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BIOSYNTHESIS OF THE SESQUITERPENOID AGERATRIOL

FRANCO BELLESIA, ROMANO GRANDI, ALESSANDRO MARCHESINI, UGO M. PAGNONI and ROBERTO TRAVE

Istituto di Chimica Organica, Università di Modena, 41100 Modena, Italy

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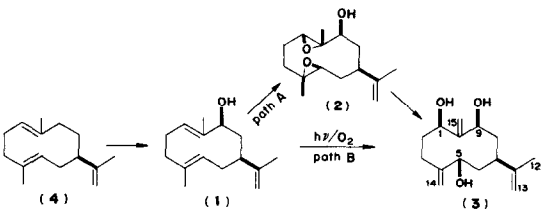
Key Word Index—*Achillea ageratum*; Compositae; germacrane sesquiterpene; terpenoids; mevalonic acid; epoxide rearrangement; biosynthesis.

Abstract—Ageratriol is biosynthesized from agerol through a diepoxide derivative. Mevalonic acid incorporation revealed that the formation of the isopropenylic double bond is not stereospecific.

INTRODUCTION

In previous papers [1,2] we put forward the hypothesis that ageratriol **3,** the main sesquiterpene [1,3] of *Achillea ageratum*, may be derived from (+)-germacrene A (**4**) by means of two biogenetic sequences which were supported by the discovery in the same plant of agerol **1** [4] and subsequently confirmed *in vitro* [2], namely:



Scheme 1.

RESULTS AND DISCUSSION

Although both biosynthetic processes (Scheme 1) thus appear possible, and although it has not as yet proved possible to isolate agerol diepoxide **2**,* as a first approach to the biosynthesis of ageratriol we tried path A, treating cut stems of *A. ageratum* both with labelled agerol and with its labelled diepoxide.

The radiochemical labelling of agerol 1 was effected by oxidation of **1** and subsequent reduction† with NaB^3H_4. [9–^{3}H]-agerol was fed to cut stems of the plant in full flower, then, after 8 days, the plants were extracted with $CHCl_3$. After dilution with unlabelled agerol diepoxide and ageratriol, the constituents were separated by repeated preparative TLC and purified by crystallization. The incorporations are given in Table 1.

The trapping of labelled agerol diepoxide enables one to conclude that path A (Scheme 1) of ageratriol biosynthesis is operative in the plant. Indeed, in a parallel experiment in which [13–^{3}H]-agerol diepoxide, obtained by ozonolysis of **2** and subsequent Wittig reaction with ^{3}H-labelled methyltriphenylphosphonium iodide, was directly administered, there was a significantly greater incorporation in ageratriol (Table 1).

The labelling sites were demonstrated by the following series of reactions. The labelled agerol diepoxide from [9–^{3}H]-agerol feeding was oxidized with CrO_3 in Me_2CO (Jones) at 0° to ketone **5,** which retains only 14% of the original radioactivity. The labelled ageratriol from the same experiment was acetylated, completely hydrogenated, saponified [1] and then oxidized with CrO_3 in acetone to the triketone **6**, which retains only 17% of radioactivity. The limited retention of radioactivity in **5** and **6** is probably due to randomization; on the other hand it was not possible to check the specificity of the labelling on the precursor **1,** because the corresponding ketone proved unstable (see Experimental) and **6** was destroyed by base treatment. Finally, the [13–^{3}H]-ageratriol from [13–^{3}H]-agerol diepoxide feeding was acetylated, ozonized to **7** and the

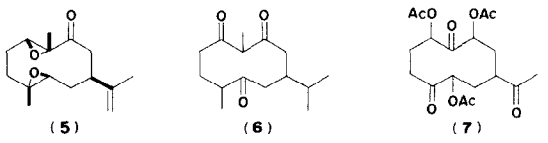
* According to TLC analysis of the crude $CHCl_3$ extract, the diepoxide **2** may be present in the plant, although in very small amounts.

† Reduction leads to a configuration of C-9 identical to that of the natural product (see Experimental).

