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TERPENE EPOXIDASES AND EPOXIDE HYDRATASES FROM CULTURES OF *JASMINUM OFFICINALE*

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Key Word Index—*Jasminum officinale*; Oleaceae; tissue culture; enzymes; oxidative metabolism; terpenes.

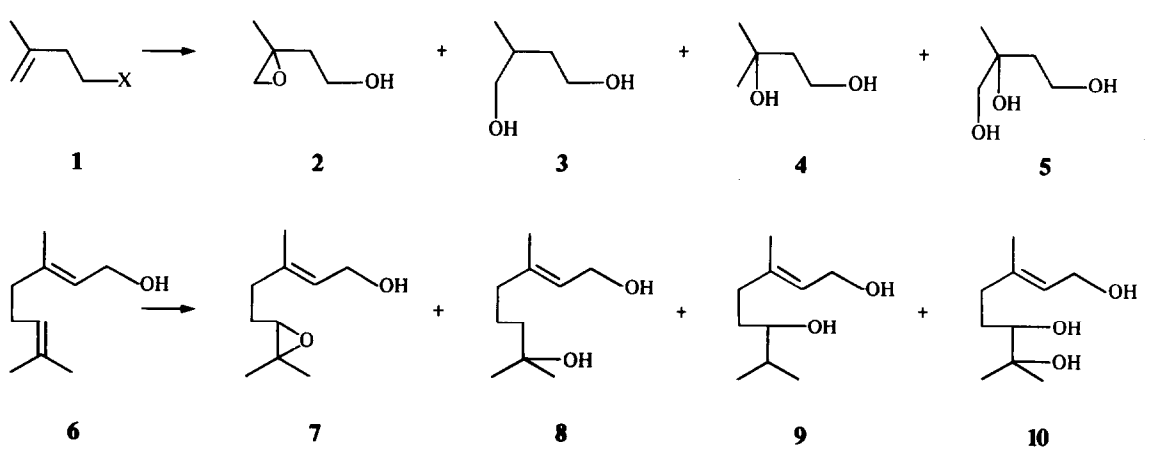
Abstract—Cell-free extracts from callus of *Jasminum officinale* contained epoxidase activities with isopentyl pyrophosphate, isopentenol, geraniol and nerol as substrates and also hydratase activities towards the resulting terpene oxides. The activities were up to 100-fold greater than those extractable from foliage of the plant.

INTRODUCTION

Epoxidase and epoxide hydratase activities towards pesticides [1, 2] and ethane [3] have been found in extracts from several plant species, and similar preparations that accept stilbene have been obtained from cultures of *Phaseolus vulgaris* [4]. Although epoxidation is a possible first step in the oxidative degradation of lower terpenoids [5], the only record of such enzymes that accept hemi- and monoterpenes refers to extracts of leaves of *Tanacetum vulgare* [6]. We here record the occurrence of the enzymes in callus cultures of *Jasminum officinale* L. (Oleaceae).

RESULTS AND DISCUSSION

Under optimum conditions, callus of *J. officinale* grew rapidly (sub-culture period *ca* 21 days) and was friable and greenish-white. Direct injection of material of various age and culture history with ^{14}C-labelled isopentenyl pyrophosphate (IPP; 3-methyl-but-3-enyl pyrophosphate), geraniol and nerol (3,7-dimethylocta-2,6-dien-1-ol; *trans* and *cis*, respectively), 3,3-dimethyallyl alcohol (3-methylbut-2-en-1-ol), isopentenol and the oxides of the last four compounds (that of IPP could not be prepared) and analysis after up to 10 days incubation provided no evidence for the products of reaction of epoxidases or epoxide hydratases. Similar results were obtained when the potential substrates were added to suspension cultures. However, incubation of IPP (**1**; X = OPPi) with cell-free extracts from callus (12–18 subcultures) gave conversions (under optimum conditions, see Experimental) into isopentenol oxide (**2**; 1.2%), the diols (**3**, **4**; 1.0%) and the triol (**5**, 3.1%) derived from isopentenol (see Scheme 1). Isopentenol (**1**; X = OH) gave conversions of 1.4, 0.9 and 1.2% respectively into these compounds. Geraniol and nerol (cf. for geraniol, **6**) were both converted 0.1, 0.3 and 0.3% into the analogous products functionalised at the 6,7-bond (**7**–**10**). Reproducibility was *ca* ±0.05%, actual value. Incorporations were acceptable, as compared with those typically obtained from extracts of higher plants sustaining secondary metabolism and they were significant.



Scheme 1. Oxidative metabolism of terpenoids.

