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BIOSYNTHETIC STUDIES IN THE COUMARIN SERIES—I

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

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Abstract—Investigations on the biosynthesis of the coumarins, umbelliprenin (1), isopimpinellin (2), alloimperatorin methyl ether (3) and isoimperatorin (4) are described. Extensive degradation studies on these systems have provided the opportunity to evaluate the various centers in these radioactive coumarins obtained from mevalonate incorporation. These results are discussed in the light of previous proposals.

The biogenesis of the two C atoms of the furan ring (C-6 and C-7) of the furanocoumarins has been a source of controversy over the years. Floss and Mothes[1] have shown that C-4 of mevalonic acid is incorporated specifically into the 7-position of the furan ring of furanocoumarins. However Caporale *et al.*[2] have reported the incorporation of radioactivity from acetate -[2-^{3}H], tyrosine -[2-^{14}C] and mevalonic acid -[2-^{14}C] into bergapten and psoralen in the leaves of *Ficus carica*. Similarly, reports of Brown[3] that mevalonic acid -[2-^{14}C] incorporates as efficiently as mevalonic acid -[5-^{14}C] into simple furanocoumarins and that acetate is a much more efficient precursor of furanocoumarins than mevalonic acid reopens the question as to the true role of mevalonic acid in the biosynthesis of these furanocoumarins. Since in the investigations of Brown[3] and Caporale *et al.*[2], no degradations were performed to determine the distribution of radioactivity in the compounds isolated, the significance of their results is questionable. Therefore, a detailed study on the biosynthesis of furanocoumarins and on the role of mevalonic acid in these systems was initiated.

It has been reported previously[4,5] that *Thamnosma montana* Torr. and Frem. contains a large array of substituted and unsubstituted coumarins and furanocoumarins. However for the purpose of these biosynthetic studies, only umbelliprenin (1), isopimpinellin (2), alloimperatorin methyl ether (3) and isoimperatorin (4) were selected as they are easily isolated and purified and are representative examples of the four main types of coumarin systems. Isopimpinellin (2) a simple furanocoumarin was selected to study the biosynthesis of the furan ring whereas alloimperatorin methyl ether (3) and isoimperatorin (4) offered an opportunity to study the biosynthesis of the alkyl and alkyl-ether side chains in these furanocoumarins. Umbelliprenin (1) a simple coumarin with a farnesyl-ether side chain, was

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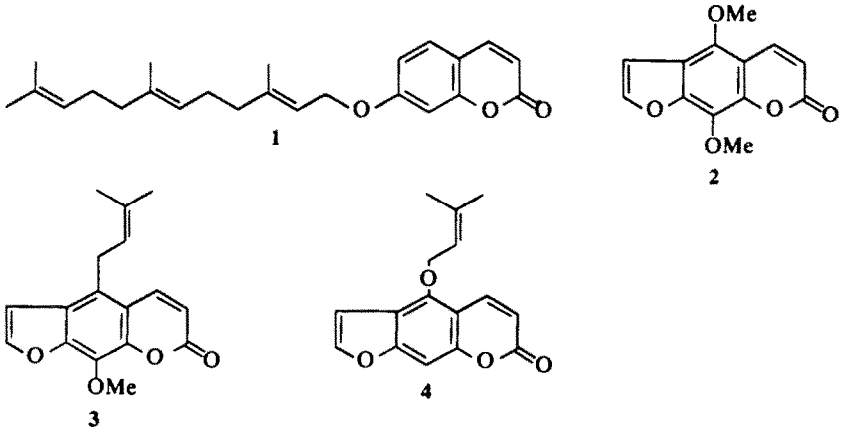
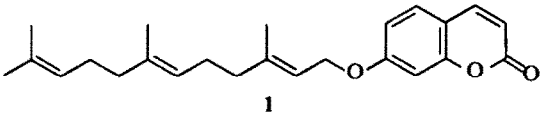


1 2 3 4

also investigated since the isoprenoid side chain made up from several C_5 units,[6,7] would allow this natural system to act as an internal standard in any subsequent mevalonic acid feeding experiments.

Prior to any biosynthetic investigations, it was essential to develop the appropriate degradation pathways which would allow evaluation of radioactivity in the relevant centres of the molecule. A brief description of these studies is now presented.

Degradation of umbelliprenin (**1**). As mentioned previously, it was of interest to determine the amount of radioactivity associated with the farnesyl side chain in the molecule isolated in the appropriate incorporation experiments. For this purpose, umbelliprenin (**1**) was hydrolyzed with hot glacial acetic acid[8] to give umbelliferone (**5**). However, all attempts to isolate farnesyl acetate failed and it was observed that under these reaction conditions, the farnesyl acetate being produced was undergoing a series of further reactions. However, since the radioactivity present in umbelliferone derived from the reaction would allow calculation of the radioactivity in the side chain by difference, it was decided to abandon further attempts to isolate the entire side chain.



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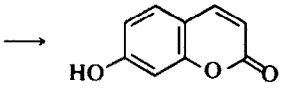

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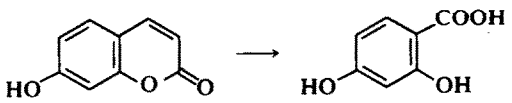
In order to determine the amount of radioactivity present (if any) in the 2, 3 and 4-positions of umbelliprenin, umbelliferone was treated with potassium hydroxide at high temperature. This reaction was previously reported to give resorcinol.[9] However, very little resorcinol could be obtained and the major product isolated proved to be 2,4-dihydroxybenzoic acid (48% yield). Thus this degradation allows the determination of radioactivity at the 2- and 3-positions in umbelliprenin.



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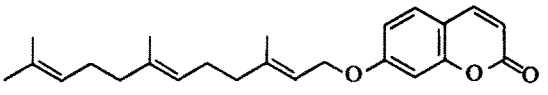
As it was of interest to determine the distribution of radioactivity in the farnesyl-ether side chain of umbelliprenin, a suitable degradation was devised. Caldwell and Jones[10] have reported the isolation of both acetone and levulinaldehyde (**6a**) (as their 2,4-dinitrophenylhydrazone derivatives) from 7-methoxy-5-geranylcoumarin by ozonolysis and steam distillation of the reaction mixture into a solution of 2,4-dinitrophenylhydrazine (2,4-DNP). However this procedure when applied in the case of umbelliprenin turned out to be unsatisfactory as the yield of levulinaldehyde-2,4-DNP (**6b**) was very poor (~ 1%). When the ozonide of **1** was decomposed under reductive conditions utilizing catalytic hydrogenation[9] and the resulting reaction mixture treated with a solution of 2,4-DNP reagent in methanolic hydrogen chloride, orange coloured crystals of **6b** precipitated (26% yield).



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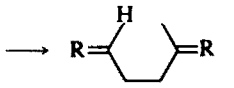


6a: R = O
6b: R = 2,4-DNP

Degradation of isopimpinellin (**2**). In order to clarify the various questions raised by the incorporation studies of Brown[3] and Caporale *et al.*[2], an extensive degradation procedure to determine the distribution of radioactivity in isopimpinellin (**2**) was needed. A summary of these degradations is given in Fig 1.

To determine the amount of radioactivity present at the 7-position, isopimpinellin was ozonized under controlled conditions. Previous workers[11] have found that by ozonolysis, furanocoumarins could be converted to the phenolic aldehydes where the furan ring has undergone degradation in preference to the pyrone ring. Thus isopimpinellin was ozonized with a 60–70% molar excess of ozone in glacial acetic acid and the product was identified as 6-formyl-7-hydroxy-5,8-dimethoxycoumarin (**7**).

However, when the ozonolysis of isopimpinellin was carried out in a similar manner but the ozonide was reduced with zinc dust over a longer period of time, no aldehyde could be isolated. The NMR spectrum of the reaction mixture revealed that the desired aldehyde (**7**) was being reduced to the corresponding alcohol (**10a**). This alcohol (**10a**) was acetylated to 6-acetoxymethyl-7-acetoxy-5,8-dimethoxycoumarin (**10b**) and the product was characterized in the usual manner.

Next it was of interest to determine the proportion of radioactivity in isopimpinellin which resided in the 6-position. For this purpose, the phenolic aldehyde (**7**) was methylated with methyl iodide and anhydrous potassium carbonate

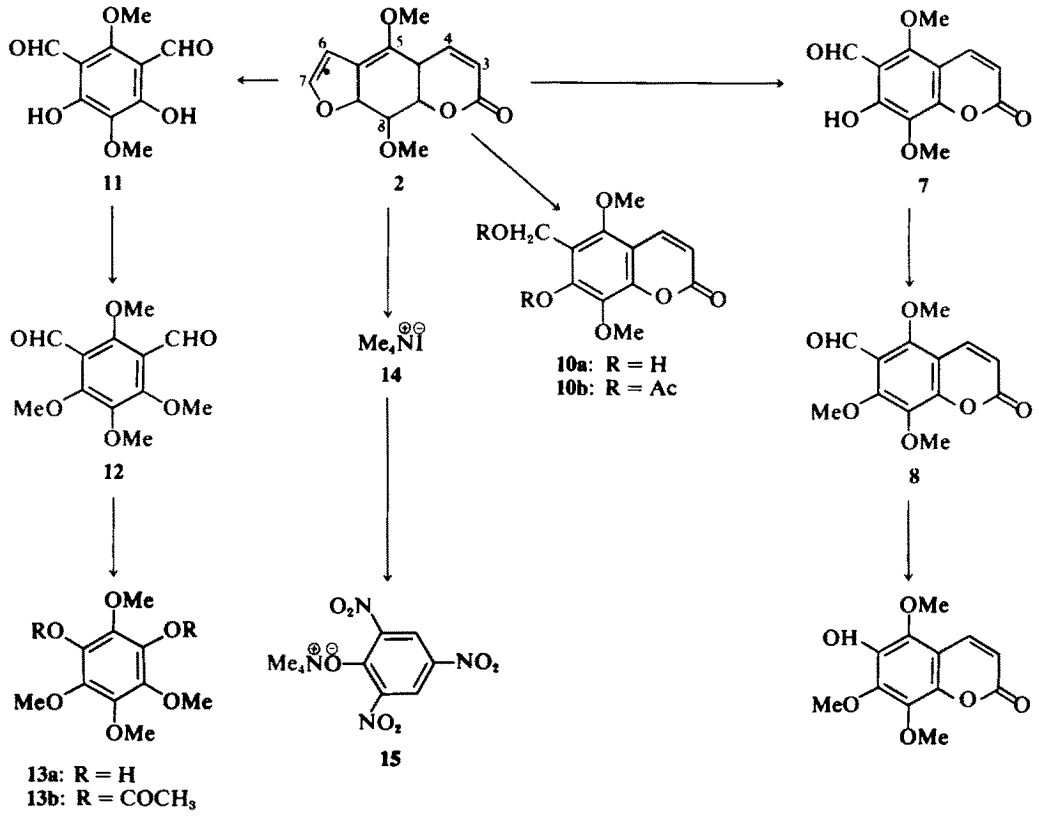


Fig 1. Degradative scheme of isopimpinellin (2).

in acetone and 6-formyl-5,7,8-trimethoxycoumarin (**8**) was isolated. The removal of the formyl group from 8 was achieved by utilizing a modified Dakin reaction.[12] Thus aldehyde **8**, in glacial acetic acid, was treated with a mixture of hydrogen peroxide and sulfuric acid and the product, 6-hydroxy-5,7,8-trimethoxycoumarin (**9**), was isolated.

Treatment of furanocoumarins with a large excess of ozone is known to cause degradation of both the furan and the pyrone rings.[13] Isopimpinellin when treated in this manner provided 1, 3-diformyl-4,6-dihydroxy-2,5-dimethoxybenzene (**11**). This reaction allowed the determination of radioactivity associated with the 2- and 3-positions of isopimpinellin.

