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FREE 3-ACETOXYFATTY ACIDS IN FLORAL GLANDS OF *KRAMERIA* SPECIES

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Key Word Index—*Krameria cuspidata*; *K. cytissoides*; *K. revoluta*; *K. parvifolia*; Krameriaceae; floral oils; 3-acetoxy fatty acids; structural determination.

INTRODUCTION

The role of lipids produced by floral glands in the pollination *Krameria* species has recently been reported [1]. These oils, like those produced by several genera scattered among at least 7 other angiosperm families are collected by bees and used with pollen as larval provisions. In the process of collecting these oils, the bees serve as pollination agents. A previous report of the floral lipids of *Calceolaria pavonii*, the only other taxon previously studied chemically stated that the oils were diglycerides of acetate and β-acetoxystearic acid [2].

We wish to report here the chemical nature of the unusual compounds secreted by floral glands in *Krameria* which we have shown to be primarily 3-acetoxyhexadecanoic, 3-acetoxyoctadecanoic and 3-acetoxyeicosanoic acids.

RESULTS AND DISCUSSION

Examination of the PMR spectra (Table 1) of lipids from the floral glands of *Krameria cuspidata* Presl., *K. revoluta* Berg, *K. cytissoides* and *K. parvifolia* Benth. reveals peaks at δ 0.87 and 1.25; these peaks are terminal Me and methylene absorptions respectively. The integral value of the methylene absorption and comparison of derived Me esters with known standards by GLC indicate chain lengths of C_{16}, C_{18}, C_{20} and in one sample C_{22}. The absence of a triplet at δ 2.5 (protons α to carbonyl group) suggests the presence of a functional group β to the carbonyl position. The presence of a sharp singlet at δ 2 and the presence of absorptions at 1745 and 1240 cm^{-1} in the IR spectrum indicate this substituent to be an acetoxy group. The 5 line pattern for the proton on the carbon which bears the acetoxy group results from splitting by two pairs of adjacent protons. The spectrum is very similar to that of β-acetoxymethyl butyrate [3]. The major peaks of the MS of *K. parvifolia* correspond to β-acetate (*m/e* 310 and 282 and 254) and β-acetate-H_2O (*m/e* 292, 264 and 236). Parent ions were not observed. IR absorptions at 3500–3050 and 1720 cm^{-1} indicate that the original compound contains a carboxyl group. TLC of these lipids suggests that they contain a polar functional group ($R_f = 0.3$) compared to triolein ($R_f = 0.8$) in the solvent system employed. Facile formation of Me esters by addition of diazomethane confirms this view. Normal glycerides are not present in most samples as evidenced by the absence of PMR absorptions at δ 4.25 and 5.4, although traces of glyceride absorptions do occur in the PMR spectra of some samples, particularly those of *K. cytissoides*. TLC of the hydroxy Me esters derived from these glycerides suggest that the fatty acid component consists of mostly acetoxy fatty acids.

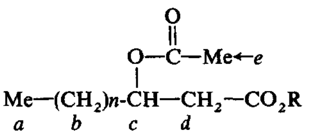
Chemical and spectral data (PMR, IR) for the β-acetoxy acid, hydroxy acid and the α,β-unsaturated fatty acid were identical to those of Vogel [2].

The percentage of impurities in lipids which were collected and stored carefully is less than 5% in most samples, but IR spectra of some extracts of whole flowers (not stored under nitrogen) show absorptions corresponding to hydroxy acid and acetic acid. The major lipid component of *K. cuspidata*, *K. cytissoides*, *K. revoluta* and *K. parvifolia*, as indicated by PMR, IR and TLC is identical.

EXPERIMENTAL

Voucher specimens of all collections are deposited in either the U.S. National Herbarium (US) or the University of Illinois Herbarium (ILL).

Table 1. PMR spectral data for 3-acetoxyhexadecanoic, 3-acetoxyoctadecanoic, 3-acetoxyeicosanoic acids and the corresponding methyl esters. All spectra were recorded at 100 MHz in $CDCl_3$ with TMS standard.



$$\underset{a}{\mathrm{Me}}-\underset{b}{(\mathrm{CH_2})_n}-\underset{c}{\overset{\overset{\overset{\mathrm{O}}{\|}}{\mathrm{O-C-Me}\leftarrow e}}{\mathrm{CH}}}-\underset{d}{\mathrm{CH_2}}-\mathrm{CO_2R}$$

1: R = H
2: R = Me←*f*

	Signal of protons					
	a	*b*	*c*	*d*	*e*	*f*
Chemical shift (δ)	0.87	1.25	5.2	2.5	2	3.65
Number of Protons	3	—	1	2	3	3
Multiplicity	distorted *t*	*s*	5 peaks	*d*	*s*	*s*
Coupling Constant (Hz)	—	—	—	7	—	—

Isolation of lipid materials. Glands were removed from flowers of the *Krameria* spp. in the field, placed in a soln of $CHCl_3$–MeOH (6:1) and stored under N_2 in sealed ampules. In a few cases, to make larger collections of lipids, whole flowers were washed with a soln of $CHCl_3$–MeOH and stored in bottles; no significant differences in PMR spectra were observed.

