ISOPRENOID-SUBSTITUTED FLAVONOIDS FROM *ARTOCARPUS* PLANTS (MORACEAE)†

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Abstract - Many phenolic compounds including flavonoids have been isolated from the plants of *Morus* and *Artocarpus* species (Moraceae). Most of *Morus* and *Artocarpus* flavonoids are characterized by the structure bearing an isoprenoid side chain at the C-3 position of flavone skeleton and 2', 4'-dioxygenated (e.g. morusin, **4**) or 2', 4', 5'-trioxygenated (e.g. artonin E, **51**) pattern in the B ring. Particularly, some of *Artocarpus* flavonoids have a unique structure having the C-C linkage between an isoprenoid side chain at the C-3 position and the 6'-carbon of the B ring. These flavones considered to be biogenetically derived from the C-3 isoprenoid-substituted flavones. Some of the *Artocarpus* flavonoids, such as artonin I (**31**), have been regarded as optically active Diels-Alder type adducts. The structure of artonin I (**31**) was established utilizing the enzyme system of *Morus alba* cell cultures which specifically produced the natural Diels-Alder type adducts. Some of the isoprenylated flavonoids from the moraceous plants showed the interesting biological activity. Artonin E (**51**) was a potent inhibitor of arachidonate 5-lipoxygenase and inhibited the release of the TNF-α from BALB/3T3 cells by treatment of okadaic acid, stronger than morusin (**4**) did. This article reviews the chemistry and biological activities of isoprenoid-substituted flavonoids isolated from the root barks and/or barks of *Artocarpus* species.

Contents

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†Dedicated to Professor Koji Nakanishi on the occasion of his 75th birthday.

I. Introduction[1 - 3]

A large number of phenolic compounds from natural sources have been studied by many investigators with structural, biological, and pharmaceutical interests. Among these compounds various isoprenoid-substituted phenolic compounds have been found in the plants. Moraceous plants are rich sources of the isoprenylated phenolic compounds, including flavonoids. Moraceae is a large family comprising sixty genera and nearly 1400 species, including important groups such as *Artocarpus*, *Morus*, and *Ficus*.

Mulberry tree, a typical plant of genus *Morus*, has been widely cultivated for its leaves which serve as indispensable food for silkworm. It's root barks have been used as a Chinese herbal medicine called "So-haku-hi" in Japan, for an antiphlogistic, diuretic, and expectorant. On the other hand, a few pharmacological studies on the mulberry tree demonstrated a hypotensive effect of the extract in rodents. Considering these reports, it was suggested that the hypotensive constituents would be a mixture of many phenolic compounds. Our interests were focused on the phenolic constituents of the mulberry tree. So, we have studied phenolic compounds of the mulberry tree and the related plants.

About seventy kinds of new phenolic compounds could be isolated from the Japanese cultivated mulberry tree and Chinese crude drug "Sang-Bai-Pi" (the root bark of Chinese mulberry tree).[1 - 3] Among them, kuwanon G (**1**) was the first isolation of the active substance exhibiting the hypotensive effect from the Japanese *Morus* root bark.[4] Furthermore, kuwanon G (**1**) is considered to be formed through an enzymatic Diels-Alder type reaction of a chalcone and dehydro-kuwanon C or its equivalent (Figure 1).[5] Since that time, about forty kinds of Diels-Alder type adducts, structurally similar to that of **1** have been isolated from *Morus* species. To confirm the biosynthetic route of the mulberry Diels-Alder type adducts, *Morus alba* cell cultures have been utilized.[6] Kuwanon J (**2**)[7] and chalcomoracin (**3**)[8] isolated from the cell cultures have been proved to be enzymatic Diels-Alder reaction products (Figure 2).[2]

Morusin (**4**), a flavone derivative, isolated from the root bark of *Morus alba* L. as a main phenolic constituents, has a structure bearing an isoprenoid moiety at the C-3 position and a 2', 4'-dioxygenated pattern in the B ring (Figure 2).[9] Furthermore, morusin (**4**) had been reported as an antitumor promoter.[10] In a two-stage carcinogenesis experiment, it inhibits tumor promotion by teleocidin on mouse skin initiated with 7, 12-dimethylbenz[*a*]anthracene (DMBA).[10]

On the other hand, the plants of *Artocarpus* species distribute over the tropical and subtropical regions, and have been used as traditional folk medicine so called "Jamu" in Indonesia against inflammation, malarial fever and so on.[11] Many kinds of isoprenoid-substituted phenolic compounds have been isolated from

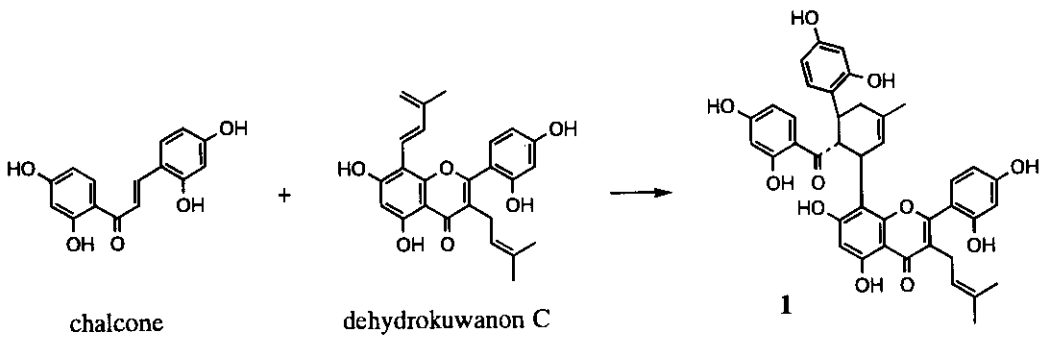


Figure 1 Formation of kuwanon G (**1**) by Diels-Alder reaction

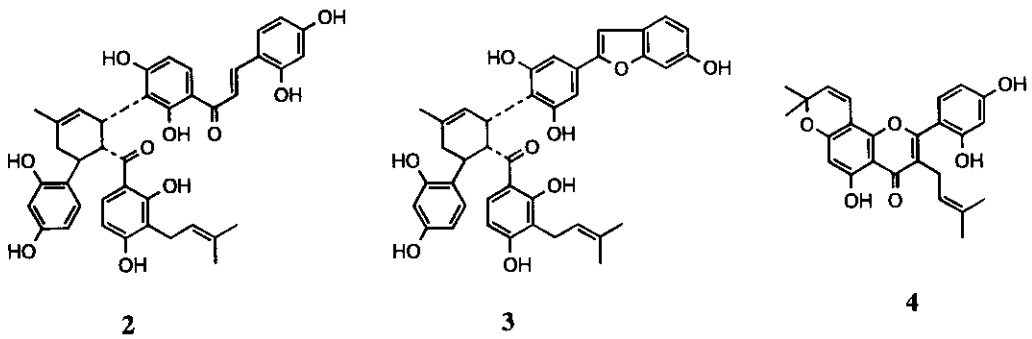


Figure 2 Structures of kuwanon J (**2**), chalcomoracin (**3**), and morusin (**4**)

Artocarpus species by Venkataraman's group[12,13] and other several groups.[14 - 16] In continuation of our works on the isoprenoid-substituted phenolic compounds from the moraceous plants, we examined the constituents of Indonesian moraceous plants, *Artocarpus heterophyllus*, *A. communis*, *A. rigida*, *Paratocarpus* (= *Artocarpus*) *venenosa*, and *A. altilis*, a moraceous plant from Sri Lanka. This article reviews the chemistry and biological activities of the isoprenoid-substituted flavonoids isolated from the root barks and/or barks of *Artocarpus* species by our group and other several groups.

II. Earlier works on the phenolic constituents of *Artocarpus* species by Venkataraman's group.[12,13]

In earlier works on the phenolic constituents of *Artocarpus* species, Venkataraman *et al.* isolated a series of flavonoids with isoprenoid-substitutents which he described in the two review articles.[12,13]

Artocarpus heterophyllus Lamk (*A. integrifolia* L.) is a large ever green tree cultivated through out India, Myanmar, and Sri Lanka for its fruits (Jack-fruit) and for its bright yellow heartwood, which is moderately resistant to decay and is used for cheap furniture and building construction.[13] Perkin and Cope isolated morin (**5**) and cyanomaclurin (**6**) in 1895 from *A. heterophyllus*,[13] it was only in 1963 that a study of the NMR spectrum of the acetate of cyanomaclurin trimethyl ether led to the structure (**6**) for cyanomaclurin.[17] *A. heterophyllus* heartwood proved to be a rich source of flavones with two unique features: the β-

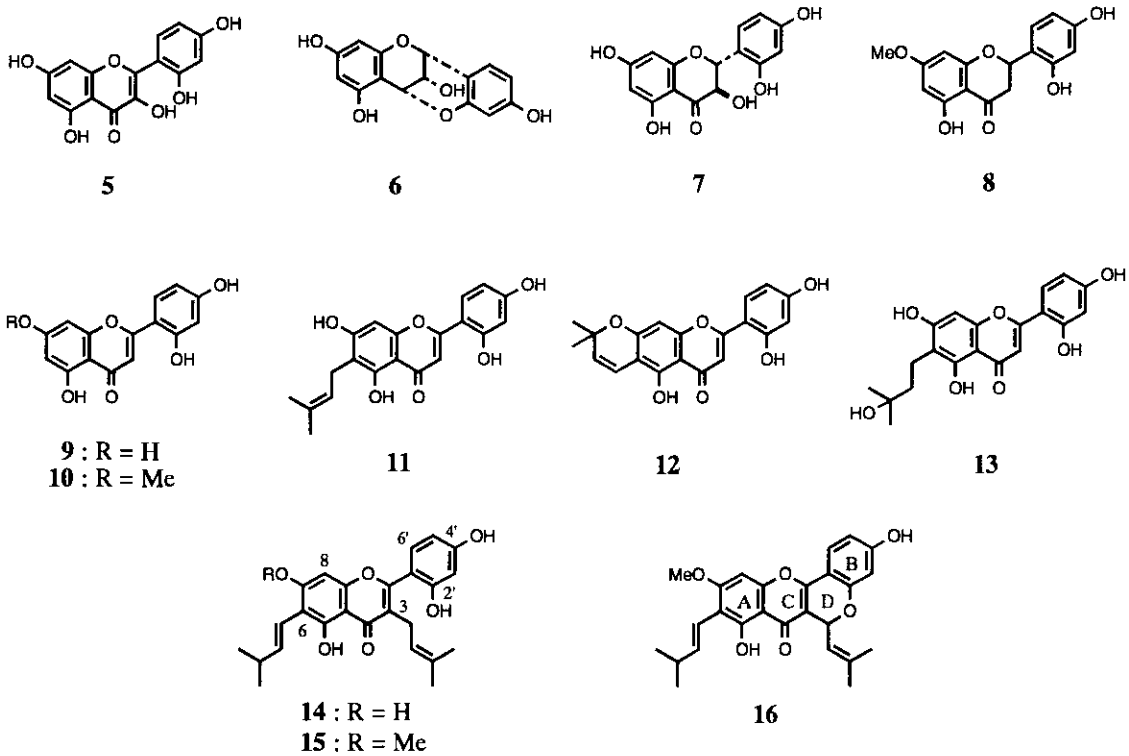


Figure 3 Structures of Flavonoids from *A. heterophyllus* by Venkataraman's group

