Phytochemistry, 1979, Vol. 18, pp. 1297–1299. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0801-1301 $02.00/0

REDOX INTERCONVERSION OF GERANIOL AND NEROL IN *ROSA DAMASCENA**

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(*Received* 19 *February* 1979)

Key Word Index—*Rosa damascena*; *Rosa dilecta*; Rosaceae; biosynthesis; geraniol; nerol.

Abstract—Use of ^{14}C, ^{3}H-labelled precursors revealed that flowerheads of *Rosa damascena* converted geraniol (3,7-dimethylocta-*trans*-2,6-dien-1-ol) into nerol (the corresponding *cis*-isomer) with loss of the *pro*-(1*S*) hydrogen, whereas the reverse isomerization involved loss of the *pro*-(1*R*) atom. The inference that the interconversion proceeded by redox reactions with the formation of the corresponding aldehydes was supported by the preparation of cell-free extracts from *R. damascena* and *R. dilecta* that sustained such processes. These reactions were $NADP^+$-NADPH dependent and had pH optima at 7.0 and 9.0, respectively.

INTRODUCTION

It is generally considered that geraniol (or its biogenetic equivalent) is an obligatory precursor for monoterpenes in higher plants. This can be converted into nerol, its *cis*-Δ^2 isomer (or the equivalent), which in turn can cyclize to form menthane derivatives and subsequently yield other monocyclic and bicyclic skeletons [1]. The mechanism of the crucial isomerization has been the subject of considerable speculation [1] and recently indirect evidence has been obtained that in certain *Tanacetum*, *Pinus*, *Mentha* and *Eucalyptus* species a redox process occurs involving the corresponding aldehydes [2]. We now report work on *Rosa damascena* cv. Versicolora and *R. dilecta* cv. Lady Seton (Rosaceae) to elucidate the nature of possible redox interconversions. Unlike the species previously studied, these roses accumulate geraniol and nerol (free or as β-glucosides) as the major monoterpenes in flowerheads [3, 4].

* Part 23 of the series "Terpene Biosynthesis". Reprints of this paper are not available. For Part 22 see Banthorpe, D. V., Modawi, B. M., Poots, I. and Rowan, M. G. (1978) *Phytochemistry* **17**, 1115.

RESULTS AND DISCUSSION

Double-label experiments

The isotope ratios in geraniol and nerol recovered after feeding flowerheads of *R. damascena* with geraniol-[^3H, ^{14}C] and nerol are presented in Table 1. The feedings (see Experimental) were carried out at a stage of opening of the buds which ensured maximum synthesis of monoterpenoids [5]. Our general procedure was to purify all biosynthetic products to constant specific radioactivity and isotope ratio by recrystallization (of derivatives, if necessary). Suitable derivatives (i.e. formed in good yield, readily crystallized and possessing good quenching properties) are not available for geraniol and nerol and it was not possible to oxidize the alcohols and derivatize the corresponding aldehydes as this would remove ^3H from C-1. Consequently, we purified both

Table 1. Isotope ratios in geraniol and nerol biosynthesized in *R. damascena*

Precursor	Product	$^3H:^{14}C$*		
		Precursor	Product	Acetate Derivative
(1*R*)-Geraniol-[^{14}C, 1-$^3H_1$]	Geraniol	1.00	0.96	0.97
	Nerol	1.00	0.98	0.96
(1*S*)-Geraniol-[^{14}C, 1-$^3H_1$]	Geraniol	1.00	0.98	0.97
	Nerol	1.00	0.01	0.02
(1*R*)-Nerol-[^{14}C, 1-$^3H_1$]	Geraniol	1.00	0.02	0.01
	Nerol	1.00	0.95	0.96
(1*S*)-Nerol-[^{14}C, 1-$^3H_1$]	Geraniol	1.00	0.92	0.94
	Nerol	1.00	0.92	0.93
Geraniol-[^{14}C, 1-$^3H_2$]	Geraniol	1.00	0.85	0.84
	Nerol	1.00	0.48	0.47
Nerol-[^{14}C, 1-$^3H_2$]	Geraniol	1.00	0.48	0.46
	Nerol	1.00	0.93	0.94

* Normalized values. In practice, $^3H:^{14}C$ in precursor varied from 1.32 to 2.11. Standard errors (estimated) for $^3H:^{14}C$ are ± 0.02; ^{14}C-radioactivity was typically 10^3–10^4 dpm and percentage incorporation of this isotope into product (via isomerization) was in the range 0.1–0.2%.

the alcohols and their acetates to constant radioactivity using GLC and TLC; the isotope ratios in products and the corresponding derivatives were essentially identical.

