Phytochemistry, Vol 23, No 1 pp 83–87, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

BIOSYNTHESIS OF NEPETALACTONE IN *NEPETA CATARIA*

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(*Received* 25 *March* 1983)

Key Word Index—*Nepeta cataria*, Labiatae, nepetalactone, iridane skeleton, monoterpene biosynthesis, hydride shift

Abstract—Iridodial is a key intermediate in the biosynthesis of nepetalactone One of the steps on the pathway prior to the lactonization is a hydride shift from C-1 to C-10 10-Hydroxycitronellol is a far more efficient precursor than the C-2/C-3 unsaturated analogue

INTRODUCTION

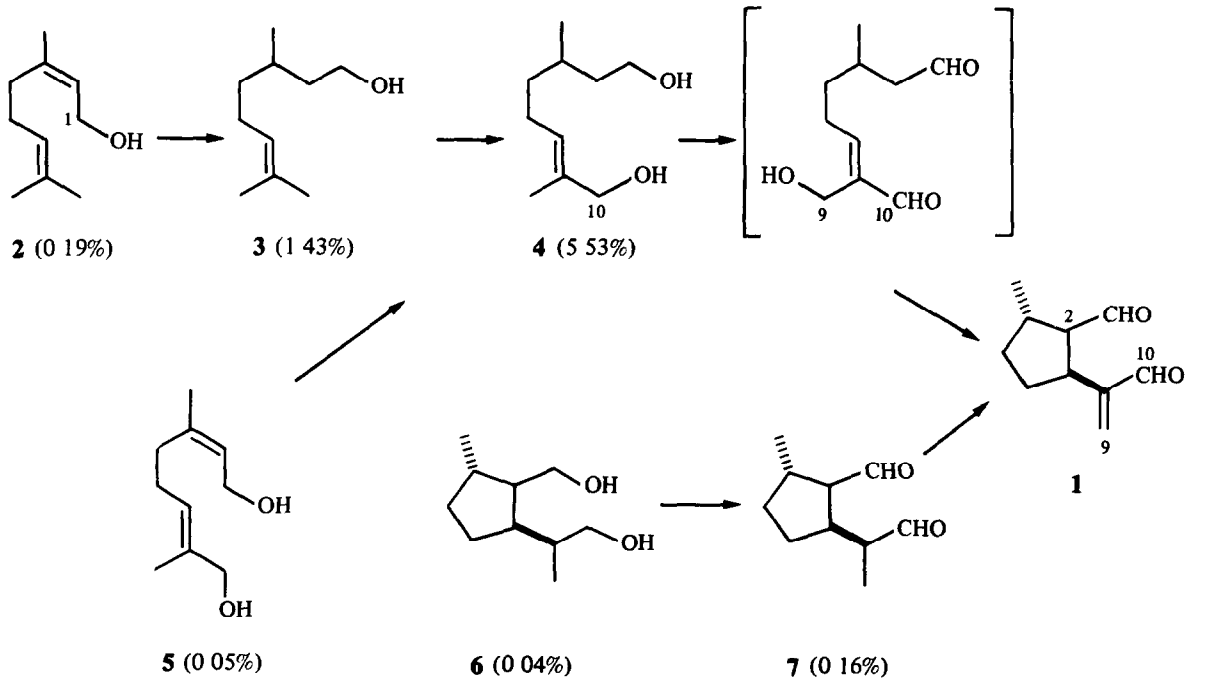
Very recently [1], we observed that the biosynthesis of dolichodial (**1**) [2] in *Teucrium marum* diverges from the biogenetic pathway suggested [3, 4] for iridoid glucosides and indole alkaloids in two important respects (a) the non-equivalency of C-9 and C-10 is retained, suggesting that the acyclic monoterpene involved in the ring-closure process has two different functions at the isopropylidene moiety, e g C-9 as an hydroxyl group and C-10 as an aldehyde group, and (b) the C-2/C-3 saturated acyclic monoterpenes seem to be preferred as precursors with respect to the unsaturated ones The results obtained with radio-labelled tracers is best accomodated by Scheme 1

