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EPICUTICULAR WAXES FROM LEAVES OF FIVE *EUPHORBIA* SPECIES

H. HEMMERS and P.-G. GÜLZ

Botanisches Institut der Universität zu Köln, Gyrhofstr.15, D-5000 Köln 41, West Germany

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Key Word Index—*Euphorbia characias*; *E. cyparissias*; *E. lathyris*; *E. niccaensis*; *E. peplus*; Euphorbiaceae; epicuticular waxes; alkanes; esters; aldehydes; alcohols; acids.

Abstract—The yield of the chloroform extracted waxes from leaves of five *Euphorbia* species grown in Europe ranged from 0.6 to 2.1% per dry weight. TLC and GC analysis showed a general similarity of wax composition. The waxes always consisted of hydrocarbons (4–17%), wax esters (8–18%), aldehydes (2–6%), free fatty acids (1–8%) and free primary alcohols (13–39%). The major carbon chain lengths ranged from C_{19} to C_{37} in hydrocarbons, C_{36} to C_{52} in wax esters, C_{24} to C_{36} in aldehydes, C_{12} to C_{30} in acids and C_{24} to C_{28} in alcohols. Saturated compounds were dominant. Differences observed were in the wax yield and in the quantitative composition of the individual wax components. Triterpenols, free and esterified, were also present in all waxes often in very high concentrations.

INTRODUCTION

The genus *Euphorbia* consists of more than 1600 species growing in nearly all types of climates throughout the world [1]. They can be found as herbs, shrubs and trees of very different appearance and all of them produce a characteristic milky-white chemically rich latex [2–8]. In the search for new plants with a high potential for the production of chemicals and liquid fuels as alternative energy sources, several *Euphorbia* species were previously examined for their economic utilization [9–13].

In our study we were interested in the amounts and compositions of epicuticular waxes from *Euphorbia characias*, *E. cyparissias*, *E. lathyris*, *E. niccaensis* and *E. peplus*. These species grow as annual or biennial herbs or perennial shrubs which are widespread in Europe. Previous papers reported latex components from whole plants of *E. characias* [14], *E. cyparissias* [15], *E. lathyris* [16–18] and *E. peplus* [19]. However only *E. peplus* has been studied previously in regard to epicuticular wax components [20].

RESULTS AND DISCUSSION

Yields of waxes ranged from 0.69 to 2.05% of the dry weight (Table 1). These amounts are similar to those reported earlier [20–23]. Only *E. cyparissias* with 2.05% showed a relatively high value in comparison with other *Euphorbia* species. In the surface waxes of all *Euphorbia* species we found hydrocarbons, wax esters, aldehydes, free primary alcohols, free fatty acids and free and esterified triterpenols. Yield and composition of the individual wax fractions differed quantitatively between the species. Alcohols and triterpenols were always the major (> 64%) wax components. The composition of triterpenols will be reported later. Alcohols were often found to be the predominant wax component in the crude wax of mono- and dicotyledons [24]. The remaining components vary between 1 and 18%. Similar results were reported from *Euphorbia esula* [23].

Hydrocarbons

Hydrocarbons were present in amounts of 3–17% of the waxes and consisted of homologous *n*-alkanes with chain lengths ranging from C_{19} to C_{37} (Table 2). The main components were always nonacosane, hentriacontane (45–63%) and tritriacontane. Similar results were reported from other *Euphorbia* species [20, 23–25]. In *Euphorbia niccaensis* the C_{27}, C_{29} and C_{31} *n*-alkanes predominated, especially nonacosane (45%); this profile seems to be a species-specific one.

Table 1. Composition and yield of epicuticular waxes from leaves of five *Euphorbia* species

Components	*Euphorbia* *niccaensis*	*cyparissias*	*characias*	*lathyris*	*peplus*
Hydrocarbons	4	6	17	10	8
Wax esters	8	12	14	13	18
Aldehydes	6	4	3	3	2
Free fatty acids	5	2	1	2	8
Free primary alcohols	39	15	15	13	36
Triterpenols and unidentified	38	61	50	59	28
Yield (% dry wt)	0.69	2.05	1.07	0.61	1.34

Table 2. Composition of hydrocarbons from epicuticular waxes of five *Euphorbia* species (peak area per cent)

Carbon no.	*Euphorbia* *niccaensis*	*cyparissias*	*characias*	*lathyris*	*peplus*
19	+	+	+	+	+
21	+	+	+	+	0.3
23	3.4	0.3	0.1	0.1	0.4
25	6.2	1.6	0.8	0.4	1.5
27	23.7	8.7	3.1	2.4	8.2
29	45.2	28.1	20.7	6.5	17.6
31	12.3	45.3	62.8	59.8	55.5
33	3.9	10.5	8.3	23.4	9.7
35	0.7	1.8	0.6	1.5	0.8
37	+	+	+	+	+
Σ even carbon no.	4.6	3.7	3.6	5.9	6.0

+, Minor components less than 0.1%.

