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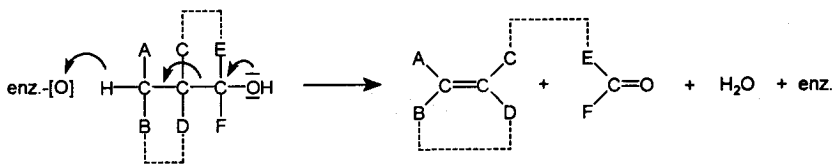
Stereoselective Syntheses of Deuterium Labelled Marmesins; Valuable Metabolic Probes for Mechanistic Studies in Furanocoumarin Biosynthesis

Volker Stanjek, Martin Miksch and Wilhelm Boland*

Max-Planck-Institut für Chemische Ökologie, Tatzendpromenade 1a, D-07745 Jena, Germany

Abstract: Stereoselective syntheses of deuterium labelled marmesins **3a-3e** as metabolic probes for biosynthetic studies are described. Starting from iodo-umbelliferone (**5**), the title compounds were readily available by copper catalysed alkylation-cyclisation and subsequent transfer-hydrogenation of the resulting furanocoumarins **6a-6e**. The bioconversion of marmesin by a microsomal preparation of *A. majus* yielded psoralene (**4**) and acetone in a single step and proceeded as a *syn*-elimination of the alkoxy-substituent together with a single hydrogen atom from a vicinal carbon atom (β-cleavage). © 1997 Elsevier Science Ltd.

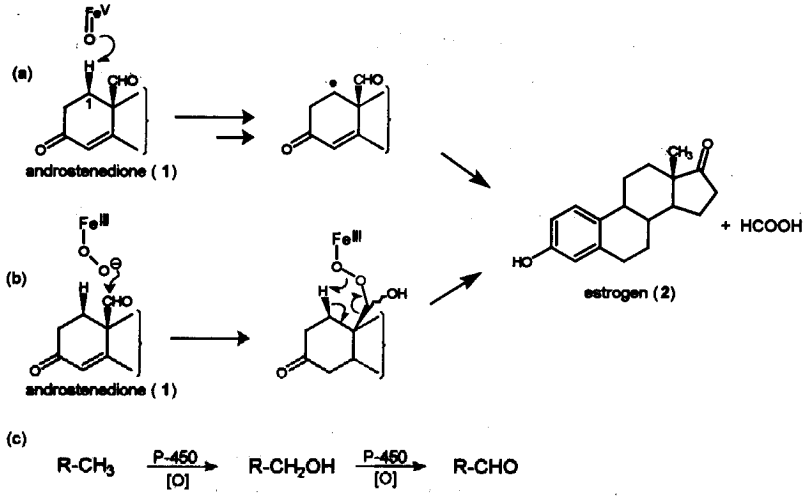
Introduction.- One of the most intriguing and least understood enzymatic reactions is the oxidative cleavage of carbon-carbon single bonds leading to olefinic products and a carbonyl compound (CO_2, HCOOH and HCHO). The typical substrates are alcohols, carbonyl compounds and their hydrates (e.g. geminal diols), respectively (cf. eqn. 1). The mechanistic course of the oxidative bond cleavage can be rationalised as an initial attack of an activated oxygen species onto a hydrogen atom attached to a carbon atom in β-position to the oxygen carrying carbon. Removal of this hydrogen atom creates a reactive intermediate which then suffers a β-cleavage yielding an olefin and a resonance-stabilised intermediate which finally is transformed to a carbonyl compound after the loss of a hydrogen atom.[1,2] In principal, the transformation can be used to cleave carbon chains, to dealkylate ring systems or to fragment (poly)cyclic structures into acyclic substructures.



eqn. 1

Common transformations which match the general concept of eqn. 1 are, for example, the demethylation reactions in the biosyntheses of steroid hormones, the production of homo- and nor-terpenoids from regular precursors, the formation of seco-compounds, the decarboxylation of fatty acids to terminally unsaturated hydrocarbons, the oxidative cleavage of hydroperoxides from unsaturated fatty acids into olefins, and, last but not least, the formation of linear and angular furanocoumarins from oxygenated precursors.[1,2] A particular well studied example of an oxidative bond cleavage reaction is the aromatase catalysed dealkylation of androstenedione (**1**) to estrogen (**2**).[3-6] The aromatase is a cytochrome P450 dependent enzyme that removes the C-19 methyl group of androstenedione (**1**) as formic acid *via* an alcohol and an aldehyde intermediate (cf. Scheme 1, path c). Several mechanistic alternatives have been proposed for the final bond cleavage reaction of the aromatase and related enzymes in the steroid metabolism.[7-12] An initial attack of an -Fe^{IV}-O$^{\bullet}$ porphyrylradicalcation

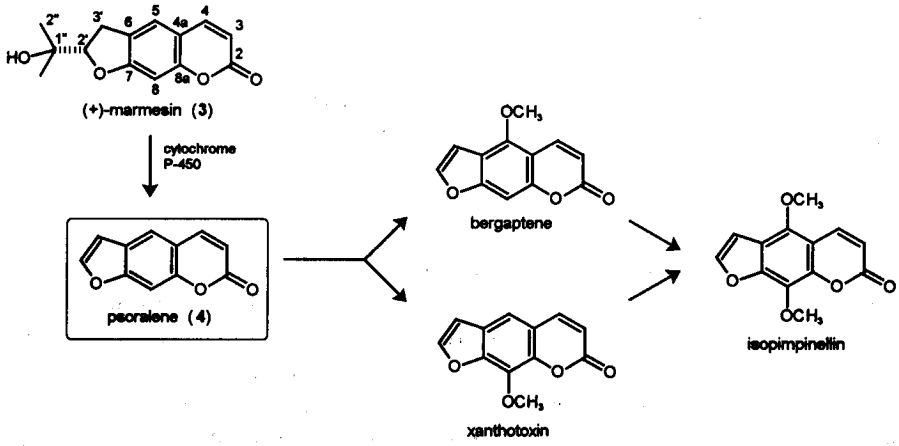
(or the canonical form of an -Fe^{V}=O) onto the *syn* hydrogen at C-1 is followed by a β-cleavage (pathway (a)) generating an olefin and formic acid; presumably *via* a geminal diol intermediate.



Scheme 1: Mechanistic alternatives of the oxidative demethylation of androstenedione (1) yielding estrogen (2).

Another proposal assumes the preceding formation of an peroxyhemiacetal from the aldehyde intermediate and a highly nucleophilic -Fe^{III}-O-OH species, followed by homolytic cleavage of the weak peroxy bond rendering an alkene and formic acid (pathway (b)) as the end products. While pathway (b), due to the cyclic nature of the transition state structure inevitably proceeds as a *syn* elimination, the course of pathway (a) is principally free from such stereochemical constraints. Although pathway (b) provides an attractive explanation for the generally observed *syn* elimination, other data strongly support pathway (a) which, of course, can also proceed as a *syn* elimination.

