Tetrahedron Letters No. 36, pp 3473 - 3476, 1973. Pergamon Press. Printed in Great Britain.

STUDIES ON THE BIOSYNTHESIS OF CHRYSANTHEMUM MONOCARBOXYLIC ACID

Gerald Pattenden[1] and R. Storer

Departments of Chemistry, University College, Cardiff, and The University, Nottingham, NG7 2RD.

(Received in UK 3 July 1973; accepted for publication 23 July 1973)

The monoterpene chrysanthemum monocarboxylic acid (CMA) (1), which occurs as esters in Chrysanthemum cinerariaefolium, is a member of a group of monoterpenes, whose carbon frameworks are not derived biogenetically by the more usual 'head-to-tail' combination of iso-pentane units[2]. With the isolation of a C_{30}-cyclopropyl alcohol ('presqualene alcohol') structurally analogous to (1) as a probable intermediate in the biosynthesis of squalene from farnesylpyrophosphate,[3] much consideration has been given to the pivotal rôle that the chrysanthemyl skeleton present in (1), might or might not play in the biosynthesis of other non 'head-to-tail' monoterpenes.[4]

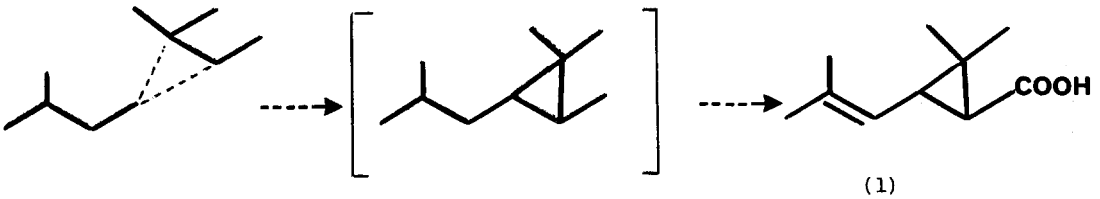
Available data[5] on the incorporation of mevalonic acids (MVA) into CMA appear consistent with the operation of a biosynthetic scheme to (1) involving a 'tail-to-middle' combination of two dimethylallyl pyrophosphate (DMAPP) molecules, as illustrated. Although proposals have been made,[5,6] no details of the mechanism for this very unusual and novel combination of C_5-units have so far been determined. As a contribution to this problem, we report a stereochemical feature of the incorporation of MVA into (1), and some data pertaining to the 'symmetry' of combination of the two C_5-units leading to the chrysanthemyl skeleton in (1).

The potassium salts of 3R,4R [4-^{3}H]-MVA and 3R, 4S [4-^{3}H]-MVA were separately fed (total activity 0.25mC each., vacuum infiltration method) to dissected achenes from C. cinerariaefolium. Elaboration of the plant material followed by separation and saponification of the esters of (1), gave radioactive CMA. Rigorous purification of the CMA's from the two feedings, and preparation of solid amide derivatives [m.p. 121°, $\nu_{max.}$ 3400, 3190, 1643, 1623 cm.$^{-1}$, τ 4.25 bd (NH_2), 5.12d(J 7.5, :CH), 7.96 dd (J6 and 7.5, :CH.CH), 8.3 (:CMe_2), 8.7 (Me), 8.9(Me)].

showed that only 3R, 4R [4-^{3}H] -MVA was incorporated into (1) (total activity 1.6 x 10^6 d.p.m. mmole.$^{-1}$, incorporation yield of 0.4%); negligible radioactivity was detected in the CMA isolated from the 3R, 4S [4-^{3}H] MVA feeding. This conclusion was verified in separate feeding experiments using doubly labelled 3R, 4R [2-^{14}C]-[4-^{3}H]-MVA (^{3}H/^{14}C 3.63) and 3R, 4S [2-^{14}C]-[4-^{3}H]-MVA (^{3}H/^{14}C 3.82); the isotope ratios in the amide derivatives of the CMA's isolated were found to be 3.64 and 0.14 respectively. These data thus showed that the 4S-hydrogen of MVA is lost during the biosynthesis of (1) from MVA. In the absence of additional data it seems clear that this hydrogen is lost, as the 2-pro-R-hydrogen (H_2) of (2), during the isomerisation 2→3 leading to DMAPP. Specific loss of the 2-pro-R-hydrogen in (2) has been observed previously in terpene biosynthesis in yeast and mammalian tissues[7] and some higher plants.[8,9]