On the basis of these results, a saturated iridodial(**7**)-like compound does not seem to be a natural intermediate of dolichodial(**1**), at least in *T marum*

RESULTS AND DISCUSSION

The retention of the non-equivalency of C-9 and C-10 and the preference for C-2/C-3 saturated acyclic monoterpenes as precursors, may represent a characteristic feature of plants producing mainly cyclopentanoid metabolites structurally much simpler than iridoid glucosides and indole alkaloids We decided, therefore, to investigate the biosynthesis of a related monoterpene, nepetalactone(**8**), in *Nepeta cataria* plants

A previous study [5] on the *in vivo* formation of nepetalactone(**8**) had demonstrated that (i) radioactivity from [2-^{14}C]MVA is incorporated (0 01 %) into **8**, and (ii) there is a randomization of label between C-9 and C-10 and also between C-4 and C-5 Our knowledge of the biogenesis of **8** was, therefore, largely incomplete and the randomization unexplained, although in part it was



Scheme 1 (The figures in parentheses are the incorporation values)

attributed to the long incubation period Iridodial(7) can be envisaged as a very close biogenetic precursor of nepetalactone(8), through an oxidation of its lactol tautomer (7a), which is present at equilibrium [6]

As we were mainly interested in the intermediates near the cyclization step, we administered to cut stalks of *N cataria*, the same substrates (Table 1), that we had previously fed to *T marum* in our study [1] of dolichodial biosynthesis In addition, [10-^{3}H]dolichodial(1) itself and its reduction product, [10-^{3}H]dolichodiol(9), were fed in a parallel series of experiments (Scheme 2) The distribution of the radioactivity in the isolated nepetalactone(8) was determined by chemical degradation of 8 to give 10 and 11 (Table 1) The results obtained indicate that the later steps in the biosynthesis of nepetalactone(8) in *N cataria* may proceed by a pathway like the one set out in Scheme 2

Thus, the sequence of acyclic precursors closely resembles the one suggested (Scheme 1) for dolichodial (1) biosynthesis, the mono-unsaturated compound (4) appearing as the more immediate precursor It is reasonable to suppose that a 1,10-dioxygenated intermediate, like 10-oxocitronellal, formed from 4, may give rise directly to a cyclopentane structure, very likely corresponding to iridodial(7)

A further important point arises from the almost complete recovery of label from C-10 of nepetalactone(8) labelled from either [1-^{3}H]nerol(2) or [1-^{3}H]citronellol(3) The hydrogen transfer from C-1 to C-10 may be explained by an intramolecular Cannizzaro-type reaction, which occurs smoothly by alkali treatment of both iridodial(7) [7] and dolichodial(1) [2], with production, on acidification, of saturated 14 and unsaturated 12 and 15 lactones, respectively

Even if the lactone 14 is the main product recovered *in vitro* from iridodial(7), the C-1 carbonyl group may be preferred as the hydride donor in the enzymatic system of the plant The effectiveness of 13 as a natural intermediate is supported by its isolation in some *N cataria* specimens [8] It can be pointed out how the above-mentioned, largely anticipated, derivation from iridodial(7) through an oxidation of its lactol tautomer(7a) and without hydride shift, does not correspond to the observed biosynthetic strategy for the formation of nepetalactone(8), at least in *N cataria*

Dolichodial(1), which is by far the main cyclopentane substance isolated from *T marum* [2], is incorporated in nepetalactone(8), but with significantly lower values In our opinion, this observation may merely mean that a route, which is normally of secondary importance in *N cataria*, is activated on introducing a closely related exogeneous product, such as dolichodial(1) The key steps of this route to nepetalactone(8) could be either the hydrogenation to iridodial(7) or a Cannizzaro-type reaction to dolicholactone(12), with a subsequent double bond shift (Scheme 2), such an olefinic isomerization would be, however, the reverse of what is observed *in vitro* on simple enollactones(16) [9], which in fact tend to change into lactones of allylic alcohols(17) However, our attempts to produce the C=C bond isomerization, on starting from either dolicholactone(12) and nepetalactone(8), failed on using either base or iodine catalysis, probably owing to the bicyclic structure of these substrates