To study the mechanistic features of such oxidative bond cleavage reactions in more detail, and to understand the reason for the often observed *syn*-elimination, the enzymatic transformation of the *t*-alcohol marmesin (3) into the furanocoumarin psoralene (4) provides another interesting example.

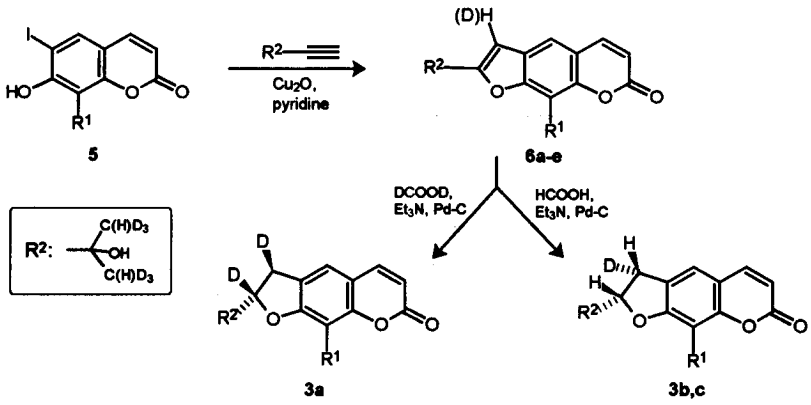


Scheme 2: Conversion of (+)-marmesin (3) into psoralene (4) and other furanocoumarins

Like the demethylations in the area of steroids the dealkylation of (+)-marmesin is achieved by a cytochrome P450-type enzyme and requires molecular oxygen and NADPH as cofactors.[13] The furanocoumarins represent a large class of compounds, and they occur widespread in Rutaceae and Umbelliferae.[14] The contact with leaves containing furanocoumarins causes blistering and erythrema, accompanied by allergic reactions, known as occupational diseases during harvesting of celery, parsley or parsnip.[15] Linear furanocoumarins like psoralene intercalate with DNA in the dark and form photoadducts upon irradiation.[16] Usually, the level of furanocoumarins is low, but after infection or damage of the plants by herbivores[17] and micro-organisms (fungi, bacteria),[18] a dramatic increase (10-100fold) of these compounds is observed within the following 2-4 days. Abiotic stress factors like UV light may have a similar effect.[19]

While the involved enzymes have been shown to belong to the large family of cytochrome P450 enzymes, no mechanistic details and no informations on the stereochemical course of the transformation of (+)-marmesin (**3**) into psoralene (**4**) (Scheme 2) are currently available. It is also unknown whether the dealkylation is achieved in a single step or proceeds sequentially via a carbonyl intermediate resembling the stepwise degradation of steroids (cf. Scheme 1, path c). To address the question of a sequential degradation and the stereochemistry of the final bond cleavage reaction generating psoralene (**4**), appropriately labelled precursors are required. For example, a marmesin with a fully deuterated *i*-propyloxy side chain may provide informations on the fate of the substituent, and substrates with stereospecific labelled methylene and methine groups within the dihydrofuran moiety may help to identify the stereochemical course of the degradation of (+)-marmesin as a *syn*- or *anti*-elimination.

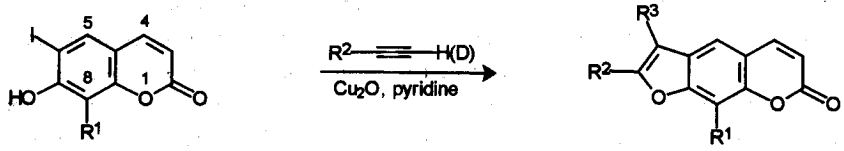
Synthesis of Deuterium Labelled Marmesins. A straightforward and highly flexible approach towards stereospecifically labelled marmesins is outlined in Scheme 3.



Scheme 3: Synthesis of stereospecifically deuterated marmesins as metabolic probes for the stereochemical analysis of the psoralene synthase

The basic skeleton of marmesin is readily built up using the copper catalysed alkynylation of (±)-6-iodo-umbelliferone (**5**) and provides the substituted furanocoumarins **6a-e** in a single operation. The reaction has no serious limitations concerning R^2, and several different free alcohols readily undergo the copper catalysed alkylation-cyclization.[20,21] The subsequent transfer-hydrogenation could be achieved without concomitant hydrogenation of the unsaturated lactone. Owing to the exclusive *syn*-addition of the two hydrogen isotopes across the double bond of the furan moiety at the surface of the Pd-catalyst, a defined *syn*- or *anti*-orientation of R^2 and a

deuterium isotope at C-3' is readily achieved. Permutation of the reaction sequence, i.e. hydrogenation of a C-3' deuterated precursor or deuteration of a substrate carrying a hydrogen at C-3' allows the synthesis *syn*- and *anti*-isomers of marmesin (3) along a uniform synthetic protocol. Best results were obtained with HCOOH ($^2HCOO^2H$) as the hydrogen (deuterium) source and Pd-C as the heterogeneous catalyst. ^1H NMR and NOE-^1H NMR experiments confirmed the stereochemical course of the hydrogenation as exclusively *syn*. A final separation of the racemic products into the pure enantiomers or an enantioselective synthesis of (+)-marmesin, the natural precursor of psoralene, was not necessary, since the psoralene synthase converted only (+)-marmesin into psoralene and was not inhibited by the (-)-enantiomer.[13] As depicted in the Table of Scheme 4, several differently substituted and differently labelled furanocoumarins were prepared according to the protocol of Scheme 4.



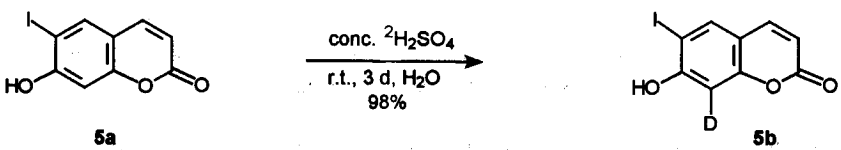
5a: $R^1 = H$
5b: $R^2 = D$

6a-e

entry		products				yield
	R^1		R^1	R^2	R^3	%
5a	H		H	$C(CH_3)_2OH$	H	ref. 20
5a	H	**6b**	H	$C(CH_3)_2OH$	D	74
5b	D	**6c**	D	$C(CH_3)_2OH$	D	91
5a	H	**6d**	H	$C(CD_3)_2OH$	H	74
5b	D	**6e**	D	$C(CD_3)_2OH$	H	87
5a	H	**7**	H	$CH(CH_3)OH$	H	80

Scheme 4: Alkylation of iodo-umbelliferones **5a** and **5b** to the furanocoumarins **6a-e** and **7**

While the iodo-umbelliferone (**5a**) was available along to standard procedures,[20] the deuterium label at C-8 of **5b** ($R^1 = {}^2H$), required as a metabolic inert isotopic marker, was introduced by stirring of **5a** with conc. $^2H_2SO_4$ at rt. for 3 d. The exchange of the hydrogen isotope proceeded instantaneously and with high efficiency (95% ^2H, 98 % yield).