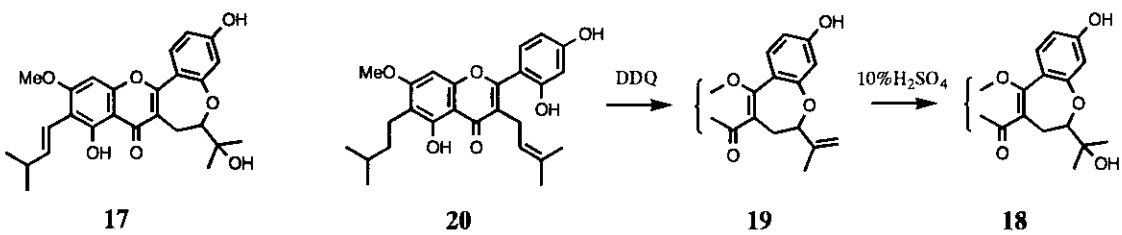


Figure 4 Structure of chaplasin (**17**)

resorcilic acid orientation of hydroxyl groups in the B ring and the presence of one or more isoprenoid groups at the 3-, 6-, and 8-positions.[12,13] In addition to the previously known dihydromorin (**7**), the flavanone, artocarpanone (**8**),[18] and the following eight flavones (**9** - **16**) were isolated;[12,13] norartocarpetin (**9**),[19] artocarpetin (**10**),[18] artocarpesin (**11**),[19] cycloartocarpesin (**12**),[20] oxydihydroartocarpesin (**13**),[20] norartocarpin (**14**),[13] artocarpin (**15**),[21] cycloartocarpin (**16**)[22] (Figure 3). The structure of artocarpin (**15**), which first became available, was proved by chemical methods involving ozonolysis and ultimately by the synthesis of tetrahydroartocarpin dimethyl ether.[23]

Two characteristic features in the UV and NMR spectra of artocarpin (**15**) and cycloartocarpin (**16**) are dependent on the substituents at the 3-position. The 3-prenyl group in **15** forces the 2-phenyl group out of plane with the chromone ring, on the other hand, the rings A, C, D, and B of **16** are coplanar. Consequently, the UV spectra of **15** and **16** showed the absorption maxima at 324 and 370 nm, respectively. In the NMR spectrum of **15** and **16**, the 6'-H signal of **16** appears at the normal position (δ 7.8 ppm), but the corresponding proton of **15** undergoes an upfield shift to δ 7.08 ppm. These spectral data is utilized to structure determination of related compounds.[12,23,24]

The heartwood of *A. chaplasha* Roxb. gave chaplashin (**17**) containing oxepine ring whose structure was derived by UV, ^{1}H-NMR, and mass spectra (MS) and by synthesis of racemic dihydrochaplashin (**18**) from the compound (**19**) obtained by the action of DDQ on dihydroartocarpin (**20**) (Figure 4).[25]

Under the bark of the main trunk of *A. heterophyllus*, specially very old tree, a deep red-brown powder was noticed. From this powder or the whole bark, two new flavones, cycloheterophyllin (**21**) and heterophyllin (**22**), were isolated in very minute quantities, and the structure (**21**) of the relatively major pigment was established. The second pigment, heterophyllin, was very probably the structure (**22**).[26] Furthermore, from the red powder, isocycloheterophyllin (**23**) having a dihydro-oxepine ring was isolated.[27] From the heartwood of *A. integer* Thunb., Indonesian species, cyclointegrin (**24**), integrin (**25**), oxyisocyclointegrin (**26**) were isolated; cyclointegrin (**24**) was the first naturally occurring flavone containing an oxocin ring system (Figure 5).[28] All of these flavonoids are characterized by β-resorcylic acid orientation of the hydroxyl groups in the B ring. From the chemotaxonomic point of view the flavonoids containing the isoprenoid at the C-3 position characterized some species of the genera *Artocarpus* and *Morus* of the family Moraceae.

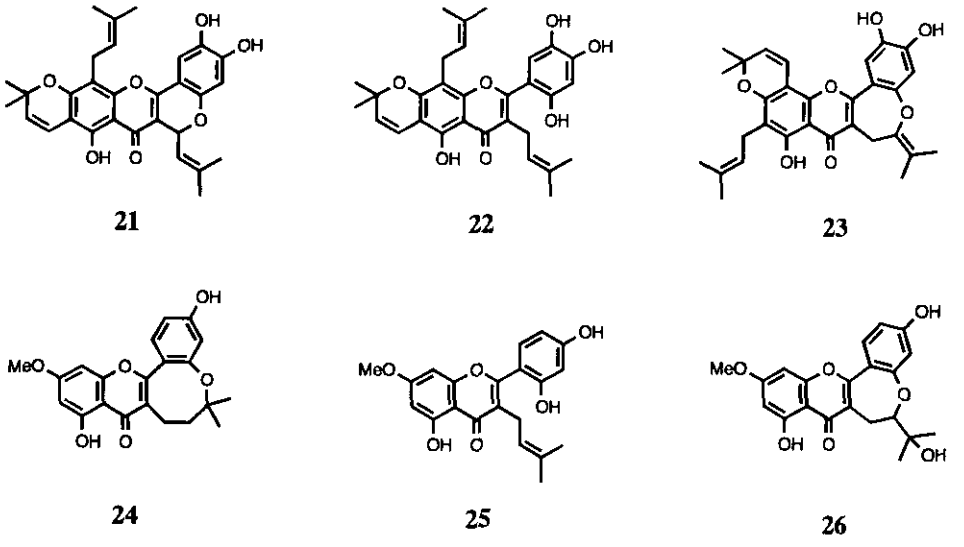


Figure 5 Structures of isoprenoid-substituted flavones

III. Structures of isoprenoid-substituted phenolic compounds from *Artocarpus* species.

III-1. Isoprenoid-substituted phenolic compounds from *A. heterophyllus*.

Eight kinds of new isoprenoid-substituted phenolic compounds, artonins A - D (**27** -**30**),[29,30] I - L (**31** -**34**),[31,32] were isolated from the root bark of *A. heterophyllus* along with two known compounds, heterophyllin (**22**),[26] and cycloheterophyllin (**21**).[26] From the bark of *A. heterophyllus*, six kinds of new phenolic compounds, artonins Q - U (**35** - **39**)[33] and artonin X (**40**),[34] along with a known compound, kuwanon R (**41**)[7] were isolated by our group (Figure 6).

On the other hand, Lin *et al.* have isolated a series of new phenolic compounds from the root bark or the root of *A. heterophyllus*, and elucidated the structures, i.e., heteroflavanones A (**42**),[35] B (**43**),[35] C (**44**),[36] cycloartocarpin A (**45**),[36] artocarpanone A (**46**),[37] artocarpetin A (**47**),[37] artocarpetin B (**48**),[15] and heteroartonin A (**49**),[15] along with a novel phenolic compound, heterophylol (**50**)[38] (Figure 7).

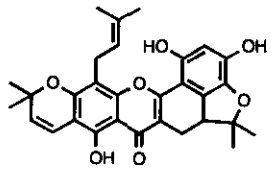
The compounds isolated by our group, except some ones, have a characteristic structures bearing an isoprenoid side chain at the C-3 position of flavone skeleton, and B ring has a 2', 4', 5'-trioxygenated pattern.

III-2. Isoprenoid-substituted phenolic compounds from *A. communis*.

From the bark of *A. communis*, our group reported the seven kinds of new isoprenoid-substituted flavonoids, artonins E (**51**),[39] F (**52**),[39] artonols A (**53**),[40] B (**54**),[40] C (**55**),[40] D (**56**),[40] and E (**57**)[40] along with three known compounds (Figure 8). Artonin E (= 5'-hydroxymorusin, **51**) exhibited the potent inhibition on arachidonate 5-lipoxygenase and the interesting biological activities. Details are described in Chapter VI. Artonols A (**53**) and B (**54**) have unique structures. Biogenetically the two compounds seem to be the derivatives from the dihydrobenzoxanthone, such as artobiloxanthone (**58**), as follows: artonol A (**53**) is assumed to be derived from dihydrobenzoxanthone hydrate (**58'**) through a *retro*-Diels-Alder reaction as shown in Figure 9. On the other hand, artonol B (**54**) is assumed to be derived from xanthone having a five membered cyclic ketone ring (**58"**) through the oxidative reaction as shown in Figure 10.[40]

Lin and Shieh's group reported nine kinds of new isoprenoid-substituted flavonoids from the root bark of this plant, i.e., cycloartomunin (**59**),[41] dihydrocycloartomunin (**60**),[41] cycloartomunoxanthone (**61**),[41] artomunoxanthone (**62**),[42] artomunoxanthentrione (**63**),[42] artomunoxanthotrione epoxide (**64**),[43] cyclocommunol (**65**),[44] cyclocommunin (**66**),[44] and dihydroisocycloartomunin (**67**)[44] (Figure 11). The cytotoxicity of these flavonoids isolated by the group was studied and the details of the results is described in Chapter VI.

As the dried flower of *A. communis* has been used as mosquito repellent in Indonesia, Fujimoto *et al.* investigated the biological activities of the alcoholic extract of this flower and found that the extract include some 5-lipoxygenase inhibitors.[45] From the extract, five new isoprenoid-substituted flavonoids, two flavanones, and three dihydrochalcones were isolated,[45] i.e., AC-3-3 (**68**), AC-5-2 (**69**); AC-3-1 (**70**), AC-3-2 (**71**), and AC-5-1 (**72**) (Figure 12). All these compounds exhibited strong inhibitory effects on 5-lipoxygenase. Details of the biological activities of these compounds are described in Chapter VI. From the dried bark of *A. communis* (named Kular), Fujimoto *et al.* reported three new isoprenoid-substituted flavonoids, KB-1, KB-2 (**73**), and KB-3 (= artonin E, **51**) along with known compound, morusin (**4**).[16] The structure proposed for KB-1 is the same one for artobiloxanthone (**58**). All these compounds (**58**, **73**, **51**) inhibited the growth of leukemia cells (L-1210) in tissue culture (IC_{50} = 0.2 - 0.5 μg/mL).



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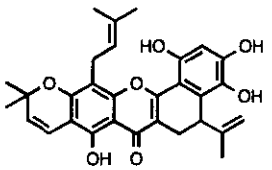

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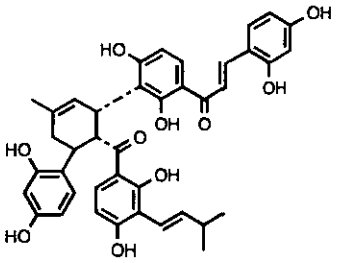

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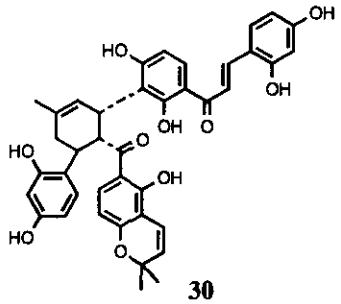

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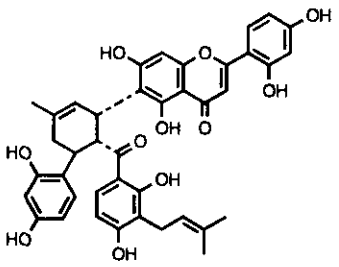

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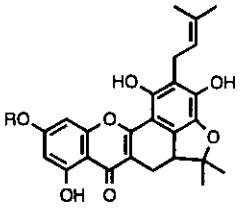
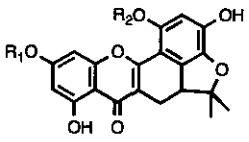
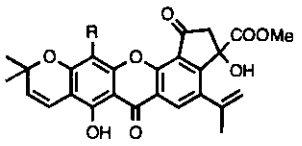


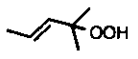
32 : R =H
38 : R = Me



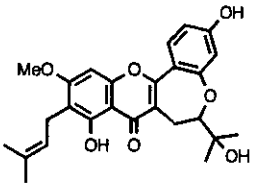
33 : R_1 = Me R_2 = H
34 : R_1 = H R_2 = Me



35: R =



36 : R =



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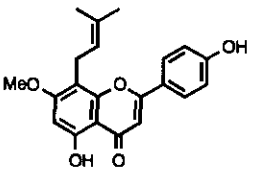