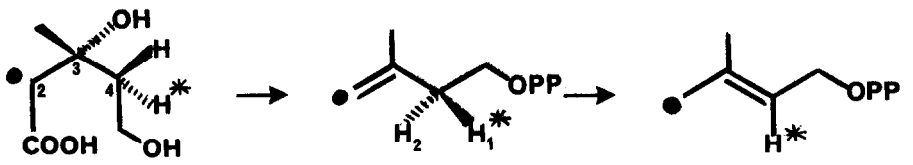
Cleavage (OsO_4-$NaIO_4$) of the side-chain in [^{3}H]-(1) from the 4R [4-^{3}H] MVA feeding, followed by separation and purification of the resulting carbonyl products (as their 2,4-dinitrophenylhydrazones) established that ∿99% of the ^{3}H-label in (1) was located in the cyclopropane ring portion (5). Ozonisation of [^{3}H]-(1) produced [^{3}H]-(-)-trans-caronic acid (6) containing only ∿5% of the original radioactivity, which suggested that the bulk of the [^{3}H]-label in (4) from the 4R [4-^{3}H]-MVA feeding was located in the side chain portion of the molecule. In separate degradation experiments, using C-2-deuterated ester (7)[10] (>98% ^{2}H), no exchange of deuterium was observed (n.m.r. and mass spectra monitoring) during conversion to (1, and amide), or to (6).

In contrast with previous studies, the present data on the incorporation of MVA into CMA show that activity from MVA is not equally distributed between the two C_5-iso-pentane units in (1) for which MVA is thought to be precursor. Unequal distribution of activity from MVA during monoterpene biosynthesis has been observed in several plants systems recently, and a number of factors have been considered to account for these observations. In the specific case of (1), one reason could be that the C_5-iso-pentane unit leading to the cyclopropane part of the molecule is not derived directly from MVA. An attractive alternative precursor might be 3,3-dimethylacrylate (8)[11] which could be involved in the biosynthesis of (1) according to Scheme 2; there is considerable in vitro analogy

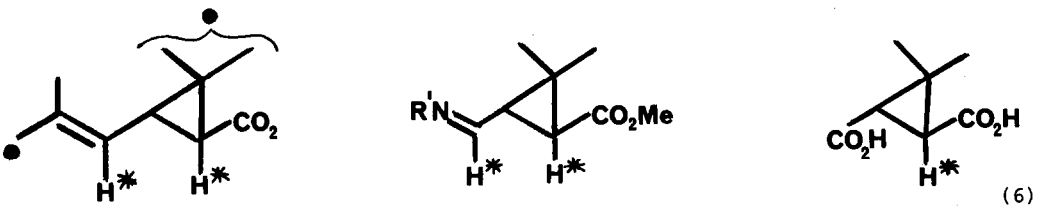


'tail-to-middle' combination

(1)

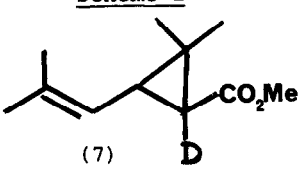


(2) (3)

