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BIOSYNTHETIC STUDIES IN THE COUMARIN SERIES—II[1]

STUDIES IN PLANTS OF *THAMNOSMA MONTANA* TORR. AND FREM. THE ROLE OF ACETATE AND GLYCINE[a]

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Abstract—The role of sodium acetate-[2-^{14}C] and glycine-[2-^{14}C] in the biosynthesis of the coumarins, umbelliprenin (1), isopimpinellin (2) and alloimperatorin methyl ether (3) has been investigated. It has been found that whereas the activity from acetate is being incorporated into the furano portion, the OMe groups and the alkyl side chain, glycine is acting as a specific precursor of the alkyl side chain of umbelliprenin (1) and alloimperatorin methyl ether (3) and of the OMe groups of isopimpinellin (2) and alloimperatorin methyl ether (3). Various plausible explanations are presented.

As mentioned in the accompanying publication,[1] there has been a lot of controversy as to the role of mevalonic acid in the biosynthesis of the furan ring of furanocoumarins. Various workers[1,2] have reported the incorporation of mevalonic acid into the furan ring of furanocoumarins but the very low levels of incorporation observed in these biosynthetic studies must raise the question as to whether mevalonic acid may act alone as the precursor of the furan ring. Brown[3] has found that acetate served as a much more efficient precursor of furanocoumarins than did mevalonic acid. Acetate is of course a well known precursor of mevalonic acid but this could not alone explain the higher incorporations observed by Brown.[3] However no degradations were performed to determine the position of radioactivity in the radioactive compounds isolated and therefore the significance of these observations is questionable. Thus a series of experiments were undertaken to learn more of the role of acetate in furanocoumarin biosynthesis.

In these experiments, sodium acetate-[2-^{14}C] was fed to young *Thamnosma montana* plants over a period of 7 days by the hydroponic feeding method and isopimpinellin (2) and alloimperatorin methyl ether (3) were isolated. The results are given in Table 1. It should be indicated at this point that the numbers assigned to the various compounds discussed here correspond to those given in the accompanying publication.[1]

These results reveal that acetate is a somewhat more efficient precursor of furanocoumarins of *Thamnosma montana* than is mevalonate although the incorporations are still frustratingly low. For isopimpinellin (2) the incorporations are 2 to 10

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Table 1. Incorporation of sodium acetate-[2-^{14}C] into *Thamnosma montana* shoots

Experiment No.	Activity fed* (dpm)	Weight of plant (g)	% Incorporation	
			Isopimpinellin (2)	Alloimperatorin methyl ether (3)†
1	$1{\cdot}11 \times 10^9$	6·2 (diluted to 16·2)	0·0015	0·00016
2	$2{\cdot}18 \times 10^9$	2·4 (diluted to 11·4)	0·0004	‡
3	$2{\cdot}16 \times 10^9$	4g (undiluted)	0·0028	0·0010

*Corrected for activity recovered outside the plant.
†Counted as alloimperatorin methyl ether diol (20).
‡Sample lost.

times greater than observed in the mevalonic acid feeding experiments[1] and for the first time measurable incorporation has been achieved into alloimperatorin methyl ether (3). However the question remained as to how the radioactivity was distributed in the molecules.

Thus isopimpinellin from both experiments 1 and 2 was degraded according to the scheme previously described (Fig 1, accompanying publication). The results are presented in Table 2.

From these results it is evident that only ~ 10% of radioactivity of isopimpinellin (2) resides in the 7-position, and approximately 0–3% resides in the 6-position. This data indicates that acetate is being incorporated specifically into the furan C atoms in a manner consistent with the acetate incorporation via mevalonate, that is in the manner previously observed.[1] Sodium acetate-[2-^{14}C] on elaboration to mevalonate would be expected to label the 4-position and not the 5-position of mevalonate.

Somewhat surprisingly the remaining radioactivity is found essentially in the methoxyl group C atoms (45–60%) at C-4 and perhaps in the benzenoid portion of isopimpinellin (2). That is, only 10–12% of the radioactivity in isopimpinellin (2) is observed to be lost in the conversion of 2 to 11. As the aldehyde (7) contains 90% of the original activity, one can conclude that only 0–2% of the activity of isopimpinellin (2) resides in the 2- and 3-positions. In the conversion of 11 to the diacetate (13b), 7–20% of the radioactivity of isopimpinellin (2) is lost. As C-6 has been shown to be essentially inactive, this result requires that C-4 must account for 7–20% of the original activity of 2. The discrepancy between the activity of the OMe groups (45–60%) and of the diacetate (70–83%)

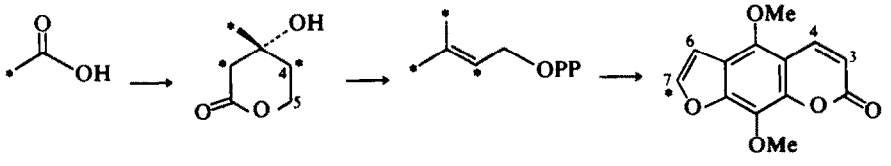

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Table 2. Degradation of active isopimpinellin (Experiments 1 and 2)

Compound	Specific activity (% total activity in isopimpinellin (2)*) Experiment 1 Trial a	Experiment 1 Trial b	Experiment 2
Isopimpinellin (2)	$1{\cdot}305 \times 10^4$ (100) dpm/mmol	$8{\cdot}04 \times 10^3$ (100) dpm/mmol	$1{\cdot}083 \times 10^4$ (100) dpm/mmol
6-Formyl-7-hydroxy-5,8-dimethoxycoumarin (7)	$1{\cdot}185 \times 10^4$ (90·6)	$7{\cdot}12 \times 10^3$ (88·6)	—
6-Formyl-5,7,8-trimethoxycoumarin (8)	—	$7{\cdot}22 \times 10^3$ (89·8)	$9{\cdot}71 \times 10^3$(89·9)
6-Hydroxy-5,7,8-trimethoxycoumarin (9)	—	$6{\cdot}95 \times 10^3$ (86·5)	$9{\cdot}83 \times 10^3$ (91·1)
1,3-Diformyl-4,6-dihydroxy-2,5-dimethoxy benzene (11)	$1{\cdot}150 \times 10^4$ (88·2)	—	$9{\cdot}70 \times 10^3$ (89·6)
1,3-Diformyl-2,4,5,6-tetramethoxybenzene (12)	—	—	$9{\cdot}73 \times 10^3$ (90·0)
1,3-Diacetoxy-2,4,5,6-tetramethoxybenzene (13b)	$9{\cdot}00 \times 10^3$ (69·0)	—	$9{\cdot}01 \times 10^3$ (83·2)
Tetramethylammonium iodide (14) Method 1	$2 \times 4{\cdot}09 \times 10^3$ (62·8)	—	$2 \times 2{\cdot}41 \times 10^3$ (44·5)
Method 2	$2 \times 3{\cdot}50 \times 10^3$(53·7)	—	$2 \times 2{\cdot}85 \times 10^3$ (52·6)
Tetramethylammonium picrate (15)	$2 \times 3{\cdot}01 \times 10^3$ (46·2)	—	$2 \times 3{\cdot}18 \times 10^3$ (58·7)

*The total activity in isopimpinellin is set at 100%.

may reflect some activity in the aromatic ring portion of isopimpinellin (2). On the other hand it is clear that there is some difficulty associated with counting tetramethylammonium iodide (14), as is reflected in the wide variation of counts obtained for this compound and its picrate (15), so this discrepancy may not be a real one.