Table 3. Composition of wax esters from epicuticular waxes of five *Euphorbia* species (peak area per cent)

Carbon no.	*Euphorbia* *niccaensis*	*cyparissias*	*characias*	*lathyris*	*peplus*
36	0.3	+	0.4	+	2.4
38	0.6	+	2.6	0.2	16.3
40	1.4	2.4	4.1	2.0	18.2
42	7.1	15.6	20.7	17.1	19.2
44	11.5	22.2	13.8	31.7	18.8
46	27.3	38.2	36.8	18.9	15.7
48	44.0	14.3	16.7	25.2	7.6
50	5.3	2.3	2.3	0.4	+
52	+	+	0.1	+	+
Σ odd carbon no.	2.5	5.0	2.5	4.5	1.8

+, Minor components less than 0.1%.

Table 4. Composition of fatty acids and primary alcohols obtained by hydrolysis of wax esters from epicuticular waxes of five *Euphorbia* species (peak area per cent)

	niccaensis		*cyparissias*		*Euphorbia* *characias*		*lathyris*		*peplus*	
Carbon no.	Acids	Alcohols	Acids	Alcohols	Acids	Alcohols	Acids	Alcohols	Acids	Alcohols
16	13.7	+	27.4	+	37.1	+	53.4	—	27.4	3.9
18	11.2	0.7	19.2	+	10.1	0.8	30.4	—	19.3	19.2
20	29.6	0.4	36.8	+	34.3	1.0	7.2	—	30.7	10.1
22	38.2	0.6	9.5	+	14.3	10.0	6.5	—	18.8	18.9
24	2.9	2.4	1.3	0.8	1.3	7.0	0.4	1.1	1.9	3.5
26	0.9	78.2	0.9	75.01	0.3	73.5	0.2	77.5	1.1	41.4
28	1.2	17.1	2.0	22.7	0.3	6.5	0.3	18.7	0.8	3.0
30	0.5	—	2.2	+	0.5	+	1.1	+	+	—
32	—	—	0.3	—	+	—	+	—	—	—
Σ unsaturated C_{16}, C_{18}, C_{20}	0.3	—	+	—	1.5	—	0.3	—	+	—
Σ odd carbon no.	1.5	0.6	0.4	1.4	0.3	1.0	0.2	2.7	+	+

+, Minor components less than 0.1%.

Table 5. Composition of free fatty acids, aldehydes and free primary alcohols from epicuticular waxes of five *Euphorbia* species (peak area per cent)

	niccaensis			*cyparissias*			*Euphorbia characias*			*lathyris*			*peplus*		
Carbon no.	Acids	Aldehydes	Alcohols	Acids	Aldehydes	Alcohols	Acids	Aldehydes	Alcohols	Acids	Aldehydes	Alcohols	Acids	Aldehydes	Alcohols
12	—	—	—	0.6	—	—	—	—	—	1.1	—	—	—	—	—
14	1.0	—	—	4.5	—	—	2.6	—	—	2.7	—	—	1.7	—	—
16	12.5	—	—	31.0	—	—	32.5	—	—	51.8	—	—	15.6	—	+
18	7.3	—	—	11.7	—	—	19.5	—	—	21.3	—	—	6.0	—	0.8
20	12.1	—	—	8.3	—	—	14.4	—	—	4.4	—	—	22.6	1.3	0.7
22	26.1	—	—	11.2	—	—	10.4			5.1	—	—	29.1	1.3	7.0
24	10.6	0.3	2.3	6.9	+	1.4	4.5	1.9	4.2	2.4	0.2	1.8	12.5	1.4	2.6
26	6.5	11.7	81.9	4.3	5.9	93.2	5.1	34.7	92.9	5.4	19.6	91.2	2.4	10.6	81.3
28	12.7	72.4	15.4	8.5	68.2	5.4	3.0	28.8	2.9	+	74.6	6.9	5.5	72.2	6.7
30	6.7	14.5	—	—	18.2	—	—	25.2	—	—	4.2	—	—	9.7	—
32	—	+	—	—	6.0	—	—	7.4	—	—	+	—	—	+	—
34	—	+	—	—	+	—	—	+	—	—	+	—	—	—	—
36		—			+			+		—	+	—	—	—	—
$\sum$ unsaturated C_{16},C_{18},C_{20} acids	1.3			12.2			6.5			5.8			4.1		
$\sum$ odd carbon No	3.2	1.1	0.4	0.8	1.7	+	1.5	2.0	+	+	1.4	+	0.5	3.5	0.9

+, Minor components less than 0.1 %.