In order to determine the amount of radioactivity associated with the 4-position of isopimpinellin, the dialdehyde[11] was methylated to give 1,3-diformyl-2,4,5,6-tetramethoxybenzone (**12**).

The treatment of the methylated dialdehyde (**12**) in acetic acid with a mixture of hydrogen peroxide and sulfuric acid under the conditions described by Schonberg[12] yielded only a complex mixture of highly coloured products. Thus it was evident that the dihydroxy derivative (**13a**) apparently being formed in the reaction was decomposing under these conditions. In order to minimize the suspected decomposition, the reaction time was reduced (from 16 hr to only 15 min) less hydrogen peroxide and a nitrogen atmosphere was used and the reaction was then quickly worked up in the cold. The NMR spectrum of the product mixture revealed no aldehydic protons but the presence of signals due to a formate ester (τ 1·62) and a phenol (τ 4·70). It was apparent that the reaction had proceeded but the hydrolysis of the intermediate formate esters was incomplete. Due to the apparent instability of **13a** it was decided to isolate it as the diacetate derivative (**13b**). However when the formate ester mixture was treated with aqueous base and acetic anhydride (to capture the resulting phenolate anion), only a complex mixture of products could be obtained.

Considering the instability of **13a** to hydrolysis, another set of conditions was devised. It was expected that a strong nucleophile such as methyllithium could be utilized to effect rapid and complete transformation of the formate ester to the dilithio salt of **13a**. Under strictly anhydrous conditions, the salt would be expected to precipitate from the organic solvent and thus as a solid, perhaps would be less prone to decomposition. Quenching such a reaction mixture with

acetic anhydride would then afford the diacetate (**13b**).

Thus the dialdehyde (**12**) was treated with a hydrogen peroxide and sulfuric acid mixture at 0° under a nitrogen atmosphere and the product mixture was dissolved in anhydrous ether and treated with excess methyllithium. As expected, a precipitate formed and after treatment with acetic anhydride and pyridine, the work up of this complex mixture yielded a near quantitative yield of 1,3-diacetoxy-2,4,5,6-tetramethoxybenzene (**13b**).

Thus by comparison of the molar activity of **13b** with that of the dialdehyde (**11**), the activity associated with the 4- and 6-positions of isopimpinellin could be obtained. Since the radioactivity of the 6-position could be determined from previous degradations, the percentage of radioactivity residing at the 4-position of isopimpinellin is thus determinable.

It was finally of interest to determine the percentage of radioactivity of isopimpinellin which might be associated with the two methoxyl groups. To accomplish this, isopimpinellin was demethylated by refluxing with hydriodic acid[14] and the resulting methyl iodide was swept from the reaction mixture with a stream of nitrogen and trapped as tetramethylammonium iodide (**14**). After scintillation counting of the latter, it was converted to its picrate derivative (**15**).

In summary the above degradation of isopimpinellin allowed evaluation of the radioactivity associated with all the C atoms attached to the benzene portion of the molecule.

Degradation of alloimperatorin methyl ether (**3**). Alloimperatorin methyl ether (**3**) contains a furan ring and a dimethylallyl side chain. Although no direct evidence is available as to the origin of these side chains in furanocoumarins, experiments with similar coumarins[6,7] have shown them to be mevalonic acid derived. Thus in order to gain some information as to the specificity of incorporation of such precursors into **3**, a series of degradations were devised which would allow the determination of the distribution of radioactivity in alloimperatorin methyl ether in the course of biosynthetic experiments.

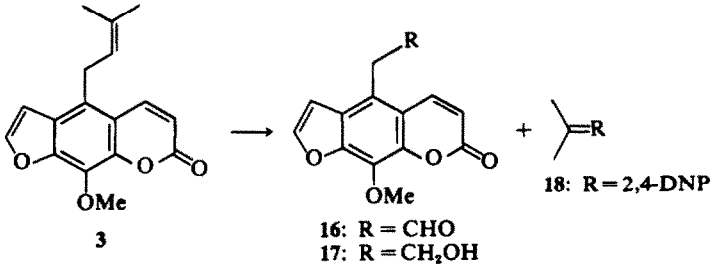
Thus to determine the distribution of radioactivity in the prenyl side chain of **3**, a cleavage reaction was indicated. It was felt that although **3** has three double bonds which would be reactive to ozone, the partial aromatic character of both the furan and the pyrone rings might allow selective ozonization of the side chain double bond. Thus alloimperatorin methyl ether was treated with 1·5 molar equivalents of ozone in acetic acid and the resultant ozonide was reductively cleaved with zinc dust. The resulting mixture was steam distilled and the 2,4-dinitrophenylhydrazone derivative of acetone was isolated in 43% yield. The non-volatile portion of the reaction mixture allowed the isolation of unreacted **3** (37% yield) and the expected aldehyde (**16**) in 49% yield. This compound appeared to be unstable to air and therefore was reduced to the corresponding alcohol (**17**) with sodium borohydride.

This degradation, while giving the desired products, was found to be undesirable when performed on radioactive **3**. The most significant problem was that **3** proved to be very difficult to obtain radiochemically pure. Also, the highly coloured nature of acetone-2,4-DNP made scintillation counting inaccurate when low levels of radioactivity were present.

Thus an alternative scheme (Fig 2) for the degradation of alloimperatorin methyl ether was considered.

As has been shown previously, ozone attacks preferentially the side chain double bond and thus it was apparent that if an ozonolysis procedure was to be used to cleave the furan and the pyrone rings, it would be necessary to first modify the side chain double bond to make it resistant to ozonolysis. Alloimperatorin methyl ether diol (**20**) was considered to be the ideal intermediate as this could be used for the cleavage of the side chain as well as for the degradation of the furan and the pyrone rings. Dreyer[15] had previously shown that alloimperatorin methyl ether could be converted to the diol **20** *vis* the epoxide **19** in good overall yield. Thus alloimperatorin methyl ether was treated with *m*-chloroperbenzoic acid and the epoxide **19** was isolated in 80% yield. Treatment of **19** with 5% oxalic acid gave the desired diol **20** in 70% yield. This compound was identical with authentic alloimperatorin methyl ether diol (**20**) kindly supplied by Dreyer.

To gain information as to the distribution of



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16: R = CHO
17: R = CH_2OH

18: R = 2,4-DNP

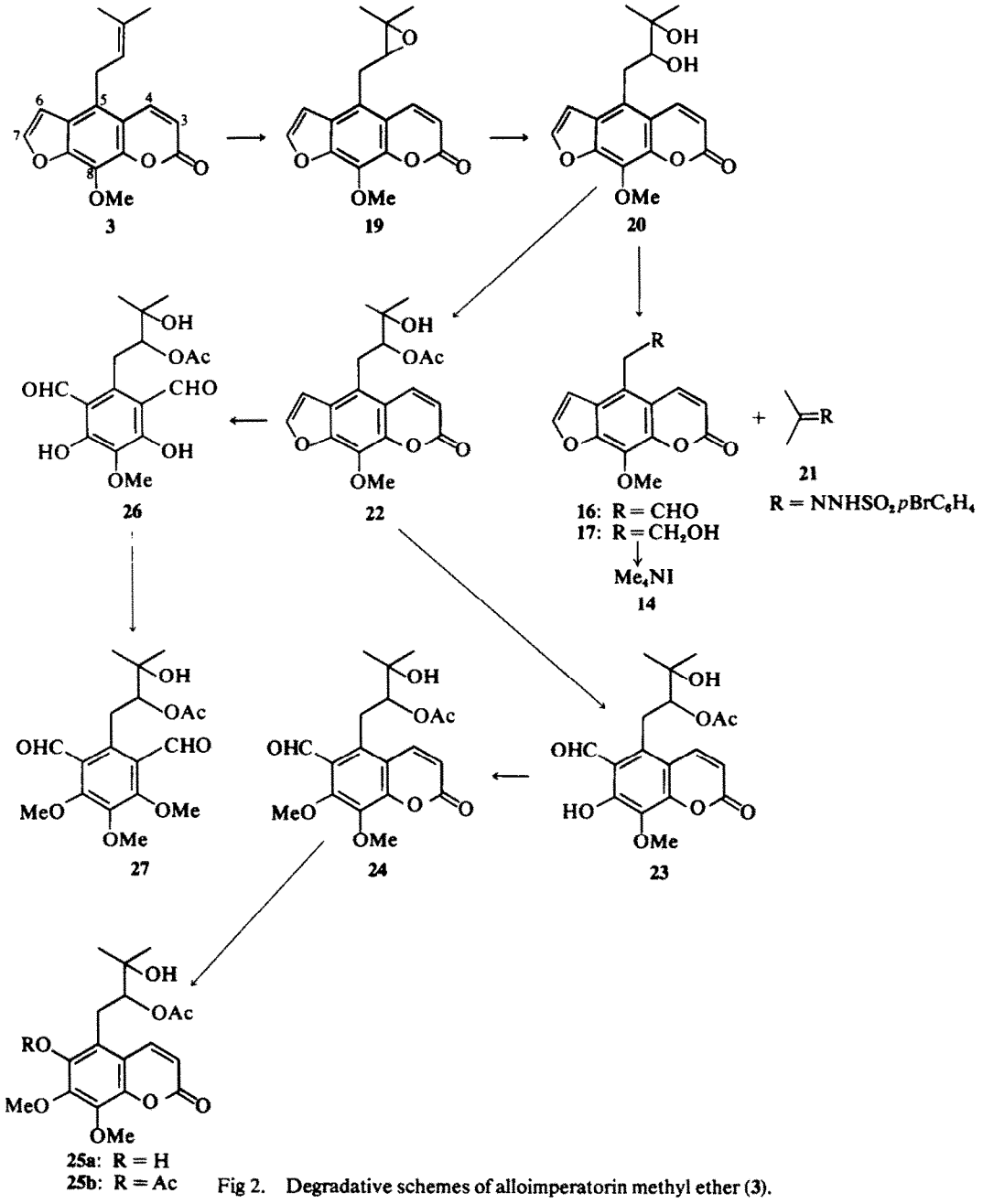


Fig 2. Degradative schemes of alloimperatorin methyl ether (3).

radioactivity in the side chain, diol **20** was treated with periodic acid and the acetone removed from the reaction mixture in the form of the colourless *p*-bromobenzenesulfonylhydrazone derivative. The non-volatile portion gave aldehyde **16** which upon reduction with sodium borohydride gave alcohol **17** in 58% yield.

Next it was of interest to determine the distribution of radioactivity in the furan portion of alloimperatorin methyl ether. For this purpose, diol **20** was acetylated with acetic anhydride in pyridine to form a monoacetate derivative[15] (**22**) in 90% yield. The monoacetate **22** was then treated with a slight excess of ozone and after reductive decomposition of the ozonide the desired phenolic aldehyde (**23**) could be isolated in 35% yield.

In order to determine the amount of radioactivity which might reside in the 6-position of **3**, the removal of the aldehyde group of **23** in the manner previously found successful in the degrada-

tions of isopimpinellin (2) was considered. For this purpose, the phenolic aldehyde (23) was methylated with methyl iodide and the methylated coumarin (24) was treated with a mixture of hydrogen peroxide and sulfuric acid. The resultant product was acetylated directly to provide 25b which could be readily purified.

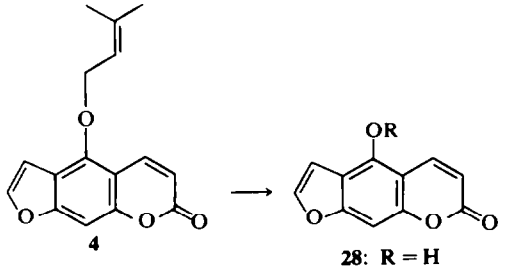
To allow determination of radioactivity associated with the pyrone ring of 3, the diol acetate 22 was ozonized in the manner described by Hegarty and Lahey[13] and the expected product 26 was isolated.