Table 1. Incorporations and distribution of radioactivity in *Achillea ageratum*

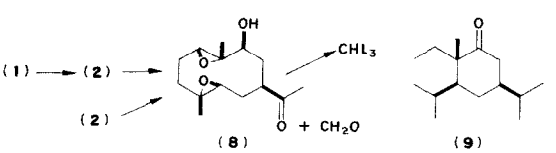
Precursor	Sp. act. 10^3, dpm/mM (Radioactivity, %) Natural products 1	2	3	Degradation products 5	6	7	8	9	CH_2O	CHI_3	Inc., %
		163 (100)		23 (14)							0·006
[9-^{3}H]-agerol (**1**)			33 (100)		5·6 (17)						0·002
[13-^{3}H]-agerol diepoxide (**2**)			6·9 (100)			0·7 (10)			6·1 (88)		0·014
	150 (100)						129 (86)		18 (12)	21 (14)	0·046
(±)-[2-^{14}C]-MVA		37 (100)					31 (84)		4·8 (13)	5·9 (16)	0·010
			10·5 (100)			8·9 (85)			1·7 (16)		0·004
	225 (100)							167 (74)			0·042
(±)-[2-^{3}H]-MVA		97 (100)		75 (77)							0·012
			42 (100)		31 (73)						0·004

formaldehyde recovered as the dimedone derivative (88% of radioactivity).



These findings prove that the sequence envisaged by us and confirmed *in vitro* by the base-promoted transformation of **2** into ageratriol is operative in the plant. However, the possibility that other biosynthetic sequences (e.g. that of photo-oxygenation, path B of Scheme 1) are involved, certainly cannot be excluded; there is ample precedence for the occurrence of more than one biosynthetic pathway to a natural product.

In view of the presence in the ageratriol molecule of the isopropenylic group, for which a lack of biosynthetic identity in the methyl and methylene groups has often been found, we fed [2-^{14}C]-MVA to cut stems of *A. ageratum*. The incorporations are listed in Table 1; the distribution of labelling in the C-12 and C-13 positions of agerol (14 and 12%, resp.) and agerol diepoxide (16 and 13%, resp.) was determined by conversion of **1** and **2** into norketone **8** and subsequent haloform reaction. In a parallel experiment in which [2-^{3}H]-MVA was fed as precursor, the percentage of radioactivity in the C-9 position of agerol **1** (26%) and its diepoxide **2** (23%) was determined by chemical transformation into **9** [5] and **5**, respectively.



Assuming that the methyl and methylenic groups were fully equilibrated, the calculated values for C-12, C-13 and C-9 would have been 16·7, 16·7 (^{14}C) and 21·4% (^{3}H), respectively*; these values are in good agreement with those found. As in the case of other biosynthetic processes, the incorporations of labelled MVA has thus revealed a lack of biosynthetic identity for C-12 and C-13.

* The results obtained for agerol and agerol diepoxide agree with those of some degradations of labelled ageratriol **3** from [2-^{14}C]-MVA and [2-^{3}H]-MVA. Very selective degradations were not possible; 27% (^{3}H) of the total radioactivity incorporated is localized at positions 9, 1 and 5 and 16% (^{14}C) at carbon atoms 13, 14 and 15 (see Experimental). On the basis of the biogenetic theories and of the previous findings the most of this radioactivity is likely to be localized at positions 9 and 13, respectively.

This equilibration, which is also observed in the biosynthesis of indole alkaloids [6] some iridoids [7] and sesquiterpenes [8], could not have predicted from current biogenetic theories. In the case of the sesquiterpenes of *A. ageratum*, this equilibration could occur during the cyclization of farnesol to the germacranic structure.

EXPERIMENTAL

Mp's were determined with a Mel-Temp apparatus and are uncorrected. NMR spectra were recorded at 60 MHz, with TMS as internal standard, chemical shifts have been recorded in δ values. All radioactive compounds were counted in a scintillation counter (Packard 314EX-2) with the samples dissolved in Instagel (Packard, 10 ml); the relative efficiencies were obtained by counting ^{3}H- and ^{14}C-toluene as internal standards.

Feeding procedure. The stems of *Achillea ageratum* from Sardinia (Italy) were cut under water [9] and immersed in the aq. soln containing the labelled precursor and Tween 80. After 8 days the stems were extracted with $CHCl_3$ and the inactive materials added to the crude extracts.

Synthesis of [9-^3H]-*agerol.* Ac_2O (0·8 ml) was added dropwise to agerol **1** (140 mg) in dry DMSO* (3·2 ml) and the mixture allowed to stand for 12 hr at room temp. After extraction with *n*-hexane (3 × 8 ml) and evaporation of the solvent, the residue (92 mg) was dissolved in aq. MeOH (95%, 4 ml) and treated with excess $NaBH_4$. Usual work-up afforded the alcohol **1** which was purified by preparative TLC and final distillation (44 mg), $[\alpha]_D^{20}+11{\cdot}4°$ (*c* 2 MeOH). The IR and NMR spectra were identical with those of an authentic sample [4].

