Pentacyclic Triterpenoids in Epicuticular Waxes from *Euphorbia lathyris* L., Euphorbiaceae

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The chemical composition of the leaf surface wax of *Euphorbia lathyris* L. was analysed using TLC, GC, GC-MS and NMR. A predominance of pentacyclic triterpenoids and primary alcohols was observed. They together constituted 60% of the total wax. Seven triterpenols: taraxerol, β-amyrin, lupeol, isomotiol, α-fernenol, simiarenol, ψ-taraxasterol and eight triterpenones: taraxerone, β-amyrinone, lupenone, isomotione, α- and β-fernenone, simiarenone and filicanone were isolated. Among them, β-amyrin and lupeol were found esterified with homologous series of fatty acids. The minor part of wax was formed by long chained and predominantly saturated alkanes, wax esters, aldehydes and free fatty acids.

Introduction

Euphorbia lathyris L. (sect. lathyris), a member of the spurge family (Euphorbiaceae), is a glabrous, glaucous, biennial plant up to 150 cm in height with numerous axillary shoots [1]. Probably native only in the eastern and central mediterranean regions, it spread from there throughout South, West and Central Europe and later has been introduced into North, Central and South America. Because of the former universal and officinal use *E. lathyris* is an old companion plant of man since ancient times [2]. At the present time this species shows a wide distribution as a ruderal plant and weed of cultivated ground.

During recent years it has attracted renewed attention with regard to its economic utilization [3–6]. This interest is based on the high content of industrially utilizable seed oil, as well as the hydrocarbon containing latex which provides a potential, renewable source for the production of liquid fuels and other chemical materials [2, 4–7]. Hence *E. lathyris* may play an important role as an energy-plant in the future.

Polycyclic triterpenes are another no less important group of chemical constituents present in the latex of *E. lathyris* in considerable quantities [8–10]. These substances are not only rich in energy but have also various biological and officinal properties [11, 12]. Our previous analyses of *E. lathyris* dealt with the constitution of the epicuticular waxes [13, 14]. They showed that the surface wax contains a noteworthy high content of pentacyclic triterpenoids beside the common wax lipids. In continuation our studies we have now investigated these triterpenoid compounds in detail. Furthermore in the present work we report on a rapid and efficient method for the separation and quantitative isolation of triterpenols and triterpenones.

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

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Materials and Methods

Plant material

E. lathyris plants were grown in the field of the Botanical Institute, University of Cologne under normal agronomic conditions. Annual shoots were

harvested in autumn. *E. lathyris* belongs to the latex producing plants. The injury of turgescent shoots led to a high extrusion of latex. To prevent wax contamination the latex was immediately absorbed with cellulose.

Wax extraction

Shoots were extracted twice by a short immersion in distilled chloroform. The wax extracts were combined, filtered, evaporated to dryness, weighed and subsequently redissolved in a small volume of distilled *n*-hexane with slight warming.

Fractionation

CC: The hexane solution of the crude wax (3630 g) was deposited on a column (5 × 40 cm) packed with silica gel 60, 0.063–0.2 mm (Merck, Darmstadt). Elution was carried out with solvents of increasing polarity as described previously [13, 15, 16]: pentane to eluate hydrocarbons; 2-chloropropane (2-CP) for esters and aldehydes; methanol (MeOH) for primary alcohols, triterpenones, triterpenols and free fatty acids (Fig. 1). The column was finally eluted with chloroform. Before use each solvent was distilled. The fractionation was veryfied on TLC-plates precoated with silica gel 60 (Merck, Darmstadt).

LPLC: Single compound classes were further separated by LPLC (Fig. 1). The columns were densely packed with silica gel 60, 0.04–0.063 mm (Merck, Darmstadt). Toluene (column 18 × 300 mm) was used for the 2-CP fraction of the initial CC and CH_2Cl_2 (column 18 × 1600 mm) for the MeOH fraction, respectively. After loading the columns were connected with a low pressure solvent pump (Duramat D80). In both cases the flow rate was 1.5 ml/min and 5 ml aliquots were collected. The fractions were immediately checked after collection by TLC (silica gel 60, Merck, Darmstadt; solvent toluene). Aliquots with the same R_f values were combined accordingly.