To determine the amount of radioactivity associated with the 4-position of 3, a sequence of reactions similar to those performed successfully on isopimpinellin was attempted. The dialdehyde 26 was methylated by standard procedures and the product (27) was obtained without difficulty. However, this material when treated under the conditions developed in the degradation of isopimpinellin (2), gave only a complex mixture of coloured products. Thus it was evident that even under highly controlled conditions of the reaction, the resultant dihydroxy derivative (or perhaps the intermediate diformate ester) was undergoing decomposition. Thus attempts to effect this conversion were abandoned.

Finally, in order to determine the amount of radioactivity residing in the methoxyl group of alloimperatorin methyl ether, alcohol 17 derived from the cleavage of diol 20, was demethylated with hydriodic acid and the resultant methyl iodide was trapped as tetramethylammonium iodide (14). Conversion to the picrate derivative was carried out as before.

Thus these series of reactions allow the determination of radioactivity associated with most of the carbon atoms of 3. It should also be noted that these degradations are equally applicable to the degradations of alloimperatorin methyl ether epoxide (19).

Degradation of isoimperatorin (4). Isoimperatorin (4) contains an alkyl ether side chain and a furan ring. Thus to study its biosynthesis, it was considered essential to determine the amount of radioactivity associated with the entire side chain and the furan ring. It was felt that an acid hydrolysis of 4 in a manner similar to that of umbelliprenin (1) would allow the determination of radioactivity associated with the C_5-alkyl side chain. Thus when 4 was refluxed with glacial acetic acid, bergaptol (28) could be isolated in 85% yield. Due to poor recovery during purification, 28 was converted to bergapten (29).



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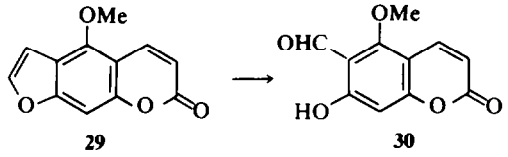
28: R = H
29: R = Me

In order to determine the amount of radioactivity associated with the furan ring of 4, bergapten (29) was treated with a slight excess of ozone in glacial acetic acid and after reductive decomposition of the ozonide, 6-formyl-7-hydroxy-5-methoxycoumarin (30) was obtained.



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Biosynthetic studies on coumarins from Thamnosma montana. As already noted earlier, several questions remain to be answered in regard to the biosynthesis of furanocoumarins. Of particular interest is the question as to what role mevalonate plays in the biosynthesis of the "extra" furan atoms in these compounds.

However, before discussing the studies performed in this regard, it is pertinent to discuss some preliminary work in which some other aspects of coumarin biosynthesis were explored. These preliminary studies were instituted with several objectives in mind; to become more familiar with the plant system under conditions of feeding precursors to small quantities of plant, to attempt to gain information as to biosynthetic interrelationships between the various coumarins of *Thamnosma montana*, and to confirm that biosynthesis of these coumarins was occurring on a regular and measurable basis in the plant.

In an attempt to gain information as to the biogenetic interrelationships between the coumarins of *Thamnosma montana*, an experiment was performed in which five samples of shoots from a single mature *Thamnosma montana* plant were each allowed to incorporate D,L-phenylalanine-[3-^{14}C] under identical growing conditions. Utilizing the hydroponic method for purposes of incorporation, the shoots were allowed to grow for different times and each sample was then extracted to obtain the pure compounds. The results are given in Table 1 and represented graphically in Fig 3. It should be noted before discussing these results that as the plant samples were all from the same plant, there can be no argument as to differing age or conditions of the plant samples. Furthermore, each experiment utilized several shoots to allow compensation for any difference in the viability of the samples during the experiments.

It is apparent from these results that the cou-

Table 1. Incorporation of D,L-phenylalanine-[3-^{14}C] into coumarins of *Thamnosma montana* shoots

Experiment No.	Feeding time (hr)	Activity Fed* (dpm)	Weight of plant (g)	% Incorporation: Isoimperatorin (4)	Alloimperatorin methyl ether (3)	Isopimpinellin (2)	Alloimperatorin methyl ether epoxide (19)
1	24	$6{\cdot}75\times10^6$	1·34	0·039	0·188	0·754	0·052
2	48	$6{\cdot}98\times10^6$	1·45	–†	–†	0·546	0·656
3	72	$6{\cdot}79\times10^6$	1·45	0·034	0·048	0·320	0·215
4	120	$7{\cdot}10\times10^6$	1·40	0·011	0·043	0·038	0·045
5	168	$7{\cdot}04\times10^6$	1·48	0·056	0·088	0·115	0·088

*The activity fed has been corrected for radioactivity recovered outside the plant.
†The samples of isoimperatorin and alloimperatorin methyl ether from this experiment could not be satisfactorily separated to allow determination of the incorporation into each coumarin. The combined value is 0·23%.

marins studied in these experiments incorporate phenylalanine rapidly with maximum incorporation after about 48 hr (except for isopimpinellin (2) which reaches an apparent maximum after 24 hr). The incorporation levels then decrease rapidly. The significance of the increase in incorporation between 5 and 7 days is questionable. It is therefore rather evident that the coumarins studied incorporate phenylalanine at approximately the same rate with the only apparent difference being that isopimpinellin (2) reaches a maximum incorporation in a shorter period of time than do the others. Another striking point is that the degree of incorporation of radioactivity into these coumarins is essentially relative to the abundance of the coumarins in the shoots. Thus isopimpinellin (2) and alloimperatorin methyl ether epoxide (19) being the most abundant coumarin constituents, incorporate to the greatest degree. In other words, the specific activity (dpm/mmole) of the coumarins would be essentially the same. These data tend to suggest that no one coumarin is being biosynthesized at the expense of another. It has been hoped that some interrelationships would be evident in these results. Thus the obvious similarity between alloimperatorin methyl ether (3) and the epoxide (19) raises speculation that one may be the progenitor of the other. However, if this was the case one would expect that the incorporation of phenylalanine would proceed first to provide radioactive 3 and the latter would then transfer its activity to 19. Thus these compounds would be expected to reach maximum incorporation values at different times. Although no such relationship is obvious from the above data, a definite lag in incorporation into 19 is observed after one day, while alloimperatorin methyl ether (3) incorporates rapidly in this time period. Thus such a relationship could well exist but not be obvious on the time scale of this experiment.

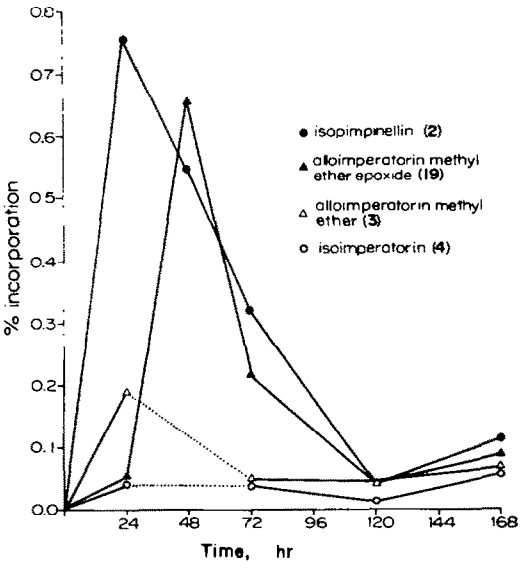


Fig 3. Incorporation of D,L-phenylalanine-[3-^{14}C] into coumarins of *Thamnosma montana* Shoots Versus Time.

These data do serve to show that the more abundant coumarins in *Thamnosma montana* shoots are being biosynthesized in the shoots and that the turnover rate is rather rapid.

In the next series of experiments, attention was focused on the role of mevalonate in the biosynthesis of the monomeric coumarins in *Thamnosma montana*. It was hoped that by incorporating mevalonate into isopimpinellin (2) and alloimperatorin methyl ether (3) it would be possible to confirm or refute some of the conflicting evidence which has been published previously.[1–3] In a series of preliminary experiments, mevalonic acid-[2-^{14}C] was administered to young *Thamnosma montana* plants which had been grown from seeds. The precursor was fed by the hydroponic method to the roots of these plants and after the desired feeding time the plants were worked up and the components were isolated in the usual manner. The pure coumarins thus isolated were

diluted with the inactive compounds, crystallized to constant activity and the radioactivity determined by the scintillation counting method. The results are depicted in Table 2.

It is immediately evident from these results that, while mevalonic acid-[2-^{14}C] is being utilized with considerable efficiency in the biosynthesis of umbelliprenin (**1**), such is not the case for the other coumarins. Except for umbelliprenin (**1**) where constant activity was achieved after five crystallizations, the amount of radioactivity present in the coumarins was not sufficient to allow purification to constant activity. Thus the incorporation of mevalonic acid-[2-^{14}C] into these coumarins is at best very low, a result also obtained by other workers.[3,7] However, the presence of significant incorporations into umbelliprenin (**1**) indicates that the precursor is being utilized by the plant in the biosynthesis of this coumarin. It was apparent that if meaningful values were to be obtained from the incorporation of C-2 labeled mevalonic acid into coumarins other than umbelliprenin (**1**), then a greater amount of radioactivity would have to be fed. Examination of the results in Table 2 leads only to the conclusion that in experiment 6, a feeding time of 2 days would appear to be inadequate to attain maximum incorporation. Incorporation into umbelliprenin (**1**) appears to be at a maximum after 10 days but the differences between the values for experiments 7–10 are not considerable. As Floss and Mothes[1] in their experiments, which employed mevalonic acid as a precursor in *Pimpinella magna*, had utilized a 14 day exposure time, and our experiment 10 also appeared to yield near optimum incorporations, it was decided that 14 days would be the feeding period in the future experiments.

In experiments 11, 12 and 13 (Table 3) D,L-mevalonic acid-]2-^{3}H] lactone was utilized as precursor. This substance was used since the commercially available tritiated precursors possessed much higher levels of activity than the C-14 labeled analogues. In these experiments alloimperatorin methyl ether (**3**) was converted to its diol (**20**) to aid in purification and isopimpinellin was further purified by sublimation. In each experiment entire young plants obtained from germinated seeds were utilized (including roots) and the hydroponic feeding method was employed. The results are presented in Table 3.

These results show that the incorporation of mevalonate-[2-^{3}H] into the two furanocoumarins is extremely small. It is notable that in these experiments it was difficult to achieve complete purification of the isolated coumarins. A combination of sublimation and successive crystallizations (up to 16 crystallizations) was necessary to obtain inactive isopimpinellin (**2**). Also, alloimperatorin methyl ether (**3**) even when converted to the diol (**20**) required 10–17 crystallizations before three

Table 2. Incorporation of mevalonic acid-[2-^{14}C] into monomeric coumarins in *Thamnosma montana*

Experiment No.	Feeding time (days)	Activity (dpm)	Weight of plant (g)	% Incorporation†		
				Umbelliprenin (**1**)	Alloimperatorin methyl ether (**3**)	Isopimpinellin (**2**)
6	2	$8{\cdot}74 \times 10^{7}$*	15	0·019	< 0·0003	—
7	4	$4{\cdot}43 \times 10^{7}$*	35	0·072	< 0·0004	< 0·0004
8	7	$9{\cdot}01 \times 10^{7}$*	16	0·045	< 0·0006	< 0·0013
9	10	$2{\cdot}47 \times 10^{8}$*	10	0·160	< 0·0009	< 0·0011
10	14	$4{\cdot}95 \times 10^{7}$‡	14	0·089	< 0·0039	< 0·0007

*Precursor administered in water as sodium salt.

†Figures preceeded by < indicate the incorporation based on the activity after the final crystallization where, due to insufficient material and insufficient specific activity in the compound, constant radioactivity could not be achieved.

‡Precursor administered in water as dibenzylethylenediamine salt.