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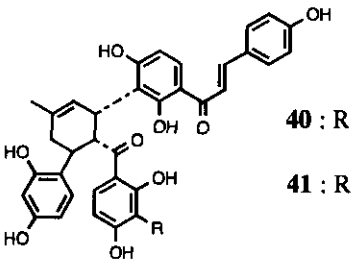


40 : R =

41 : R =

Figure 6 Structures of isoprenoid-substituted flavonoids from *A. heterophyllus*

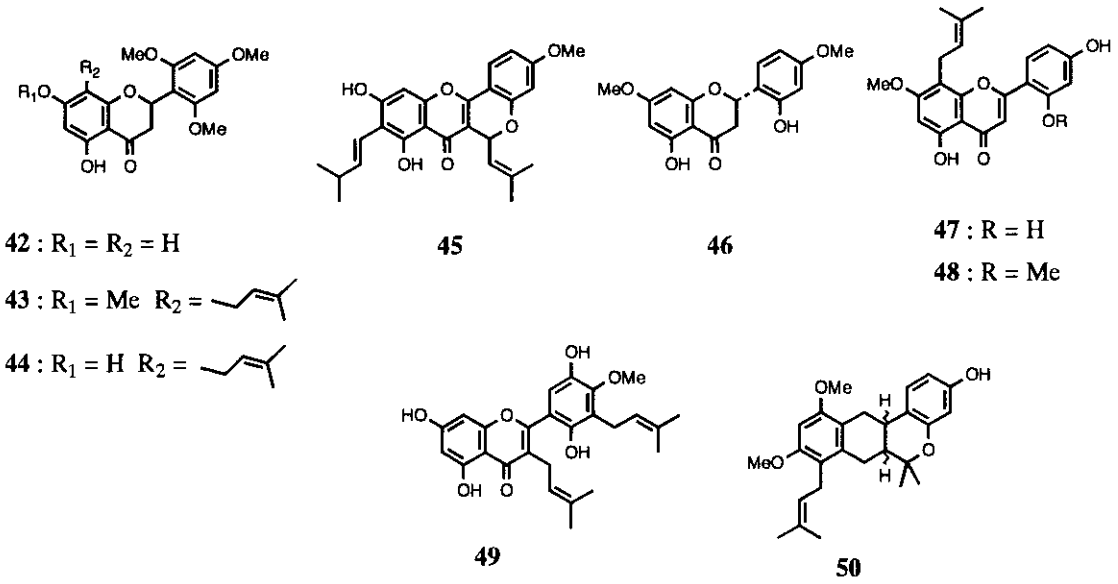


Figure 7 Structures of isoprenoid-substituted phenolic compounds from *A. heterophyllus*

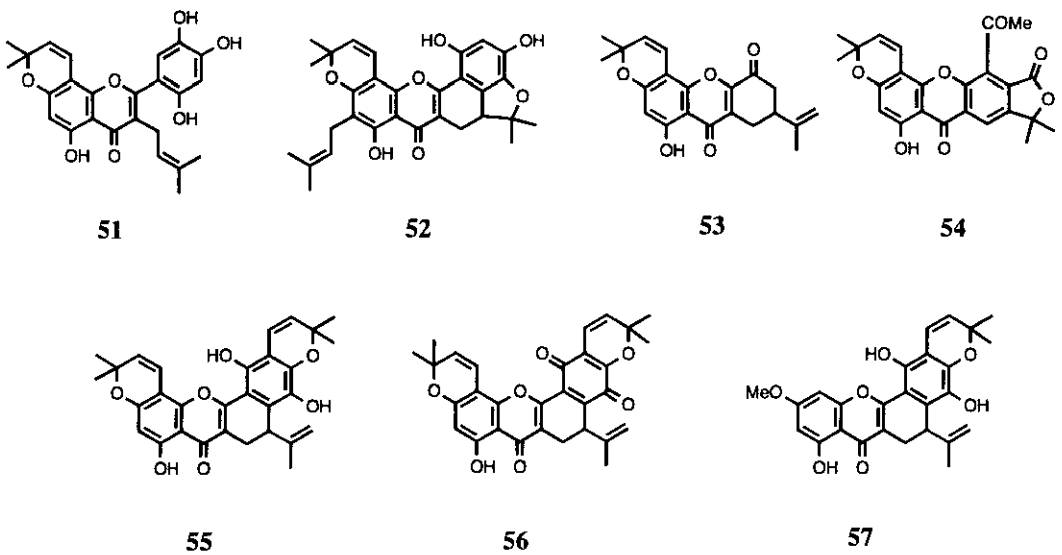


Figure 8 Structures of isoprenoid-substituted phenolic compounds from *A. communis* by our group

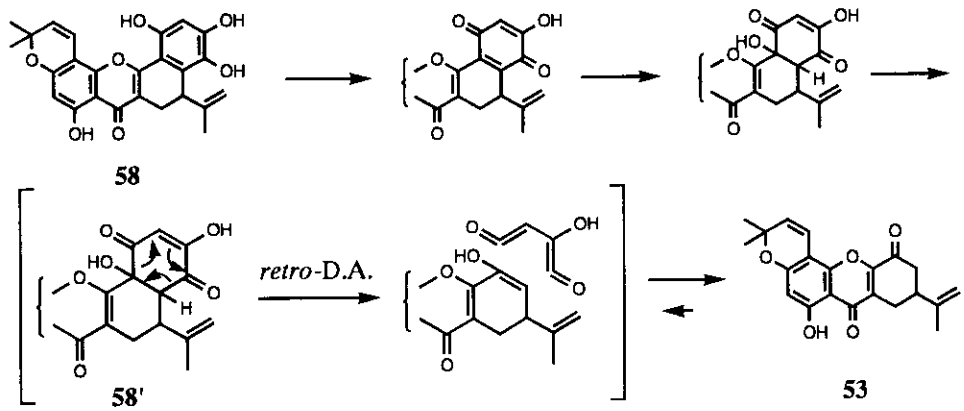


Figure 9 Hypothesis of the biogenetic route to artonol A (**53**) from artobiloxanthone (**58**)

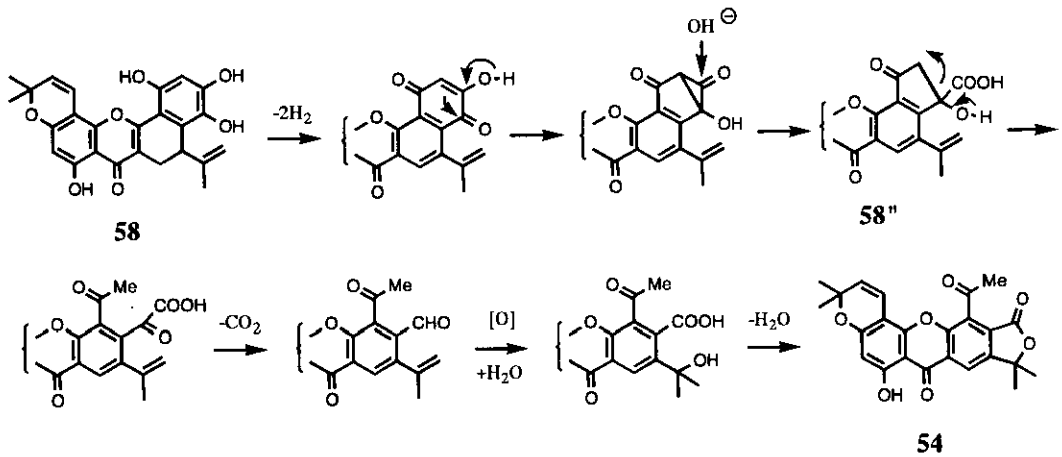


Figure 10 Hypothesis of the biogenetic route to artonol B (**54**) from artobiloxanthone (**58**)

III-3. Isoprenoid-substituted phenolic compounds from *Artocarpus nobilis*

A. nobilis is the only endemic species of the genus *Artocarpus* belonging to the family Moraceae found in Sri Lanka. Earlier investigations of the species yielded five chromeno-flavonoids, which were characterized and named artobilochromen (**74**), chromanoartobilochromen *b* (**75**), (-)-dihydrofuranoartobilochromen *a* (**76**), (-)-dihydrofuranoartobilochromen b_1 (**77**), and dihydrofuranoartobilochromen b_2 (**78**).[46] Artobilochromen (**74**) with formic acid gave chromanoartobilochromens *a* (**75'**) and *b* (**75**), and with DDQ gave (±)-dihydrofuranoartobilochromen *a* (**76**) (Figure 13).[46]

On the other hand, our group studied the ^{1}H-NMR examination of the prenylated flavonoids and reported that the presence of the prenyl group at the C-3, C-6, or C-8 position of flavone can be deduced from the chemical shift of methylene protons of prenyl group measured in acetone-d_6.[47] The application of this ^{1}H-NMR method to the identification of these *Artocarpus* flavonoids was carried out.[47] The methylene proton signal of the prenyl group of artobilochromen (**74**) appears at δ 3.16 (in acetone-d_6). The chemical shift of the compound indicates that the prenyl group exists at the C-3 position. The ^{1}H and ^{13}C-NMR data of

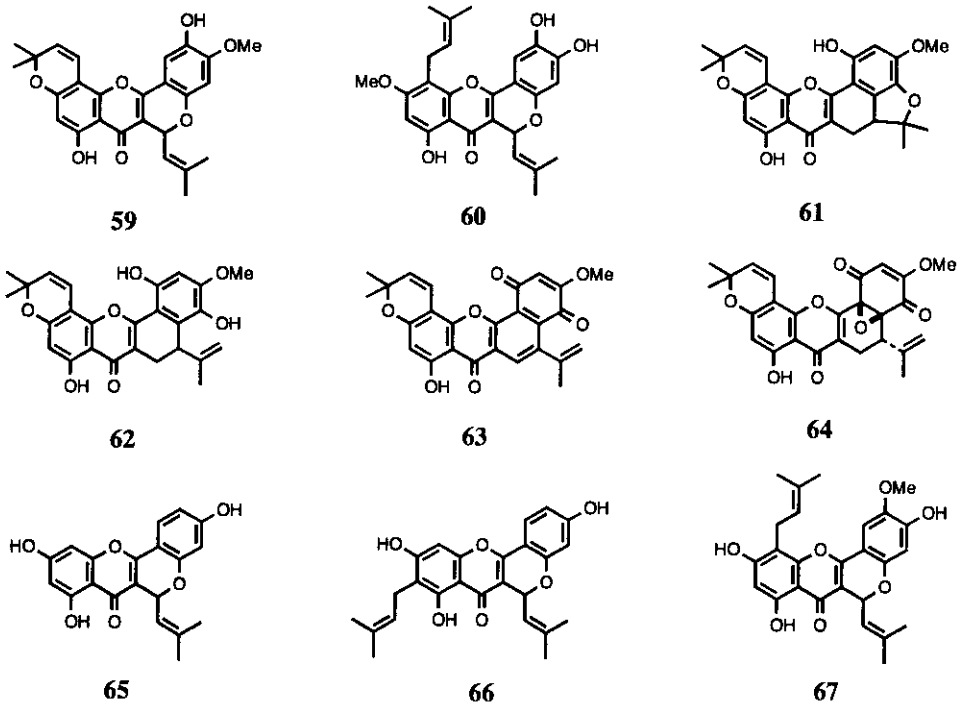


Figure 11 Structures of isoprenoid-substituted flavones from *A. communis* by C. -N. Lin's group

