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BIOSYNTHESIS OF ARTEMISIA KETONE IN HIGHER PLANTS*

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Key Word Index—*Artemisia annua; Santolina chamaecyparissus; Chrysanthemum cinerariaefolium;* Compositae; monoterpene biosynthesis; artemisia ketone.

Abstract—MVA-[2-^{14}C], IPP-[4-^{14}C] and DMAPP-[4-^{14}C] were incorporated (optimum 0.04%–0.8%) into artemisia ketone by *Artemisia annua* in a position-specific manner so that the C-5 moiety not containing the carbonyl group was preferentially (87–95%) labelled. IPP and DMAPP, but not MVA, were similarly utilised in *Santolina chamaecyparissus*. Feeding of geraniol-[2-^{14}C] to *A.annua* resulted in artemisia ketone being labelled in a position indicating extensive degradation of the precursor. ^{14}C-labelled *cis* and *trans*-chrysanthemyl alcohols and chrysanthemates or DMVC were negligibly ($< 5 \times 10^{-4}$%) incorporated into artemisia ketone in both species over a range of feeding conditions. (+)-*trans*-Chrysanthemyl alcohol-[Me^{14}C] was an effective (*ca* 2% incorporation) precursor of the terpenoid part of pyrethrins I and II in flowers of *Chrysanthemum cinerariaefolium* but ^{14}C-labelled artemisyl alcohol (3, 3, 6-trimethylheptan-1, 5-dien-4-ol) or (±)-*cis*-chrysanthemyl alcohol were not detectably incorporated. Although some of the negligible incorporations are probably attributable to compartmentation effects preventing access of precursors to biosynthetic sites, the experiments indicate some limitation of the previously proposed pathways of biogenesis of artemisia ketone and related irregular monoterpenes.

INTRODUCTION

Artemisia ketone (**1**) is probably the most widely distributed monoterpene of the irregular type that does not obey the classical isoprene rule [1, 2]. Moreover, its skeletal-type does not fit into the 'biogenetic' isoprene rule [3, 4] (i.e. that all monoterpenes are derived from geranyl pyrophosphate or its immediate isomers) in any obvious manner. An attractive suggestion [5] is that the artemisyl (**2**) and related irregular skeletons result from cleavage of 1*R*,3*R*-(+)-*trans*-chrysanthemyl alcohol (**3**) which has recently been discovered in nature [6]. **3** is an analogue of presqualene alcohol and prephytoene [13], but the proposed scissions leading to **2** and related skeletons have no counterpart in those leading to the corresponding tri- or tetra-terpene. Furthermore, nothing is known of how **3** is biosynthesised. Other proposals are that the skeleton **2** results from (a) condensation of two DMAPP molecules [7] or their allylic isomers [8] perhaps by means of a sigmatropic rearrangement at a sulphonium centre of an enzyme [9]; (b) cleavage of car-3-ene (**4**) or a cyclopropane derivative (**5**) derived from linalol [10, 11], or (c) from Wagner–Meerwein rearrangement of an ion (**6**) derived from linalol [12]. A proposal [3] that the artemisyl skeleton could be derived from appropriate cleavage of the fenchane skeleton is unlikely on biochemical grounds and has never been developed.

No experimental tests of any of these proposals have appeared. Model systems have demonstrated the formation of **2** and other irregular skeletons from chrysanthemyl compounds [14–17] and from C-5 precursors [9, 18–19] but the relevance of these (involving exotic leaving groups and reaction conditions) to the situation *in vivo* is far from obvious. We now describe studies on the biosynthesis of artemisia ketone in foliage of *Artemisia annua L.* and *Santolina chamaecyparissus L.* (both Compositae). Some results have appeared in preliminary form [20].