Table 3. Incorporation of D,L-mevalonic acid-[2-^{3}H] lactone into *Thamnosma montana*

Experiment No.	Activity fed* (dpm)	Weight of plant (g)	% Incorporation		
			Isopimpinellin (**2**)	Alloimperatorin methyl ether (**3**)	Umbelliprenin (**1**)
11	$4{\cdot}35 \times 10^{9}$	5	inactive	0·00012	—
12	$6{\cdot}66 \times 10^{9}$	5	inactive	0·00003	0·024
13	$1{\cdot}11 \times 10^{10}$	5	inactive	< 0·00008	—

*Corrected for activity isolated outside the plant

consistent counts could be obtained. It is perhaps not surprising that this precursor should not incorporate into isopimpinellin (2). Although other workers[2,3] have reported incorporation of C-2 labeled mevalonic acid into simple furanocoumarins, it is clear that if the formation of the furan ring were to follow the normally accepted mechanism, then C-4 and C-5 of mevalonic acid should be utilized while C-1, C-2 and C-3 should be lost.

As alloimperatorin methyl ether (3) contains a five carbon isoprene-like side chain, mevalonic acid could well serve as the precursor of this side chain, thereby accounting for the observed incorporations. Unfortunately, the radioactivity present in 3 was so low that degradation could not be expected to yield meaningful results. Indeed the low incorporations observed raised doubts as to their overall significance. Perhaps most notable is the observed incorporation into umbelliprenin (1) in experiment 12. Unfortunately, at this time inactive umbelliprenin (1) was in short supply and detailed investigation was precluded. However partial degradation of 1 was conducted according to the scheme already described, i.e. conversion of 1 to umbelliferone (5) and levulinaldehyde-2,4DNP (6b).

Umbelliprenin (1) (with specific activity of $1{\cdot}47 \times 10^6$ dpm/mmol) yielded umbelliferone which was shown to have a specific activity of less than $3{\cdot}43 \times 10^3$ dpm/mmol or 0·23% of the original radioactivity of 1. Unfortunately, the small quantity of umbelliferone (5) isolated was not sufficient to crystallize to constant activity. However it was clear that mevalonate-[2-^{3}H] was being incorporated essentially exclusively into the farnesyl side chain.

It was also of interest to gain information as to how the radioactivity present in the farnesyl side chain of umbelliprenin (1) was distributed. To this end umbelliprenin (1) ($4{\cdot}19 \times 10^5$ dpm/mmol) from experiment 12 was ozonized as previously described and levulinaldehyde *bis*-2,4-dinitrophenylhydrazone (6b) was isolated. This compound was purified and shown to have a specific activity of $7{\cdot}22 \times 10^4$ dpm/mmol or 17·2% of the original radioactivity of 1. As two molar equivalents of levulinaldehyde (6a) should be produced in this reaction, this result indicates that only 34% 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 method is difficult to determine at this time as the lack of umbelliprenin (1) precludes further experimentation.

As Table 3 reveals we were unable to demonstrate any incorporation of mevalonate-[2-^{3}H] into isopimpinellin (2). This is particularly interesting as it tends to contradict the results of other workers[2,3] who have reported positive incorporation of C-2 labeled mevalonic acid into furanocoumarins. Taken by themselves these results would be tenuous at best in refuting this previous work, but when considered in conjunction with the next series of experiments, their importance is greatly enhanced.

In this series of experiments mevalonic acid-[3R,4R-4-^{3}H,3S,4S-4-^{3}H] lactone was administered to young *Thamnosma montana* plants by the hydroponic method and the plants were allowed to grow for a period of 14 days. Isolation of isopimpinellin and alloimperatorin methyl ether was achieved in the normal manner. The latter compound was converted to its diol (20) for counting purposes and in experiment 14, after 8 crystallizations, the diol (20) was converted to its monoacetate (22) for further counting. The results are presented in Table 4.

The results in Table 4 reveal that again the incorporations into alloimperatorin methyl ether (3) are extremely low. However definite positive incorporation has been achieved into isopimpinellin (2). Although these incorporations are very low there can be no doubt as to the fact that definite and reproducible incorporation of mevalonate-[4-^{3}H] has been obtained. In experiment 14, isopimpinellin (2) was shown to have constant activity over the course of 6 crystallizations.

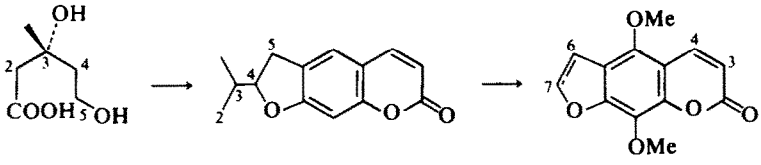
To determine the location of the radioactivity, isopimpinellin (2) ($5{\cdot}02 \times 10^3$ dpm/mmol) from experiment 14 was converted to 6-formyl-7-

Table 4. Incorporation of mevalonic acid-[3R,4R-4-^{3}H, 3S,4S-4-^{3}H] lactone into *Thamnosma montana*

Experiment No.	Activity fed* (dpm)	Weight of plant (g)	% Incorporation	
			Alloimperatorin methyl ether (3)	Isopimpinellin (2)
14	$1{\cdot}11 \times 10^9$	2 (diluted to 17)	~ 0·00007†	0·00032
15	$0{\cdot}79 \times 10^9$	2 (diluted to 10)	inactive‡	0·00024

*Corrected for activity recovered outside the plant.
†Counted as alloimperatorin methyl ether diol monoacetate (22).
‡Counted as alloimperatorim methyl ether diol (20).

hydroxy-5,8-dimethoxycoumarin (7) by ozonolysis as described previously. This material was shown to be essentially inactive. In a similar manner isopimpinellin ($4{\cdot}47 \times 10^3$ dpm/mmol) from experiment 15 was converted to 7 which was shown to lack any measurable activity. Thus it is evident that mevalonate has incorporated into isopimpinellin in a specific manner, such that C-4 of mevalonate becomes C-7 of isopimpinellin.



2

The results of experiments 14 and 15 in conjunction with the complete lack of incorporation of mevalonate-[2-^{3}H] into isopimpinellin (2) in experiments 11, 12 and 13 clearly indicate that mevalonate can act as a specific precursor for the furan ring atoms of isopimpinellin (2). It is felt that the very low incorporations observed into alloimperatorin methyl ether (3), in experiments 14 and 15 at least, must simply reflect an even lower incorporation rate than was observed for isopimpinellin (2).

The results of experiments 11–15 represent a confirmation of the results of Floss and Mothes[1] and thus support Seshadri's[16] proposal for furanocoumarin biosynthesis (i.e. that C-4 and C-5 of mevalonic acid serve as precursors of C-7 and C-6 of furanocoumarin). However, the very low levels of incorporation observed must raise questions as to whether mevalonate alone may act as the precursor of the furan ring. Additional and in some respects more satisfying data on the role of mevalonate is presented in the accompanying publication in which tissue cultures were employed.

As mentioned previously, Brown[3] 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]

Thus a series of experiments were undertaken to learn more of the role of acetate and glycine in furanocoumarin biosynthesis and these results are presented in the accompanying publication.

EXPERIMENTAL

M.ps were determined on a Kofler block and are uncorrected. The UV spectra were recorded in MeOH soln utilizing a Cary 11 or a Unicam, model SP800 spectrophotometer. The IR spectra were recorded on Perkin-Elmer model 20 or 457 spectrometers utilizing a KBr disc. The position of the absorption maxima are quoted in wave numbers (cm^{-1}). NMR spectra were recorded in $CDCl_3$ soln (unless otherwise indicated) at 100 MHz on a Varian HA-100 or a Varian XL-100 instrument and at 60 MHz on a Varian T-60 spectrometer. Chemical shifts are given in the Tiers τ scale with reference to TMS as the internal standard. Mass spectra were recorded on an Atlas CH-4 mass spectrometer and high resolution mass spectra were carried out on an AEI-MS 902 instrument. Woelm neutral alumina and silica gel G (acc. to Stahl) containing 1% by weight electronic phosphor were used for analytical and preparative layer chromatography, unless otherwise noted. Woelm neutral alumina (activity IV-unless otherwise indicated) was used for column chromatography. The TLC plates were activated in an oven at 90° for 1 hr. For qualitative chromatography, layers of 0·3 mm thickness were used and spots were visualized by viewing under UV light. For preparative TLC, large (20 × 20 cm) plates with a thicker layer (0·5 mm) were used. Developing solvents were used; A, anhydrous ether-hexane (1 : 1) or B, EtOAc-chloroform (1 : 1), unless otherwise noted.

Microanalysis were performed by Mr. P. Borda, Microanalytical Laboratory, University of British Columbia.

MeOH was made acetone-free by treatment with I_2 and NaOHaq.[17a] Chloroform was made acetone-free by flushing through a column of Celite impregnated with 2,4-dinitrophenyl hydrazine and the eluent was distilled.[17b]

Radioactivity was measured with a Nuclear Chicago Mark I or Mark II Liquid Scintillation counter in counts per min (cpm). The radioactivity of the sample in disintegration per min (dpm) was subsequently calculated using the counting efficiency which was determined for each sample by the external standard technique[18] utilizing the built-in barium-133 gamma source. The organic scintillator soln used with the counter was made up of the following components: toluene (1 l), 2,5-diphenyloxazole (4 g) and 1,4-bis[2-(5-phenyloxazolyl)]-benzene (0·05 g). In practice, a sample was dissolved in benzene (1 ml) or in methanol (1 ml) if the compound was not sufficiently soluble in benzene, in a counting vial. The volume was then made up to 15 ml with the above scintillator soln. In case of water soluble counting samples, an aqueous scintillator solution was utilized made up of the following components: toluene (385 ml), dioxane (385 ml), methanol (230 ml), naphthalene (80 g), 2,5-diphenyloxazole (5 g) and 1,4-bis[2-(5-phenyloxazolyl)] benzene (0·0625 g). In practice, a sample was dissolved in water (as required) and methanol (1 ml) in the counting vial. The solution was made up to 15 ml with the aqueous scintillator soln. For each sample counted, the background was determined for the counting vial to be used by filling the vial with the appropriate solvent and scintillator solution and counting (3 × 40 min: 3 × 100 min: 2 × 100 min). The difference in the cpm between the background count and the sample count was used for subsequent calculations. Unless otherwise noted, radioactivity was determined by scintillation counting with organic scintillator solution. Deviation from these

normal counting procedures will be discussed in the specific instances in which they arise.

Preparation of a standard solution of ozone in glacial acetic acid. Glacial AcOH was placed in a flask equipped with a bubbler and O_3 enriched O_2 was allowed to bubble through the soln for 30 min at room temp, at which time the soln had a definite blue tinge. The bubbler was then removed and the flask was tightly stoppered. Aliquots (20 ml) of this soln were added to a soln of KI (1 g) in water (20 ml) and the I_2 which was liberated was titrated with a standard soln of $Na_2S_2O_3$ using starch as an indicator. The $Na_2S_2O_3$ soln was standardized against a standard $K_2Cr_2O_7$ soln. In a typical experiment, glacial AcOH was saturated with O_3 as described above, and two aliquots (20 ml) were removed and added individually to aqueous solns of KI (1 g per flask in two flasks). The I_2 liberated was titrated with 0·0125 N $Na_2S_2O_3$ soln requiring respectively 17·7 and 16·8 ml to reach the end point. Thus the average of these two values (16·95 ml) required that the O_3 concentration at room temp be 0·106 mmole per 20 ml glacial AcOH.

