	s
24	1.22	s	1.05	s	1.05	s
25	0.80	s	0.86	s	0.85	s
26	1.09	s	1.09	s	1.10	s
27	1.02	s	1.01	s	1.01	s
28	1.16	s	1.17	s	1.17	s
29	0.94	s	0.95	s	0.96	s
30	0.97	s	0.99	s	0.99	s
OAc	–	–	–	–	2.02	s

* Overlapping signals.

Table II. Results of NOE-experiments on glutinone (**1**).

Irradiation in proton	NOE
6	23
7	25, 26
8	27
23	6
24	10
25	26, 1, 7
26	28, 25, 18, 7
27	8
28	18, 26
30	28

Table III. ^{13}C-NMR of glutinone (**1**), glutine (**2**) and glutinacetate (**3**) ($CDCl_3$; δ in ppm, $CDCl_3$ = 77.0 ppm).

Carbon	**1**	**2**	**3**	
1	21.6	18.2	18.9	t
2	38.1	27.8	25.5	t
3	215.5	76.3	78.6	s/d
4	50.0	40.8	39.1	s
5	142.4	141.6	141.9	d
6	121.3	122.0	120.0	t
7	23.6	23.6	23.5	t
8	47.0	47.4	47.3	d
9	35.0	34.8	34.8	s
10	50.6	49.6	49.8	d
11	35.9	36.0	36.0	t
12	30.3	30.3	30.4	t
13	39.3	39.3	39.3	s
14	37.8	37.8	37.8	s
15	31.9	32.1	31.9	t
16	35.1	35.1	35.1	t
17	28.2	28.2	28.2	s
18	43.1	43.0	43.1	d
19	38.9	38.9	38.9	t
20	30.1	30.3	30.1	s
21	34.1	33.1	33.1	t
22	35.9	34.6	34.6	t
23	28.5	28.9	29.1	q
24	24.3	25.4	25.0	q
25	15.6	16.2	16.0	q
26	19.3	19.6	19.5	q
27	18.4	18.4	18.4	q
28	32.4	32.4	32.4	q
29	34.5	34.5	34.5	q
30	32.0	32.0	32.0	q
Ac	–	–	21.2	q
	–	–	170.9	s

The leaf wax composition of *Senecio cuneatus* Schultz Bip. and *Senecio ficoides* Schultz Bip. appeared identical. From various fractions we obtained three major crystalline materials, identified as friedelin, a mixture of ca 70% of glutin with 30% of taraxerol, and a mixture of ca 70% of taraxerol with 30% of taraxasterol. Taraxerol occurs rather rarely in Compositae, while taraxasterol is abundant in this family, but mostly known as a latex constituent (Hegnauer, 1964).

The wax layer of *Senecio kleinia* Less. exhibits a series of terpenoids. We obtained four crystalline products which were identified as glutinyl acetate (**3**), friedelin, 3-epilupeol, and a mixture of some 60% of lupeone with 40% of friedelin.

Table IV. Major triterpenoids in the leaf wax of *Kalanchoe-* and *Senecio*-species.

	β-Amyrenone	β-Amyrinacetate	3-Epilupeol	Friedelin	Glut-5-en-3β-ol	Glutin (**2**)	Glutinacetate (**3**)	Glutinone (**1**)	Lupeone	Taraxerol	Taraxasterol
Kal. fedtschenkoi				×	×						
Kal. gastonis-bonnieri	×										
Kal. marnieriana				×		×					
Kal. miniata		×						×			
Kal. pumila	×										
Kal. thyrsiflora	×										
Sen. cuneata				×		×				×	×
Sen. ficoides				×		×				×	×
Sen. kleinia			×	×			×		×		

Although glut-5-en-3β-ol, the corresponding acetate and the ketone glut-5-en-3-one are already known from different plant species, no assigned NMR-data have been reported so far. The ^{1}H-NMR data of glutinone (Table I) could partly be assigned by double resonance and NOE experiments (Table II). The conformation of glutinone in solution (determined by NOE) is the same as in solid state (determined by X-ray; Ohki *et al.*, 1981). The assignment of ^{13}C-NMNR data (Table III) was done by 2D hetero correlated technics (HMQC) and comparison with those of friedelin derivatives (Patra and Chaudhuri, 1987; Prakasch *et al.*, 1987).

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