Biosynthesis of Angular Furanocoumarins: Mechanism and Stereochemistry of the Oxidative Dealkylation of Columbianetin to Angelicin in *Heracleum mantegazzianum* (Apiaceae)

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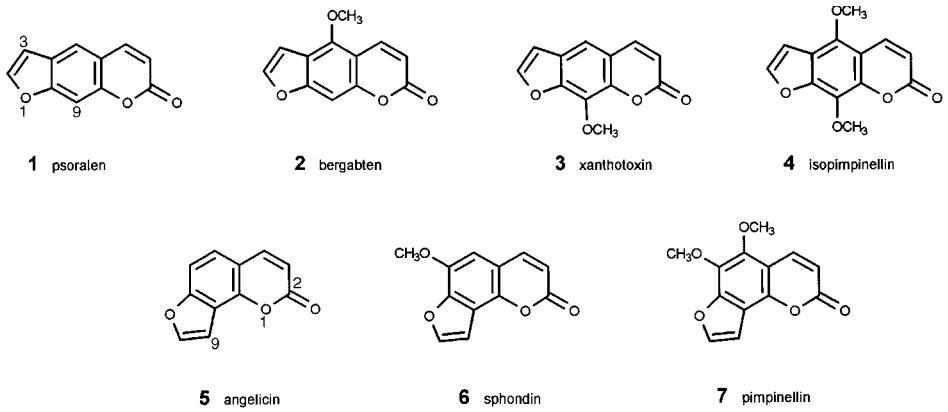
Deuterium-labelled 5-fluorocolumbianetin **13** was synthesized as a metabolic probe to examine the stereochemical course of the bioconversion of (+)-columbianetin (**12**) into the angular furocoumarin angelicin (**5**). In leaves of *Heracleum mantegazzianum*, oxidative dealkylation of the specifically deuterated fluorocolumbianetin **13** proceeded by *syn*-elimination of a D-atom, from C(9), and the vicinal 1-hydroxy-1-methylethyl substituent, yielding 5-fluoroangelicin (**23**). This matches the stereochemical course of the related reaction converting (+)-marmesin (**10**) into the linear furocoumarin psoralen (**1**). Key steps in the synthesis of 5-fluorocolumbianetin (**13**) were the copper-catalysed alkynylation/cyclization of 5-fluoro-8-iodoumbelliferone (**15**) followed by a transfer hydrogenation, which established the *cis*-orientation of the D-Atom and the 1-hydroxy-1-methylethyl substituent.

1. Introduction. – Plants have evolved many traits, both physical and chemical, that protect them from damage by pathogens and herbivores. Unlike physical traits like spines, chemical defences may be either constitutively present or inducible. Induced responses to herbivory may be localized, restricted to a small area around the damage site, or systemic, causing effects far distant from the original injury [1]. One of the most intensively studied constitutive and/or inducible chemical defenses is the production of furocoumarins, *e.g.*, **1**–**7**. Furocoumarins (= furanocoumarins) represent a large class of compounds with widespread occurrence in the Apiaceae and Rutaceae [2]. They are typical phytoalexins, being (photo)toxic to a wide variety of organisms, including bacteria, fungi, insects, and mammals [3]. Furocoumarin biosynthesis can be induced by insect herbivory [4], infection with pathogens [5], light stress [6], and even by airborne methyl jasmonate [7]. In some plants such stimulation not only increases the amount of furocoumarins within the leaf tissue, but also leads to higher titres on the leaf surface [8]. Contact with induced leaves causes blistering and erythrema, accompanied by allergic reactions, well-known occupational diseases during the harvesting of celery, parsley, or parsnip [9]. All furocoumarins are able to intercalate with DNA, but only linear furocoumarins of type **1**–**4** will cross-link the single-strands, by forming [2 + 2]-photoadducts between appropriately located nucleobases [10].

Unlike the widely distributed linear furocoumarins, in which the furan moiety is attached to the atoms C(6) and C(7) of the 1-benzopyran-2-one moiety, angular furocoumarins like, *e.g.*, angelicin (**5**) and sphondin (**6**) are much less common. In many plants, they are found as minor components in mixtures dominated by linear furocoumarins. Since no plants are known which exclusively produce angular furocoumarins, their biosynthesis may be considered as a more recently evolved trait. The naturally occurring mixtures of both linear and angular furocoumarins are more



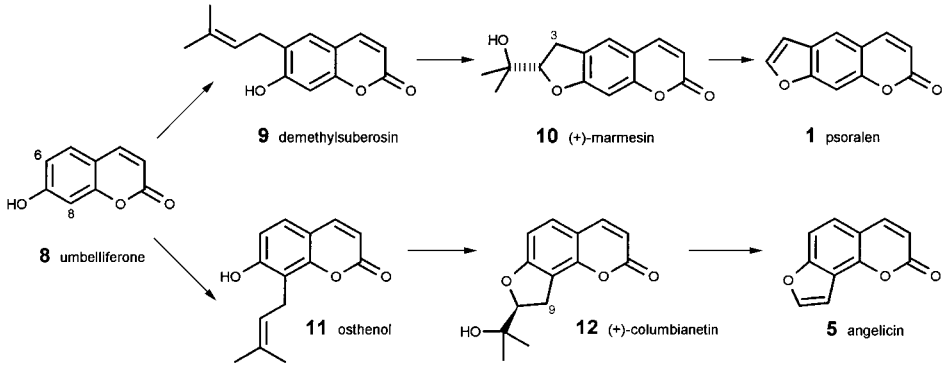
toxic to herbivorous insects, especially in the absence of light, than pure psoralen (**1**) or xanthotoxin (**3**) [3][11]. As shown recently, this synergistic effect is apparently due to a strong inhibition of the detoxifying P450-type enzymes of the insects by the angular furocoumarins [3][12]. Thus, plants which produce mixtures of both types of furocoumarins are generally better protected against even those herbivores which are adapted to plants containing large amounts of linear furocoumarins. For example, angelicin seriously interferes with the growth and reproduction of the black swallowtail (*Papilio polyxenes*), a specialist that is adapted to feed on plants containing the linear furocoumarin xanthotoxin (**3**) [13].

These findings suggest that the biosynthesis of angular furocoumarins may have evolved from the pathway leading to linear furocoumarins in response to selection pressure by herbivores [3][14]. The required diversification of the primordial pathway could easily have been achieved by selection of an enzyme catalysing the prenylation of umbelliferone (**8**) at C(8), instead of C(6) as is the case in the biosynthesis of the linear furocoumarins (*Scheme 1*).

By subsequent evolutionary optimization of the other enzymes of the ancient pathway, a new set of biocatalysts, adapted to the angular regioisomers, may have developed. Accordingly, both pathways share the same type of intermediates like **9** and **11**, or **10** and **12**, and in both pathways, the unfunctionalized parent systems **1** and **5** are further modified by oxidations and methylations [15]. Although several enzymes from the linear furocoumarin pathway have been isolated and studied in detail [16], almost nothing is known about the corresponding enzymes from the angular furocoumarin pathway. A most remarkable transformation in the biosynthesis of both types of furocoumarins is the oxidative dealkylation of (+)-marmesin (**10**) or (+)-columbianetin (**12**). In the case of the psoralen (**1**) biosynthesis, the reaction is catalysed by a cytochrome P450 and requires molecular oxygen and NADPH as cofactors [17]. Recently, the mechanism of this bioconversion was established as a single-step transformation cleaving (+)-marmesin (**10**) into psoralen (**1**) and acetone [18] [19].