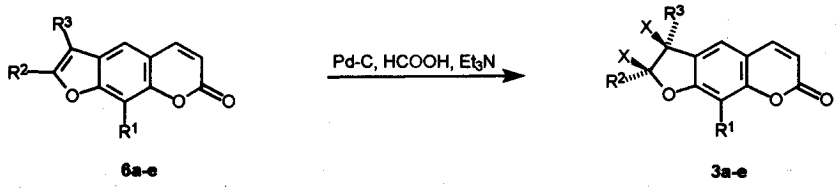
5a **5b**

Scheme 5: Synthesis of deuterium labelled 6-iodoumbelliferone

The introduction of a deuterium atom at C-3' of **6b** and **6c** was achieved by using terminally deuterated alkynes for alkylation. However, a high degree of deuterium labelling (>95%) at this position required an absolutely dry solvent (pyridine), a rigorous exclusion of moisture and a previous exchange of the phenolic proton of **5a/b** by a deuterium atom (by stirring with CH_3O^2H). The furanocoumarins **6d/e** possessing a fully deuterated *i*-propyloxy

side were synthesised from **5a** or **5b** by alkylation with [$^2H_3$]methyl-[1,1,1-$^2H_3$]but-3-yn-2-ol, obtained by reaction of ethynylmagnesium bromide with acetone-d_6.

Attempts to reduce the furan double bond with e.g. Pt-C or Pd-C and hydrogen gas failed due to concomitant hydrogenation of the unsaturated lactone. Following the protocol of Scheme 6, high yields of the marmesins **3a-3e** were, however, obtained by transfer hydrogenation with Pd-C using HCOOH or $^2HCOO^2H$ as the source of the hydrogen isotope.



6a-e **3a-e**

entry	products					yield
		R^1	R^2	R^3	X	%
6a	**3a**	H	$C(CH_3)_2OH$	H	D	58
6b	**3b**	H	$C(CH_3)_2OH$	D	H	68
6c	**3c**	D	$C(CH_3)_2OH$	D	H	62
6d	**3d**	H	$C(CD_3)_2OH$	H	H	50
6e	**3e**	D	$C(CD_3)_2OH$	H	H	70
7	**8**	H	$CH(CH_3)OH$	H	D	80

Scheme 6: Transfer-hydrogenation of furanocoumarins to marmesin derivatives

Bioconversion of labelled marmesins 3a-e. To unravel the degradative sequence and the stereochemical course of the bond cleavage reaction, the labelled marmesins **3a-e** were incubated with a microsome preparation from a cell culture of *Ammi majus*. Prior to the isolation of the microsomes the cell culture was elicited with a cell wall preparation from the plant pathogenic fungus *Phytophthora megasperma* f. sp. *glycinea*[22] to induce a *de novo* biosynthesis of the cytochrome P450 enzymes responsible for the dealkylation of (+)-marmesin to the furanocoumarin psoralene (cf. Scheme 2).[13]

Two remarkable results followed from the biotransformations of **3a-e**. First of all, the oxidative dealkylation of the specifically labelled marmesin **3b** or **3c** yielded psoralene (**4**) with a quantitative loss of the vicinal deuterium atom from the dihydrofuran moiety as demonstrated in the mass spectrum of Figure 1. If the deuterium atom at C-3' of the precursor was located in an *anti* position to the alkoxy substituent, as for example in **3a**, the isotope was not lost. These findings unambiguously characterised the bond cleavage reaction as stereospecific transformation proceeding as a *syn*-elimination of the alkoxy side chain and a single hydrogen atom from C-3' of the dihydrofuran substructure. Secondly, analysis of the side chain cleavage products of **3d** or **3e** revealed that the *i*-propyloxy substituent was cleaved off intact and could be identified as labelled acetone. The qualitative and quantitative analysis of the side chain fragment was most conveniently performed by GLC-MS after derivatisation of the carbonyl fragment with pentafluorobenzylhydroxylamine (Figure 2).[23,24] According to the molecular ion of the resulting pentafluorobenzylhydroxylimine (**9**) at m/z = 259 *Da*, all deuterium atoms of the *i*-propyloxy substituent, e.g. from **3d** or **3e**, were still present in the product. The corresponding derivatives of acetone and acetone-d_6 served as references. Since psoralene (**4**) and acetone-d_6 were produced in equimolar amounts, the

dealkylation of marmesin has to be considered as a single step transformation rather than a sequential process (cf. Scheme 1).

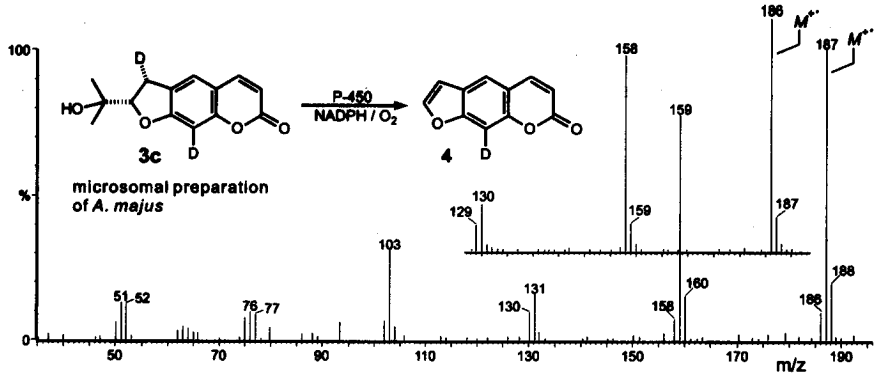


Figure 1: Mass spectrum of psoralen-d_1 resulting from the enzymatic transformation of the dideuterated marmesin 3c. Insert: molecular ion and first fragments of unlabelled, natural psoralene (4).

