Tetra- and Pentacyclic Triterpenoids from Epicuticular Wax of *Euphorbia cyparissias* L., Euphorbiaceae

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The epicuticular wax of *Euphorbia cyparissias* contains pentacyclic triterpenoids (34%) and primary alcohols (31%) as major components. The major triterpenoids are triterpenols (23%) consisting of α- and β-amyrin, glutinol, 24-methylenecycloartenol and ψ-taraxasterol. The triterpenones (10.5%) are composed of taraxerone, α- and β-amyrinone, lupenone, glutinone, ψ-taraxasterone, β-fernenone and three further unidentified triterpenes. α-Amyrin was found esterified with homologous series of fatty acids. Minor components are alkanes, wax esters, aldehydes and free fatty acids.

Introduction

Triterpenoids apparently are the major constituents of many *Euphorbia* latices [1–5]. These latex triterpenoids have been investigated extensively, because they proved to be useful as a tool in the taxonomic grouping of the species concerned.

Another reason may be the suggestion that the latices provide a renewable source for the production of liquid fuels and other chemical materials [6–9]. Less attention has been given to the triterpenoids accumulated in the epicuticular waxes of the plants. Recent wax studies, carried out on several *Euphorbiaceae* have demonstrated that these waxes also contain a great number of pentacyclic triterpenoids, partly in considerable amounts [10–14]. With regard to the triterpenoids we have continued our studies on epicuticular waxes from *Euphorbiaceae*. The wax triterpenoids from *Euphorbia cyparissias* have now been analyzed.

E. cyparissias L. (sect. esula) is a glabrous perennial weed up to 50 cm in height, usually unbranched at the base but with up to 16 axillary non-flowering branches and 0–7 axillary rays. It grows in most of Europa except the extreme north and the extreme south, but only as an alien in most of the north [15].

Materials and Methods

E. cyparissias plants were grown in the field of the Botanical Institute, University of Cologne under normal agronomic conditions. Annual shoots were harvested in autumn and extracted immediately twice by short time immersion in distilled chloroform. Care was taken that no latex contaminates the extract. The wax extracts were combined, filtered, evaporated to dryness, weighed and subsequently redissolved in a small volume of distilled *n*-hexane with slight warming.

Wax was fractionated by CC on silica gel 60, 0.063–0.2 mm (Merck, Darmstadt). Elution was carried out with pentane, 2-chloropropane and methanol [10, 11, 13, 14]. Component classes were separated by LPLC on silica gel 60. 0.04–0.063 mm (Merck, Darmstadt) using toluene as eluent for triterpenol esters, wax esters and aldehydes and dichloromethane for triterpenones, triterpenols, primary alcohols and fatty acids [13, 14]. The flow rate was 1.5 ml/min and 5 ml aliquots were collected. Fractions were examined by TLC, GC, GC-MS and chemical reactions (ethanolysis, acetylation, deacetylation, reduction, oxidation) as before [10, 13, 14].

Abbreviations: CC, column chromatography; GC, gas chromatography; GC-MS, gas chromatography-mass spectrometry; LPLC, low pressure liquid chromatography; TLC, thin-layer chromatography; RP, reversed phase.

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TLC and RP 18 TLC: Silica gel 60 precoated plates (Merck, Darmstadt); solvent systems: toluene (Rf_1), $AgNO_3$ impregnated plates CH_2Cl_2/EtOAc 24:1 (Rf_2) [10]; RP 18 plates acetonitrile/acetone 65:35 (triterpenones Rf_3, triterpenols Rf_4) [14]; spray reagents used: bromothymol-blue (Rf_1, Rf_2), carbazole (Rf_1, Rf_3, Rf_4) [13, 14, 16].

GC: Hewlett Packard 5710, equipped with FID and integrator 3380 S; fused silica capillary column 10 m OV-1 CB; temp. program: 180 °C–280 °C, 4 °C/min, 2 min isotherm at 180 °C; nitrogen carrier gas 1.0 bar.

