Phytochemistry, Vol. 22, No. 12, pp. 2723–2726, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

BIOSYNTHESIS OF DOLICHOLACTONE IN *TEUCRIUM MARUM*

ROMANO GRANDI, UGO M. PAGNONI, ADRIANO PINETTI and ROBERTO TRAVE

Istituto di Chimica Organica dell'Università, Via Campi 183, I-41100 Modena, Italy

(*Received* 11 *April* 1983)

Key Word Index—*Teucrium marum*; Labiatae; dolicholactone; monoterpene biosynthesis; iridane skeleton; hydride shift.

Abstract—Iridodial is a very efficient precursor of dolicholactone in *Teucrium marum*. In the biogenetic formation of the lactone ring a hydride shift from C-1 to C-10 is observed. Citronellol and its 10-hydroxy derivative are preferred as precursors with respect to the C-2/C-3 unsaturated analogues.

INTRODUCTION

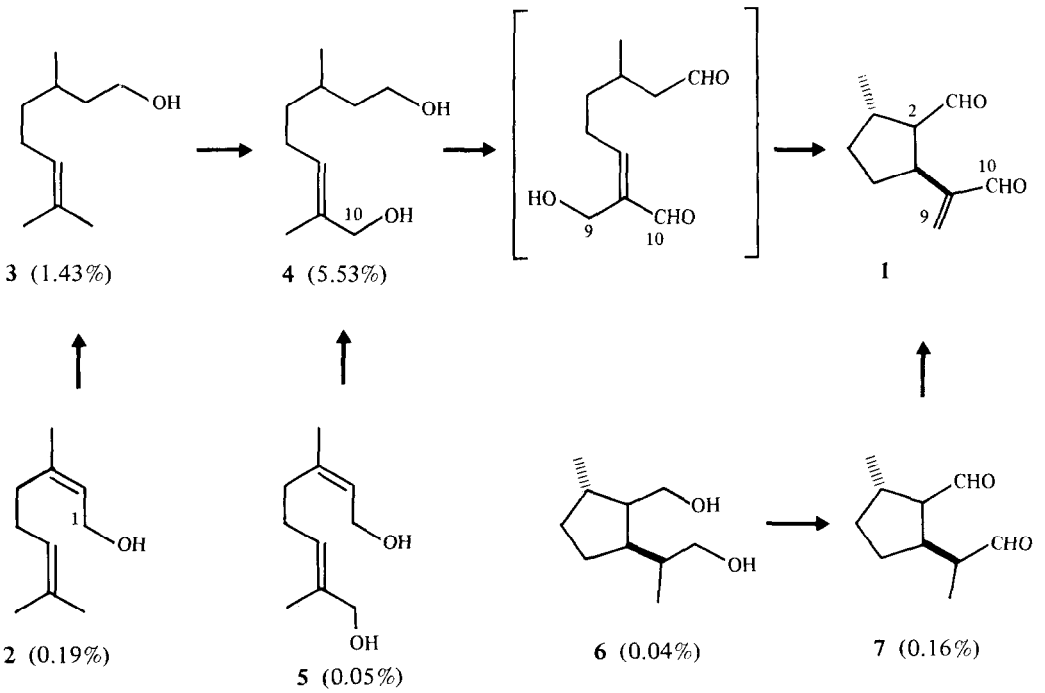
The main monoterpene component of *Teucrium marum*, a wild plant that grows in the Mediterranean area, is dolichodial (**1**) [1], a cyclopentane dialdehyde isolated as a mixture of epimers at C-2. Very recently [2], the biosynthesis of **1** was investigated and the following scheme (Scheme 1) was consistent with incorporation data (reported in parentheses).

With respect to the biogenetic pathway suggested [3,4] for iridoid glucosides and indole alkaloids, two important differences must be outlined: (i) C-9 and C-10 retain their non-equivalency; and (ii) the C-2/C-3 saturated acyclic monoterpenes seem to be better precursors than the unsaturated ones.