Acid catalyzed hydrolysis of umbelliprenin (**1**). Umbelliprenin (**1**) (39·5 mg; 0·108 mmol) was dissolved in glacial AcOH (4 ml) and the soln was refluxed for 8 hr. After cooling, water (10 ml), was added and the soln was extracted with ether (4 × 20 ml). The ether extract was washed with water (20 ml), dried over anhyd Na_2SO_4 and the solvent was removed under reduced pressure. The residue (41 mg) was chromatographed on preparative TLC (eluting with chloroform-EtOAc, 1:2). The more polar band (blue; UV) was isolated to yield umbelliferone (**5**) (12·6 mg; 72% yield). Crystallization from EtOAc yielded pure **5**, m.p. 230–231° (lit.[10] m.p. 232°), mixed m.p. with authentic umbelliferone (**5**), 230–231°. The less polar band (dark, UV indicator) was isolated from the TLC plate as a colourless oil (6 mg), IR (film) 2950 (C—C). Analysis by GLC (column: 20% SE 30, on 60/80 mesh chromosorb W, ¼" × 10', helium flow rate 100 ml/min, 165°), 8 distinct but overlapping peaks between retention times 2 and 16 min.

2,4-*Dihydroxybenzoic acid.* Umbelliferone **5** (18·0 mg; 0·111 mmol) was added to a soln of KOH (260 mg) in water (20 drops) and the mixture was heated in a nickel crucible under a N_2 stream, at 280° for 75 min.[9] After cooling, the soln was neutralized with dil H_2SO_4, water (20 ml) was added and the mixture was extracted with ether (6 × 35 ml). The ether extract was dried over $NaSO_4$ and the solvent was removed under reduced pressure to yield a white solid (15 mg). Sublimation of this material (170°; 0·01 mm) afforded 2,4-dihydroxybenzoic acid (7·5 mg; 47% yield), m.p. 230° (lit.[19] m.p. 235–236°); NMR (60 MHz) in $CDCl_3$-DMSO-d_6, 1·7 (3H, broad s, disappears on addition of D_2O, COO<u>H</u> and two phenolic O<u>H</u>), 2·32 (1H, d, J = 9 Hz, H (6)), 3·70 (2H, m, H (3) and H (5)); mixed m.p. with authentic 2,4-dihydroxybenzoic acid (available commercially), 230°; TLC properties identical. (Found: C, 54·66; H, 3·92. Calcd. for $C_7H_6O_4$: C, 54·55; H, 3·92%).

Levulinaldehyde bis-2,4-*dinitrophenylhydrazone* (**6b**) *from umbelliprenin* (**1**). Umbelliprenin **1** (24 mg; 0·066 mmol) was dissolved in EtOAc (1 ml) and subjected to a stream of O_3 enriched O_2, at −78°, for 1·5 hr. The mixture was allowed to warm to room temp, then was transferred to Parr Hydrogenator Flask. The ozonization vessel was rinsed with MeOH (1 ml) and this was added to the soln for hydrogenation. 5% Pd-$CaCO_3$ (50 mg) was added and the mixture hydrogenated at 45 psi for 1·5 hr. The mixture was filtered immediately into 2,4-dinitrophenylhydrazine reagent (2,4-dinitrophenylhydrazine (300 mg) in freshly prepared methanolic HCl (1 ml)). The yellow ppt which formed immediately was collected by filtration (45·8 mg) and was recrystallized from DMF and was washed with MeOH to yield **6b** (19 mg; 26% yield), m.p. 241–242·5°, mixed m.p. with authentic (prepared from 2-methylfuran[20]) **6b**, 242–244°, (lit.[10] m.p. 233°). (Found: C, 44·48; H, 3·62; N, 23·95. Calcd. for $C_{17}H_{16}N_8O_8$: C, 44·3; H, 3·55; N, 24·2%).

6-*Formyl*-7-*hydroxy*-5,8-*dimethoxycoumarin* (**7**). Isopimpinellin **2** (45 mg; 0·183 mmol) was treated with O_3 saturated glacial AcOH (60 ml: 0·30 mmol) and the mixture stirred for 1 hr at room temp. Zn dust (100 mg) was then added and stirring continued for further 10 min. The mixture was then filtered and solvent was then evaporated *in vacuo*. The residue (~ 70 mg) was dissolved in chloroform-MeOH mixture (3 ml) and was chromatographed on silica gel (6 g). The fractions eluted with benzene and benzene-chloroform contained isopimpinellin (**2**) and a more polar compound (yellow spot; UV and visible). These fractions were combined (33 mg) and crystallized from acetone to yield **7** (23 mg: 50% yield), m.p. 214–216°; IR (KBr) 1758, 1730, 1625, 1592 (α-pyrone), 1640 (aldehyde C=O); UV λ_{max}^{MeOH} (ε) 275 (27,100); UV λ_{max}^{MeOH} (ε) (+NaOH) 238 (19,200), 269 (16,600), 299 (12,900), 360 (14,200); UV λ_{max}^{MeOH} (ε) (+HCl) 208 (29,000), 226 (sh) (15,600), 263 (12,800), 320 (15,600); NMR (100 MHz) in $CDCl_3$, TMS lock, −2·03 (1H, s, disappears on addition of D_2O, phenolic OH), −0·23 (1H, s, aromatic CHO), 2·17 (1H, d, J = 10 Hz, H (4) of coumarin), 3·73 (1H, d, J = 10 Hz, H(3) of coumarin), 6·00, 6·02 (6H, two s, two aromatic OCH_3); mass spectrum *m/e* 250 (M), 235 (M-15), 221 (M-29), 207 and 179. (Found: C, 57·38; H, 4·07. Calcd. for $C_{12}H_{10}O_6$: C, 57·61; H, 4·03%). High resolution molecular weight determination: Calcd. for $C_{12}H_{10}O_6$: 250·048. Found: 250·046.

6-*Acetoxymethyl*-7-*acetoxy*-5,8-*dimethoxycoumarin* (**10b**). Isopimpinellin **2** (23·5 mg; 0·095 mmol) was treated with O_3 saturated glacial AcOH (32 ml; 0·160 mmol) and the mixture stirred for 3 hr at room temp. Zn dust (400 mg) was added and stirring continued for a further hr. The soln was filtered and the solvent was evaporated *in vacuo*. The residue (30 mg) showed no aldehyde on TLC plate. The NMR of the residue revealed it to be the corresponding alcohol (**10a**). This **10a** was treated with Ac_2O and pyridine and the soln was stirred for 10 hr. The solvent was evaporated *in vacuo* and the residue gave a single spot on TLC. It was separated on preparative TLC and crystallized from EtOAc to yield **10b** (21 mg; 66% yield), m.p. 139–141°; IR (KBr) 1780 (aromatic acetate C=O), 1735 (aliphatic acetate C=O) 1598 (α-pyrone); UV λ_{max}^{MeOH} (ε) 209 (23,000), 225 (sh) (15,000), 251 (6,940), 293 (11,670); NMR (100 MHz) in $CDCl_3$, TMS lock, 2·06 (1H, d, J = 10 Hz, H (4) of coumarin), 3·59 (1H, d, J = 10 Hz, H (3) of coumarin), 4·81 (2H, s, C<u>H</u>$_2$OCOCH$_3$), 6·00, 6·08 (6H, two s, two aromatic OCH_3), 7·65 (3H, s, aromatic $OCOCH_3$), 7·98 (3H, s, aliphatic C<u>H</u>$_2$$OCOCH_3$) mass spectrum *m/e* 336 (M), 294 (M-42), 251 (M-85), 234 (base peak), 219 and 205. (Found: C, 56·99; H, 4·88. Calcd. for $C_{16}H_{16}O_8$: C, 57·14; H, 4·76%). High resolution molecular weight determination: Calcd. for $C_{16}H_{16}O_8$: 336·084. Found: 336·086.

6-*Formyl*-5,7,8-*trimethoxycoumarin* (**8**). To a soln of **7** (24 mg; 0·096 mmol) in acetone (20 ml) was added

anhyd K_2CO_3 (1 g) and MeI (2 ml). The mixture was refluxed for 1·5 hr, stirred a further 1 hr at room temp then water (20 ml) was added. The soln was acidified with conc HCl, extracted with chloroform (3 × 20 ml); the chloroform extracts were washed with water (20 ml), dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield a crystalline residue (25·8 mg), which was observed to be essentially one compound on TLC. Crystallization from EtOAc yielded **8** (18·4 mg; 73% yield) as white needles, m.p. 152·5–154°; IR (KBr) 1745, 1720, 1610, 1578 (α-pyrone), 1689 (HC=O); UV λ_{max}^{MeOH} (ε) 206 (20,300), 267 (14,600), 300 (sh) (10,200); NMR (100 MHz) in $CDCl_3$, TMS lock, −0·37 (1H, s CHO), 2·03 (1H, d, J = 10 Hz, H (4) of coumarin), 3·65 (1H, d, J = 10 Hz, H (3) of coumarin), 5·91, 6·02, 6·08 (9H, three s, three aromatic OCH_3); mass spectrum *m/e* 264 (M, base peak), 249 (M-15), 235 (M-29), 221. (Found: C, 58·91; H, 4·73. Calcd. for $C_{13}H_{12}O_6$: C, 59·09; H, 4·58%). High resolution molecular weight determination: Calcd. for $C_{13}H_{12}O_6$: 264·063. Found: 264·063.

6-*Hydroxy*-5,7,8-*trimethoxycoumarin* (**9**). Compound **8** (16·8 mg; 0·064 mmol) was dissolved in glacial AcOH (1·5 ml) and cooled in an ice bath until it began to solidify. An ice-cold mixture of 30% H_2O_2 (0·20 ml) and 50% H_2SO_4 (0·75 ml) was added and the mixture was allowed to stand in a refrigerator for 20 hr.[12] The mixture was then poured into cold brine (20 ml). The soln was extracted with chloroform (3 × 20 ml) and the extract was washed with brine (20 ml), dried over Na_2SO_4 and the solvent removed under reduced pressure to yield a residue (15·4 mg) shown to be essentially one component by TLC. Preparative TLC (eluting with solvent A) followed by sublimation of the isolated material (140°, 0·02 mm), allowed isolation of **9** (12·3 mg; 77% yield). Crystallization from 95% EtOH yielded pure **9** as fine needles, m.p. 198·5–199·5°; IR (KBr) 3525 (OH), 1720, 1611, 1570 (α-pyrone); UV λ_{max}^{MeOH} (ε) 208 (24,500), 308 (10,350); UV λ_{max}^{MeOH} (+NaOH) 248 (33,800), 317 (21,600); UV λ_{max}^{MeOH} (+HCl) 209 (22,500), 308 (10,100); NMR (100 MHz) in $CDCl_3$, TMS lock, 2·06 (1H, d, J = 9·5 Hz, H (4) of coumarin), 3·70 (1H, d, J = 9·5 Hz, H (3) of coumarin), 4·38 (1H, broad, disappearing on addition of D_2O, phenolic OH), 5·93, 6·04, 6·06 (9H, three s's, three aromatic OCH_3); mass spectrum *m/e* 252 (M, base peak), 237 (M-15), 209, 181, 153. (Found: C, 57·24; H, 4·90. Calcd. for $C_{12}H_{12}O_6$: C, 57·14; H, 4·80%). High resolution molecular weight determination: Calcd. for $C_{12}H_{12}O_6$: 252·063. Found: 252·063.