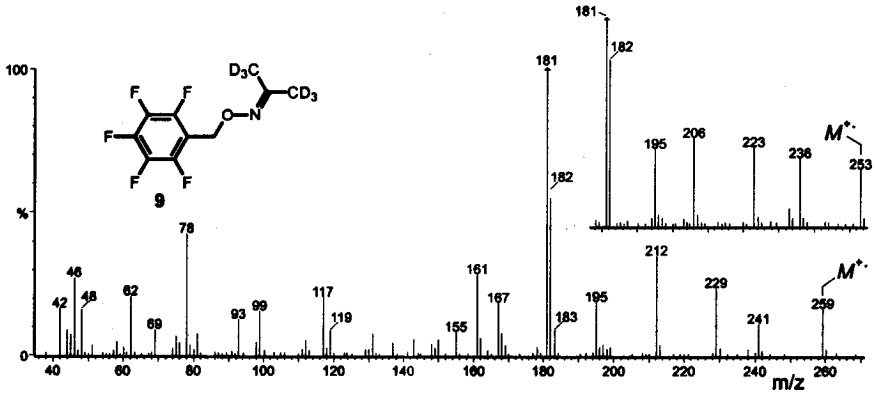
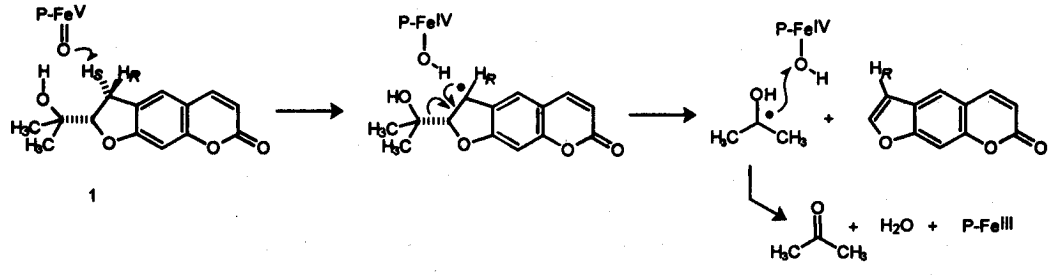


Figure 2: Mass spectrum of acetone-d_6 produced in incubation experiments of 3e with microsomes from *A. majus*. The carbonyl compound was converted into the pentafluorobenzylhydroxylimine (9) prior to GLC-MS. Insert: molecular ion and first fragment ions of an unlabelled synthetic reference.

In conclusion of the experimental findings the dealkylation of marmesin to psoralene has to be considered as a single step process and may be rationalised by the mechanistic sequence outlined in Scheme 7.



Scheme 7: Mechanistic rationale for the P450 catalysed dealkylation of marmesin (3) into psoralene (4) and acetone.

Thus, marmesin is attacked by the reactive Fe(V)=O species of the enzyme, and a benzylic radical is formed. The reactive intermediate stabilises by β-cleavage to yield psoralene (**4**) an *i*-propyloxy radical. The latter, then, attacks the directly neighboured porphyryl-Fe(IV)-OH species to yield acetone, water and the reduced cytochrome catalyst. In this model, the facial arrangement of the reactive Fe(V)=O is responsible for the observed *syn*-elimination of the hydrogen atom H_S and the alkoxy substituent. The arrangement also prevents the escape of long-living radicals from the active centre, since the close, cage-type arrangement of all reactive partners secures a rapid interception of the emerging radicals. The mechanistic sequence proposed in Scheme 7 could be already independently confirmed by a chemical model system based on Fe- or Mn-porphinato complexes which were also able to convert marmesin into psoralene and acetone. A detailed discussion of the stereochemical aspects of the enzymatic process, isotope effects of the C-^{2}H bond cleavage and the results from the P450 model system will be published elsewhere.[25]

Experimental

General: Reactions were performed under Ar; solvents were dried according to standard methods. IR: Perkin-Elmer Series 1600 FTIR Spectrophotometer. ^{1}H- und ^{13}C NMR: Bruker AC 250 or Bruker AC 400 Spectrometer; $CDCl_3$ as solvent. Chemical shifts of ^{1}H and ^{13}C NMR are given in ppm (δ) downfield relative to TMS as internal standard. GC-MS (70eV): Fisons MD 800 or Finnigan ITD 800 coupled with a Carlo Erba GC 6000, Model Vega, equipped with a fused silica, coated with DB1 or SE 30 (15m x 031mm); hydrogen served as carrier gas. HR-MS: Kratos MS 20. Silica gel, Si 60 (0.200-0.063 mm, E. Merck, Darmstadt, Germany) was used for chromatography. Thin layer chromatography was performed with silica gel plates Polygram Sil G_{F254}, from Macherey & Nagel, Düren.

6-Iodo-[8-^{2}H]-umbelliferone (5b)

A suspension of 6-iodo-umbelliferone (**5a**) (1.00 g, 3.47 mmol) was refluxed in methanol-d_1 (40 ml) containing a catalytic amount of ^{2}HCl until a clear soln. was obtained. After cooling, the solvent was removed i.v. and the residue was dissolved in conc. $^2H_2SO_4$ (9.0 ml). Stirring was continued for 3 d at r.t. until GLC-MS indicated virtually complete exchange (>95%) of the acidic proton at C-8 (compounds were analysed as methyl ethers after hydrolysis, extraction and derivatisation with diazomethane). After prolonged reaction times a decrease of the regioselectivity of the exchange was observed. The reaction mixture was slowly(!) poured into ice water (400.0 ml) and the resulting suspension was extracted with ethyl acetate (3 x 50 ml). The combined organic layers were washed with a sat. $NaHCO_3$ solution and dried over anhydr. $MgSO_4$. Evaporation of solvent i.v. afforded virtually pure **5b**. Yield: 0.98 g (98 %). IR [cm^{-1}]: 3142, 1683, 1602, 1386, 1227, 1107, 931, 894, 822, 768, 706, 625. ^{1}H NMR (acetone-d_6, 400 MHz) δ: 8.00 (s, 1H-C(5)), 7.81 (d, 1H-C(4)), 6.15 (d, 1H-C(3)), 2.90 (s, 1H-OH). ^{13}C NMR (acetone-d_6 100 MHz) δ: 160.5, 160.3, 156.8, 143.6, 139.3, 115.1, 113.9, 79.5. MS (70 eV) *m/z*: 289 (100, $M^{+\bullet}$), 261 (48), 134 (15), 106 (13). 88 (10). HR-MS *m/z* calcd. for $C_9H_4DO_3I$: 288.9346, found: 288.9346.

2-[$^2H_3$]-Methyl-[1,1,1-$^2H_3$]-but-3-yn-2-ol

A soln. of ethynylmagnesium chloride in THF (10.0 ml, 0,5 M soln.) was slowly added with stirring to a soln. of acetone-d_6 (0.37 ml, 5.0 mmol). Stirring was continued at r.t. for 3 h, and, then, the solvent was removed i.v.. Ether (20.0 ml) was added to the residue, followed by hydrolysis with water and addition of sat. aq. soln. of NH_4Cl to dissolve the resulting precipitate. Extractive work-up with ether (2 x 20 ml) and removal of solvents afforded the pure product. Yield: 0.24 g (54 %). IR [cm^{-1}]: 3301, 2238, 1383, 1121, 1048, 1011, 820, 647. ^{1}H

NMR ($CDCl_3$, 250 MHz) δ: 2.42 (s, 1H-C(4)), 2.05 (s, 1H-O<u>H</u>). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 88.7, 70.2, 64.7. MS (70 eV) *m/z*: 90(2, $M^{+\bullet}$), 88 (6, M-D^+), 72 (100), 46 (48). HR-MS *m/z* calcd. for C_5H_8O: 90.0952, found: 90.0946.

