A Novel Way of determining the Structure of Artonin I, an Optically Active Diels–Alder Type Adduct, with the Aid of an Enzyme System of *Morus alba* Cell Cultures

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The structure of artonin I, an optically active Diels–Alder type adduct from *Artocarpus heterophyllus*, an Indonesian moraceous plant, was established utilizing the enzyme system of *Morus alba* cell cultures which specifically produce the natural Diels–Alder type adducts, as well as spectroscopic evidence.

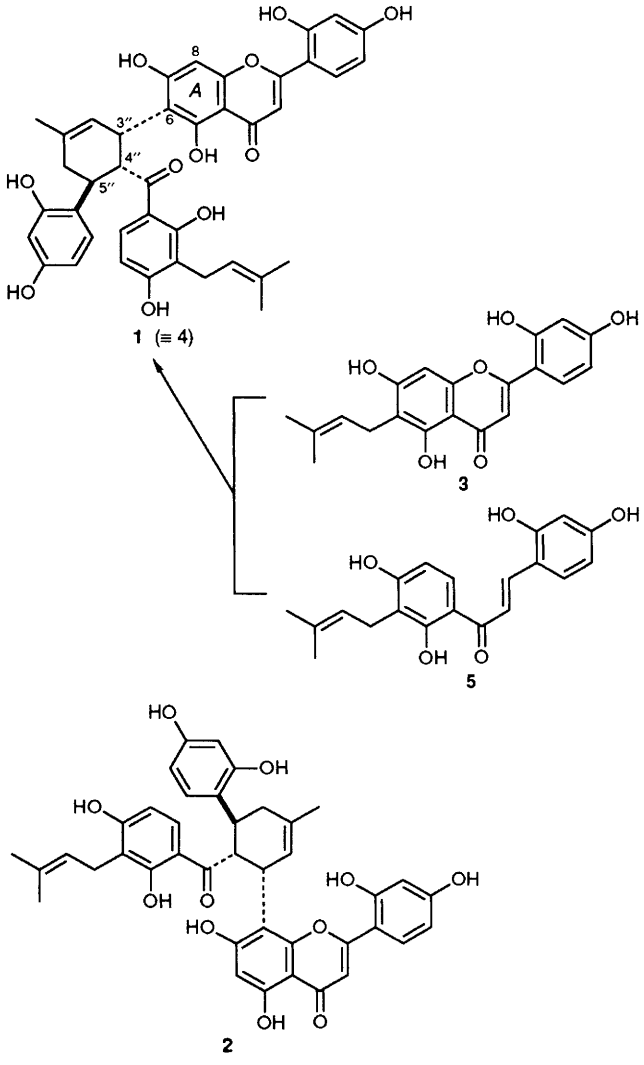
In our continuing studies on a series of isoprenoid-substituted phenolic compounds from moraceous plants, many optically active Diels–Alder type adducts have been isolated from Japanese and Chinese *Morus* plants and also from an Indonesian *Artocarpus* plant.[1,2] Further extensive investigation on the biosynthesis of the Diels–Alder type adducts using *M. alba* cell cultures revealed that the adduct is composed of two molecules of cinnamoylpolyketide-derived skeletons,[3] and that an enzymic Diels–Alder reaction is the pivotal step in the biosynthesis of the adducts.[4] *M. alba* cells induced from the seedlings or the leaves were subjected to selection, giving rise to cell lines which produce optically active Diels–Alder type adducts in high levels.[5] Furthermore, the cell cultures produce various *O*-methylated Diels–Alder type adducts by aberrant metabolism of *O*-methylated precursors.[4]

This paper describes a novel structural determination of an optically active Diels–Alder type adduct, artonin I, isolated from an Indonesian moraceous plant, *A. heterophyllus*, with the aid of the enzymic synthesis of the title compound as an aberrant metabolite through the administration of a putative precursor **3** to the *M. alba* cell cultures.

Artonin I **1** {a yellow powder, $[\alpha]_D$ + 95 (*c* 0.05, acetone)} was isolated as a minor component (0.7 mg, 3.5×10^{-6}% yield from the root bark) from the methanol extract of the root bark of *A. heterophyllus* by successive purification on silica gel column chromatography and HPLC. It showed MH^+ at *m/z* 693 in its fast-atom bombardment mass spectrum (FAB MS). In its ^1H NMR spectrum in [$^2H_6$]acetone, signals ascribable to 6- or 8-substituted 2′,4′,5,7-tetrahydroxyflavone, 2,4-dihydroxyphenyl, 3-(3,3-dimethylallyl)-2,4-dihydroxybenzoyl, and methylcyclohexene ring† moieties were observed, suggesting artonin I to be a typical Diels–Alder type adduct of an isoprenylated flavone derivative and an isoprenylated chalcone derivative. From these data and studies on a series of optically active mulberry Diels–Alder type adducts, two possible formulae (**1** and **2**) may be proposed for artonin I. The co-occurrence of artocarpesin **3**[6] in the same plant led us to the presumption that formula **1** is preferable to formula **2** on the basis of biogenetic speculation.

In order to confirm this presumption, artocarpesin **3** (50.3 mg), as a precursor, was added to the *M. alba* cell cultures

† Selected spectroscopic data for **4**: ^1H NMR ([$^2H_6$]acetone): methylcyclohexene ring, δ 1.93 (3H, br s), 2.25, 2.49 (each 1H br d, *J* 18 Hz), 3.83 (1H, m), 4.15 (1H, br), 4.67 (1H, t, *J* 5 Hz), 5.66 (1H, br s).



suspended in sterilized water and Tween 80. The suspension was shaken in the dark at 25 °C for 7 days. The cells were harvested, lyophilized and extracted with methanol. Conventional work-up of the methanolic extract yielded an aberrant metabolite **4** (8 mg) along with the usual constituents, chalcomoracin[7,8] and kuwanon J.[5]

The metabolite **4** {a yellow powder, $[\alpha]_D$ + 91 (*c* 0.075, acetone)} showed MH^+ at *m/z* 693 in its FAB MS and the ^1H NMR spectrum coincided with that of artonin I. This result indicates that artocarpesin **3** fed into *M. alba* cells reacted as a diene with 3′-(3,3-dimethylallyl)-2,2′,4,4′-tetrahydroxychalcone **5** which is produced in the cells as the dienophile, resulting in the formation of the $[4_\pi + 2_\pi]$ cycloadduct **4** (≡**1**).

The ^{13}C NMR spectrum of **4** indicated the presence of 40 carbon atoms consisting of 24 aromatic, 14 aliphatic and two carbonyl. Of these, the carbon signal at δ 95.7 due to C–H in the *A* ring of the flavone skeleton can be assigned to C-8 from the chemical shift value, indicating that a methylcyclohexene ring is located at the C-6 position. The above ^{13}C NMR spectrum of **4** also supported the formula **1** for the structure of artonin I.

The stereochemistry of the methylcyclohexene ring of **1** was concluded to be 3″*S*,4″*R*,5″*S* from the relative configuration (*cis–trans*) of the three substituents on the ring and the positive optical rotatory value.[9] Thus, the formula **1** including the absolute configuration was confirmed for the structure of artonin I.

This is the first example of the elucidation of the stucture of an organic natural product by application of an enzymatic synthesis of the target substance with the aid of the cell cultures of a related plant.

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