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WAX ESTERS FROM *LARREA DIVARICATA**

DAVID S. SEIGLER, JANET JAKUPCAK† and TOM J. MABRY

Department of Botany, University of Illinois, Urbana, IL 61801, U.S.A. and
Department of Botany, University of Texas at Austin, TX 78712, U.S.A.

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Abstract—The wax on the stems and leaves of *Larrea divaricata* contains a mixture of even carbon-numbered alkyl esters (C_{48}–C_{56}) consisting of fatty acids (C_{22}–C_{32}) and primary alcohols (C_{24}–C_{30}).

INTRODUCTION

THE GENUS *Larrea* (Zygophyllaceae) is composed of 4 species distributed in the arid and semi-arid areas of North and South America. One of the species, *Larrea divaricata*, has a disjunct distribution with subspecies *tridentata* and *divaricata* occurring in North and South America, respectively.[1,2] Although *Larrea divaricata* is a major plant species of the south western U.S.A. and northern Mexico, there are comparatively few reports of its secondary chemical constituents. Nordihydroguaiauretic acid and at least two flavonoids have been isolated, but the flavonoids were incompletely characterized.[3,4] Recently, a series of eleven flavonoids, mostly kaempferol and quercetin methyl ethers, and nordihydroguaiauretic acid were isolated from *Larrea cuneifolia* from Argentina.[5] Most of the resinous material on the leaves and stems of the plant is comprised of phenolics,[4] but the presence of a waxy substance has also been observed.[3] Waller and Gisvold[3] reported physical properties and the chemical composition of the wax, which is similar to carnauba wax in its properties. These workers also saponified the wax and, on the basis of limited data (primarily m.ps.) concluded it was composed of C_{26} and C_{28} fatty acids and a C_{30} primary alcohol.

In the course of an I.B.P. project involving ecosystems in which *Larrea* species are among the dominant vegetation, we have re-investigated the waxy material from the stems and leaves of *L. divaricata* and report our findings here. Waxes of this type are commonly found in plants and insects, but relatively few have been characterized to date.

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* This is the second in a planned series of papers concerning the natural products chemistry of *Larrea* in connection with an International Biological Program (I.B.P.) Integrated Research Project, entitled "The Origin and Structure of Ecosystems"; see Ref. 5 for Paper I.

† Present address: Department of Biology, University of Missouri Columbia, MO 65201, U.S.A.

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RESULTS AND DISCUSSION

The NMR and IR spectra of the fraction obtained from the petroleum ether extract of *Larrea divaricata* wax esters after recrystallization at 0° showed no absorptions other than those corresponding to alkyl esters consisting of long chain primary alcohols and fatty acids. The following IR absorptions were observed: 2950, 1720, 1450, 1160 and 720 cm^{-1}. The protons in the NMR spectrum were assigned as follows:

$$\underset{(d)}{CH_3}-\underset{(c)}{(CH_2)_x}-\underset{(a)}{CH_2}-O-CO-\underset{(b)}{CH_2}-\underset{(c)}{(CH_2)_y}-\underset{(d)}{CH_3}$$

(a) 4·10, overlapping triplets; (b) 2·30, overlapping triplets; (c) 1·27, broad, intensive peak; (d) 0·95, overlapping distorted triplets.

Analyses of wax ester mixtures using MS by Aasen *et al.*[6] showed that this method tends to give larger percentages of lower MW esters, probably because of their greater volatility. In this instance, however, analysis of *Larrea divaricata* wax by GLC generally confirms the data derived from the MS.

The MS of the fraction showed parent ions at 816·8, 788·7, 760·8, 732·6 and 704·7 corresponding to $C_{56}H_{112}O_2$, $C_{54}H_{108}O_2$, $C_{52}H_{104}O_2$, $C_{50}H_{100}O_2$ and $C_{48}H_{96}O_2$ respectively. The MS of eicosanyl eicosanoate, $Me(CH_2)_{18}-CO_2-(CH_2)_{19}Me$ recorded[7] under similar conditions exhibits a parent ion at m/e 592 and a base peak at m/e 313; the latter ion could be assigned to the acid moiety of the ester, $Me(CH_2)_{18}CO_2H_2^+$. The base peak in the MS of the wax esters from *Larrea divaricata* appeared at 424·5;* this corresponds to a C_{28} acid moiety. Other major fragment ions appeared in the MS at m/e 368·3, 396·5, 452·6 and 480·4 corresponding with C_{24}, C_{26}, C_{30} and C_{32} fatty acids in the esters. The MS data indicated that the acid composition of the original esters was: C_{24}, 13·1%; C_{26}, 18·8%; C_{28}, 45·0%; C_{30}, 16·4%; and C_{32}, 6·6%. Fragments corresponding to the acyl ions of these acids were also observed.

In the MS of tetradecyl palmitate measured under similar conditions a peak derived from the alcohol portion of the molecule by cleavage of the bond between the carbonyl carbon and the remainder of the acyl of group was detected: $Me-(CH_2)_x-CH_2-O-C{\equiv}O^+$. Analogous peaks were observed in the MS for the waxes from *Larrea* at m/e 354·3, 382·3, 410·8, 438·4, and 466·5; these ions correspond to C_{22} to C_{30} (even-numbered) alcohols respectively attached to the carbonyl carbon.