It is difficult to explain these observed incorporations on the basis of the normally invoked theories of acetate metabolism in plants. Acetate is not normally considered to be a "C_1" source but it is definitely acting in such a manner in this case. One possible explanation could be that acetate is being converted to glyoxylic acid (31) *via* the glyoxylate cycle (presented in part in Fig 1). Glyoxylic acid (31) is a well known donor of "active formate" and as such could serve, in conjunction with folic acid, as a source of carbon for the OMe C atoms, for C-4 and some of the carbon atoms in the benzenoid portion of isopimpinellin (2).

The three C atoms of the lactone ring present in the coumarins arise from phosphoenol pyruvate.[5] C-3 of phosphoenol pyruvate is known to arise from "active formate" *via* folic acid[6] and thus C-4 of isopimpinellin (2) could arise from the formate pool (Fig 2). Since it is well known that phosphoenol pyruvate is also involved in the biosynthesis of the benzenoid system, via the shikimate pathway, it would be expected that radioactive pyruvate would label the benzene portion of 2. Such a result is actually observed if the difference in activity between the OMe groups and the diacetate mentioned above is considered.

It should be mentioned that acetate, in addition to entering the pathway via the glyoxalate cycle mentioned above, could also enter at the citrate synthesis level. In this instance the sequence, citrate → succinate →→ oxaloacetate → phosphoenol pyruvate, would label both C-2 and C-3 of the latter, a result which is not in accord with the experimental data.

Although these proposals would explain the

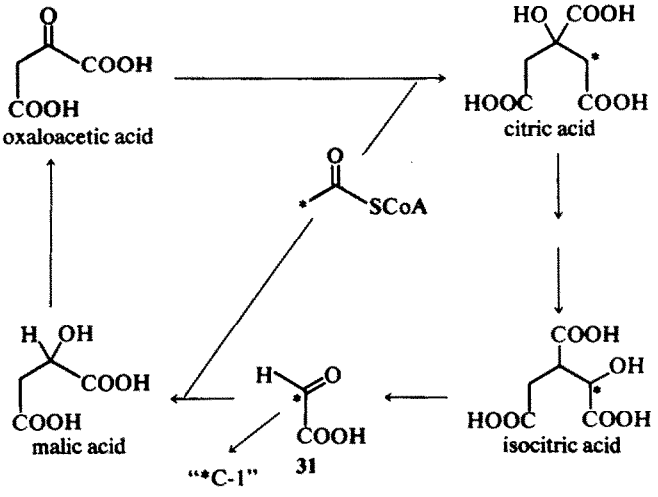

Fig 1. The glyoxylate cycle (in part)[4]

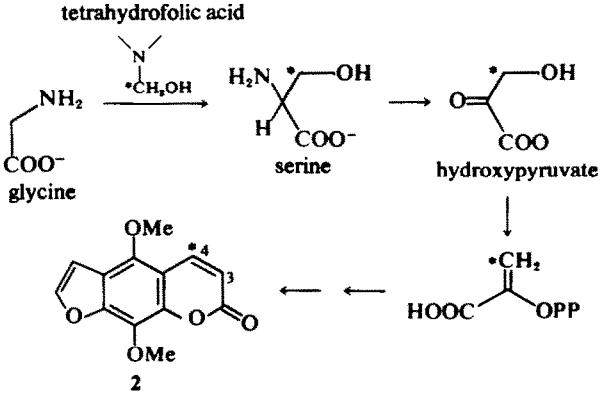

Fig 2. Proposed incorporation of "active formate" into C-4 of isopimpinellin (2).

observed distribution of activity in isopimpinellin (2), they must be considered as conjecture at this moment as acetate is a key precursor of many plant metabolites, any number of which could enter into coumarin biosynthesis. The results do however offer some explanation of the more efficient incorporation of acetate relative to mevalonate into isopimpinellin (2). It is notable that if one considers only the incorporation of acetate into the furan portion of isopimpinellin (2), the level of incorporation is actually lower in experiments 1 and 2 than was observed for mevalonate.[1]

As noted previously, low incorporation was also achieved into alloimperatorin methyl ether (3) in experiments 1 and 3. In these experiments 3 was converted to the diol (20) in the usual manner and then purified to constant radioactivity. Unfortunately the radioactivity present in the diol (20), after constant activity was achieved, was not sufficient (in experiment 1) to perform a complete degradation. As it was expected that the furanocoumarin portion of the molecule would be biosynthesized in a manner similar to isopimpinellin (2), attention was directed primarily to the dimethylallyl side chain. The results of these degradations are presented in Table 3.

These experiments show that acetate is indeed a precursor of the dimethylallyl side chain of 3. The incorporation into the terminal three carbon atoms of the side chain is considerable (48–62%) and as the side chain can be thought of as being formed from three acetate units (if it were mevalonate derived) one could reasonably conclude that ~ 25% of the original activity of 3 should reside in the remaining two C atoms of the side chain. With ~ 6% of the radioactivity in the OMe group, this suggests that only 10–20% of the original activity of 3 resides in the furanocoumarin portion of the molecule, presumably in the 4- and 7- positions. The incorporation of acetate into 3 would appear to be considerably greater than has been observed for mevalonate. However the significance of this increase is unclear. That the side chain is acetate derived would appear to be in keeping with mevalonate serving as the vehicle for incorporation. It is hoped that future experiments will provide more definite information in this regard.

The results reinforce the statement, made earlier, that for incorporation studies to yield truly meaningful information, degradations must be performed to determine the distribution of the labels in the molecules under study. Thus although one might have reasonably assumed that if acetate were to incorporate into simple furanocoumarins, it would incorporate exclusively into the furan portion of the molecule, this has been shown to be an invalid assumption. The mechanism by which acetate incorporates into C-4 and the OMe groups of isopimpinellin (2) is unclear, but such incorporation has definitely been observed.