RP 18-LPLC: The triterpenol- and triterpenone mixtures, as obtained through LPLC, were subsequently separated into their individual components by RP 18-LPLC (see Fig. 1). The column used was a 24 × 250 mm glass column with teflon caps perforated by a stainless steel capillary. It was densely packed with dry silica gel RP 18, 0.063 mm (preparated by the method of Glatz [17]), closed, connected with the solvent pump mentioned above, degassed with MeOH and finally equilibrated with the solvent used for the following chromatography. For each further elution only the column was reequilibrated. The sample was evaporated to dryness, redissolved in 2 ml of tetrahydrofuran and injected with a glass syringe. Mobile phases were MeOH for triterpenols and acetonitrile for triterpenones (flow rate 5 ml/min). Each solvent was degassed separately by applying a mild vacuum for 15 min. Detection was performed at 212 nm using a 80 µl quartz flow cell.

Identification

Compound classes and individual components were characterized and identified by chemical reactions (ethanolysis, acetylation, deacetylation, reduction, oxidation), TLC, GC, GC-MS and NMR as described [15, 16].

Oxidation: Triterpenols were oxidized with pyridinium-chlorochromate (PCC) [18]. Dry samples were dissolved in CH_2Cl_2, PCC was added and the solution stirred for 1 h at room temp. After removing of the solvent, the reaction product was redissolved in *n*-hexane, filtered and the filtrate concentrated. The reaction was verified by TLC, GC and GC-MS.

TLC: The qualitative detection of triterpenoids was carried out on silica gel 60 plates (Merck, Darmstadt) using the following solvent systems: 1. toluene (R_{f1}); 2. more polar compounds CH_2Cl_2-EtOAc (24:1) (R_{f2}); 3. $AgNO_3$ impregnated plates, CH_2Cl_2-EtOAc (24:1) (R_{f3}). Spray reagents employed: bromothymolblue ($R_{f1} - R_{f3}$), carbazole as selective indication of triterpenoids (R_{f1}, R_{f2}) [19].

RP 18 TLC: The TLC separation of individual triterpenols and triterpenones gave good results on TLC-plates precoated with RP 18 (Merck, Darmstadt). The TLC was performed following a modified form of passage-chromatography as described previously [20, 21] using the solvent system acetonitrile-acetone (65:35) and β-amyrine as internal standard. The procedure was exactly terminated after 4 h for triterpenones (R_{f4}), after 6 h for triterpenols (R_{f5}), respectively. In both cases the compounds were visualized by spraying with carbazole (0.8% w/v in ethanol-conc. sulfuric acid (5:1).

GC: Hewlett Packard 5710, equipped with FID and integrator 3380 S; fused silica capillary column 10 m OV-1 CB; temp. program: 180–280 °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.

NMR: Bruker WM 400 ($CDCl_3$), the chemical shifts (δ) are given in ppm.

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

Results

The epicuticular wax from *E. lathyris* contained a large number of pentacyclic triterpenoids which amounted to 32% of the total wax (Table I). Among the free triterpenoids, triterpenols were predominant (17%) followed by triterpenones (13%). Triterpenol esters (2%), however, were present in much lower concentration. Similar high amounts of triterpenoids were also observed in the surface waxes of other *Euphorbia* species [16, 22, 23]. Primary alcohols constituted the second important class present in notable amounts of 28%. The value reported in an earlier investigation [13] had to be corrected since the analytical method used for the quantitative determination of primary alcohols has improved considerably. Beside primary alcohols, other long chain and predominantly saturated lipid components were hydrocarbons, wax esters, aldehydes and free fatty acids (Table I). With small deviations, yields and compositions corresponded to those reported previously [13, 14].

Isolating procedure

After CC of the crude wax, the methanol fraction was subsequently separated by LPLC into component classes and single components the R_f values of which showed unequivocal differences (Fig. 1, Table I and II). Thus triterpenols and primary alcohols, which are quite difficult to separate from one another, were separated from the compounds mentioned above but eluted together. Different HPLC methods have been applied for the quantitative separation of triterpenoids [21, 24, 25]. However the low column capacities often makes these methods difficult to apply. During this investigation we found that RP 18 LPLC proved to be a very effective tool for the quantitative isolation of primary and triterpenoic alcohols as well as of individual triterpenols and triterpenones. With methanol as eluent the primary alcohols and 6 triterpenols were isolated. Subsequently, a partial separation of the triterpenone mixture was achieved with acetonitrile (see Fig. 1). This technique is not suitable for quantitative determinations of triterpenoid mixtures.

