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# Polyketide Folding in Higher Plants: Biosynthesis of the Phenylanthraquinone Knipholone

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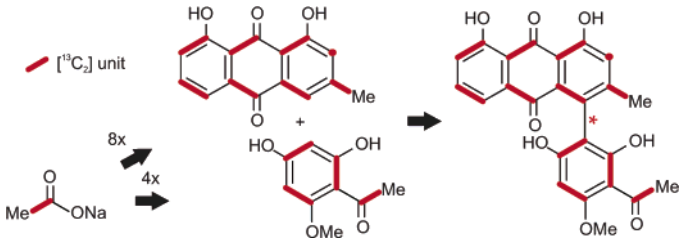


The biosynthesis of knipholone, as an axially chiral phenylanthraquinone, in higher plants was examined by feeding experiments with [$^{13}C_2$]-labeled precursors. [$^{13}C_2$]-Acetate and advanced synthetic intermediates were fed to sterile cultures of *Kniphofia pumila* (Asphodelaceae), with subsequent NMR analysis on the isolated natural product involving 2D INADEQUATE and SELINQUATE experiments. Due to its uneven number of carbon atoms, and because of its uncertain decarboxylation site, the "northern" part of the molecule (i.e., the chrysophanol portion) might originate from four different cyclization modes. According to the labeling pattern of the product isolated after incorporation, this anthraquinone part of knipholone is formed by the so-called F folding mode (originally established for fungi). The acetophenone part of the molecule, which does not undergo a decarboxylation reaction, originates from four acetate units. The surprising lack of randomization of the intact [$^{13}C_2$] units in this "southern" part reveals the absence of a free symmetric intermediate as initially anticipated. This is in agreement with the intact incorporation of the "authentic" southern molecular portion, 4,6-dihydroxy-2-methoxyacetophenone, while the corresponding symmetrical candidate trihydroxyacetophenone was clearly not incorporated, showing that the *O*-methylation of the freshly cyclized tetraketide is the step that prevents symmetrization of the acetophenone.

## Introduction

Acetogenic secondary metabolites constitute a vast class of structurally diverse bioactive natural products. They are formed through the action of polyketide synthases (PKSs),[1] of which three classes are known to date. For the regiocontrolled cyclization of the reactive $\beta$-polycarbonyl chain to give oligocyclic acetogenic natural products basically two different folding types are known:[2] mode F, referring to its occurrence in fungi, and mode S, which is used by filamentous bacteria of the genera *Streptomyces* and *Actinomyces*. Reports on the biogenesis of polyketides in higher plants, by contrast, are still rare,[2−7] and the question of whether plants synthesize their polyketidic secondary metabolites only via the mode F, or possibly also via S, is thus presently of great evolutionary interest.

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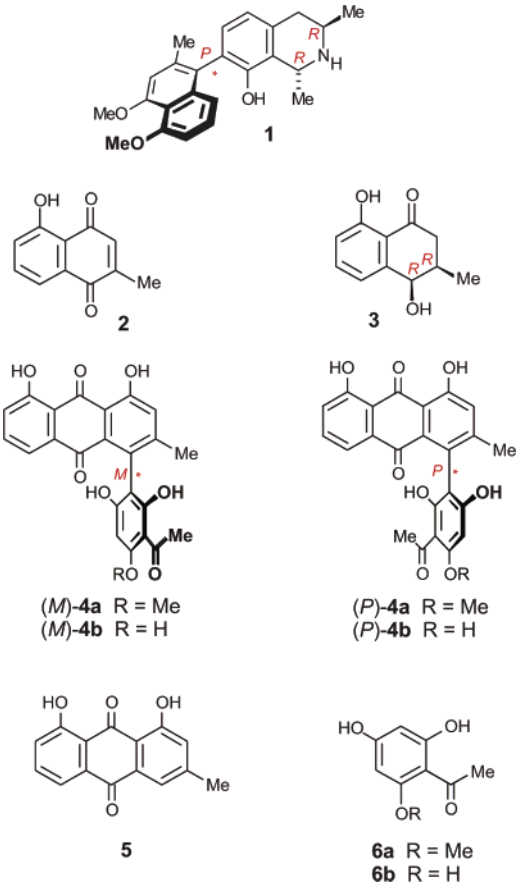


**FIGURE 1.** Acetogenic natural products from plants: dioncophylline A (**1**), plumbagin (**2**), isoshinanolone (**3**), knipholone (**4a**), and 4-*O*-demethylknipholone (**4b**), and the two molecular moieties of **4a**, chrysophanol (**5**) and 2,4-dihydroxy-6-methoxyacetophenone (**6a**), along with the structure of the other imaginable precursor, the symmetrical compound 2,4,6-trihydroxyacetophenone (**6b**).

