Molecular Diversity and Biological Activity of Natural Products from Indonesian Moraceous Plants

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Summary: Moraceae is a large family consists of about sixty genera, including *Morus, Artocarpus* and *Ficus*, and over 1400 species. Plants of this family elaborate many interesting secondary metabolites, including prenylated flavonoid, arylbenzofuran, and stilbene derivatives. Prenylated flavonoids from plants in this family exhibit diverse biological activities, including inhibitory effect on arachidonate 5-lipoxygenase, antiplatelet, antibacterial and cytotoxic activities. As part of an ongoing search for novel natural products of plant origin with potential biological activity, a number of species of *Artocarpus* collected in Indonesia have been investigated. In this paper a brief review of our recent work on some of these species that has led to the isolation of a host of many types of prenylated flavonoids and their cytotoxic activity against murine leukemia P388 cell lines will be presented.

Introduction

Large sections of population living in rural areas of the Asian countries rely on the indigenous medicinal systems for the treatment of diseases. These are largely based on the use of roots, leaves, fruits and flowers from various species of plants [1-2]. Isolation, structural, synthetic and pharmacological studies have accor-dingly been directed to the discovery of new and useful natural products in many laboratories, including ours. Our interest is, however, not limited to the traditional medicinal plants but we also pay attention to any new leads encountered in other natural sources. In this regards terrestrial tropical forest plants are an invaluable source of novel secondary metabolites, many of which possess a wide diversity of structures and pharmacological properties which match those obtained from other organisms.

Moraceae forms a significant element in the flora of tropical region of Souheast Asia. It is a large family of about sixty genera and approximately 1600 species, including the important groups *Morus, Artocarpus* and *Ficus* [3]. *Morus* or mulberry is a small genus, found primarily in temperate and subtropical regions of the northern hemisphere, and has been widely cultivated in China and Japan, because the leaves serve as indispensable food for silkworms. However, *Artocarpus* is one of the most commonly encountered representatives of Moraceae in the lowland forest of tropical Southeast Asia, apart from the ubiquitous *Ficus*. The genus *Artocarpus* consists of about 50 species, the greatest diversity of which is in the Indonesian archipelago [4]. Recent studies on the phyto-chemistry of Moraceae has clarified a number of outstanding features and there is now a fairly good picture that this family of plants is primarily a good source of 3-prenylflavone derivatives, an unusual feature of which is the presence of isoprenyl substituent at C-3 positon of the flavone skeleton. During the last decade it has become increasingly recognised that these natural products also showed a wide range of biological activities, including inhibitory effect on arachidonate 5-lipoxygenase, antiplatelet, antibacterial and cytotoxic activities [5-6]. Thus, during the last few years, as part of our continuing investigation of Indonesian tropical rainforest plants, a systematic evaluation of a number ofmoraceous plants of the genus *Artocarpus* commonly found in Indonesia had been carried out [7-9]. Some of rhe first species to be studied in our laboratory were *A. champeden, A. rotunda, A. maingayu* and *A, scortechinii*. Our examination of these endemic taxons had led to the isolation of a host of many types of prenylated

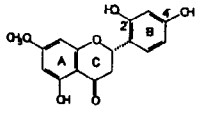
flavonoid, including novel compounds, which revealed many facets of the biosynthetic capacity *of Artocarpus,* with regard to transformation of the secondary metabolites arising from 3-prenylflavones. In this paper we report the discovery of a number of novel and interesting compounds and their in vitro cytotoxic activity against murine leukemia P388 cell lines.