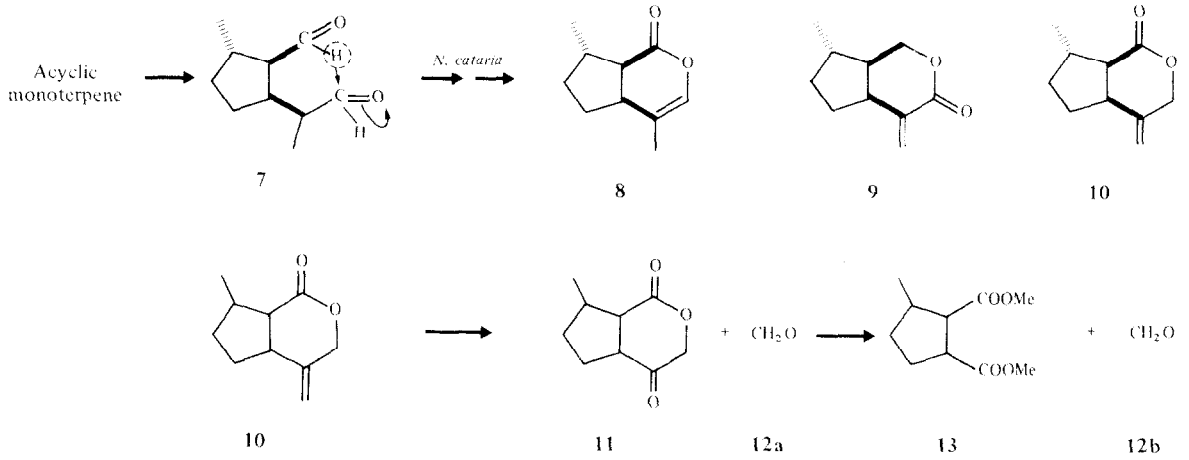
These features also hold for the biosynthesis [5] of nepetalactone (**8**) in *Nepeta cataria* plants. Whereas iridodial (**7**) cannot be considered to be a natural intermediate of dolichodial (**1**) (Scheme 1), at least in *T. marum*, it proved to be a key intermediate in the biosynthesis of nepetalactone (**8**) [5]. A hydride shift from C-1 to C-10 through a Cannizzaro-type reaction was the next important step to the bicyclic structure (Scheme 2).

RESULTS AND DISCUSSION

Besides dolichodial (**1**), we isolated [1] [6–7% of dolichodial (**1**)] from the *n*-hexane extracts of *T. marum* two cyclopentanoid monoterpene lactones, **9** and **10**



Scheme 1.



Scheme 2.

(Scheme 2) the latter being by far the more abundant (1 : 20).

It is possible to exclude that **9** and **10** are artefacts produced in the isolation procedure, because under many different alkaline conditions, dolichodial (**1**) affords [1] mixtures of these two lactones in a ratio opposite (15 : 1) to that found in the plant extracts. A biological Cannizzaro-like reaction in the plant may, however, favour the nucleophilic attack of water on C-1 and the subsequent displacement of a hydride ion towards C-10..

In order to understand the 'natural' origin of the main lactone **10** ('dolicholactone'), we fed to *T. marum* the same acyclic and cyclic compounds that we previously [2] administered in the biosynthetic study of dolichodial (**1**), e.g. [1-^{3}H]nerol (**2**), [1-^{3}H]citronellol (**3**) and their 10-hydroxy-[10-^{3}H] derivatives **5** and **4**, [10-^{3}H]iridodial (**7**) and [10-^{3}H]iridodiol (**6**). The localization of the radioactivity was determined by submitting the isolated dolicholactone (**10**) to the degradation procedure shown in Scheme 2.

The observed incorporation values are reported in Table 1 and suggest for the formation of dolicholactone (**10**) in *T. marum* a biogenetic pathway such as that outlined in Scheme 3.

The following main biogenetic aspects can be pointed out from the present administration experiments. (1) In keeping with analogous observations on the structurally related dolichodial [2] (**1**) (in *T. marum*) and nepetalactone [5] (**8**) (in *N. cataria*), (i) the C-2/C-3 saturated acyclic monoterpenes appear to be significantly better precursors with respect to the corresponding unsaturated ones, and (ii) the C-9/C-10 non-equivalency is retained. (2) Iridodial (**7**), which was poorly incorporated into dolichodial (**1**) [2], now proves to be an exceedingly efficient precursor

