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Indonesian Rainforest Plants – Chemodiversity and Bioactivity

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ABSTRACT Discovery of natural products for pharmaceutical and agrochemical industries is in rapid development during the last decades. This increased interest is primarily due to the diversity of chemical structures found in nature, and it can be expected that these natural compounds will become more important in bioindustries. To date only a small percentage of the living organisms in the biosphere have been described, and there exists an enormous and still undiscovered natural compounds of obviously unlimited structural diversity, particularly in the tropical rainforests. This paper will present the recent status of part of our works on natural products derived from the Indonesian rainforest plants, covering substantial structural diversity and biological activity, from a number of species of *Artocarpus* (Fam. Moraceae) largely distributed in Indonesia.

INTRODUCTION

The genus *Artocarpus* (Moraceae) is widely distributed in the Southeast Asian region. It has been reported that there are over 40 species of *Artocarpus* in this region and a large number of members of this genus are present and endemic to the Indonesian archipelago [1], where they had been used for their timber, fruits and herbal medicine against malarial fever, inflammation, etc [2]. Plants of this family elaborate mainly prenylated flavonoids and related compounds, arylbenzofurans, and stilbenes [3, 4]. The prenylated flavonoids from these family exhibit diverse biological activities, including inhibitory effect on arachidonate 5-lipoxygenase, antiplatelet, antibacterial and cytotoxic activities.

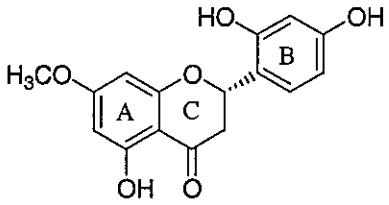
During the last few years, a systematic evaluation of the chemistry of a number of Indonesian *Artocarpus* had been carried out in our laboratory [5-12]. The work involved collection of suitable plant materials, followed by laboratory investigations for chemical isolation, characterization, structure elucidation and biological evaluation of the isolated compounds. It is intended that this present paper will discuss the structural features of interesting compounds discovered in our laboratories during the last two years, and will be an extension of our last report in this series [13]. This paper will also report the cytotoxic activity of these compounds against murine leukemia P-388 cell lines.

RESULTS AND DISCUSSIONS

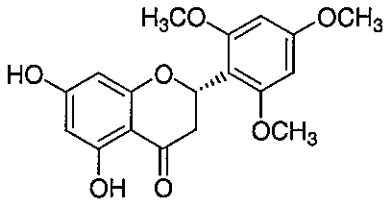
Several species of *Artocarpus* collected in West Java, West Sumatera and South Sulawesi had been investigated. The appropriate tissues of each of these plants were extracted exhaustively by cold percolation with organic solvents, hexane and methanol, followed by liquid-liquid partitions with benzene, chloroform, acetone and finally with ethyl acetate. Chromatographic separations of the fractions thus obtained, using vacuum-liquid, collumn and centrifugal partition chromatography techniques, yielded pure compounds. The structure of each of these compounds was elucidated based on UV, IR, ^{1}H and ^{13}C NMR, including 2D ^{1}H-^{1}H COSY, HMQC and HMBC, and MS spectroscopic analysis. While, cytotoxic assay against P-388 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, Sulawesi, 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. Our work on this particular species was prompted, because examination of this species had not been reported previous to our work. Thus, the chloroform soluble fraction of

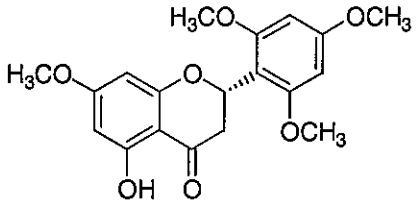
the methanol extract of the heartwood of *A. champeden* yielded artocarpanone **1**, a 2',4'-dioxygenated flavanone derivative, while the benzene soluble fraction of the methanol extract of the tree bark of this species afforded artoindonesianin E **2** and heteroflavanone A **3**, possessing 2',4',6'-trioxygenated pattern of Ring B. The ethyl acetate soluble fraction of the methanol extract also yielded norartocarpetin **4**, a 2',4'-dioxygenated flavone derivative [14, 15].