Finally, it may be proposed that the dihydronepetalactones(18), which have been found also in extracts of *Actinidia polygama* [8], are biogenetically derived directly from 7, by a C-1 → C-10 hydride shift and lactonization of the resulting hydroxy acid

EXPERIMENTAL

Plant material N cataria plants were collected in July from a wild bush in Lombardia and contained about 0 1 % (w/w leaf and

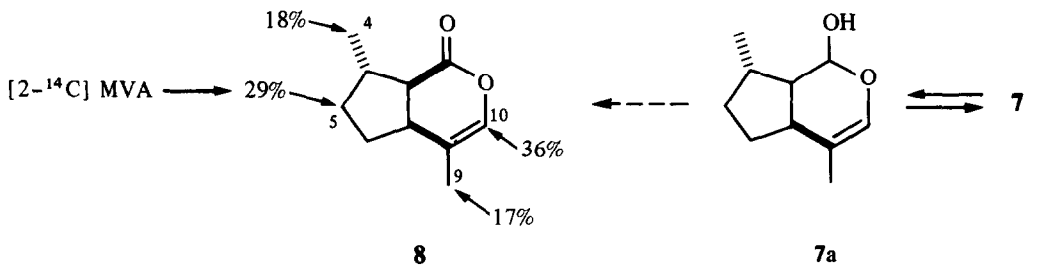


Fig 1

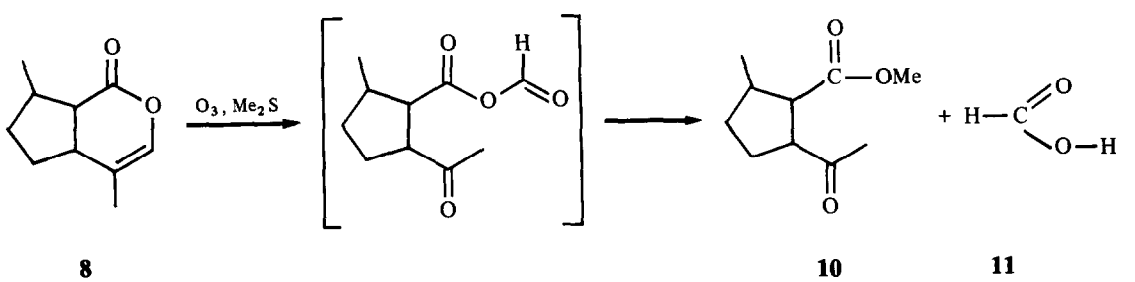


Fig 2

Table 1 Results of administration of labelled compounds to *N cataria*

Compound fed			Nepetalactone (**8**)			Degradation products (dpm/mmol)*	
	Tot act (dpm)*	Sp act (dpm/mmol)*	Tot act (dpm)*	Sp act † (dpm/mmol)*	Incorp ‡ (%)	**10**	**11**§
[10-^{3}H]-**1**	1149	6806	0 517	1 738	0 09	0 040 (2 3)‖	1 632 (93 9)‖
[1-^{3}H]-**2**	1745	7238	1 091	4 398	0 25	0 031 (0 7)	4 380 (99 6)
[1-^{3}H]-**3**	179	1201	0 058	0 221	0 13	0 005 (2 3)	0 206 (93 4)
[10-^{3}H]-**4**	157	980	1 103	3 990	2 81	0 072 (1 8)	3 699 (92 7)
[10-^{3}H]-**5**	4282	25 160	0 310	1 104	0 03	0 032 (2 9)	1 041 (94 3)
[10-^{3}H]-**6**	7709	47 644	0 385	1 124	0 02	0 035 (3 1)	1 035 (92 1)
[10-^{3}H]-**7**	620	4704	3 720	15 056	1 20	0 241 (1 6)	14 499 (96 3)
[10-^{3}H]-**9**	3385	28 220	0 068	0 215	0 008	0 009 (4 2)	0 193 (89 6)

* $\times 10^{-4}$

†After addition of cold nepetalactone (see Experimental)