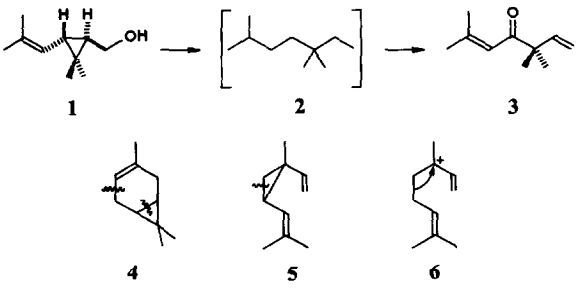

Fig. 1. Possible intermediates in biogenesis of artemisia ketone.

RESULTS AND DISCUSSION

Biosynthesis of artemisia in A. annua

Our specimens yielded a steam-volatile oil (*ca.* 3.0% w/wet wt. foliage) containing 30–40% (from different batches) of artemisia ketone. A typical analysis was: artemisia ketone 36.2, borneol 19.5; pinocamphone

*Part 16 of series "Terpene Biosynthesis". For part 15, see D. V. Banthorpe, E. Cardemil and M. del Carmen Contreras, (1976) *Phytochemistry* **15**, 391.

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‡Abbreviations used: MVA, mevalonic acid; IPP, isopentenyl pyrophosphate; DMAPP, 3,3-dimethylallyl pyrophosphate; DMVC, 3-methylbutyl-1-en-3-ol (dimethylvinylcarbinol).

14.5; 1:8-cineole 10.3; isothujone 8.6; α-pinene 7.1; β-pinene 1.0; isoartemisia ketone 1.0 and sesquiterpenes 0%: this represents an almost unique selection of skeletal types for one species. Artemisia ketone was shown (NMR) to have the structure **1**. This has been previously demonstrated [21] but the original conclusion [22] that it is the 1,6-diene is still often accepted cf. [2, 23, 24].

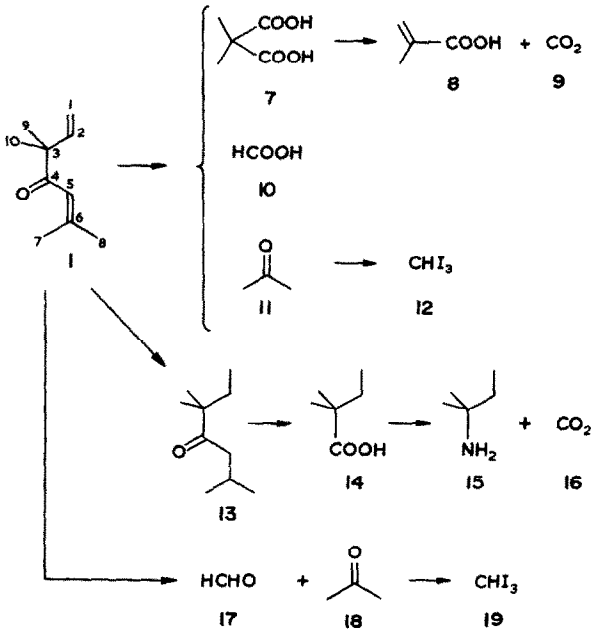

Fig. 2. Degradation schemes for artemisia ketone.

After experiments to determine the optimum (seasonal; metabolism period) conditions for incorporation of tracer into the ketone, various ^{14}C-labelled precursors were fed (cut-stem or vacuum-infiltration of leaf discs) and the labelled ketone was extracted, and purified and degraded after addition of carrier. In all cases, solids or solid derivatives of liquid products were recrystallised to constant specific radioactivity and isotope balances in starting materials and degradation products were established. Degradative procedures are shown in Fig. 2 and results are given in Table 1.