The qualitative separation within a class of triterpenoids has usually been achieved by argentation TLC which discriminates different compounds by number and position of their double bonds. Thus, α- and β-amyrin could not be separated by this method as they have identical positions of the double bond.

Table I. Composition, yield and R_f values of the leaf epicuticular wax components from *Euphorbia lathyris*.

Component class	mg	% Wax	% dry wt.	Carbon No.	R_{f1}*	R_{f2}*
n-Alkanes	320	8.8	0.05	$^{H}C_{19}-C_{37}$	0.71	–
Wax esters	420	11.6	0.06	$^{H}C_{18}-C_{52}$	0.65	–
Aldehydes	150	4.1	0.03	$^{H}C_{24}-C_{36}$	0.42	0.79
pr. Alcohols	1006	27.7	0.16	$^{H}C_{24}-C_{23}$	0.06	0.30
Fatty acids	30	0.8	<0.01	$^{H}C_{12}-C_{28}$	0.01	0.15
Triterpenol esters	80	2.2	0.01	$^{H}C_{18}-C_{52}$	0.67	–
Triterpenones	460	12.7	0.07	$^{S}C_{30}H_{48}O$	0.20	0.55
Triterpenols	624	17.2	0.09	$^{S}C_{30}H_{50}O$	0.06	0.30
Unidentified	140	3.8	0.02			
Lost on column	400	11.1	0.06			
Yield	3630		0.55			

*, Except simiarenol and filicanone; R_f values see Table II; ^{H}, homologous series; ^{S}, single components.

Table II. Characterization of triterpenes from epicuticular wax of *Euphorbia lathyris*.

Component	TLC						GC	MS	Σ%
	R_{f1}	R_{f2}	R_{f3}	R_{f4}	R_{f5}	Ca*	rrt[@]	M^+	
Triterpenols esterified									
β-Amyrin	0.06	0.30	0.30		0.46	+	1.000	426	50
Lupeol	0.06	0.30	0.19		0.60	+	1.043	426	50
									100
Triterpenones									
Taraxerone	0.20	0.55	0.49	0.71		+	0.954	424	22
β-Amyrinone	0.20	0.55	0.52	0.70		+	0.979	424	+
Lupenone	0.20	0.55	0.40	0.81		+	1.024	424	+
Isomotione	0.20	0.55	0.52	0.53		+	1.053	424	1
α-Fernenone	0.20	0.55	0.52	0.53		+	1.088	424	8
Simiarenone	0.20	0.55	0.52	0.53		+	1.128	424	1
β-Fernenone	0.20	0.55	0.52	0.53		+	1.142	424	1
Not identified	0.20	0.55	0.52	0.53		+	1.185	424	+
Filicanone	0.17	0.52	0.49	0.58		−	1.223	426	6
Triterpenols									
Taraxerol	0.06	0.30	0.28		0.46	+	0.962	426	1
β-Amyrin	0.06	0.30	0.30		0.46	+	1.000	426	22
Lupeol	0.06	0.30	0.19		0.60	+	1.043	426	16
Isomotiol	0.06	0.30	0.30		0.34	+	1.063	426	1
α-Fernenol	0.06	0.30	0.30		0.34	+	1.095	426	10
Simiarenol	0.13	0.41	0.03		0.52	+	1.147	426	11
ψ-Taraxasterol	0.06	0.30	0.30		0.43	+	1.171	426	+
									100

*, Carbazole colour reaction; [@], relative retention time (10 m OV 1 CB, β-amyrin = 1.000).

