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SHORT REPORTS

SECONDARY DIOLS OF *PINUS RADIATA* NEEDLE EPICUTICULAR WAX

ROBERT A. FRANICH*, ANN P. GOWAR† and JOHN K. VOLKMAN†
* Forest Research Institute, New Zealand Forest Service, P.B., Rotorua, New Zealand;
† Organic Geochemistry Unit, School of Chemistry, University of Bristol, Bristol, BS8 1TS, U.K.

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INTRODUCTION

Recently, the composition of *Pinus radiata* needle epicuticular wax was described [1]. Among the various classes of compounds isolated was a mixture of long-chain secondary diols, which are novel constituents of plant waxes. The diol fraction, isolated by column chromatography, followed by PLC, was analysed by GLC and GC–MS of the bis-TMSi ether derivatives using packed columns, which, however, only partially resolved the mixture [1]. The compounds identified were heptacosane-5,10-diol, nonacosane-10,13-, 4,10- and 5,10-diols. The diol mixture has now been re-examined using high resolution capillary column GC–MS with fast scan times.

RESULTS AND DISCUSSION

The R_t (relative to 1-hexadecanol TMSi ether) of the diol bis-TMSi ether GLC peaks and the relative amount of each are summarized in Table 1. The 6 new diols identified from their GC–MS analysis, were nonacosane-6,10-, 7,10-, 10,16-diol, pentacosane-5,10-diol, octacosane-5,10-diol and hentriacontane-10,13-diol. The primary fragment ions formed by cleavage α to the OTMSi groups are summarized in Table 2. The most favoured fragmentation of the diol bis-TMSi ethers is that giving the shorter OTMSi fragment (**a** in Table 2).

Table 1. R_t of diol bis-TMSi ether GLC peaks relative to 1-hexadecanol OTMSi ether and the relative amount of each diol ether

C No.	Substitution	R_t rel. C_{16} OTMSi (min)	Amount (%)
25	5,10	29.2	0.5
27	5,10	34.4	7.5
28	5,10	37.4	0.7
29	10,13	38.6	21.9
	10,16	39.0	11.7
	7,10	39.2	10.2
	6,10	39.4	2.9
	5,10	40.0	36.5
	4,10	40.6	8.0
31	10,13	44.8	0.1

While the major C_{29} diols have substituents at 5,10 and 10,13, the C_{25}, C_{27} and C_{28} compounds have only 5,10, and the C_{31} compound only 10,13 OH substituents. Octacosane-5,10-diol is the first even carbon secondary diol identified in a plant wax.

When packed OV-17 GLC columns were used for analysis of the diol mixture, quantitative data different from that presented here in Table 1 were obtained [1]. This is attributed to the co-elution of nonacosane-4,10-diol bis-TMSi ether with residual nonacosane-5,10-diol bis-TMSi ether and other C_{29} diol derivatives identified in this report. Consequently the GLC peak integral assigned to nonacosane-4,10-diol bis-TMSi ether was larger than that measured for the fully-resolved peak obtained using capillary columns.

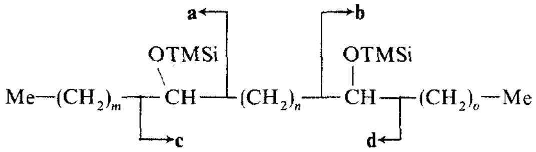
The MS described for nonocosane-4,10-diol bis-TMSi ether in the previous study [1] was taken near the tail of the last eluting GLC peak (in order to eliminate ions corresponding to nonacosane-5,10-diol bis-TMSi ether) and did not show a base peak due to the shorter OTMSi fragment. However, the MS obtained for nonacosane-4,10-diol bis-TMSi ether in the present case was taken at the top of its total ion current peak, and gave *m/e* (**a** in Table 2, 100%); 369 (**b**, 52%); 541 (**c**, 1%); and 317 (**d**, 45%).

Whereas the diol fraction contains the C_{25}, C_{27}, C_{28}, C_{29} and C_{31} compounds, the secondary alcohol fraction from the wax contains solely nonacosan-10-ol [1], identified from the MS of the TMSi ether derivative [2]. In plants, secondary alcohols are synthesized from the parent hydrocarbon, the oxidation being catalysed by a mixed-function oxidase [3]. The long-chain diols may arise through a similar oxidation, the position of substitution depending on the substrate chain-length.

EXPERIMENTAL

Plant material. *Pinus radiata* seedlings were grown for 3 months using conditions described previously [4]. One-year-old secondary needles were harvested from 5-year-old forest trees [5].

Table 2. Primary fragment ions in the MS of bis-TMSi ethers of C_{25}, C_{28}, C_{29} and C_{31} diols



C No.	OTMSi position (left to right in above structure)	Ion assignment: *m/e* (rel. int. %) a	b	c	d
25	5,10	159 (100)	313 (67)	—	317 (48)
28	5,10	159 (100)	355 (46)	—	317 (50)
29	6,10	173 (100)	369 (25)	—	317 (28)
	7,10	187 (100)	369 (25)	499 (1)	317 (8)
	10,16	229 (100)	285 (72)	457 (8)	401 (15)
31	10,13	229 (100)	335 (65)	—	—*

* Ion **d** not observed, but **d** − 90, *m/e* 269 (90).

Extraction and fractionation. Epicuticular wax from primary needles of seedlings and secondary needles from trees was obtained by extracting needle surfaces with $CHCl_3$ at 20° [5]. The wax was freed from fatty and diterpene acids by ion-exchange chromatography, and the diol fraction isolated by column chromatography of the neutral fraction on deactivated Al_2O_3, followed by PLC on Si gel [1].

Analysis. GLC was carried out on a 20 m × 0.3 mm OV-1 WCOT glass capillary column, programmed from 100 to 270° at 3.5°/min, and using splitless injection. Carrier gas was He at 1.5 ml/min. Injector temp. and FID detector temp. 300°. GC–MS was carried out using a quadrupole filter instrument operating at 400 μA, 40 eV electron energy and an ion source temp. of 250°. The capillary column was coupled directly to the MS ion source. Chromatographic conditions were similar to those above. Spectra were taken at 1.5 sec intervals. The diol fraction was derivatized by dissolving a weighed portion in dry C_5H_5N, adding BSTFA, and heating at *ca* 60° for 30 min. The solvent and excess reagent were removed in a stream of dry N_2, and the residue was dissolved in dry CH_2Cl_2.

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