2-Methyl-[4,2*O*-$^2H_2$]-but-3-yn-2-ol.

A soln. of 2-Methylbut-3-yn-2-ol (0.30 ml, 3.09 mmol) in ether (10.0 ml) was slowly added with stirring to a soln. of ethylmagnesium bromide (9.27 mmol) in the same solvent (10.0 ml) and, then, refluxed for 1 h. After cooling, the reaction was quenched with ^2H_2O (2.5 ml) and ^2HCl (1.0 ml). Extractive work-up with ether afforded the crude [2*O*,4-$^2H_2$]-2-methylbut-3-in-2-ol after evaporation of solvents i.v. The crude alkynol was used for the copper catalysed alkylation (see below) without purification to avoid loss deuterium atoms.

Copper Catalysed Alkylation of 6-Iodo-umbelliferones (5a/b); General Procedure.[20]

0.30 g (1.04 mmol) of the appropriate 6-iodo-umbelliferone (**5a/b**) and the respective alkyne (0.168 g, 2.0 mmol) were dissolved in dry pyridine (12.0 ml). Copper(I)oxide (0.20 g, 1,40 mmol) was added, and the mixture was refluxed for 2 h. After cooling the reaction mixture was filtered through a pad of silica gel and diluted with ethyl acetate. The solution was washed with 1.0 N HCl, saturated $NaHCO_3$ and brine and dried over anh. $MgSO_4$. Following evaporation of solvents the residue was purified by chromatography on silica gel using pentane: ethyl acetate, 1:2, v:v for elution.

2'-(1"Hydroxy-1"-methylethyl)-psoralene (6a)

6a was prepared as described previously. Yield and spectroscopic data of **6a** agreed with literature data.[20]

2'-(1"Hydroxy-1"-methylethyl)-[3'-^{2}H]-psoralene (6b)

Prepared from **5a** (0.30 g, 1.04 mmol) and 2-methyl-[4,2*O*-$^2H_2$]-but-3-in-2-ol (0.17 g, 2 mmol) as described above. To warrant a high degree of isotopic labelling at C-3' of the resulting psoralene **6b**, the 6-iodo-umbelliferone (**5a**) was refluxed in methanol-d_1 with a cat. amount of ^2HCl to replace the phenolic hydrogen by a deuterium atom. Following removal of solvents i.v. the copper catalysed alkylation was carried out as described above. All solvents had to be thoroughly dried prior to use. Yield: 0.19 g (74 %). IR [cm^{-1}]: 3405, 3343, 2979, 1733, 1708, 1629, 1573, 1448, 1378, 1284, 1165, 1136, 1109, 889, 845, 823. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.77 (d, 1H-C(4)), 7.57 (s, 1H-C(5)), 7,38 (s, 1H-C(8)), 6.35 (d, 1H-C(3)), 1.70 (s, 6H-C<u>H</u>$_3$)). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 165.5, 161.2, 156.3, 151.8, 144.2, 125.8, 119.5, 115.2, 114.4, 99.7, 69.2, 28.7. MS (70 eV) *m/z*: 245 (28, $M^{+\bullet}$), 230 (100), 227 (68), 199 (40), 188 (23), 184 (13), 116 (11), 79 (12), 52 (12), 51 (13). HR-MS *m/z* calcd. for $C_{14}H_{11}DO_4$: 245.0798, found: 245.0798.

2'-(1"Hydroxy-1"-methylethyl)- [3',8-$^2H_2$]-psoralene (6c)

Prepared from **5b** (0.30 g, 1.04 mmol) and 2-methyl-[4,2*O*-$^2H_2$]-but-3-in-2-ol (0.17 g, 2 mmol) as described above. To warrant a high degree of isotopic labelling at C-3' of the resulting psoralene **6c**, the 6-iodo-umbelliferone (**5a**) was refluxed in methanol-d_1 with a cat. amount of ^2HCl to replace the phenolic hydrogen by a deuterium atom. Following removal of solvents i.v. the copper catalysed alkylation was carried out as described above. All solvents had to be thoroughly dried prior to use. Yield: 0.233 g (91 %). IR [cm^{-1}]: 3405, 3342, 2981, 1732, 1703, 1624, 1564, 1431, 1378, 1284, 1163, 1112, 889, 841, 825, 774. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.78 (d, 1H-C(4)), 7.59 (s, 1H-C(5)), 6.37 (d, 1H-C(3)), 2.15 (s, 1H-O<u>H</u>), 1.70 (s, 6H-C<u>H</u>$_3$)).^{13}C NMR ($CDCl_3$, 100 MHz) δ: 165.4, 161.2, 156.3, 151.8, 144.2, 125.8, 119.5, 115.3, 114.5, 69.2. 28.7. MS (70 eV) *m/z*: 246 (35, $M^{+\bullet}$), 231 (100), 189 (19), 46 (32). HR-MS *m/z* calcd. for $C_{14}H_{10}D_2O_4$: 246.0861, found: 246.0864.

2'-(1"Hydroxy-1"-methylethyl)- [2",2",2",3",3",3"-$^2H_6$]-psoralene (6d)
Prepared from **5a** (0.30 g, 1.04 mmol) and [$^2H_3$]-methyl-[1,1,1-$^2H_3$]-but-3-in-2-ol (0.17 g, 2.0 mmol) as described. Yield: 0.194 g (74 %). IR [cm^{-1}]: 3370, 3061, 2230, 1723, 1707, 1631, 1592, 1577, 1446, 1423, 1385, 1185, 1123, 1054, 1002, 915, 877, 831, 808, 738, 669. ^1H NMR ($CDCl_3$, 250 MHz) δ: 7.80 (d, 1H-C(4)), 7.62 (s, 1H-C(5)), 7.42 (s, 1H-C(8)), 6.63 (s, 1H-C(3')), 6.38 (d, 1H-C(3)). MS (70 eV) *m/z*: 250 (30, $M^{+\bullet}$), 232 (100), 188(18), 93 (9), 46 (50). HR-MS *m/z* calcd. for $C_{14}H_6D_6O_4$: 250.1112, found: 250.1109.