After the metabolism times chosen in our experiment, some 0.1 to 0.2% of the precursor was converted into its isomer and *ca* 90% of the original radioactive precursor was recovered. The conversion of geraniol and nerol involved the loss of the *pro*-(1*S*) hydrogen of the former; whereas the reverse reaction involved loss of the *pro*-(1*R*) hydrogen of nerol. The pattern was clear, but nevertheless the isotope ratios were uniformly lower than the theoretical value in the cases when strict stereospecificity of hydrogen-loss would lead to the ratio being preserved in products. These reductions (up to 16%) were outside the experimental error and can hardly reflect the reversibility of the overall redox process (with opposing stereospecificity in each direction) because of the low percentage conversions. They may be the result of (i) side processes comprising reversible formation of geranyl or neryl pyrophosphates with C—O cleavage involving inversion at C-1 and hence scrambling of the epimeric hydrogens at that atom (cf. Discussion in ref. [2]), or (ii) the reversibility of the formation of geranial from geraniol (and correspondingly for neral and nerol). All plant feedings were made on flowerheads in approximately the same physiological state of development over the course of two days in July, 1978, but the slight variations in isotope ratio may reflect unavoidable differences in levels of metabolism that could influence levels of oxidized and reduced coenzymes that in turn control the redox processes.

It has previously been shown that geraniol is converted into isothujone (*trans*-thujan-3-one) in *Tanacetum vulgare* (presumably with intermediate formation of nerol) with loss of its *pro*-(1*S*) hydrogen [2]; and also the conversion of 2-*trans*-farnesol and its *cis*-isomer in *Andrographis paniculata* shows the same stereospecificity for hydrogen loss in both forward and back reactions [6] as we have now found in *R. damascena*. In contrast, the conversion of 2-*trans*-farnesol into its *cis*-isomer in a fungus resulted in the loss of the *pro*-(1*R*) hydrogen [7]. However, there is recent evidence (based on preservation of $^{3}H:^{14}C$ ratios from mevalonate-[2-^{14}C, 5-$^{3}H_2$] in cyclic monoterpenes formed in several species) that an alternative non-redox route exists for the conversion of geraniol into nerol in higher plants [8].

Our present results are consistent with the suggestion that the sequence geraniol → geranial → neral → nerol exists in *Rosa* species [9]. Although we did not detect radioactive aldehydes, other workers have detected such compounds as presumed intermediates in the interconversions of farnesol in both higher plants and fungi [7, 10, 11]. Recently, an unexpected redox interconversion involving stereospecific loss of hydrogen from C-1 of farnesyl pyrophosphate has been discovered in *A. paniculata* [12]. Our results are consistent with the phosphate esters of geraniol and nerol being substrates for the redox reactions in *R. damascena*, but whereas in *A. paniculata* it makes good sense to have such an isomerase system since 2-*cis* farnesyl pyrophosphate is required for cyclization to other sesquiterpene skeletons [12], it is reasonable that *Rosa* species should have an isomerase system involving the alcohols as the latter are the main constituents of the oil and cyclic monoterpenes and sesquiterpenes only occur in negligible proportions.

Cell-free extracts

Acetone powders that could be reconstituted to display the enzymic activities recorded in Table 2 were prepared from both *R. damascena* and *R. dilecta*. A brief report has been published on the preparation of a similar system [13]. Conditions were not optimised to give maximum conversions, but it is clear that geraniol and nerol are oxidized by a $NADP^+$-dependent system at *ca* pH 9.0, whereas citral requires a NADPH-dependant system at *ca* pH 7.0. Terpene-β-glucosides, phosphates or pyrophosphates were not detected as products, nor was citronellol (2,3-dihydrogeraniol) although a reductase producing the latter has been reported in extracts of *R. damascena* [14]. These pH optima are close to those reported for a redox system interconverting geraniol and nerol in a *Citrus* species [15].

