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Wax Components of *Asparagus officinalis* L. (Liliaceae)

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Chloroform-extracted epicuticular wax from cladophylls of *Asparagus officinalis* was investigated. Pentane, 2-chloropropane, and methanol fractions were eluted from a silica gel column. These fractions were separated by TLC and GC into individual alkane (31.5%), wax ester (20.1%), ketone (6.4%), aldehyde (13.5%), alcohol (16.4%), and fatty acid (8.5%) components. Catalytic hydrogenation, esterification, reduction, and acetylation were employed for component identification or derivatization for GC injection.

Asparagus officinalis L. has been cultivated since ancient times. According to Pliny, the Romans, as early as 200 B.C., wrote gardening instructions for the cultivation of asparagus and were well aware of quality differences in the spears (Hexamer, 1908). The Greeks, unlike the Romans, did not cultivate asparagus but used the short-branched, more or less prostrate plant parts collected from wild stands (Boswell, 1949). Today, this perennial, dioecious plant is grown in most parts of the world. The commercial lines that are cultivated today in the United States are improved forms of the indigenuous plants originally found along the seacoasts of Europe, North Africa, and Asia. Formerly used also for its diuretic properties and for heart afflictions (Hexamer, 1908), *A. officinalis* is now grown for consumption of its edible spring shoots. In some areas, however, like the mountains of Sabah, Borneo, it is harvested during the entire year.

While much effort was made to identify its gene resources, and to study its sex inheritance mechanisms and expressions, relatively little was done to investigate the phytochemical nature of this species which is considered an important commercial vegetable. Proteins and vitamins A and C contents were determined (USDA, 1981), as well as its steroid β-glycosides (Goryanu et al., 1976). Also determined were its steroid saponins (Goryanu and Kintya, 1976), the bitter principle and structure of furostanol saponin (Kawano et al., 1975), and the fructooligosaccharides (Shiomi et al., 1976). Foliar isozymes (Roux, 1980), as well as asparagusate dehydrogenase (Yanagawa, 1976), and the formation of sulfur-containing acids as flavor components were also investigated (Tressl et al., 1977).

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We report in this paper on the individual components of the epicuticular wax, so important in the plant's natural defense mechanism to invasion by parasitic organisms, in the deposition of agricultural spray chemicals, and in the water economy of plants, especially those grown in semiarid climates.

EXPERIMENTAL SECTION

Fully grown cladophylls (fernlike leaves) were harvested in Aug 1985 at the Agricultural Experiment Station, University of California, Riverside. The soluble cuticular lipids were extracted by dipping the fresh material consecutively into three beakers of $CHCl_3$ (800 mL) for a total of 3 min. The extract was taken to dryness and the raw wax redissolved in 50 mL of warm n-C_5H_{12}. After cooling, the supernatant was fractionated on a Si gel CC (Type 60, Merck, 70–230 mesh). Hydrocarbons were eluted with pentane, esters and aldehydes with 2-chloropropane, and free alcohols and free acids with methanol. Yield and composition of the individual fractions are reported in Table I; fractionation and separation systems, in Figure 1 (Gülz, 1984).

The eluted individual fractions were streaked on TLC plates coated with silica gel 60 (Merck). The solvent system was benzene for hydrocarbons, aldehydes, wax esters, and methyl esters and 2-chloromethane–ethyl acetate (24:1) for fatty acids and alcohols. The detection reagent for both was bromothymol blue. The band extracts were purified and injected into a Hewlett-Packard 5750 gas chromatograph with fid and integrator 3380 S. The columns used were fused silica gel capillary OV-101, 25 m, temperature programmed from 160 to 280 °C with a 4 °C/min advance, and for wax esters a 12-m glass capillary column DUHT OV-101 temperature programmed from 160 °C to a maximum of 340 °C at a 4 °C/min advance was used. The components were characterized by comparing their retention times to those of standards.