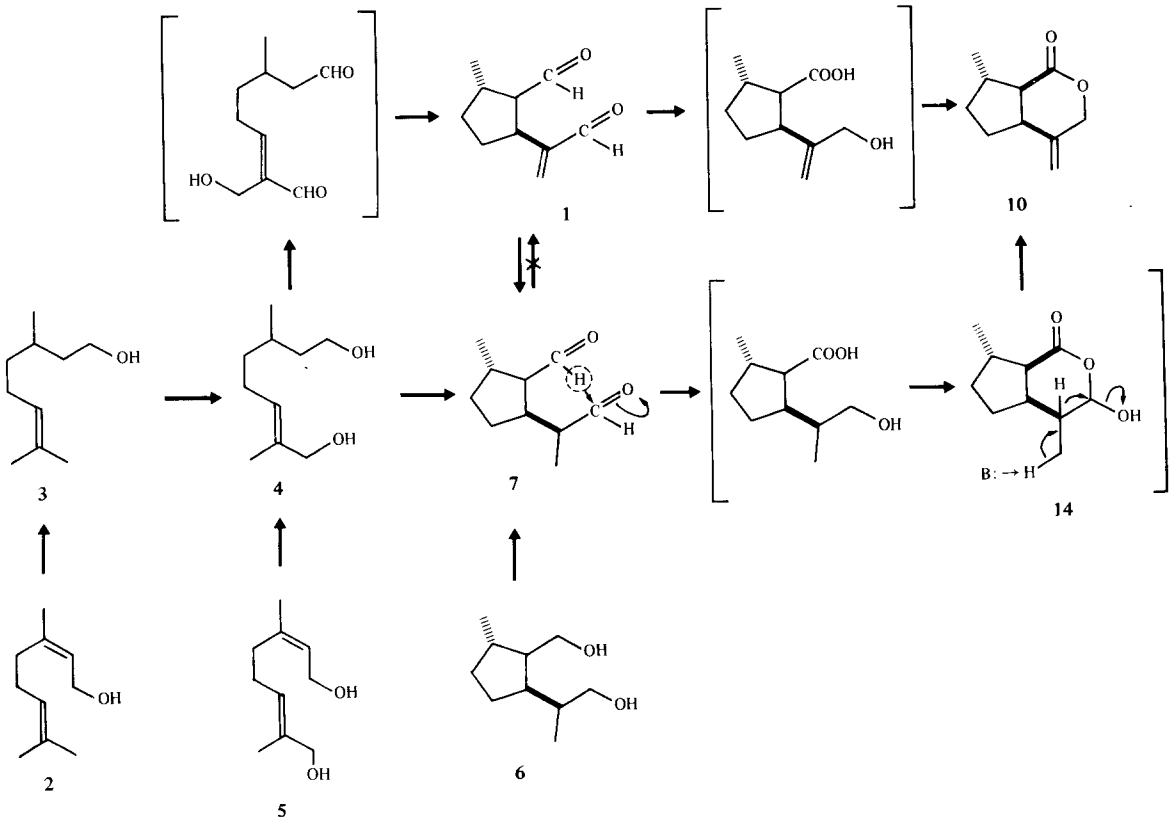
Table 1. Results of administration of labelled compounds to *T. marum*

Compound fed			Dolicholactone (**10**)			Degradation products Sp. act. (dpm/mM)* (radioactivity as % of total incorp. into **10**)			
	Tot. act. (dpm)*	Sp. act. (dpm/mM)*	Tot. act. (dpm)*	Sp. act. (dpm/mM)*	Incorp.† (%)	**11**	**12a**‡	**13**	**12b**‡
[1-^{3}H]-**2**	818	8115	0.014	0.088	0.007	0.085	—	0.002	0.084
						(96.6)		(2.3)	(95.5)
[1-^{3}H]-**3**	177	1694	0.057	0.222	0.13	0.219	—	—	0.225
						(98.7)			(101.3)
[10-^{3}H]-**4**	160	2203	0.284	1.644	0.71	1.614	—	0.025	1.609
						(98.2)		(1.5)	(97.9)
[10-^{3}H]-**5**	1855	15970	0.116	0.736	0.025	0.665	0.017	0.003	0.641
						(90.3)	(2.3)	(0.4)	(87.1)
[10-^{3}H]-**6**	2112	24712	0.238	1.650	0.045	1.605	0.005	0.045	1.584
						(97.3)	(0.3)	(2.7)	(96.0)
[10-^{3}H]-**7**	287	4360	5.580	31.643	3.89	32.023	—	0.127	31.295
						(101.2)		(0.4)	(98.9)

* $\times 10^4$.

† Incorporation values have been corrected for loss of 75 % for ^{3}H for all compounds listed except [10-^{3}H]-7, where a correction for 50 % loss was made.

