Phytochemistry, Vol. 25, No. 6, pp. 1333–1335, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

VOLATILE CONSTITUENTS OF *ZINGIBER OFFICINALE* RHIZOMES PRODUCED BY *IN VITRO* SHOOT TIP CULTURE*

FUKIKO SAKAMURA†, KAZUHITO OGIHARA‡, TAKAYUKI SUGA‡¶, KENJI TANIGUCHI§ and RYUSO TANAKA§||

†Department of Food, Oshimo Women's College, Gion-cho, Asaminami-ku, Hiroshima 731-01, Japan; ‡Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Naka-ku, Hiroshima 730, Japan; §Laboratory of Plant Chromosome and Gene Stock of the Faculty of Science, Hiroshima University, Higashisenda-machi, Naka-ku, Hiroshima 730, Japan; ||Department of Botany, Faculty of Science, Hiroshima University, Higashisenda-machi, Naka-ku, Hiroshima 730, Japan

(*Received* 3 *September* 1985)

Key Word Index—*Zingiber officinale*; Zingiberaceae; ginger; shoot tip culture; plantlets; rhizomes; volatile constituents.

Abstract—Plantlets with rhizomes were produced *in vitro* from shoot tips of *Zingiber officinale* grown in both modified Gamborg's B5 (B5) and Murashige–Skoog (MS) media supplemented with various levels of growth regulators. The rhizomes accumulated the volatile oils similar to those formed in the original rhizome. In the oil from the rhizome grown in the modified B5 medium, the acyclic oxygenated monoterpenes predominated, while the oil from the rhizome grown in the modified MS medium consisted mainly of sesquiterpenes.

INTRODUCTION

Ginger cultivar 'Oshoga', *Zingiber officinale* Rosc., is widely used as a herb, spice and additive in foods and beverages in Japan. Previously, Sakamura *et al.* [1, 2] reported on volatile constituents from the freshly harvested rhizome of the cultivar 'Oshoga' and on changes in the constituents during storage of the rhizome. *Zingiber officinale* 'Oshoga' annually propagates itself by rhizomes stored for several months under high humidity prior to planting. However, storage of the rhizome of this cultivar is more difficult than that of other cultivars because the rhizome suffers from cold injury. Therefore, an alternative propagation technique for *Z. officinale* is needed. Recently, Tanaka *et al.* [3] established the culture of shoot primordia. The shoot primordia not only undergo rapid propagation, but also form oil bodies. Application of the shoot primordia method to 'Oshoga', however, resulted in the formation of plantlets with rhizomes. As a continuation of the previous work [1, 2], we examined the essential oils from these rhizomes.

RESULTS AND DISCUSSION

Shoot tip explants of *Z. officinale* 'Oshoga' were planted in both modified Gamborg's B5 (B5) medium [4] and Murashige–Skoog (MS) medium [5] containing various levels of 1-naphthaleneacetic acid (NAA) and 6-benzyladenine (BAP). According to the shoot primordia production method [3], the explants had been cultured for 100 days at 22° in the above media by rotating slowly under continuous fluorescent light of 9000 lux. However, the culture only produced precocious branches including plantlets with rhizomes. The plantlets were produced in the modified B5 media supplemented with 0.2 and 2.0 ppm of BAP and with 0.2 and 2.0 ppm of BAP in addition to 0.2 ppm of NAA. The plantlets were also formed in the modified MS media supplemented with 0.2 ppm of BAP and with 0.2 and 2.0 ppm of BAP in addition to 0.2 ppm of NAA. The essential oils from the rhizomes of these plantlets were analysed by GC and GC/MS. Similarly, the essential oil from the original rhizome was analysed for comparison.

The essential oils from these cultures contained the same constituents as in the original rhizome (Table 1). However, there were considerable quantitative differences. The essential oil from the rhizome grown in the modified B5 medium consisted mainly of monoterpenes. Acyclic oxygenated compounds, such as geraniol, geranial and geranyl acetate, together composed between 42 and 52% of the oil. The oil pattern is similar to those in the original rhizome and in other Japanese fresh rhizomes [1]. On the other hand, the essential oil from the rhizome grown in the modified MS medium consisted mainly of sesquiterpenes, among which zingiberene predominated. Such an oil composition is similar to those in the ginger rhizomes from other countries [6–12]. In addition, the major oil constituents, probably diterpenes, were present in the rhizome formed in the modified MS medium.

Obviously, the oil composition in the culture depended on the composition of the basal culture medium, but it was influenced by the concentration of the growth regulators added. Thus, a change in the composition of the medium provides a means of producing cultures with different aromas. In the case of the rhizome produced in the modified MS medium, monoterpene biosynthesis may be depressed. Such a depression might result from an excess of glycine and/or from a deficiency of thiamine hydrochloride in comparison of the composition of MS medium with that of B5 medium.

*Part 2 in the series "Constituents of Essential Oils from *Zingiber officinale*".