2'-(1"Hydroxy-1"-methylethyl)- 8,[8,2",2",2",3",3",3"-$^2H_7$]-psoralene (6e)
Prepared from **5b** (0.30 g,1.04 mmol) and [$^2H_3$]-methyl-[1,1,1-$^2H_3$]-but-3-in-2-ol (0.17 g, 2.0 mmol) as described. Yield: 0.23 g (87 %). IR [cm^{-1}]: 3366, 2924, 2231, 1732, 1707, 1623, 1566, 1430, 1382, 1284, 1176, 1127, 1031, 1000, 807, 769. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.78 (d, 1H-C(4)), 7.59 (s, 1H-C(5)), 6.63 (s, 1H-C(3')), 6.36 (d, 1H-C(3)), 2.20 (s, 1H-O<u>H</u>). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 165.5, 161.2, 156.3, 151.8, 144.2, 125.9, 119.5, 115.3, 114.5, 100.0, 69.0. MS (70 eV) *m/z*: 251 (32, $M^{+\bullet}$), 233 (100), 205 (5), 189 (24), 46 (44). HR-MS *m/z* calcd. for $C_{14}H_5D_7O_4$: 251.1175, found: 251.1176.

Transfer-Hydrogenation of 2'-(1"Hydroxy-1"-methylethyl)-psoralene (6a-e). General procedure.
Acetone (2 ml) and formic acid (0,11 ml, 3.0 mmol) were added to palladium (10 %) on charcoal (0.15 g) and 0.15 g (0.82 mmol) of the respective 2'-(1"-hydroxy-1"-methylethyl)-psoralene (**6a-e**). After stirring for 15 min triethylamine (0.55 ml, 3.94 mmol) was added slowly at rt. Stirring was maintained for 2 h, and, then, the catalyst was removed by filtration. Evaporation of solvents i.v. and chromatography on silica gel (pentane:ethyl acetate, 1:2, v:v) afforded the pure products.

(±)-*trans*-[2',3'-$^2H_2$]-Marmesin {= (±)-*trans*-(2-(1-Hydroxy-1-methylethyl)-[2,3-$^2H_2$]-2,3-dihydro-7H-furo-[3,2-g]chromen-7-one} (3a)
Acetone-d_6 and formic acid-d_2 were used instead of the non-labelled reagents for the transfer of deuterium. Transfer-deuteration of **6a** (0.15 g, 0.82 mmol) afforded **3a**. Yield: 89.0 mg (58 %). IR [cm^{-1}]: 3483, 3042, 2980, 2970, 2928, 1701, 1630, 1573, 1481, 1446, 1402, 1385, 1368, 1342, 1311, 1287, 1262, 1189, 1143, 1033, 987, 961, 943, 927, 834, 821, 788, 772, 753, 725, 612. ^1H NMR ($CDCl_3$, 250 MHz) δ: 7.58 (d, 1H-C(4)), 7.19 (s, 1H-C(5)), 6.70 (s, 1H-C(8)), 6.18 (d, 1H-C(3)), 3.20 (s, 1H-C(3')), 1.98 (s, 1H-O<u>H</u>)), 1.35 (s, 3H-C<u>H</u>$_3$)), 1,22 (s, 3H-C<u>H</u>$_3$)). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 163.2, 161.6, 155.7, 143.8, 125.1, 123.5, 112.8, 112.2, 97.9, 91.1, 71.6, 29.4, 26.1, 24.3. MS (70 eV) *m/z*: 248 (48, $M^{+\bullet}$), 247 (33), 215 (19), 214 (14), 190 (60), 189 (97), 188 (100), 187 (34), 176 (20), 162 (26), 161 (27), 160 (12), 132 (15), 133 (15), 59 (64). HR-MS *m/z* calcd. for $C_{14}H_{12}D_2O_4$: 248.1017, found: 248.1012.

(±)-*cis*-[3'-^2H]-Marmesin {= (±)-*cis*-(2-(1-Hydroxy-1-methylethyl)-[3-^2H]-2,3-dihydro-7H-furo-[3,2-g]-chromen-7-one (3b)
Transfer-hydrogenation of **6b** (0.15 g, 0.82 mmol) afforded **3b**. Yield: 0.103 g (68 %). IR [cm^{-1}]: 3480, 3041, 2969, 2928, 1701, 1630, 1573, 1481, 1403, 1366, 1268, 1181, 1137, 963, 928, 818. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.59 (d, 1H-C(4)), 7.22 (s, 1H-C(5)), 6.72 (s, 1H-C(8)), 6.20 (d, 1H-C(3)), 4.73 (d, 1H-C(2')), 3.17 (d, 1H-C(3')), 1.92 (s, 1H-O<u>H</u>)), 1.30 (s, 3H-CH_3), 1.17 (3H-CH_3)). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 163.2, 161.5 155.7, 143.7, 125.1, 123.5, 112.8, 112.2, 97.9, 91.1, 71.7, 29.2 (t), 26.1, 24.3. MS (70 eV) *m/z*: 247 (61, $M^{+\bullet}$), 213 (20), 189 (100), 188 (99), 176 (17), 161 (35), 132(18), 59 (58). HR-MS *m/z* calcd. for $C_{14}H_{13}DO_4$: 247.0955, found: 247.0950.

(±)-*cis*-[8,3'-$^2H_2$]-Marmesin {= (±)-*cis*-(2-(1-Hydroxy-1-methylethyl)-[3,9-$^2H_2$]-2,3-dihydro-7H-furo-[3,2-g]-chromen-7-one} (3c)

Transfer-hydrogenation of **6c** (0.15 g, 0.82 mmol) afforded **3c**. Yield: 0.096 g (62 %). IR [cm^{-1}]: 3480, 3040, 2969, 2928, 1696, 1627, 1564, 1471, 1441, 1399, 1366, 1309, 1269, 1252, 1142, 1038, 958, 934, 916, 823, 768. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.59 (d, 1H-C(4)), 7.22 (s, 1H-C(5)), 6.20 (d, 1H-C(3)), 4.73 (d, 1H-C(2')), 3.17 (d, 1H-C(3')), 1.82 (s, 1H-OH)), 1.30 (s, 3H-CH_3), 1.17 (s, 3H-CH_3)). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 163.2, 161.5 155.6, 143.7, 125.0, 123.4, 112.8, 112.3, 91.1, 71.7, 29.2 (t), 26.1, 24.3. MS (70 eV) *m/z*: 248 (68, $M^{+\bullet}$), 214 (21), 190 (99), 189 (100), 177 (16), 162 (30), 133 (12), 59 (57). HR-MS *m/z* calcd. for $C_{14}H_{12}D_2O_4$: 248.1018, found: 248.1019.

