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SHORT COMMUNICATION

INCORPORATION OF MEVALONOLACTONE INTO *PETASITES HYBRIDUS*: EFFECT OF SYNTHETIC INHIBITORS ON SESQUITERPENOID AND STEROL PRODUCTION

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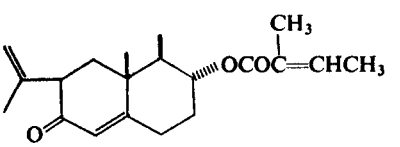
Abstract—Incorporation of activity from DL-[2-^{14}C]-mevalonolactone (fed to whole plants of *Petasites hybridus*) into petasin (assayed as isopetasyl acetate) was increased by previous feeding of SK and F 7997-A_3.

INTRODUCTION

OUR INITIAL investigations[1] on the incorporation of DL-[2-^{14}C]-mevalonolactone (MVAL) into *Petasites hybridus* showed that a major proportion of the radioactivity appeared in the fraction containing sterols and triterpenoid alcohols, and only a very minor proportion in the sesquiterpenoid fraction. As our primary interest is in the biosynthesis of petasin (I) we have explored a different approach with the aim of obtaining higher incorporation of [2-^{14}C]-MVAL into this sesquiterpenoid.



(I) Petasin

RESULTS AND DISCUSSION

There have been several reports in the literature on the use of synthetic inhibitors of sterol formation. The original work of Holmes and DiTullio[2] showed that with the inhibitors SK & F 3301-A [2,2-diphenyl-1-(β-dimethylaminoethoxy)-pentane hydrochloride] and SK & F 525-A [β-diethylaminoethyldiphenylpropyl acetate hydrochloride] inhibition occurred (in rat-liver homogenates) at the stage of isomerization of isopentenyl pyrophosphate (IPP) to dimethylallyl pyrophosphate (DMAPP). With SK & F 7732-A_3 [tris-(2-dimethylaminoethyl)-phosphate trihydrochloride] and SK & F 7997-A_3 [tris-(2-diethylaminoethyl)-phosphate trihydrochloride] inhibition occurred at a later stage and caused the accumulation of a non-polar triterpenoid intermediate. A related result was obtained by Reid[3] who also observed the accumulation of a non-polar triterpenoid intermediate when he

[1] C. J. W. BROOKS, J. A. ZABKIEWICZ, R. A. B. KEATES and A. M. M. BERRIE, paper in preparation.
[2] W. L. HOLMES and N. W. DITULLIO, *Am. J. Clin. Nutr.* **10**, 310 (1963).
[3] W. W. REID, *Biochem. J.* **100**, 13P (1966).

fed SK & F 7997-A_3 and [2-^{14}C] MVAL to *Nicotiana tabacum* slices. In further work, Reid[4,5] demonstrated increased incorporation of label into squalene and 2,3-oxido-squalene, as well as into other unidentified terpenoids, while the biosynthesis of β-amyrin and phytosterols was inhibited.

Investigations by Bonner, Heftmann and Zeevaart[6] into the effect on plants of these and other inhibitors showed that there was powerful inhibition of floral induction in certain of the species studied. These authors suggested that flower hormone synthesis was the process inhibited by SK & F 7997-A_3, indicating that this hormone may be a terpenoid or steroid. Reduced incorporation of label from [2-^{14}C]-MVAL into the major phytosterols was also observed.

The above results indicated that biosynthesis of the sterols could be inhibited without necessarily affecting the lower terpenoids. Separate trials with two inhibitors, SK & F 525-A and 7997-A_3 were therefore undertaken. The inhibitor was fed, together with [2-^{14}C]-MVAL, to mature leaves of *Petasites hybridus*, by a wick technique. After what was considered to be an appropriate interval, the leaves were harvested and the radioactivity in petasin [assayed as isopetasyl acetate] was determined. The results are given in Table 1.

TABLE 1. EFFECT OF VARIOUS CONCENTRATIONS OF SK & F 525-A AND 7997-A_3 ON THE INCORPORATION OF RADIOACTIVITY FROM DL-[2-^{14}C]-MEVALONOLACTONE INTO PETASIN

Inhibitor	p.p.m.	Wet leaf weight (g)	Isopetasyl acetate (mg)	dpm	Specific activity (dpm/μmole)	Incorporation	
						p.p.m.	Percentage
SKF 525-A	25	32·1	6·54	1785	75	81	0·0081
	50	34·0	5·02	1452	79	66	0.0066
	100	31·9	1·85	773	115	35	0·0035
SKF 7997-A_3	25	33·8	3·90	1708	144	94	0·0094
	50	28·3	8·18	3254	135	179	0·0179
	100	49·0	12·66	4939	130	271	0·0271
None		29·7	4·66	1041	61	47	0·0047

Counting efficiency was 82·4 per cent for ^{14}C. Background 27 cpm.

