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MONOTERPENE SYNTHESIS IN SHOOTS REGENERATED FROM CALLUS CULTURES

JUDITH K. WEBB, DEREK V. BANTHORPE* and DAVID G. WATSON*

Welsh Plant Breeding Station, Aberystwyth, Dyfed, U.K.; *Chemistry Department, University College, London, WC1H-OAJ, U.K.

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Key Word Index—*Lavandula angustifolia*; *Rosmarinus officinalis*; Labiatae; lavender; rosemary; tissue culture; biosynthesis; monoterpenes.

Abstract—Shoots regenerated from two-year old callus cultures of *Lavandula angustifolia* and *Rosmarinus officinalis* accumulated monoterpenes characteristic of the parent tissue. No such compounds could be detected in undifferentiated callus maintained under a variety of conditions.

INTRODUCTION

Cell cultures of higher plants rarely produce essential oils even when the latter are the main secondary metabolites of the parent tissue [1, 2]. Organogenesis in culture may be necessary for the formation of secondary metabolites in general [3–6], and in particular small amounts of oils (containing some monoterpenes) have been detected in shoots regenerated from callus of *Pimpinellum anisum* that had been two years in culture [7] and also recently in roots regenerated from cultures of *Eucalyptus* species [8]. We here report on the synthetic capability of regenerated shoots from callus of *Rosmarinus officinalis L.* (rosemary) and *Lavandula angustifolia* Mill. (≡ *L. officinalis* Chaix. ≡ *L. vera* D. C.; English lavender). Previously no essential oil was detected in cultures of the former species [7] whereas the latter has not been investigated in culture.

RESULTS AND DISCUSSION

Callus of *R. officinalis* was compact containing tracheids and meristematic cells with up to 0.6% of the pigment (chlorophyll + carotenoid) content of the parent tissue. Assay over a period of two years of cultures grown under a variety of media, light and temperature regimes [9] revealed no detectable accumulation of monoterpenes (either free or conjugated as esters or glycosides) within the tissue or excreted into the medium. However, after *ca* 10 sub-cultures (*ca* 8 months in culture), GC/MS revealed the accumulation of some 10 μg/g of alkanes (mainly $C_{15}H_{32}$, $C_{16}H_{34}$ and $C_{17}CH_{36}$, all with a MS base peak at *m/z* 57 suggesting terminal branching) within the callus. The parent plant contains *ca* 80 μg/g of alkanes (mainly C_{29} and above [10]) and callus of *Pogostemon cablin* contained up to 10^4 μg/g of C_{15} and C_{17} alkanes [6]. Such

Table 1. Monoterpene composition of tissues

Plant	Tissue	Monoterpene (μg/g fr.wt)				
		α-Pinene	β-Pinene	1,8-Cineol	β-Ocimene	$C_{10}H_{18}O$†
R. officinalis	Culture‡	0.18	0.30	—*	—	2.0
	Seedling§	4.9	24	6.2	—	—
	Leaf‖	140	150	690	—	—
L. angustifolia	Culture	—	0.7	—	0.7	—
	Seedling	—	5	67	—	—
	Leaf	—	60	850	350	—

*No value entered indicates $< 10^{-4}$ μg/g, if any. The recorded monoterpenes were formed by the culture. The other tissues produced, in addition, camphor, borneol, bornyl acetate, camphene and limonene in total quantity *ca* 20% of the monoterpene fraction. Duplicate GC/MS assays were performed.

†Unidentified monoterpene alcohol, possibly pinan-2-ol.

‡Excised shoot from callus (0.5–1.0 cm).

§Seedlings (5 weeks old).

‖Mature leaves.

compounds occur in the leaf wax of many plants. In contrast, callus of *L. angustifolia* was friable and homogeneous with neither apparent differentiation nor pigmentation. No volatile secondary metabolites could be detected up to 2 years in culture (25 sub-cultures), but after the second sub-culture the callus excreted an unidentified blue pigment (a complex involving an anthocyanin and a high MW lipid) into the medium.

