Biosynthesis of Optically Active Diels–Alder Type Adducts revealed by an Aberrant Metabolism of *O*-Methylated Precursors in *Morus alba* Cell Cultures

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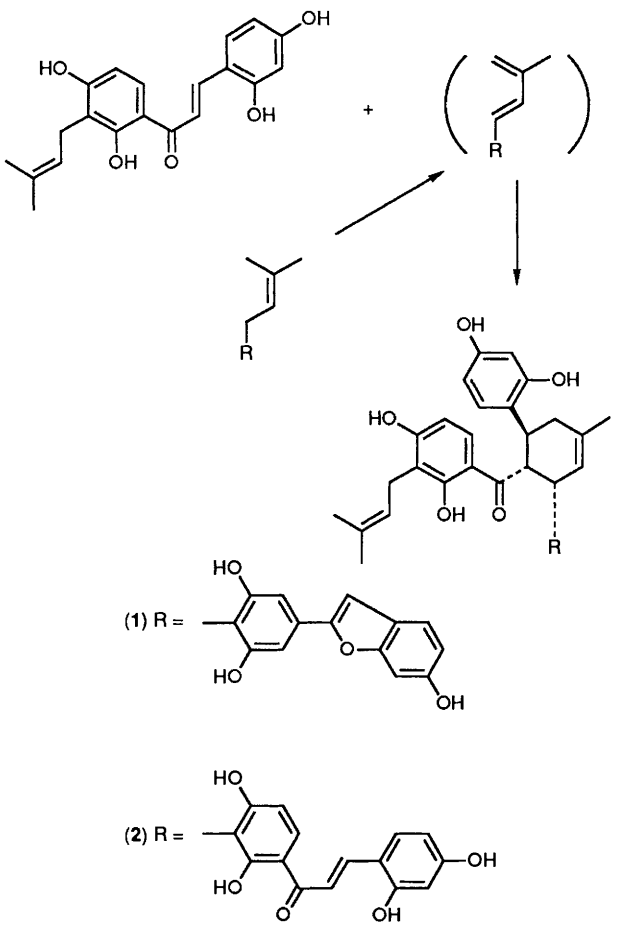
Addition of *O*-methylated chalcone, as precursors, to *Morus alba* cell cultures has resulted in the formation of optically active *O*-methyl derivatives of chalcomoracin (**1**) and kuwanon J (**2**), corroborating the pivotal step of the biosynthesis of (**1**) and (**2**) to be the enzymic Diels–Alder reaction.

Chalcomoracin (**1**),[1,2] kuwanon J (**2**),[3] and their congeners[4] from *Morus alba* L., brosimone A[5] from a Brazilian Moraceous plant *Brosimopsis oblongifolia*, and (−)-flavoskylin[6] produced by *Penicillium sp.* have been considered to be intermolecular Diels–Alder type adducts.

A preliminary study[7] in which [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetates were added to *M. alba* cell cultures, which produce Diels–Alder type adducts including (**1**) and (**2**) in very high yields,[2] revealed that both optically active Diels–Alder type adducts are formed through the condensation of two



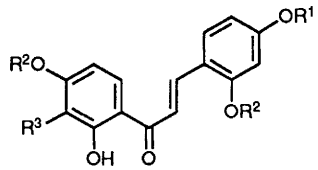
Scheme 1. Enzymic Diels–Alder type cycloaddition reaction yielding chalcomoracin (**1**) and kuwanon J (**2**).

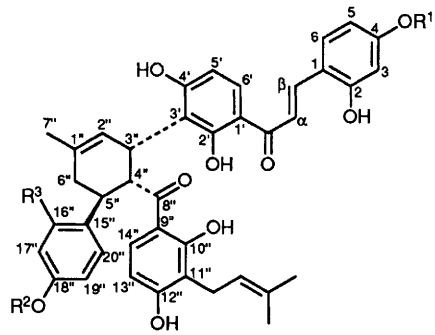
cinnamoylpolyketide-derived skeletons. Recently, solanapyrones from *Alternaria solani* have been found to be biosynthesized *via* an intramolecular Diels–Alder reaction.[8]

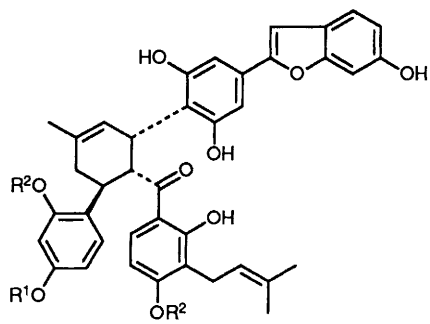
This paper describes one of the later stages in the biosynthesis of optically active mulberry Diels–Alder type adducts. *O*-Methylated chalcones, as precursors, were added with Tween 80 to *M. alba* cell cultures [in which there were no detectable *O*-methylated chalcones (**3**), (**4**), and (**9**) or Diels–Alder type adducts] suspended in sterilized water and the suspension was shaken in the dark at 25 °C. After incubation for 7 days, the cells were harvested and lyophilized. Extraction of the dry cells with methanol followed by the usual work up yielded the metabolites.