‡Incorporation values have been corrected for loss of 75% for ^{3}H for all compounds listed except [10-^{3}H]-**1** and [10-^{3}H]-**7**, where a correction for 50% loss was introduced

§Isolated and purified as *p*-bromophenacyl ester

‖Radioactivity as % total incorporation

stem) of nepetalactone–epinepetalactone mixture (49 1)

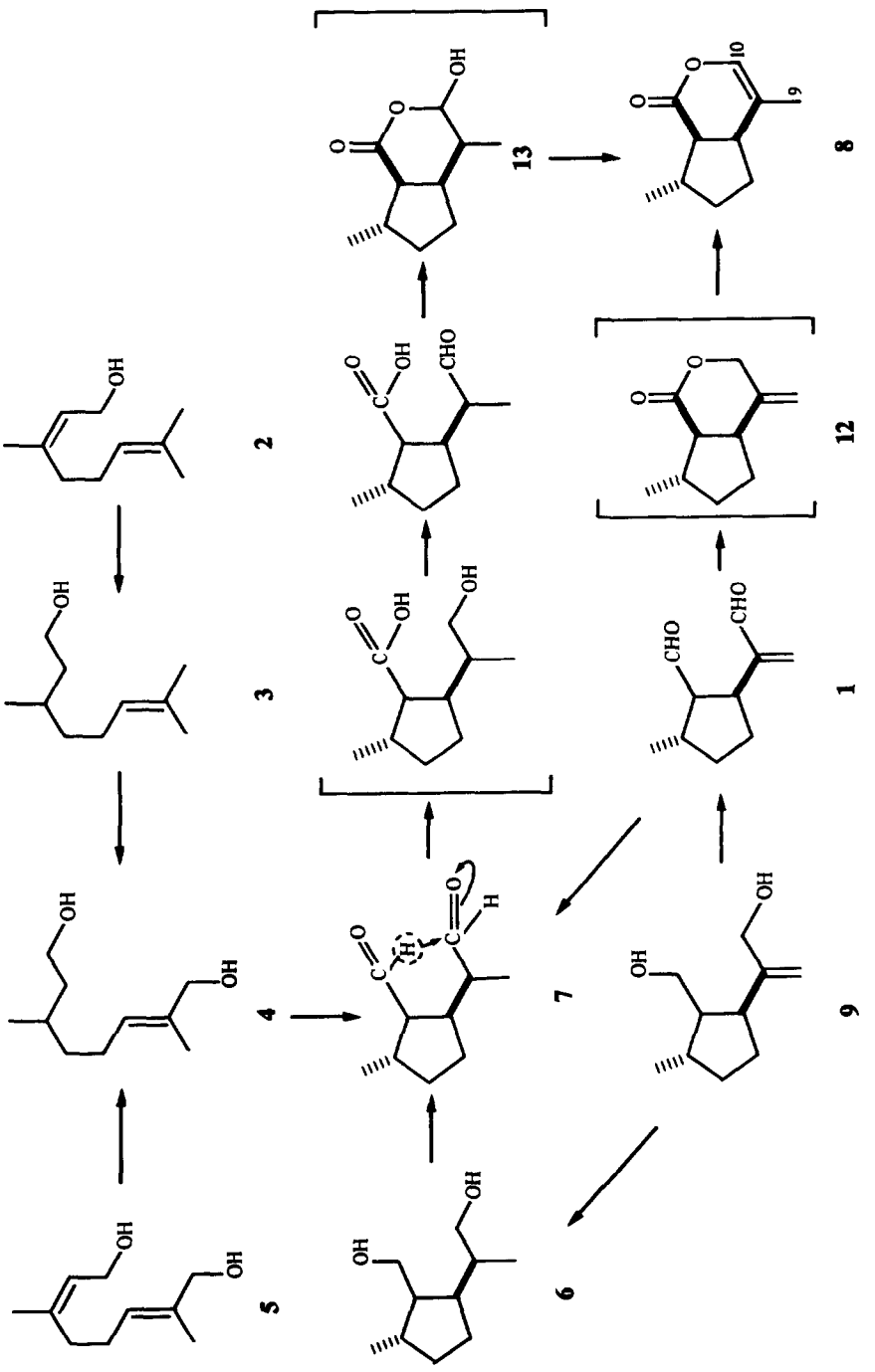
Isotopic analysis Two methods were used for determining the radioactivity of labelled compounds *p*-Bromophenacyl formate and [7-^{3}H]pentacosane (internal standard) were counted by liquid scintillation in Instagel (Packard) The other compounds were assayed for radioactivity in a gas proportional counter (Packard, Mod 894) suitably modified in our laboratory by placing the first (oxidizing) furnace directly into the GC oven, sample loss by polymerization was thus reduced to a minimum and efficiency for ^{3}H was 59% Absolute counting values were obtained by adding [7-^{3}H]pentacosane to homogeneous (TLC) isolated compounds and carrying out GC analysis by the internal standard method with the FID and the proportional counter detectors in parallel GC conditions glass column (2 5 m × 4 mm) of 1% OV-17, 2% OV-210 on Chromosorb W 80–100 mesh, carrier gas He 65 ml/min, temps oven 90° for 3 min, then to 230° at 10°/min, injector 260°, FID 250° Quenching gas for proportional counter propane at 6 ml/min Mass and radioactivity peaks were recorded simultaneously in a two channel instrument and areas were estimated by cutting out and weighing the peaks Calibration analyses were previously performed to calculate the relative (to internal standard) response factors