Artocarpanone **1**

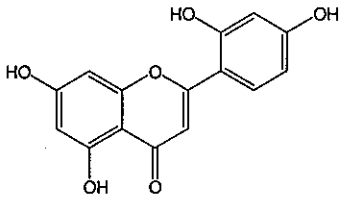


Artoindonesianin E **2**

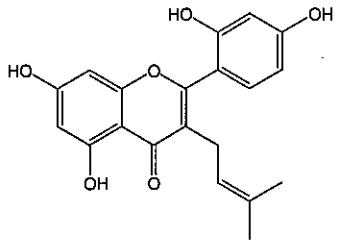


Heteroflavanone A **3**

From the chloroform soluble fraction of the methanol extract of the heartwood of *A. champeden*, four flavone derivatives with 3-prenylated 2',4'-dioxygenated functionalities had also been isolated, namely cyclocomunol **5**, cudraflavone C **6**, norartocarpin **7** in addition to artocarpin **8** [6, 9].

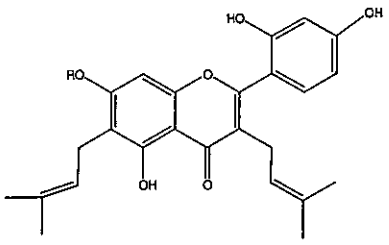


Norartocarpetin **4**

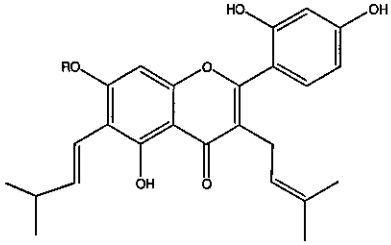


Cyclocomunol **5**

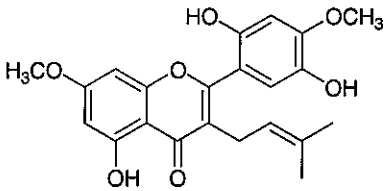
A. champeden also yielded a series of 3-prenylated flavones with 2',4',5'-trioxygenated B ring, such as artoindonesianin Q **9**, artoindonesianin R **10** and heterophyllin **11**.



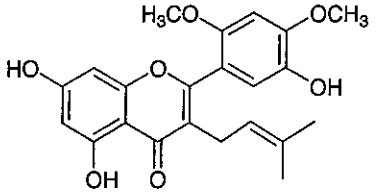
Cudraflavone C **6**



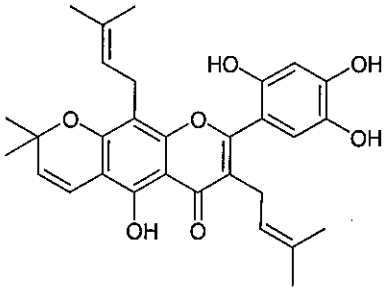
R = H : Norartocarpin **7**
R = Me : Artocarpin **8**



Artoindonesianin Q **9**

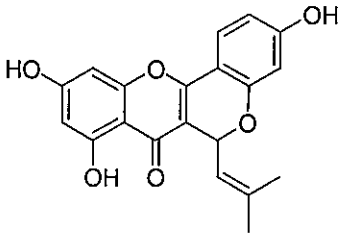


Artoindonesianin R **10**

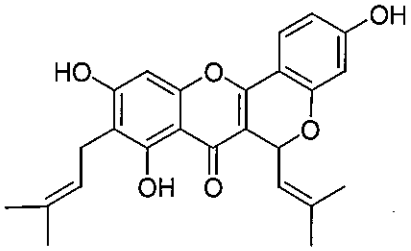


Heterophyllin **11**

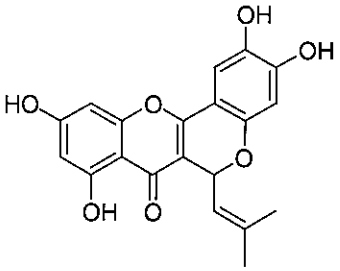
The species *A. champeden* also yielded novel pyranoflavone derivatives having 2',4'-dioxygenated or 2',4',5'-trioxygenated systems in ring B of the flavone skeleton, exemplified by the following metabolites: albanin A **12**, cyclocomunin **13**, artoindonesianin **14**, 5-hydroxycudraflavone **15** and cycloheterophyllin **16** [3].