1,3-*Diformyl*-4,6-*dihydroxy*-2,5-*dimethoxybenzene* (**11**). Isopimpinellin **2** (52 mg; 0·211 mmol), dissolved in glacial AcOH (5 ml) and EtOAc (2 ml), was subjected to an O_3 enriched stream of O_2 at 0° for 0·5 hr. Zn dust (100 mg) was added, the mixture was stirred for 20 min at room temp. Filtration of the mixture and removal of the solvent *in vacuo* yielded an orange oil which was treated with hot chloroform. The chloroform soluble portion (57 mg) was crystallized from EtOAc to yield **11** (17 mg; 35% yield). Recrystallization from EtOAc yielded an analytical sample of **11**; m.p. 162–164°; IR (KBr) 1625 (C=O, H-Bonded); UV λ_{max}^{MeOH} (ε) 258 (30,280), 325 (sh) (6080); UV λ_{max}^{MeOH} (ε) (+NaOH) 302 (49,800), 358 (9,450) UV λ_{max}^{MeOH} (ε) (+HCl) 213 (11,600), 243 (sh) (6,800), 268 (23,100); NMR (100 MHz) in $CDCl_3$, TMS lock, −2·44 (2H, s, disappearing on addition of D_2O, two equiv phenolic OH) −0·08 (2H, s, two equiv CHO), 5·94, 6·13 (6H, two s's, two aromatic OCH_3); mass spectrum *m/e* 226 (M, base peak), 211 (M-15) 198 (M-28), 183, 165, 152. (Found: C, 53·06; H, 4·45. Calcd. for $C_{10}H_{10}O_6$: C, 53·10; H, 4·46%). High resolution molecular weight determination: Calcd. for $C_{10}H_{10}O_6$: 226·048. Found: 226·048.

1,3-*Diformyl*-2,4,5,6-*tetramethoxybenzene* (**12**). Compound **11** (8·5 mg: 0·0335 mmol) was dissolved in acetone (5 ml) and refluxed with K_2CO_3 (500 mg) and MeI (2 ml) for 4 hr. After cooling, water (10 ml) was added and the mixture was acidified with conc HCl and then extracted with chloroform (3 × 15 ml). After washing with water (10 ml) and drying over Na_2SO_4, the chloroform was removed under reduced pressure to yield a residue (9·5 mg) which was chromatographed by preparative TLC (eluting with solvent A) to yield **12** (8·2 mg; 86% yield). Crystallization from anhyd ether-hexane yielded **12** as white needles, m.p. 49–50° (lit.[13] m.p. 109–111°); IR (KBr) 1686 (C=O, aldehyde); UV λ_{max}^{MeOH} (ε) 214 (14,100), 257 (14,900), 314 (2770) NMR (100 MHz) in $CDCl_3$, TMS lock, −0·37 (2H, s, two CHO), 5·96 (6H, s, two OCH_3), 6·09, 6·15 (6H, s, two OCH_3); mass spectrum *m/e* 254 (m, base peak), 239 (M-15), 225 (M-29), 211. (Found: C, 56·58; H, 5·48. Calcd. for $C_{12}H_{14}O_6$: C, 56·69; H, 5·55%).

1,3-*Diacetoxy*-2,4,5,6-*tetramethoxybenzene* (**13b**). Compound **12** (12·5 mg; 0·04 mmol) was dissolved in glacial AcOH (1 ml), 50% H_2SO_4 (0·25 ml) was added and the mixture was cooled at 0° in an ice bath. The soln became pale yellow. With the system closed to air, N_2 was bubbled through the soln for 10 min and then 30% H_2O_2 (0·03 ml; 0·26 mmol) was added and the soln was allowed to stand at 0° with N_2 passage continued for 20 min, during which time no discernable colour change took place. The mixture was then quickly poured into ice cold brine (10 ml) and extracted with chloroform (3 × 15 ml). The chloroform extract was washed with brine (2 × 10 ml), dried over Na_2SO_4 and the solvent removed *in vacuo*. The residue was dissolved in anhyd ether (5 ml) and transferred to a dry flask and the soln was cooled in an ice bath. MeLi (2·1 M in ether, 0·25 ml, 0·505 mmol) was added with stirring and a fine white ppt was observed to form immediately. The mixture was allowed to come to room temp and stirring was continued for 5 min. Ac_2O (0·5 ml) and pyridine (1 ml) were then added and stirring was continued for 3 hr. Water (5 ml) was added and the mixture was extracted with chloroform (3 × 15 ml). The chloroform extract was washed with brine (10 ml), dried over Na_2SO_4 and the solvent removed *in vacuo* to yield a colourless oil (16 mg). Preparative TLC (eluting with solvent A) allowed isolation of **13b** (14·9 mg, 97% yield) as a colourless oil b.p. ~ 110° at 0·5 mm. Microdistillation provided an analytical sample. Crystallization from anhyd ether–hexane yielded **13b** as colourless plates, m.p. 57–58°; IR (film) 1740 (aromatic acetate, C=O); UV λ_{max}^{MeOH} (ε) 202·5 (40,000), 272 (992); NMR (100 MHz) in $CDCl_3$, TMS lock, 6·14 (3H, s, aromatic OCH_3), 6·17 (6H, s, two aromatic OCH_3), 6·28 (3H, s, aromatic OCH_3), 7·68 (6H, s, two aromatic $OCOCH_3$); mass spectrum *m/e* 314 (M), 272 (M-42), 230 (M-84), 215. (Found: C, 53·77; H, 4·89. Calcd. for $C_{14}H_{18}O_8$: C, 53·50; H, 4·77%). High resolution molecular weight determination: Calcd. for $C_{14}H_{18}O_8$: 314·098: Found: 314·100.

Demethylation of isopimpinellin (**2**).[14] Isopimpinellin **2** (20·8 mg; 0·084 mmol) was treated with HI according to the published procedure[14] to yield tetramethylammonium iodide **14** (28·8 mg, 82·5% yield).

Tetramethylammonium picrate (**15**). This derivative was prepared according to the published procedure.[14]

Selective ozonolysis of alloimperatorin methyl ether (**3**). Compound **3** (100 mg; 0·352 mmol) was treated with O_3 sat glacial AcOH (100 ml; 0·530 mmol O_3) and the mixture was stirred for 5 hr at room temp. Zn dust (200 mg) was added and stirring continued for 12 hr more. The soln was filtered to remove Zn and the filtrate was steam distilled for 10 min into 2,4-dinitrophenylhydrazine reagent (2,4-dinitrophenylhydrazine (500 mg) in conc H_2SO_4 (10 ml) and diluted to 60 ml with water). The 2,4-dinitrophenylhydrazine soln was extracted with benzene (3 × 30 ml), the extract was washed with water, dried over Na_2SO_4, concentrated to a small volume (2 ml) and chromatographed on Woelm acid washed alumina (activity 1, 30 g). The fractions eluted with benzene contained only acetone 2,4-dinitrophenylhydrazone (**18**; by TLC). Crystallization of the residue of these fractions from MeOH yielded **18** (36 mg; 43% yield) as yellow needles, m.p. 123–125° (lit.[19] m.p. 128°), mixed m.p. with **18** prepared from acetone, 123–125°. The non-volatile portion of the mixture was extracted with chloroform (3 × 20 ml). The extracts were dried over Na_2SO_4 and the soln was concentrated to a small volume and was applied to a preparative TLC plate. Elution with solvent B allowed isolation of **3** (41 mg; 41% recovery) and a less polar band (yellow, UV), the expected aldehyde **16** (45·5 mg; 53% yield). Crystallization from EtOAc yielded **16** as an amorphous reddish powder, m.p. 195–200°; UV λ_{max}^{MeOH} 219, 245 (sh), 250, 265, 307; NMR (100 MHz) in $CDCl_3$-DMSO-d_6, TMS lock, 0·21 (1H, m, CHO), 1·85 (1H, d, $J = 10$ Hz, H (4) of furanocoumarin), 2·04 (1H, d, $J = 2$ Hz, H (7) of furanocoumarin), 2·89 (1H, d, $J = 2$ Hz, H (6) of furanocoumarin), 3·67 (1H, d, $J = 10$ Hz, H(3) of furanocoumarin) 5·64 (2H, d, $J = 1$ Hz, CH_2CHO), 5·84 (3H, s aromatic OCH_3); mass spectrum *m/e* 258 (M), 229 (base peak, M-29), 214, 201, 186, 158.

5-(2'-Hydroxyethyl)-8-methoxypsoralen (**17**). To a soln of **16** (19·8 mg; 0·077 mmol) in MeOH-$CHCl_3$ mixture (5 ml) at 0°, was added dropwise an ice cold soln of $NaBH_4$ (100 mg; 2·6 mmol) in MeOH (5 ml). The mixture was stirred at 0° for 2 hr at which time, water (5 ml) was added to destroy the excess reagent. The soln was extracted with $CHCl_3$ (5 × 15 ml), the extract was dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield **17** (17·5 mg; 88% yield). Crystallization from EtOAc followed by sublimation (145°, 0·03 mm) yielded an analytical sample of **17**, m.p. 167·0–169·0°; IR (KBr) 3450 (OH), 1704, 1690, 1585 (α-pyrone); UV λ_{max}^{MeOH} (ε) 220 (24,600) 245 (sh) (18,600). 251 (20,800), 265 (17,500), 306 (12,770); NMR (100 MHz) in $CDCl_3$, TMS lock, 1·93 (1H, d, $J = 10$ Hz, H (4) of furanocoumarin), 2·33 (1H, d, $J = 2$ Hz, H (7) of furanocoumarin), 3·12 (1H, d, $J = 2$ Hz, H (6) of furanocoumarin), 3·76 (1H, d, $J = 10$ Hz, H (3) of furanocoumarin), 5·79 (3H, s, aromatic OCH_3), 6·07 (2H, broad tr becoming a sharp tr on addition of D_2O, $J = 6$ Hz, CH_2—$\underline{CH_2}$—OH), 6·75 (2H, tr $J = 6$ Hz, $\underline{CH_2}$—CH_2—OH), 8·38 (1H, broad, disappearing on addition of D_2O, CH_2—CH_2—OH); mass spectrum *m/e* 260 (M), 242 (M-18), 229 (base peak, M-31), 214 (M-46), 201, 186, 158. (Found: C, 64·66; H, 4·61. Calcd. for $C_{14}H_{12}O_5$: C, 64·62; H, 4·62%). High resolution molecular weight determination: Calcd. for $C_{14}H_{12}O_5$: 260·068. Found: 260·069.

Synthesis of 5-(2',3'-epoxy-3'-methylbutyl)-8-methoxypsoralen (**19**).[5,15] This compound, m.p. 103–104°, was prepared according to the published procedure.[5,15] Its identity was established by comparison (IR, mixed m.p. 104–106°) with an authentic sample.

5-(2',3'-Dihydroxy-3'-methylbutyl)-8-methoxypsoralen (**20**) (*Alloimperatorin methyl ether diol*).[15] This compound, m.p. 176–177°, was prepared according to the published procedure[15] and compared with an authentic sample kindly provided by Dr. D. L. Dreyer (mixed m.p. 176–177°).

Reaction of 5-(2',3'-dihydroxy-3'-methylbutyl)-8-methoxypsoralen (**20**) *with periodic acid.* Compound **20** (48·8 mg; 0·153 mmol) was dissolved in acetone-free MeOH (5 ml), periodic acid (100 mg; 0·437 mmol) in water (5 ml) was added and the mixture was stirred for 1 hr. The mixture was then heated to ~ 30° in a water bath, with stirring, while a stream of N_2 was blown over the surface of the soln and the effluent gases were bubbled through a soln of *p*-bromobenzenesulfonylhydrazide (125 mg) in glacial AcOH (10 ml) and water (10 ml), for 0·5 hr. The hydrazone mixture was extracted with Acetone-free chloroform (3 × 15 ml). The chloroform extract was washed with water (15 ml), dried over Na_2SO_4 and the solvent was removed under pressure to yield a white residue (110 mg) from which acetone *p*-bromobenzenesulfonylhydrazone **21** (15 mg; 35% yield) was isolated on an alumina preparative layer chromatography plates (eluting with $CHCl_3$-MeOH, 20:1). It was crystallized from chloroform-light petroleum as colourless needles, m.p. 146–148° (lit.[21] m.p. 145–146°); IR (KBr) 3220 (NH), 1342, 1180 (RSO_2N); UV λ_{max}^{MeOH} (ε) 235 (14,000); NMR (100 MHz) in $CDCl_3$, TMS lock, 2·28 (4H, A_2B_2 m, *para*-disubstituted benzene), 3·22 (1H, broad, disappears on addition of D_2O, NH), 8·10, 8·22 (6H, two s, $NHN{=}C(CH_3)_2$ *cis* and *trans*); mass spectrum *m/e* 292, 290 (M), 221, 219 (M-71), 205, 203 (M-87), 157, 155 (M-135), 71 (base peak). (Found: C, 36·86; H, 3·88; N, 9·40. Calcd. for $C_9H_{11}N_2SO_2Br$: C, 37·1; H, 3·80; N, 9·62%).