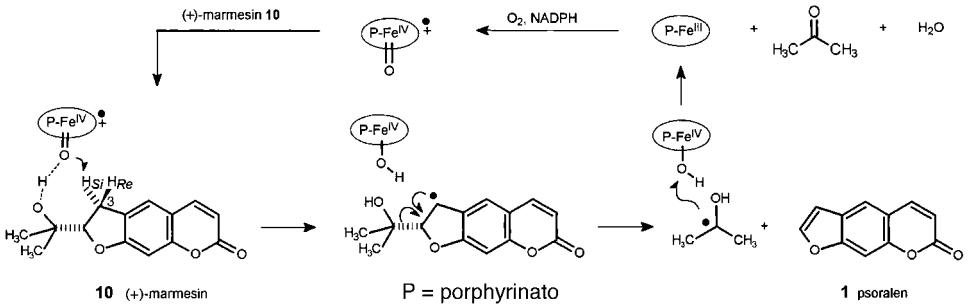
As outlined in *Scheme 2*, the H_{Si} atom at C(3) of (+)-marmesin (**10**) is attacked by the electrophilic $[Fe^{IV}(porphyrinato)(=O)]^{\cdot+}$ species, and a benzylic radical is formed. The reactive intermediate is stabilized by β-cleavage generating psoralen (**1**) and a 1-hydroxy-1-methylethyl radical. Subsequent reaction with neighbouring $[Fe^{IV}$(porphyr-

Scheme 1. *Biosynthesis of the Linear Furocoumarin Psoralen* (**1**) *and the Angular Furocoumarin Angelicin* (**5**) *from the Common Precursor Umbelliferone* (**8**)



inato)(OH)] yields acetone, presumably *via* a geminal diol, together with the reduced cytochrome catalyst. Although the dealkylation of (+)-columbianetin **12**) is formally related, no mechanistic or stereochemical information is available yet. We now report *i*) the synthesis of D-labelled 5-fluorocolumbianetin **13**, a valuable metabolic probe for the mechanistic analysis of angular furocoumarin biosynthesis, and *ii*) the first data on the stereochemical course of oxidative dealkylation of columbianetin to angelicin in leaves of *Heracleum mantegazzianum*.

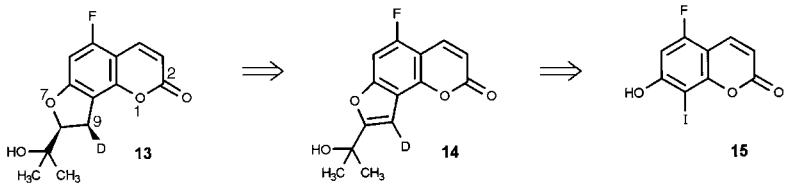
Scheme 2. *Mechanistic Rationale for the P450-Catalysed Dealkylation of (+)-Marmesin* (**10**) *to Psoralen* (**1**) *and Acetone*



Results and Discussion. – 1. *Synthesis of* cis-*5-Fluoro(9-D)columbianetin* (**13**) *as a Metabolic Probe.* While mechanistic studies towards linear furocoumarins can rely on microsomal preparations from well-established cell cultures of several Apiaceae, in particular *Ammi majus* [17] [18], no such system is available for the production of angular furocoumarins. Therefore, the experiments and the metabolic probe(s) were designed for the use with intact plants. To secure identification of the expected metabolites, undiluted by the large amounts of natural furocoumarins, we decided to use a C(5)-fluorinated columbianetin **13** as a metabolic probe. Due to the similar size of F- and H-atoms, space filling of the probe and the natural precursor will be almost identical; negative effects from the electron-withdrawing substituent are not expected to be strong enough to prevent the oxidative dealkylation. To gain insight into the

stereochemistry of the transformation, the precursor has to be additionally labelled by a D-atom at C(9) in a configurationally defined manner, either *cis* or *trans* to the hydroxy(methyl)ethyl substituent. These structural demands are easily fulfilled following a synthetic route developed previously for the preparation of configurationally defined, deuterium-labelled (±)-marmesins [19]. As outlined in *Scheme 3*, exclusive *cis*-orientation of the D-atom at C(9) and the side chain, as in **13**, is feasible *via* transfer hydrogenation of **14** in the presence of Pd/C using formate as a H_2 source [20].

Scheme 3. retro-*Synthetic Route to 5-Fluoro-8-iodoumbelliferone* (**15**)



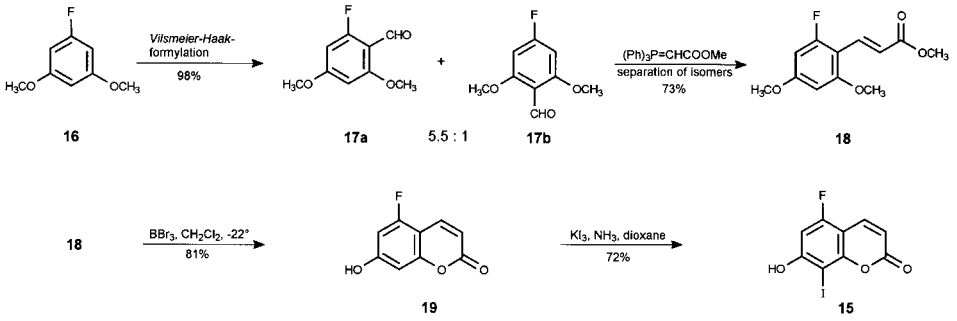
The substituted furocoumarin **14** should be accessible from dihalide **15** by a copper-catalysed alkynylation with 2-methylbut-3-yn-2-ol [20]. Thus, only the synthesis of the dihalide remained to be developed.

Although direct fluorination of the readily available 8-iodoumbelliferon [21] should be possible, in principle, the dihalide **15** was synthesized utilizing the protocol shown in *Scheme 4*. The symmetric precursor **16** already containing the F-atom was converted to **13** in only a few steps and high overall yield. Dangerous manipulations with elemental F_2 or unstable fluorinating reagents were, thus, not required.

Dissymmetrization of commercial **16** was achieved by a *Vilsmeier-Haak* formylation, yielding the two regioisomeric aldehydes **17a** and **17b** in almost quantitative yield. Due to the lower steric hindrance next to the F-atom, the desired regioisomer **17a** was formed in excess (**17a**/**17b** 5.5 : 1). Since it proved difficult to separate the two regioisomers by column chromatography, the mixture was directly subjected to a *Wittig-Horner* olefination. The reaction proceeded virtually quantitatively, and after chromatographic separation (SiO_2), the configurationally pure (*E*)-ester **18** was obtained in 73% overall yield. Subsequent ether cleavage with BBr_3 proceeded instantaneously and was accompanied by an (*E*) → (*Z*) isomerization of the acrylic side chain with the (*Z*)-isomer being trapped by cyclization to the 5-fluoroumbelliferone (**19**) in a single operation. Introduction of the I-atom at C(8) was achieved with exceptionally high positional preference using I_2 and aqueous ammonia in dioxan. Following the sequence of *Scheme 4*, the required 5-fluoro-8-iodoumbelliferone (**15**) was obtained in *ca.* 40% overall yield from **16**.