Negligible ($< 5 \times 10^{-4}$ %; if any) incorporations of tracer from ^{14}C-labelled (+)-*trans*, (±)-*trans* and (±)-*cis*-chrysanthemyl alcohols; (±)-*trans* and (±)-*cis*-chrysanthemates; linalol; and DMVC into artemisia ketone occurred in feeding experiments with seedlings and mature plants during March–August. Some (2 to 30%) tracer entered chlorophyll of which up to 60% resided in the phytol moiety. Smaller amounts (0·1–2%) of tracer from chrysanthemyl alcohols and chrysanthemates were located in a polar (TLC) compound which was not the corresponding aldehyde and was not further examined. This metabolite was not detectable when the plant was exposed to CO_2-[^{14}C] at physiological concentrations of the gas although under these conditions up to 0·02% tracer entered artemisia ketone. The unidentified metabolite may be a salvage product (epoxide or polyol?) formed by detoxification processes induced by the unphysiological levels of the additives [cf. 25].

The incorporations of MVA, IPP and DMAPP were larger than those usually found in feeding experiments with higher plants [26] and eliminate the suggestion [inferred from the non-incorporation of tracer from MVA-[2-^{14}C] into artemisia ketone in *S. chamaecyparissus* [15, 27]) that the ketone was formed by a non-mevalonoid pathway. The degradations carried out (Fig. 2) established that with either MVA-[2-^{14}C], IPP-[4-^{14}C] or DMAPP-[4-^{14}C] as precursors, 87–95% of the incorporated tracer resided in **8** ($C_3 + C_9 + C_{10}$), and the balance was in **11** ($C_7 + C_8$). On biogenetic grounds we can reasonably infer that C_3 was unlabelled and that the label in each pair of methyl groups ($C_9 + C_{10}$), ($C_7 + C_8$) was specific at one atom. The preferential labelling of the C-5 moiety of artemisia ketone *not* containing the carbonyl group rules out biogenetic routes involving condensations of similar C-5 units from the same metabolic pool cf. [7–9] and implies that one such

Table 1. Tracer distribution in degradation products of artemisia ketone biosynthesised by *A. annua*

Precursor	Month	Period (hr)*	%; α †‡	Products (sp. act. %) §
MVA-[2-^{14}C]	May	36	0.01; 7200	**7** 6740(94); **8** 6650(93) **9** 200(2); **10** 25(0); **11** 550(8)
MVA-[2-^{14}C]	March	48	0.04; 27300	**7** 25100(90); **8** 25000(90) **9** 720(2); **10** 90(0); **11** 2760(11) **12** 1510(6)
MVA-[2-^{14}C]	May	48	0.02; 17540	**13** 17540(100); **14** 16760(96) **15** 15920(91); **16** 10(0)
Geraniol-[2-^{14}C]	June	40	0.06; 13870	**7** 9780(70); **8** 4650(34) **9** 4140(30); **10** 3000(22) **11** 1580(11); **12** 970(7)
IPP-[4-^{14}C]	June	24	0.81; 187200	**7** 179710(96); **8** 177800(95) **9** 80(0); **10** 825(0); **11** 9300(5)
DMAPP-[4-^{14}C]	June	24	0.12; 80000	**7** 71200(89); **8** 69500(87) **9** 100(0); **10** 80(0); **11** 8230(10)

* Metabolic period after uptake of tracer. These times were chosen after extensive preliminary studies (semi-quantitative by PC and TLC-radiochromatography) to assess the time of maximum incorporation into artemisia ketone. † % incorporation into artemisia ketone (based on 3*R*-isomer for MVA). ‡ Specific radioactivity (dpm mmol^{-1}) of material recovered after dilution with varying (in different experiments) amounts of unlabelled material. § Specific activity (dpm mmol^{-1}) in degradation products; % label in these. All values are independent.

unit occurs in a largely unlabelled pool [26, 28]. Similar asymmetry of labelling of the IPP-derived unit of many regular monoterpenes biosynthesised from the same precursors as here used has been attributed to the existence of a protein-bonded pool of DMAPP [29]. Thus, the regular and irregular monoterpenes may share building bricks from common metabolic pools. On these grounds we can rule out a biogenetic route involving car-3-ene as an intermediate to artemisia ketone since if the former is labelled asymmetrically as in *Pinus* species [30], this would lead to the predominant labelling of the carbonyl-containing C-5 group in the ketone. Furthermore, car-3-ene has not been recorded to co-occur with artemisia ketone.