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Table I. Qualitative Composition (% of Raw Wax) of Epicuticular Wax Components of *A. officinalis*

no. C atoms	alkanes (31.5)	ketones (6.4)	aldehydes (13.5)	alcohols (16.4)		fatty acids (8.5)		no. C atoms	wax esters (20.1)
				esterified	free	esterified	free		
10						1.5	3.0	35	
11						+		36	
12						0.5	3.0	37	
13						+	0.4	38	+
14						1.0	2.6	39	+
15						+	0.6	40	1.0
16						17.1	26.8	41	0.2
17						+	0.2	42	5.5
18						14.6	13.1	43	0.9
19						+	1.0	44	13.5
20				+		45.5	6.0	45	1.9
21	+[a]			+		+	0.3	46	34.2
22	+		+	0.4	1.0	12.8	6.6	47	2.4
23	0.2		0.1	+	0.8	0.3	0.6	48	25.1
24	0.4		2.3	5.7	5.9	3.6	5.1	49	1.4
10						1.5	3.0	35	
25	1.6	19.1	1.5	1.1	1.0	+	1.8	50	8.7
26	1.3	–	37.8	45.7	51.7	1.2	7.2	51	0.4
27	9.3	36.2	2.6	2.2	2.9	+	2.2	52	3.6
28	4.0	–	34.1	32.8	28.2	0.6	7.8	53	+
29	49.3	31.9	2.7	2.4	1.4	+	1.3	54	1.2
30	3.2	–	9.7	7.7	6.1	+	4.8	55	+
31	29.3	12.8	1.1	+	+	+	+	56	+
32	0.7		5.9	0.4	0.8	+	3.4		
33	0.3		0.1						
34			+						

[a] + = present in minute quantities.

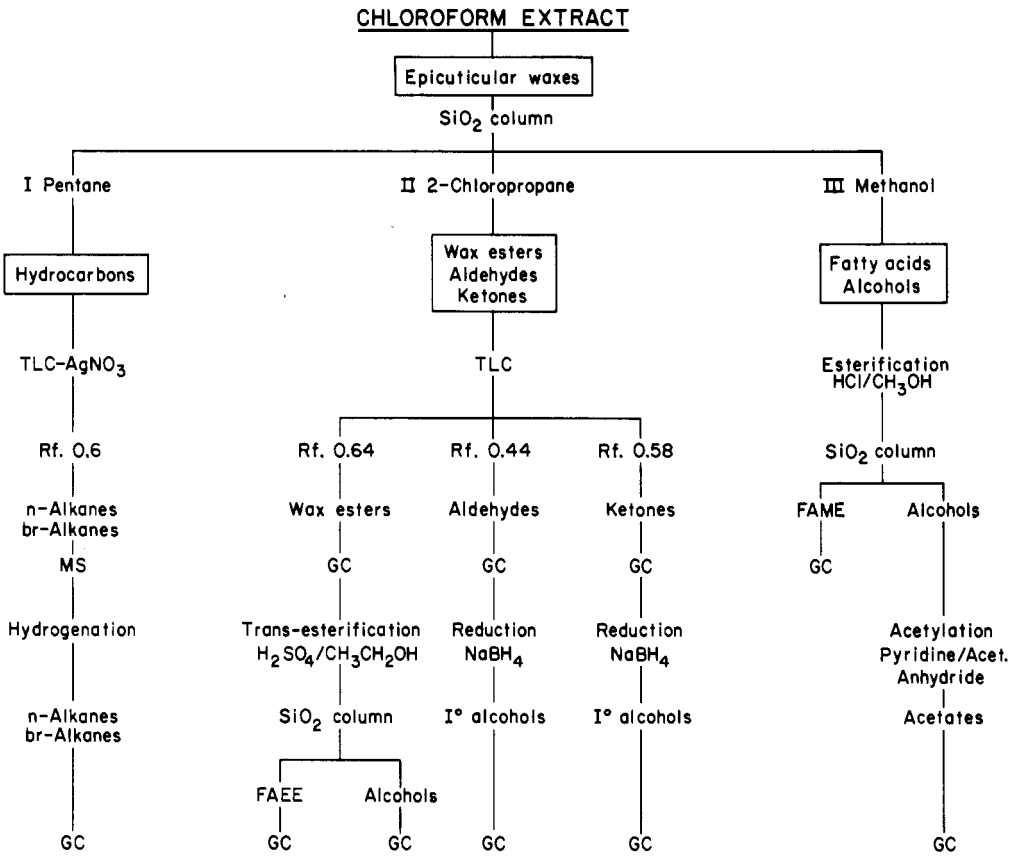

Figure 1. Chloroform extract of epicuticular waxes on an SiO_2 column.

Aldehydes, alcohols, and fatty acids were detected also by GC–MS, Finnigan-Mat 4510, 70 eV, EJ.

Catalytic hydrogenation with platinum oxide was employed to determine whether branched alkanes or alkenes were present (Morrison and Boyd, 1962).