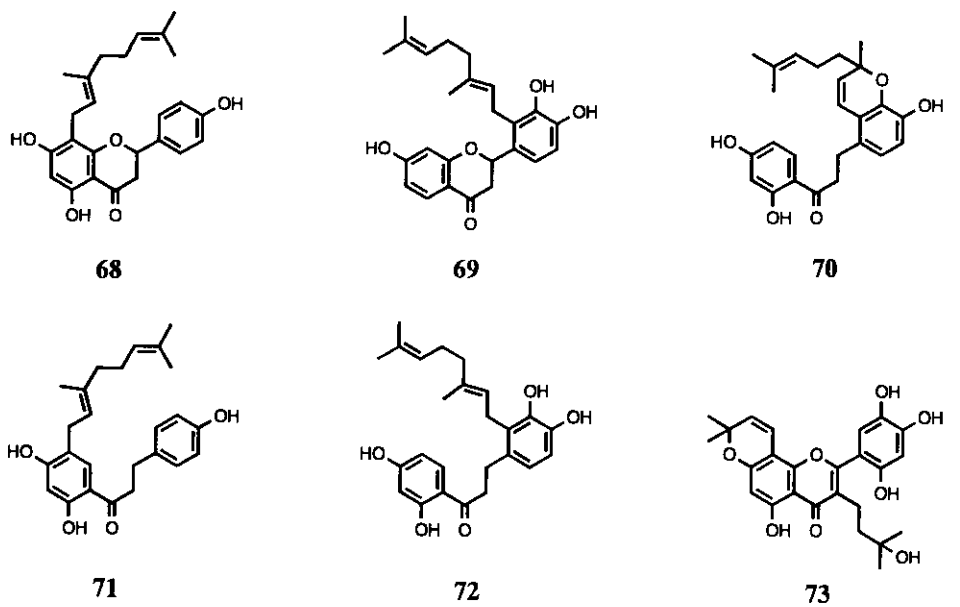


Figure 12 Structures of isoprenoid-substituted flavonoids from *A. communis* by Fujimoto's group

artobilochromen are similar to those of artonin E (= KB-3, **51**). Thus, they are same compounds, and the formula (**74**) must be revised to the structure of artonin E (**51**). As (-)-dihydrofuranoartobilochromen *a* (**76**) and chromanoartobilochromen *b* (**75**) were correlated with artobilochromen (**74**) by chemical methods, the structures of these compounds must be reinvestigated.

Sultanbawa's group reported two novel pyranodihydrobenzoxanthones, artobiloxanthone (**58**) and cycloartobiloxanthone (**79**), along with artobilochromen (artonin E, **51**), and proposed a feasible biosynthetic route for the formation of these dihydrobenzoxanthones from a simple flavonoids as shown in Figure 14.[14] This is the first report of the occurrence of dihydrobenzoxanthone in plants.

On the other hand, our group isolated two prenylflavones, artonins A (**27**) and B (**28**) from *A. heterophyllus* as described in III-1, the structures were determined on the basis of the X-Ray crystallographic analysis, spectroscopic data, and chemical evidence. These two compounds have the unique structure in which the C-C linkage takes place between the C-6' position of the B ring and the C-10

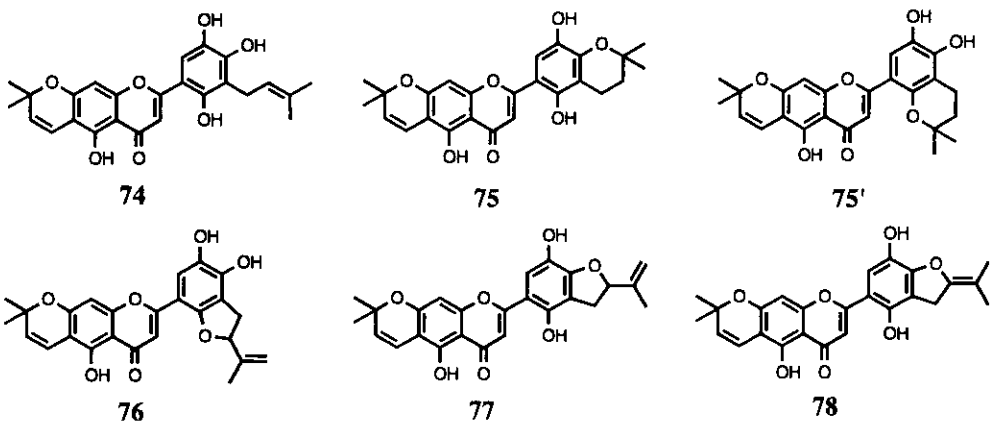


Figure 13 Structures of isoprenoid-substituted flavones from *A. nobilis* in earlier investigations

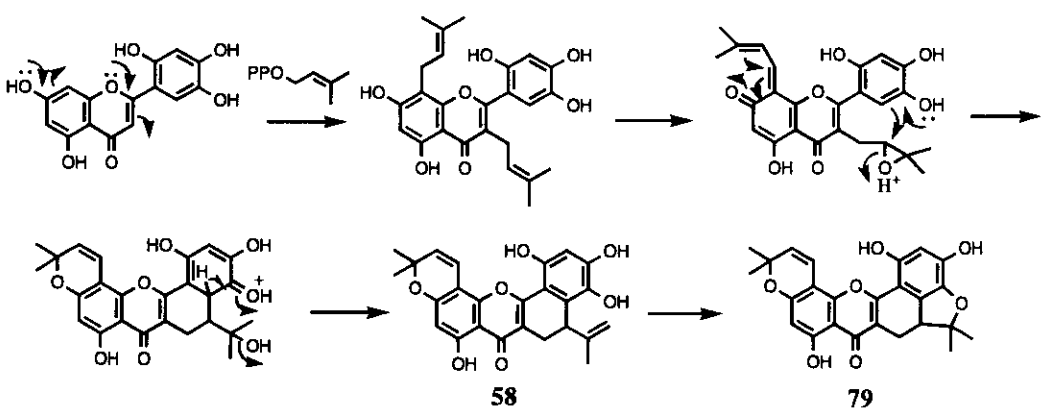


Figure 14 Feasible biosynthetic route for the formation of artobiloxanthone (**58**) and cycloartobiloxanthone (**79**) by Sultanbawa *et al.*

position of isoprenoid moiety located at the C-3 position. Taking no optical activities into the account, artonins A (**27**) and B (**28**) are biogenetically assumed to be derivatives from heterophyllin (**22**) through the oxidative coupling reaction as shown in Figure 15.[29]

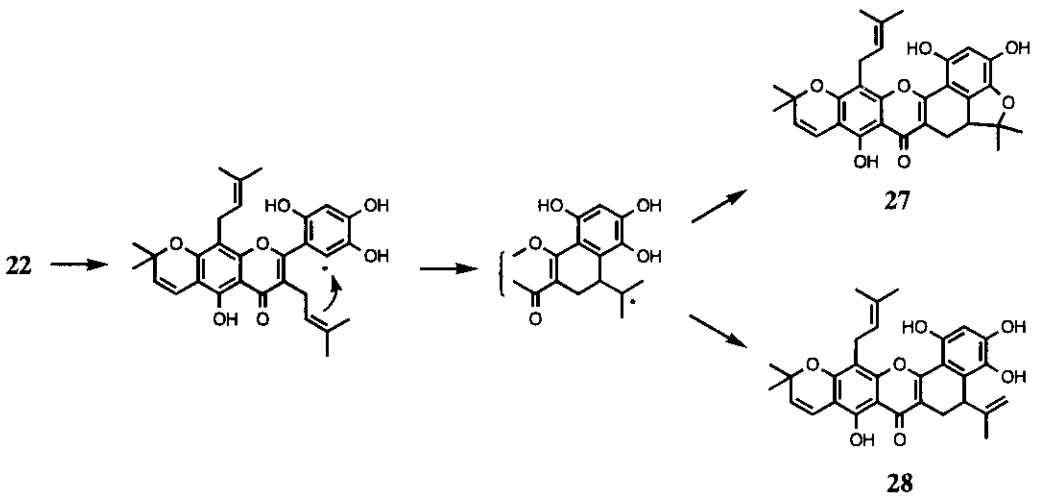


Figure 15 Hypothesis of biogenetic route to artonins A (**27**) and B (**28**) by our group

III-4. Isoprenoid-substituted flavonoids from *Artocarpus rigida*

From the bark of *A. rigida*, six kinds of new compounds, artonins G (**80**),[48] H (**81**),[48] M (**82**),[49] N (**83**),[49] O (**84**),[49] and P (**85**)[49] along with three known compounds, artonin E (**51**), cycloartobiloxanthone (**79**), and artobiloxanthone (**58**) were isolated. On the structures of artonins O (**84**) and P (**85**), the B rings of these compounds were suggested to be *p*-benzoquinone structure. This assumption was supported by the addition reaction of artonin O (**84**) with diazomethane. Fieser *et al.* reported that the reaction of *p*-benzoquinone with one mole of diazomethane gives the adduct, a pyrazol and/or a indazol, in good yield while *o*-benzoquinone gives much poor results in the same reaction.[50] The reaction of **84** with diazomethane afforded a pyrazol product (**84a**) quantitatively, in the structure of which the orientation of the diazomethane addition to the C-1' and 6' positions is not clear. This fact substantiated the B ring of **84** to be *p*-benzoquinone structure (Figure 16).[48,49]

III-5. Isoprenoid-substituted flavonoids from *Paratocarpus* (= *Artocarpus*) *venenosa*

The latex from the seeds of *A. venenosa* Zoll contains poison and it has been used for eradication of rats. From the bark of the plant, seven new isoprenoid-substituted chalcones, paratocarpins A (**86**),[51] B (**87**),[51] C (**88**),[51] D (**89**),[51] E (**90**),[51] F (**91**),[52] and G (**92**),[52] along with five new isoprenoid-substituted flavanones, paratocarpins H (**93**),[52] I (**94**),[52] J (**95**),[52] K (**96**),[52] and L (**97**)[52] (Figure 17).

III-6. Isoprenoid-substituted flavonoids from other *Artocarpus* species (*A. altilis*, *A. elasticus*, and *A. champeden*)

