Journal of Natural Products
Vol. 46, No. 5, pp. 606-608, Sep-Oct 1983

SEED WAX COMPOSITION OF *ARUNDO DONAX*

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ABSTRACT.—The analysis of *Arundo donax* L. seed wax by means of a simplified analytical procedure is reported. Main components in the wax are hydrocarbons (14.9%; C_{29} and C_{31} are main constituents) and esters (26.7%).

The chemical analysis of plant epicuticular waxes has advanced to the point that the most interesting problems are related to the relationship between chemical composition and the biological function of the epicuticular wax layer (1-3).

To continue our work on Gramineae seed waxes (4,5), we adapted methods that have been applied recently to other lipid classes (3,6), developing a procedure that has enabled us to obtain reproducible results in a simple and efficient way.

RESULTS

The epicuticular seed wax of *Arundo donax* L. was extracted by the usual procedures (4,5), using $CHCl_3$ as solvent. The yield, on the basis of dry seed weight, was 0.2%.

An aliquot of the crude wax extract was separated into component fractions by preparative tlc; the plate was developed in two successive runs: the first with a polar mixture ($CHCl_3$-MeOH, 98:2), was stopped after a 8 cm run, and the second with an apolar solvent mixture (petrol ether-benzene, 80:20). The evenness of the run was confirmed by uv light.

The procedure resulted in a plate showing six main fractions, three fractions present as traces (below 0.2%), and an insoluble residue that remained at the origin and that could not be fractionated further by tlc, either after acetylation or methylation. The fractions so separated were scraped and eluted, (in some cases derivatized), and to each was added a measured amount of a solution of *n*-tetracosane in $CHCl_3$ (100 μg/ml) as internal standard. The FID response factors (Rf) (7) were determined for normal free alkanols (0.94) and normal methyl esters (0.98), using *n*-tetracosane as reference (1.00; Rf for hydrocarbons is 1.00).

The tlc fractions 1-9 had progressively lower Rfs. Fraction 2 was non-hydrolyzable, had a polarity higher than *n*-hydrocarbons (fraction 1) but lower than esters (fraction 3), and is presumed to represent cyclic or unsaturated hydrocarbons. Using a Rf of 1.00 as an approximation, it represents less than 0.3% of the original crude wax. By glc it could be resolved into three components, in the relative proportion of 1:1:14. Fractions 5 and 6, of polarities intermediate between esters and free *n*-alkanols, and Fraction 8, intermediate between free *n*-alkanols and free acids, could not be identified either. Each represented less than 0.2% of the crude wax.

The main fractions were analyzed by glc, and their weight percentages, as determined by this method, are summarized in table 1.

DISCUSSION

The procedure described collects our experience on wax analysis. The two successive runs have the advantage of good separations both for the polar and nonpolar components of the wax in only one plate. The nonmigrating products at the origin are mainly

TABLE 1. Composition of wax fractions from *Arundo donax* L. seed wax (weight % determined by glc).*

No. of carbon atoms	Fraction[a] 1(14.9%)[b] hydrocarbons	3(26.7%) esters acids	Alkanols	4(1.2%) esters[c] acids	7(2.6%) free alkanols	9(3.9%) free acids
14		7		3		
15		4				
16		34		27		19
17		2				
18		22		22		18
19		tr				
20		19		18		6
21						2
22		8	11	13		14
23	2					1
24	tr	4	20	12	4	10
25	9					tr
26	2		17	2	8	10
27	27					tr
28	4		10	2	10	10
29	49					tr
30	1		14	2	23	11
31	7					
32	tr		6			
A			7		11	
B			15		43	

*Figures presented are an average of five different determinations. Standard deviations were: for hydrocarbons 0.69; for esters 3, 1.69; for esters 4, 0.14; for free alkanols, 0.24 and for the free acids, 0.31

[a]All fractions are mixtures of the normal homologous series, except for alcohols A and B (probably terpenic). Quantitation for A and B was done on the basis of a Response Factor similar to other alkanols.

[b]Figures in brackets correspond to % weight of fraction refered to crude wax.