Transesterification of wax esters was accomplished with H_2SO_4 and ethanol. The resulting fatty acid ethyl esters and alcohols are reported in Table I.

Presence of aldehydes was verified by reduction with $NaBH_4$ to primary alcohols and of ketones to secondary alcohols.

Esterification of free fatty acids to fatty acid methyl esters was accomplished with HCl and CH_3OH. Alcohols were converted to their acetates with pyridine and acetic

anhydride.

RESULTS AND DISCUSSION

Significant differences were observed for the amounts and composition of the constituent classes of lipids.

In the pentane fraction of *A. officinalis*, we detected primarily saturated hydrocarbons, the *n*-alkanes. In a series of 13 homologues ranging from C_{21} to C_{33}, the dominant component was nonacosane ($C_{29}H_{60}$) with 49% (Table I). The even-numbered homologues comprised about 10% and the uneven-numbered ones about 90%. On TLC with $AgNO_3$-impregnated plates, the band containing these homologues eluted at R_f 0.6 (Figure 1). Branched alkanes were also detected with C_{30} and C_{32}, yielding 0.3. Catalytic hydrogenation of the hydrocarbon fraction with platinum oxide, in order to see whether these latter ones are alkenes, was negative.

In the 2-chloropropane fraction, aldehydes were present at R_f 0.44, ranging from C_{20} to C_{34} with C_{26} and C_{28} as the dominant amounts at 38% and 34%. Reduction with $NaBH_4$ yielded primary alcohols. The adjacent TLC band (R_f 0.58) consisted of an additional series of homologues. These substances were accompanied by aldehydes and wax esters that could not be separated. TLC R_f values and GC retention times indicate the presence of ketones. In addition, these substances were reducible with $NaBH_4$. Thus, we believe this series to be composed of ketones. The third band of the TLC fraction consisted of wax esters (R_f 0.64). These ranged from C_{38} to C_{56}, with C_{46} yielding the highest percentage of 34.2% and C_{48} with 25.1%.

Transesterification resulted in fatty acid ethyl esters (FAEE) ranging from C_{10} to C_{32}, with C_{20} comprising 45.5%, and also primary alcohols ranging from C_{20} to C_{32}. The largest component of the primary alcohols was C_{26} with 45%, followed by C_{28} with 32.8%.

The third, methanol fraction was clear, thus indicating that no flavanoids were present that would otherwise have tinged the solution yellow. Similar retention times of the free fatty acids and free alcohols and the nonfeasibility to gas chromatograph the fatty acids in their free form necessitated their esterification into methyl esters (FAME). This was accomplished by heating the fraction with 2 N HCl/methanol. These saturated fatty acid methyl esters ranged from C_{10} to C_{32}, with C_{16} yielding the highest amount of 26.8%. Two or three peaks appeared in the FAME reaction between $C_{18:0}$ and $C_{20:0}$. They were unidentified diterpenes. GC–MS spectra showed molecular ions m/z 334 (M^+). After hydrogenation, they could be separated as five peaks by GC, each with m/z 336 (M^+). They comprised about 2% of the injected fraction. There were also small unidentified peaks in front of the higher uneven-numbered FAME. Hydrogenation suggested that they were not unsaturated FAME. They did not constitute a homologuous series.

For further identification, the alcohols from the methanol fraction were acetylated. These ranged from C_{22} to C_{32}, with C_{26} comprising 51.7%. Unidentified diterpenes occurred also in the GC chromatogram before C_{22}.

While even-carbon-numbered constituents predominate in the aldehydes, wax esters, fatty acids, and alcohols, odd-numbered homologues are the main components of the hydrocarbon fraction, and of the ketones. The occurrence of aldehydes as one of the major components, 13.5%, of the epicuticular wax contrasts with the leaf waxes of many plants in which they have been reported as minor constituents (Freeman et al., 1979; Hemmers and Gülz, 1986). Only in sugarcane (Lamberton and Redcliffe, 1959), cranberry fruit (Croteau and Fagerson, 1971), *Brassica* (Baker, 1974), lemons and mandarins (Baker et al., 1975), and sorghum (Bianchi et al., 1978), as well as in the moss *Pogonatum urnigerum* (Haas, 1982), have they been described as major components. All other components fall into the common ranges as reported by the extensive literature on plant waxes (Tulloch, 1976). All epicuticular wax components of *A. officinalis* show homologous series of only saturated compounds with very long chain lengths.

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