Results and Discussion

Several species of *Artocarpus* had been investigated, including A *champeden* and *A. rotunda,* collected in the Indonesian Provinces of West Java, *A. maingayii* and *A. scortechinii,* collected in West Sumatera. The appropriate tissues of each of the plants were extracted exhaustively by cold percolation with organic solvents, hexane and methanol, followed by liquid-liquid partitions with benzene, chloroform or dichloromethane, acetone and finally with ethyl acetate. Chromatographic separations of the fractions thus obtained, using vacuum-liquid, column and centrifugal partition chromatography techniques, yielded pure compounds, the structures of which were determined based on UV, IR, ^{1}H and ^{13}C NMR, including 2D ^{1}H-^{1}H COSY, HMQC and HMBC, and MS spectros-copic analyses. While, cytotoxic assay against P388 cell-lines was carried out using the cells supplied by the Japan Foundation for Cancer Research and maintained in RPMI-1640 medium supplemented with 5% fetal calf serum and kanamycin.

Artocarpus champeden (Lour.) Stokes is a large evergreen tree, up to 20 m tall, common in primary and secondary lowland rainforest. It is widely distributed in Indonesia, including Sumatera, Borneo, Celebes, Moluccas, Irian Jaya, as well as in Java, and known locally as "cempedak". This species is one of the famous folk medicines in Indonesia, and has been used as antimalaria drug [1]. Our work on this particular species was prompted because examination of this species had not been reported previous to our work.

In our early work on *A. champeden* it was found that the heartwood contained some flavanone derivatives, such as artocarpanone **(1)**, artoindonesianin E **(2)** and heteroflavanone A **(3)**, which formed the simplest class of the flavonoid of *Artocarpus,* characterised by a 2',4'-dioxygenated ring B, in the case of **(l)**, or possessing a 2',4',6'-trioxygenated pattern of ring B of the flavanone backbone, as exemplified by **(2)** and **(3)**. The heartwood of *A. champeden* also yielded a flavone derivative, norartocarpetin **(4)**, characterised by a 2',4'-dioxygenated ring B of the flavone skeleton [10,11].



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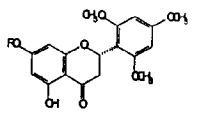
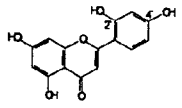


2 : R = H
3 : R = CH_3



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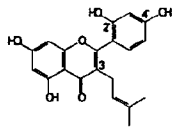
A. champeden also produced a series of 3-prenylflavone and related compounds, such as cyclocommunol **(5)**, norartocarpin **(6)**, artocarpin **(7)**, artoindonesianins R **(8)** and Q **(9)**, characterised by a prenyl substituent at C-3 position and 2',4'-dioxygenatcd or 2\4\5'-trioxygenated ring B of the flavone skeleton [10,12]



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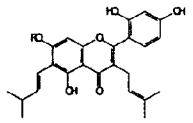
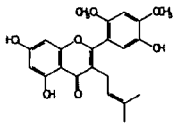


6: R = H
7: R = CH_3



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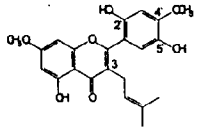

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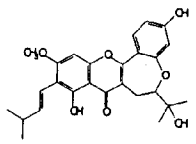
The heartwood of *A. champeden* also yielded a series of oxepinoflavones derivatives, exemplified by chaplasin **(10)** and artoindonesianin B **(11)** [13]. This type of compounds has a novel skeleton, characterised by an oxepin ring fused to rings B and C, involving the isoprenyl substituent attached to C-3 and the oxygen function at C-2' positions of the flavone backbone.



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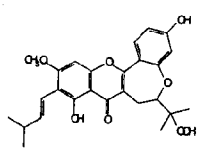

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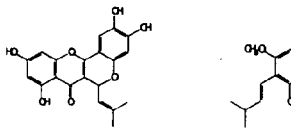
Both compounds **(10)** and **(11)** were isolated together with a series of pyranoflavone derivatives, exemplified by cyclochampedol (or artoindonesianin) **(12)**, cycloartocarpin **(13)** and artoindonesianin D **(14)** [14]. The pyranoflavone type of compounds also possess a novel skeleton, characteri-sed by a pyran ring fused to the rings B and C involving the isoprenyl substituent at C-3 and the oxygen function at C-2' of the flavone skeleton.