Feeding experiments involving C-10 compounds did not lead to conclusive results concerning the immediate precursors of artemisia ketone. The tracer pattern in the ketone after feeding geraniol-[2-^{14}C] indicated non-specific positional incorporation. Each carbon contained at least 6% of the total activity although (C_2 + C_4) were by far the most heavily labelled and accounted for about a half of the total. This suggests extensive degradation of the materials fed and reincorporation of labelled C-2 or C-5 fragments. Geraniol-[2-^{14}C] is incorporated intact into 1:8-cineole in *Eucalyptus* [31] and into camphor in *Salvia* sp. [31a], but our results provide no evidence for geraniol or the related linalol as intermediates for artemisia ketone. Such negative results may however result from the inability of geraniol to penetrate to the sites of terpene synthesis, although autoradiography showed that tracer from geraniol-[^{14}C] was widely distributed through the plant tissue very shortly (*ca* 2 hr) after uptake of tracer, and so translocation of tracer was unimpaired. Similarly, there was no evidence for incorporation (either intact or of fragments) of DMVC (3-methylbut-1-en-3-ol) or of the chrysanthemyl derivatives.

Biosynthesis of artemisia ketone in S. chamaecyparissus

Artemisia ketone comprised 85–95% of the essential oil produced in *ca* 2% w/wet wt of foliage of our specimens. A typical analysis was: artemisia ketone 90; β-phellandrene 3.8; myrcene 3.3; *p*-cymene 1.0%. In contrast with the results for *A. annua*, feeding experiments with *S. chamaecyparissus* using MVA-[2-^{14}C] in June to December with metabolic periods 2–163 hr gave negligible ($<10^{-4}$ %) incorporations of tracer into artemisia ketone, although parallel feedings with CO_2-[^{14}C] under physiological conditions showed that significant (up to 0.03% incorporation) synthesis of the ketone was occurring. Similar non-incorporations of MVA had been previously found [15, 27]. Linalol, DMVC and the *cis*- and *trans*-chrysanthemyl compounds were also found to be not precursors of the ketone under a wide range of conditions. IPP-[4-^{14}C] and DMAPP-[4-^{14}C] were, however, incorporated 0.2–0.8% into the ketone in 24 hr periods and degradations (Fig. 1) indicated that 90–95% of the tracer was asymmetrically situated (mainly at C_9 + C_{10}) as in the experiments with *A. annua*. At first sight these results suggest that linalol, chrysanthemyl alcohol and so on, are not precursors of artemisia ketone, but there is always the possibility that compartmentational effects occur at several levels and lead to observed negligible incorporations of obligatory precursors. The results of this and the previous section indicate the possible limitations of feeding experiments on whole plants and a continuation of this study using cell-free extracts is described in the following paper. [31b]

Incorporations of chrysanthemyl and artemisyl alcohols into meroterpenoids of Chrysanthemum cinerariaefolium

The negligible or zero incorporation of prospective precursors with the chrysanthemyl skeleton in the previous experiments stimulated studies on the biosynthesis of the *trans*-chrysanthemyl mono- and dicarboxylic acid moieties of the meroterpenoids pyrethrin I and II formed in flower-heads of *C. cinerariaefolium*. (+)-*trans*-Chrysanthemate was purified from natural pyrethrin and was converted into (+)-*trans*-chrysanthemyl alcohol-[Me-^{14}C]. This alcohol and synthetic (±)-*cis*-chrysanthemyl-alcohol-[^{14}C] were fed separately to flowerheads under conditions reported to give optimum formation of pyrethrins [32]. Although tracer from the synthetic compound was not incorporated, the former was an excellent precursor for both pyretheins I and II (2.1 and 0.2% respectively). Degradation of pyrethrin I by hydrolysis to liberate the terpenoid portion followed by oxidative cleavage of the latter showed that >99% label had been incorporated in a position-specific manner and that the non-terpenoid part of the meroterpene was not radioactive. (+)-*trans*-Chrysanthemate is reported [32–33] to be significantly incorporated (*ca* 1%) in similar feeding experiments. Our present results indicate the occurrence *in vivo* of an oxidation system for the formation of this acid from *trans*-chrysanthemyl alcohol [cf. 33]