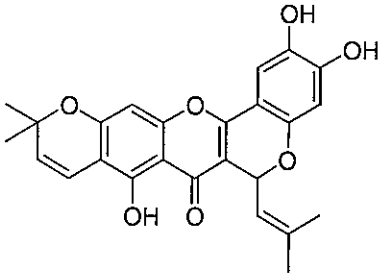
Albanin A **12**



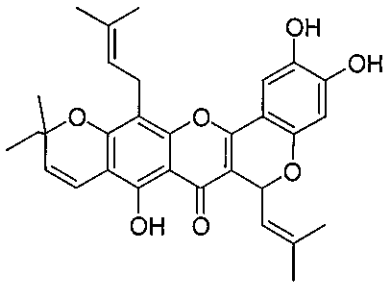
Cyclocomunin **13**



Artoindonesianin **14**

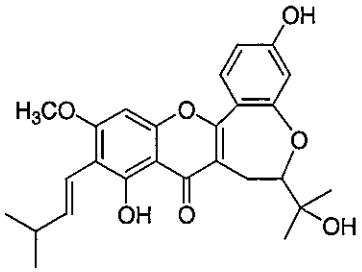


5-Hydroxycudraflavone **15**

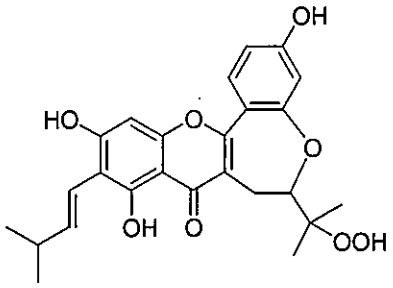


Cycloheterophyllin **16**

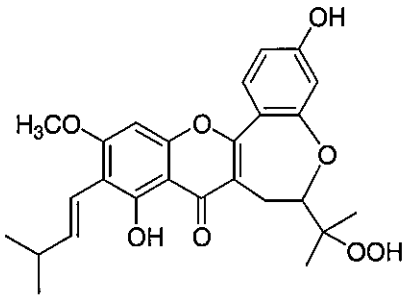
Furthermore, *A. champeden* also produced a number of novel oxepinoflavone derivatives possessing 2',4'-dioxygenated B ring, such as chaplasin **17**, artoindonesianin K **18** and artoindonesianin B **19** [9].



Chaplasin **17**

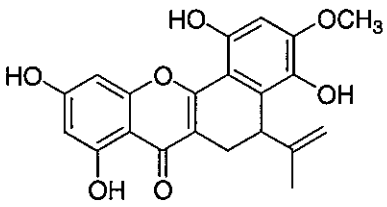


Artoindonesianin K **18**

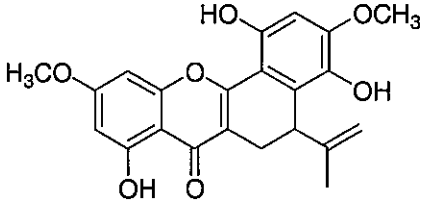


Artoindonesianin B **19**

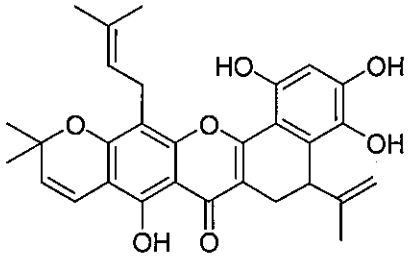
Another type of flavonoids, namely the dihydrobenzoxanthone derivatives, posessing 2',4',5'-trioxygenated ring B of the flavone backbone, had also been isolated from the chloroform soluble fraction of the acetone extract of the tree bark of *A. champeden*, such as artoindonesianin T **20**, artoindonesianin S **21** and artonin B **22** [16].



Artoindonesianin T **20**

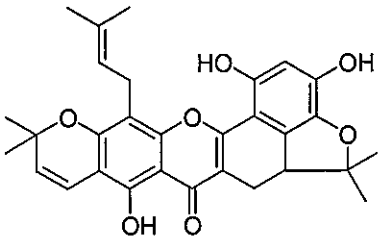


Artoindonesianin S **21**

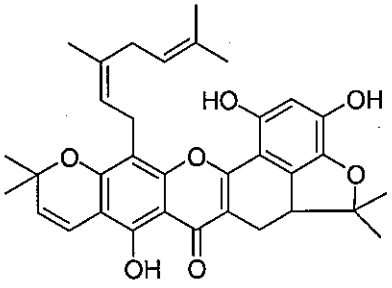


Artonin B **22**

Our investigation of *A. champeden* had also yielded some pyranodihydro-benzoxanthone derivatives, exemplified by such compound as artonin A **23** and artoindonesianin A **24**, from the benzene soluble fraction of the methanol extract of the root bark of *A. champeden* [9].