GC-MS: Finnigan-MAT 4510, 70 eV, EI; fused silica capillary column 15 m DB-1.

Authentic samples of triterpenoids were obtained from various sources: α-amyrin, β-amyrin, lupeol, lupenone (Roth, Karlsruhe); α-fernenol, simiarenol (Dr. A. P. Tulloch, Prairie Regional Laboratory, Saskatoon, Canada); taraxasterol acetate (Dr. P. G. Waterman, Phytochemistry Research Laboratories, University of Strathclyde, England). 24-Methylenecycloartenol acetate (Prof. Dr. G. Willuhn, University of Düsseldorf, Germany).

Results and Discussion

The composition of *E. cyparissias* epicuticular wax is shown in Table I. Triterpenoids (34%) were the largest group of components. They mainly appeared as triterpenols (23%) whereas the corresponding ketones were present in smaller amounts (10.5%) and only 0.5% of the wax consisted of triterpenol esters. Primary alcohols (31%, corrected value) constituted another prominent component class. The other lipid components were alkanes, wax esters, aldehydes and fatty acids. Yields and compositions were in good agreement with those previously observed [17, 18]. As has been noted before, triterpenoids and primary alcohols are the principal components of *Euphorbia* waxes [10–14]. In *E. peplus* they amounted to 45% of the crude wax [13], *E. nicaeensis* wax contained 50% [13], *E. dendroides* 54% [10], *E. lathyris* 60% [14], *E. characias* 69% [13] and *E. aphylla* even contained 73% [11]. On the contrary to these results in *E. esula,* however, triterpenoids are reported as minor wax constituents [19]. The triterpenoids in *E. cyparissias* wax are found to be: α-amyrin, α-amyrinone and α-amyrin esters, β-amyrin, β-amyrinone, glutinol and glutinone, 24-methylenecycloartenol, ψ-taraxasterol and -sterone, lupenone and β-fernenone (Table II).

α- and β-amyrin, as well as their ketones, were identified by RP 18 TLC, GC and GC-MS analysis as before (Table II) [13, 14]. From both alcohols only α-amyrin was found to be esterified with fatty acids of different chain length. Amyrins and their derivatives are the principal triterpenes in all *Euphorbia* waxes examined [10–14] but their proportions between the species varied considerably. β-Amyrin, a major triterpenol in *E. dendroides* [10], *E. aphylla* [11] and *E. lathyris* [14], was a minor component in *E. characias, E. nicaeensis, E. peplus* [13] and *E. cy-*

Table I. Composition and yield of *E. cyparissias* epicuticular leaf wax.

Component Class	mg	% Wax	% dry wt	Carbon No.	Rf_1*
n-Alkanes	223	5.3	0.11	$^{H}C_{19}-C_{37}$	0.71
Wax esters	332	7.9	0.17	$^{H}C_{36}-C_{52}$	0.65
Aldehydes	309	7.4	0.15	$^{H}C_{24}-C_{36}$	0.42
pr. Alcohols	1287	30.8	0.65	$^{H}C_{24}-C_{28}$	0.06
Fatty acids	53	1.3	0.03	$^{H}C_{12}-C_{28}$	0.01
Triterpenol esters	19	0.5	0.01	$^{H}C_{48}-C_{52}$	0.67
Triterpenones	442	10.5	0.22	$^{E}C_{30}H_{48}O$	0.20
Triterpenols	973	23.3	0.49	$^{E}C_{30}H_{50}O$	0.06
Unidentified	119	2.8	0.06		
Lost on column	433	10.2	0.21		
Yield	4190		2.10		

* Except glutinol; R_f value see Table II.
[H] Homologous series.
[E] Single components.

Table II. Composition and characterization of triterpenoids from *E. cyparissias* epicuticular wax.