There are as yet only a few experimentally established examples of polyketidic folding modes in higher plants; the octaketidic anthraquinone, chrysophanol (**5**),[3] and both hexaketidic molecular "halves" of the naphthylisoquinoline alkaloids, e.g., dioncophylline A (**1**, Figure 1),[4,5] and the related naphthoquinones,[5−7] e.g., plumbagin (**2**) and tetralones isoshinanolone (**3**). They all originate from identical polyketide precursors, and all follow the type F cyclization mode.

Axially chiral biaryls like **1** that consist of two different aromatic portions are quite rare in nature compared to the numerous homocoupled dimers[8] of phenolic compounds from a range of classes of natural products.[9] Even the two molecular portions of **1**, which are, at first sight, structurally so different, originate from identical − and even equally folded − polyketide chains.[4,5] A naturally occurring biaryl compound that consists of two substantially different aromatic moieties, hence formed via apparently divergent polyketidic pathways, is knipholone (**4a**, Figure 1), which is thus a rewarding subject for biosynthetic investigations. This axially chiral phenylanthraquinone, first discovered in the monocotyledonic plant *Kniphofia foliosa* (Asphodelaceae),[10] has since also been isolated from a dicotyledonic *Cassia* (a.k.a. *Senna*) species of the Caesalpiniaceae family.[11] More recently, an enantiodivergent total synthesis has been developed[12−14] to this antimalarial,[15] antitumoral,[16] and antiinflammatory[17] compound. Furthermore, protocols for the in vitro propagation of knipholone-producing plants have been established.[18−20]

Biosynthetically, both molecular portions of knipholone (**4a**) are of high interest: The "northern" half, in its free form the long-known anthraquinone chrysophanol (**5**),[21] is widely distributed in plants and fungi, but only little work has been reported on its biosynthetic origin. In the early 1970s, feeding experiments with [$^{14}$C]-acetate proved the acetogenic nature of chrysophanol (**5**) in plants,[22,23] but since no NMR-active nuclei (like $^{13}$C) were used, no information about the folding type was acquired. Due to the loss of one C-atom in the course of its biosynthesis, the structure of **5** might, in principle, be compatible with different folding modes. We have recently shown that **5** can indeed be formed via both an F- and an S-type folding mode, depending on the producing organism.[3]

The "southern" part of **4a** is the likewise well-known, but biosynthetically as yet unexplored 2,4-dihydroxy-6-methoxyacetophenone (**6a**), which has been isolated from plants,[24] but not as yet from fungi or filamentous bacteria. From its substitution pattern, only one folding type[25] seems imaginable for the biosynthetic formation of **6a**; however, it could then be incorporated into knipholone (**4a**) in two different ways (see below).

In this paper, we describe feeding experiments on the plant metabolite knipholone (**4a**) with [$^{13}C_2$]-acetate and with more advanced possible precursors of the "southern" part of **4a**, i.e., the acetophenones **6a** and **6b**, in a specifically [$^{13}C_2$]-acetyl labeled form. The knipholone (**4a**) subsequently isolated was investigated by NMR methods like 2D INADEQUATE[26,27] and

(8) Strictly speaking, these biarylic compounds should rather be addressed as *dehydro* dimers, formed through phenol-oxidative coupling.