The non-volatile portion of the mixture was extracted with chloroform (3 × 20 ml), the extracts were washed with $NaHCO_3$aq, dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield a residue (43·5 mg) which was dissolved in MeOH-$CHCl_3$ (5 ml), cooled to 0°. To it was added $NaBH_4$ (100 mg; 2·6 mmol) in ice-cold MeOH (5 ml). The mixture was stirred at 0° for 0·5 hr then water (10 ml) was added and the mixture was extracted with chloroform (3 × 20 ml). The chloroform extract was washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield a residue which was chromatographed on preparative TLC (solvent B) to yield **17** (19·0 mg; 47% yield). Crystallization from EtOAc yielded needles, m.p. 167–169°.

5-(2'-Acetoxy-3'-hydroxy-3'-methylbutyl-8-methoxypsoralen (**22**).[15] This compound, m.p. 184–186°, was prepared according to the published procedure[15] (lit.[15] m.p. 183–185°).

5-(2'-Acetoxy-3'-hydroxy-3'-methylbutyl)-6-formyl-7-hydroxy-8-methoxycoumarin (**23**). The monoacetate **22** (250 mg; 0·69 mmol) in glacial AcOH (5 ml) was stirred with O_3 saturated glacial AcOH (200 ml; 0·780 mmol O_3) for 3 hr at room temp. Zn dust (400 mg) was then added and stirring was continued for 2 hr more. The soln was filtered to remove Zn and solvent was removed *in vacuo* to yield a residue, which was treated with hot chloroform. The chloroform soln was filtered, concen-

trated and chromatographed by preparative TLC (eluting with $CHCl_3$-EtOAc, 2:1). The major yellow (UV) band which appeared to contain some starting material was thus isolated. This material was partitioned between $CHCl_3$ (30 ml) and 1% KOHaq (30 ml). The nonbase soluble material (~ 100 mg) appeared to contain some starting acetate (**22**) and a less polar component (blue spot; UV) but was not examined farther. The aqueous layer was acidified and extracted with $CHCl_3$ (4 × 15 ml) and EtOAc (2 × 15 ml). The extracts were combined, washed with water (20 ml), dried over Na_2SO_4 and the solvent was removed under pressure to yield a residue (151 mg) which was crystallized from EtOAc to yield **23** (69 mg; 27·3% yield), m.p. 162–164°; IR (KBr) 3520 (OH), 1745 (C=O), 1730, 1625, 1595 (α-pyrone); UV λ_{max}^{MeOH} (ε) 273 (25,800), 345 (sh) (7,940); UV λ_{max}^{MeOH} (ε) (+NaOH) 236 (20,900), 267·5 (14,450), 300 (11,550), 357 (11,950); UV λ_{max}^{MeOH} (ε) (+HCl) 205 (44,100), 263 (10,150), 326 (13,700); NMR (100 MHz) in $CDCl_3$, TMS lock, −2·37 (1H, broad, disappears on addition of D_2O, phenolic OH), −0·39 (1H, s, CHO), 2·00 (1H, d, J = 10 Hz, H(4) of coumarin), 3·70 (1H, d, J = 10 Hz, H (3) of coumarin), 5·04 (1H, d of doublets, J = 4 Hz and 10 Hz, CH_2-CH(R)-$OCOCH_3$), 6·02 (3H, s, aromatic OCH_3), 6·3–6·8 (2H, AB of ABX m, J_{AB} = 15 Hz, J_{AX} = 10 Hz, J_{BX} = 4 Hz, CH_2-CH(R)-O), 7·94 (1H, broad disappears on addition of D_2O, OH), 8·29 (3H, s, —$COCH_3$), 8·68 (6H, s, —C(OH) $(CH_3)_2$); mass spectrum *m/e* 364 (M), 346 (M-18), 245 (base peak, M-119). (Found: C, 59·59; H, 5·51. Calcd. for $C_{18}H_{20}O_8$: C, 59·34; H, 5·49%). High resolution molecular weight determination: Calcd. for $C_{18}H_{20}O_8$: 364·116. Found: 364·114.

5-(2′-*Acetoxy*-3′-*hydroxy*-3′-*methylbutyl*)-6-*formyl*-7,8-*dimethoxycoumarin* (**24**). The aldehyde **23** (23 mg; 0·063 mmol) from the previous reaction was dissolved in acetone (7 ml) and K_2CO_3 (1·1 g) and MeI (2·2 ml) were added. The mixture was refluxed for 40 min, allowed to cool, then water (10 ml) was added and the soln was acidified with conc HCl. The soln was extracted with $CHCl_3$ (3 × 15 ml), the extract was dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield a residue (35 mg) which was observed to be essentially one component by TLC. Preparative TLC (solvent A) allowed isolation of pure **24** (19 mg; 80% yield), which crystallized from anhyd ether-hexane as colourless plates, m.p. 116–118°; IR (KBr) ~3500 (OH), 1735 (C=O, acetate and pyrone), 1675 (C=O, aldehyde); UV λ_{max}^{MeOH} (ε) 219 (16,900), 266 (23,600), 305 (sh) (9,400); NMR (100 MHz) in $CDCl_3$, TMS lock, −0·62 (1H, s, CHO), 1·93 (1H, d J = 10 Hz, H (4) of coumarin), 3·66 (1H, d, J = 10 Hz, H (3) of coumarin), 4·96 (1H, d of doublets, J = 3 Hz, $CH_2CH(R)OCOCH_3$), 5·97, 6·05 (6H, two s's, two aromatic OCH_3), 6·18 (1H, d of d's, J = 3 Hz and 14 Hz, HCH—CH(R)—O), 6·81 (1H, d of d's, J = 10 Hz and 14 Hz, HCH—CH(R)—O), 8·1 (1H, broad, disappears on addition of D_2O, OH), 8·34 (3H, s, $OCOCH_3$), 8·66, 8·74 (6H, two s's, C(OH)-$(CH_3)_2$); mass spectrum *m/e* 378 (M), 318 (M-60), 303, 277, 259 (base peak, M-119), 245. (Found: C, 60·00; H, 5·61. Calcd. for $C_{19}H_{22}O_8$: C, 60·31; H, 5·86%).

5-(2′-*Acetoxy*-3′-*hydroxy*-3′-*methylbutyl*)-6-*acetoxy*-7,8-*dimethoxycoumarin* (**25b**). Compound **24** (20 mg; 0·053 mmol) from the previous reaction, was dissolved in glacial AcOH (2 ml) and the soln was cooled in an ice bath until the liquid began to solidify. An ice cold mixture of 50% H_2SO_4 (0·75 ml) and 30% H_2O_2 (0·20 ml: 1·77 mmol) was then added and the soln was stirred at ice temp for a few min. The mixture was placed in a refrigerator and allowed to stand at 4° for 18 hr. Water (10 ml) was then added and the mixture was extracted with $CHCl_3$ (3 × 15 ml). The $CHCl_3$ extract was washed with brine (10 ml), dried over Na_2SO_4 and the solvent was removed *in vacuo* to yield a residue (25 mg) which was chromatographed on a preparative TLC plate (solvent B). Extraction of the major band from the plate yielded **25a** (18 mg; 93% yield); which resisted crystallization; NMR (60 MHz) in $CDCl_3$ 2·01 (1H, d, J = 10 Hz, H (4) of coumarin), 3·63 (1H, d, J = 10 Hz, H (3) of coumarin), 3·86 (1H, broad, disappears on addition of D_2O, phenolic OH), 4·95 (1H, d of d's, J = 3·5 Hz and 9 Hz, $CH_2CH(R)OCOCH_3$), 5·90, 5·98 (6H, two s's, two aromatic OCH_3), 6·5–7·1 (2H, AB of ABX m, $CH_2CH(R)O$—), 7·98 (1H, broad, disappears on addition of D_2O, OH), 8·18 (3H, s, $OCOCH_3$), 8·67 (6H, s, $C(OH)(CH_3)_2$). This material was treated with Ac_2O (1 ml) in pyridine (2 ml) for 12 hr. Removal of the solvents *in vacuo* yielded an oily residue which crystallized on standing. Recrystallization from EtOAc yielded **25b** (9·5 mg; 44% overall yield), m.p. 143–144°; IR (KBr) 1770 (aromatic acetate C=O), 1740 (aliphatic acetate C=O), 1705, 1592 (α-pyrone); UV λ_{max}^{MeOH} (ε) 208 (36,200), 248 (sh) (4,600), 303 (11,800); NMR (100 MHz) in $CDCl_3$, TMS lock, 2·05 (1H, d, J = 10 Hz, H (4) of coumarin), 3·65 (1H, d, J = 10 Hz, H (3) of coumarin), 5·04 (1H, d of d's, J = 5 Hz and 8 Hz, CH_2CH-(R)$OCOCH_3$), 6·01, 6·06 (6H, two s's, two aromatic OCH_3), 6·99 (2H, AB of ABX m, J_{AB} ~ 16 Hz, J_{AX} = 8 Hz, J_{BX} = 5 Hz, CH_2—CH(R)—O—), 7·67 (3H, s, aromatic $OCOCH_3$), 8·17 (4H, becoming 3H on addition of D_2O, s, aliphatic $OCOCH_3$ and OH), 8·74 (6H, s, $C(OH)(CH_3)_2$); mass spectrum *m/e* 408 (M), 366 (M-42), 351 (M-57), 348 (M-60), 306 (M-102), 288, 273 (base peak, $C_{15}H_{13}O_5$), 235, 149. (Found: C, 59·08; H, 5·88. Calcd. for $C_{20}H_{24}O_9$: C, 58·82; H, 5·92%). High resolution molecular weight determination: Calcd. for $C_{20}H_{24}O_9$: 408·142. Found: 408·145.

1-(2′-*Acetoxy*-3′-*hydroxy*-3′-*methylbutyl*)-2,6-*diformyl*-3,5-*dihydroxy*-4-*methoxybenzene* (**26**). The monoacetate **22** (100 mg; 0·278 mmol) was dissolved in glacial AcOH (5 ml) and EtOAc (2 ml) and the soln was subjected to a stream of O_3 enriched O_2 at −78° for 0·5 hr. Zn dust (150 mg) was then added and the mixture was stirred at room temp for a further 0·5 hr. The soln was then filtered and the solvents were removed *in vacuo* to yield a residue which was treated with hot chloroform (50 ml). The chloroform soln was filtered and the solvent was removed under reduced pressure to yield an oily residue (115 mg). Crystallization of this material from EtOAc afforded **26** (51·6 mg; 54% yield) as plates, m.p. 188–190°; IR (KBr) 3535 (OH), 1735 (C=O, acetate), 1630 (C=O, aldehyde); UV λ_{max}^{MeOH} (ε) 270 (32,400); UV λ_{max}^{MeOH} (ε) (+NaOH) 266 (sh) (14,700), 292·5 (25,300), 340 (9,330); UV λ_{max}^{MeOH} (+HCl) 224 (10,200), 270 (19,200); NMR (100 MHz) in $CDCl_3$, TMS lock, −2·82 (2H, s, disappears on addition of D_2O, two equiv phenolic OH), −0·28 (2H, s, two equiv CHO), 5·00 (1H, d of d's, J = 4 Hz and 10 Hz, $CH_2CH(R)OCOCH_3$), 6·09 (3H, s, aromatic OCH_3), 6·36 (2H, AB of ABX m, J_{AB} = 15 Hz, J_{AA} = 10 Hz, J_{BX} = 4 Hz), 8·04 (1H, broad, disappears on addition of D_2O, OH), 8·16 (3H, s, $OCOCH_3$), 8·70 (6H, s, $C(OH)(CH_3)_2$); mass spectrum *m/e* 340 (M) 322 (M-18), 298, 280, 262, 221 (base peak).