[c]Fraction 4 corresponds to esters, for its yields normal acids on hydrolysis. The alkanols are short-chained, and from the retention time of the intact esters could be calculated (8) to be C_6 or C_7.

extraneous matter that could also be eliminated by previous chromatography through a short silica gel column chromatography. With the method described certain components (such as Fractions 2 and 4) can be observed, that are usually lost when using single-solvent procedures. Sulfuric acid spray, was used as it is the only universal visualizing agent for all wax components.

n-Tetracosane as internal standard has the advantage of a glc retention time that allows its use with all fractions. Being even-numbered, it is found only in minimal percentages as a natural component of the wax, and the exact percentage of indigenous *n*-tetracosane can be calculated from a second glc run of the hydrocarbon fraction using no internal standard.

Arundo donax L. is a cane introduced into Uruguay (9). It belongs to the tribe Arundineae. Previous work (10) on *A. donax* leaf-wax composition described the presence of triacontane and dotriacontane, although this is doubtful from what is known on the biosynthesis of these compounds, and could not be borne out in our related results. Triacontanol, also (10) described in the leaf-wax, was found present both free and combined in the ester fraction of the seed wax.

EXPERIMENTAL

MATERIALS.—Precoated chromatoplates were used for preparative tlc (Merck 5765-20 x 20 cm x 0.25 mm thickness; Kieselgel 60 GF 254). Gas chromatography was performed with a Pye-Unicam 104 and a Shimadzu GC-6AM with dual FID. Columns were OV-17 5% on Chromosorb 80-100 mesh; 1.5 m length, 3 mm id. A program of 200-370° (6°/min) was used.

PREPARATION OF THE CRUDE WAX.—*A. donax* seeds were collected in Montevideo, Uruguay, in June 1979, from wild plants. A voucher sample was kept at the Museo José Arechavaleta (Facultad de Química, Montevideo) as MVFQ No. 3140. Complete seeds (318 g) were extracted with $CHCl_3$ to yield 0.63 g wax (0.2%), using the usual procedure (4,5).

PREPARATIVE TLC.—The chromatoplates were prepared as follows: a free margin of 1 cm; 7.50 mg crude wax dissolved in $CHCl_3$ were spread over 15 cm; a 2-cm free band; 0.50 mg wax in $CHCl_3$ spread over 1 cm; a free margin of 1 cm.

The plate was developed up to 8 cm with $CHCl_3$-MeOH (98:2), the solvent evaporated at room temperature, and the plate re-run up to 16 cm using petroleum ether-benzene (80:20). The second solvent was evaporated, and the plate cut along the 2-cm empty strip. The 3-cm wide side-strip was visualized by charring after H_2SO_4-spray.

Ten fractions could be observed, including the band at the origin. Each fraction was scraped into a test tube. Fractions 1, 2, and 7 were eluted with $CHCl_3$-MeOH (9:1), and gas chromatographed directly after addition of a known volume of internal standard solution. Fractions 3 and 4 were hydrolyzed (NaOH 0.5 N in EtOH), and, after addition of a known volume of internal standard solution, were acidified and extracted with $CHCl_3$. The solvent was evaporated, the residue redisolved in Et_2O, and methylated with diazomethane. Fraction 9 was eluted with Et_2O and methylated with diazomethane. The solvent was evaporated and the residue dissolved in a known volume of internal standard solution and gas chromatographed.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the invaluable support of the International Foundation for Science (Stockholm), which made their work possible. PM and ES thank an equipment grant from the Japanese Embassy in Montevideo that enabled them to complete the research.

LITERATURE CITED

1. P.E. Kolattukudy, *Lipids*, **5**, 259 (1970).
2. W.G. Williams, G.G. Kennedy, R.T. Yamamoto, J.D. Thacker, and J. Bordner, *Science*, **207**, 888 (1980).
3. I. Richter and H. Krain, *Lipids*, **15**, 580 (1980).
4. E. Soler, P. Moyna and G. Ramos, *Lloydia*, **41**, 71 (1978).
5. P. Moyna, G. Ramos, and J. Toth, *Phytochemistry*, **17**, 556 (1978).
6. J.F. Pernes, T. Nurit, and M. De Heaulme, *J. Chromatogr.*, **181**, 254 (1980).
7. W.A. Dietz, *J. Gas Chromatogr.*, **5**, 68 (1967).
8. A.P. Tulloch, *J. Chromatogr. Sci.*, **13**, 403 (1975).
9. B. Rosengurtt, B. Arrillaga de Maffei, and P. Izaguirre de Artucio, "Gramíneas Uruguayas". Univ. República, Montevideo, Uruguay, 1970.
10. R.K. Chaudhuri and S. Ghosal, *Phytochemistry*, **9**, 1895 (1970).

Received 5 May 1982