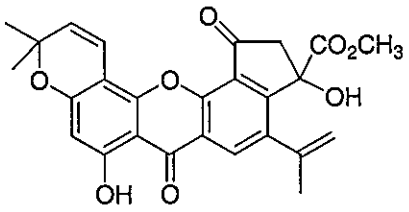


Artonin A **23**

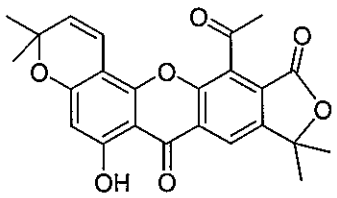


Artoindonesianin A **24**

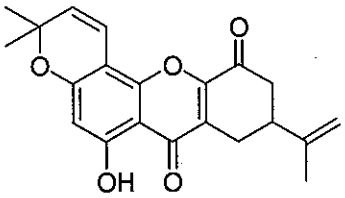
***Artocarpus scortechinii* King.** is a large evergreen tree, distributed in Sumatera and Peninsular Malaysia, and common in primary and secondary lowland rainforest. The chloroform extract of the tree bark of this species yielded among others norartocarpetin **4**, artonin A **23** and 5-hydroxycudraflavone **15**, while the benzene extract of the same plant material produced artoindonesianin C **25**, together with artonol B **26** and artonol A **27**, possessing modified flavonoid skeletons [17, 18, 19].



Artoindonesianin C **25**

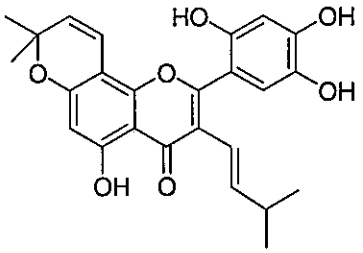


Artonol B **26**

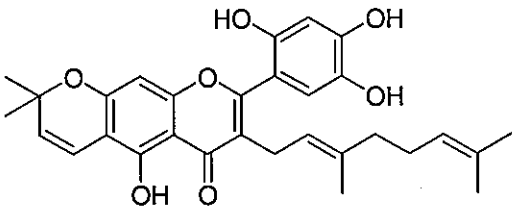


Artonol A **27**

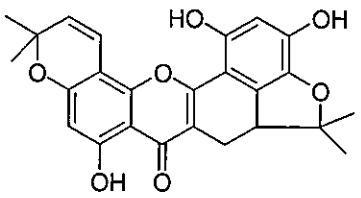
***Artocarpus rotundus* (Houtt.) Panzer**, was investigated for the first time in our laboratory. The plant samples for this investigation was collected from the Reserved Forest of Bengkulu, Sumatera. Artonin E **28** and a new compound trivially named artoindonesianin L **29** were the only 3-prenylflavones possessing 2',4',5'-trioxygenated system of ring B isolated from this species, together with related furanodihydro-benzoxanthone derivatives cycloarthobiloxanthone **30** and artonin M **31** 20, 21].