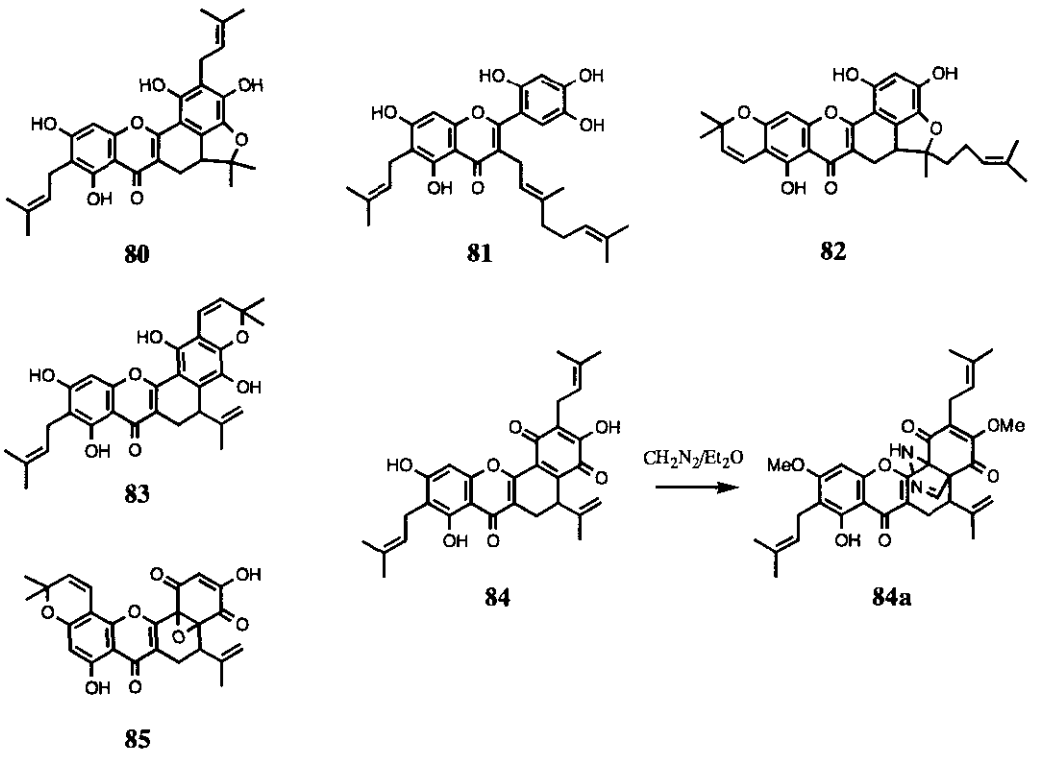


Figure 16 Structures of isoprenoid-substituted flavones from *A. rigida*

The stems and roots of *A. altilis* Fosberg have been used traditionally for the treatment of liver cirrhosis and hypertension in Taiwan, and the plant also has been reported to possess anti-inflammatory and detoxifying effects.[53] From the stem of the plant collected in Taiwan, Chen *et al.* reported three new isoprenoid-substituted flavones, isocyclomorusin (= cudraflavone A,[54] **98**), isocyclomulberrin (= cyclocommunin, **66**), and cycloaltilisin (**99**), along with three known flavonoids, cyclomorusin, cyclomulberrin, and engeletin.[53] On the other hand, our group reported artonin V (**100**) isolated from the root bark of *A. altilis* collected in Sri Lanka.[55] From the wood of *A. elasticus*, Kijjoa *et al.* reported three new isoprenoid-substituted flavones, artelastin (**101**), artelastochromene (**102**), and artelasticin (**103**), along with the known artocarpesin (**11**).[56] Achmad *et al.* reported a new isoprenoid-substituted flavone, cyclochampedol (**104**), isolated from *A. champeden*. The compound (**104**) is active in the brine shrimp lethality assay.[57] While nonisoprenoid-substituted phenolic compound, Yamazaki *et. al.* isolated (±)-catechin (**105**) as an active principle with an inhibitory effect on stress ulcer formation in restrained and water-immersed mice from the leaves of *A. integra*, which is used as a herbal drug in Indonesia[58] (Figure 19).

IV. Formation of dihydrobenzoxanthone skeleton from 3-isoprenylated 2', 4', 5'-trioxygenated flavone.

As described in III-3, *Artocarpus* flavones, some flavones such as artobiloxanthone (**58**), cycloartobiloxanthone (**79**) and artonin M (**82**), have a unique structure involving a dihydrobenzoxanthone

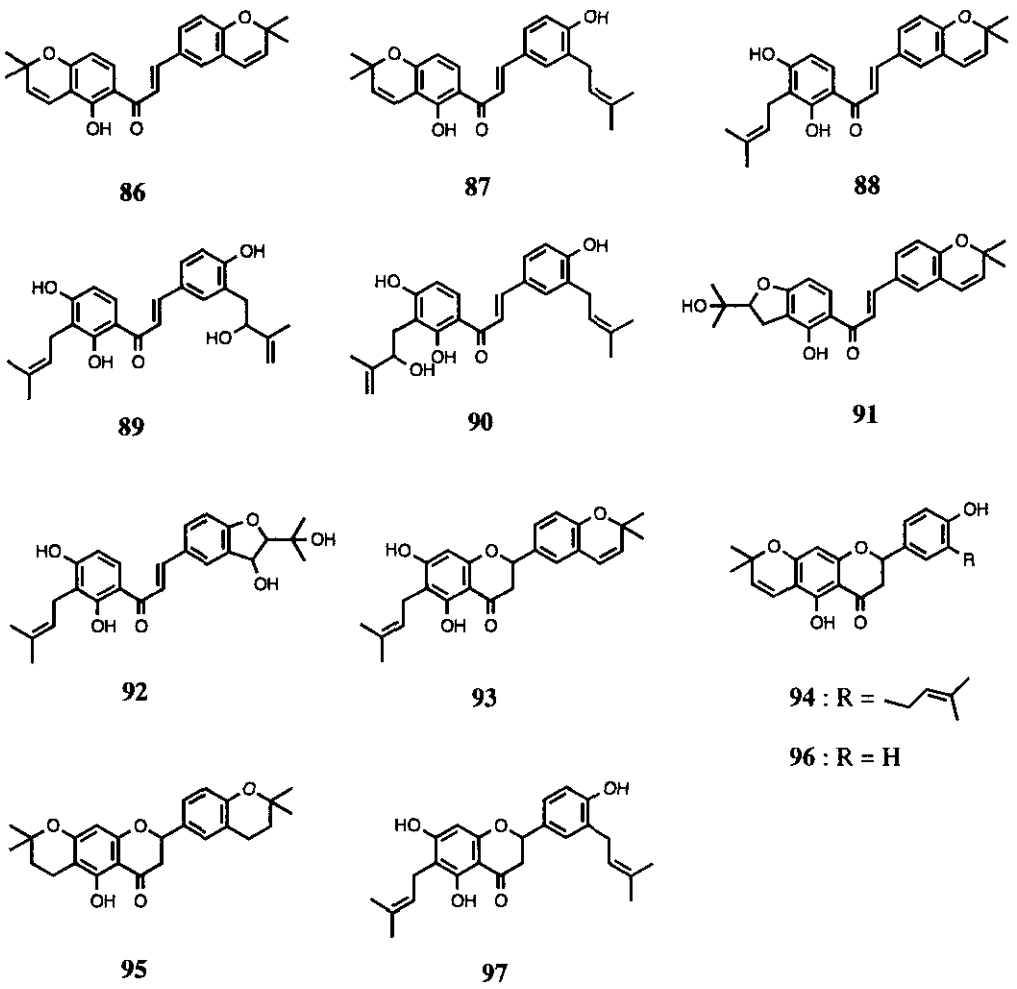


Figure 17 Isoprenoid-substituted chalcones and flavanones from *Paratocarpus* (*Artocarpus*) *venenosa*

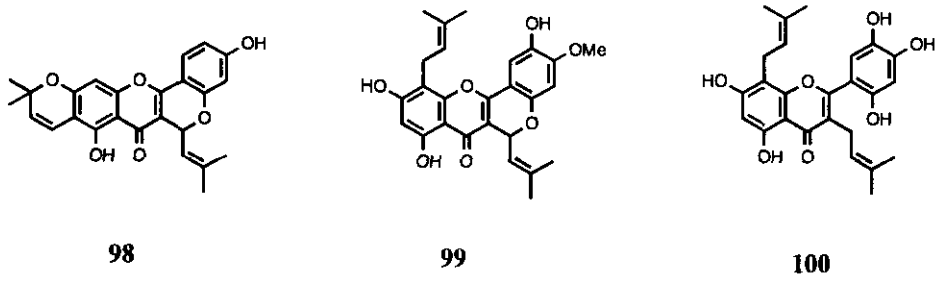
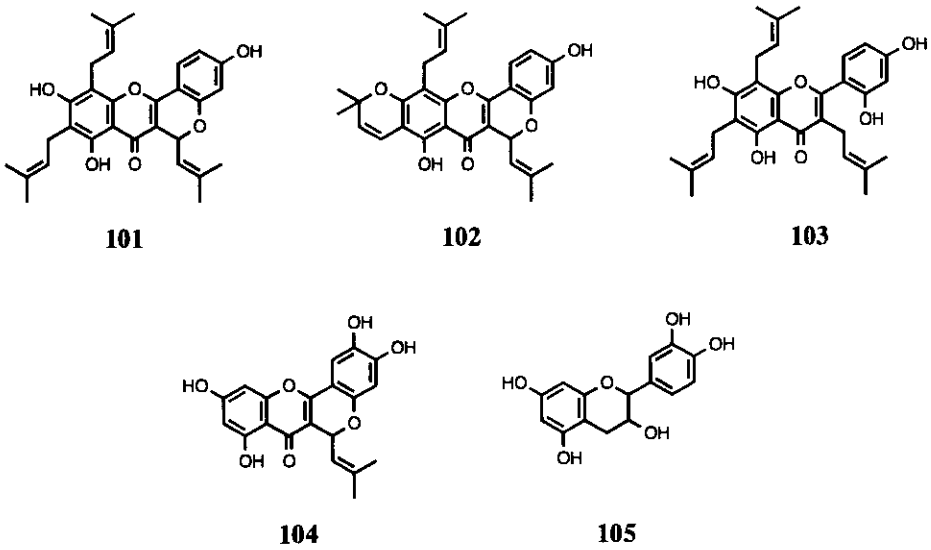


Figure 18 Structures of isoprenoid-substituted flavones from *A. altilis*



101 **102** **103**

104 **105**

Figure 19 Structures of isoprenoid-substituted flavonoids from plants of *Artocarpus* species

skeleton, in which the C-C linkage takes place between the C-6' position of the B ring and the C-10 position of isoprenoid moiety located at the C-3 position. Taking no optical activities into the account, the flavones having the dihydrobenzoxanthone skeleton are seemed to be biogenetically derived from the 3-isoprenylated 2', 4', 5'-trioxygenated flavones through the phenol oxidative cyclization.[29] We attempted to derive the flavone having the dihydrobenzoxanthone skeleton from the 3-isoprenylated 2', 4', 5'-trioxygenated flavone. Photoreaction of artonin E (**51**) in chloroform solution with high-pressure mercury lamp produced artobiloxanthone (**58**) and cycloartobiloxanthone (**79**). Furthermore, the treatment of artonin E (**51**) with radical reagent (diphenyl picryl hydrazyl: DPPH) resulted in the same products (Figure 20). These results suggest that the photo-oxidative cyclization of artonin E (**51**) may proceed through phenol oxidation *via* the semiquinone radicals described in Figure 21.[59]

On the other hand, the similar oxidative cyclization had been reported by our group.[1,60] When a solution of morusin (**4**) in chloroform was irradiated using high-pressure mercury lamp, morusin hydroperoxide (**106**) was obtained in *ca.* 80 % yield. The reaction mechanism was suggested as follows: morusin (**4**) in the ground state interacts with an oxygen molecular to form a contact charge transfer complex. On irradiation, the complex gives an excited charge transfer state that presumably leads to reactive species such as free radicals as described in Figure 22. Considering these results, the possible reaction mechanism of photoreaction of artonin E (**51**) can be sketched as follows: artonin E (**51**) in the ground state interacts with an oxygen molecule to form a contact charge transfer complex as in the case of morusin (**4**). Irradiation of the complex produces the reaction species such as semiquinone radical, and artobiloxanthone (**58**) and cycloartobiloxanthone (**79**) are derived from the radicals as described in Figure 21.