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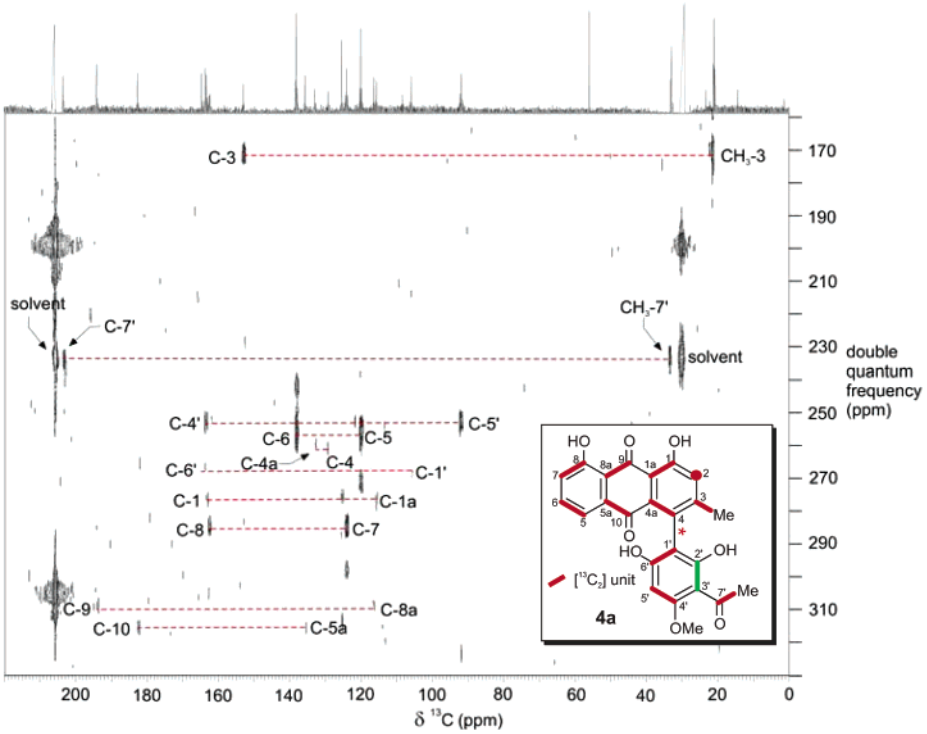


**FIGURE 2.** 2. 2D INADEQUATE NMR spectrum of knipholone (**4a**) in $(CD_3)_2CO$ isolated after administration of [$^{13}C_2$]-labeled acetate to *K. pumila*. The incorporation pattern of the intact [$^{13}C_2$] units is marked in red (the only such unit that has remained unproven, viz. the one between C-2′ and C-3′, is marked by a green line).

SELINQUATE[28] in combination with cryoprobe methodology. It was shown that the "northern" part of the molecule, chrysophanol (**5**), is built up following the folding mode F. The "southern" portion, the 3-fold oxygenated acetophenone, originates from four $C_2$ units. The unexpected lack of randomization of the $C_2$ units incorporated into the aromatic part of the phloroglucinol portion reveals that its origin does not involve symmetrical intermediate, which is in agreement with the specific incorporation of the *O*-methylated substrate **6a**, while the symmetric 2,4,6-trihydroxyacetophenone (**6b**) was not incorporated.

## Results and Discussion

**Acetate Feeding.** For the feeding experiments, sterile whole plants instead of tissue or suspension cultures of *K. pumila* were chosen because they permitted easy handling of the acetate feeding and produced knipholone (**4a**),[29] while the cell cultures that we had likewise succeeded in establishing[19] did not contain any **4a**. To these sterile plants, [$^{13}C_2$]-labeled acetate was fed by administration to the roots over a period of 2 months. The $^{13}C$ NMR spectrum of the isolated knipholone (**4a**) showed partly overlapping signals and an insufficient signal-to-noise ratio, obscuring some of the $^{13}C$ doublets, so that it was impossible to draw final conclusions from their $^{13}C,^{13}C$ coupling constants. The problems were partially overcome by using the 2D INADEQUATE[26,27] NMR experiment for the identification of direct C,C-connectivities.[30] The signal-to-noise ratio, which suffered from the small quantity (ca. 1 mg) of the knipholone **4a** isolated, was further substantially improved by acquiring the data with a cryoprobe.[31] Thus, an incorporation of 0.6% was observed for the labeled acetate, permitting identification of nearly all of the $^{13}C_2$ units.