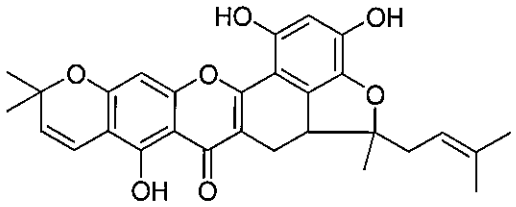
Artonin E **28**



Artoindonesianin L **29**

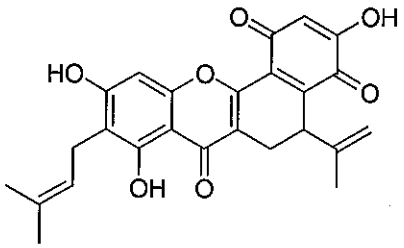


Cycloartobiloxanthone **30**



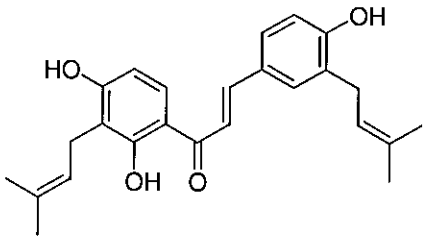
Artonin M **31**

Artonin O **32**, was the only quinonodihydrobenzoxanthone derivative so far isolated in our laboratory from *A. rotundus*. The presence of this unusual benzoquinone system can explain the occurrence of the flavonoid modified skeleton of xanthone derivatives such as artoindonesianin C **25**, artonol B **26** and artonol A **27**, which were isolated from *A. scortechinii* mentioned above.

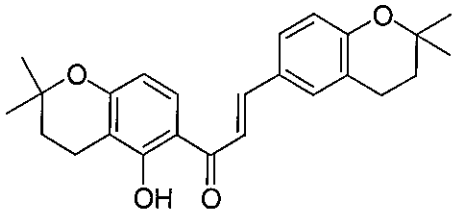


Artonin O **32**

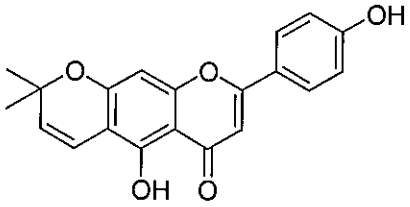
***Artocarpus bracteata* Hook.**, a species which was investigated for the first time in our laboratory, yielded two prenylated chalcone derivatives, kanzonol C **33** and a new chalcone named artoindonesianin J **34** together with another flavone derivative carpachromene **35**. It is interesting to note that the number and positions of the oxyaryl fungtionalities are the same on the A and B rings of all these flavonoids [22, 23].



Kanzonol C **33**



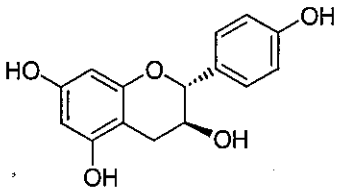
Artoindonesianin J **34**



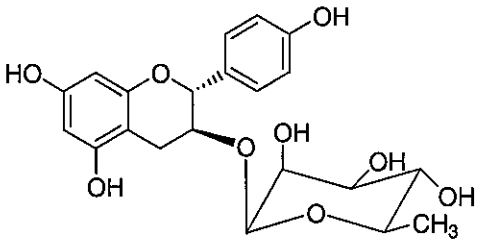
Carpachromene **35**

Artocarpus reticulatus Miq. is a large evergreen tree, up to 30 m tall. It is a rare species native to Sulawesi, and known locally as maumbi. It was investigated for the first time in our laboratory. This species yielded a series of flavan-3-ol derivatives, namely afzelechin **36**, afzelechin-3-O-α-L-rhamnoside **37** and catechin **38**. It should be noted that, differ from flavone derivatives these compounds possess 4'-monohydroxy or 3',4'-dihydroxy system of ring B of the flavan skeleton. Oxyresveratrol or 2,4,3',5'-

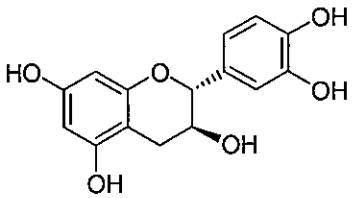
tetrahydroxy-*trans*-stilbene **39** had also been isolated from the same species [24].