These findings support that the flavone having the dihydrobenzoxanthone skeleton, such as artobiloxanthone (**58**), cycloartobiloxanthone (**79**), and artonin M (**82**), are biogenetically derived from the 3-isoprenylated 2', 4', 5'-trioxygenated flavone, such as artonin E (**51**) and artonin H (**81**), through the phenol oxidative cyclization (Figure 21). On the other hand, the 3-isoprenylated 2', 4'-dioxygenated flavones, such as morusin (**4**), give the hydroperoxide (**106**) having a dihydrooxepine ring under the same

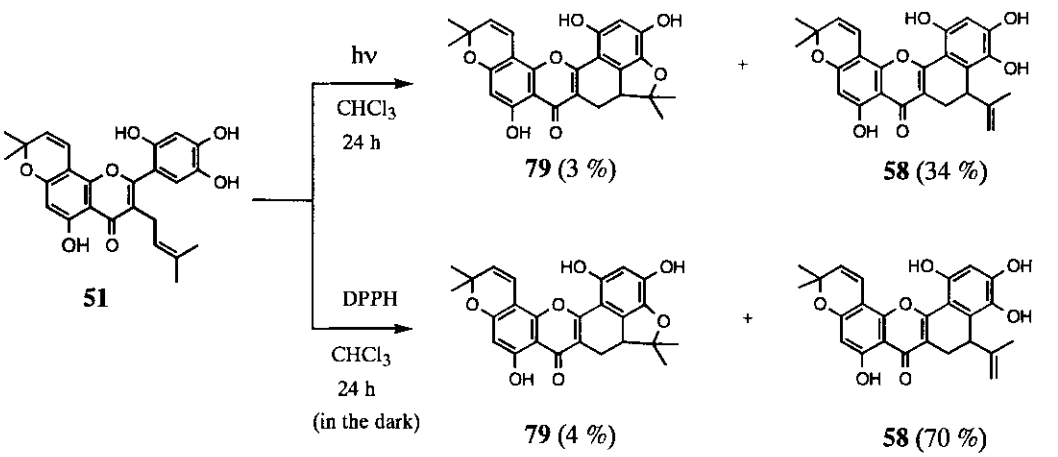


Figure 20 Photoreaction of artonin E (**51**) and the reaction with radical reagent

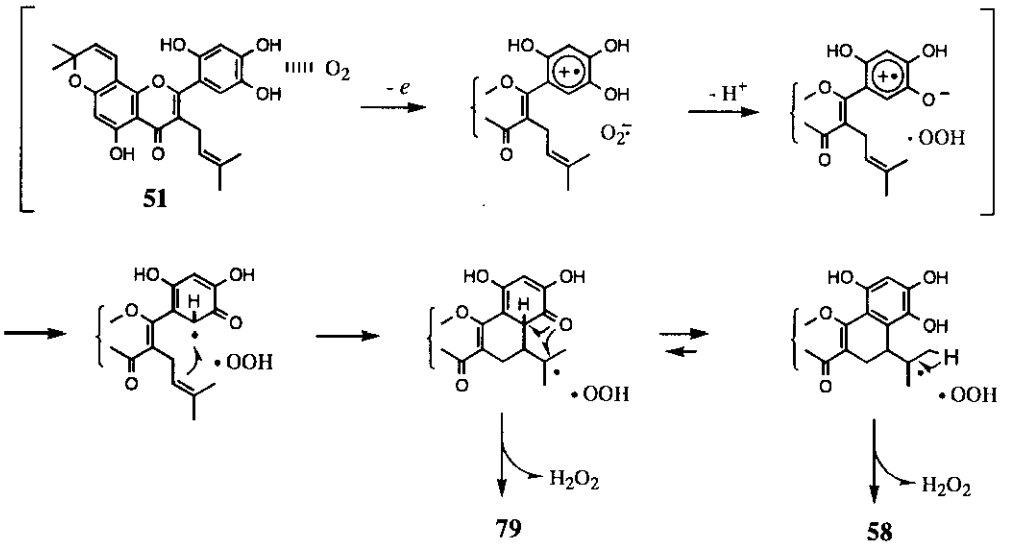


Figure 21 Plausible mechanism for the formation of artobiloxanthone (**58**) and cycloartobiloxanthone (**79**) from artonin E (**51**)

condition (Figure 22).[59] The flavones having the dihydrobenzoxanthone skeleton are characteristic constituents of *Artocarpus* species, since they have never been observed in other species.[59]

V. Diels-Alder type adducts from *Artocarpus* species.

Some of the *Artocarpus* flavonoids, such as artonins C (**29**), D (**30**), I (**31**), X (**40**), and R (**41**), have been regarded as intermolecular [4 + 2] cycloaddition products from the isoprenyl portion of a dehydroprenyl-phenol, as a diene, and the α, β-unsaturated bond of a chalcone skeleton, as a dienophile.[1-3] In the case of adducts from higher plants, the mulberry Diels-Alder type adducts, such as kuwanon G (**1**), are the most characteristic components of *Morus* species. Recently, from the other species belonging

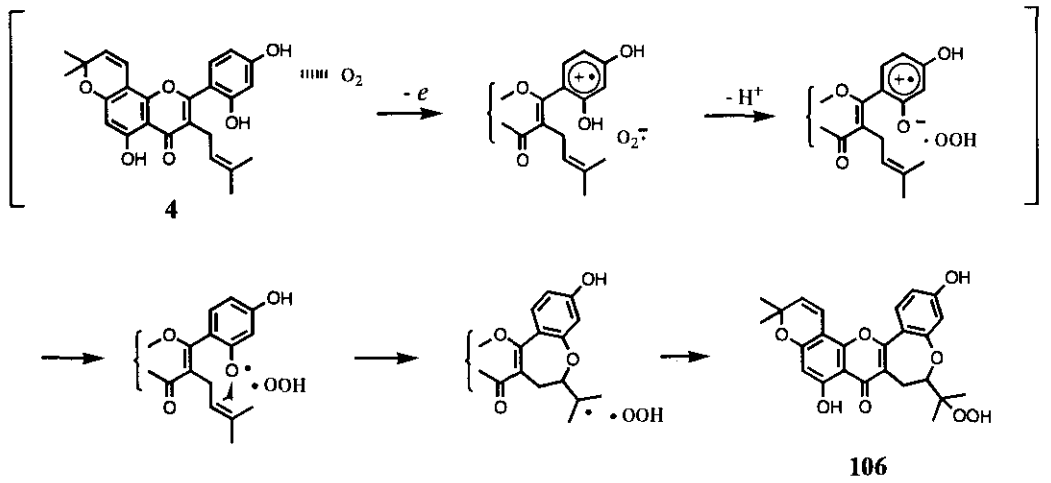


Figure 22 Photoreaction of morusin (**4**) in $CHCl_3$

to Moraceae, *Brosimopsis*, *Sorocea*, and *Artocarpus*, about ten kinds of Diels-Alder type adducts were isolated by our group and other groups.[2,3,61]

The confirmation of the biosynthesis of the mulberry Diels-Alder type adducts was obtained from the following results; as precursors, *O*-methylated chalcones (**107**, **108**) were added to the *Morus alba* cell cultures, which luck in detectable *O*-methylated chalcones or *O*-methylated Diels-Alder type adducts. After incubation, the cells were harvested and lyophilized. Usual work-up of the lyophilized cell cultures afforded four Diels-Alder type metabolites (**111** - **114**) originated intact from the *O*-methylated precursory chalcone (Figure 23). The four Diels-Alder type metabolites were all optically active, having the same stereochemistries as those of kuwanon J (**2**) and chalcomoracin (**3**). From these results, kuwanon J (**2**) and chalcomoracin (**3**) have been proved to be enzymatic Diels-Alder type reaction products.[62]

Then we attempted the synthesis of natural Diels-Alder type adduct, artonin I (**31**), with the aid of an enzyme system of *Morus alba* cell cultures. As artonin I (**31**) was considered to be formed through the Diels-Alder type reaction of a chalcone derivative, morachalcone A (**109**) and artocarpesin (**11**), as a precursor, artocarpesin (**11**) was added to the *Morus alba* cell cultures, and which were treated by usual way. The metabolite isolated from the cells was identical with artonin I (**31**) by direct comparisons (Figure 24).[31] This is the first example of the elucidation of the structure of an organic natural product by application of an enzymatic synthesis of the target substance with the aid of the cell cultures of the related plants.

VI. Biological activities of *Artocarpus* flavonoids

VI-1. Inhibitory effects of artonin E (**51**) and related compounds on 5-lipoxygenase and mouse TNF-α release.

As described in the introduction, *Artocarpus* plants have been used as traditional medicine in Indonesia for a swelling and malarial fever. The usage seems to be expecting for effect of anti-inflammation. So the inhibitory effect of the *Artocarpus* flavonoids against arachidonate 5-lipoxygenase was examined.[63]

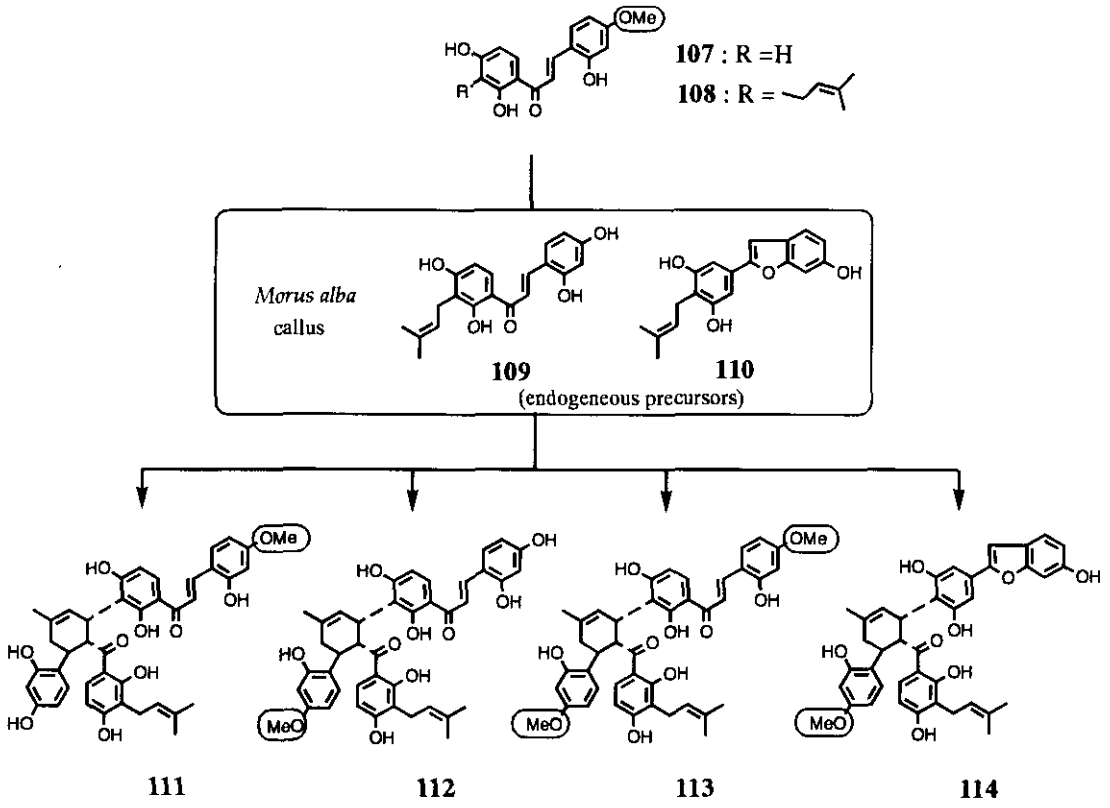


Figure 23 Bioconversion of exogenous chalcones to Diels-Alder type adducts with the aid of *Morus alba* cell cultures

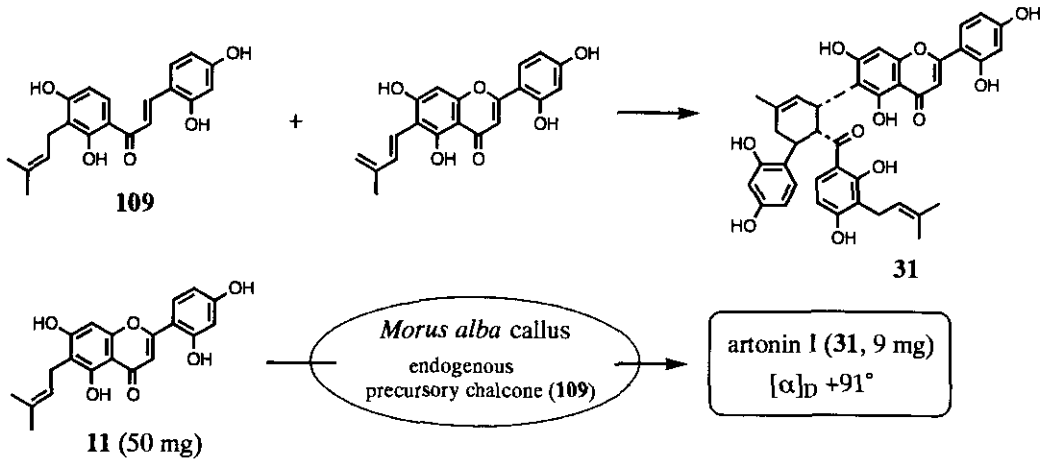


Figure 24 Formation of artonin I (**31**) by Diels-Alder reaction and bioconversion of artocarpesin (**11**) to artonin I (**31**) with the aid of *Morus alba* cell cultures