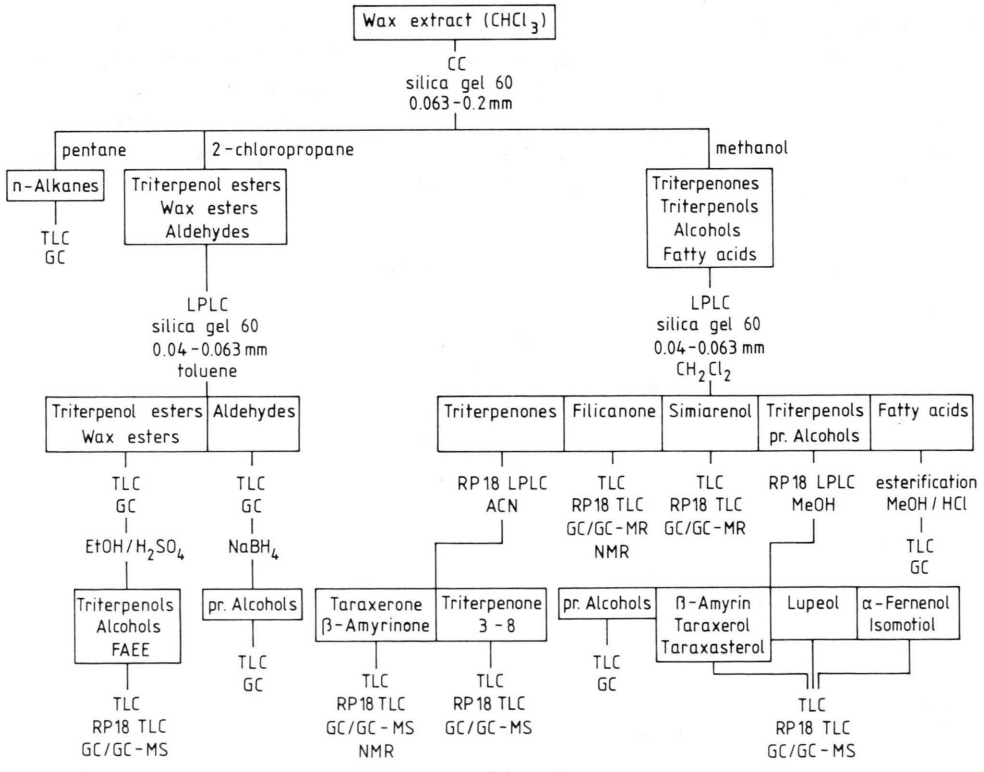

Fig. 1. Diagram for fractionation, separation and identification of epicuticular wax from *Euphorbia lathyris*.

Their separation was only possible by GC. Using capillary columns, GC is a very powerful technique for the separation and identification of triterpenoids [11, 26, 27]. However, the GC method used in this investigation failed to distinguish α-amyrin and lupeol. The direct comparison of these three compounds was possible on RP 18 TLC plates with acetonitrile/acetone 65:35 as solvent and allowed a comprehensive characterization of the triterpenoids by TLC (Table II). Fig. 1 shows the whole isolating procedure and Table II summarizes the composition and individual values of the isolated triterpenols and triterpenones.

Compounds identified

β-Amyrin (22%) was a major constituent of the triterpenol mixture of *E. lathyris* wax. It belongs to the Δ12 oleanene type and is one of the most common naturally occurring pentacyclic triterpenes [28, 29] (Fig. 2). In *Euphorbia* surface waxes it has been found in all the species examined [16, 22, 23, 30]. Amyrins are readily recognizable by mass spectrometry, since the molecular ion undergoes a retro-Diels-Alder reaction resulting in the prominent fragment ion *m/z* 218 [22, 31, 32] (Table III). α-Amyrin, frequently present in surface waxes of *Euphorbiaceae* [22, 23, 30], however, was not detected in this species.

β-Amyrinone was only present in traces. The MS spectra of both generally show the same fragmentation pattern with peaks of similar intensity differing in the molecular ion (*m/z* 424) due to the two-mass unit difference in their molecular weights. In *Euphorbia* epicuticular waxes β-amyrinone has also been found in *E. dendroides* [16], *E. characias* [22, 30], *E. peplus* [22] and *E. aphylla* [23]. But in all these waxes this ketone was also a minor constituent and in *E. nicaeensis* was completely absent [22]. β-Amyrin but no β-amyrinone has been found in surface waxes of *E. esula* [33].