Recently Shah and Rogers[7] have postulated the intermediacy of glycollate via glycine and serine

Table 3. Degradation of active alloimperatorin methyl ether (3) (Experiments 1 and 3)

Compound	Specific activity (% of alloimperatorin methyl ether (3))		
		Experiment 3	
	Experiment 1	Trial a	Trial b
Alloimperatorin methyl ether diol (20)	$7{\cdot}02 \times 10^3$ dpm/mmol (100)	$2{\cdot}29 \times 10^4$ dpm/mmol (100)	
5-(2′-Hydroxyethyl)-8-methoxypsoralen (17)	$3{\cdot}86 \times 10^3$ (5)	$1{\cdot}022 \times 10^4$ (43)	
Acetone *p*-bromobenzenesulfonylhydrazone (21)	$3{\cdot}38 \times 10^3$ (48)	$1{\cdot}49 \times 10^4$ (62·7)	
Tetramethylammonium iodide (14) Method 1			
Method 2	$4{\cdot}95 \times 10^2$ (7)*	$1{\cdot}07 \times 10^3$ (4·5)*	
5-(2′-Acetoxy-3′-hydroxy-3′-methylbutyl)-8-methoxypsoralen (22)			$1{\cdot}605 \times 10^4$ dpm/mmol (100)
5-(2′-Acetoxy-3′-hydroxy-3′-methylbutyl)-6-formyl-7-hydroxy-8-methoxy-coumarin (23)			—
5-(2′-Acetoxy-3′-hydroxy-3′-methylbutyl)-6-acetoxy-7,8-dimethoxycoumarin (25b)			$1{\cdot}44 \times 10^4$ (89·6)
1-(2′-Acetoxy-3′-hydroxy-3′-methylbutyl)-2,6-diformyl-3,5-dihydroxy-4-methoxybenzene (26)			$1{\cdot}39 \times 10^4$ (87·0)

*Insufficient 14 obtained to convert to picrate.

in the biosynthesis of acetyl-CoA. This proposal was based on the work with greening etiolated maize seedlings and involved intra- and extra-chloroplastidic terpenoid synthesis. The authors felt that their work offered strong evidence for a number of proposals, specifically the relatively direct synthesis of amino acids from carbon dioxide by-passing the carbohydrate intermediates and the involvement of these amino acids in terpenoid biosynthesis. Both of these proposals are supported in the literature.[8,9]

In an attempt to determine a specifically incorporated (i.e., non-randomized) precursor of mevalonate and the C_9 unit of cephaeline, Gear and Garg[10] administered labelled glycollic acid and glycine to three-year-old *Cephalis ipecacuanha* plants. The [1-^{14}C]-labelled forms of both these compounds gave either inactive or only slightly active cephaeline. This was not unexpected as Shah and Rogers[7] had likewise reported negative incorporation for glyoxylic acid-[1-^{14}C] into β-carotene. The rationale for this finding can be seen in the biogenesis of acetyl-CoA as postulated by these authors. When, however, glycine-[2-^{14}C] and glycollic acid-[2-^{14}C] were fed to the aforementioned plant system, active cephaeline was isolated. It was found that glycollic acid-[2-^{14}C] was being randomized whereas glycine-[2-^{14}C] was acting as a specific precursor of the C_9 unit of cephaeline.

In subsequent publication, glycine-[2-^{14}C] and acetate-[2-^{14}C] were fed to *C. acumentata*[11] and *R. serpentina*[12] plants. In the former, cephaeline and the phytosterol, β-sitosterol, were isolated and examined. It was found that, whereas acetate was specifically incorporated into the sterol, its activity was randomized in cephaeline whereas glycine-[2-^{14}C] was again reported to be specifically incorporated into the C_9 unit of cephaeline but not into the phytosterol. This result was taken to indicate that glycine and acetate "act as specific and exclusive precursors of different monoterpene moieties, in different compounds, in the same plant." Therefore it was felt that if glycine-[2-^{14}C] can act as a specific precursor of the C_9 unit of cephaeline, which has been known to be mevalonate derived, it might also act as a specific precursor of the alkyl side chains found in many natural coumarins. Therefore it was decided to study the role of glycine in the biosynthesis of these alkyl groups.

In a series of experiments, glycine-[2-^{14}C] was administered to young *Thamnosma montana* plants by the hydroponic method and the plants were allowed to grow for a period of 7 days. The plants were worked up in the normal manner and alloimperatorin methyl ether (**3**) was converted to the diol (**20**) for counting purposes. The results are presented in Table 4.

Thus the results in Table 4 indicate that glycine-[2-^{14}C] is being incorporated into all three coumarins and the incorporation level is reasonable.

To determine the distribution of radioactivity in umbelliprenin (**1**), it was degraded according to the scheme previously described.[1] Thus umbelliprenin (**1**) ($2{\cdot}23 \times 10^5$ dpm/mmole) from experiment 4 was subjected to acid hydrolysis as previously described and umbelliferone (**5**) was isolated and shown to have a specific activity of $2{\cdot}43 \times 10^4$ dpm/mmole or 10·9% of the original radioactivity of **1**. In a similar experiment, umbelliprenin (**1**) ($2{\cdot}16 \times 10^5$ dpm/mmole) from experiment 5 was degraded and umbelliferone (**5**) ($3{\cdot}24 \times 10^4$ dpm/mmole) was shown to have 15% of the original activity of umbelliprenin (**1**). Thus it is clear that between 85–90% of the radioactivity of **1** resides in the farnesyl side chain and only 10–15% is present in the rest of the coumarin molecule.

It was also of interest to gain information as to the distribution of radioactivity present in the farnesyl side chain of umbelliprenin (**1**). To this end, umbelliprenin (**1**) ($2{\cdot}23 \times 10^4$ dpm/mole) from experiment 5 was ozonized as previously described and levulinaldehyde *bis*-2,4-dinitrophenylhydrazone (**6b**) was isolated. This compound was shown to have a specific activity of $4{\cdot}55 \times 10^3$ dpm/mmole or 20·4% of the original activity of **1**. As two molar equivalents of levulinaldehyde (**6a**) should be produced in this reaction, this result indicates that only 41% of the radioactivity in the side chain resides in the internal ten carbon portion. Whether this represents an unequal labeling of the farnesol or reflects some error in the counting method is difficult to determine at this time as the lack of nonactive umbelliprenin (**1**) precludes further experimentation.

To determine the distribution of radioactivity in

Table 4. Incorporation of glycine-[2-^{14}C] into *Thamnosma montana*

Experiment no.	Activity[a] fed (dpm)	Weight of plant (g)	% Incorporation		
			Umbelliprenin (**1**)	Alloimperatorin methyl ether (**3**)	Isopimpinellin (**2**)
4	$8{\cdot}8 \times 10^7$	15	0·031	0·073	0·082
5	$2{\cdot}53 \times 10^8$	20	0·019	0·029	0·15
6	$5{\cdot}55 \times 10^8$	20	0·001	0·01	0·022

[a]Corrected for activity recovered outside the plant.
[b]Counted as alloimperatorin methyl ether diol (**20**).

isopimpinellin (2) this compound from experiments 4, 5 and 6 was degraded according to the scheme previously described[1] and the results are presented in Table 5.