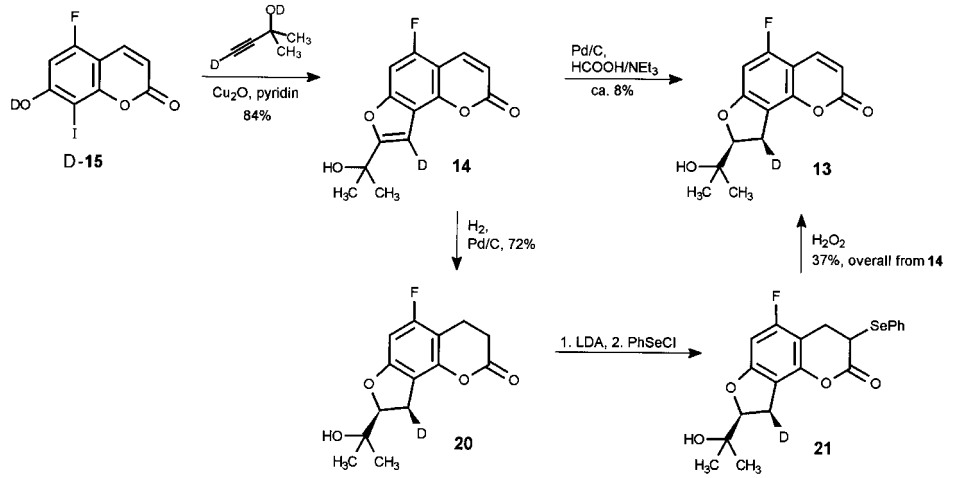
The *C*-skeleton of 5-fluorocolumbianetin **13** was completed by copper-catalysed alkynylation of **15** with deuterium-labelled 2-methylbut-3-yn-2-ol as depicted in *Scheme 5*. The introduction of a D-atom at C(9) of **14** was achieved by employing an alkynol possessing a D-atom at the terminal C-atom and at the hydroxy group. Moreover, a high degree of D-labelling (> 98%) at C(9) of **14** required an absolute solvent (pyridine), a rigorous exclusion of moisture, and a previous exchange of the phenolic proton of **15** by a D-atom (by stirring with MeOD in the presence of a catalytic amount of DCl) [19]. The final transfer hydrogenation placed the two H-atoms in a *cis*-

Scheme 4. *Synthesis of 5-Fluoro-8-iodoumbelliferone* (**15**)



fashion across the C=C bond of the furan moiety [19] [20]. However, the yields were low (*ca.* 5–8%) and could not be optimized, since the reduction of the unsaturated lactone proceeded faster. To overcome the low yield, both C=C bonds were reduced yielding **20**. Then, the unsaturated lactone substructure was regenerated by successive deprotonation and selenenylation of **20** with lithium diisopropylamide and phenylselenenyl chloride [22]. Oxidation of the selenide with H_2O_2 and final elimination of phenylselenenic acid provided **13** in 37% overall yield from **14**.

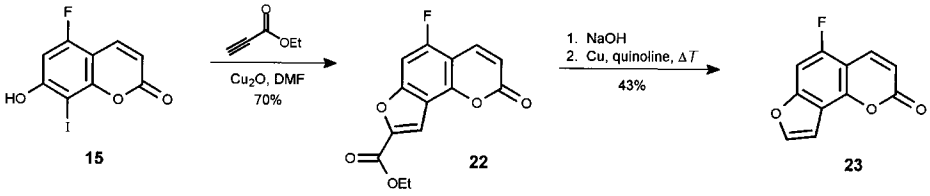
Scheme 5. *Synthesis of Stereospecifically Deuterated Fluorocolumbianetin* **13** *as a Metabolic Probe for Analysis of the Stereochemical Course of Angular Furocoumarin Biosynthesis*



Fluorinated angelicin **23**, required as an authentic reference for the biotransformation of the *cis*-5-fluoro(9-D)columbianetin (**13**) was synthesized along a similar protocol (*Scheme 6*). Key step of the sequence was the copper-catalysed decarboxylation of the readily available angelicin-type ester **22** [21]. Thus, copper-catalysed alkynylation of **15** with ethyl propiolate furnished ester **22**. Hydrolysis and thermal decarboxylation of the resulting acid for 1 h in quinoline/copper at 210° afforded the required 5-fluoroangelicin (**23**) in 43% yield.

2. *Incubation Experiments with Deuterium-Labelled 5-Fluorocolumbianetin* **13**. The labelled *cis*-5-fluoro(9-D)columbianetin (**13**), *Triton X 100*, and jasmonic acid

Scheme 6. *Synthesis of 5-Fluoroangelicin* (**23**)



(required for elicitation of the biosynthesis of the furocoumarins [8]) were dispersed in tap water and sonicated until a stable emulsion resulted. Freshly detached leaves of two- to three-month-old plants of *Heracleum mantegazzianum* were immediately placed into the emulsion. After five days, the nonpolar surface compounds including the furocoumarins and the metabolites of **13** were collected from the leaf surface by briefly dipping the leaves in CH_2Cl_2. The constituents were analysed by GLC/MS and identified by comparison with reference compounds [8]. Analysis of the surface lipids was preferred, because of the ease of extraction and the simplicity of the compound spectrum, compared to that of corresponding leaf-tissue extracts.

Alternatively, the leaf surfaces of young plants (two- to three-week-old) of *H. mantegazzianum* were repeatedly sprayed with the above emulsion at four-day intervals. After 3–4 weeks, the surface lipids were collected as described above, and the constituents were analysed by GLC/MS. Unlike the direct administration experiment which resulted in *ca.* 50% mortality of the treated plants, surface application had no visible negative impact on the plants.

As a matter of fact, both application modes resulted in the formation of 5-fluoroangelicin **23** from the administered 5-fluorocolumbianetin, albeit in very low yield. About 0.5–1% of the total angelicin isolated from the surface of the leaves proved to be 5-fluoroangelicin (**23**). However, as shown in *Fig. 1*, the mass spectra of the metabolite and the synthetic reference were fully congruent, as were their retention times.

The molecular ion of the metabolite at *m/z* 204 Da indicated complete loss of D-labelling and, hence, the oxidative dealkylation of *cis*-5-fluoro(9-D)columbianetin (**13**) had proceeded exclusively by *syn*-elimination. This observation was of great importance, since exclusive removal of the D-atom, despite a potential isotope effect (*vide infra*), characterized the metabolite as a product of an enzymatic transformation. Moreover, *syn*-elimination of D–C(9) and the 1-hydroxy-1-methylethyl substituent is in perfect agreement with the stereochemical course of the corresponding oxidative dealkylation of (+)-marmesin **10** to the linear furocoumarin psoralen (**1**) [18] [20]. Thus, it is reasonable to assume that the oxidative degradation of (+)-columbianetin (**12**) to angelicin (**5**) is also catalysed by a cytochrome P450, similar to that involved in the biosynthesis of psoralen (**1**).

3. *Biomimetic Dealkylation of 5-Fluoro(9-D)columbianetin.* Recently, we described the first chemical model system for the oxidative dealkylation of marmesin to psoralen using (tetraphenylporphyrinato)manganese and iodosylbenzene [18]. This reagent combination has been frequently used to simulate cytochrome P450 catalysed

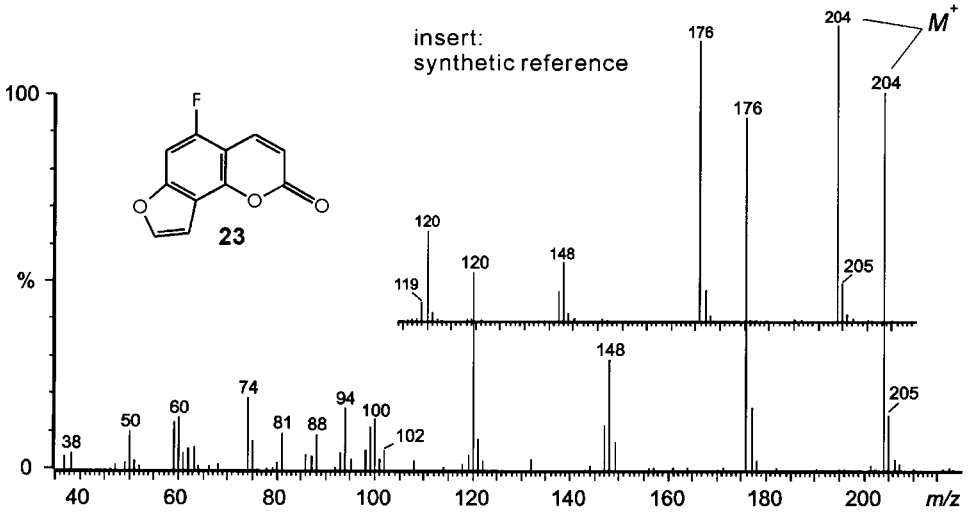


Fig. 1. *Mass spectrum of fluoroangelicin* **23** *resulting from the enzymatic transformation of deuterated fluorocolumbianetin* **13**. Insert: molecular ion and first fragments of the synthetic reference. Both spectra are congruent, *i.e.*, the vicinal *cis*-arranged D-atom removed from C(9) of **13** during the oxidative dealkylation.