Lupeol and lupenone are further common triterpenoid constituents of epicuticular waxes. Thus, lupene derivatives (alcohols, ketones, acetates, es-

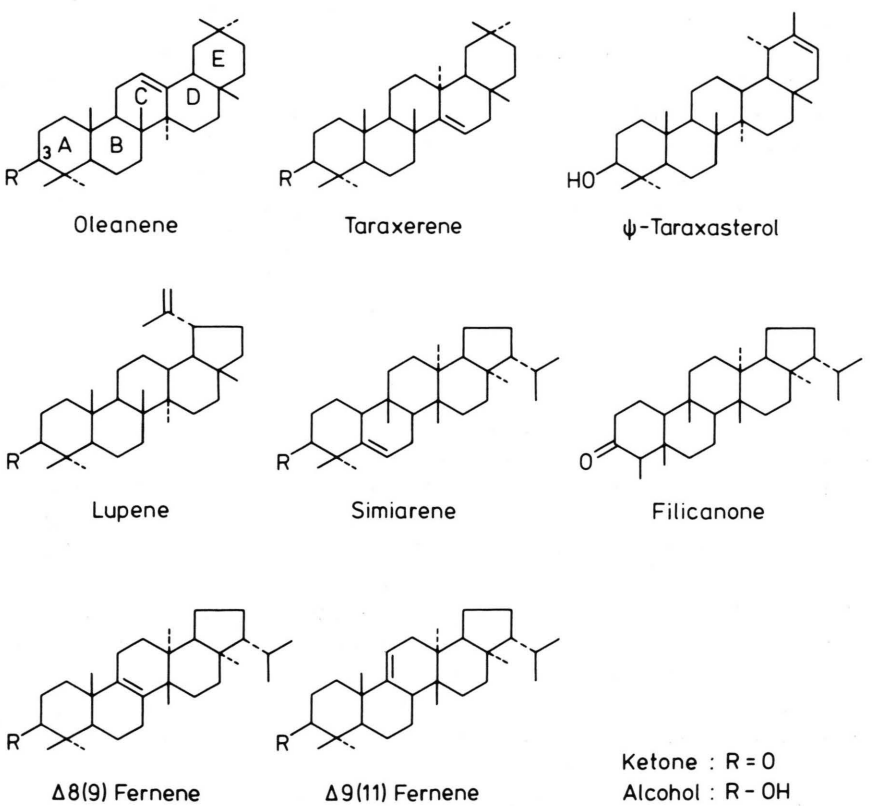

Fig. 2. Structure of triterpenoids from epicuticular wax of *Euphorbia lathyris*.

Table III. Mass fragmentation patterns of triterpenes isolated from epicuticular wax of *Euphorbia lathyris**.

Component	Characteristic *m/z* (relative intensity)
Taraxerol	426 (1.5), 411 (0.5), 302 (10), 287 (8), 269 (5), 218 (10), 204 (65), 189 (15), 175 (8), 163 (10), 147 (15), 135 (45), 121 (40), 107 (45)
β-Amyrin	426 (2), 411 (1), 257 (1), 243 (0.5), 218 (100), 203 (40), 189 (15)
Lupeol	426 (3), 411 (0.5), 315 (1.5), 257 (2), 218 (20), 207 (30), 189 (35)
Isomotiol	426 (1.5), 411 (3.5), 393 (1), 273 (2), 259 (25), 247 (3), 241 (10)
α-Fernenol	426 (0.5), 411 (1), 273 (0.5), 259 (40), 255 (5), 247 (5), 241 (18), 229/205 (3), 191 (8), 173 (5), 163/147 (10), 137 (35), 119 (40)
Simiarenol	426 (1), 408/393/286 (0.5), 274 (50), 259 (40), 245 (5), 231 (15), 205/204/189 (10), 175/161 (15), 152 (60), 134 (100)
Taraxerone	424 (4), 409 (1.5), 300 (25), 285 (15), 272/257 (5), 218/175 (10), 204 (70), 189 (20), 161 (15), 149 (23), 133 (70), 121 (40)
β-Amyrinone	424 (3), 409 (1), 257 (1), 243 (0.5), 218 (100), 203 (22), 189 (18)
Lupenone	424 (0.6), 409/257 (0.3), 313 (1), 218 (5), 205 (20), 189 (10)
Isomotione	424 (5), 409 (10), 271 (10), 257 (95), 245 (10), 205 (8), 187/173 (5), 159/147 (10), 137 (50)
α-Fernenone	424 (5), 409 (10), 271 (10), 257 (55), 245 (10), 205/191 (8), 173 (5), 163 (15), 147 (12), 137 (45)
Simiarenone	424 (1), 409 (0.5), 274 (60), 259 (40), 245 (5), 231/189 (15), 175/163 (12), 151 (25), 137 (40)
Filicanone	426 (2.5), 411 (2), 341 (5), 286 (0.5), 273 (2), 257 (1), 231/219 (5), 205 (10), 191 (40), 179 (30), 164/149 (15), 135 (20)