From these results it is evident that more than 90% of the activity in isopimpinellin (2) resides in the two OMe groups and very little (less than 10%) activity is present in the rest of the molecule. The incorporation of glycine into the OMe groups of isopimpinellin (2) is explicable in terms of the degradation of glycine to a C_1-unit, a process observed previously. Byerrum *et al*[13] showed that in plant systems, C-2 of glycine could function as a C_1-unit as efficiently as the methyl groups of methionine and choline, and ten times as efficiently as formate whereas the carboxyl carbon of glycine showed no such activity. Our studies are consistent with these findings.

To determine the distribution of radioactivity in alloimperatorin methyl ether (3) from experiments 4, 5 and 6, it was degraded according to the scheme already described[1] and the results are given in Table 6.

These results indicate that glycine-[2-^{14}C] is being incorporated into the dimethylallyl side chain of alloimperatorin methyl ether (3). Also it is clear from these results that most of the activity in 3 is present in the OMe groups. It is significant to note that very little activity could be found in the furanocoumarin portion of the molecule indicating that glycine-[2-^{14}C] is acting as a specific precursor of the C_1-pool and the C_5-alkyl side chain. This result is in contrast to the experiments with acetate-[2-^{14}C] where it was found that acetate-[2-^{14}C] was being incorporated into the furanocoumarin portion of the molecule as well as in the OMe groups and the alkyl side chain.

It is pertinent to note that while the above experiments were being conducted, studies in the plant, *Vinca rosea L*, on the incorporation of glycine into the indole alkaloid ajmalicine were being pursued in our laboratory. Although the alkaloid bears an identical C_{10} unit to that present in cephaeline we were unable to demonstrate any incorporation of glycine into this portion of the molecule.[14] Similar findings were also reported from another laboratory in which *V. rosea* and *Strychnos nux vomica* plant systems were utilized to evaluate glycine incorporation into the alkaloids vindoline and strychnine.[15] These latter authors also conclude that in these two plant systems, glycine-2-^{14}C does not function as a precursor of the $C_{9\text{-}10}$ unit but contributes to the plant C_1 pool, a situation in accord with our published findings[14] and with the present results. In the meantime, a detailed account of the experiments by Garg and Gear has appeared.[16]

Table 5. Degradation of radioactive isopimpinellin (2) (Experiments 4, 5 and 6)

Compound	Specific activity (% total activity in isopimpinellin)[a]		
	Experiment 4	Experiment 5	Experiment 6
Isopimpinellin (2)	$3{\cdot}208 \times 10^5$ (100%) dpm/mmole	$2{\cdot}80 \times 10^4$ (100%) dpm/mmole	$1{\cdot}00 \times 10^5$ (100%) dpm/mmole
6-Formyl-7-hydroxy-5,8-dimethoxycoumarin (7)	$3{\cdot}12 \times 10^5$ (97·5%)	$2{\cdot}57 \times 10^4$ (91·8%)	$8{\cdot}2 \times 10^4$ (82%)
6-Formyl-5,7,8-trimethoxy-coumarin (8)	—	—	—
6-Hydroxy-5,7,8-trimethoxy-coumarin (9)	$2{\cdot}91 \times 10^5$ (90·5%)	—	—
1,3-Diformyl-4,6-dihydroxy 2,5-dimethoxybenzene (11)	$3{\cdot}03 \times 10^5$ (94·5%)	$2{\cdot}57 \times 10^4$ (91·8%)	$9{\cdot}27 \times 10^4$ (92·7%)
1,3-Diformyl-2,4,5,6-tetra methoxybenzene (12)	$3{\cdot}06 \times 10^5$ (95·5%)	—	—
1,3-Diacetoxy-2,4,5,6-tetra-methoxybenzene (13b)	$3{\cdot}08 \times 10^5$ (96%)	—	$9{\cdot}17 \times 10^4$ (91·7%)
Tetramethylammonium iodide (14)	$2 \times 1{\cdot}44 \times 10^5$ (89·8%)	$2 \times 1{\cdot}44 \times 10^5$ (100%)	$2 \times 4{\cdot}75 \times 10^4$ (95%)
Tetramethylammonium picrate (15)	$2 \times 1{\cdot}38 \times 10^5$ (86%)	$2 \times 1{\cdot}26 \times 10^5$ (90·5%)	—

[a]The total activity in isopimpinellin is set at 100%.

Table 6. Degradations of radioactive alloimperatorin methyl ether (3)

Compound	Specific activity (% of alloimperatorin methyl ether)			
	Experiment 4	Experiment 5	Experiment 6	
			Trial a	Trial b
Alloimperatorin methyl ether diol (20)	$3{\cdot}29 \times 10^4$ (100%) dpm/mole	$1{\cdot}10 \times 10^4$ (100%) dpm/mmole	$1{\cdot}22 \times 10^4$ (100%) dpm/mmole	
5-(2'-Hydroxyethyl)-8-methoxypsoralen (17)	—	$1{\cdot}00 \times 10^4$ (91·5%)	$1{\cdot}11 \times 10^4$ (92%)	
Acetone *p*-bromobenzenesulfonylhydrazone (21)	$2{\cdot}19 \times 10^3$ (6·7%)	$5{\cdot}72 \times 10^2$ (5·2%)	—	
Tetramethylammonium iodide (14)	—	$7{\cdot}7 \times 10^3$ (70%)	$1{\cdot}12 \times 10^4$ (92%)	
Tetramethylammonium picrate (15)	—	—	$1{\cdot}18 \times 10^4$ (97·5%)	
5-(2'-Acetoxy-3'-hydroxy-3'-methylbutyl)-8-methoxypsoralen (22)	$3{\cdot}29 \times 10^4$ (100%)	$1{\cdot}10 \times 10^4$ (100%)	$1{\cdot}22 \times 10^4$ (100%)	$4{\cdot}85 \times 10^4$ (100%)
5-(2'-Acetoxy-3'-hydroxy 3'-methylbutyl)-6-formyl-7-hydroxy-8-methoxycoumarin (23)	$2{\cdot}91 \times 10^4$ (88·5%)	$1{\cdot}10 \times 10^4$ (100%)	$1{\cdot}18 \times 10^4$ (96·6%)	$4{\cdot}75 \times 10^4$ (97·5%)
5-(2'-Acetoxy-3'-hydroxy 3'-methylbutyl)-6-acetoxy-7,8-dimethoxycoumarin (25b)	—	$1{\cdot}06 \times 10^4$ (96·5%)	—	
1-(2'-Acetoxy-3'-hydroxy-3'-methylbutyl)-2,6-diformyl-3,5-dihydroxy-4-methoxybenzene (26)	—	$1{\cdot}04 \times 10^4$ (94·5%)	—	

EXPERIMENTAL

For detailed description of experimental procedure employed in the various degradations, see accompanying publication.[1]

Feeding experiments with sodium acetate-[2-^{14}C]. In these experiments, sodium acetate-[2-^{14}C] (obtained from Amersham/Searle Corp.) was administered to cut shoots of 2–3 year old *Thamnosma montana* plants by hydroponic method. The precursor obtained as solid salt was dissolved in distilled water (~ 2 ml) and administered in this form. After the soln was absorbed by the plants, the original vials containing the precursor were rinsed with distilled water and these washes were allowed to be absorbed by the plants. The plants were allowed to grow for 7 days under continuous fluorescent illumination. After the preselected time period, the plants were worked up as described previously[1] and 2 and 3 were isolated. In each case, 3 was converted to the diol 20 before counting. Where necessary, dilutions were performed to provide sufficient sample for purification and, if necessary, for degradation.