Addition of 2,2′,4′-trihydroxy-4-methoxychalcone (**3**) (42.6 mg) yielded 2,2′,4′-trihydroxy-4-methoxy-3′-prenylchalcone (**4**) (2 mg), 4-*O*-methylkuwanon J (**5**) (3 mg), 4-*O*-methylkuwanon Q (**6**) (3 mg), 4,18″-di-*O*-methylkuwanon J (**7**) (3 mg), and 18″-*O*-methylchalcomoracin (**8**) (4 mg) as metabolites. The metabolite (**4**)† was identified by comparison with a known sample of (**4**). The structures of optically active Diels–Alder type metabolites (**5**), (**6**), (**7**), and (**8**)† were obtained by comparison of their spectroscopic data with those of (**1**) and (**2**).

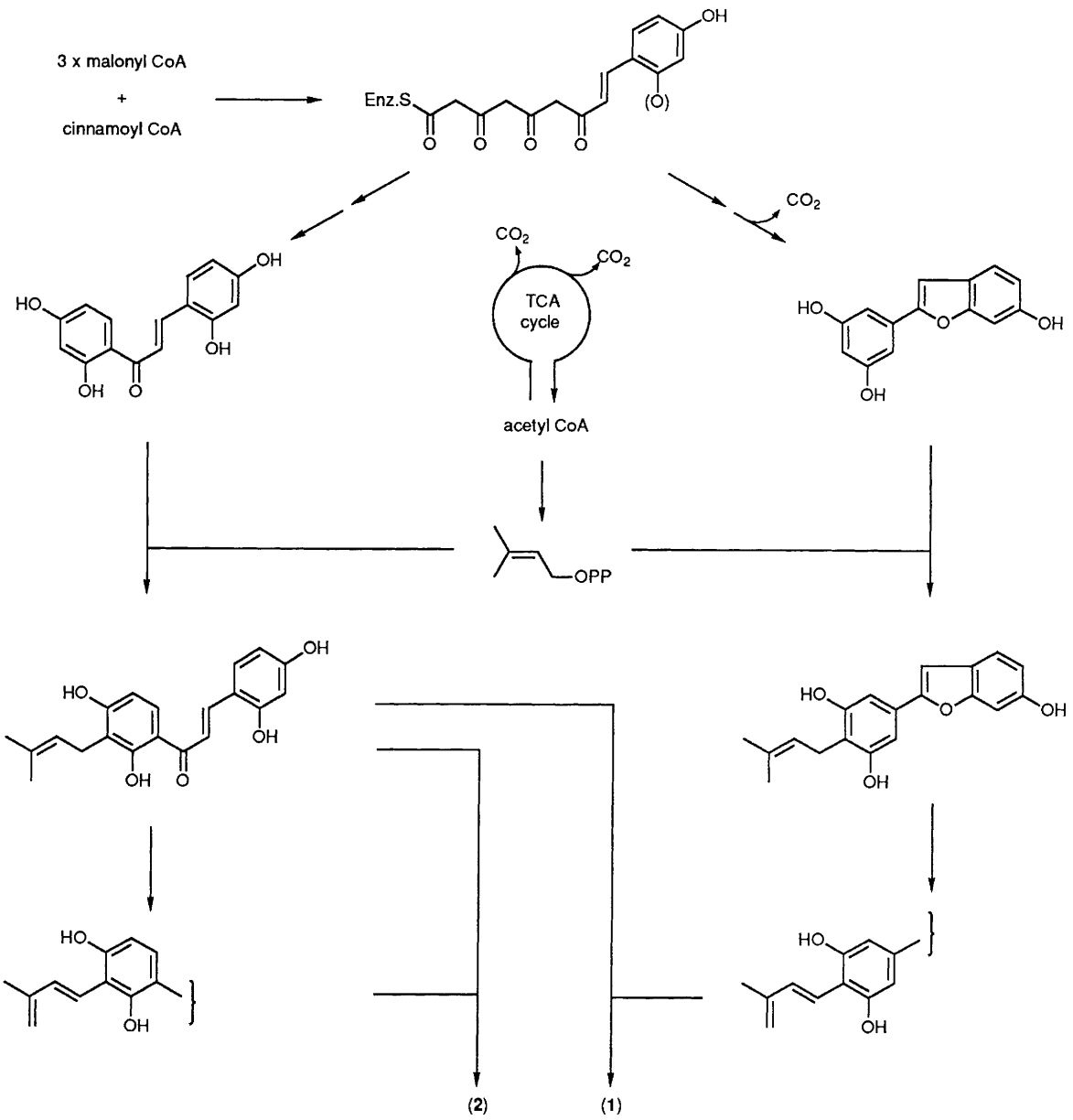
The structure of (**4**) indicates that prenylation takes place after aromatization of the cinnamoylpolyketide-derived chalcone skeleton. Metabolites (**5**), (**6**), (**7**), and (**8**) revealed that the two molecules of precursory chalcone (**3**) were incorporated intact into the optically active Diels–Alder type adducts.