In the anthraquinone part of **4a**, all of the expected [$^{13}C_2$]-connectivities had thus become apparent in the 2D INADEQUATE spectrum (Figure 2), giving rise to a complete picture of a continuous series of six endocyclic (C-1/C-1a, C-9/C-8a, C-8/C-7, C-6/C-5, C-5a/C-10, C-4a/C-4) acetate-derived $C_2$ units and an exocyclic one (3-Me), leaving only C-2 without a coupling partner, which reveals this C atom to be the one where the decarboxylation must have occurred. The $^{13}C$ signal of this carbon atom was likewise enhanced. From this unambiguously established pattern of intact acetate-derived $C_2$ units incorporated, without bond cleavage, and, in particular, due to the fact that the first ring (the "western" one) contains two (not three) intact $C_2$ units, the chrysophanol part of the phenylanthraquinone **2a** clearly follows the *F*-type folding mode.[23]

Under these optimized conditions, three of the four $C_2$ units of the phloroglucinol part of **4a** were clearly identified: the coupling between the two exocyclic carbon atoms of the acetyl group (i.e., the assumed starter unit), C-5′/C-4′, and to a smaller extent also C-1′/C-6′ (see Figure 2). The only connectivity not clearly visible was the one expected to join the two quaternary carbon atoms C-2′ and C-3′. Their signal intensities were too small for determination of a $^{13}C,^{13}C$ coupling constant and furthermore did not show any correlation in the 2D INADEQUATE spectrum.

(28) Berger, S. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1196−1197.
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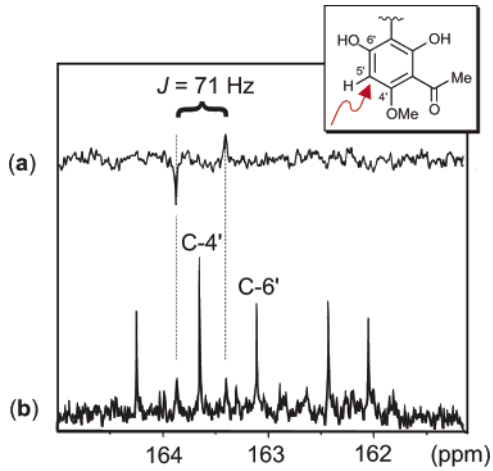
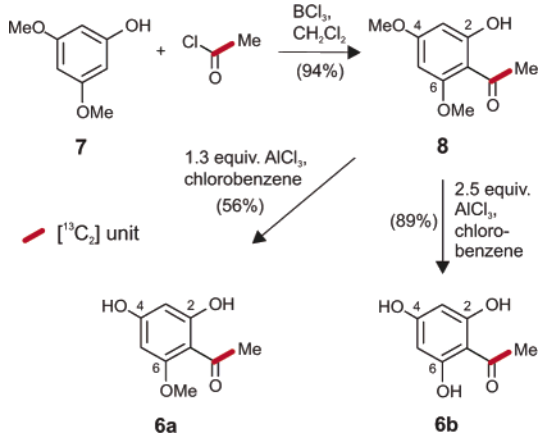



**FIGURE 3.** 3. SELINQUATE spectrum of knipholone (**4a**), as isolated after the [$^{13}C_2$]-acetate feeding, obtained upon selective coherence transfer carried out by a selective 270° Gauss pulse (7 ms pulse length) at 91.89 ppm (i.e., the resonance of C-5′, see arrow) in the SELINQUATE pulse sequence;[28] the only coherence transfer observed leads to a signal with the chemical shift of C-4′ (a); "normal" $^{13}$C NMR spectrum of **4a** for comparison (b).

From the three intact acetate units unambiguously identified, the folding in the phloroglucinol part of **4a** was still already fully evident. This result is remarkable with respect to the orientation of the above-mentioned pairwise interactions in the phloroglucinol part: Their particular specific, nonrandom array was entirely unexpected, since the most favored candidate as a precursor to the "southern" part of the molecule had been the symmetric 2,4,6-trihydroxyacetophenone (**6b**). This compound would have been expected to be either the direct substrate for the C,C-linking phenol-oxidative coupling (to give *O*-demethylknipholone (**4b**), a natural product, e.g., from *Bulbine capitata*, Asphodelaceae)[32] or *O*-methylated *ortho* to the acetyl group to give 2,4-dihydroxy-6-methoxyacetophenone (**6a**) as the coupling substrate. Since **6b** is a constitutionally symmetric compound, its occurrence in the biosynthetic formation of **4a** would—although leaving the $C_2$ units as such intact—make the [$^{13}C_2$] entities C-6′/C-1′, C-2′/C-3′, and C-4′/C-5′ undistinguishable from those involving C-6′/C-5′, C-4′/C-3′, and C-2′/C-1′, respectively, while the *exo*-acetyl should remain fully and "undilutedly" labeled. However, no such randomization was observed, which excludes 2,4,6-trihydroxyacetophenone as a (free) biosynthetic precursor to **4a**.