oxygenation reactions [23]. Although only minor amounts (< 1%) of the D-labelled 5-fluorocolumbianetin **13** were dealkylated to 5-fluoroangelicin **23**, the mass spectrum of the product clearly indicated that in this case, unlike in the enzymatic conversion (see above), the *trans*-arranged H-atom was preferentially removed, leading to a product carrying a D-atom (70% D–C(9); see *Fig. 2*). The rather low conversion rate of **13** was largely due to the electronic effect of the F-substituent, since the corresponding non-fluorinated columbianetin was converted to angelicin in much higher yield (*ca.* 10%).

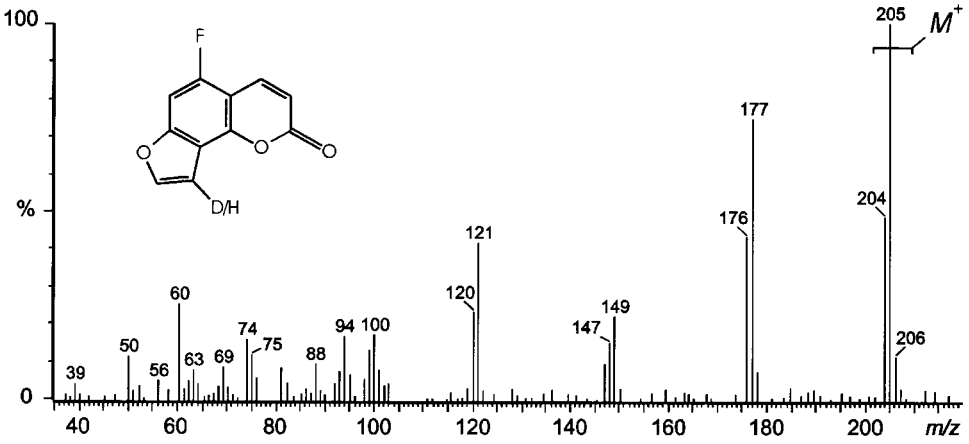


Fig. 2. *Mass spectrum of fluoroangelicin* **23** *resulting from the biomimetic dealkylation of fluorocolumbianetin* **13** *with (tetraphenylporphyrinato)manganese(III) and iodosobenzene.* The isotopomer of the deuterated fluoroangelicin prevails, indicating a predominant loss of the *trans*-arranged H-atom at C(9) of **13**.

On the other hand, the amount of *syn*-elimination (*ca.* 30%) in the biomimetic dealkylation of **13** was remarkably high and may be due to a preorientation of the porphyrinato system and the 5-fluorocolumbianetin *via* a H-bond between the substrate and the [Mn^{V}(porphyrinato)(=O)] complex as outlined in *Scheme 2*. In the enzymatic transformation, the facial arrangement of the reactive [Fe^{IV}(porphyrinato)(=O)]$^{+}$ complex is responsible for the observed complete *syn*-elimination of the H_{Si} atom at C(9) and the hydroxyalkyl substituent. This peculiar arrangement at the same time prevents the escape of long-lived reactive radicals from the active centre, since the close, cage-type arrangement of all interacting species secures almost immediate trapping of the ensuing radicals.

Conclusions. – The stereochemical course of the bioconversion of (+)-columbianetin (**12**) into angelicin (**5**), as shown above, is identical to the stereochemical course of the related conversion of (+)-marmesin (**10**) into the linear furocoumarin psoralen (**1**) [18]. In both cases, the oxidative dealkylation proceeds by *syn*-elimination of an H-atom and a vicinal 1-hydroxy-1-methylethyl substituent (*cf. Scheme 1*). Although the present study was performed with intact plants, which did not allow for a direct analytical proof of the acetone fragment, its formation is reasonable because of the perfect stereochemical analogy with psoralen synthase [18]. As with psoralen synthase, angelicin synthase catalyses the oxidative dealkylation of stereospecifically deuterated columbianetin **13** against a potential kinetic isotope effect with a quantitative loss of the vicinal *cis*-located D-atom. This is especially remarkable, since the biomimetic dealkylations with D-labelled marmesin and (tetraphenylporphyrinato)-manganese, activated by iodosylbenzene, exhibit a kinetic isotope effect of $k_H/k_D \approx 4$ ($T = 20°$) [18] suggesting that the removal of the H radical from C(3) is rate-contributing (*cf. Scheme 2*).

The perfect mechanistic and stereochemical analogies between the two pathways leading to linear and angular furocoumarins strongly support the idea that the pathway to angular furocoumarins indeed originated from a primordial route to linear furocoumarins [24]. Considering the negative impact of angelicin on the detoxification of linear furocoumarins [13] in insects as an important evolutionary driving force, accidental appearance of angular furocoumarins may have been selected immediately as a trait for chemical protection and may explain its subsequent phylogenetic inertia.

Although today's prenyl transferases are enzymes of high regiospecificity [24], it is tempting to speculate that a simple switch of the selectivity of the prenylation of umbelliferone from C(6) to C(8) has initiated the evolutionary shift from linear to angular furocoumarins. Probably due to a certain structural tolerance of the ancient enzymes processing the compounds downstream of demethylsuberosin (**9**; *Scheme 1*), also osthenol (**11**) may have been further processed to give angelicin (**5**), albeit with low efficiency. However, subsequent evolutionary adaptation of the enzymes may have created a new set of biocatalysts optimized to the molecular architecture of the angular compounds. The current work supports such an evolutionarily driven development in as much as the stereochemical and mechanistic aspects of the crucial oxidative dealkylation are identical in both pathways. However, further comparative studies on the enzymes of these two pathways are urgently needed to establish more parallels between the transformations and their biocatalysts.

Experimental Part

General. Angelicin (**5**) was purchased from *Roth*, Karlsruhe, Germany. Reactions were performed under Ar; solvents were dried according to standard procedures. Column chromatography (CC): silica gel *Si60* (0.200–0.063 mm; *E. Merck*, Darmstadt, Germany). TLC: Silica-gel plates *Polygram Sil* G_{F254}, from *E. Merck*. GLC: *Carlo Erba,* series *4100*, equipped with fused silica capillaries coated with *SE30* (15 m × 0.31 mm) from *Macherey & Nagel* (Düren, Germany). IR: *Perkin-Elmer-1600-FTIR* spectrophotometer, ν in cm^{-1}. ^1H- and ^{13}C-NMR (^1H-decoupled): *Bruker-AC-250* or *Bruker-AC-400* spectrometer; $CDCl_3$ or (D_6)acetone as solvent, chemical shifts δ in ppm downfield from $SiMe_4$ (= 0 ppm), *J* in Hz. GLC/MS (70 eV): *Finnigan ITD 800* coupled with a *Carlo Erba GC 6000*, model *Vega* or *Fisons MD 800* GLC-MS system. HR-MS: *Kratos MS 50*.