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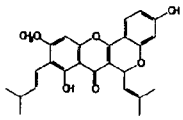

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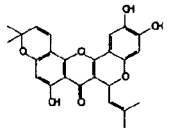

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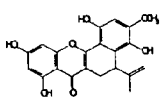
A. champeden was also found to produce a number of dihydrobenzoxanthone derivatives, such as artoindonesianins T **(15)** and S **(16)** together with artonin B **(17)** [12]. This type of compound is characterized by a carbon-carbon bond between C-6' of ring B and C-10 of the isoprenyl substituent at C-3.



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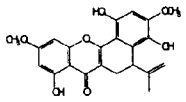

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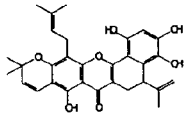

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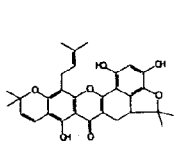
In addition, some furanodihydrobenzo-xanthone derivatives, such as artonin A **(18)** and artoindonesianin A **(19)**, had also been isolated from the benzene soluble fraction of the methanol extract of the root bark of A *champeden* [13-14].



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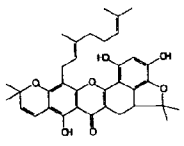

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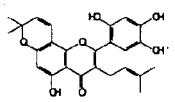
Artocarpus rotunda (Hout) Panzer is a large evergreen tree, common in primary and secondary lowland rainforest. Examination of this species had also not been reported previous to our work. Extraction of the root bark *of A. rotunda* yielded among others 3-prenylflavone derivatives, artonin E **(20)** and artoindonesianin L **(21)** together with a furanodihydrobenzoxanthone derivatives, cycloarto-biloxanthone **(22)** and artonin M **(23)**, together with a novel quinonodihydrobenzoxanthone derivative, artonin O **(24)**, possessing a modified flavonoid skeleton [15-17].



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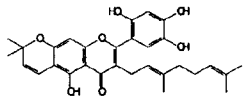

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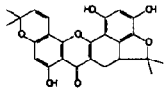

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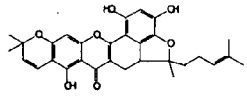

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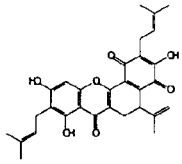

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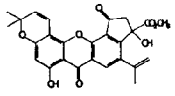
Artocarpus teysmanii Miq. is a large ever-green tree up to 45 m tali, distributed in Sumatera, Borneo, Celebes and Mollucas, and occurs in lowland evergreen forest up to 300 m altitude. The benzene soluble fraction of the methanol extract of the dried root bark of *A. teysmanii* yielded artoindonesianin C **(25)**, a cyclopentenoxanthone derivative, together with artonol B **(26)**, a xanthonolide, two different flavonoid derived xanthone derivatives [18].



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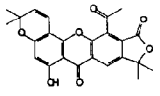

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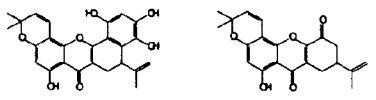
Artocarpus scortechinii King. is a large ever-green tree, distributed in Sumatra, and common in primary and secondary lowland rainforest. This particular species had also not been reported previous

to our work. The chloroform extract of the tree bark of the species yielded among others norartocarpetin **(4)**, a 3-prenylflavone derivative, artonin E **(20)** and a dihydrobenzoxanthone derivative, artobiloxanthone **(27).** While, the benzene extract of the same plant material produced flavonoid derived xanthone derivatives, namely artonol B **(26)**, together with another type of xanthone derivative, artonol A **(28)** [19-20].