It has been suggested that the artemisyl skeleton may be the precursor of the chrysanthemyl skeleton *in vivo* [34]. However, artemisyl alcohol-[4-$^{3}H_1$] was not incorporated into the terpenoid part of pyrethrins in *C. cinerariaefolium* [33]. Apart from problems associated with compartmentation, this result may be invalid since we have demonstrated a redox system involving the alcohol and its ketone in acetone powders prepared from flower heads of this species. However, we also found that artemisyl alcohol-[G-^{14}C] was similarly not ($<10^{-4}$%, if any) incorporated so a further explanation is required.

EXPERIMENTAL

Methods. Preparative-scale GLC was carried out on columns (6m × 1cm) packed with either Carbowax 20M (30% w/w on acid–alkali washed G-Cell, 60–80 mesh) or FFAP (10% w/w on similar G-Cell) at 120–70° with N_2 flow rate 3.5–5 $l.hr^{-1}$. Analytical GLC was on Carbowax 20M (5% w/w on G-Cell; 6m × 0.5cm). TLC sprays used were $KMnO_4$ (0.3%) in H_2SO_4 (0.05% aq. w/w); rhodamine B (0.05%); bromocresol purple (0.1% in 0.001M NaOH); and phosphomolybdic acid (5% in EtOH: heated 110° for 5 min to develop). TLC separations could be scaled up to the column size if 'material for TLC' was used. All products had NMR, IR and MS consistent with the proposed structures and mps agreed ±1° with literature values unless indicated.

Materials. Specimens of *A. annua* were gifts from Dr. P. Yeo (Cambridge) or were grown from seed (Royal Botanic Gardens, Kew). They were cultivated indoors in Central London and used for feeding expts when 10–15 cm high (typically 3 months after germination). *S. chamaecyparissus* was obtained as 18-month-old plants from Chelsea Physic Garden, and were pruned to ground level, cultivated outdoors and used 3 mths later. All plants were wintered in pots at 20° in a south-facing aspect. Specimens of *C. cinerariaefolium* were obtained from Kew and similarly treated. Geraniol-[2-^{14}C] was prepared by Reformatsky-type coupling of ethyl bromoacetate-[2-^{14}C] (0.50 g;