To further check whether the—initially anticipated—randomization of the $C_2$-units of the "southern" aromatic ring, i.e., C-6′/C-1′, C-2′/C-3′, C-4′/C-5′ vs C-6′/C-5′, C-4′/C-3′, C-2′/C-1′, might still be occurring to a minor degree, a SELINQUATE[28] experiment seemed to be the method of choice (Figure 3). This efficient technique for the determination of selected C,C-connectivities offers a particularly good sensitivity in the presence of a hydrogen-bearing C-atom.[33] Therefore, and because of a strong signal overlap in the chemical shift range of C-4′ and C-6′, the irradiation was performed selectively on C-5′ (91.89 ppm), showing that this tertiary carbon atom displays a C,C-correlation only to C-4′.

**SCHEME 1. Synthesis of 6a and 6b in a Specifically [$^{13}C_2$]-Labeled Form**




Exemplarily for this $C_2$ unit, the experiment thus confirms the presence of the above-mentioned labeling pattern of the "southern" molecular moiety of **4a** to an even greater extent (>80%), i.e., without any major randomization.

This result clearly excluded the existence of any free symmetric monocyclic precursor, in particular, trihydroxyacetophenone **6b** (cf. Figure 1), down to a degree of <20%. The monomethyl ether **6a**, by contrast, i.e., the authentic "southern" portion of **4a**, might be considered as a possible substrate for the phenol oxidative biaryl coupling, due to its unsymmetrical structure. In order to investigate whether **6a** was indeed a precursor, comparative feeding experiments of **6a** and — as a control — **6b** seemed rewarding. This required the availability of the two trioxy-substituted acetophenones **6a** and **6b** in a specifically [$^{13}C_2$]-labeled form.

**Labeling Synthesis and Feeding of Advanced Precursors.** For a directed labeling, the introduction of the exocyclic acetyl function by an electrophilic aromatic acylation reaction with [$^{13}C_2$]-acetyl chloride into an appropriately protected phloroglucinol derivative seemed the method of choice. The synthesis, as shown in Scheme 1, followed a known (albeit nonlabeled) pathway described previously.[12,34,35] Thus, treatment of 3,5-dimethoxyphenol (**7**) with [$^{13}C_2$]-acetyl chloride gave the acetophenone **8** with the expected high and specific degree of [$^{13}C_2$] labeling (>98% by NMR) in the introduced acetyl portion. Regioselective mono-*O*-demethylation of **8** was brought about by using only a slight excess of $AlCl_3$ (1.3 equiv), while a larger amount of the same Lewis acid (2.5 equiv) gave the fully *O*-demethylated (and thus symmetrical) phloroglucinol derivative **6b**.

The two precursors **6a** and **6b** were then administered in three portions, at intervals of two weeks, to sterile whole plants of *Kniphofia pumila* with concentrations of 0.50 and 0.43 mg/mL, respectively, over a total period of 6 weeks. The results of the comparative feeding experiment with **6a** and **6b** clearly showed incorporation of the unsymmetric precursor **4a**. Thus, the $^{13}$C NMR spectrum (see the Supporting Information) revealed two doublets at 33.1 ($^{13}CH_3$) and 204.4 ($^{13}C_{carbonyl}$) ppm by $^1J_{C,C}$-couplings in the [$^{13}C_2$]-enriched fragment, while the symmetric intermediate was not incorporated. Both compounds, **6a** and