Degradations of isopimpinellin (2) *from experiment* 1

Radioactive 6-formyl-7-hydroxy-5,8-dimethoxycoumarin (7). (a) Isopimpinellin 2 (47 mg, $1{\cdot}305 \times 10^4$ dpm/mmole) from Experiment 1 was selectively ozonized as described previously[1] and 7 (5·0 mg) was isolated and shown to have specific activity $1{\cdot}185 \times 10^4$ dpm/mmole or 90·6% of the original activity of 2. (b) Isopimpinellin 2 (49 mg, $8{\cdot}04 \times 10^3$ dpm/mmole) from experiment 1 was selectively ozonized as described previously[1] and crystalline 7 (12·0 mg) was isolated and shown to have specific activity $7{\cdot}12 \times 10^3$ dpm/mmol or 88·6% of the activity of the original 2.

Radioactive 6-formyl-5,7,8-trimethoxycoumarin 8. The mother liquors from the crystallizations of 7 above (b) were purified by preparative TLC and crude 7 (12 mg) was isolated. This material was methylated as previously described[1] and 8 (5·5 mg) was isolated. Pure 7 (6·8 mg) from the above reaction was methylated in the same manner and 8 (5·0 mg) was isolated. The two samples were combined and a count revealed it had a specific activity of $7{\cdot}22 \times 10^3$ dpm/mmole (89·8% of the original activity of 2).

Radioactive 6-hydroxy-5,7,8-trimethoxycoumarin (9). Compound 8 (10·2 mg, $7{\cdot}22 \times 10^3$ dpm/mmole) from the above reaction was degraded as previously described[1] and 9 (4·6 mg) was isolated and shown to have a specific activity of $6{\cdot}95 \times 10^6$ dpm/mmole or 86·5% of the original activity of 2.

Radioactive 1,3-diformyl-2,6-dihydroxy-2,5-dimethoxybenzene (11). Isopimpinellin 2 (52 mg, $1{\cdot}305 \times 10^4$ dpm/mmole) from experiment 1 was ozonized as previously

described[1] and crystalline **11** (9·5 mg) was isolated and shown to have a specific activity of $1·150 \times 10^4$ dpm/mmole or 88·2% of the original activity of **2**.

1,3-Diformyl-2,4,5,6-tetramethoxylbenzene (**12**). Pure **11** (8·5 mg) was methylated as previously described and **12** (8·2 mg) was isolated. The mother liquors from the crystallizations of **11** in the above reaction were methylated in the same manner and **12** (5·2 mg) was isolated. The two samples were combined and crystallized (11·8 mg) and this material was used in the next reaction.

Radioactive 1,3-diacetoxyl-2,4,5,6-tetramethoxybenzene (**13b**). Compound **12** (11·8 mg, $1·150 \times 10^4$ dpm/mmole) from the above reaction was degraded as previously described[1] and **13b** (8·1 mg) was isolated and shown to have a specific activity of $9·00 \times 10^3$ dpm/mmole or 69·0% of the original activity of **2**.

Radioactive tetramethylammonium iodide (**14**). Isopimpinellin **2** (20·8 mg, $1·305 \times 10^4$ dpm/mmole) from experiment 1 was demethylated as described previously[1] and **14** (28·8 mg) was isolated. This material was counted in two ways. In Method 1 the salt (~ 0·5 mg was dissolved in water (10 drops) and MeOH (1 ml) and the soln was made up to 15 ml with aqueous scintillator soln. However iodine appeared to be produced in this mixture causing the soln to be coloured and thus lowering counting efficiency (to about 35%).

In Method 2 the salt (~ 2 mg) was dissolved in 0·1N $Na_2S_2O_3$ aq (10 drops) and MeOH (1 ml) and the soln was made up to 15 ml with aqueous scintillator soln. In this method the soln remained colourless and a counting efficiency of ~ 65% was achieved. In both cases a blank sample of the same constitution (except that non-radioactive **14** was used) was counted in the same vial to determine background. By method 1, **14** indicated a specific activity of $5·09 \times 10^3$ dpm/mmole or 31·4% of the original activity of **2**. By method 2, **14** indicated a specific activity of $3·50 \times 10^3$ dpm/mmole or 26·8% of the original activity of **2**. The iodide **14** (12 mg) was converted to the picrate **15** and this was counted by the following method. The derivative **15** (~ 2 mg) was dissolved in glacial AcOH (5 drops) and Ac_2O (5 drops) and enough Zn dust was added to decolourize the soln. Sodium metabisulfite (~ 100 mg) was added and the mixture was then filtered directly into the counting vial. The original container was washed with MeOH (1 ml) and this wash was also filtered into the counting vial. The soln was made up to 15 ml with organic scintillator soln and then counted after standing at least 1 hr in the cold and in the dark. As before, an inactive sample of **15** was counted in the same manner to determine background prior to counting the radioactive sample. By this method the picrate **15** indicated a specific activity of $3·01 \times 10^3$ dpm/mmole or 23·1% of the original activity of **2**.

Degradations of isopimpinellin from experiment 2

Radioactive 6-formyl-7-hydroxy-5,8-dimethoxycoumarin (**7**). Isopimpinellin **2** (66·2 mg, $1·08 \times 10^4$ dpm/mmole) from experiment 2 was selectively ozonized as previously described and **7** (15·5 mg) was isolated. This material was not counted but used immediately in the next reaction.

Radioactive 6-formyl-5,7,8-trimethoxycoumarin (**8**). Compound **7** (15·5 mg) from the above reaction was methylated as previously described and **8** (10·2 mg) was isolated and shown to have specific activity ($9·71 \times 10^3$ dpm/mmole or 89·9% of the original activity of **2**.

Radioactive 6-hydroxy-5,7,8-trimethoxycoumarin **9**. Compound **8** (8·2 mg, $9·71 \times 10^3$ dpm/mmole) from the above reaction was degraded as previously described and **9** (2·6 mg) was isolated. This material was shown to have a specific activity of $9·83 \times 10^3$ dpm/mmole or 91·1% of the original activity of **2**.

Radioactive 1,3-diformyl-4,6-dihydroxy-2,5-dimethoxybenzene (**11**). Isopimpinellin **2** (60·0 mg, $1·08 \times 10^4$ dpm/mmole) from experiment 2 was ozonized as previously described and **11** (15·0 mg) was isolated and shown to have specific activity of $9·70 \times 10^3$ dpm/mmole or 89·6% of the original activity of **2**.