Products were identified by comparison with authentic standards and all were purified by recrystallisation of derivatives (after addition of carrier), to constant specific radioactivity and the aliquots used for assay enabled meaningful counting statistics to be achieved (see Experimental). Thus the terpene epoxidase and epoxide hydratase activities were unequivocally demonstrated, but they appeared to be sequentially linked as addition of the terpene oxides (alone, or with a variety of prospective cofactors) gave no detectable products of ring-opening. Cell-free extracts prepared from leaves of *J. officinale* harvested in either June or December (there is a marked seasonal variation of epoxidase activity in *T. vulgare* [6]) gave interconversions of IPP into oxidised products some 100-fold lower than that obtained from the cultures.

Screening of the extracts from the cultures led to the following conclusions (for the metabolism of IPP): (i) Addition of Mg^{2+} or Mn^{2+} (up to 40 mM); NAD^+, $NADP^+$ (or their reduced forms), FAD, FMN (all 1 mM); EDTA or 2-CEPA (2 mM) were all without effect. (ii) Fe^{2+} (1 mM) increased the levels of diols and triols by up to 5-fold at the expense of the oxide. (iii) Cu^{2+} (1 mM) alone or in the presence of Fe^{2+} or Mg^{2+} inhibited (> 98%) all activity. (iv) Oct-1-ene (a potent inhibitor of epoxidases from liver [7]) was ineffective. (v) *p*-Aminobenzoic acid or PVP had no effect, although they are claimed to enhance the oxidation of pesticides [8]. (vi) Activity was lost at −20° after 48 hr.

Further attempts to demonstrate these novel enzyme systems using extracts from available cultures of *T. vulgare*, *Rosa damascena* and various *Mentha* species failed. The specificity of the epoxidases and hydratases that metabolise pesticides has not been recorded. Unfortunately, our attempts (cf. [4]) to establish cultures of the appropriate *Phaseolus* and *Pisum* species were unsuccessful. However, extracts of germinating seeds of *Phaseolus vulgaris* gave small but significant conversions of geraniol into its 2,3-oxide (*ca* 0.2%) and geraniol and nerol were converted into their 6,7-oxides and 6,7-diols (total *ca* 0.4%) by similar extracts from *Pisum sativum*. No ($< 10^{-4}$%) corresponding conversions of IPP could be demonstrated. These low levels of activity are in contrast with good (*ca* 5%) conversions of aldrin into dieldrin diol by the same homogenates from *P. vulgaris*. Either the enzymes that oxidase the pesticides do not appreciably accept the terpenes or derivatives of the latter (e.g. glucosides?) are required as substrates.

Although any enzyme involved in terpenoid metabolism that occurs in tissue cultures is so rare as to be interesting, the metabolic significance of these enzymes is uncertain. Vacuolar epoxidases and epoxide hydratases involved in xenobiotic metabolism are known [1–3] and such may be isolated here. On the other hand, there is some evidence that similar enzymes that accept terpenoids play a specific role in the metabolism of these substrates [6].

EXPERIMENTAL

[1–^{14}C]-Isopentenol and [1–^{14}C]-3,3-dimethylallyl alcohol (both 280 MBq/mmol), their pyrophosphates (148 MBq/mmol) and [2–^{14}C]-geraniol and nerol (7.4 MBq/mmol); all chemically (> 99% GLC; TLC) and radiochemically pure (> 99%), were prepared by reported methods [9]. Epoxides for standards were obtained by catalytic oxidation [10] or use of peroxyoctanoic acid [11]. Ring-opened products from these (again for authentic standards) resulted from treatment with H_2O_2–HCO_2H [12] or hydration of the parent alcohols [13]. Characterization and chromatographic and spectroscopic properties of all these (mainly new) compounds have been recorded [14, 15].

Leaves (5–10 cm) or internodal parts of stems of *J. officinale* (nodal points often developed shoots in culture) were explanted in agar (0.9% w/w) made up in a defined medium [16] adjusted (aq. Na_2CO_3) to pH 5.8 and supplemented with kinetin (0.5 mg) and coconut milk (10% v/v). Stem sections (*ca* 0.8 × 1 mm) were cut longitudinally and were scored on the curved surface. Circular leaf sections (10–25 mm^2) were similarly treated. After incubation (27°; natural illumination with protection from direct sunlight) for 50 days about 15% of explants developed callus. Coconut milk was weaned at first subculture (callus 0.5–1 cm^3; 160–180 days) and thereafter subculture was at 21 day intervals. Suspension cultures (100 rpm) were established on the same liquid medium under the same conditions and subculture period.