The wax had an R_f value of 0·95 by TLC on silica gel G with Et_2O–hexane (1 : 1); a trace impurity was also observed at R_f 0·6. Co-chromatography of the wax with tetradecyl palmitate on silica gel G with hexane-ether (99 : 1) gave spots of idetical R_f values (0·22).

After saponification of the wax esters and subsequent GLC of the mixture obtained on an OV1 column, four major peaks were observed which corresponded with C_{24}, C_{26}, C_{28} and C_{30} primary alcohols (5, 65, 26·6 and 3·5% respectively). After *trans*-esterification of the wax esters using MeOH and subsequent GLC of the mixture, peaks were observed which corresponded to compounds with even-numbered carbon atoms between C_{20}–C_{30}.

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* The acid moiety ions observed at 10 eV from the *Larrea* waxes appeared to correspond most closely to even-MW fragments in contrast to the m/e 313 reported for eicosanyl eicosanoate at 70 eV.

[6] AASEN, A. J., HOFSTETTER, H. H., IYENGAR, B. T. R. and HOLMAN, R. T. (1971) *Lipids* **6**, 502.

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GLC gave four major peaks with retention times which corresponded with C_{22}, C_{24}, C_{26}, and C_{28} fatty acid methyl esters and primary alcohols, the two classes of compound not being resolved. MS of this mixture showed it to be comprised of the expected methyl esters and alcohols. Peaks were observed at *m/e* 354·2, 382·4, 410·3 and 438·2 corresponding to the four methyl esters noted above as well as other peaks corresponding to the primary alcohols and a C_{20} methyl ester.

From these data, it can be concluded that the wax esters from *Larrea divaricata* are composed of several compounds most of which correspond to those possibilities summarized in Table 1.

TABLE 1. RELATIVE PERCENTAGE COMPOSITION OF *Larrea divaricata* WAX AS CALCULATED FROM GLC AND MS

Wax esters	Acid	Alcohol	*m/e*	Relative percentages GLC	(MS)
C_{46}	C_{22}	C_{24}	676·7	4·8	
C_{48}	C_{22}	C_{26}	704·7	16·5	14
	C_{24}	C_{24}			
C_{50}	C_{22}	C_{28}	732·6	27·0	27
	C_{24}	C_{26}			
	C_{26}	C_{24}			
C_{52}	C_{22}	C_{30}	760·8	29·0	27
	C_{24}	C_{28}			
	C_{26}	C_{26}			
	C_{28}	C_{24}			
C_{54}	C_{24}	C_{30}	788·7	16·9	21
	C_{26}	C_{28}			
	C_{28}	C_{26}			
	C_{30}	C_{24}			
C_{56}	C_{26}	C_{30}	816·8	6·15	11
	C_{28}	C_{28}			
	C_{30}	C_{26}			
	C_{32}	C_{24}			

EXPERIMENTAL

NMR spectra were measured in $CDCl_3$ solution using TMS as an internal standard an a 60-MHz instrument and IR spectra were determined in CCl_4 solution. GLC analysis of methyl esters and alcohols were made using FID on a 3% OV1 column (3 m × 3 mm stainless steel) programmed 50–250°/min (6 min initial hold). The wax esters themselves were separated on the same column by programming 250–350° at 2·5°/min (20 min initial hold). MS were measured at both 10 and 70 eV with a source temp. of 140° and a solid probe temp. of 200°.

Isolation of wax esters. Ground leaves and stems of *Larrea divaricata* Cav. from Langtry, Texas (1966 g; collection No. David Seigler 950) were extracted with 30–60° petrol for 2 days. When the petrol extract was cooled to 0°, a dense white ppt. formed. This ppt. was removed by filtration and washed with cold Et_2O to yield a white solid (m. p. 75–76°, uncorrected) (17·8 g, 0·9% yield).*

Trans-*esterification of the esters.* A sample of wax esters (100 mg, 2 × cryst. from hexane) was refluxed with MeOH (100 ml) and H_2SO_4 (2 ml) for 6 days during which time the material dissolved. The sample was concentrated under vacuum to small vol., then H_2O (15 ml) and Et_2O (15 ml) were added and the phases separated. The aq. phase was washed 2 × with Et_2O, the Et_2O phases combined and dried over anhyd. $MgSO_4$. The mixture was filtered and the solvent removed under vacuum. This procedure yielded a light yellow oil.

Saponification of the wax esters. Wax esters (100 mg) were added to a soln. of KOH (50 mg), C_6H_6 (10 ml), dibenzo-18-crown-6 ether (Aldrich Chemical Co.) (20 mg) and sufficient MeOH to dissolve the mixture; the soln was refluxed for 2 days. Et_2O (5 ml) and H_2O (5 ml) were added to the mixture remaining after the

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* Later, it was established that the bulk of the wax esters occurred on the external surface of the stems. (We thank Dr. Nobuji Nakatani for this information.)

C_6H_6 was allowed to evaporate. The phases were separated and the aq. phase washed 2× with Et_2O. Ethereal phases were combined and dried over anhyd. $MgSO_4$. The mixture was filtered, and the Et_2O removed to yield a white solid.

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