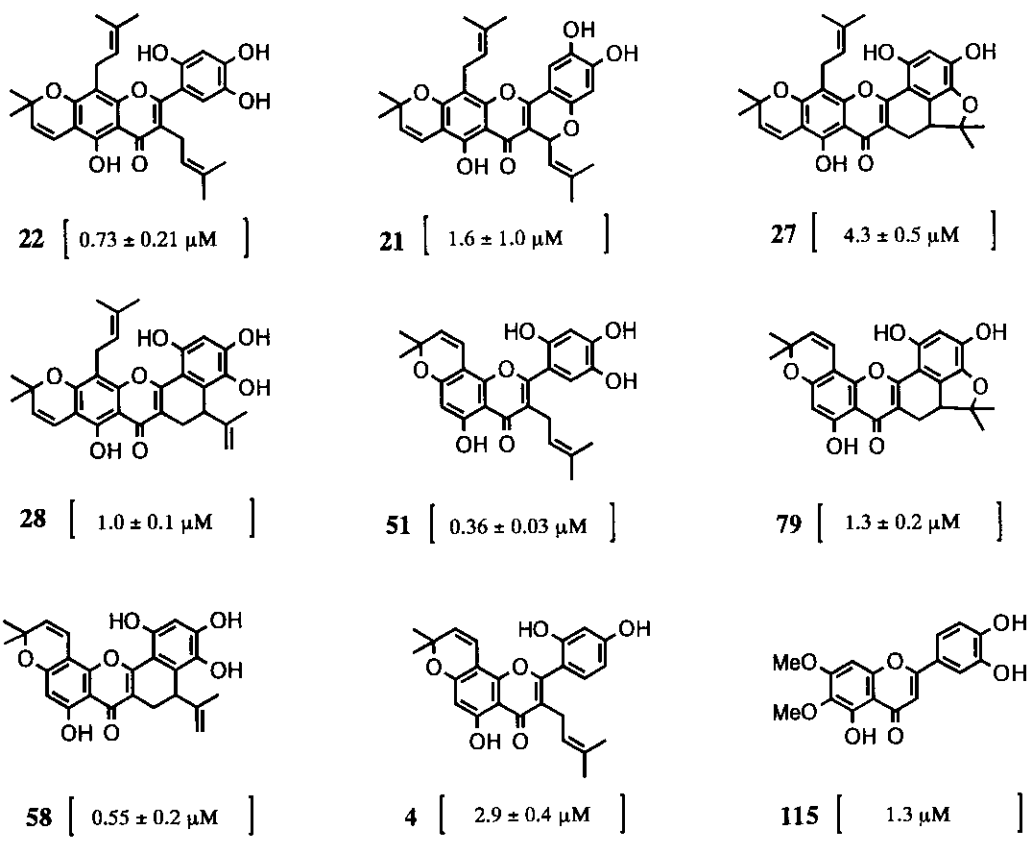


Figure 25 The inhibitory effect (IC_{50} ± S.D.) on arachidonate 5-lipoxygenase activity

Yamamoto *et al.* proposed two structural factors of the flavonoids for the specific inhibitory activity, one is catecohol type of the B ring and the other is the presence of an alkyl-like side chain at the C-3 position.[64 - 66] Seven *Artocarpus* flavonoids and morusin (**4**) were tested for their inhibitory actions on arachidonate 5-lipoxygenase purified from porcine leukocyte.[63] Of the compounds tested, artonin E (**51**) exhibited the most potent inhibition on arachidonate 5-lipoxygenase (IC_{50} = 0.36 μM) as shown in Figure 25. The IC_{50} values varied depending on the structural modification of the compound. Compounds with 4', 5'-vicinal diol in the B ring of the flavone skeleton (compounds **51**, **58**, **22**, and **28**) showed lower IC_{50} values. Thus, the vicinal diol was important for 5-lipoxygenase inhibition. Artonin E (**51**) was significantly more potent than cirsiliol (**115**), which was reported as a 5-lipoxygenase inhibitor. This finding was consistent with the reports that the inhibitory activity of cirsiliol (**115**) with 5-lipoxygenase was enhanced by introducing a lipophilic alkyl group at the C-3 position of the flavone skeleton.[64 - 66]
Inhibitory actions of artonin E (**51**) and morusin (**4**) on other mammalian arachidonate oxygenases were examined. Artonin E (**51**) inhibited two 12-lipoxygenases from porcine leukocytes and human platelets, 15-lipoxygenase from rabbit reticulocytes, and fatty acid cyclooxygenase from bovine vesicular glands (IC_{50} = 2.3, 11, 5.2, and 2.2 μM, respectively). However, IC_{50} values for these oxygenases were higher by one order of magnitude than that for 5-lipoxygenase. These results indicated that artonin E (**51**) was a relatively specific inhibitor for 5-lipoxygenase. Morusin (**4**) also inhibited these enzymes (except for

human platelet 12-lipoxygenase) with IC_{50} values of micromolar order. Thus, the selectivity for 5-lipoxygenase was not observed with morusin (**4**).[63]

The inhibitory effect of the *Artocarpus* flavonoids on the 5-lipoxygenase activity might be accountable for the usage in Indonesian traditional medicine.

On the other hand, morusin (**4**) has been found to be anti-tumor promotor in a two-stage carcinogenesis experiment with teleocidin.[1,10] Considering the similarity of the structures between morusin (**4**) and artonin E (**51**), artonin E (**51**) was expected to be an anti-tumor promotor. Recently, Fujiki *et al.* proposed a new tumor-promotion mechanism applicable to human cancer development on the basis of the experiment with okadaic acid.[67 - 70] The new idea suggests that tumor-necrocis factor-α (TNF-α) induced by okadaic acid acts as a mediator of human carcinogenesis. As briefly summarized in Figure 26,[71] okadaic acid inhibits the action of protein phosphatase type 1 and 2A, resulting in the accumlation of phosphorylated proteins. Recently, it has been shown that TNF-α acts as a tumor promotor in BALB/3T3 cell transformation in vitro.[68,69] So we examined the inhibitory effect of the *Artocarpus* flavonoids on TNF-α-release stimulated by okadaic acid using BALB/3T3 cells. This experiment was carried out in co-operation with Dr. Fujiki and his co-workers (Saitama Cancer Center Research Institute, Japan).

All the compounds tested inhibit the TNF-α release stimulated by okadaic acid at suitable lower concentration. This result suggests that several *Artocarpus* flavonoids act as anti-tumor promotor against to the okadaic acid type promotion. However, the detailed mechanism is not clear at present (Figure 27).

The comparison of the inhibitory effects of the *Artocarpus* flavonoids against the TNF-α release and arachidonate 5-lipoxygenase was carried out. Artonin E (**51**) was the most potent inhibitor on the both tests and the other compounds, artobiloxanthone (**58**) and heterophyllin (**22**), inhibited stronger than cycloartobiloxanthone (**79**), cycloheterophyllin (**21**), and morusin (**4**). The compounds showing stronger activity all have three hydroxyl groups in the B ring. This characteristic feature might be important factor for both biological activities.[72]

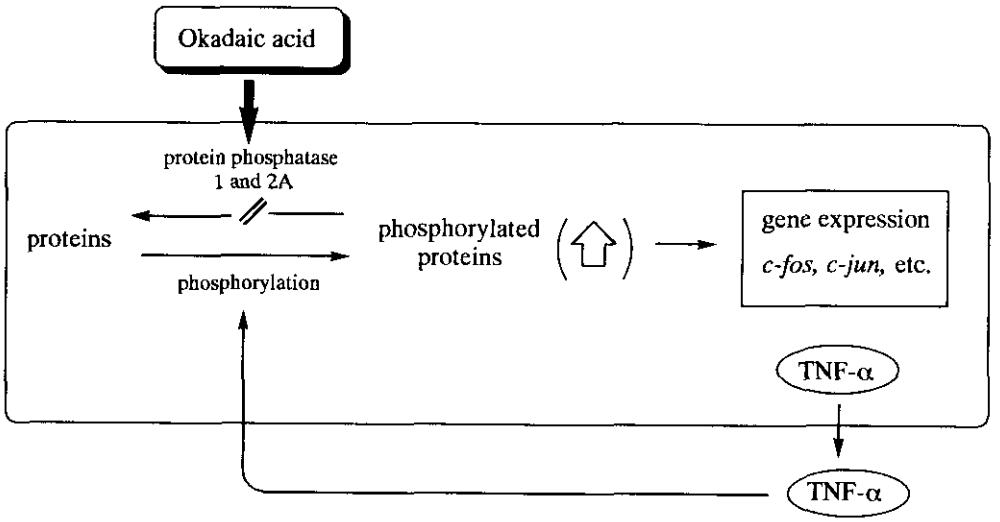


Figure 26 Tumor-promotion by okadaic acid through TNF-α-release

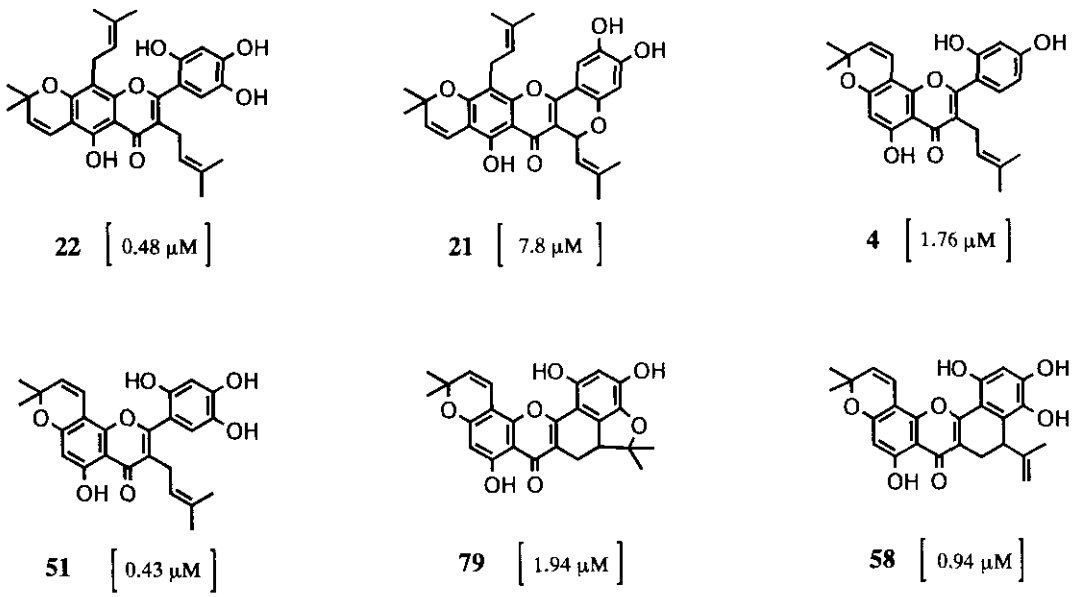


Figure 27 The inhibitory effect (IC_{50}) on mouse TNF-α release from BALB/3T3 cells induced by okadaic acid

VI-2. Cytotoxic activities of artonin E (**51**) and related compounds

We examined the cytotoxic activities of the *Artocarpus* flavonoids, artonin E (**51**), A (**27**), B (**28**), H (**81**), heterophyllin (**22**), and cycloheterophyllin (**21**), against cancer cells, mouse L-1210 and colon 38. All compounds tested showed the cytotoxic activities against both cancer cells. Among them, cyctotoxicity of heterophyllin (**22**), artonins B (**28**) and E (**51**) were stronger than the critical drug, TFU (Figure 28).[73]

VI-3. Inhibitory effect of an isoprenoid-substituted dihydrochalcone, AC-5-1 (**72**), from *Artocarpus communis*, on 5-lipoxygenase

Isoprenoid-substituted phenolic compounds from *A. communis* inhibited 5-lipoxygenase of cultured mastocytoma cells. One of the five compounds isolated from the plant, AC-5-1 (**72**), strongly inhibits 5-lipoxygenase with a half-inhibition dose of $5 \pm 0.12 \times 10^{-8}$ M. However, prostaglandin synthesizing activity is not inhibited until 10^{-5} M. AC-5-1 (**72**) is a highly selective inhibitor for 5-lipoxygenase. The AC-5-1 at 10^{-5} M inhibits 96 % of leukotriene C_4 synthesis of mouse peritoneal cells facilitated by calcium-ionophore. Arachidonic acid induced ear edema of mice, an *in vivo* inflammatory model, involving leukotriene induction, is strongly inhibited by AC-5-1 (**72**) in a dose-dependent manner. The inhibition is the strongest of any inhibitors of 5-lipoxygenase reported previously.[74]

VI-4. Cytotoxicity and antiplatelet activity of Formosan Moraceae plants, and antibacterial activity against cariogenic bacteria.