* First peak is M^+.

ters) were found in all *Euphorbia* waxes, particularly in waxes of *E. dendroides* [16], *E. characias* [22, 30] and *E. aphylla* [23], where they are the principal triterpenes. In wax of *E. lathyris* lupeol accounted for 16% of the free triterpenes whereas lupenone was only present in traces. The two compounds were identified by TLC, GC and GC-MS analyses (Table II and III).

Taraxerol and taraxerone

The proportions of taraxerol and taraxerone were exactly the opposite of those for the amyrins (Table II). Taraxerenes are Δ14 unsaturated pentacyclic triterpenoids (Fig. 2). For these molecules a similar retro-Diels-Alder reaction of ring D is described as has been mentioned above for ring C of the Δ12 unsaturated derivatives [31, 32]. According to this process the mass spectrum of taraxerone shows three important peaks *m/z* 300, 285 (300-Me) and 204 which are the most characteristic fragments of this molecule [31] (Table III). Only the latter was also found in the spectrum of taraxerol. Instead of the ions at *m/z* 300 and 285, in this spectrum peaks of similar intensity appeared at *m/z* 302 and 287. The ^{13}C NMR spectrum confirmed the presence of this molecular structure. On the basis of the whole data taraxerol and taraxerone were unambigiously identified (Table II and III). Taraxerenes have previously been found in epicuticular waxes from leaves of the ouricuri palm *Syagrus coronata* (free and acetylated) [34] and in extracts of whole leaves and stem bark in the Apocynaceae and Myricaceae [35, 36]. Taraxerone is also a major constituent in waxes of several *Dudleya* species [37]. However, it only occurs in *E. lathyris* the Euphorbiaceae so far analyzed.

Simiarenol and simiarenone

Simiarenol (11% of the free triterpenes) is a Δ5 unsaturated pentacyclic triterpenol first isolated from whole leaves of *Rhododendron simiarum* [38, 39] (Fig. 2). Significant identification data were afforded by TLC and GC-MS analysis (Table II). The mass spectrum contained the molecular ion *m/z* 426 ($C_{30}H_{50}O$) and the diagnostic ions *m/z* 274 (50%), 259 (40%) and 231 (15%) for simiarenol (Table III). This was confirmed by previous data [38, 39]. The spectrum of the corresponding ketone (1%) showed a molecular ion *m/z* 424 ($C_{30}H_{48}O$) and exhibited the same three major peaks observed for the alcohol. Simiarenol was a major triterpenol in *E. peplus* [22]

and also present in *E. aphylla* [23] whereas simiarenone was absent in both species. Although simiarenol is a more unusual wax constituent it has frequently been found as a minor compound in several panicoid and eragrostoid grasses [40–42].

