the buffer containing 0.2 M NaCl to give the xylan-containing fraction Since the preliminary experiment revealed that the xylan became partly water-insoluble after freeze-drying, the isolation procedures were performed without lyophilization. The 0.2 M NaCl fraction gave, on complete acid hydrolysis, L-arabinose, D-xylose and D-galactose in the molar ratio 38 3 . 33.4 . 28.3 with trace amounts of D-mannose and D-glucose as neutral sugars. This fraction, after dialysis against H_2O, was applied to a column (2 3 × 12 cm) of cellulose powder (Whatman, CF 11), which was first washed with water to give crude AGP and then eluted with 1 M NaOH to give acidic xylan. The alkaline fraction was dialysed against H_2O and then centrifuged. The supernatant gave L-arabinose, D-xylose, D-mannose, D-glucose and D-galactose in the molar ratio 4.0 : 89.5 : 1.3 : 3.1 : 2.1. The yield of the acidic xylan was *ca* 50 mg from 1 l of ECP soln.

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VOLATILE COMPONENTS IN CELL SUSPENSION CULTURES OF *CRYPTOMERIA JAPONICA*

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Key Word Index—*Cryptomeria japonica*; Taxodiaceae; cell suspension culture; volatiles; diterpenes; aldehydes; fatty acids and their esters.

Abstract—The cell suspension culture of *Cryptomeria japonica* contains volatile oils, the yield of which was 0.005–0.01 % of the fresh cells. In the volatiles, five aldehydes, ten fatty acids and their esters, and two diterpenes of abietatriene and ferruginol have been found. Of these, palmitic acid is present as the most predominant component, amounting to *ca* 40 % of the volatiles.

INTRODUCTION

Cryptomeria is a rich source of volatile oils. *C. japonica* (Japanese cedar) is known to yield *ca* 0.7 % of volatiles from the fresh leaves, in which diterpenes of kaurene, isokaurene, 9-ketoferruginol (sugiol) and the others have been found [1]. Recently, many callus tissues and suspension cells have also been found to produce mono- and sesquiterpenes [2, 3] As one of us recently succeeded in establishing the cell culture of Japanese cedar [4], we thus examined the volatile oil in the suspension cultured cells.

RESULTS AND DISCUSSION

The cell suspension culture of Japanese cedar was maintained in a modified liquid medium based on that of Schenk and Hildebrandt [6] From this, the 14th generation of suspension cells was harvested at a 14-day culture (the stationary growth phase) and subjected to extraction of volatiles. The yield of volatile oils obtained by a simultaneous distillation extraction [7] was 0.005–0.01 % of the fr. wt. The cells at this growth stage contained a

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Table 1. Volatile components in cell suspension cultures of *Cryptomeria japonica*

Compounds	GC R_t (min)	Approx. % of volatiles*
Aldehydes		
Nonanal	1.6	0.5
(2*E*,4*Z*)-2,4-Heptadienal	2.2	1.2
(2*E*,4*E*)-2,4-Heptadienal	2.3	1.0
2,4-Nonadienal	4.3	0.6
(2*E*,4*Z*)-2,4-Decadienal	5.2	1.2
(2*E*,4*E*)-2,4-Decadienal	5.5	3.2
Acids		
Myristic acid	15.5	0.6
Palmitic acid	17.6	38.5
Linoleic acid	22.0	2.1
Linolenic acid	23.3	0.5
Esters		
Methyl palmitate	11.1	3.5
Ethyl palmitate	11.4	4.4
Methyl linoleate	13.6	0.6
Ethyl linoleate	14.2	4.5
Methyl linolenate	14.3	0.8
Ethyl linolenate	14.6	5.1
Diterpenes		
Abietatriene	13.1	1.6
Ferruginol	26.2	1.6

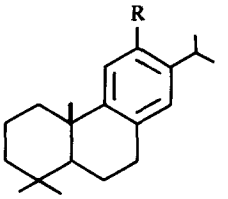
*Calculated from relative peak height by GC.

maximal level of volatiles, and also, as reported in a previous study [4], they contained a small amount of chloroplasts and a large amount of catechin and procyanidins.