Liou *et al.* studied the cytotoxicity of prenylflavonoids, cyclomorusin (**116**), cycloartomunin (**59**), dihydrocycloartomunin (**60**), dihydroisocycloartomunin (**67**), artomunoxanthone (**62**), artomunoxantho-

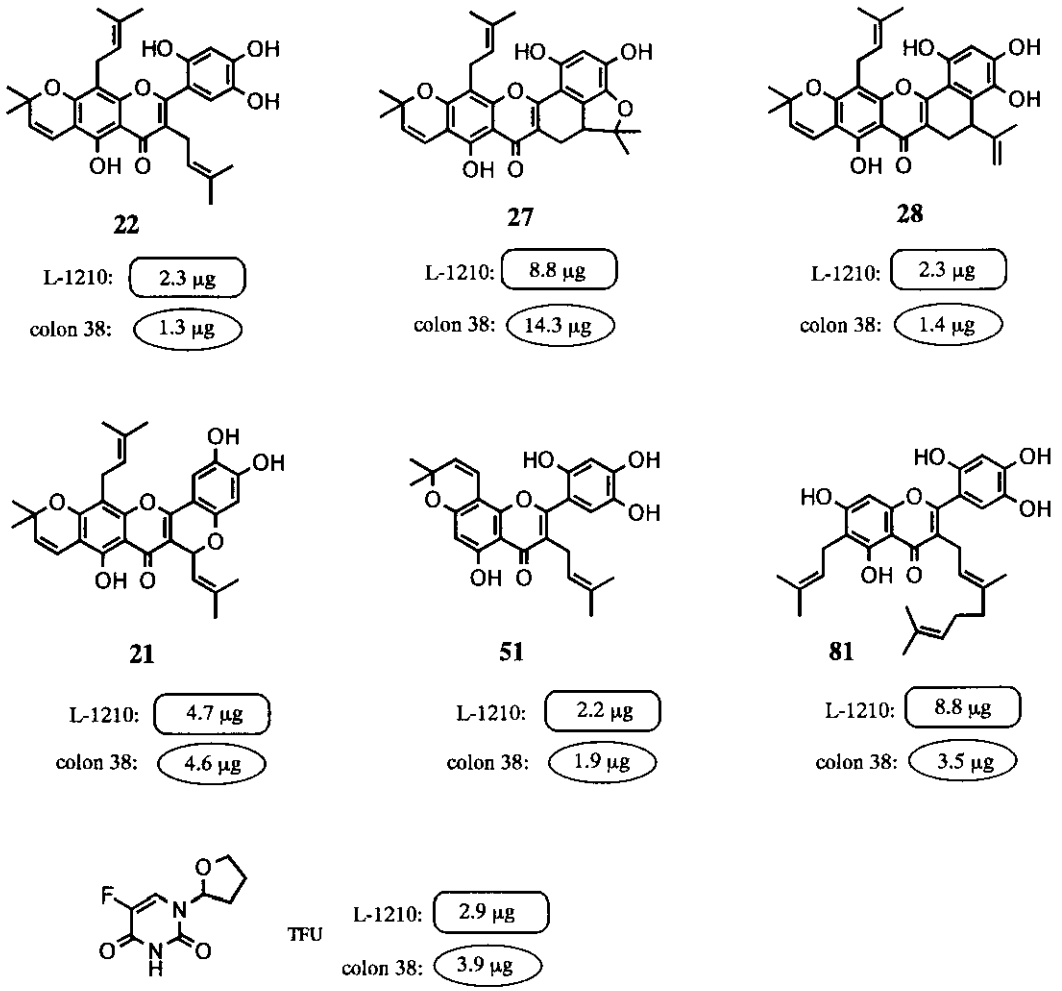
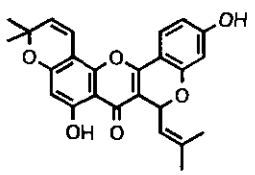


Figure 28 Cytotoxic activities (IC_{50}: μg/mL) against L-1210 and colon 38

trione epoxide (**64**), cyclocommunol (**65**), cyclomulberrin (**117**), and cyclocommunin (**66**), isolated from Formosan *Artocarpus communis*, against human hepatoma PLC/PRF/5 and KB cells *in vitro*.[75] The results are listed in Table 1. Artomunoxanthotrione epoxide (**64**), cyclocommunol (**65**), cyclomulberrin (**117**), and cyclocommunin (**66**) showed cytotoxic activities against human hepatoma PLC/PRF/5 and KB cells *in vitro*. A prenyl group substituted at C-6 or C-8 of cyclocommunol (**65**) enhanced the cytotoxic effect against KB cells *in vitro*. Cyclomorusin (**116**) and cycloartomunin (**59**) did not show cytotoxic effects against human hepatoma PLC/PRF/5 and KB cells *in vitro* except for cyclomorusin against KB cells *in vitro*. This indicates a chromene ring substituted at C-7 and C-8 of prenylflavones decreased the cytotoxic effects, but the cleavage of the chromene ring at the ether linkage enhanced the cytotoxic effects against human hepatoma PLC/PRF/5 and KB cells *in vitro*. Artomunoxanthotrione epoxide (**64**) showed cytotoxic effects against the human hepatoma PLC/PRF/5 and KB cells *in vitro*, but artomunoxanthone (**62**) only showed significant cytotoxic effect against KB cells *in vitro*, indicating that a xanthone with an epoxide ring enhanced the cytotoxic effects.

Table 1 Cytotoxicity (ED_{50} : μg/mL) of γ-pyrones isolated from *A. communis*

compound	cell line	
	PLC/PRF/5	KB
cyclomorusin (**116**)	6.51	3.67
cycloartomunin (**59**)	11.64	10.30
dihydrocycloartomunin (**60**)	5.59	2.82
dihydroisocycloartomunin (**67**)	3.67	1.28
artomunoxanthone (**62**)	5.74	3.47
artomunoxanthotrione epoxide (**64**)	1.06	2.09
cyclocommunol (**65**)	2.03	2.11
cyclomulberrin (**117**)	2.50	0.73
cyclocommunin (**66**)	2.05	0.71
cisplatin	5.29	0.16



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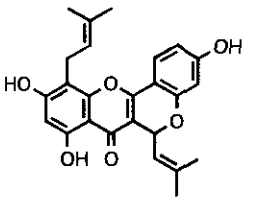

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Lin *et al.* reported that prenylflavonoids, isolated from Formosan *Artocarpus communis*, cyclomulberrin (**117**), dihydroisocycloartomunin (**67**), cyclocommunol (**65**), and cyclocommunin (**66**) had antiplatelet actions on collagen- and arachidonic acid-induced platelet aggregation with little or no effect on PAF-induced platelet aggregation (Table 2). Cyclomorusin (**116**) and artomunoxanthone (**62**) showed inhibition on the platelet aggregation induced by PAF.[76] Furthermore, they reported the antiplatelet activities of a series of isoprenylated flavonoids, isolated from Formosan *Artocarpus heterophyllus* and *Broussonetia papyrifera*.[77] Cycloartocarpin A (**45**, IC_{50} = 18.5 μM) and cycloheterophyllin (**21**, IC_{50} = 10.9 μM) showed inhibition of arachidonic acid-induced platelet aggregation. Of the compounds tested, 5'-(γ, γ-dimethylallyl)-2', 3, 4, 4'-tetrahydroxychalcone (= broussochalcone A) exhibited the most potent inhibition of platelet aggregation induced by arachidonic acid (IC_{50} = 6.8 μM), and the mechanisms of action are investigated.[77]

Sato *et al.* reported that the extract of *Artocarpus heterophyllus* showed the intensive antibacterial activity against cariogenic bacteria. Artocarpin (**15**) and artocarpesin (**11**) isolated from the extract inhibited the growth of primary cariogenic bacteria at 3.11 - 12.5 μg/mL. They also exhibited the growth inhibitory effects on plaque-forming *streptococci*. These phytochemical isoprenylflavones would be potent compounds for the prevention of dental caries.[78]

Table 2 IC_{50} values (μM) of prenylflavonoids on the platelet aggregation induced by arachidonic acid (AA) and collagen

compound	IC_{50} (μM)	
	AA	collagen
cyclomorusin (**116**)	> 300	113.4
dihydroisocycloartomunin (**67**)	44.1	72.8
artomunoxanthone (**62**)	180.1	-
cyclocommunol (**65**)	57.3	63.5
cyclomulberrin (**117**)	67.1	128.2
cyclocommunin (**66**)	12.5	14.4

VII. Conclusion

Many kinds of isoprenoid-substituted flavonoids have been isolated from the plants of *Artocarpus* species (Moraceae). The compounds, except some ones, have a characteristic structure bearing an isoprenoid side chain at the C-3 position of flavone skeleton, and the B ring has a 2', 4', 5'-trioxygenated pattern. Particularly , the structure of artonin E (**51**) corresponds to 5'-hydroxymorusin. In addition to the feature, some of the flavones, such as artobiloxanthone (**58**) and cycloartobiloxanthone (**79**), have a unique structure having the C-C linkage between an isoprenoid side chain at the C-3 position and the 6'-carbon of the B ring. This C-C linkage was considered to be biogenetically synthesized through a phenol oxidation in the plants. To confirm the assumption for the formation of the C-C linkage, photoreaction of artonin E (**51**) was carried out to produce artobiloxanthone (**58**) and cycloartobiloxanthone (**79**). Furthermore, the treatment of artonin E (**51**) with a radical reagent resulted in the same products. These findings led to an assumption that the unique structure having the C-C linkage is formed through a phenol oxidation. These flavonoids are characteristic constituents of *Artocarpus* species.

Some of the *Artocarpus* flavonoids, such as artonins C (**29**) and I (**31**), have been regarded as intermolecular [4 + 2] cycloaddition product from the isoprenyl portion of a dehydroprenylphenol, as a diene, and the α,β-unsaturated bond of a chalcone skeleton, as a dienophile. As artonin I (**31**) was considered to be formed through the Diels-Alder type reaction of a chalcone derivative, morachalcone A (**109**) and artocarpesin (**11**), as a precursor artocarpesin was added to the *Morus alba* cell cultures to produce artonin I (**31**). Some of the isoprenoid-substituted flavonoids from Artocarpus species showed interesting biological activities, such as inhibitory effect on mouse TNF-α release and 5-lipoxygenase, cytotoxicity, antiplatelet activity, and antibacterial activity against cariogenic bacteria. Particularly, artonin E (**51**) was the potent inhibitor of arachidonate 5-lipoxygenase and mouse TNF-α release, and showed cytotoxic activities against mouse L-1210 and colon 38, which was stronger than TFU. Thus, the *Artocarpus* plants were found to be important medicinal resources.

ACKNOWLEDGEMENT

We are grateful to Dr. H. Fujiki, Saitama Cancer Center Research Institute, for his valuable guidance and advise for the bioassay on inhibitory activity of TNF-α release.

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Received, 12th September, 1997