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**) and [10-^{3}H]iridodiol (**6**) were obtained as previously [1] reported [10-^{3}H]Dolichodial (**1**) was obtained from dolichodial-1-ethyleneacetal [2] as follows, (i) NaB^3H_4 in MeOH–H_2O (4 1), (ii) MnO_2 in *n*-hexane, (iii) Me_2CO–H_2O (2 1) and cat *p*-TsOH [10-^{3}H]Dolichodiol (**9**) was prepared from [10-^{3}H]-dolichodial (**1**) by $NaBH_4$ in MeOH–H_2O (4 1) Usual work-up afforded homogeneous (TLC, GC) intermediates which had consistent IR, NMR and mass spectra [10-^{3}H]-**1** and [10-^{3}H]-**9**, after silica gel chromatography and distillation, were radio-GC pure (> 99%) [7-^{3}H]Pentacosane was prepared as reported, sp act $7\,18 \times 10^4$ dpm/mg

Feeding procedure For each administration expt 7–9 stalks (15–20 cm long) of *N cataria* were cut under H_2O and the cut ends immediately immersed in the aq soln containing the labelled substrate and Tween 80 (1%) as an emulsifier After 3 days, the immersed portions (2–3 cm) of the stems were cut away and the remaining plant material extracted by percolation with $CHCl_3$ After the addition to the $CHCl_3$ extract of cold nepetalactone (40–60 mg) for dilution, silica gel chromatography using a *n*-hexane–Et_2O gradient afforded homogeneous (TLC, GC) nepetalactone (**8**), which was distilled (93–96°/0 5 mm) An aliquot (8–10 mg) was dissolved in Et_2O (1–2 ml), [7-^{3}H]pentacosane (10–12 mg) added and 5–8 μl of this soln was subjected to radio-GC

Degradation procedures (a) Nepetalactone (**8**) (20–25 mg), isolated as described in the feeding procedure, was ozonized in CH_2Cl_2 soln at −40° After Me_2S addition to decompose the ozonide, the solvent was evaporated under red pres The residue was treated with 0 5 ml H_2O at 60° for 90 min, then the soln was evaporated to dryness under vacuum Esterification with excess CH_2N_2 afforded the ketomethylester **10** Cold **10** (85–90% yield from **8**) had bp 96–99°/0 5 mm, IR (film) 1715 (*br*) cm^{-1}, ^{1}H NMR ($CDCl_3$) δ 0 93 (3H, *d*, Me–CH), 2 00 (3H, *s*, Me–CO), 2 50 (1H, *t*, CH–CO), 3 14 (1H, *dd*, CH–CO) and 3 52 (3H, *s*, Me–OCO), GC/MS *m/z* 184 $[M]^+$ After addition of the internal standard to an aliquot of **10** dissolved in Et_2O, the soln was analysed by radio-GC