It will be seen that for single feedings of individual plants with SK & F 525-A there is a decrease in activity incorporated and in weight of isopetasyl acetate recovered, as the level of inhibitor increases. Suppression of sesquiterpenoid biosynthesis is consistent with the known site of action of SK & F 525-A.[2] The observed variation in specific activity is too small to warrant any comment.

The results from the SK & F 7997-A_3 feedings show that the activity incorporated into petasin increases with increasing amounts of inhibitor. This is consistent with existing evidence that this inhibitor acts at a stage beyond squalene, and probably by the inhibition of cyclization of 2,3-oxido-squalene.[4] Weights of recovered isopetasyl acetate clearly depend on many factors, and their values are not interpretable.

Experiments intended to verify the latter results from the SK & F 7997-A_3 feedings are in hand, and initial results show the same pattern of inhibition. Radio-TLC scanning of the

[4] W. W. REID, *Phytochem.* **7**, 451 (1968).
[5] W. W. REID, Abstract No. C56, 5th Internat. Symp. on Chem. Nat. Products, I.U.P.A.C., London (1968).
[6] J. BONNER, E. HEFTMANN and J. ZEEVAART, *Plant Physiol.* **38**, 81 (1963).

total benzene extracts from these trials indicates accumulation of radioactivity into squalene and 2,3-oxido-squalene, together with a decrease of incorporation into the sterol fraction. A more detailed investigation of the effect of this inhibitor on the sterols and triterpene alcohols is under way.

Of related interest are the results of Dennis, Upper and West[7] and Graebe[8] who have shown that synthesis of kaurene (a precursor of the gibberellins) is inhibited by AMO 1618 [2′-isopropyl-4′-(trimethylammonium chloride)-5′-methylphenyl piperidine-1-carboxylate] after the acyclic precursor is formed. It is possible that physiological effects such as those observed by Bonner *et al.*[6] may result indirectly from the action of the inhibitor on terpenoid hormones, notably gibberellins and abscisic acid.

EXPERIMENTAL

Chemicals

DL-[2-^{14}C]mevalonolactone (specific activity 4·8 mc/mmole) was obtained from the Radiochemical Centre, Amersham. SK & F 525-A and 7997-A_3 (Smith Kline and French, Philadelphia, U.S.A.) were kindly supplied by Dr. C. G. Elliott, Botany Department, University of Glasgow. Isopetasol was obtained by extraction of *Petasites hybridus* rhizomes.[9,10]

Plant Material

The source and propagation of *P. hybridus* plants were as described elsewhere.[1,9] All had a minimum of three mature leaves and were left in the growth cabinet (8-hr day at 20°) for 4 days before commencement of feeding in order to standardize their growth.

Feedings

Each plant was fed a total of 1 ml of an aqueous solution containing 25, 50 or 100 p.p.m. of SK & F 525-A or 7997-A_3. Each of two separate leaves on every plant was fed via a wick dipping into a vial containing 0·5 ml of the solution. Later (after complete assimilation), 0·5 ml solutions of [2-^{14}C]-MVAL (each containing 5 μc) were fed in a similar way to each leaf, followed 24 hr later by 0·5 ml of water. The plants were left for a further 48 hr: they were then removed and the leaves excised.

Extraction

The leaves were weighed, homogenized (Silverson blender) in benzene and left to steep for 48 hr. The benzene extract was concentrated and an aliquot (80 per cent) of the oil was hydrolysed with ethanolic KOH (0·5 g KOH/2 ml H_2O/18 ml EtOH) for 2 hr. Extraction and subsequent purification of the isopetasol were as described.[1]

Assay Method

The mass of the isopetasol (as the acetate) was quantitated by gas–liquid chromatography. A calibration curve was constructed from measurements made on standard solutions of isopetasyl acetate (0·1–1 mg per ml of methyl acetate). Radioactivity was estimated by scintillation counting in a Packard Tri-Carb Scintillation Counter (10 ml toluene solution containing PPO and DiMe-POPOP; standard error ± 2 per cent).

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[7] D. T. Dennis, C. D. Upper and C. A. West, *Plant Physiol.* **40**, 948 (1965).
[8] J. E. Graebe, *Phytochem.* **7**, 2003 (1965).
[9] G. H. Draffan, Ph.D. Thesis, Glasgow Univ. (1967).
[10] C. J. W. Brooks and G. H. Draffan, *Tetrahedron*, in press.