2-Fluoro-4,6-dimethoxybenzaldehyde (**17a**) *and 4-Fluoro-2,6-dimethoxybenzaldehyde* (**17b**). $POCl_3$ (1.8 ml, 19.3 mmol) was added slowly to a well-stirred mixture of 1-fluoro-3,5-dimethoxybenzene (2.6 ml, 19.25 mmol) and *N*-methylformanilid (2.5 ml, 20 mmol) while the temp. was kept below −5°. Stirring was continued at r.t. for 1.5 h and at 60° for another 2 h. Then, the chilled mixture was hydrolysed with ice-water (60.0 ml), and the resulting suspension neutralized by addition of 5N aq. NaOH. Following extraction with AcOEt (2 × 30 ml), the aq. phase was adjusted to pH *ca.* 10 by 5N NaOH and reextracted with AcOEt (2 × 30 ml). The combined org. phase was washed with sat. aq. $NaHCO_3$ soln. (30 ml) and brine (10 ml), dried ($MgSO_4$), and evaporated. CC (silica gel, hexane/AcOEt 1:1) afforded a colourless solid consisting of **17a/b** (5.5:1) which were separated only for anal. purposes. Yield of **17a/b**: 3.47 g, 98%.

Data of **17a**. M.p. 86–87°. IR (KBr): 2861, 2784, 1679, 1628, 1572, 1478, 1457, 1410, 1344, 1311, 1208, 1157, 1095, 824, 802. ^1H-NMR ($CDCl_3$, 400 MHz): 10.26 (*d*, *J* = 1.2, CHO); 6.25 (*dd*, *J* = 12.8, 2.2, H–C(3)); 6.25 (*J* ≈ 2.0, H–C(5)), 3.89 (*s*, 1 MeO); 3.85 (*s*, 1MeO). ^{13}C-NMR ($CDCl_3$, 100 MHz): 185.8 (*d*, *J* = 3.1, CHO); 166.0 (*d*, *J* = 15.2, C(4)); 165.2 (*d*, *J* = 260.6, C(2)); 163.3 (*d*, *J* = 8.2, C(6)); 108.1 (*d*, *J* = 9.2, C(1)); 94.3 (*d*, *J* = 3.6, C(5)); 94.0 (*d*, *J* = 25.3, C(3)); 56.2 (*s*, MeO); 55.9 (*s*, MeO). MS (70 eV): 184 (100, $M^{+\cdot}$), 183 (71), 169 (10), 168 (22), 167 (56), 166 (37), 155 (13), 153 (40), 140 (20), 139 (37), 138 (34), 127 (12), 124 (25), 112 (10), 110 (13), 97 (13), 95 (10), 82 (12), 81 (13), 63 (20). HR-MS: 184.0535 ($C_9H_9FO_3^+$; calc. 184.0535).

*Methyl (*E*)-3-(2′-Fluoro-4′,6′-dimethoxyphenyl)prop-2-enoate* (**18**). A stirred soln. of **17a/b** (3.4 g, 18.5 mmol) in MeCN (200.0 ml) was treated with methyl(triphenylphosphoranylidene)acetate (8.0 g, 23.9 mmol) at r.t. The resulting suspension was kept at 55–60° for 12 h. After cooling, AcOEt (200 ml) was added and the org. layer washed with a sat. $NaHCO_3$ soln. (100 ml). The aq. phase was reextracted with AcOEt (2 × 50 ml) and the combined org. extract dried ($MgSO_4$) and evaporated. The regioisomer mixture was submitted to CC (silica gel, hexane/AcOEt 2.5:1): 3.25 g (73%) of **18** as a colourless solid. M.p. 76°. IR (KBr): 2951, 1711, 1613, 1431, 1314, 1293, 1194, 1170, 1090, 992, 858, 809. ^1H-NMR ($CDCl_3$, 400 MHz): 7.79 (*d*, *J* = 16.2, H–C(3)); 6.59 (*d*, *J* = 16.3, H–C(2)); 6.16–6.20 (*m*, H–C(3′), H–C(5′)); 3.79 (*s*, 1 MeO); 3.73 (*s*, 1 MeO); 3.71 (*s*, 1 MeO). ^{13}C-NMR ($CDCl_3$, 100 MHz): 168.6 (*s*, C(1)); 163.6 (*d*, *J* = 250.0, C(2′)); 162.4 (*d*, *J* = 17.2, C(4′)); 160.6 (*d*, *J* = 8.9, C(6′)); 133.4 (*d*, *J* = 3.3, C(3)); 119.0 (*d*, *J* = 10.2, C(2)); 105.4 (*d*, *J* = 13.6, C(1′)); 94.5 (*d*, *J* = 3.0, C(5′)); 93.6 (*d*, *J* = 27.4, C(3′)); 55.9 (*s*, ArOMe); 55.6 (*s*, ArOMe); 51.5 (*s*, CO_2Me). MS (70 eV): 240 (63, $M^{+\cdot}$), 209 (100), 194 (21), 180 (9), 179 (9), 167 (29), 166 (27), 151 (16), 139 (14). HR-MS: 240.0800 ($C_{12}H_{13}FO_4^+$; calc. 240.0798).

5-Fluoroumbelliferone (= *5-Fluoro-7-hydroxy-2*H*-1-benzopyran-2-one;* **19**). A stirred soln. of **18** (3.1 g, 12.9 mmol) in CH_2Cl_2 (250 ml) at −22° was gradually treated with 1M BBr_3 in hexane (110 ml). After 2 h, the mixture was slowly allowed to come to r.t., and after 36 h at r.t., the red soln. was hydrolysed with ice-water (100 ml). The aq. layer was extracted with AcOEt (3 × 70 ml), the combined org. extract washed with sat. aq. $NaHCO_3$ soln. (50 ml) and brine, dried ($MgSO_4$), and evaporated, and the residue submitted to CC (silica gel, hexane/AcOEt 1:1): 1.88 g (81%) of **19**. Colourless solid. M.p. 245–246°. IR (KBr): 3191 (br.), 1700, 1629, 1569, 1466, 1394, 1334, 1286, 1246, 1157, 1113, 1059, 899, 837, 705, 657. ^1H-NMR ((D_6)acetone, 400 MHz): 9.74 (br. *s*, OH); 7.81 (*d*, *J* = 9.7, H–C(4)); 6.47–6.52 (*m*, H–C(6), H–C(8)); 6.10 (*d*, *J* = 9.7, H–C(3)). ^{13}C-NMR ((D_6)acetone, 100 MHz): 162.5 (*d*, *J* = 13.9, C(7)); 160.4 (*d*, *J* = 251.0, C(5)); 160.4 (*s*, C(2)); 157.2 (*d*, *J* = 7.4, C(8a)); 137.2 (*d*, *J* = 3.9, C(4)); 113.3 (*s*, C(3)); 102.8 (*d*, *J* = 19.8, C(4a)); 100.1 (*d*, *J* = 23.16, C(6)); 99.9 (*d*, *J* = 3.0, C(8)). MS (70 eV): 180 (82, $M^{+\cdot}$), 152 (100), 124 (10), 123 (17), 96 (43), 95 (13), 83 (8), 75 (16), 69 (11). HR-MS: 180.0231 ($C_9H_5FO_3^+$; calc. 180.0223).