Radioactive 1,3-diformyl-2,4,5,6-tetramethoxybenzene (**12**). Compound **11** (13 mg) from the above reaction was mixed with the crystallization mother liquors of **11** and methylated as previously described. The product, **12** (13·5 mg) was isolated. A count of this material indicated a specific activity of $9·73 \times 10^3$ dpm/mmole or 90·0% of the original activity of **2**.

Radioactive 1,3-diacetoxy-2,4,5,6-tetramethoxybenzene (**13b**). Compound **12** (11·7 mg, $9·73 \times 10^3$ dpm/mmole) from the previous reaction was degraded as previously described and **13b** (9·5 mg) was isolated and shown to have a specific activity of $9·01 \times 10^3$ dpm/mmole or 83·2% of the original activity of **2**.

Radioactive tetramethylammonium iodide (**14**). Isopimpinellin **2** (20·0 mg, $1·083 \times 10^3$ dpm/mmole) from experiment 2 was demethylated as previously described and **14** (28·3 mg) was isolated. Counting by method 1 (see previous demethylation) indicated a specific activity of $2·41 \times 10^3$ dpm/mmole or 22·3% of the original activity of **2**. Method 2 indicated a specific activity of $2·85 \times 10^3$ dpm/mmole or 26·3% of the original activity of **2**. The iodide **14** (12 mg) was converted to the picrate **15** and this material was shown to have specific activity $3·18 \times 10^3$ dpm/mmole or 29·4% of the original activity of **2**.

Degradations of alloimperatorin methyl ether (**3**) *from experiment 1*

Periodic acid cleavage of radioactive 5-(2',3'-dihydroxy-3'-methylbutyl) 8-methoxypsoralen (**20**) (*alloimperatorin methyl ether diol*). Compound **20** (46 mg, $7·02 \times 10^3$ dpm/mmole) from experiment 1 was cleaved with periodic acid as described previously[1] and **17** (19·2 mg) was isolated and shown to have a specific activity of $3·86 \times 10^3$ dpm/mmole or 55% of the original activity of **20**. Compound **21** (11·8 mg) was also isolated and was shown to have specific activity $3·38 \times 10^3$ dpm/mmole or 48% of the original activity of **20**.

Demethylation of radioactive 5-(2'-hydroxyethyl)-8-methoxypsoralen (**17**). Compound **17** (15·9 mg, $3·83 \times 10^3$ dpm/mmole) from the previous reaction was demethylated as described previously[1] and **14** (6·2 mg) was isolated. When counted by method 2 this material had specific activity $4·95 \times 10^2$ dpm/mmole or 7·05% of the original activity of **20**.

Degradations of alloimperatorin methyl ether (**3**) *from experiment 3*

Periodic acid cleavage of 5-(2',3'-dihydroxy-3'-methylbutyl)-8-methoxypsoralen (**20**) (*alloimperatorin methyl ether diol*). Compound **20** (59 mg, $7·38 \times 10^4$ dpm/mmole) from experiment 3 was cleaved with periodic acid as described previously[1] and **17** (23 mg) was isolated and shown to have a specific activity $1·022 \times 10^4$ dpm/mmole or 43% of the original activity of **20**. Compound **21** (28 mg) was also isolated and was shown to have a specific activity of

$1{\cdot}49 \times 10^3$ dpm/mmole or 62·7% of the original activity of **20**.

Demethylation of radioactive 5-(2′-*hydroxyethyl*)-8-*methoxypsoralen* (**17**). Compound **17** (18·2 mg, $1{\cdot}022 \times 10^4$ dpm/mmole) from the previous reaction was demethylated as described previously[1] and **14** (10·5 mg) was isolated and shown by counting method 2 to have a specific activity $1{\cdot}07 \times 10^3$ dpm/mmole or 4·5% of the original activity of **20**.

Radioactive 5-(2′-*acetoxy*-3′-*hydroxy*-3′-*methylbutyl*)-8-*methoxypsoralen* (**22**). Compound **20** (12·1 mg, $1{\cdot}40 \times 10^5$ dpm/mmole) from experiment 3 was diluted with non-radioactive **20** (89·2 mg) and this material was converted to **22** (98·0 mg) which was shown to have a specific activity of $1{\cdot}605 \times 10^4$ dpm/mmole.

Radioactive 5-(2′-*acetoxy*-3′-*hydroxy*-3′-*methylbutyl*)-6-*formyl*-7-*hydroxy*-8-*methoxycoumarin* (**23**). Compound **22** (63·5 mg, $1{\cdot}605 \times 10^4$ dpm/mmole) from the above reaction was selectively ozonized as previously described[1] and after crystallization, **23** (15·0 mg) was isolated. This material was not counted but used directly in the next reaction.

Radioactive 5-(2′-*acetoxy*-3′-*hydroxy*-3′-*methylbutyl*)-6-*formyl*-7,8-*dimethoxycoumarin* (**24**). Compound **23** (15·0 mg) from the above reaction was methylated as previously described[1] and **24** (9·0 mg) was isolated and shown to have specific activity $1{\cdot}44 \times 10^4$ dpm/mmole or 89·6% of the original activity of compound **22**.

Radioctive 1-(2′-*acetoxy*-3′-*hydroxy*-3′-*methylbutyl*)-2,6-*diformyl*-3,5-*dihydroxy*-4-*methoxybenzene* (**26**). Compound **22** (30·8 mg, $1{\cdot}605 \times 10^4$ dpm/mmole) from experiment 3 was ozonized as previously described[1] and **26** (9·8 mg) was isolated and shown to have a specific activity of $1{\cdot}39 \times 10^4$ dpm/mmole or 87·0% of the original activity of **22**.

Feeding experiments with glycine-[2-^{14}C]. In these experiments, glycine-[2-^{14}C] (obtained from New England Nuclear Corp., Boston, Mass) was administered to young whole plants (2–3 years old) by hydroponic technique. The precursor, obtained as 0·1N HCl, was used as such and the plants were allowed to grow for 7 days. The plants were worked up as before and compound isolated. In each case, **3** was converted to **20**.

Degradations of umbelliprenin (**1**) *from experiments* 4 *and* 5

Acid-catalyzed hydrolysis of umbelliprenin (**1**). Umbelliprenin **1** (40 mg, $2{\cdot}23 \times 10^5$ dpm/mmole) from experiment 4 was hydrolyzed with AcOH as described previously[1] and **5** (12 mg) was isolated and shown to have a specific activity of $2{\cdot}43 \times 10^4$ dpm/mmole or 10·9% of the original activity of **1**.

Acid-catalyzed hydrolysis of umbelliprenin (**1**). Radioactive **1** (50 mg, $2{\cdot}16 \times 10^5$ dpm/mmole) from experiment 5 was degraded as described previously[1] and **5** (14 mg) was isolated and shown to have a specific activity of $3{\cdot}24 \times 10^4$ dpm/mmole or 15% of the total activity of **1**.