EXPERIMENTAL

Materials. Rosa cultivars were obtained from Messrs. Hillier (Winchester, Hants.) and were cultivated outdoors in central London. Flowerheads (5 cm stems) were harvested at the pre-perfect stage, 8 days after initial splitting of calyx [3, 5]. [^{14}C, ^{3}H]-Labelled geraniol and nerol were prepared as previously described [2].

Plant feeding. All feedings were carried out in duplicate over

Table 2. Redox activity in cell-free extracts of *R. damascena**

Substrate (0.5 μM)	Cofactor (0.5 μM)†	Products‡				
		pH 6.0	7.0	8.0	9.0	10.0
Geraniol	$NADP^+$	0.0	0.0	0.0	C (1.5)	0.0
Nerol	$NADP^+$	0.0	0.0	0.0	C (1.4)	0.0
Citral§	NADPH	0.0	G (2.7) N (1.2)	0.0	0.0	0.0

* Details of incubation, extraction, etc. are in Experimental. Similar results were obtained with *R. dilecta*.

† At pH 7.0, incubation of citral with NADPH + $NADP^+$ (1.5 μM; 0.15 μM) gave similar conversion, but with NADPH + $NADP^+$ (0.15 μM; 1.5 μM) the reduction was completely suppressed. At pH 9.0, incubation of geraniol with NADPH + $NADP^+$ (0.5 μM, 1.5 μM) gave similar conversion, but with NADPH + $NADP^+$ (1.5 μM, 0.15 μM) the oxidation was completely suppressed.

‡ % Conversion in standard incubation system at differing pH. Zero conversion signifies < 0.1%, if any, reaction. G = geraniol; N = nerol; C = citral.

§ Equilibrium mixture of geranial and neral (70:30).

2 days in July, 1978. Flowerheads were cut under sterile H_2O after sterilization (0.1 M NaOCl; EtOH) of the stem, and plastic tubing (1.0 cm) containing H_2O was tightly fitted over the cut end. The whole was then inverted and after the tube had nearly been emptied by transpiration, a soln of geraniol- or nerol-[^{14}C, ^{3}H] (10 mg, 0.5 μCi) solubilized by Tween 80 (20 mg) in Pi buffer (pH 7.0, 0.1 M, 0.3 ml) was added. After this soln was taken up, the tube was kept topped-up with Pi buffer for the duration of the experiment. Geraniol and nerol are known [3] to be rapidly metabolized in *Rosa* species and so a series of experiments were carried out to determine the period for maximum incorporation of tracer from exogenous geraniol or nerol into extractable products. This was found to be at *ca* 75 min after initial uptake of tracer.

Isolation of products. Flowerheads were ground in liq. N_2 and the powder was extracted with Et_2O (20 ml × 2; 36°/10 hr). The organic layer was separated and the residue was incubated with β-glucosidase (100 mg; as almond emulsin) in Ac buffer (pH 5.0, 0.1 M, 0.5 ml) at 37°/2 hr and extracted with C_6H_{12} (5 ml × 2). The organic extracts were dried (Na_2SO_4) and reduced in vol. (to 10 μl) in a slow stream of N_2 at 20°; controls showed that 15% of geraniol or nerol was lost during this procedure. The final product was then chromatographed (TLC) in turn on (*a*) Si gel H with Et_2O, and (*b*) Si gel H with EtOAc–C_6H_6 (15:85) to obtain geraniol and nerol; one spot, R_f 0.87 in (a); 0.33 in (b). Aliquots (5 μl) of each alcohol were then added as carrier and the alcohols were separated by GLC on 10% FFAP (2m × 0.5 cm; N_2 flow 60 μl/min) at 100° for 10 min followed by 100–150° for 30 min (R_t: geraniol 1.00; nerol 0.91). The components were collected in Et_2O at 0° and were separately rechromatographed on 10% Carbowax 20 M (2m × 0.5 cm; N_2 flow 60 ml/min) at 130° (R_t: geraniol 1.00; nerol 0.88). The products obtained were >98% pure (by GLC) and were assayed for radioactivity. Part of each purified product was diluted with carrier (5 μl) and converted (Ac_2O, 200 μl; Py, 200 μl; 20° for 12 hr) into its acetate. This was chromatographed on FFAP at 80° for 10 min followed by 80–150° for 70 min. The collected compounds (R_t: geranyl acetate 1.00; neryl acetate 0.91) were assayed as before.