¶To whom correspondence should be addressed.

Table 1. Percentage relative abundances of essential oil constituents from *Zingiber officinale* rhizomes produced by shoot tip culture

	Culture												
	Gamborg's B5 medium						Murashige-Skoog medium						
	NAA*	BAP†	NAA	BAP	NAA	BAP	NAA	BAP	NAA	BAP	NAA	BAP	
Constituents	0	0.2	0.2	0.2	0.2	2.0	0	0.2	0.2	0.2	0.2	2.0	Original rhizome‡
Monoterpenes§	65.2		60.0		63.3		31.3		23.4		44.7		72.1
β-Pinene	2.2		1.1		2.1		0.9		1.4		1.6		tr
Myrcene	0.5		0.2		0.7		0.2		0.2		0.3		tr
1,8-Cineole	14.2		5.6		17.6		5.8		5.5		9.4		1.8
Linalool	2.3		2.0		2.3		1.2		0.8		1.3		1.0
Isoborneol	0.5		0.3		0.3		tr		tr		0.3		0.1
α-Terpineol	0.8		0.6		0.7		0.8		0.7		0.7		0.9
Citronellol	1.0		0.6		1.0		0.5		0.7		0.7		2.1
Nerol	2.3		1.6		1.0		1.1		0.7		1.1		0.9
Neral	7.5		4.4		4.4		3.8		2.4		5.2		8.1
Geraniol	10.5		8.9		9.1		2.8		1.7		5.0		20.8
Geranial	16.7		12.1		12.0		8.5		8.0		13.7		25.2
Geranyl acetate	6.7		22.6		12.0		5.7		1.3		5.1		11.2
Sesquiterpenes§	29.0		32.0		29.8		50.4		61.1		40.4		12.6
α-Copaene	0.1		0.4		tr		0.4		0.2		0.2		0.1
β-Bisabolene	2.7		1.7		1.4		2.9		2.8		1.7		0.3
Zingiberene	8.5		9.0		8.5		16.4		26.6		15.4		3.2
α-Curcumene	4.0		4.8		4.4		5.2		6.2		5.3		1.6
β-Sesquiphellandrene	2.6		1.6		2.4		4.3		4.4		2.4		0.7
Nerolidol	0.8		1.2		1.2		1.8		0.9		0.9		1.0
β-Sesquiphellandrol	0.4		0.6		0.6		0.9						
Others‖	5.8		8.0		6.9		18.3		15.5		14.9		—

*1-Naphthaleneacetic acid (ppm).
†6-Benzyladenine (ppm).
‡The rhizome of 8-week-old plants.
§Order of elution from an OV-101 GC column.
‖Other higher boiling compounds. tr: trace (< 0.1%).

EXPERIMENTAL

Shoot tip cultures from ginger. Shoot tip explants (0.3 mm) of *Z. officinale* Rosc. 'Oshoga' were obtained under stereo-microscope by dissecting pale yellow segments of stems of 5-week-old plants (10–25 cm high). Each explant composed of the apical dome with 1–2 of the youngest leaf primordia was planted in a liquid medium (25 ml) in a growth vial (200 mm × 27 mm), and cultured at 22° for 100 days on a gyrated drum rotated at 2 rpm under continuous fluorescent light of 9000 lx [3]. Culture media used were modified B5 [4] and MS [5] media containing 0, 0.02, 0.2, 2.0 and 4.0 ppm of NAA and 0.2 and 2.0 ppm of BAP, as shown in Table 1. Plantlets were produced in the modified B5 media supplemented with 0.2 and 2.0 ppm of BAP and with 0.2 and 2.0 ppm of BAP in addition to 0.2 ppm of NAA. Plantlets were also produced in the modified MS media supplemented with 0.2 ppm of BAP and with 0.2 and 2.0 ppm of BAP in addition to 0.2 ppm of NAA. The rhizome of the plantlets produced per explant weighed 1.75–2.30 g in the modified B5 medium and 1.60–3.12 g in the modified MS medium.

Analysis of volatile constituents from shoot tip cultures. The cultured rhizomes and the original rhizome, after being sliced, were subjected to steam distillation. The steam distillate was extracted by a liquid–liquid continuous extractor for 6 hr with Et_2O. The Et_2O soln, dried over anhydrous Na_2SO_4, was concd to a small vol. under red. pres. The concentrate was assayed by a combination of GC and GC/MS under the conditions as follows. GC: a 50 m × 0.25 mm i.d. WCOT glass capillary column coated with 2% OV-101, column temp. 100–250° at 2°/min, injector temp. 250°, detector temp. 250°; GC/MS: a 50 m × 0.25 mm i.d. WCOT glass capillary column coated with 2% OV-101, connected to a Shimazu QP-1000 mass spectrometer, column temp. 100–250° at 2°/min, ion source temp. 250°, ionization voltage 70 eV.

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