5-Fluoro-8-iodoumbelliferone (= *5-Fluoro-7-hydroxy-8-iodo-2*H*-1-benzopyran-2-one;* **15**). A soln. of **19** (1.00 g, 5.56 mmol) in dioxan (9.0 ml) was treated with 20% aq. NH_3 soln. (25.0 ml). Then, a soln. of I_2 (1.6 g, 6.10 mmol) and KI (2.5 g, 15 mmol) in H_2O (38.0 ml) was slowly added with stirring at 0°. After 1 h, the chilled mixture was slightly acidified with 2.5N H_2SO_4 and extracted with AcOEt (1 × 80 ml, 2 × 30 ml). The org. layer was washed with sat. aq. $NaHCO_3$ soln. (50 ml) and brine (20 ml), dried ($MgSO_4$), and evaporated and the

beige solid purified by CC (silica gel, hexane/AcOEt 1:1): 1.21 g (72%) of **15**. M.p. 220° (dec.). IR (KBr): 3200 (br.), 1705, 1618, 1561, 1503, 1436, 1387, 1326, 1240, 1172, 1115, 1065, 929, 839, 823, 751, 712, 686, 656. ^{1}H-NMR ((D$_6$)acetone, 400 MHz): 10.55 (br. *s*, OH); 7.94 (*d*, *J* = 9.5, H–C(4)); 6.83 (*d*, *J* = 10.6, H–C(6)); 6.29 (*d*, *J* = 9.4, H–C(3)). ^{13}C-NMR ((D$_6$)acetone, 100 MHz): 161.8 (*d*, *J* = 13.4, C(7)); 160.7 (*d*, *J* = 252.1, C(5)); 160.1 (*s*, C(2)); 156.6 (*d*, *J* = 8.3, C(8a)); 137.2 (*d*, *J* = 3.7 C(4)); 113.8 (*d*, *J* = 1.3, C(3)); 103.6 (*d*, *J* = 19.6, C(4a)); 99.1 (*d*, *J* = 23.5, C(6)); 68.7 (*d*, *J* = 3.9, C(8)). MS (70 eV): 306 (100, $M^{+\cdot}$), 278 (57), 151 (16), 123 (23), 95 (12), 94 (13), 75 (14). HR-MS: 305.9195 ($C_9H_4FIO_3^+$; calc. 305.9189).

2-Methyl(4-^{2}H)but-3-yn-2-(^{2}H)ol. A soln. of 2-methylbut-3-yn-2-ol (0.75 ml, 7.73 mmol) in Et$_2$O (25.0 ml) was slowly added with stirring to EtMgBr (22.5 mmol) in Et$_2$O (22.5 ml). The mixture was refluxed for 1 h, chilled, and hydrolysed with ^{2}H$_2$O (2.5 ml) and conc. ^{2}HCl (1.0 ml). Extractive workup with Et$_2$O and evaporation afforded the crude 2-methyl(4-^{2}H$_2$)but-3-yn-2-(^{2}H)ol which was used for the copper-catalysed alkynylation/cyclization without further purification to avoid loss of the isotopes.

5-Fluoro-8-(1-hydroxy-1-methylethyl)(9-^{2}H)angelicin (= *5-Fluoro-8-(1-hydroxy-1-methylethyl)(9-^{2}H)-2*H-*furo[2,3-*h*]-1-benzopyran-2-one;* **14**). A suspension of **15** (0.75 g, 2.45 mmol) in MeO^{2}H (15 ml) was treated with one drop of conc. ^{2}HCl soln. and refluxed until a clear soln. was obtained. Then, the solvent was evaporated and the remaining solid dissolved in absolutely dry pyridine (12 ml). Then, 2-methyl(4-^{2}H)but-3-yn-2-(^{2}H)ol (7.73 mmol), copper(I) oxide (0.30 g, 2.1 mmol), and ^{2}H$_2$O (0.10 ml) were added and refluxed for 2 h. After cooling to r.t., the mixture was filtered through a small pad of silica gel, diluted with AcOEt (50 ml), and acidified with 2N HCl, the org. phase washed with sat. aq. NaHCO$_3$ soln. (50 ml) and brine (10 ml), dried (MgSO$_4$), and evaporated, and the residue submitted to CC (silica gel, pentane/AcOEt 1:1): 0.54 g (84%) of **14**. Faintly yellow solid. Deuterium incorporation: > 98%. M.p. 148–149°. IR (KBr): 3488, 3056, 2984, 1711, 1628, 1570, 1465, 1371, 1330, 1290, 1259, 1196, 1121, 1018, 976, 934, 882, 835, 771. ^{1}H-NMR (CDCl$_3$, 400 MHz): 7.93 (*d*, *J* = 9.8, H–C(4)); 7.07 (*d*, *J* = 8.9, H–C(6)); 6.33 (*d*, *J* = 9.8, H–C(3)); 2.26 (br. *s*, OH); 1.63 (*s*, 2 Me). ^{13}C-NMR (CDCl$_3$, 100 MHz): 164.4 (*d*, *J* = 3.5, C(8)); 160.1 (*s*, C(2)); 156.1 (*d*, *J* = 250.1, C(5)); 156.0 (*d*, *J* = 15.5, C(6a)); 147.6 (*d*, *J* = 7.1, C(9b)); 137.7 (*d*, *J* = 4.5, C(4)); 114.0 (*d*, *J* = 1.3, C(3)); 113.7 (*d*, *J* = 2.9, C(9a)); 104.5 (*d*, *J* = 21.0, C(4a)); 95.7 (*d*, *J* = 26.7, C(6)); 69.1 (*s*, Me$_2$*C*); 28.6 (*s*, *Me*$_2$C). MS (70 eV): 263 (26, $M^{+\cdot}$), 248 (100), 246 (6), 220 (9), 206 (17), 150 (6). HR-MS: 263.0711 ($C_{14}H_{10}{}^2HFO_4^+$; calc. 263.0704).

(±)-cis-*5-Fluoro-3,4,8,9-tetrahydro-8-(1-hydroxy-1-methylethyl)(9-^{2}H)angelicin* (= *(±)*-cis-*5-Fluoro-3,4,8,9-tetrahydro-8-(1-hydroxy-1-methylethyl)(9-^{2}H)-2*H-*furo[2,3-*h*]-1-benzopyran-2-one;* **20**). A suspension of **14** (0.50 g, 1.90 mmol) and 10% Pd/C (500.0 mg) in acetone (15 ml) was stirred for 4 h at r.t. under H$_2$. Evaporation and chromatography (silica gel, pentane/AcOEt 1:1) yielded pure **20** (0.38 g, 72%). Colourless solid. M.p. 131°. IR (KBr): 3515, 3010, 2926, 1754, 1645, 1616, 1488, 1366, 1256, 1237, 1177, 1145, 1092, 1057, 1008, 974, 907, 834, 755, 635. ^{1}H-NMR (CDCl$_3$, 400 MHz): 6.28 (*d*, *J* = 9.1, H–C(6)); 4.62 (*d*, *J* = 9.5, H–C(8)); 3.08 (*d*, *J* = 9.5, H–C(9)); 2.87 ('*t*', *J* = 7.0, 2 H–C(4)); 2.70 ('*t*', *J* = 7.1, 2 H–C(3)); 1.78 (br. *s*, OH); 1.27 (*s*, 1 Me); 1.14 (*s*, 1 Me). ^{13}C-NMR (CDCl$_3$, 100 MHz): 167.6 (*s*, C(2)); 160.2 (*d*, *J* = 14.2, C(6a)); 159.4 (*d*, *J* = 243.4, C(5)); 148.5 (*d*, *J* = 10.2, C(9b)); 110.1 (*d*, *J* = 3.4, C(9a)); 102.1 (*d*, *J* = 24.5, C(4a)); 93.8 (*d*, *J* = 27.8, C(6)); 91.0 (*s*, C(8)); 71.8 (*s*, Me$_2$*C*); 28.6 (*s*, C(3)); 27.3 (*t*, *J* = 20.7, C(9)); 26.1 (*s*, Me); 23.9 (*s*, Me); 17.0 (*d*, *J* = 3.2, C(4)). MS (70 eV): 267 (29, $M^{+\cdot}$), 233 (12), 209 (70), 208 (29), 194 (7), 191 (7), 181 (10), 167 (31), 166 (23), 154 (11), 138 (8), 110 (10), 59 (100), 55 (9). HR-MS: 267.1015 ($C_{14}H_{14}{}^2HFO_4^+$; calc. 267.1017.