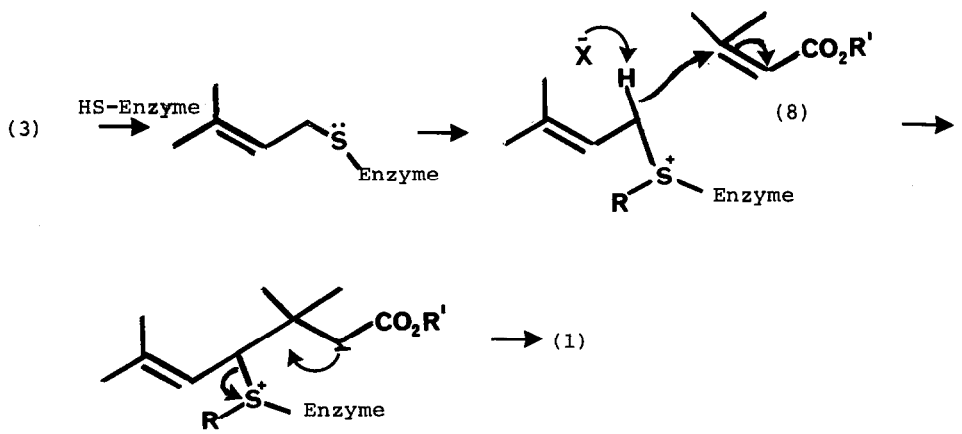


(4) (5)

Scheme 1



(7)



Scheme 2

for such a scheme.[12] In a preliminary investigation of this proposal, the incorporation of the K-salt of [1-^{14}C]-3,3-dimethylacrylic acid[13] into C. cinerariaefolium was examined; data on the radioactive amide of CMA from this feeding showed an incorporation yield of 3.5 x 10^{-2}%, when MVA was incorporated to the extent of 5 x 10^{-2}% (same plants fed at the same time during same season etc.,), and now further experiments are in progress to examine the precursor role of (8,R' = H) in the biosynthesis of (1) in greater detail.

We thank the S.R.C. for financial support.

References

1. Address correspondence to this author at The University, Nottingham.

2. For recent review of monoterpene biosynthesis see D. V. Banthorpe, B. V. Charlwood and M. J. O. Francis, in 'The Biosynthesis of Monoterpenes', Chemical Reviews, 72, 115 (1972).

3. H. C. Rilling, J. Biol. Chem., 241, 3233 (1966). H. C. Rilling and W. W. Epstein, J. Amer. Chem. Soc., 91; 1041 (1969), J. Biol. Chem., 245, 4597 (1970); J. Edmond, G. Popjak, S. M. Wong, and V. P. Williams, ibid, 246, 6254 (1971).

4. For review and leading references see W. W. Epstein and C. D. Poulter, Phytochemistry 12, 737 (1973).

5. M. P. Crowley, P. J. Godin, H. S. Inglis, M. Snarey and E. M. Thain, Biochem. Biophys. Acta, 60, 312 (1962).

6. C. F. Pattenden, Ph.D. Thesis, Univ. Wales, 1971. E. E. van Tamelen and M. A. Schwartz, J. Amer. Chem. Soc., 93, 1780 (1971).

7. J. W. Cornforth, Quart. Rev. Chem. Soc., 23, 125 (1969).

8. M. J. O. Francis, D. V. Banthorpe, and G. N. J. Le Patourel, Nature, 228, 1005 (1970).

9. Similar observations were made during studies of the biosynthesis of Chrysanthemum dicarboxylic acid. C. F. Pattenden, Ph.D. Thesis, Univ. Wales.

10. This ester was prepared from the corresponding cis-chrysanthemic ester following treatment with MeOD containing NaOMe and esterification with CH_2N_2.

11. cf. D. V. Banthorpe, B. V. Charlwood, and M. R. Young, J. Chem. Soc., Perkin Trans. I, 1532 (1972).

12. J. Martel, C. Huynh, E. Toromanoff and G. Nomine, Bull, Soc. Chim. France, 982, 985 (1967); L. Velluz, J. Martel and G. Nomine, Compt. rend., 268 (C), 2199 (1969); M. Julia, and A. Guy-Rouault, Bull. Soc. Chim. France, 1411(1967)

13. D. L. Williams and D. G. Ott, in 'Org. Synthesis with Isotopes', ed. A. Murray and D. Williams, Interscience, New York (1958). We are most grateful to Dr. D. W. Young (Univ. Sussex) for providing us with a sample of ^{14}C-acid.