Fernenes

Two pentacyclic triterpenoid alcohols and three ketones of the fernene type were detected in *E. lathyris* surface wax. Although the sublimation with RP 18 LPLC yielded no individual fernene components, comparative TLC and, in particular, GC and GC-MS analyses indicated their presence. On the basis of these data and comparison with previously published data α-fernenol (fern-9(11)-ene) and isomotiol (fern-8(9)-ene), as well as their corresponding ketones and additionally β-fernenone were identified (Fig. 2). The three ketones had identical R_f values in all TLC systems used but they could be easily differentiated on GC (Table II). The two alcohols also showed the same R_f values and were distinguishable only by GC. Characteristic MS fragments of Δ8 and Δ9(11) unsaturated pentacyclic triterpenols are reported to be *m/z* 259 and 241, the former being the base peak or most prominent peak, accompanied by *m/z* 273, 255 and 247 of lesser intensity [43–45]. Since mass spectra of Δ9(11) arborenes have also been described to contain these typical fragments [27, 46, 47] the identification of single compounds is extremely aggravated. α- and β-fernenone have indistinguishable mass spectra which are characterized by a molecular peak at *m/z* 424 ($C_{30}H_{48}O$) and strong peaks at *m/z* 257 (55%), 271/245 (10%) and 163 (15%). Isomotione shows the same M^+ at 424 and peaks at *m/z* 271 and 245 (10%) but here the ion at *m/z* 257 is much more prominent. Instead of *m/z* 163 fragments at *m/z* 159 (10%) and 147 (10%) were present. The peaks of the corresponding alcohols were two mass units higher (see Table III). The key evidence in the identification of the three fernenones was provided by the relative retention times in GC analysis. The order of emergence from an OV 1 or comparable stationary GC phase is generally similar: β-amyrin – isoarborinol – α-amyrin – lupeol – α-fernenol – simiarenol – β-fernenol [40, 48]. The rrt from the corresponding ketones is slightly shorter but the order of emergence is the same. The rrt of the fernenes from *E. lathyris* are listed in Table II. The oxidation product of an authentic sample of α-fernenol had identical TLC, GC and GC-MS data as the isolated α-fernenone. Traces of another triterpenone (rrt 1.185) were visible in the ketone mixture but remained unidentified. Pentacyclic alcohols and ketones of the fernene type have been found mainly in epicuticular waxes and roots of Gramineae [27, 40, 44] but were also detected in the stem bark of Ulmaceae [46, 47]. Among the Euphorbiaceae free and esterified α-fernenol appeared in epicuticular wax of *E. peplus* [22].

Filicanone

During LPLC on silica gel 60 (0.04–0.063 mm) an unusual compound was isolated. On the basis of chemical, chromatographic and spectroscopic evidence it was identified as a triterpene consisting of a pentacyclic saturated ring skeleton with a keto group in position C_3, filican-3-one. It accounted for 6% of the free triterpenes. The R_f values, rrt and the MS fragmentation were distinctly different from any other pentacyclic triterpenones previously isolated from *Euphorbia* species (Table II). Moreover, there was no reddish brown carbazole reaction. The mass spectrum of filicanone contained the molecular ion *m/z* 426 for $C_{30}H_{50}O$, confirming the saturated ketone (Table III). Two significant ions *m/z* 191 (40%) and 179 (25%) were found at lower mass. The molecular formula was finally elucidated by NMR analysis. The ^{13}C NMR spectrum exhibited signals of 6 singlets with one singlet at δ 212.7 (C-3), 6 doublets, 10 triplets and 8 quaternary carbons establishing the structure of the saturated pentacyclic ketone. The characteristic low-field peak at δ 6.8 indicates there is only one methyl group at C-4 [49] in contrast to the other triterpenoids examined which possessed geminal methyl substitutions at C-4. The 2D- ^1H spectrum and the nOe's of the methyl groups allowed an unambiguous identification of the structure of ring A with a keto function at C-3, and two methyl groups at C-4 and C-5, the former being equatorial and the latter axial. The ^{13}C chemical shifts of ring A were 41.3 t (C-1), 41.6 t (C-2), 212.7 s (C-3), 60.0 d (C-4), 42.2 s (C-5), 35.7 t (C-6), 6.8 q (C-4-Me) and 22.0 q (C-5-Me). The other ^{13}C shifts and ^1H data indicated the molecule contained an isopropyl group, probably in the E ring, and four additional methyl groups. Filicanone and closely related compounds have been prepared previously from filicene, found in several ferns [50, 51] but it has not been described as natural epicuticular wax constituent.

ψ-Taraxasterol

In addition to the above alcohols a triterpenol with rrt 1.171 was detected. It was not clearly identified but the TLC, GC and GC-MS data indicated a taraxasterene structure (Fig. 2), probably ψ-taraxasterol. Besides a positive carbazole reaction it also had identical R_f values in all TLC systems applied with an authentic sample of taraxasterol, obtained from taraxasterol acetate (Table II). The rrt of the GC analysis was also close to that of taraxasterol. The mass spectrum of the isolated compound and that of authentic taraxasterol contained the same important peaks but with slightly different fragmentation patterns. The most abundant fragments reported for ψ-taraxasterol are *m/z* 189 and 207, the former being the base peak [32, 41, 52]. The isolated component was not pure and occurred only in traces, thus NMR analysis was not possible.