Component	TLC Rf_1	Rf_2	Rf_3	Rf_4	Ca[1]	GC rrt[2]	MS M^+	Σ[3]
Triterpenols esterified								
α-Amyrin	0.06	0.30	0.44		+	1.041	426	
Triterpenones								
Taraxerone	0.20	0.49	0.71		+	0.954	424	2
β-Amyrinone	0.20	0.52	0.70		+	0.979	424	5
α-Amyrinone	0.20	0.52	0.66		+	1.024	424	16
Lupenone	0.20	0.40	0.81		+	1.024	424	10
Glutinone	0.20	0.52	0.53		+	1.061	424	20
ψ-Taraxasterone	0.20	0.52	0.66		+	1.091	424	2
not identified	0.20	–	–		÷	1.099	424	2
β-Fernenone	0.20	0.52	0.53		+	1.142	424	3
not identified	0.20	–	–		÷	1.185	424	40
								100
not identified	0.17	0.37	0.37		+	1.173	426	
Triterpenols								
β-Amyrin	0.06	0.30		0.46	+	1.000	426	1
α-Amyrin	0.06	0.30		0.44	+	1.041	426	6
Glutinol	0.13	0.03		0.58	+	1.071	426	86
24-Methylene-cycloartenol	0.06	0.30		–	+	1.129	440	6
ψ-Taraxasterol	0.06	0.30		0.43	+	1.171	426	1
								100

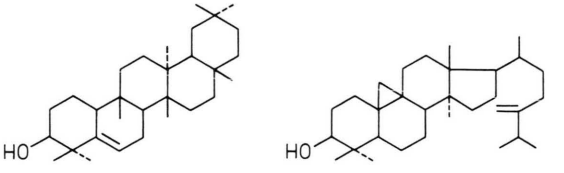
[1] Carbazole colour reaction.
[2] Relative retention time (10 m OV 1 CB, β-amyrin 1.000).
[3] Relative concentration.

parissias. The ketone and esters were found in all species except *E. nicaeensis* (no ketone), *E. characias* and *E. cyparissias* (no esters). α-Amyrin, its corresponding ketone and esters were present in *E. cyparissias* and *E. aphylla* but were completely absent in *E. dendroides, E. lathyris* and *E. peplus*, whereas *E. characias* and *E. nicaeensis* wax only contained the free and esterified alcohol. In *E. aphylla* additionally α- and β-amyrin acetate was found [11].

Lupeol and lupenone are also frequent wax triterpenes. Thus lupene derivatives (alcohols, ketones, acetates, esters) were present in the species examined, particularly in waxes of *E. dendroides* [10], *E. characias* [13] and *E. aphylla* [11]. In *E. cyparissias*, however, only the ketone could be detected (characteristic values see Table II).

β-Fernenone was the only fernene type triterpene. It was a minor constituent of the triterpenones but has been readily identified by TLC, GC and GC-MS analyses (Table II) [14]. This was also present in the waxes of *E. lathyris* and *E. peplus* as free and esterified material [13, 14].

The principal triterpenoids of *E. cyparissias* wax were glutinol and glutinone with 86% and 20% (relative values) respectively (Table II). Their R_f-values were comparable to those of simiarenol and simiarenone, identified in *E. lathyris* wax [14]. Glutinol is a Δ5 unsaturated pentacyclic triterpenol consisting of five cyclohexane rings (Fig. 1). Simiarenol consists of four cyclohexane rings with a double bond in Δ5, too, and one cyclopentane ring with an isopropyl group at C_{21}. The two components have very similar mass spectra but ion *m/z* 231 is



Glutinol 24-Methylene-cycloartenol

Fig. 1. Molecular structures of glutinol and 24-methylene-cycloartenol.

present only in the spectrum of simiarenol, due to the much easier loss of its isopropyl group (C_3H_7) from ion *m/z* 274 [20, 21]. Important fragments in the mass spectrum of glutinol are *m/z* (rel. int.): 426 (M^+), 408 (M^+-18), 274 (65), 259 (50), 245 (5), 205 (30), 191 (10), 173 (20), 163 (15), 152 (40), 134 (90). The ketone showed the same fragmentation pattern with the molecular ion *m/z* 424. Glutinol but no glutinone has been found previously in waxes of panicoid grasses [22]. However, the two were found only in *E. cyparissias* of the Euphorbia species so far studied.