The above procedure was repeated using NaB^3H_4 (0·6 Ci/mM), obtaining [9-^{3}H]-agerol (6 × 10^9 dpm/mM).

Incorporation of [9-^3H]-*agerol.* After feeding (8 mg, 0·3 × 10^9 dpm), the crude $CHCl_3$ extract of the stems was diluted with inactive agerol diepoxide and ageratriol (30 and 50 mg, respectively). Pure labelled compounds were isolated by preparative TLC and purified by crystallization.

The agerol diepoxide (27 mg, 16·3 × 10^4 dpm/mM, 0·006% inc.) was subjected to Jones oxidation at 0°. The crude ketone **5** was purified by preparative TLC and crystallization (diisopropyl ether), (20 mg 2·3 × 10^4 dpm/mM), mp 191–3°, $[\alpha]_D^{20}$ −64·4° (*c* 0·4 MeOH). IR (nujol): 1710 cm^{-1} NMR ($CDCl_3$): 1·37, 1·62 (3H each, s, Me–C–O), 1·80 (3H, *s*, Me–C=), 2·9–3·3 (2H, *m*, H—C(O)—C) and 4·8 δ (2H, br. *s*, CH_2=C). (Found: C, 71·83; H, 8·94. $C_{15}H_{22}O_3$ requires: C, 71·96; H, 8·86%).

The ageratriol (46 mg, 3·3 × 10^4 dpm/mM, 0·002% inc.) was acetylated, hydrogenated and then saponified to hexahydroageratriol [1], which was subjected to Jones oxidation. The triketone **6** (mp 40–42°; IR (nujol): 1700 cm^{-1}, NMR ($CDCl_3$): 0·8–1·25δ (12H, Me–C), was purified by repeated preparative TLC (18 mg, 0·56 × 10^4 dpm/mM). (Found: C, 71·60; H, 9·39. $C_{15}H_{24}O_3$ requires: C, 71·39; H, 9·58%).

Synthesis of [13-^3H]-*agerol diepoxide.* The agerol diepoxide **2** (246 mg) in CH_2Cl_2 (20 ml) was ozonized (at −70°) in the presence of tetracyanoethylene (TCNE) (107 mg). Usual work-up afforded the crude ketone **8** which was purified by preparative TLC and crystallization (diisopropyl ether) (120 mg), mp 186–8°; IR (nujol): 3450 and 1710 cm^{-1}; NMR ($CDCl_3$): 1·27 (3H, *s*, Me–C–O); 1·33 (3H, *s*, Me–C(O)), 2·24 (3H, *s*, Me–C=O), 2·65 (1H, *d*(*J* 8·5 Hz), H—C(O)—C), 2·93 (1H, *d* (J 9·7 Hz), H—C(O)—C) and 3·25 δ (1H, *dd*, H–C–O). (Found: C, 66·02; H, 8·84. $C_{14}H_{22}O_4$ requires: C, 66·11; H, 8·72%).

Dry DMSO (0·3 ml) was added dropwise to NaH (28 mg, 80% oily dispersion), previously washed with *n*-pentane under N_2, then the mixture warmed at 75–80° for 40 min. After cooling to 0°, $(C_6H_5)_3PC^3H_3I$ (378 mg, 3 × 10^7 dpm/mM) in DMSO (1 ml) was added dropwise. The red solution was stirred for 10 min, the ketone **8** (58 mg) added and the mixture allowed to stand at room temp. for 5 hr under stirring. After addition of water (5 ml), extraction with Et_2O and evaporation of the solvent afforded the crude [13-^{3}H]-agerol diepoxide **2** which was purified by preparative TLC and crystallization (29 mg, mp 186°; 44 × 10^7 dpm/mM) identified by mmp, IR and NMR spectra [2].

Incorporation of [13-^3H]-*agerol diepoxide.* After dilution with inactive material (100 mg), the ageratriol (**3**) (96 mg, 6·9 × 10^3 dpm, 0·014% inc.) from the feeding experiment using [13-^{3}H]-**2** (10·9 mg, 1·9 × 10^7 dpm) as precursor was acetylated and then ozonized in the presence of TCNE. The formaldehyde, recovered as dimedone derivative (6·1 × 10^3 dpm/mM), was separated (preparative TLC) from the triketone **7** (0·7 × 10^3 dpm/mM), bp 130–2°/0·05 mm; IR (film): 1730–1700, 1250 cm^{-1}; NMR ($CDCl_3$); 2·08 (3H, *s*, Me–COO), 2·10 (6H, *s*, Me–COO), 2·22 (3H, *s*, Me–C=O) and 4·6–5·6 δ (3H, *m*, H–C–O). (Found: C, 56·43; H, 6·11. $C_{18}H_{24}O_9$ requires: C, 56·24; H, 6·29%).