(±)-[2",2",2",3",3",3"-$^2H_6$]-Marmesin {= (±)-(2-(1-Hydroxy-1-[$^2H_3$]-methyl-[2,2,2-$^2H_3$]-ethyl)-2,3-dihydro-7H-furo-[3,2-g]-chromen-7-one} (3d)

Transfer-hydrogenation of **6d** (0.15 g, 0.82 mmol) afforded **3d**. Yield: 0.076 g (50 %). IR [cm^{-1}]: 3483, 3042, 2972, 2928, 2221, 1706, 1631, 1571, 1486, 1446 1440, 1404, 1370, 1338, 1312, 1271, 1246, 1204, 1183, 1135, 1059, 1034, 979, 929, 835, 823. ^1H NMR ($CDCl_3$, 250 MHz) δ [ppm]: 7.58 (d, 1H-C(4)), 7.18 (s, 1H-C(5)), 6.68 (s, 1H-C(8)), 6.18 (d, 1H-C(3)), 4.72 (t, 1H-C(2')), 3.20 (m, 2H-C(3')), 2.02 (s, 1H-OH)).^{13}C NMR ($CDCl_3$, 100 MHz) δ: 163.2, 161.6, 155.6, 143.8, 125.2, 123.5, 112.8, 112.2, 97.9, 91.2, 71.4, 29.5. MS (70 eV) *m/z*: 252 (48, $M^{+\bullet}$), 216 (27), 188 (93), 187 (100), 175 (13), 160 (38), 131 (25), 65 (47). HR-MS *m/z* calcd. for $C_{14}H_8D_6O_4$: 252.1268, found: 252.1266.

(±)-[8,2",2",2",3",3",3"-$^2H_7$]-Marmesin {= (±)-(2-(1-Hydroxy-1-[$^2H_3$]-methyl-[2,2,2-$^2H_3$]-ethyl)-[9-^2H]-2,3-dihydro-7H-furo-[3,2-g]-chromen-7-one} (3e)

Transfer-hydrogenation of **6e** (0.15 g, 0.82 mmol) afforded **3e**. Yield: 0.106 g (70 %). IR [cm^{-1}]: 3480, 3040, 2924, 2223, 1694, 1626, 1565, 1471, 1437, 1253, 1134, 1043, 923, 822, 769. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.59 (d, 1H-C(4)), 7.22 (s, 1H-C(5)), 6.21 (d, 1H-C(3)), 4.73 (t, 1H-C(2')), 3.21 (m, 2H-C(3')),1.56 (s, 1H-OH)). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 163.2, 161.5 155.6, 143.7, 125.1, 123.4, 112.8, 112.3, 91.1, 71.3, 29.5. MS (70 eV) *m/z*: 253 (54, $M^{+\bullet}$) 217 (25), 189 (92), 188 (100), 176 (11), 161 (33), 132 (17), 65 (45). HR-MS *m/z* calcd. for $C_{14}H_7D_7O_4$:253.1331, found: 253.1338.

2'-(1-Hydroxyethyl)-psoralene (7)

The psoralene 7 was prepared from **5a** (0.30 g, 1.04 mmol) and but-3-in-2ol (0.15 ml, 1.94 mmol) as described for **6a**.[20] Yield: 0.191 g (80 %). IR [cm^{-1}]: 3471, 3112, 3062, 2974, 2918, 2669, 2367, 2340, 1991, 1825, 1723, 1700, 1633, 1600, 1575, 1451, 1392, 1375, 1340, 1290, 1265, 1237, 1224, 1190, 1160, 1149, 1136, 1099, 1065, 1021, 928, 888, 860, 832, 821, 754, 736, 721, 668, 619, 607; ^1H NMR ($CDCl_3$, 250 MHz) δ: 7.82 (d, 1H-C(4)), 7.65 (s, 1H-C(5)), 7.45 (s, 1H-C(8)), 6.70 (s, 1H-C(3')), 6.40 (d, 1H-C(3)), 5.05 (m, 1H-(C1")), 1.70 (d, 3H-C(2")). MS (70 eV) *m/z*: 230 (47, $M^{+\bullet}$), 216 (11), 215 (100), 213 (14), 187 (11), 149 (9), 131 (8), 43 (9). HR-MS *m/z* calcd. for $C_{13}H_{10}O_4$: 230.0579, found: 230.0558.

(±)-[2',3'-$^2H_2$]-1''-*nor*-Marmesin {= 2-(1-Hydroxy-ethyl)-2,3-dihydro-furo[3,2-g]chromen-7-one} (8)

Transfer-hydrogenation of 7 (0.17 g, 0.74 mmol) with acetone-d_6 and formic acid-d_2 as described for **3a** afforded **8**. Yield: 0.13 g (75 %). IR [cm^{-1}]: 3470, 3092, 3042, 2977, 2936, 2883, 2655, 2193, 1711, 1631, 1570, 1488, 1445, 1399, 1340, 1284, 1264, 1235, 1190, 1128, 1066, 1025, 1010, 971, 920, 888, 871, 819, 791, 772,

751, 717. ^{1}H NMR (THF-d_8, 250 MHz) δ: 7.68 (d, 1H-C(4)), 7.28 (s, 1H-C(5)), 6.60 (s, 1H-C(8)), 6.08 (d, 1H-C(3)), 4.22 and 4.15 (d (each), 1H-OH) (diastereomers), 3.91 and 3.78 (m (each), 1H-C(1")), 3.20 (s, 1H-C(3')), 1.18 and 1.15 (d (each), 3H-C(2")). ^{13}C NMR (THF-d_8, 50 MHz) δ: 164.5, 160.5, 156.8, 144.1, 125.9, 124.3, 113.3, 112.5, 97.7, 89.9 and 89.7 (diastereomers), 69.0, 30.7 and 29.5, 19.3 and 18.8. MS (70 eV) *m/z*: 234 (100, $M^{+\bullet}$), 233 (56), 207 (28), 202 (13), 201 (35), 200 (24), 190 (30), 189 (60), 188 (57), 187 (29), 177 (27), 176 (50), 175 (17), 162 (31), 161 (42), 160 (36), 159 (22), 134 (14), 133 (34), 132 (20), 69 (15), 43 (12), 40 (25). HR-MS *m/z* calcd. for $C_{13}H_{10}D_2O_4$: 234.0861, found: 234.0852.