Wax esters

Wax esters were present in amounts of 8–18%. Most esters (95%) were of even numbered chain lengths ranging from C_{36} to C_{52} (Table 3). From C_{42} to C_{48} all even chain lengths occurred as major components. Resulting wax ester patterns were thus species-specific. *Euphorbia peplus* showed additionally large amounts of short chain esters ranging from C_{38} to C_{42}. In the GC-chromatograms we observed peaks with higher retention times than those of C_{48} esters. These however did not belong to the homologous series of wax esters. They indicated the presence of triterpenol esters [22, 26], which was confirmed by saponification. Ethanolysis of the wax esters yielded fatty acids ranging from C_{16} to C_{32} (Table 4) but only the fatty acids ranging from C_{16} to C_{22} were present in high concentrations. As with the esters, no uniformly dominant component could be found among these acids. Each species showed a characteristic distribution pattern. Chain lengths of even carbon numbers were predominant and unsaturated components appeared only in very small amounts.

In contrast, combined alcohols had a smaller carbon range containing only one main (>70%) component, hexacosanol (see Table 4). Only *E. peplus* contained short chain alcohols similar to those observed previously in the wax esters. The wax esters in all five species are based on fatty acids which differ from species to species. This is in contrast to that of the alcohols which are much more uniform. Similar results were reported from wax esters isolated from *Cistus* epicuticular waxes [27, 28].

Aldehydes

Aldehydes ranging from C_{26} to C_{30} in chain length were found in all species, the yield varying between 2 and 6% of the wax (Table 5). Four species have octacosanal as the main component. These results are similar to those reported for *E. esula* [23]. By contrast, *E. characias* contains equal amounts of hexacosanal, octacosanal and triacontanal. Small percentages of short chain aldehydes could be observed in *E. peplus*.

Alcohols

Table 5 shows the composition of free alcohols (13–39%) and free acids (1–8%). Alcohols were found in concentrations up to 39% of the wax yield. The chain lengths range from C_{24} to C_{28}. Hexacosanol appeared in all waxes > 81% of total alcohol fraction. These alcohol patterns were very similar to those found previously for combined alcohols. Again *E. peplus* was the only species that possessed small percentages of short chain alcohols. While the alcohols consist primarily of hexacosanol, the aldehydes are based on octacosanal. The differences in chain length suggest that the alcohols and aldehydes arise from different pathways [29], rather than that the alcohols are formed directly from the reduction of the aldehydes [30].

Fatty acids

Free fatty acids were found only in low concentrations of 1–8% with chain lengths ranging from C_{12} to C_{30} (Table 5). The resulting distribution patterns were species-specific and were similar to those of the acids obtained by saponification of their wax esters. Unsaturated C_{16}, C_{18} and C_{20} acids appeared in low amounts of about 6%. Only *E. cyparissias* showed higher concentrations up to 12%.

EXPERIMENTAL

All *Euphorbia* species were one-year-old plants, grown from seed in the field of the Botanical Institute in Cologne under the same environmental and horticulture conditions.

Plants were harvested in October (635 g *E. niccaensis*, 747 g *E. cyparissias*, 1724 g *E. characias*, 2076 g *E. lathyris* and 305 g *E. peplus*) and extracted by dipping the shoots into $CHCl_3$. The $CHCl_3$ extract was decanted and concd by evaporation. The crude wax was fractionated by CC on silica gel. One g crude wax was deposited on the column (3 × 25 cm), packed with Merck 60 silica gel. This column was successively eluted with 1000 ml *n*-pentane for hydrocarbons, with 1500 ml 2-chloropropane for esters and aldehydes, and 1500 ml MeOH for free alcohols and fatty acids [31, 32]. Yields and compositions are summarized in Table 1.

The ester aldehyde mixture was analysed by GC before separation by prep. TLC on silica gel plates into the two classes of components. The solvent was C_6H_6. Wax esters were then subjected to acid ethanolysis with 5% H_2SO_4 in EtOH for 12hr under reflux. Saponification products were separated by TLC, with C_6H_6 as eluant. After separation, the alcohols were acetylated (Ac_2O–pyridine) and each fraction analysed by GC. Aldehydes were identified by reduction with $NaBH_4$ in dioxane–H_2O (4:1) for 1.5 hr at room temp.