Acetone powders [14]. Petals (60 g) were ground in liq. N_2 and stirred (10 min) with Me_2CO (300 ml) at −78°. The powder was filtered, extracted with Me_2CO (300 ml × 8) until washings were colourless, and then stirred (1 hr) with Et_2O (200 ml). After filtration and extensive pumping down at 0.1 mm Hg, the powder was dried over P_2O_5 *in vacuo* (2 days, −20°) and then stored in sterile tubes at −20°. Under these conditions, enzymic activities were retained for some weeks. Extraction of the powder (C_6H_{12}) and assay (GLC) showed no residual terpene to be present. The powder (1g) was reconstituted by stirring (20° for 4 min) with the appropriate buffer (0.1 M, 2 ml) containing 2-mercaptoethanol (1 mM) followed by centrifugation (10 000 *g* for 15 min). ^{14}C-Labelled geraniol, nerol or citral (the last made by MnO_2 oxidation of geraniol) (0.1 mg, 0.1 μCi) was then added to the supernatant (1 ml) and the mixture was solubilized (Tween 80, 1 mg). $NADP^+$ and/or NADPH were added at the concns shown in Table 2 and the soln was then incubated (27° for 1 hr) and afterwards extracted with Et_2O (1 ml × 2) following saturation with NaCl. After drying and concn, the soln was subjected to TLC on Si gel H with EtOAc–toluene (15:85); R_f: geraniol and nerol 0.33; citral 0.46. The compounds were then eluted and geraniol and nerol were separated by GLC on 10% Carbowax (2m × 0.5 cm) at 130° (R_t: geraniol 1.00, nerol 0.90). Na-MeS, Tris–HCl and borate buffers were used for the range pH 6.0–10.0. Boiled enzyme controls were carried out in all experiments.

Radiochemical methods. These were routine [2].

Acknowledgement—We thank the SRC for a studentship to I.P. and Dr. M. G. Rowan (Bath University) for advice.

REFERENCES

1. Banthorpe, D. V., Ekundayo, O. and Rowan, M. G. (1978) *Phytochemistry* **17**, 1111.
2. Banthorpe, D. V., Modawi, B. M., Poots, I. and Rowan, M. G. (1978) *Phytochemistry* **17**, 1115.
3. Francis, M. J. O. and Allcock, C. (1969) *Phytochemistry* **8**, 1339.
4. Ohno, Y. and Tanaka, S. (1977) *Agric. Biol. Chem.* **41**, 399.
5. Francis, M. J. O. and O'Connell, M. (1968) *Phytochemistry* **8**, 1705.
6. Overton, K. H. and Roberts, F. M. (1974) *Biochem. J.* **144**, 585.
7. Imai, K. and Marumo, S. (1974). *Tetrahedron Letters* 4401.
8. Suga, T., Shishibori, T. and Hirata, T. (1977) *Chem. Letters* 937.
9. Banthorpe, D. V., Francis, M. J. O. and Le Patourel, G. N. J. (1972) *Biochem. J.* **30**, 1045.
10. Chayet, L., Pont-Lezicka, R., George-Nascinento, C. and Cori, O. (1973) *Phytochemistry* **12**, 95.
11. Suzuki, Y. and Marumo, S. (1972) *Tetrahedron Letters* 5101.
12. Mackie, H. and Overton, K. H. (1977) *Eur. J. Biochem.* **77**, 101.
13. Dunphy, P. J. (1973) *Phytochemistry* **12**, 1512.
14. Dunphy, P. J. and Allcock, C. (1972) *Phytochemistry* **11**, 1887.
15. Potty, V. H. and Bruemmer, J. H. (1970) *Phytochemistry* **9**, 1003.