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Biogenesis of Flavonoids from *Artocarpus*

Summarizing the data described above, we can conclude that the phenolic constituents isolated from *Artocarpus* species in our laboratory may be differentiated into the flavonoid and the modified flavonoid classes of compounds. The structures of the flavonoid class of compounds, which include the structure of normal flavanone and flavone, 3-prenyl-flavone, oxepinoflavone, pyranoflavone, dihydro-benzoxanthone and furanodihydrobenzoxa-nthone, are characterised by two unique features, namely the presence of an isoprenoid group at C-3 position and the 2',4' or 2',4',5' orientations of hydroxyl groups in the B ring of the flavone backbone. It may be suggested that the first reaction step leading to the formation of flavone derivatives, such as norartocar-petin **(4),** are followed by isoprenylation and further hydroxylation of the flavone skeleton, to produce 3-prenylated flavone derivatives, such as cyclocommu-nol **(5),** norartocarpin **(6),** artocarpin **(7),** artoindo-nesianin R **(8)** and Q **(9),** aitonin E **(20)** and artoindonesianin L **(21).** Biogenetically, the 3-prenylated flavones arise by prenylation of the C-3 position of flavone. As simple as this pathway is, however, the 3-prenylated flavones still form a unique class of natural products only found in this family of plant. The 3-prenylated flavone type of compounds is a central branch point in the biogenesis of the phenolic compounds produce by *Artocarpus,* serving as precursors for several different structural types, namely the oxepinoflavone type of compounds exemplified by chaplalsin **(10)** and artoindonesianin B **(11),** and the pyranoflavone type, exemplified by cyclochampedol or artoindone-sianin **(12),** cyclo-artocarpin **(13)** and artoindonesianin D **(14).** Bio-genetically, the oxepinoflavones and pyranoflavone s can be regarded as arising by intra-molecular oxidative coupling of the phenolic group at C-2' position and the methylene carbon (C-9) or the olefmic carbon (C-10) of the isoprenyl substituent at C-3 position, respectively. The other flavonoids isolated from *Artocarpus* are represented by the dihydrobenzoxanthone type of compounds, such as artoindonesianins T **(15)** and S **(16),** artonin B **(17),** and artobiloxanthone **(27),** and the furanodihydro-benzoxanthone type structure, represented by artonin A **(18),** artoindonesianin A **(19),** and cycloartobilo-xanthone **(22).** Biogeneti-cally, it may be suggested that the fundamental step toward formation of the dihydrobenzoxanthone and furanodihydrobenzoxan-thone type structures is the formation of the C-C bond between C-10 position of the isoprenyl moiety located at C-3 and C-6' position of the B ring posessing 2',4',5' orientation of the hydroxyl groups. [21] had proposed a feasible biosyn-thetic route for the formation of the dihydro-benzoxanthone and furanodihydrobenzoxanthone, from a simple flavone. While, it had also been proposed that the C-C bond in both structures is formed through a phenol oxidative coupling reaction of the 3-isoprenoid 2',4',5'-trioxygenatedflavone-type of compounds [6]. The dihydrobenzoxanthone type of compounds is again a branch point interme-diates in the biogenesis of modified flavonoid type of compounds produced by *Artocarpus.* It serves as exclusive precursor for the type of structures, namely the quinonodihydroxan-thones, exemplified by artonin O **(24),** cyclopenteno-xanthones type of compounds, such as artoindone-sianin C (25), the xanthonolides structure represented by artonol B **(26),** and the dihydroxanthone structure exemplified by artonol A **(28).** The biogenetic schemes for compounds possessing these type of structures had also been proposed, in which, artonol A **(28)** may be derived from the dihydrobenzo-xanthone derivative, such as artobiloxanthone **(27)** through a *retro*-Diels-Alder reaction of an inter-mediate quinonodihydro-benzoxanthone hydrate obtained from the quinonodi-hydrobenzoxanthone, such as artonin O **(24)** [6]. While, artonol B **(26)** had been assumed to be derived from xanthone having a five membered cyclic ketone ring, exemplified by the artoindonesianin C **(25),** through decarboxylation, oxidation and lactonization re-actions. From the chemotaxonomic point of view, these flavonoids containing the isoprenoid substituent at C-3 position characterize some species of the genera *Artocarpus* and *Morus* of the family Moraceae.