By GC/MS analysis of the volatile oils from the suspension cells (Table 1), five aldehydes, 10 fatty acids and their esters, and two diterpenes were identified as the major components. In particular, the cells yielded large amounts of palmitic acid, which comprised *ca* 40% of the volatiles. We also found that the cells produced seven diterpenes, of which abietatriene (**1**) and ferruginol (**2**) were identified. By a preliminary examination, both compounds also have been confirmed to be present in the green leaves of Japanese cedar. 9-Ketoferruginol, which have been reported as a characteristic component in the leaves, could not be detected in the suspension cells.

EXPERIMENTAL

Plant material. C. japonica cv 'kumotôshi' used for the present study is a tall tree growing in Japan and produces valuable timber. As described previously [4], the callus was induced from the tip section of a young shoot with Murashige and Skoog medium [5] containing 2,4-D (1 mg/l), kinetin (0.1 mg/l.), sucrose (30 g/l.) and agar (0.9% w/v) The callus tissues were then subcultured every 3 weeks for *ca* 5.5 months at 25° in the dark. From calluses at the 8th generation, the cell suspension culture was derived and maintained in a modified Schenk and Hildebrandt medium [6] containing 2,4-D (0.5 mg/l.), kinetin (0.01 mg/l.) and sucrose (30 g/l.). A portion (1.2–1.4 g) of the suspension cells in a Erlenmeyer flask (200 ml) was regularly transferred to fresh medium (50 ml each) every 2 weeks. Flasks were agitated on a



R
1 H
2 OH

reciprocal shaker at 62 strokes per min with horizontal excursion of 5.5 cm at 25° under white light (350 lx). As estimated from the increase in fr. wt, the doubling time of the cells in the logarithmic growth phase (9 days after the 3–4 days lag growth phase) was *ca* 48 hr. The suspension cells of the 14th generation (the 22nd generation after callus formation) were harvested at a 14-day culture (the stationary growth phase) and subjected to extraction of volatiles.

Extraction and GC and GC/MS. Volatile oils from suspension cells were separated by a simultaneous distillation extraction [7]. The cells (41.5 g) harvested were homogenized in H_2O (300 ml) at *ca* 0° with a blender for 5 min. The homogenate was transferred into the vessel for a simultaneous distillation extraction. The soln obtained was extracted with 30 ml of Et_2O for 90 min. Five nl of tridecane in 5 μl of Et_2O was then added to the Et_2O extract. After concn of the Et_2O extract under N_2, and under red. pres. to *ca* 30 μl, the volatile components were analysed by GC and GC/MS as follows. For GC analysis, the column used was a 30 m × 0.3 mm WCOT glass capillary column coated with Carbowax 20 M. Column temp. 70–150° at 5°/min, injector temp. 170°, detector temp. 170°, N_2 carrier at 0.78 ml/min, split ratio 1.85. For GC/MS analysis, the column used was a WCOT glass capillary column coated with FFAP (50 m × 0.25 mm i.d.). Column temp. 40–210° at 3°/min, He carrier at 1.0 ml/min, split ratio 1·20. Ionizing voltage for both EIMS and CIMS was 70 eV; reagent gas for CIMS was CH_4.

Volatile components. Volatile components listed in Table 1 were identified by direct comparison with authentic specimens using GC/MS. MS of diterpenes were as follows. Abietatriene EIMS *m/z* (rel. int.): 270 $[M]^+$ 255 (100), 213 (7), 199 (11), 185 (35), 173 (60), 159 (63), 143 (17), 141 (12), 129 (20), 117 (15), 91 (10), 83 (9), 69 (45), CIMS *m/z*. 271 $[M+H]^+$ (70), 270 $[M]^+$ (80), 269 $[M-H]^+$ (70). Ferruginol EIMS *m/z* (rel. int.): 286 $[M]^+$ (95), 272 (20), 271 (100), 229 (16), 215 (16), 201 (39), 189 (66), 175 (64), 69 (51), 55 (16); CIMS *m/z*: 287 $[M+H]^+$ (70), 286 (100).

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