(±)-cis-*5-Fluoro(9-^{2}H)columbianetin* (= *(±)*-cis-*5-Fluoro-8,9-dihydro-8-(1-hydroxy-1-methylethyl)(9-^{2}H)-2*H-*furo[2,3-*h*]-1-benzofuran-2-one;* **13**): A soln. of **20** (0.38 g, 1.41 mmol) in THF (10 ml) was added to a well-stirred, cold (– 78°) LDA soln. in THF (20 ml, 4.20 mmol), followed by an immediate and rapid addition of a phenylselenenyl chloride (0.804 g, 4.20 mmol) soln. in THF (10 ml). After stirring at – 78° for 1 h, the mixture was allowed to come to 0°, and an AcOH (1.5 ml) soln. in H$_2$O (5 ml) was added, followed by slow addition of a 30% H$_2$O$_2$ soln. (5.0 ml). Stirring was continued for 30 min at 0° and 30 min at r.t., followed by extraction with AcOEt (3 × 30 ml). Hexane (50.0 ml) was added to the combined org. extract, the resulting soln. washed with sat. aq. NaHCO$_3$ (30 ml) and NaCl soln. (20 ml), dried (MgSO$_4$), and evaporated, and the crude solid purified by CC (silica gel, hexane/AcOEt 1:1): 0.194 g (52%) of **13**. M.p. 171°. IR (KBr): 3448 (br.), 3072, 2970, 1718, 1627, 1467, 1398, 1357, 1292, 1267, 1171, 1120, 1074, 1034, 1008, 955, 906, 851, 827, 758. ^{1}H-NMR (CDCl$_3$, 400 MHz): 7.75 (*d*, *J* = 9.8, H–C(4)); 6.43 (*d*, *J* = 9.7, H–C(6)); 6.14 (*d*, *J* = 9.7, H–C(3)); 4.74 (*d*, *J* = 9.6, H–C(8)); 3.21 (*d*, *J* = 9.7, H–C(9)); 1.80 (br. *s*, 1 OH); 1.29 (*s*, 1 Me); 1.17 (*s*, 1 Me). ^{13}C-NMR (CDCl$_3$, 100 MHz): 163.7 (*d*, *J* = 14.6, C(6a)); 160.4 (*s*, C(2)); 159.4 (*d*, *J* = 253.1, C(5)); 150.8 (*d*, *J* = 7.6, C(9b)); 137.2 (*d*, *J* = 4.0, C(4)); 112.1 (*d*, *J* = 1.6, C(3)); 109.6 (*d*, *J* = 3.0, C(9a)); 102.9 (*d*, *J* = 20.2, C(4a)); 94.4 (*d*, *J* = 25.3, C(6)); 92.1 (*s*, C(8)); 71.8 (*s*, Me$_2$*C*); 26.9 (*t*, *J* = 20.5, C(9)); 26.0 (*s*, Me); 24.1 (*s*, Me). MS (70 eV): 265 (41, $M^{+\cdot}$), 231 (13), 209 (14), 208 (17), 207 (76), 206 (66), 205 (18), 195 (10), 194 (15), 179 (32), 178 (14), 150 (20), 102 (9), 59 (100), 43 (17). HR-MS: 265.0855 ($C_{14}H_{12}{}^2HFO_4^+$; calc. 265.0861).

8-(Ethoxycarbonyl)-5-fluoroangelicin (= *Ethyl 5-Fluoro-2-oxo-2*H-*furo[2,3-*h*]-1-benzofuran-8-carboxylate*; **22**). Ethyl propiolate (0.4 ml, 3.94 mmol) and a soln. of **15** (0.27 g, 0.88 mmol) in dry DMF (4.0 ml) were added to copper(I) oxide (0.15 g, 1.05 mmol) suspended in dry DMF (4.0 ml). The mixture was heated to 110° for 24 h, then filtered through a small pad of silica gel, and diluted with AcOEt (20 ml). The org. soln. was washed with 1N HCl, sat. aq. $NaHCO_3$ soln. (50 ml) and brine, dried ($MgSO_4$), and evaporated. The residue was submitted to CC (silica gel, hexane/AcOEt 1:1): pure **22** (0.17 g, 70%). Faintly yellow solid. IR (KBr): 3054, 2985, 1729, 1621, 1563, 1473, 1368, 1290, 1207, 1143, 1014, 864, 847, 760, 642. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.95 (*d*, *J* = 9.8, H–C(4)); 7.71 (*d*, *J* = 0.7, H–C(9)); 7.18 (*dd*, J_1 = 8.9, 0.8, H–C(6)); 6.40 (*d*, *J* = 9.8, H–C(3)); 4.39 (*q*, *J* = 7.1, CH_2); 1.37 (*t*, *J* = 7.1, Me). ^{13}C-NMR ($CDCl_3$, 100 MHz): 159.2, 158.5 (2*s*, C(2), CO_2Et); 158.2 (*d*, *J* = 253.8, C(5)); 156.6 (*d*, *J* = 15.3, C(6a)); 149.1 (*d*, *J* = 7.7, C(9b)); 147.0 (*d*, *J* = 4.4, C(8)); 137.3 (*d*, *J* = 5.7, C(4)); 115.0 (*s*, C(3)); 113.3 (*d*, *J* = 2.4, C(9a)); 110.5 (*d*, *J* = 1.5, C(9)); 105.5 (*d*, *J* = 21.8, C(4a)); 96.2 (*d*, *J* = 25.8, C(6)); 62.0 (*s*, MeCH_2); 14.3 (*s*, *Me*CH_2). MS (70 eV): 276 (100, $M^{+\cdot}$), 248 (23), 231 (30), 220 (48), 204 (18), 176 (12), 175 (10), 147 (15), 119 (11), 99 (10). HR-MS: 276.0435 ($C_{14}H_9FO_5^+$, calc. 276.0434).

5-Fluoroangelicin (= *5-Fluoro-2*H-*furo[2,3-*h*]-1-benzofuran-2-one*; **23**). A suspension of **22** (0.13 g, 0.47 mmol) in 20% aq. NaOH soln. (15.0 ml) was refluxed for 2 h. After cooling and acidification with conc. HCl soln., the mixture was extracted with AcOEt (1 × 100 ml, 2 × 50 ml). The combined org. layer washed with sat. aq. $NaHCO_3$ soln. (40 ml), dried, and evaporated and the remaining solid taken up in quinoline (6.0 ml). Copper powder (20.0 mg, 0.31 mmol) was added and the suspension heated to 210° for 1 h. After cooling, cold 1N HCl (50.0 ml) was added and the aq. layer extracted with AcOEt. The org. layer was washed with sat. aq. $NaHCO_3$ soln. (20 ml) and brine (10 ml), dried ($MgSO_4$), and evaporated. CC (silica gel, hexane/AcOEt 3:2) yielded **23** (40.0 mg, 43%). Colourless solid. M.p. 155°. IR (KBr): 3080, 3066, 1737, 1631, 1533, 1470, 1370, 1309, 1255, 1189, 1167, 1141, 1113, 1085, 1074, 990, 829, 749, 630. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.95 (*d*, *J* = 9.9, H–C(4)); 7.60 (*d*, *J* = 2.2, H–C(8)); 7.11 (*dd*, J_1 = 9.1, 1.0, H–C(6)); 7.01 (*dd*, *J* = 2.2, 0.8, H–C(9)); 6.35 (*d*, *J* = 9.7, H–C(3)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 160.0 (*s*, C(2)); 156.4 (*d*, *J* = 249.9, C(5)); 156.3 (*d*, *J* = 15.9, C(6a)); 147.9 (*d*, *J* = 7.3, C(9b)); 146.0 (*d*, *J* = 3.8, C(8)); 137.7 (*d*, *J* = 4.5, C(4)); 114.1 (*d*, *J* = 1.4, C(3)); 113.3 (*d*, *J* = 2.0, C(9a)); 104.6 (*d*, *J* = 20.7, C(4a)); 104.0 (*d*, *J* = 1.72, C(9)); 95.8 (*d*, *J* = 26.3, C(6)). MS (70 eV): 204 (100, $M^{+\cdot}$), 176 (71), 148 (20), 120 (27). HR-MS: 204. 0221 ($C_{11}H_5FO_3^+$; calc. 204.0223).