0.5 mCi) with 6-methyl-5-hepten-2-one (0.38 g) in Et_2O (2 ml) to give a product that was saponified, dehydrated with Ac_2O (0.43 g) and reduced with sodium dihydro-*bis*-(2-methoxyethoxy)-aluminate to give geraniol and nerol (20%; 1.1 v/v). These were separated by GLC on Carbowax 20M at 150° and TLC on Si gel with EtOAc–C_6H_6 (15:85) to give products with 126 μCi mmol^{-1}. A portion of geraniol-[2-^{14}C] was converted into its bromide with Ph_3P and CBr_4 and hydrolysed with moist Ag_2O in C_6H_6 [35] to give products that were separated by TLC on silicic acid (Biosil A, Biorad Labs; Richmond, CA). Si gel GF_{254} (2.1 w/w) developed (4 ×) with EtOAc–C_6H_{12} (15:85) to give linalol-[2-^{14}C] (10%; R_f 0.72; 72 μCi mmol^{-1}) and nerol and geraniol (R_f 0.55; 0.47). These were further purified by GLC on FFAP at 150°. Linalol-[2-^{14}C] was also prepared in poor (*ca* 3%) yields by isomerisation of geranol-[2-^{14}C] with BF_3 [36]. IPP-[4-^{14}C] and DMAPP-[4-^{14}C] (both 17 mCi mmol^{-1}) were prepared [25] from 3RS-MVA-[2-^{14}C] (50 μCi; 5 μmol) and 3-methylbut-1-en-3-ol-[Me-^{14}C] was obtained from reaction of Me_2CO-[1, 3-^{14}C] (0.1 mCi) with vinyl lithium [37]. Artemisyl alcohol-[G-^{14}C] was obtained by reduction of labelled artemisia ketone obtained from *A. annua* that had been exposed to CO_2-[^{14}C] (0.3 mCi) in a 10 l vessel for 10 days. The product (2 mg; 0.8 μCi) was separated on Si gel H (6 × 2 cm) with EtOAc–C_6H_6 /15:85), followed by TLC on the same material at 4° with CH_2Cl_2 and preparative GLC on Carbowax 20M at 130°. (+)-*trans*-Chrysanthemic acid; bp 105°(1.3 mm, $[\alpha]_D$ + 24(c6,$CHCl_3$); was isolated [38] from natural pyrethrins (Bush Boake Allen, London), and was converted into its methyl ester. This was oxidised with OsO_4–$NaIO_4$ to give the (+)-*trans*-aldehyde (80%) and tracer was introduced by coupling this (Wittig Reaction) with Me_2CO-[1, 3-^{14}C] (150 μCi) to give methyl(+)-*trans*-chrysanthemate [Me-^{14}C] [39] in overall 1% yield. This was reduced to (+)-*trans*-chrysanthemyl alcohol [Me-^{14}C] with sodium dihydro-*bis*-(2-methoxylthoxy)aluminate. A mixture of (±)-*cis* and (±)-*trans* chrysanthemic acids (and thence of the corresponding alcohols) was also labelled by the above method starting with (±)-*trans,cis*-ethyl chrysanthemate (BDH, London). A more efficient method involved addition of ethyl diazoacetate-[1-^{14}C] (20 mg; 250 μCi) to 2,5-dimethylhexa:2,4-diene [40–1] to give ethyl esters of (±)-*cis,trans*-chrysanthemic acids-[COOH-^{14}C], which were hydrolysed and the acids were separated by GLC on polypropyleneglycol sebecate (10% w/w on chromosorb W) at 160° [42]. Alternatively, acids were separated on a Si gel H column (20 × 1.5 cm) using iPrOAc (150 ml), EtOAc (200 ml; to elute the *cis*-acid) and MeOH (100 ml; to elute the *trans*-acid). Eluted fractions were neutralised and extracted (× 3) with 20 ml (Et_2O); layers were then adjusted to pH5, saturated with NaCl and re-extracted with Et_2O to give the (±)-*cis*-acid, mp 114% and the (±)-*trans*-acid, mp 54°. The two isomeric acids were also separated by fractional crystallisation [43]: the yield of *trans*-acid in the initial mixture being increased by equilibration of the esters with NaOtAm [44]. Separated acids were reduced (>95%) to the alcohols with sodium dihydro *bis*-(2-methoxyethoxy)aluminate, and overall yields based on ethyl diazoacetate were about 10%. All chrysanthemyl derivatives had NMR spectra as previously recorded [45] and radiochemical purity of alcohols was checked by conversion into 3,5-dinitrobenzoates and recrystallisation of these to constant specific radioactivity. (±)-*trans* and *cis*-Chrysanthemyl alcohols for use as carrier, were prepared by reduction of (±)-*cis, trans*-ethyl chrysanthemate and the product (R_f 0.32) was separated from unreacted ester (R_f 0.67) by TLC on Si gel H (EtOAc–C_6H_6; 15:85) and the *cis* and *trans* alcohols were then separated by GLC on Carbowax 20M at 162°. Artemisia ketone was prepared from 2, 2-dimethylbut-3-enoic acid chloride and isobutene [46–7] with the modification that the intermediate ethyl-2,2-dimethyl-3-hydroxybutanoate was dehydrated with P_2O_5. The product was distilled (Buchi spinning-band column) to give 2 fractions (9:1 v/v) at 166–75° and 175–80°. GLC (150 μ aliquots) of the first (Carbowax 20M) at 150° yielded artemisia ketone (bp 180°/760 mmHg; ν 1685*s*, 920*s*, 885*m* cm^{-1}; λ_{max} (EtOH) 239 nm (ε 9, 830); *m/e* 152 (M^+, 0.4%), 83 (100), 55 (28), 29 (11); τ (100 MHz) 8.82 *s* 6H, 8.12 *s* 3H, 7.87 *s* 3H, 4.94–3.54 *m* 4H) and isoartemisia ketone (10.1; the latter is the 1, 6-diene isomer of the former; τ (100 MHz) 8.81 *s* 6H, 8.41 *s* 3H, 6.83 *bd.s* 2H, 5.00–4.1 *m* 5H; M^+ 152); both >99% pure (GLC, TLC). Similar treatment of the second fraction yielded largely cycloartemisia ketone(3,3-dimethyl-5-isopropylidene-cyclopent-1-one)that was identical with an authentic specimen cf [48].