Triterpenol esters

As with other Euphorbia waxes *E. lathyris* contained triterpenol esters (2%) as minor components accompanied by wax esters. They showed the same carbazole reaction as with free triterpenes. Ethanolysis gave alcohols consisting of triterpenols and alkanols and fatty acid ethyl esters. The analysis of the combined triterpenols was carried out in the same manner as described for the free triterpenes. The triterpenol esters consisted exclusively of β-amyrin and lupeol esterified with fatty acids of different chain lengths.

Discussion

As indicated earlier, waxes from Euphorbiaceae consist of the two biochemically different compound classes, the common lipids and triterpenoids [16, 22, 23, 30]. Analyses of *E. dendroides* [16, 30], *E. aphylla* [23], *E. characias* [22, 30], *E. nicaeensis* [22], *E. peplus* [22] and *E. lathyris* presented here have shown that triterpenoids and primary alcohols are the major components (45–73%) of these waxes. On the other hand, however, triterpenoids are less prominent in waxes of several *E. esula* biotypes [33]. Whereas the quantitative and qualitative composition of the common lipids alkanes, wax esters, aldehydes, primary alcohols and fatty acids is generally the same within the species examined, their triterpenoid content differs drastically. In *E. lathyris* wax the situation is complicated by the presence of an extraordinary manifold of triterpenoid compounds. Seven triterpenols (free and esterified) and eight triterpenones were identified. There is as yet no indication of a comparable complex triterpenoid composition in surface waxes of other Euphorbiaceae. To distinguish these triterpenoids, the use of a combination of chromatographic and spectroscopic techniques is necessary. With the application of various stationary phases, solvent systems and spray reagents, TLC proved to be an important method for the characterization of triterpenoids or triterpenoid mixtures. LPLC was an effective and quicker tool for the complete or at least partial quantitative separation of individual triterpenols and triterpenones. For qualitative separation and quantitation GC is exceedingly capable whereas for identification and structure elucidation GC-MS and NMR are important methods.

The most common wax triterpenoids β-amyrin and lupeol, found in all Euphorbia waxes examined, were also present in *E. lathyris*, as well as their esters and corresponding ketones. Wax of *E. aphylla* also contained the acetates of both [23]. α-Amyrin, usually a frequent wax triterpenoid [22, 30], was absent in *E. lathyris*. In contrast taraxerol as well as taraxerone were only present in *E. lathyris* wax. Thus, these components occur in a number of other plant parts including surface waxes [34–37]. Among the fifteen free triterpenoids identified five fernenes were found, namely α-fernenone, β-fernenone, isomotione, α-fernenol and isomotiol but no β-fernenol. Although fernenols and their derivatives are widespread in waxes [27, 40] and in whole plants of the Gramineae [53, 54] and in Pteridophyta [44, 50, 51, 54] the corresponding ketones are rare. In waxes of Euphorbiaceae fernenols, as well as fernenones seemed to be less common constituents as they only appeared in *E. lathyris*, although α-fernenol is found in *E. peplus* [22]. The distribution of simiarenol and simiarenone is comparable to that of the fernenes. Simiarenol has been described in Gramineae [27, 40, 42, 53] and was previously observed in *E. peplus* [22] and *E. aphylla* [23] waxes. Only *E. lathyris* wax contained simiarenone. The unusual compound, filicanone, was first mentioned as a derivative obtained from filicene which was found as a fern constituent [50, 51]. It is the first time that this saturated pentacyclic ketone has been identified in epicuticular waxes. Although a range of free triterpenols was present only β-amyrin and lupeol appeared to be esterified with fatty acids of different chain length.

Thus, the present study of *E. lathyris* wax compared with other species of the same genera [16, 22, 23, 33] agrees with the conclusion that their triterpenoid patterns show very distinctive variations. There are also appreciable differences regarding the latex triterpenoids. In the latex of *E. lathyris* euphol, various sterols and compounds belonging to the 9,19-cycloartene group predominate [9, 55], together with a number of triterpenols esterified with conjugated fatty acids [10]. Both results confirm the assumption that the latex system and the wax system behave as metabolically independent systems in the plant.

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