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GERANYLGERANYL ESTERS IN NORWAY SPRUCE WOOD

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Diterpenoids are considered to be biogenetically derived from geranylgeraniol and closely related compounds such as geranyllinalol which has been isolated from the oleoresin of Norway spruce, *Picea abies* (L.) Karst. [1]. Esters of isoprenoid alcohols in higher plants have been proposed to be located in the chloroplasts [2], but oligoprenyl esters of fatty acids have also been detected in the wood of silver birch [3, 4]. The occurrence of geranylgeranyl esters of fatty acids in the wood of coniferous trees has not been reported.

The presence of geranylgeranyl esters in the lipophilic extractives of Norway spruce wood was indicated by the following finding: saponification of the terpenoid ester fraction (TLC) gave sterols, triterpene alcohols, fatty acids and a diterpene alcohol, proved to be identical with an authentic sample of geranylgeraniol (GC–MS of the alcohol, its hydrogenation product and their TMSi ethers). Attempted GC–MS of the unhydrolysed fraction was not successful due to decomposition of the esters at the high temperatures required. Phytyl esters have been found to behave similarly [5]. However, hydrogenation of the fraction followed by GC–MS gave normal shaped peaks and mass spectra corresponding to phytanyl esters of saturated C_{16}–C_{20} fatty acids, the expected hydrogenation products of the geranylgeranyl esters assumed to be present. The GC–MS data for synthetically prepared phytanyl heptadecanoate and phytanyl 14-methylhexadecanoate (*anteiso*-heptadecanoate) were in complete agreement with those of the corresponding esters in the investigated sample.

Table 1. Fatty acid composition of geranylgeranyl esters from *P. abies* wood

Fatty acid*	Percentage composition	ECL value BDS, 190°
anteiso 16:0	1.0	15.71
16:0	14.3	16.00
7-16:1	1.7	16.34
9-16:1	2.1	16.41
anteiso 17:0	22.2	16.72
17:0	2.0	17.00
iso 18:0	1.0	17.55
anteiso 18:0	1.9	17.72
18:0	3.6	18.00
9–18:1	5.3	18.32
11-18:1	5.4	18.39
anteiso 19:0	13.7	18.73
9,12–18:2	12.4	18.90
anteiso 9–19:1	1.5	18.97
5,9,12–18:3	4.0	19.16
20:0	4.4	20.00
11-20:1	2.2	20.29
11,14–20:2	1.2	20.87

* Analysed as the methyl ester.

Isolation of the geranylgeranyl esters was achieved by preparative TLC. Saponification of the fraction facilitated GC–MS identification of the fatty acid residues (Table 1). The high proportion of saturated (64%) and *anteiso* (40%) acids esterified with geranylgeraniol is noteworthy. Total fatty acids in Norway spruce wood are composed of the same fatty acids, but the contribution of saturated (10%) and *anteiso* (4%) acids is significantly lower [6]. The amount of geranylgeranyl esters was 0.7% of total petrol-soluble extractives from the investigated wood sample.

EXPERIMENTAL

A debarked disc from the trunk of a 45-year-old *P. abies* tree was freeze-dried and ground to wood meal. Extraction of 50 g dry sample in a Soxhlet apparatus with petrol (bp 40–60°) for 6 hr gave 450 mg of dry extractives. One mg of phytanyl heptadecanoate, prepared by mixing the acid chloride and hydrogenated phytol, was added as internal standard.

The extractives were dissolved in Et_2O and chromatographed on PLC (Si gel) plates with petrol (bp 40–60°)-Et_2O (17:3). I_2 vapour was used as general detection reagent and a broad fraction at hR_f 50–70 mainly representing terpenoid esters was isolated.

Approximately one half of the crude ester fraction was dissolved in *iso*-octane and hydrogenated with a slow stream of H_2 for 2 hr at room temp. using 10% Pd/C as catalyst. The resulting mixture was analysed on GLC and GC–MS (LKB-9000 instrument) using a 25 m × 0.3 mm id glass capillary column coated with SE-30, column temp. 285°. The amount of hydrogenated geranylgeranyl esters was calculated from peak areas with phytanyl heptadecanoate as quantitative standard.

The untreated portion of the crude ester fraction was rechromatographed on TLC (Si gel). With petrol (bp 40–60°)-C_6H_6 (2:3), the geranylgeranyl esters at hR_f 35 could be separated from triterpenoid esters and phytanyl heptadecanoate which were located in the hR_f 40–50 region.

Saponification of the geranylgeranyl esters was achieved by 0.5 N KOH in 90% EtOH at 70° for 3 hr. After 1:1 dilution with H_2O and neutralization with 0.5 N H_2SO_4 the neutral and acid residues were extracted with Et_2O. The acids in the mixture were methylated with CH_2N_2 and analysed on GLC and GC–MS using a 30 m × 0.3 mm id glass capillary column coated with 1,4-butanediol succinate (BDS) at 190°.

The alcohol residue was isolated from the mixture by TLC (Si gel) with petrol (bp 40–60°)-Et_2O (3:2). The alcohol at hR_f 20 and authentic geranylgeraniol, their hydrogenation products (*iso*-octane, H_2, 10% Pd/C, 2 hr) and the TMSi ethers of these were analysed on GLC and GC–MS with a 2 m × 1.8 mm id glass column packed with 1% XE-60 and operated at 110–190°, 4°/min.

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