Spectral determinations. All PMR spectra were measured on a 100 MHz instrument in $CDCl_3$ with TMS as an int. stand. Lipid solns were concd under N_2, redissolved in $CDCl_3$ and PMR samples flushed with N_2 to avoid decomposition. MS were obtained at 70 eV. IR spectra were determined as a thin film on NaCl discs. The PMR spectra of lipids from *K. cuspidata* (BBS 7091, 7090, 7113), *K. revoluta* (DS 9487, BBS 7100), *K. parvifolia* (DS-9213) and *K. cytissoides* (BBS 7030) had PMR absorptions: δ 0.87 (3H, *t*); 1.25 (*s*), 5.2 (1H, 5 peak *m*), 2.5 (2H, *d*, $J = 7$ Hz), 2 (3H, *s*) and 3.65 (3H, *s*). The spectrum of *K. cytissoides* (BBS 9030) also had *m* at δ 4–4.3 and 5.3. The lipid of *K. cytissoides* (BBS 7033) had IR absorptions at 3600–3050 (*br*, OH stretch); 2960, 2920 and 2850 (CH stretch): 1745 (*s*, acetate carbonyl) and 1720 (*s*, acetate carbonyl); 1375 (*m*), 1260 and 1240 (*s*) 1080 and 1045 cm^{-1}. The MS of *K. parvifolia* (DS-9213) floral lipid had major peaks at *m/e* 310, 299, 292, 282, 264, 254, 238, 236, 222, 220 and other fragments of lower mass.

TLC. Lipids were chromatographed on Si gel G with petrol–Et_2O–HoAc (70:30:1) and visualized with H_2SO_4 and charring or under UV light following spraying with 2′,7′-dichlorofluorescein. The major lipid component has $R_f = 0.3$ in this system. In one sample of *K. cytissoides* (BBS 7030) an additional spot (R_f 0.77) was observed.

Me esters of β-hydroxy fatty acids. Me esters of the β-acetoxy acids were prepared by treatment of the sample with BF_3–MeOH using the method of ref. [4] or by refluxing with 2% H_2SO_4 in MeOH. These methods resulted in loss of the acetoxy group and formation of the corresponding hydroxy ester. PMR: δ 0.9 (*t*), 1.25 (*s*), 2.25–2.7 (*m*) 3.67 (*s*), 3.9–4.2 (*m*), 5.4–5.7 (*br*); IR 3430 (*br*), 2930 (*s*), 2850 (*m*), 1735 (*s*), 1465, 1440, 1375, 1255, 1170, 1080, 1040 (*s*) and 875 cm^{-1}; TLC (Si gel G, hexane–Et_2O–HOAc (70:30:1) R_f 0.4). Me esters of fatty acids without OH groups have R_f 0.8 in this system.

Me β-acetoxy fatty acids. Me esters with the β-acetoxy group unchanged were prepared by treating solns of β-acetoxy fatty acids with CH_2N_2–Et_2O. The Et_2O was removed *in vacuo* to yield a light yellow oil. The PMR spectrum was unchanged except for the appearance of a sharp singlet at δ 3.67. IR 2930 (*s*), 2850 (*m*), 1735 (*s*), 1200–1240 (*s*), 1020 cm^{-1}.

β-Hydroxy fatty acids. Hydrolysis of the lipids with methanolic NaOH (10 ml 3N NaOH in 90 ml MeOH, refluxing 1 hr) followed by acidification (6N HCl) yielded a compound which appeared (by TLC) to be the hydroxy acid ($R_f = 0.2$). PMR: δ 0.87 (*t*), 1.35 (*s*), 2.3–2.6 (*m*), 3.8–4.2 (*m*), 4.9–5.6 (*br*).

α,β-Unsaturated fatty acids. Other attempts to hydrolyze the β-acetoxy fatty acids by the above method as well as attempts to acetylate the hydroxy acid with refluxing Ac_2O and Py produced unsaturated fatty acids. These were converted to Me esters and hydrogenated by the method of ref. [5] with Pt_2O catalyst. GLC of the derived Me esters was performed on a 2 m × 3.5 mm glass column (10% DEGS on chromosorb from 170–190°). R_ts were compared with those of authentic standards. The percentages of C_{16}, C_{18}, C_{20} and (in one sample) C_{22} fatty acids varied among samples both within and between species. The chain lengths observed were confirmed by both PMR and MS measurements.

β-Acetoxy fatty acid glycerides. The lipid fraction (R_f 0.77) which occurred in *K. cytissoides* (BBS 7030) was isolated by PLC. Conversion of the hydrolysate to the corresponding Me esters by BF_3–MeOH and TLC indicated that the fatty acid component of these glycerides appeared to consist of acetoxy-fatty acids, (R_f 0.4 compared to R_f 0.8 for Me esters of fatty acids without OH group).

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