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(33) This is also the reason why the SELINQUATE experiment could not be used to make that one last missing $C_2$ unit between the two quaternary C-atoms C-2′ and C-3′ visible.
(34) Cunningham, B. D. M.; Lowe, P. R.; Treadgill, M. D. *J. Chem. Soc., Perkin Trans. 2* **1989**, *9*, 1275−1282.
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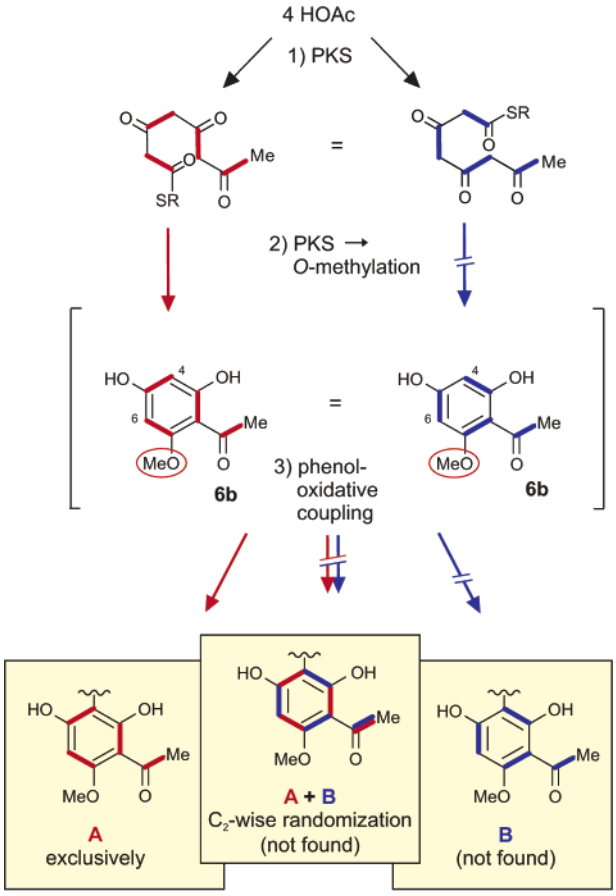
**SCHEME 2. Proposed Biogenesis of the "Southern" Moiety of the Phenylanthraquinone Knipholone (4a)**




**6b**, were no longer detectable after the feeding experiment, neither in the plant nor in the medium. This clearly showed that they had been taken up and metabolized and had thus been fully available to the biological system. The lack of incorporation of the symmetric acetophenone **6b** thus unambiguously proved that it is not a biosynthetic precursor to knipholone (**4a**), which again corroborated the above assumption that no symmetric intermediate can be involved, as drawn from the nonrandomization of the acetate labeling pattern. The (unsymmetric) methyl ether **6a**, by contrast, clearly is a precursor and thus apparently the direct coupling substrate (Scheme 2). The results furthermore showed that the *O*-methylation reaction — and not the likewise imaginable biaryl coupling — is the desymmetrizing (or, rather, non-symmetry-conserving) step.

Even this assumption seems contradictory at first glance, since the methyl ether **6a** can, in principle, only have become nonsymmetrical by the *O*-methylation of a symmetric precursor, viz. the trihydroxyacetophenone **6b**. Still, a possible explanation for the nonrandomization may be that the desymmetrizing *O*-methylation step happens so early and in such close cooperation with the polyketide synthase (PKS) that the initially resulting cyclization product never occurs in a free form. Such an immediate, symmetry-preventing scavenging reaction might be imaginable either chemically, by *O*-methylation of an early, not yet aromatic cyclization product, e.g., **A** (see Figure 4, left), or more likely, in a way that the trihydroxyacetophenone (**6b**) is indeed formed, but initially still embedded in the PKS enzyme pocket, i.e., by a "topological differentiation". The compound **6b** would thus be de facto unsymmetric, partially shielded by the enzyme, and would therefore be *O*-methylated only at one

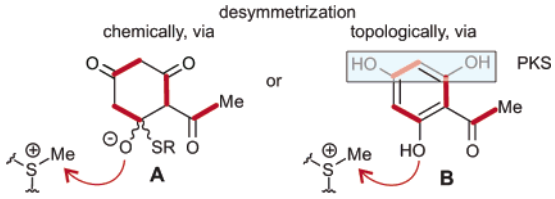



**FIGURE 4.** Hypothetical desymmetrization by regioselective in situ *O*-methylation of the acetophenone precursor to knipholone (**4a**)— chemically, via **A**, or by a "topological differentiation", via **B**.

of the phenolic OH groups (see **B**, Figure 4, right). In both cases, the *O*-methyl transferase should act as a tailoring enzyme, in close cooperation with the PKS.

## Conclusions

The first investigation on the biosynthetic origin of knipholone (**4a**), as described in this paper, shows that the "northern" portion of the molecule, the anthraquinone moiety, is built up from eight acetate units, according to the folding mode F. After previous results for naphthylisoquinoline alkaloids and related naphthoquinones in dicotyledonous plants and free chrysophanol (**5**) in monocotyledonous plants, this is a further hint at a possible general use of mode F by higher plants.