Biological Activity of Flavonoids from *Artocarpus*

In conjunction with our studies directed toward the discovery of natural product cancer chemotherapeutic agents, preliminary evaluation of some selected flavonoid derivatives mentioned above had been conducted against murine leukemia P388 cells in vitro. The flavanone type compounds, artocarpanone **(1)** and heteroflavanone A **(3),** were no very active with the P388 cell lines, demonstrated LC_{50} 19.3 and 16,7 μg/mL, respectively. Some examples of 3-prenylated flavones derivatives, such as cyclocommunol **(5),** artoindonesianin Q **(9)** and artonin E **(20),** were found to be moderately cytotoxic to the cultured P388 cells, demonstrated LC_{50} 9.0, 10.3 and 3.0 μg/mL, respectively, however artocarpin **(7)** showed strong cytotoxicity (LC5o 1.8 μg/mL). While, the oxepinoflavone derivatives, namely chaplasin **(10)** and artoindonesianin B **(11)** also showed significant cytotoxic against the cultured cells, with LC_{50} 2.0 and 3.9 μg/mL, respectively. The pyranoflavone derivatives, such as artoindonesianin (12) with LC_{50} 0.2 μg/mL, was found to possess very strong activity in this assay, while cycloartocarpin (13), showed significant activity with LC_{50} 3.9 μg/mL, against the P388 cell lines. In addition, the dihydrobenzoxanthone derivative, artobiloxanthone **(25)** was also strongly active in this assay, possessing LC_{50} 1.7 μg/mL. Furthermore, the furanodihydrobenzoxanthone derivatives, namely artonin A **(18),** artoindonesianin A **(19),** cycloartobiloxanthone **(22)** and artonin M **(23)** exhibited moderate cytotoxicity with the P388 cell lines, demonstrated LC_{50} 21.0, 4.6 and 7.9 μg/mL, respectively, except artonin A **(18)** which showed very strong cytotoxicity with this cultured cells, with LC_{50} 0.2 μg/mL. The modified flavonoid type compounds isolated from *Artocarpus* as summariized above, namely artonin O **(24),** artoindonesianin C **(25)** and artonol B **(26),** in this assay demonstrated LC_{50} values of 1.6, 6.2 and >100 /μg/mL, respectively. Artonol B (26) [LC_{50} >100 μg/mL) showed no activity, compared with the strongly active artonin 0 **(24)** (LC_{50} 1.6 μg/mL) and moderately active artoindonesianin C **(25)** (LC_{50} 6.2 μg/mL). This preliminary assessment illustrate the biological potential of these natural products as cancer chemotherapeutic agents. In general, it may be concluded that the flavonoid type compounds exhibited considerable cytotoxic activity, and the presence of an isoprenyl moiety attached to C-3 position together with free *ortho* hydroxyl groups in ring B of the flavone backbone are among the important factors for the activity. Some of these compounds, artoindonesianin **(12),** heteroflavanone A **(3)** and artocarpin **(7),** strongly inhibited leucine uptake into brush border membrane vesicles from *Bombyx mori* larval midgut [22].

Conclusions

The chemical constituents of several species of *Artocarpus* (family Moraceae) endemic to Indonesia had been examined It had been found that this genus, produces a wide variety of structurally unique flavonoids and modified flavonoid type compounds. A prevalent structural theme of metabolites isolated from *Artocarpus* is a 3-prenylated flavonoids and related flavonoid derived xanthones. It is noteworthy that these unique structural features correlate significantly with the significant cytotoxic activity of these compounds against murine leukemia P388 cell lines. There is little doubt that many more chemicals of these and other related structural variations will be disclosed when more *Artocarpus* species are examined.

Acknowledgements

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