Feeding and purification techniques. ^{14}C-labelled precursors (20–100 μCi dissolved in min. H_2O) were fed via cut stems with illumination and forced transpiration [49] or by vacuum-infiltration of leaf discs of *A. annua* at a level of 10–15 μCi g^{-1} plant material. 3RS-MVA-lactone was hydrolysed prior to feeding by incubation (1h) with sat. aq. $NaHCO_3$. The most effective method of feeding *S. chamaecyparissus* was by wick-injection into a sterile (EtOH; NaOCl-washed) incision in the woody stem that was subsequently sealed with vinyl aerosol (Fisons, Cambridge). Uptake of tracer in the various feeding techniques usually took *ca* 1h and the plant was then left (for cut stems or discs) in H_2O of Pfeffers' medium [49] over the period of metabolism. Distribution of tracer *in vivo* was checked by autoradiography [49]. Water-insolubles were supplied as solutions in H_2O–DMSO (80:20) or H_2O–EtOH (70:30) or as suspensions (1:10) in Tween 80 in H_2O (total 1 ml). These methods caused no apparent necrosis of tissue and when applied to water-solubles gave incorporations identical with those in controls when organic solvents and emulsifiers were absent. After the metabolism period, the frozen (liq. N_2) pulverised tissue (*ca* 25g) was extracted either by trituration (10 min) with CH_2Cl_2 (50 ml × 2) or by steeping in $(Me)_2CO$ (250 ml; 12 hr): either method extracted all monoterpenes and most (>95%) of incorporated tracer. After concentration, and in the first case partition with H_2O, artemisia ketone (25 mg) was added as carrier and pigments were removed by passage through a column (12 × 1.5 cm) of MgO with C_6H_{12} as eluant, followed by a similar column of Si gel H (with CH_2Cl_2) at 10°. The fraction containing the ketone was further purified by TLC on silicic acid (Silicar CC7, Mallinckrodt, St Louis, Mo) with EtOAc–C_6H_{12} (1:9) and Si gel H_{254} with Et_2O–petrol, 40–60 (1:9). No suitable derivative of artemisia ketone was found for recrystallisation to constant specific radioactivity, but the material obtained was shown to be radiochemically pure by the constant specific activity of segments across GLC and TLC traces, and comparison with the activity of artemisyl alcohol prepared from the ketone and purified by recrystallisation (4-fold, ex eq. $(Me)_2CO$) of its 3,5-dinitrobenzoate. Feeding experiments with *C. cinerariaefolium* were carried out when 2–4 rows of disc florets were fully open [32]. After 3 days metabolic period, flowerheads (100 g) were ground with $(Me)_2CO$ and pyrethrins (I and II; 200, 93 mg) extracted, purified [50], and hydrolysed (ethanolic NaOH) to yield (+)-*trans*-chrysanthemic acid which was purified as described beforehand. Chlorophyll and phytol were recovered and purified by standard methods [49]. GLC analyses of oils used material steam-distilled from leaf-tissue (100–150g) and isolated components compared spectrally with authentic samples.