‡ Isolated and purified as methylene-bis-dimedone.



Scheme 3.

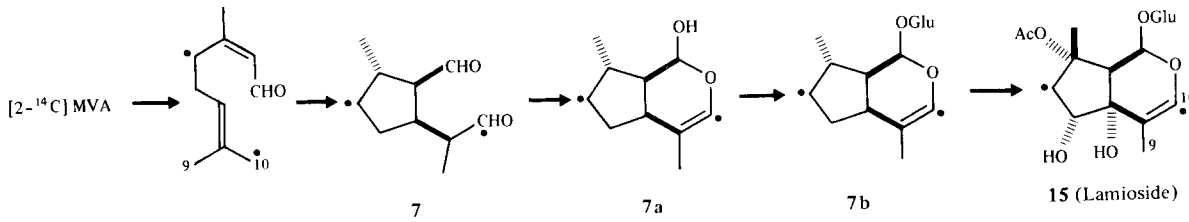
(see Table 1) of dolicholactone (**10**), very likely being the cyclization process product in its biosynthesis.

The retention of the C-9/C-10 non-equivalency and the high incorporation value of iridodial (**7**) are consistent with the pathway proposed (Scheme 4) by Inouye *et al.* [6] for the biosynthesis of iridoid glucosides (e.g. lamioside **15**) processed by some plants (e.g. *Lamium amplexicaule*). Iridodial (**7**) and glucoside **7b** of its lactol tautomer **7a** [7] were, in fact, proposed [6] as key intermediates in the biosynthesis of these glucosides, by contrast with 9-oxo-iridodial (**3a**) (likely formed from 9,10-dioxoneral) in the so far established biosynthetic route to loganin, secoiridoids and indole alkaloids. However, a hydride shift from C-1 to C-10 was observed on starting either from [1-^{3}H]-**2** or from [1-^{3}H]-**3**, which strictly parallels the analogous shift in the biosynthesis of nepetalactone (**8**) [5] in *N. cataria* plants (see Introduction). In both lactones **8** and **10**, therefore, the heterocyclic ring is not formed through oxidation of the lactol tautomer (**7a**) of iridodial, which at first sight appears to be a fairly obvious precursor, especially so in the case of nepetalactone (**8**); a biological Cannizzaro-like reaction seems, on the contrary, to be preferred for the lactonization process.

Hydroxydiidronepetalactone (**14**) is suggested as the closer intermediate to **10** in the biogenetic pathway from **7**; **14** has in fact been found [8] as a natural (minor) product in *N. cataria* plants, where it may represent the immediate biological precursor of nepetalactone (**8**).

As a chemical remark, it is noteworthy that, although nepetalactone (**8**) and dolicholactone (**10**) are very close olefinic isomers, their interconversion failed in our hands on using a variety of conditions, including those described [9] for the isomerization of simple enol-lactones into lactones of allylic alcohols.

The intermediacy of dolichodial (**1**) in a biosynthetic shunt cannot be excluded. In fact, even if the *in vivo* formation of **1** from iridodial (**7**) appears unlikely on the basis of previous experimental evidence, its biosynthesis from **4** has been well established [2]; a hydride shift from C-1 to C-10 in dolichodial (**1**) could produce dolicholac-



Scheme 4.

tone (**10**), as outlined in Scheme 3.

Double-labelled (^{14}C and ^{3}H) substrates could be useful in the elucidation of this particular point; in fact, in agreement with the pathways proposed in Scheme 3, it may be expected that the route through iridodial (**7**) requires the loss of half the label present on C-10 of dolicholactone (**10**), with respect to the route through dolichodial (**1**). Further work on this subject will involve the preparation of these substrates and their administration, together with [^{3}H]dolichodial (**1**), to *T. marum*. An attempt will also be made to try to trap labelled iridodial (**7**) after feeding labelled acyclic precursors.

EXPERIMENTAL

Plant material. T. marum plants were collected in July from a wild bush in northern Sardinia and contained, besides dolichodial (**1**) (0.65% wt/wt leaf and stem), the two isomeric unsaturated lactones (0.04%), dolicholactone (**10**) and allodolicholactone (**9**) (20:1).

Isotopic analysis. The radioactivity of compounds was determined by liquid scintillation (methylene-bis-dimedone) spectrometry and by GLC-gas proportional counting (a modified version of Packard, Mod. 894), using the experimental directions of refs. [2] and [5].