In the "southern" part of **4a**, which originates from four acetate units, the $^{13}C_2$ units were found to be localized, not $C_2$-wise randomized as expected. In agreement with this result, the symmetric [$^{13}C_2$]-labeled trihydroxyacetophenone (**6b**) was not incorporated, while the unsymmetric methyl ether **6a** proved to be a specific precursor and is the immediate substrate in the biaryl coupling reaction. Therefore, the *O*-methylation step (and not the phenol-oxidative coupling reaction) must be the desymmetrizing step, which hence must occur at an early stage in the biosynthesis of **4a**. The results suggest at a close organizational cooperation of the PKS with the *O*-methyl transferase as a tailoring enzyme. More detailed investigations on the biochemistry of this efficient and early cooperation, and on the enzymes involved, will be rewarding in terms of combinatorial biosynthesis and metabolic diversity.

## Experimental Section

**Plant Material and Sterile Cultures.** For metabolic investigations, sterile whole plants of *K. pumila* (Asphodelaceae) (average size: 20 cm) were established in 300-mL Erlenmeyer flasks as previously reported.[18] For feeding experiments, these plants were cultivated on the same solid medium (half-strength MS medium) as used for general maintenance. For reasons of reproducibility, the plants used were not more than 2 years old.

**Feeding Experiments.** An aqueous sterile-filtered solution (4 mg/mL) of 0.05 M sodium [$^{13}C_2$]-acetate in distilled water, adjusted to pH 5.8, was used for the feeding experiments. This solution was fed to the roots of the sterile plants (1 mL/flask once a week) over a period of 2 months; in total, 224 mg of [$^{13}C_2$]-NaOAc was administered to 3.39 g (dry weight) of the roots. The medium was replaced after 4 weeks as usual.

For the feeding experiments of the labeled acetophenone derivatives **6a** and **6b**, the plants were cultivated as mentioned above but only for 6 weeks. A 10.4 mg portion of the [$^{13}C_2$]-labeled 2,4-dihydroxy-6-methoxyacetophenone (**6a**) was administered in total over 6 weeks to the roots of seven sterile plants (3.89 g dry weight), while in the case of 2,4,6-trihydroxyacetophenone (**6b**), a total of 9.03 mg of the labeled precursor was fed over 6 weeks to 3.41 g of root material (seven cultures).

**Knipholone (4a).** The sterile plants were rinsed with water to remove residues of the medium. The cut roots were lyophilized, ground, and extracted with $H_2O$/acetone (5/95) at room temperature for 48 h. The solvent was removed by rotary evaporation, and the extract was purified by preparative HPLC (Waters Symmetry RP 18 19 × 300 mm column, 12 mL/min, water (A)/acetonitrile (B) (each with 0.05% TFA); 0 min 5% B, 30 min 70% B, 40 min 100% B; 4a: $t_R$ = 33.4 min) giving knipholone (4a), identical in its chromatographic and physical data to the literature[9] (except for the $^{13}C$ NMR spectrum, see the Supporting Information): mp 221 °C dec (water/acetonitrile) (lit.[10] mp 225 °C); $[\alpha]^{22}_D = +24$ (*c* 0.01, MeOH) (lit.[35] $[\alpha]^{22}_D = +22$, *c* 0.04, MeOH).