Degradation or artemisia ketone and chrysanthemic acid (Fig. 2). Artemisia ketone (**1**) was oxidised [51] with aq. $KMnO_4$ to give dimethylmalonic acid (**7**, 70%), HCOOH (**10**; 32%) and $(Me)_2CO$ (**11** 53%). The last was distilled out and purified as its 2, 4-dinitrophenylhydrazone, mp 128°. Acids were separated (R_f 0.12; 0.45) by TLC on cellulose (MN 300HR grade, Macherey–Nagel GmBH, Düren, W. Germany) with EtOH–H_2O–NH_4OH (85:11.4) and further purified as the *S*-benzylisothiuronium chlorides (ex aq. EtOH) mps 150° (lit. 148°) and 123°. Dimethylmalonic acid was decarboxylated to isobutanoic acid (**8**; 98%, bp 154°; *S*-benzylisothiuronium chloride mp 135°) on gentle heating and the CO_2 (**9**) was collected in ethanolamine. Acetone 2,4-dinitrophenylhydrazone was converted into $(Me)_2CO$ (70% recovery) by refluxing with an excess of MeCOCOOH (70% aq.) and thence converted into iodoform, **12** mp 120° (32%). Artemisia ketone (50 mg) in C_6H_{12} (5 ml) was converted into its tetrahydro derivative (**13**; 90%) with Adam's catalyst [52] and the product was purified by TLC on Si gel H

at 4° with EtOAc–C_6H_{12} (8:92; ketone R_f 0·54; tetrahydro compound R_f 0·13). A solution of the pure product (50 mg) in C_6H_{12} (5 ml) was shaken with $Na_2Cr_2O_7$ (75 ml), conc. H_2SO_4 (0·5 g) and H_2O (2 ml) at 20° until a negative ketone test was obtained. The product was worked-up by TLC on cellulose with EtOH–H_2O–NH_4OH (85:11:4) to give 2,2-dimethylbutyric acid (**14**; 60% R_f 0.54; bp 187°; S-benzylisothiuronium chloride mp 93°) which was in turn decarboxylated by the Schmidt procedure to yield CO_2 (95%; collected in ethanolamine) and 1,1-dimethylpropylamine (**15**; 62%; tetraphenylboron salt ex. aq. $(Me)_2CO$, mp 197° d.). Artemisia ketone (50 mg) in MeOH (0.5 ml) was ozonised at −40° for 3h (0.14% O_3; 31 hr^{-1}) and the product decomposed by refluxing with H_2O (1·0 ml) for 1 hr, and then (as **17**, **18**) distilled into 2,4-dinitrophenylhydrazine. HCHO and $(Me)_2CO$ derivatives were separated by TLC on Si gel H with MeOH–C_6H_{12} /1:99); R_f 0.18 and 0.39; mps 128, 166° respectively. The truncated skeleton formed a polymeric ozonide which was not further investigated. (+)-*trans*-Chrysanthemic acid was similarly ozonised or oxidised with OsO_4–KIO_4 and $(Me)_2CO$ was distilled out as before. This $(Me)_2CO$ contained all the tracer: the crude aldehyde–acid concomitantly formed was shown by autoradiography not to be radioactive.

Radioactive methods. All solids were recrystallised at least thrice to constant specific radioactivity. Counting techniques, statistics etc. were as previously described [53]. CO_2 evolved in degradation was passed into ethanolamine (2 ml) and 2-methoxyethanol (4 ml) (controls indicated >96% absorption) and the aliquots were added to the scintillation medium [54].

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