† *Selected spectroscopic data* for (**4**): EI MS, *m/z* 354 (M^+); ^{1}H NMR δ 3.82 (4-OMe). For (**5**): FAB MS, *m/z* 693 (MH^+); $[\alpha]_D^{23}$ +26° (*c* 0.16, EtOH); ^{1}H NMR δ 3.80 (4-OMe). For (**6**): FAB MS, *m/z* 677 (MH^+); $[\alpha]_D^{23}$ +133° (*c* 0.075, EtOH); ^{1}H NMR δ 3.80 (4-OMe). For (**7**): FAB MS, *m/z* 707 (MH^+); $[\alpha]_D^{23}$ + 28° (*c* 0.085, EtOH); ^{1}H NMR δ 3.80 (4-OMe), 3.74 (18″-OMe). For (**8**): FAB MS *m/z* 663 (MH^+); $[\alpha]_D^{23}$ +152° (*c* 0.088, EtOH); ^{1}H NMR δ 3.73 (18″-OMe). For (**10**): FAB MS, *m/z* 691 (MH^+), $[\alpha]_D^{23}$ +104° (*c* 0.083, EtOH); ^{1}H NMR δ 3.78, 3.93, 4.02 (16″-, 18″-, and 12″-OMe).



TCA = tricarboxylic acid; CoA = coenzyme

Scheme 2. Proposed biosynthetic pathway of chalcomoracin (**1**) and kuwanon J (**2**).

Furthermore, in an analogous experiment employing 2′-hydroxy-2,4,4′-trimethoxy-3′-prenylchalcone (**9**) (70.7 mg) as a putative precursor, a small amount (0.5 mg) of an optically active Diels–Alder type adduct (**10**)† was obtained as the metabolite.

These results strongly suggest that a molecule of prenylated chalcone is recognized as a dienophile at the α,β-double bond in the skeleton, while another molecule of the chalcone acts as a diene at the prenyl portion. This is reinforced by the results of the administration of the synthesized prenylchalcone (**4**) to the *M. alba* cell cultures. Incubation of the cell cultures with the *O*-methylated prenylchalcone (**4**) (79.7 mg) gave the Diels–Alder type metabolites (**5**) (3 mg), (**6**) (4 mg), (**7**) (22 mg), and (**8**) (13 mg) as in the case of the experiment using (**3**). This result strongly indicates that dehydrogenation at the prenyl portion of (**4**) followed by the [4π + 2π]cycloaddition reaction with the α,β-double bond of another molecule of prenylchalcone has led to the formation of the adducts described. The *O*-methylated Diels–Alder type adducts (**5**), (**6**), (**7**), (**8**), and (**10**) obtained from *M. alba* cell cultures incubated with the *O*-methylated chalcones are all optically active, having the same stereochemistries as those of chalcomoracin (**1**) and kuwanon J (**2**), as shown by their identical CD spectra. This confirms that the cycloaddition reaction in the cell cultures is enzymatic.

In conclusion, the aberrant biosynthesis of Diels–Alder type adducts, methylated at specified positions, from precursor *O*-methylated chalcones in *M. alba* cell cultures has shown the pivotal process in the formation of optically active mulberry constituents chalcomoracin (**1**) and kuwanon J (**2**) to

be an enzymic Diels–Alder type cycloaddition reaction (Scheme 1). From these findings and the results of the incorporation of acetates labelled with ^{13}C, the biosynthesis of (**1**) and (**2**) are envisaged as shown in Scheme 2.

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