Ozonolysis of radioactive umbelliprenin (**1**). Umbelliprenin **1** (25 mg, $2{\cdot}23 \times 10^4$ dpm/mmole) from experiment 5 was ozonized under optimum conditions as described previously[1] and **6b** (20 mg) was isolated after crystallization. This material **6b** was counted in the following manner. The derivative **6b** (~ 2 mg) was dissolved in the counting vial in a mixture of glacial AcOH (10 drops), Ac_2O (5 drops) and DMF (20 drops). The mixture was then heated to complete dissolution and Zn dust (~ 50 mg) was added to decolourize the soln. Sodium metabisulfite (100 mg) was added, then benzene (~ 0·5 ml) and the soln was made up to 15 ml with organic scintillator soln. After standing in the cold and dark for 1 hr, the sample was counted. Due to the unorthodox counting soln employed, counting efficiency was determined by adding an accurately weighed sample of ^{14}C-hexadecane standard to the already counting sample and it was counted again. The ratio of the expected dpm to found cpm for hexadecane determined the counting efficiency (~ 64%). In each case a blank sample containing an equal amount of inactive levulinaldehyde *bis*-2,4-DNP (**6b**) was counted first to determine the accurate background. In this manner, the radioactive **6b** was shown to have a specific activity of $4{\cdot}55 \times 10^3$ dpm/mmole or 20·4% of the total activity of **1**.

Degradations of isopimpinellin (**2**) *from experiment* 4

Radioactive 6-*formyl*-7-*hydroxy*-5,8-*dimethoxycoumarin* (**7**). Radioactive **2** (50 mg, $3{\cdot}208 \times 10^5$ dpm/mmole) was selectively ozonized as previously described[1] and **7** (20 mg) was isolated. It was shown to have a specific activity of $3{\cdot}12 \times 10^5$ dpm/mmole or 97·5% of the total activity of **2**.

Radioactive 6-*formyl*-5,7,8-*trimethoxycoumarin* (**8**). Compound **7** (20 mg, $3{\cdot}12 \times 10^5$ dpm/mmole) from the above reaction was methylated and **8** (18 mg) was isolated by preparative layer chromatography. It was not counted but was used as such in the next reaction.

Radioactive 6-*hydroxy*-5,7,8-*trimethoxycoumarin* (**9**). Radioactive **8** (18 mg) from the previous reaction was treated with H_2O_2 and H_2SO_4 and **9** (10 mg) was isolated and shown to have a specific activity of $2{\cdot}99 \times 10^5$ dpm/mmole or 90·5% of the total activity of **2**.

Radioactive 1,3-*diformyl*-4,6-*dihydroxy*-2,5-*dimethoxybenzene* (**11**). Isopimpinellin **2** (60 mg, $3{\cdot}208 \times 10^5$ dpm/mmole) was ozonized as described previously[1] and crystalline **11** (23 mg) was isolated and shown to have a specific activity of $3{\cdot}03 \times 10^5$ dpm/mmole or 94·5% of the total activity of **2**.

Radioactive 1,3-*diformyl*-2,4,5,6-*tetramethoxybenzene* (**12**). Radioactive **11** (23 mg, $3{\cdot}03 \times 10^5$ dpm/mole) from the above reaction was methylated and **12** (19 mg) was isolated. This was shown to have a specific activity of $3{\cdot}06 \times 10^5$ dpm/mmole or 95·5% of the total activity of **2**.

Radioactive 1,3-*diacetoxy*-2,4,6-*tetramethoxybenzene* (**13b**). Compound **12** (19 mg, $3{\cdot}06 \times 10^5$ dpm/mmole) from the previous reaction was degraded as described previously[1] and **13b** (12 mg) was isolated and shown to have a specific activity of $3{\cdot}08 \times 10^5$ dpm/mmole or 96% of the total activity of isopimpinellin.

Radioactive tetramethylammonium iodide (**14**). Radioactive **2** (40 mg, $3{\cdot}208 \times 10^5$ dpm/mmole) was demethylated with HI and **14** (48 mg) was isolated. It was counted as previously described and was shown to have a specific activity of $2 \times 1{\cdot}44 \times 10^5$ dpm/mmole or 89·8% of the total activity of isopimpinellin. The iodide **14** was converted to the picrate **15** and this was counted as previously described and was shown to have a specific activity of $2 \times 1{\cdot}38 \times 10^5$ dpm/mmole or 86% of the total activity of **2**.

Degradations of isopimpinellin (**2**) *from experiment* 5

Radioactive 6-*formyl*-7-*hydroxy*-5,8-*dimethoxycoumarin* (**7**). Isopimpinellin 2(50 mg, $2{\cdot}80 \times 10^4$ dpm/mmole) from experiment 5 was ozonized and **7** (20 mg) was isolated and shown to have a specific activity of $2{\cdot}57 \times 10^4$ dpm/mmole or 91·8% of the total activity of **2**.

Radioactive 1,3-*diformyl*-4,6-*dihydroxy*-2,5-*dimethoxy*-

benzene (**11**). Isopimpinellin **2** (43 mg, $2{\cdot}80\times10^4$ dpm/mmole) was ozonized as described previously[1] and **11** (8·5 mg) was isolated and shown to have a specific activity of $2{\cdot}57\times10^4$ dpm/mmole or 91·8% of the total activity of **2**.

Radioactive tetramethylammonium iodide (**14**). Isopimpinellin **2** (23 mg, $2{\cdot}80\times10^4$ dpm/mmole) was demethylated and **14** (25 mg) was isolated and shown to have a specific activity of $2\times1{\cdot}44\times10^4$ dpm/mmole or all the original activity of **2**. The iodide **14** was converted to the picrate **15** and this was shown to have a specific activity of $2\times1{\cdot}26\times10^4$ dpm/mmole or 90% of the activity of isopimpinellin.

Degradations of isopimpinellin **2** *from experiment* 6

6-Formyl-7-hydroxy-5,8-dimethoxycoumarin (**7**). Radioactive isopimpinellin (35 mg, $1{\cdot}00\times10^5$ dpm/mmole) was selectively ozonized and **7** (15 mg) was isolated and was shown to have a specific activity of $8{\cdot}2\times10^4$ dpm/mmole or 82% of the total activity of **2**.

Radioactive 1,3-diformyl-4,6-dihydroxy-2,5-dimethoxybenzene (**11**). Isopimpinellin (55 mg, $1{\cdot}00\times10^5$ dpm/mg) was ozonized as described previously[1] and **11** (19·5 mg) was isolated and shown to have a specific activity of $9{\cdot}27\times10^4$ dpm/mmole or 92·7% of the total activity of **2**.

Radioactive 1,3-diformyl-2,4,5,6-tetramethoxybenzene (**12**). Radioactive **11** (19·5 mg, $9{\cdot}27\times10^4$ dpm/mmole) from the previous reaction was methylated and **12** (16 mg) was isolated. It was not counted but was used as such in the next reaction.