Incorporation of sodium (±)-[2-^{14}C]-*mevalonate* (26 × 10^7 dpm). Inactive agerol (**1**), agerol diepoxide (**2**) and ageratriol (**3**) (101, 92 and 131 mg, respectively) were added to the crude $CHCl_3$ extract before preparative TLC separation. (a) The labelled agerol (**1**) (87 mg, 15 × 10^4 dpm/mM, 0·046% inc.) was transformed into the diepoxide **2** and then ozonized. The formaldehyde was recovered as dimedone-derivative (12 mg, 1·8 × 10^4 dpm/mM); the ketone **8** (48 mg, 12·9 × 10^4 dpm/mM) was treated with I_2/OH^- [10] and the CHI_3 recovered (9 mg, 2·1 × 10^4 dpm/mM). (b) The labelled agerol diepoxide (**2**) (89 mg, 3·7 × 10^4 dpm/mM, 0·010% inc.) was ozonized in the presence of TCNE and the formaldehyde separated as dimedone derivative (17 mg, 0·48 × 10^4 dpm/mM) from the ketone **8** (61 mg, 3·1 × 10^4 dpm/mM). **8** was then treated with I_2/OH^- and the CHI_3 recovered (13 mg, 0·59 × 10^4 dpm/mM). (c) The labelled ageratriol (**3**) (124 mg, 1·05 × 10^4 dpm/mM, 0·004% inc.) was acetylated and then ozonized in the presence of TCNE. The formaldehyde–dimedone compound (19 mg, 0·17 × 10^4 dpm/mM) and the triketone **7** (88 mg, 0·89 × 10^4 dpm/mM) were separated by preparative TLC and further purified by crystallization and distillation, respectively.

Incorporation of sodium (±)-[2-^3H]-*mevalonate* (0·8 × 10^9 dpm). (a) After diln, the labelled agerol (**1**) (22·5 × 10^4 dpm/mM) was transformed into β-elemen-9β-ol, then hydrogenated and oxidated to **9** [5] (16·7 × 10^4 dpm/mM). (b) After diln, the labelled agerol diepoxide (**2**) (9·7 × 10^4 dpm/mM) was oxidated, as previously described, to the ketone **5** (7·5 × 10^4 dpm/mM). (c) After diln, the labelled ageratriol (**3**) (4·2 × 10^4 dpm/mM) was acetylated, hydrogenated, saponified [1] and then oxidated, as previously described, to **6**, which was isolated and purified by preparative TLC (3·1 × 10^4 dpm/mM).

* More than 20 standard oxidation procedures failed to give the expected ketone.

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REFERENCES

1. Garanti, L., Marchesini, A., Pagnoni, U. M. and Trave, R. (1972) *Tetrahedron Letters* **15**, 1397.
2. Bellesia, F., Pagnoni, U. M. and Trave, R. (1974) *Tetrahedron Letters* **14**, 1245.
3. Grandi, R., Marchesini, A., Pagnoni, U. M., Trave, R. and Garanti, L. (1974) *Tetrahedron*, **30**, 3821.
4. Grandi, R., Marchesini, A., Pagnoni, U. M. and Trave, R. (1973) *Tetrahedron Letters* **20**, 1765.
5. Grandi, R., Marchesini, A., Pagnoni, U. M. and Trave, R. (1972) *Phytochemistry* **11**, 3363.
6. Battersby, A. R., Brown, R. T., Kapil, R. S., Plunkett, A. O. and Taylor, J. B. (1966) *Chem. Commun.* 46.
7. Coscia, C. J. and Guarnaccia, R. (1967) *J. Am. Chem. Soc.* **89**, 1280; Inouye, H., Ueda, S. and Nakamura, Y. (1967) *Tetrahedron Letters* **33**, 3221.
8. Biollaz, M. and Arigoni, D. (1969) *Chem. Comm.* 633.
9. Loewus, F. and Baig, M. M. (1970) *Methods Enzymol.* **18**, 22.
10. Grandi, R., Pagnoni, U. M., Trave, R. and Garanti, L. (1974) *Tetrahedron*, **30**, 4037.