Fractions containing free acids and alcohols were generally refluxed with 5% H_2SO_4 in MeOH. Subsequently esters and alkanols were separated by CC and TLC as described above. Triterpenols were usually isolated together with alkanols and analysed by TLC and carbazole as spray reagent [33]. All substances, except triterpenols, were identified by GC comparison with authentic compounds. For GC analysis 25 m glass-capillary column coated with silicone OV 101, was used with detection by FID and a 3380s integrator. The temp. program ranged from 180 to 280° for hydrocarbons, aldehydes, alcohols, triterpenols, and for methyl- and ethyl esters. For wax esters the temp. program ranged from 180 to 340°. A 12 m quarz capillary column coated with FFAP, and temp. program ranging from 140 to 220° was used for detection of unsaturated compounds.

REFERENCES

1. Hegnauer, R. (1966) *Chemotaxonomie der Pflanzen*, Vol. 4, p. 103. Birkhäuser, Basel.
2. Marston, A. and Hecker, E. (1983) *Planta Med.* **47**, 141.
3. Fürstenberger, G. and Hecker, E. (1977) *Experientia* **33**, 986.
4. Baas, W. J. (1977) *Planta Med.* **32**, 1.
5. Folsone, G. and Schneider, C. (1985) *Z. Naturforsch.* **40b**, 553.
6. Mahlberg, P. G. and Pleszcynska, J. (1983) in *Numerical Taxonomy* (Felsenstein, J., ed.) p. 500. Springer, Berlin.
7. Busboer, D. D., D'Amour, P., Wilson, S. R. and Mahlberg, P. (1982) *Phytochemistry* **21**, 1115.
8. Wernaar, F. (1976) *Lipids* **12**, 707.
9. Nielsen, P. E., Nishimura, H., Otnos, J. W. and Calvin, M. (1977) *Science* **198**, 942.
10. Nishimura, H., Philip, R. P. and Calvin, M. (1977) *Phytochemistry* **16**, 1048.
11. Calvin, M. and Calvin G. J. (1979) *The Sciences* **4**, 24.

12. Calvin, M. (1983) *Science* **219**, 24.
13. Sternburg, C. and Rodriguez, E. (1982) *Am. J. Botany* **69**, 214.
14. Floris, G., Medda, R. and Rinaldi, A. (1983) *Arch. Biochem. Biophys.* **220**, 623.
15. Ponsinet, G. and Ourisson, G. (1968) *Phytochemistry* **7**, 89.
16. Nielsen, P. E., Nishimura, H., Liang, Y. and Calvin, M. (1979) *Phytochemistry* **18**, 103.
17. Adolf, W. and Hecker, E. (1971) *Experientia* **27**, 1393.
18. Lynn, K. R. and Clevette-Radford, N. A. (1983) *Biochim. Biophys. Acta* **746**, 154.
19. Riz, A. M., Hammouda, F. M., El-Missiry, M. M., Radwan, H. M. and Evans, F. J. (1985) *Phytochemistry* **24**, 1605.
20. Eglinton, G., Gonzales, A. G., Hamilton, R. J. and Raphael, R. A. (1962) *Phytochemistry* **1**, 89.
21. Tulloch, A. P. and Bergter, L. (1981) *Phytochemistry* **20**, 2711.
22. Tulloch, A. P. and Hoffman, L. L. (1982) *Phytochemistry* **21**, 1639.
23. Manners, G. D. and Davis, D. G. (1984) *Phytochemistry* **23**, 1059.
24. Tulloch, A. P. (1976) in *Chemistry and Biochemistry of Natural Waxes* (Kolattukudy, P. E., ed.) p. 235. Elsevier, Amsterdam.
25. Herbin, G. A. and Robins, P. A. (1969) *Phytochemistry* **8**, 1985.
26. Tulloch, A. P. (1984) *Phytochemistry* **23**, 1619.
27. Gülz, P.-G., Latza, E., Köhlen, E. and Schütz, R. (1978) *Z. Pflanzenphysiol.* **89**, 59.
28. Gülz, P.-G. and Dielmann, G. (1980) *Z. Pflanzenphysiol.* **100**, 175.
29. Avato, P., Bianchi, G. and Salamini, F. (1985) *Phytochemistry* **24**, 1995.
30. Kolattukudy, P. E., Croteau, R. and Buckner, J. S. (1976) in *Chemistry and Biochemistry of Natural Waxes* (Kolattukudy, P. E., ed.) p. 289. Elsevier, Amsterdam.
31. Gülz, P.-G. (1982) *Z. Naturforsch.* **37c**, 1053.
32. Gülz, P.-G. (1985) *Proceedings of the 6th International Conference on Jojoba and its Uses* (Wisniak, J. and Zabicky, J., eds) p. 185. Ben-Gurion University of the Negev, Beer-Sheva, Israel.
33. Gosh, P. and Thakur, S. (1982) *J. Chromatogr.* **240**, 215.