Induction Experiments Plants: Young plants of *Heracleum mantegazzianum* were bought from a garden centre (*Hof Berg-Garten*, Lindenweg 17, D-79737 Herrischried) and grown in 49-cm^2 diameter pots filled with potting compost. The plants were kept at r.t. and illuminated with daylight fluorescent tubes (*ca.* 270 μE s^{-1} m^{-2}, using a regime of 14 h light and 10 h dark period). Plants used for induction experiments were about two- or three-month-old.

Application of the Test Compounds: A soln. of *cis*-5-fluoro(9-^{2}H)columbianetin (**13**; 0.8 mg/ml), *Triton X 100* (*ca.* 4 mg per ml), and jasmonic acid (0.2 mg per ml) were sonicated (*ca.* 5–10 min) until a stable emulsion resulted. The emulsified test soln. was stable for the time of the induction experiment (5 days) without noticeable decomposition. Freshly detached leaves were immediately placed into the emulsions. About 50% of the directly treated plants were lost during the first 3 days due to shrinking of the stem and subsequent necrotization of the leaf. No 5-fluoroangelicin **23** was found among the surface lipids of the necrotized plant leaves.

Surface Treatment of Leaves: The above emulsion containing *cis*-5-fluoro(9-^{2}H)columbianetin (**13**; 1 mg per ml), *Triton X 100* (*ca.* 4 mg per ml), and jasmonic acid (0.2 mg per ml) was repeatedly sprayed onto the leaves of the intact plants (four-days intervals). This treatment had no visible impact onto the plants. About three weeks after the beginning of the treatment, the leaves were detached and their surface lipids were analysed for metabolites.

Collection of the Surface Lipids: The nonpolar surface compounds were obtained by brief dipping (2 × 30 s) of the pretreated, but undamaged leaves into CH_2Cl_2 (*ca.* 10 ml) [25]. After filtration and controlled evaporation at *ca.* 300 Torr; the residue was dissolved in AcOEt (100–200 μl). The resulting soln. was used for GLC/MS without further purification.

Mass Spectroscopic Analysis of Surface Compounds: The solns. containing the surface lipids of the pretreated plants or from the leaves of untreated control plants were directly analysed by GLC/MS by injecting (1 μl) onto a fused silica column coated with *DB 1* (15 m × 0.25 mm, 0.25 μm). Compounds were separated under programmed conditions (50° for 2 min, then to 280° at 20°/min). Transfer line: 250°. Scan range: 35–350 Da/s. Authentic references were used for the unequivocal identification of all furocoumarins and the fluorinated metabolites.

Biomimetic Dealkylation of cis-*5-Fluoro(9-^{2}H)columbianetin* (**13**): A soln. of (tetraphenylporphyrinato)-manganese(III) (1.4 mmol), 5-fluoro(9-^{2}H)columbianetin (**13**; 8.1 mmol) and iodosylbenzene (22.0 mmol) in

CH_2Cl_2 (3.0 ml) was stirred at 4° for 8 h. The resulting products were analysed by GLC/MS as above. The yield of 5-fluoroangelicin **23** was *ca.* 1–3%. Due to the low conversion, no attempts were made to trap acetone as the second fragment, as has been done previously for the comparable transformation of (+)-marmesin (**10**) to psoralen (**1**).

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REFERENCES

[1] R. A. Creelman, J. E. Mullet, *Annu. Rev. Plant. Mol. Biol.* **1997**, *48*, 355.
[2] R. D. H. Murray, J. Mendés, S. A. Brown, in 'The Natural Coumarins: Occurrence, Chemistry and Biochemistry', Wiley and Sons, Chichester – New York, 1982.
[3] M. R. Berenbaum, A. R. Zangerl, *Recent Adv. Phytochem.* **1996**, *30*, 1.
[4] A. R. Zangerl, M. R. Berenbaum, *Ecology* **1990**, *71*, 1933.
[5] K. D. Hauffe, K. Hahlbrock, D. Scheel, *Z. Naturforsch., C* **1986**, *41*, 228.
[6] R. C. Beier, E. H. Oertli, *Phytochemistry* **1983**, *22*, 2595.
[7] M. Miksch, W. Boland, *Experientia* **1996**, *52*, 739.
[8] V. Stanjek, C. Herhaus, U. Ritgen, W. Boland, E. Städler, *Helv. Chim. Acta* **1990**, *80*, 1408.
[9] L. D. Scheel, V. B. Perone, R. L. Larkin, R. E. Kupel, *Biochemistry* **1963**, *2*, 1127.
[10] L. Musajo, G. Rodighiero, G. Columbo, V. Torlone, F. Dell'Acqua, *Experientia* **1967**, *23*, 335.
[11] M. M. Diawara, J. T. Trumble, K. K. White, W. G. Carson, L. A. Martinez, *J. Chem. Ecol.* **1993**, *19*, 2473.
[12] M. R. Berenbaum, P. Feeney, *Science (Washington, D.C.)* **1981**, *212*, 927.
[13] M. R. Berenbaum, A. R. Zangerl, *Oecologia* **1993**, *95*, 370.
[14] M. R. Berenbaum, A. R. Zangerl, *Evolution* **1992**, *46*, 1373.
[15] U. Matern, P. Lüer, D. Kreusch, in 'Comprehensive Natural Products Chemistry', Ed. U. Sankawa, Elsevier, Oxford, in press.
[16] A. Estevéz-Braun, A. G. Gonzales, *Nat. Prod. Rep.* **1997**, *14*, 465.
[17] H. Wendorff, U. Matern, *Eur. J. Biochem.* **1986**, *161*, 391.
[18] V. Stanjek, M. Miksch, U. Matern, P. Lüer, W. Boland, *Angew. Chem.*, **1998**, in press.
[19] R. A. W. Johnstone, A. H. Wilby, I. D. Entwistle, *Chem. Rev.* **1985**, *85*, 129.
[20] V. Stanjek, M. Miksch, W. Boland, *Tetrahedron* **1997**, *53*, 17699.
[21] E. Zubía, F. R. Luis, G. M. Massanet, I. G. Collado, *Tetrahedron* **1992**, *48*, 4239.
[22] H. J. Reich, *Acc. Chem. Res.* **1979**, *12*, 22.
[23] T. J. McMurry, J. T. Groves, in 'Cytochrome P450: Structure, Mechanism and Biochemistry', Ed. P. R. Ortiz de Montellano, Plenum Press, New York, 1986.
[24] D. S. Dillon, S. A. Brown, *Arch. Biochem. Biophys.* **1976**, *177*, 74.
[25] E. Städler, H. R. Buser, *Experientia* **1984**, *40*, 1157.

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