Callus of both species that had been maintained for *ca* 2 years in culture (25–30 sub-cultures) regenerated shoots after 4 weeks on transfer to a medium containing benzyladenine [11]. Success occurred in *ca* 10 and 30% of explants (for *Lavandula* and *Rosmarinus* respectively). Regeneration was preceded by loss of pigmentation (if present) followed by outgrowth of an intense node of green tissue which eventually formed the shoot that grew into and became embedded in the agar support. The shoots remained small (0.5–1 cm) and did not enlarge after sub-culture of an excised part of the callus carrying them. Root formation from callus could be easily induced by standard additions to the medium [12] but similar attempts to induce root formation from explants containing the shoot led to its reversion to callus. Assay of callus and of root outgrowth by GC/MS gave no indication of monoterpene formation ($< 10^{-4}$ μg/g fr. wt; if any). But excision and assay of the shoots gave the results in Table 1 where monoterpene accumulation in regenerated tissue is compared with that in the parent plants. No monoterpenes or volatiles other than those recorded were detected in the shoots. Accumulation of the products, which are the main components of the essential oil of the whole plants, was low, e.g. for *Lavandula ca* 12 and 0.1% respectively of that found in 5-week seedlings and in leaves from mature plants; but for both species was at least 10^4-fold greater than in the parent callus. Thus the necessity for organogenesis for induction of monoterpene formation in these cultures, as well as the totipotency of the callus, was demonstrated.

EXPERIMENTAL

Callus was initiated from stem material on Murashige and Skoog's medium [12] in 0.6% (w/w) agar initially containing coconut milk (10% v/v). The latter was reduced to 2% or 0% (for *Rosmarinus* and *Lavandula* respectively) after the first sub-culture. *Rosmarinus* cultures grew well on media not containing the additive, but more vigorously in its presence. Sub-culture was at 2 to 3-week intervals over a period of up to 27 months under a regime of natural daylight and a constant temp. of 28°. Assay of material grown under other regimes gave similar results. Shoot regeneration occurred after transfer to the medium supplemented with benzyladenine (20 mg/l) and was optimum with a 16 hr photoperiod (Thorn 'white' light 1500 lux, 30°; dark, 25°). Shoots appeared within 4 weeks. For comparison experiments, 5-week seedlings were grown in trays (natural light June, 25°) and leaves were collected from mature potted plants (outdoors, June).

Plant material (10 g) was pulverized in liquid N_2, extracted with Et_2O (100 ml; Soxhlet, 48 hr) and the product decolourized with activated charcoal before concentration (below 0°). Internal standards were used in all cases to correct for evaporation losses. The concentrate (1 ml) was assayed by GC [10% FFAP on Chromosorb W 60–80 mesh (3.5 m × 4 mm); 90° to 135°; FID; N_2 60 ml/min]. Subsequent analysis was by GC/MS with the same column connected to a Kratos MS-2S mass spectrometer linked to a Kratos 65–505 data system and computer.

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REFERENCES

1. Dougal, D. K. (1981) in *Biochemistry of Plants* (Stumpf, P. K. and Conn, E. E., eds) Vol. 7, p. 21. Academic Press, New York.
2. Banthorpe, D. V. and Branch, S. A. (1983) in *Terpenoids and Steroids* (Hanson, J., ed.) Vol. 12, p. 1. The Chemical Society, London.
3. Krikorian, A. D. and Steward, F. C. (1969) in *Plant Physiology* (Steward, F. C., ed.) Vol. 5B, p. 227. Academic Press, New York.
4. Tabata, M., Yamamoto, H., Hiraoka, N., Marumoto, Y. and Konoshima, M. (1971) *Phytochemistry* **10**, 723.
5. Ikuta, A., Syono, K. and Furuya, T. (1974) *Phytochemistry* **13**, 2175.
6. Hart, J., Woodcock, G. J. and Wilson, L. (1970) *Ann. Botany* **34**, 789.
7. Becker, H. (1970) *Biochem. Physiol. Pflanzen* **161**, 425.
8. Gupta, P. K. and Mascarenhas, A. F. (1983) *Basic Life Sci.* **22**, 299.
9. Watson, D. G. (1982) *Ph.D. Thesis*, University of London.
10. Brieskorn, C. H. and Beck, K. R. (1970) *Phytochemistry* **9**, 1633.
11. Murashige, T. (1974) *Ann. Rev. Plant Physiol.* **25**, 135.
12. Murashige, T. and Skoog, F. (1962) *Physiol. Plant.* **15**, 473.