**[1′,2′-$^{13}C_2$]-4,6-Dimethoxy-2-hydroxyacetophenone (8).** To a solution of $BCl_3$ (0.68 g, 4.90 mmol) in 10 mL of dry $CH_2Cl_2$ was added dropwise 3,5-dimethoxyphenol (**7**, 0.75 g, 4.90 mmol) in 10 mL of $CH_2Cl_2$ at −10 °C. After further stirring for 10 min at room temperature, the [$^{13}C_2$]-labeled acetyl chloride (0.50 g, 6.35 mmol) in 15 mL of $CH_2Cl_2$ was added over 20 min, and the reaction mixture was refluxed for a further 3 h. After hydrolysis with 1 M HCl, the aqueous phase was extracted with $CH_2Cl_2$. The organic layer was dried ($MgSO_4$) and the solvent evaporated in vaccuo. Column chromatography (silica gel, $CH_2Cl_2$/MeOH = 100:1) afforded **8** (0.91 g, 0.46 mmol, 94%) as colorless needles: mp 77 °C ($CH_2Cl_2$/MeOH) (lit.[34] mp 74 °C); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 2.44−2.76 (dd, $J_{C,H}$ = 129 Hz, $J_{C,C}$ = 6.19 Hz, 3H, $^{13}Me$), 3.81 (s, 3H, OMe), 3.87 (s, 3H, OMe), 5.92 (m, 1H), 6.06 (d, $J$ = 2.14 Hz, 1H), 14.02 (s, 1H, OH); $^{13}C$ NMR (101 MHz, $CDCl_3$) $\delta$ 32.8 (d, $J_{C,C}$ = 42.6 Hz, $^{13}Me$), 55.2 (OMe), 55.4 (OMe), 90.7 ($C_{arom}$), 93.4 ($C_{arom}$), 105.9 ($C_{arom}$), 162.8 ($C_{arom}$), 166.0 ($C_{arom}$), 167.5, 203.0 (d, $J_{C,C}$ = 42.6 Hz, $^{13}C_{carbonyl}$).

**[1′,2′-$^{13}C_2$]-2,4-Dihydroxy-6-methoxyacetophenone (6a).** A solution of **8** (0.50 g, 2.52 mmol) in 10 mL of chlorobenzene was treated with $AlCl_3$ (0.44 g, 3.28 mmol) at room temperature and then refluxed for 2 h. The reaction mixture was carefully treated with 1 M HCl at 0 °C. The aqueous phase was extracted with $CH_2Cl_2$, and the combined organic layers were dried with $MgSO_4$. Evaporation of the solvent and column chromatography (silica gel, $CH_2Cl_2$/MeOH = 100:1) afforded **6a** (256 mg, 1.14 mmol, 56%) as colorless crystals: mp 143 °C ($CH_2Cl_2$/MeOH) (lit.[37] mp 142 °C); $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$ 2.43−2.79 (dd, $J_{C,H}$ = 129 Hz, $J_{C,C}$ = 6.19 Hz, 3H, $^{13}Me$), 3.92 (s, 3H, OMe), 5.92 (dd, $J$ = 2.28 Hz, $J$ = 0.75 Hz, 1H), 6.01 (t, 1H); $^{13}C$ NMR (101 MHz, $CD_3OD$) $\delta$ 33.1 (d, $J$ = 42.2 Hz, $^{13}Me$), 56.1 (OMe), 82.8 ($C_{arom}$), 91.9 ($C_{arom}$), 96.7 ($C_{arom}$), 164.9 ($C_{arom}$), 166.2 ($C_{arom}$), 167.8 ($C_{arom}$), 203.0 (d, $J_{CC}$ = 42.6 Hz, $^{13}C_{carbonyl}$).

**[1′,2′-$^{13}C_2$]-2,4,6-Trihydroxyacetophenone (6b).** A solution of **8** (0.20 g, 1.01 mmol) in 10 mL of chlorobenzene was treated with $AlCl_3$ (0.34 g, 2.50 mmol) at room temperature and then refluxed for 2 h. The reaction mixture was carefully hydrolyzed with 1 M HCl. The solvent was evaporated, and the residue was dissolved in 5 mL of isopropyl alcohol. Filtration of the mixture and subsequent column chromatography (silica gel, $CH_2Cl_2$/MeOH = 95:5) gave **6b** (150 mg, 0.90 mmol, 89%) as colorless crystals: mp 221 °C ($CH_2Cl_2$) (lit.[38] mp 218 °C); $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$ 2.49 − 2.81 (dd, $J_{C,H}$ = 123 Hz, $J_{C,C}$ = 4.19 Hz, 3H, $^{13}Me$), 5.96 (d, $J$ = 1.02 Hz, 2H); $^{13}C$ NMR (101 MHz, $CD_3OD$) $\delta$ 32.9 (d, $J_{C,C}$ = 42.2 Hz, $^{13}Me$), 92.1 ($C_{arom}$), 96.8 ($C_{arom}$), 166.1 ($C_{arom}$), 166.5 ($C_{arom}$), 205.0 (d, $J_{C,C}$ = 42.2 Hz, $^{13}C_{carbonyl}$).

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**Supporting Information Available:** Experimental details and NMR spectra for obtained compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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