(Found: C, 56·24; 5·91. Calcd. for $C_{16}H_{20}O_8$: C, 56·47; H, 5·92%). High resolution molecular weight determination: Calcd. for $C_{18}H_{20}O_8$: 340·116. Found: 340·120.

1-(2'-Acetoxy-3'-hydroxy-3'-methylbutyl)-2,6-diformyl-3,4,5-trimethoxybenzene (**27**). Compound **26** from the previous reaction (22·7 mg; 0·0628 mmol) was dissolved in acetone (5 ml) and to it was added K_2CO_3 (0·5 g) and MeI (2 ml). The mixture was refluxed for 1·5 hr, then water (10 ml) was added and the mixture was stirred 10 min more. The mixture was then acidified with conc HCl and extracted with chloroform (3 × 15 ml). The chloroform extract was washed with saturated brine (10 ml), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The resultant residue was chromatographed on preparative TLC (solvent A) and the major band, **27** (19·3 mg; 81·5% yield) was thus isolated. Crystallization from anhyd ether-hexane yielded pure **27** as plates, m.p. 79·5–80·5°; NMR (100 MHz) in $CDCl_3$, TMS lock, −0·39 (2H, s, two equiv CHO), 5·00 (1H, d of d's, $J = 5$ Hz and 10 Hz, $CH_2CH(R)OCOCH_3$), 6·00 (6H, s, two equiv aromatic OCH_3), 6·15 (3H, s, aromatic OCH_3), 6·2–6·6 (2H, AB of ABX m, $CH_2CH(R)OCOCH_3$), 7·90 (1H, broad disappears on addition of D_2O, OH), 8·22 (3H, s, $-OCOCH_3$), 8·70, 8·74 (6H, two s's, $C(OH)(CH_3)_2$). (Found: C, 58·63; H, 6·34. Calcd. for $C_{18}H_{24}O_8$: C, 58·69; H, 6·59%).

Treatment of **27** *with hydrogen peroxide and sulfuric acid.* A soln of **27** (19 mg; 0·0516 mmol) in glacial AcOH (1 ml) was cooled in an ice bath and 50% H_2SO_4 (0·25 ml) was added and the mixture was cooled to 0° while N_2 was bubbled through the soln to remove O_2 from the system. After 10 min of such treatment 30% H_2O_2 (0·03 ml; 0·265 mmol) was added and the N_2 flow was continued for 15 min at 0° at which time the soln began to take on an orange colour. The mixture was then poured into ice cold brine (10 ml) and extracted with chloroform (4 × 10 ml). The chloroform extract was washed with brine (10 ml), dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield an orange oil which was dried *in vacuo*. This material was then dissolved in anhyd ether (10 ml), MeLi (0·50 ml; 1·05 mmol) was added and the mixture was stirred at 0° for 15 min. Ac_2O (1 ml) and pyridine (2 ml) was then added and the mixture was stirred at room temp for 4 hr. Water (10 ml) was then added and the soln was extracted with chloroform (3 × 15 ml). The chloroform extract was washed with brine (10 ml), dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield an orange coloured residue which appeared to contain 4 components by TLC. Preparative TLC (solvent B) allowed separation of two apparently major bands which constituted only ~ 4 mg and were thus examined no further.

Demethylation of 5-(2'-*hydroxyethyl*)-8-*methoxypsoralen* (**17**).[14] The alcohol **17** (27 mg; 0·104 mmol) was demethylated with HI according to the published procedure.[14] The liberated MeI was trapped as tetramethylammonium iodide in the manner described for the demethylation of **2**.

Acid catalyzed hydrolysis of isoimperatorin (**4**). Isoimperatorin **4** (21 mg; 0·078 mmole) was hydrolyzed in refluxing AcOH in the manner described previously for **1**. The product obtained was **28** (11·8 mg, 85% yield), m.p. 275° (lit[22] m.p. 278°).

Methylation of bergaptol (**28**). Bergaptol **28** (11·8 mg; 0·054 mmole) from the previous reaction was dissolved in acetone (10 ml) and anhyd K_2CO_3 (500 mg) and MeI (3 ml) were added. The mixture was refluxed for 15 min and the workup as described in the preparation of **8**, yielded **29** (12 mg, 90% yield) which was crystallized from EtOAc as colorless plates, m.p. 186–188° (lit.[22] m.p. 191°), mixed m.p. with authentic bergapten obtained from Dr. D. L. Dreyer, 186·5–187·5°.

6-Formyl-7-hydroxy-5-methoxycoumarin (**30**). Bergapten **29** (24 mg; 0·111 mmole) was dissolved in glacial AcOH (4 ml) and 30 ml of O_3 saturated glacial AcOH was added and the mixture was stirred for 1 hr. Zn dust (50 mg) was added and stirring continued for another 10 min. Soln was filtered and the solvent was evaporated *in vacuo*. The residue (50 mg) was dissolved in $CHCl_3$–MeOH mixture and was chromatographed on silica gel (6 g). Elution with benzene and benzene-$CHCl_3$ mixture gave the desired **30** (14 mg) which was crystallized from acetone to give pure **30** (8 mg: 33% yield), m.p. 220–221° (lit.[23] m.p. 222–223°); IR (KBr) 1742, 1592 (α-pyrone), 1697 (aldehyde C=O); UV λ_{max}^{MeOH} (ε) 205·5 (5,870), 225 (sh) (2,690), 266 (19,900), 312 (3,180), 340 (sh) (1,345); UV λ_{max}^{MeOH} (ε) (+NaOH) 206 (18,850), 238 (13,700), 262 (8,940), 285 (sh) (4,160) 347 (8,800), 394 (9,800); UV λ_{max}^{MeOH} (ε) (+HCl) 207 (11,370), 222 (sh) (5,740), 267 (15,400), 314 (5,630); NMR (100 MHz) in $CDCl_3$, TMS lock, −1·96 (1H, s, disappears on addition of D_2O, phenolic OH), −0·23 (1H, s, aromatic CHO), 2·14 (1H, d of d's, $J = 9·75$ Hz and 0·6 Hz, H(4) of coumarin), 3·35 (1H, m, H(8) of coumarin), 3·72 (1H, d, $J = 9·75$ Hz, H (3) of coumarin), 5·95 (3H, s, aromatic OCH_3); mass spectrum *m/e* 220 (M), 202 (M-18), 191 (M-29), 174, 146 (base peak). (Found: C, 60·06; H, 3·71. Calcd. for $C_{11}H_8O_5$: C, 60·00; H, 3·64%). High resolution molecular weight determination: Calcd. for $C_{11}H_8O_5$: 220·037. Found: 220·036.

The *Thamnosma montana* plants used in this study were collected in summer as seeds and mature plants from the north facing slopes of small hills in the vicinity of Joshua Tree National Monument, in the Mojave Desert area of Southern California. Some seeds could be propagated by Dr. P. Salisbury of our department. Of mature plants collected, some could be successfully transplanted and continued to grow. Small *Thamnosma montana* plants (2–4 years old) were obtained from Molecular Biochemical Corporation, Tempe, Arizona.

Isolation of constituents of Thamnosma montana. *Thamnosma montana* plants were ground to a coarse powder in a Warring blender and extracted with acetone in a Soxhlet extractor. The acetone extract was reduced to dryness and the residue was treated with hot chloroform. The chloroform soluble portion was filtered and the solvent removed under reduced pressure. The residue was preabsorbed on alumina (neutral, activity IV) and the preabsorbed material was chromatographed on alumina (neutral, activity IV). The compounds were isolated by preparative layer chromatography. A detailed experimental procedure for the isolation of the various compounds has already been described.[5]

Feeding technique. In the phenylalanine experiments, freshly cut shoots from a *single* mature plant were immersed in a solution of the radioactive precursor (hydroponic feeding method) contained in small conical centrifuge tubes.

In the mevalonic acid experiments, the whole plant was removed from the soil, the roots washed with water and the plant was immersed immediately into the solution of the radioactive precursor.

Feeding experiments. The radioactive compounds were fed to the plants by the hydroponic feeding method. D,L-Phenylalanine-[3-^{14}C] was obtained from New England Nuclear Corp., Boston, Massachusetts. D,L-Mevalonic acid [2-^{14}C] lactone (obtained from New England Nuclear Corp.,) was administered as the Na salt by dissolving the lactone in Na_2CO_3aq. D,L-Mevalonic acid -[2-^{3}H] lactone (obtained from Amersham/Searle Corp. of Des Plaines, Illinois) obtained as a benzene solution was reduced to dryness and the residue was administered as an aqueous soln. D,L-Mevalonic acid -[4-^{3}H] lactone (obtained from Amersham/Searle Corp.) as a benzene soln was again administered as an aqueous soln. The plants were kept under continuous fluorescent illumination and were allowed to grow for the preselected time period. The plants were then worked up in the normal manner and the compounds were isolated by preparative layer chromatography. In the case of D,L-phenylalanine-[3-^{14}C] feeding experiments, the radioactivity of the compounds isolated was determined by the Nuclear-Chicago Actigraph Chromatogram Scanner equipped with an analytical count ratemeter. In all other feeding experiments, the compounds isolated were crystallized and the radioactivity determined by liquid scintillation counting. In a typical experiment, 16 g of wet plant material gave umbelliprenin (3·7 mg), alloimperatorin methyl ether (4·6 mg) and isopimpinellin (10·7 mg). The compounds were diluted with the cold material wherever necessary to allow sufficient quantities to perform degradations.

Degradation of radioactive umbelliprenin (**1**)

(a) Umbelliprenin **1** (39·5 mg, $1{\cdot}47 \times 10^6$ dpm/mmol) from feeding experiment 12 was hydrolysed with glacial AcOH as described previously to yield **5** (12·6 mg). This material was sublimed and although crystallized repeatedly, constant radioactivity could not be obtained. The final activity ($5{\cdot}04 \times 10^3$ dpm/mmol) represented 0·34% of the activity of **1** used.

(b) Umbelliprenin **1** (24 mg, $4{\cdot}19 \times 10^5$ dpm/mmol) from feeding experiment 12 was ozonized under optimum conditions as described previously and **6b** (19·0 mg) was isolated after recrystallization. This material 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, 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 (6×10 or 6×20 min). If the individual counts did not vary significantly then an average was taken to determine the total cpm. Due to the unorthodox counting soln employed counting efficiency was determined by adding an accurately weighed sample of tritiated hexadecane standard to the already counted sample and it was counted again. The ratio of expected dpm to found cpm for hexadecane determined the counting efficiency (~ 16%). In each case a blank sample containing an equal amount of inactive **6b** was counted first to determine the accurate background cpm. In this manner the radioactive **6b** was shown to have a specific activity of $7{\cdot}22 \times 10^4$ dpm/mmol or 17·2% of the activity of **1** used.

Degradation of isopimpinellin (**2**) *from experiment* 14. Isopimpinellin **2** (57 mg, $4{\cdot}97 \times 10^3$ dpm/mmol) from experiment 14 was selectively ozonized as described previously and **7** (15·0 mg) was isolated. This substance was shown to have a specific activity of less than $1{\cdot}0 \times 10^2$ dpm/mmol or less than 2% of the original activity of **2**.

Degradation of isopimpinellin (**2**) *from experiment* 15. Isopimpinellin **2** (42 mg; $4{\cdot}47 \times 10^3$ dpm/mmol) from experiment 15 was selectively ozonized as described previously and **7** (11 mg) was isolated. This substance was shown to have a specific activity of less than $1{\cdot}0 \times 10^2$ dpm/mmol or less than 3% of the original activity of **2**.

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