Beside these major components a small amount of 24-methylenecycloartenol, a tetracyclic triterpene containing a 9,19-cyclofunction (see Fig. 1), was observed. Its R_f- and *rrt* values (see Table II) gave no significant indication to the molecular structure but the mass spectrum showed the characteristic fragmentation of this compound with the ions *m/z* (rel. int.): 440 (M^+ $C_{31}H_{52}O$), 422 (3), 425 (3), 407 (4), 379 (3), 353 (2), 315 (1), 300 (5), 175 (20) [23]. The mass spectrum obtained from 24-methylenecycloartenol acetate (authentic sample) exhibited the same important fragment peaks of similar intensities. In *Euphorbia* surface waxes 24-methylenecycloartenol was also found in *E. esula* [19]. Concerning *Euphorbiaceae,* tetracyclic triterpenes and especially cycloartane derivatives are mainly described as latex constituents [2, 24–29]. Furthermore, a great number of cycloartane triterpenoids and allied compounds have been reported to be fern constituents [30]. Taraxerone is a less frequent component in *Euphorbia* waxes. It previously has only been found in *E. lathyris* [14]. But in this species a very small amount of the corresponding alcohol was also detected. It was identified by its characteristic R_f and *rrt* values (Table II) and in particular by the fragmentation pattern during MS analysis {*m/z* (rel. int.): 424 (M^+), 409 (1), 300 (30), 285 (15), 272/257 (8), 218/175 (10), 204 (70), 189 (20), 161 (15)} [14, 31, 32]. Concerning other plant families taraxerenes are described in waxes of *Syagrus coronata* (free and acetylated) [33] and in extracts of whole leaves and stem bark in the *Apocynaceae* and *Myricaceae* [34, 35]. Taraxerone is also a major constituent in waxes of several *Dudleya* species [36].

Beside the triterpenes mentioned above a not clearly identified alcohol and ketone were observed. The TLC and GC data indicated a taraxasterene structure, probably ψ-taraxasterol and -sterone (Table II). Their mass spectra contained the same important fragment ions with comparable intensities reported for ψ-taraxasterenes [22, 32, 37]. The most abundant fragments were *m/z* (rel. int.) 426 (M^+, ketone 424), 218 (10), alcohol 207 (45), ketone 205 (20), 189 (100). Three further triterpenes, partly in considerable amounts were present but remained unidentified (Table II).

Thus, surface wax composition from *Euphorbia cyparissias* shows a very species specific triterpenoid pattern whereas the composition of the common lipid components resembles that obtained by analysis of other members of this genera [10–14]. The presence of the amyrins as well as of lupenone indicate once more that these triterpenoids are widespread among *Euphorbia* species; they have now been found in all the surface waxes examined but with considerably different quantities. Principal triterpenoids of this wax are glutinol and glutinone beside a further triterpenone which remained unidentified. Although the alcohol 24-methylenecycloartenol may be a common component of latices from *Euphorbia* species [24–29] it only occurred in *E. cyparissias* and *E. esula* [19] epicuticular waxes. Taraxerone, ψ-taraxasterol and -sterone previously have been found in *E. lathyris* [14]. In extracts of the whole plant of *E. cyparissias* the presence of β-amyrin, glutinol, 24-methylenecycloartanol and euphol has been reported [2]. Therefore it appears that the two last-mentioned compounds may be limited to the latex. The results of this and other analyses of *Euphorbia* waxes give a strong indication that each species is provided with a very specific triterpenoid composition.

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