(b) An aliquot of isolated nepetalactone (**8**) (20–25 mg) was ozonized as just described and treated with Me_2S The residue obtained after evaporation of the solvent was stirred with H_2O–EtOH (1 2, 1 ml) containing Na_2CO_3 (100 mg) for 30 min,



Scheme 2

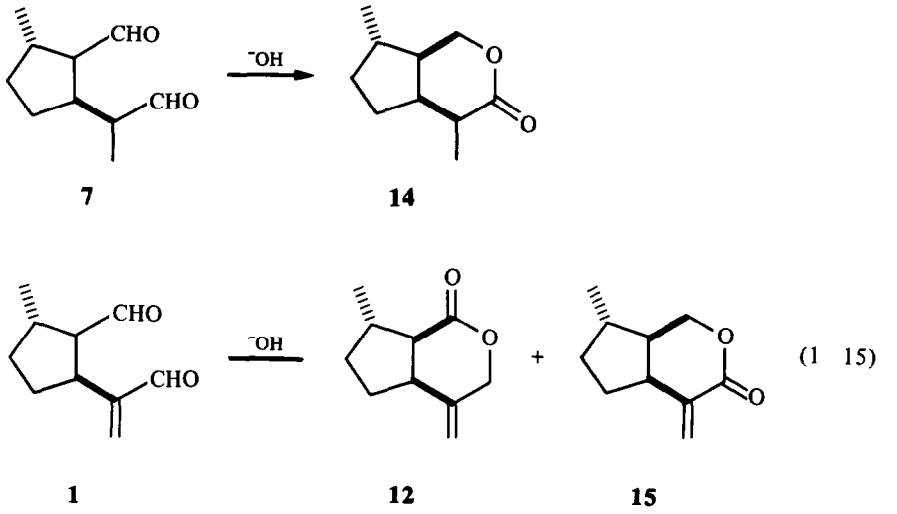


Fig 3

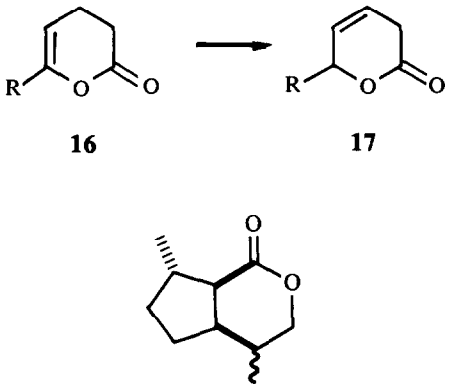


Fig 4

then *p*-bromophenacyl bromide (100–150 mg) was added and stirring continued until complete reaction (TLC monitoring) *p*-Bromophenacyl formate (GC/MS *m/z* 242 $[M]^+$ was isolated (75–84% yield from **8**) by extraction with CH_2Cl_2 and prep TLC (silica gel, Et_2O–*n*-hexane, 1 2) The isolated ester (**11**) was crystallized (mp 138–139°) from diisopropyl ether to constant sp radioactivity and then analysed by liquid scintillation counting

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

REFERENCES

1 Bellesia, F, Pagnoni, U M, Pinetti, A and Trave, R (1983) *Phytochemistry* **22**, 2197
2 Pagnoni, U M, Pinetti, A, Trave, R and Garanti, L (1976) *Aust J Chem* **29**, 1375
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 Regnier, F E, Waller, G R, Eisenbraun, E J and Auda, H (1968) *Phytochemistry* **7**, 221
6 Cavill, G W K and Ford, D L (1960) *Aust J Chem* **13**, 296
7 Clark, K J, Fray, G I, Jaeger, R H and Robinson, R (1959) *Tetrahedron* **6**, 217
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 *Rodd's Chemistry of Carbon Compounds* (1965) 2nd edn, pp 272–273 Elsevier, Amsterdam