(±)-2-Acetyl-2,3-dihydro-furo[3,2-g]chromen-7-one (10)

Pyridinium dichromate (50.0 mg, 0.13 mmol) was added to a solution of the *nor*-marmesin **8** (20.0 mg, 0.08 mmol) in dichloromethane (6.0 ml), and the suspension was stirred for 72 h at r.t. After removal of the solvent i.v. the crude product was purified by chromatography on silica gel using pentane:ethyl acetate, 1:2, v:v for elution. Yield: 18,8 mg (95 %). IR [cm^{-1}]: 3408, 3038, 2962, 2193, 2010, 1719, 1632, 1575, 1489, 1443, 1397, 1357, 1338, 1311, 1290, 1262, 1237, 1210, 1186, 1141, 1102, 1051, 1030, 1009, 985, 958, 911, 885, 871, 821, 774, 749, 717, 671, 643, 610. ^{1}H NMR (acetone-d_6, 250 MHz) δ: 7.82 (d, 1H-C(4)), 7.45 (s, 1H-C(5)), 6.78 (s, 1H-C(8)), 6.15 (d, 1H-C(3)), 3.35 (s, 1H-C(3')), 2.22 (s, 3H-C(2")). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 207.2, 162.4, 161.2, 155.9, 143.6, 123.7, 122.9, 113.4, 113.0, 98.6, 87.1, 31.4, 26.5. MS (70 eV) *m/z*: 232 (68, $M^{+\bullet}$), 231 (43), 218 (29), 217 (69), 216 (45), 190 (43), 189 (100), 188 (72), 162 (33), 161 (83), 160 (57), 133 (46), 134 (17), 132 (32), 104 (11), 78 (20), 43 (35). HR-MS *m/z* calcd. for $C_{13}H_8D_2O_4$: 232.0704 found: 232.0697.

Preparation of Microsomes from *Ammi majus* Cell Cultures.

The microsomes from cell cultures of *Ammi majus*, pre-treated with an elicitor from cell walls of the plant pathogenic fungus *Phytophthora megasperma* f. sp. *Glycinea*,[22,25] were kindly provided by Prof. Dr. Ulrich Matern, Marburg, Germany.

Biotransformations of Labelled Marmesins: General Procedure:

Samples of the labelled marmesins **3a-e** (40 nmol) in ethanol (2.0 µl) were placed in an Eppendorf micro test tube. Then, the solvent was removed in a gentle stream of argon, tris/HCl-buffer (25.0 µl of a 50.0 mM soln., pH 7.5) containing 1.0 mM EDTA, NADPH in the same buffer (10.0 µl of a 15.0 mM soln.) and the microsomal suspension (20.0 µl) were added, and the test-mixture was incubated for 30 min at 20 °C. Extraction of the products was achieved by simply inserting an SPME-fibre (polymethyldisiloxane) into the reaction mixture. Following 20 min of equilibration, the absorbed compounds were directly evaporated from the fibre within the heated injection port (250 °C) of the GLC-MS.[26] In general, ca. 5 % of the test compound were transformed into the corresponding labelled psoralene. Acetone and acetone-d_6 were analysed and quantified after derivatisation with pentafluorobenzylhydroxylamine.[23,24] To achieve a complete derivatisation, an excess of the reagent (5.0 µl of a 0.1 M soln.) in the above tris/HCl-buffer was added to the microsomal suspension prior to the extraction by SPME. The formation of the imine was complete after 30 min. According to GLC-MS equal amounts of psoralene and acetone were produced by the oxidative dealkylation of marmesin (calibration of the mass spectroscopic responses with authentic references).

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References

1. Boland, W., Feng, Z., Donath, J. in: *'Flavor Precursors'* (P. Schreier, Ed.), Allured Publ. Comp., Wheaton, IL., USA. **(1993)** 123.
2. Boland, W.; *Pure & Appl. Chem.* **1993**, *65*, 1133.
3. Akhtar, M; Wright, J. N. *Nat. Prod. Rep.* **1991**, 527.
4. Dawson, J. D.; Sono, M.; *Chem. Rev.* **1987**, *87*, 1255.
5. Cole, P. A.; Robinson, C. H.; *J. Med. Chem.* **1990**, *33*, 2933.
6. Lee-Robichaud, P.; Shyadehi, A. Z.; Wright, J. N.; Akhtar, M. E.; Akhtar, M.; *Biochemistry* **1995**, *34*, 14104.
7. Shyadehi, A. Z.; Lamb, D. C.; Kelly, S. L.; Kelly, D. E.; Schunck, W.-H.; Wright, J. N.; Corina, D.; Akhtar, M.; *J. Biol. Chem* **1996**, *271*, 12445.
8. Fischer, R. T.; Trzaskos, J. M.; Magolda, R. L.; Ko, S. S.; Brosz, C. S.; Larsen, B.; *J. Biol. Chem.* **1991**, *266*, 6124.
9. Mak, A. Y.; Swinney, D. C.; *J. Am. Chem. Soc.* **1992**, *114*, 8309.
10. Akhtar, M.; Wright, J. N.; Shyadehi, A. Z; Robichaud, P.; *Pure & Appl. Chem.* **1994**, *66*, 2387.
11. Lee-Robichaud,P.; Wright, J. N.; Akhtar, M; *J. Chem. Soc., Chem. Commun.* **1994**, 1501.
12. Roberts, E. S.; Vaz, A. D. N.; Coon, M. J.; *Proc. Natl. Acad. Sci* **1991**, *88*, 8963.
13. Wendorff, H.; Matern, U.; *Eur. J. Biochem.* **1986**, *161*, 391.
14. Murray, R. D. H.; Méndez, J.; Brown, S. A.; *The Natural Coumarins* John Wiley & Sons LTD, Chichester, 1982.
15. Scheel, L. D.; Perone, V. B.; Larkin, R.; Kupel, R. E., *Biochemistry* 1963, *2*, 1127.
16. Kanne, D.; Straub, K. , Rapoport, H.; Hearst, J. E., *Biochemistry* **1982**, *21*, 861.
17. Zangerl, A. R.; Berenbaum, M. R.; *Ecology* **1990**, *71*, 1933.
18. Johnson, C.; Brannon, D. R.; Kuc, J.; *Phytochemistry* **1973**, *12*, 2961.
19. R.C. Beier, E.H. Oertli, *Phytochemistry* **1983**, *22*, 2595.
20. Zubía, E.; Luis, F. R.; Massanet, G. M.; Collado, I. G., *Tetrahedron* **1992**, *48*, 4239.
21. Doad, G. J. S.; Barltop, J. A.; Petty, C. M.; Owen, T. C.; *Tetrahedron Lett.* **1989**, *30*, 1597.
22. Hamerski, D.; Beier, R. C.; Kneusel, R. E.; Matern, U.; Himmelspach, K.; *Phytochemistry* **1990**, *29*, 1137.
23. Cancilla, D. A.; Que Hee, S. S.; *J. Chromatogr.* **1992**, *627*, 1.
24. Glaze, W. H.; Koga, M.; Cancilla, D.; *Environ. Sci. Technol.* **1989**, *23*, 838.
25. Stanjek, V.; Miksch, M.; Lüer, P.; Matern U.; Boland, W.; *Angew. Chem.*, submitted.
26. Pawliszyn, J.; Yang, M. J.; Zhang, Z.; *Z. Anal. Chem.* **1994**, *66*, 844.

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