^{14}C-Labelled precursors (*ca* 1 kBq; 1–30 μg variously) solubilized if necessary with Triton X-100, (1:1, v/v) were injected into callus (2 sets and 2 controls; 15–21 day old cultures that had been subcultured for 2–22 cycles) and worked-up after 1–10 days. Products derived from IPP were extractable with difficulty from H_2O and were obtained by extraction of the solid material derived from addition of Plaster of Paris to the incubation soln as previously described in detail [6]. Those derived from the monoterpenes were extracted with Et_2O in a 3-step process [6]. In either case, the solvent was removed (0°) and the residue taken up in MeOH (0.5 ml), carrier added (5 mg), and the extract passed through a column of silica gel H (10 × 1 cm) with Me_2CO–toluene (1:1, v/v) as eluant when the relative elution volumes of the alcohols and epoxides were *ca* 1:2:6; the crude fractions were separated by TLC using the methods previously described in detail [6]. The purified products were identified by comparison of ^{1}H NMR and ^{13}C NMR spectra with those of authentic standards. Boiled-enzyme controls and controls under anaerobic conditions (Thunberg tubes) were carried out in all cases.

^{14}C-Labelled additives were introduced to suspension cultures that had passed through 15 cycles of callus culture and then six cycles of suspension culture. The cells were harvested and worked up after 15 days. Cell-free extracts (*ca* 0.3 mg/ml protein) from callus (10 g: ex. 12–18 subcultures) were prepared and incubated with substrates as described [6]. Seedlings of legumes (14–21 days after germination) were harvested and soluble fractions prepared [4]. Products from all feedings were purified and assayed as outlined above.

Radiochemical assays were made using aliquots containing 10^3 to 10^4 dpm; and 4×10^4 disintegrations were accumulated so that 2σ error was ±1%.

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THE IDENTITY OF THE IRIDOID GLUCOSIDE TARPHETALIN WITH IPOLAMIIDE

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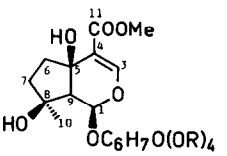
Key Word Index—*Stachytarpheta jamaicensis*; Verbenaceae; iridoid glucoside; tarphetalin; ipolamiide.

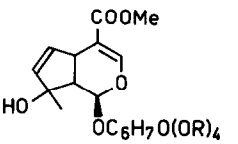
Abstract—^{1}H, ^{13}C NMR and MS data as well as chemical evidence show that the structure of tarphetalin should be corrected to that of ipolamiide.

As a part of our biosynthetic investigations on iridoid glucosides, we have studied the later stages in the biosynthesis of the main iridoid in *Stachytarpheta jamaicensis* (L.) Vohl (Verbenaceae). It has been reported [1] that *S. jamaicensis* contains ipolamiide (**1**), whereas later work [2] reports the occurrence of tarphetalin (**2**) as the main constituent of this plant. Other species of *Stachytarpheta* contain ipolamiide (**1**) [1, 3–5].

In the present work we isolated the glucosides from 123 g of *S. jamaicensis*. One iridoid glucoside **A** (635 mg, 0.5%) was obtained by reversed phase chromatography. The ^{1}H NMR spectrum (90 MHz, D_2O, DSS) of **A** was identical with that of authentic **1**. Some of its features are: δ 7.52 (*s*, H-3), 5.81 (*d*, $J_{1,9}$ = 0.8 Hz, H-1), 3.74 (*s*, CO_2Me), 2.49 (*d*, $J_{1,9}$ = 0.8 Hz, H-9), and 1.15 (*s*, 10-Me). The ^{13}C NMR spectrum of **A**: 169.0 (C-11), 153.0 (C-3), 113.8 (C-4), 99.2 (C-1′), 94.4 (C-1), 79.0 (C-8), 77.1 (C-5′), 76.1 (C-3′), 73.2 (C-2′), 71.3 (C-5), 70.4 (C-4′), 61.5 (C-6′), 60.6 (C-9), 52.6 (OMe), 39.4 (C-7), 37.9 (C-6), and 22.7 (C-10). The ^{13}C NMR spectrum of **1** has been published [4] but contains some misassignments. The physical data of **A** (given in Table 1) are virtually identical to those of **1** [6], and we thus conclude that *A* is ipolamiide (**1**).



R
1 H
1a Ac



R
2 H
2a Ac

As the physical data of **1** and **1a** are very similar to those of **2** and **2a** (Table 1) it was indicated that tarphetalin is identical with ipolamiide. The ^{1}H NMR (60 MHz,

Table 1. Physical data for **A**, **1**, **2**, **1a**, and **2a**

	A	**1** [6]	**2** [2]	**1a** [6]	**2a** [2]
mp	142–3°	144–5°	140–150.5°	173–174.5°	166.7°
$[\alpha]_D$	−139°	−136°	120.4°*	−107°	
	(*c* 1.2, MeOH)	(*c* 0.5, dioxane)	(*c* 1.16, EtOH)	(*c* 0.8, dioxane)	

*No sign is given for $[\alpha]_D$.