Preparation of labelled compounds. [1-^{3}H]nerol (**2**), [1-^{3}H]citronellol (**3**), 10-hydroxy[10-^{3}H]nerol (**5**), 10-hydroxy[10-^{3}H]citronellol (**4**), [10-^{3}H]iridodial (**7**), [10-^{3}H]iridodiol (**6**) and [7-^{3}H]pentacosane (sp. act. 7.18×10^{4} dpm/mg) were obtained as previously described [2].

Feeding procedure. For each administration expt, 7–10 stalks (15–18 cm long) of *T. marum* were cut under H_2O and the cut ends immediately immersed in aq. soln containing the labelled substrate and Tween 80 (1%) as an emulsifier. After 3 days, the immersed portions (1–2 cm) of the stems were cut away and the remaining plant material was extracted by percolation with *n*-hexane (100 ml). After evapn of the solvent and addition of cold dolicholactone (**10**) (30–45 mg) for dilution, silica gel chromatography using a *n*-hexane–Et_2O gradient afforded homogeneous (TLC) dolicholactone (**10**), which was distilled (80–82°/0.5 mm). An aliquot of the distilled product was dissolved in Et_2O (1–2 ml) and [7-^{3}H]pentacosane (7–12 mg) added. 3–8 μl of this soln was injected into the GLC proportional counter for simultaneous recording of mass and radioactivity peaks.

Degradation procedure. The purified (silica gel chromatography and distillation) dolicholactone (**10**) (25–35 mg) was ozonized in CH_2Cl_2 soln at −70°. After Me_2S addition to decompose the ozonide, H_2O extraction afforded HCHO which was transformed into the dimedone derivative (70% yield). The latter was repeatedly crystallized for liquid scintillation counting. From the CH_2Cl_2 phase the keto-lactone (**11**) was isolated and purified by silica gel prep. TLC; after addition of the int. standard ([7-^{3}H]pentacosane) to an aliquot of the compound dissolved in Et_2O, the soln was analysed by the radiogas-chromatograph. Remaining **11** was treated (1 hr) with 1 M KOH in MeOH–H_2O (3:1, 3 ml), and then with HIO_4. After 3 hr at room temp., HCHO was trapped with dimedone; the adduct was repeatedly crystallized and analysed by liquid scintillation counting. Acidification of the mother liquid and extraction with CH_2Cl_2 isolated a crude product which was esterified with excess CH_2N_2. The dimethyl ester (**13**) was purified by silica gel prep. TLC; cold **13** (55–60% yield from **10**) had: bp 82–85°/0.1 mm; IR (film) cm^{-1}: 1730; ^{1}H NMR (CCl_4): δ 1.10 (3H, *d*, $J = 6.5$ Hz, CH_3–C); 3.20 (2H, *m*, CH–COO) and 3.69 (6H, *s*, CH_3–O); GC/MS *m/z*: 200 $[M]^+$. After addition of int. standard, the ester was assayed for activity by radiogas-chromatography.

Acknowledgements—We thank the Consiglio Nazionale delle Ricerche, Rome, and the Ministero della Pubblica Istruzione for financial assistance.

REFERENCES

1. Pagnoni, U. M., Pinetti, A., Trave, R. and Garanti, L. (1976) *Aust. J. Chem.* **29**, 1375.
2. Bellesia, F., Pagnoni, U. M., Pinetti, A. and Trave, R. (1983) *Phytochemistry* **22**, 2197.
3. Escher, S., Loew, P. and Arigoni, D. (1970) *Chem. Commun.* 823.
4. Battersby, A. R., Brown, S. H. and Payne, T. G. (1970) *Chem. Commun.* 827.
5. Bellesia, F., Grandi, R., Pagnoni, U. M., Pinetti, A. and Trave, R. (1983) *Phytochemistry* (in press).
6. Inouye, H., Ueda, S. and Uesato, S. (1977) *Phytochemistry* **16**, 1669.
7. Cavill, G. W. K. and Ford, D. L. (1960) *Aust. J. Chem.* **13**, 296.
8. Sakan, T., Isoe, S., Hyenon, S. B., Katsumura, R., Maeda, T., Wolinsky, J., Dickerson, D., Slabaugh, M. and Nelson, D. (1965) *Tetrahedron Letters* **46**, 4097.
9. (1965) *Rodd's Chemistry of Carbon Compounds*, 2nd edn, pp. 272–273. Elsevier, Amsterdam.