Radioactive 1,3-diacetoxy-2,4,5,6-tetramethoxybenzene (**13b**). Compound **12** (16 mg) from the previous reaction was degraded to **13b** (10 mg). This was shown to have a specific activity of $9{\cdot}17\times10^4$ dpm/mmole or 91·7% of the total activity of **2**.

Radioactive tetramethylammonium iodide (**14**). Isopimpinellin **2** (25 mg, $1{\cdot}00\times10^5$ dpm/mmole) from experiment 6 was demethylated and **14** (28 mg) was isolated and shown to have a specific activity of $2\times4{\cdot}75\times10^4$ dpm/mmole or 95% of the total activity of isopimpinellin.

Degradations of alloimperatorin methyl ether (**3**) *from experiment* 4

Periodic acid cleavage of 5-(2',3'-dihydroxy-3'-methylbutyl)-8-methoxyposoralen (**20**) (*alloimperatorin methyl ether diol*). Radioactive **20** (67·5 mg, $3{\cdot}29\times10^4$ dpm/mmole) from experiment 4 was cleaved with periodic acid and **21** (41 mg) was isolated and shown to have a specific activity of $2{\cdot}19\times10^3$ dpm/mmole or 6·7% of the total activity of **20**.

Radioactive 5-(2'-acetoxy-3'-hydroxy-3'-methylbutyl)-8-methoxypsoralen (**22**). Compound **20** from experiment 4 was acetylated to **22**.

Radioactive 5-(2'-acetoxy-3'-hydroxy-3'-methylbutyl)-6-formyl-7-hydroxy-8-methoxycoumarin (**23**). Radioactive **22** (56 mg, $3{\cdot}29\times10^4$ dpm/mmole) was selectively ozonized to **23** (26 mg). This was not counted but was used as such in the next reaction.

Radioactive 5-(2'-acetoxy-3'-hydroxy-3'-methylbutyl)-6-formyl-7,8-dimethoxycoumarin (**24**). Compound **23** (36 mg) from the previous reaction was methylated to give **24** (15 mg). This was shown to have a specific activity of $2{\cdot}91\times10^4$ dpm/mmole or 88·5% of the total activity of **22**.

Degradations of alloimperatorim methyl ether (**3**) *from experiment* **5**

Periodic acid cleavage of alloimperatorim methyl ether diol (**20**). Radioactive **20** (55 mg, $1{\cdot}10\times10^4$ dpm/mmole) was cleaved with periodic acid as previously described[1] and **17** (25·8 mg) was isolated and was shown to have a specific activity of $1{\cdot}00\times10^4$ dpm/mmole or 91·5% of the total activity of **20**. Compound **21** (11 mg) was also isolated. This was shown to have a specific activity of $5{\cdot}72\times10^2$ dpm/mmole or 5·2% of the total activity of **20**.

Demethylation of radioactive 5-(2'-hydroxyethyl)-8-methoxypsoralen (**17**). Radioactive **17** (23 mg, $1{\cdot}00\times10^4$ dpm/mmole) from the previous reaction was demethylated and **14** (10 mg) was isolated and was shown to have a specific activity of $7{\cdot}70\times10^3$ dpm/mmole or 70% of the total activity of **20**.

Radioactive 5-(2'-acetoxy-3'-hydroxy-3'-methylbutyl)-8-methoxypsoralen (**22**). Radioactive **20** from experiment 5 was acetylated to **22**.

Radioactive 5-(2'-acetoxy-3'-hydroxy-3'-methylbutyl)-6-formyl-7-hydroxy-8-methoxycoumarin (**23**). Radioactive **22** (67 mg, $1{\cdot}10\times10^4$ dpm/mmole) was selectively ozonized and **23** (32 mg) was isolated. This was shown to have a specific activity of $1{\cdot}10\times10^4$ dpm/mmole or all the activity of **22**.

Radioactive 5-(2'-acetoxy-3'-hydroxy-3'-methylbutyl)-6-formyl-7,8-dimethoxycoumarin (**24**). Radioactive **23** (32 mg, $1{\cdot}10\times10^4$ dpm/mmole) from the previous reaction was methylated and **24** (18·5 mg) was isolated. This was not counted but was used as such for the next reaction.

Radioactive 5-(2'-acetoxy-3'-hydroxy-3'-methylbutyl)-6-acetoxy-7,8-dimethoxycoumarin (**25b**). Radioactive **24** (18·5 mg) from the above reaction was degraded and **25b** (8·3 mg) was isolated and shown to have a specific activity of $1{\cdot}06\times10^4$ dpm/mmole or 96·4% of the total activity of **22**.

Radioactive 1-(2'-acetoxy-3'-hydroxy-3'-methylbutyl)-2,6-diformyl-3,5-dihydroxy-4-methoxybenzene (**26**). Radioactive **22** (50 mg, $1{\cdot}10\times10^4$ dpm/mmole) from experiment 5 was ozonized as described previously[1] and **26** (15 mg) was isolated. This was shown to have a specific activity of $1{\cdot}04\times10^4$ dpm/mmole or 99·5% of the total activity of **22**.

Degradations of alloimperatorin methyl ether (**3**) *from experiment* 6

Periodic acid cleavage of alloimperatorin methyl ether diol (**20**). Radioactive **20** (50 mg, $1{\cdot}22\times10^4$ dpm/mmole) from experiment 6 was cleaved with periodic acid and **17** (32 mg) was isolated and shown to have a specific activity of $1{\cdot}11\times10^4$ dpm/mmole or 92% of the total activity of **20**.

Demethylation of radioactive 5-(2'-hydroxyethyl)-8-methoxypsoralen (**17**). Radioactive **17** (20 mg, $1{\cdot}11\times10^4$ dpm/mmole) from the previous reaction was demethylated and **14** (12 mg) was isolated and was shown to have a specific activity of $1{\cdot}12\times10^4$ dpm/mmole or 92% of the original activity of **20**. The iodide **14** was converted to the picrate **15** which was shown to have a specific activity of $1{\cdot}18\times10^4$ dpm/mmole or 96·6% of the total activity of **20**.

Radioactive 5-(2'-acetoxy-3'-hydroxy-3'-methylbutyl)-6-formyl-7-hydroxy-8-methoxycoumarin (**23**)—*trial a*. Radioactive **22** (60 mg, $1{\cdot}22\times10^4$ dpm/mmole) was selectively ozonized and **23** (25 mg) was isolated and was shown to have a specific activity of $1{\cdot}18\times10^4$ dpm/mmole or 96·6% of the total activity of **22**.

Radioactive 5-(2'-acetoxy-3'-hydroxy-3'-methylbutyl)-6-formyl-7-hydroxy-8-methoxycoumarin (**23**)—*trial b*.

Radioactive **22** (60 mg, $4{\cdot}85 \times 10^4$ dpm/mmole) from experiment 6 was selectively ozonized and **23** (15 mg) was isolated and shown to have a specific activity of $4{\cdot}73 \times 10^4$ dpm/mmole or 97·5% of the total activity of **22**.

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