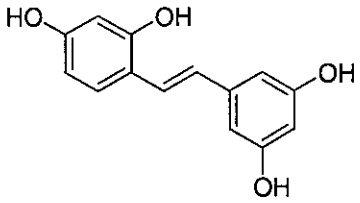
Afzelechin **36**



Afzelechin-3-a-L-rhamnoside **37**

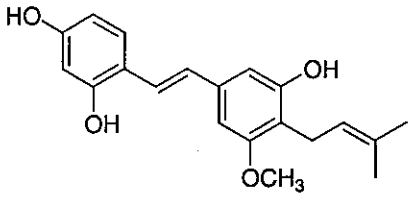


Catechin **38**

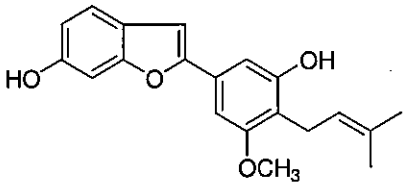


Oxyresveratrol **39**

***Artocarpus gomezianus* Wallich ex Trecul** is a timber tree. This species which was investigated for the first time in our laboratory yielded primarily different groups of phenolic compounds. In addition to a flavan derivative catechin **38**, and the stilbene oxyresveratrol **39**, this species also yielded a new stilbene derivative, a 4'-prenyl-2,4,3',5'-tetrahydroxystilbene, trivially named artoindonesianin N **40**, together with a new arylbenzofuran derivative, called artoindonesianin O **41** [25, 26].

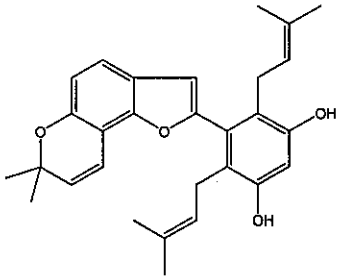


Artoindonesianin N **40**

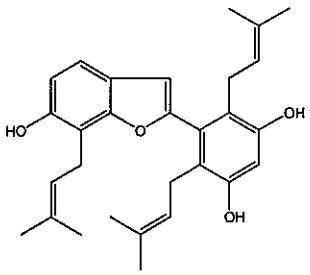


Artoindonesianin O **41**

***Artocarpus fretessi* Hassk.** is native to South Sulawesi and was also investigated for the first time in our laboratory. In addition to the flavanols afzelechin **36**, afzelechin-3-O-α-L-rhamnoside **37** and catechin **38**, *A. fretessi* also yielded two new prenylated arylbenzofuran derivatives, named artoindonesianin X **42** and artoindonesianin Y **43** [27, 28].

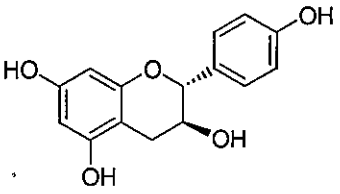


Artoindonesianin X **42**

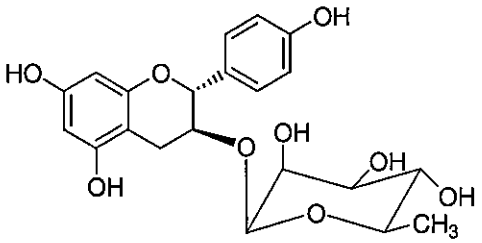


Artoindonesianin Y **43**

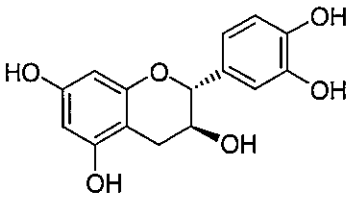
tetrahydroxy-*trans*-stilbene **39** had also been isolated from the same species [24].



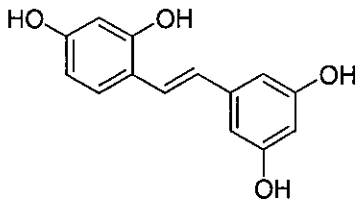
Afzelechin **36**



Afzelechin-3-a-L-rhamnoside **37**

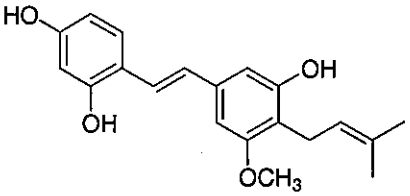


Catechin **38**

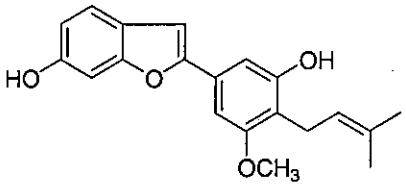


Oxyresveratrol **39**

***Artocarpus gomezianus* Wallich ex Trecul** is a timber tree. This species which was investigated for the first time in our laboratory yielded primarily different groups of phenolic compounds. In addition to a flavan derivative catechin **38**, and the stilbene oxyresveratrol **39**, this species also yielded a new stilbene derivative, a 4'-prenyl-2,4,3',5'-tetrahydroxystilbene, trivially named artoindonesianin N **40**, together with a new arylbenzofuran derivative, called artoindonesianin O **41** [25, 26].

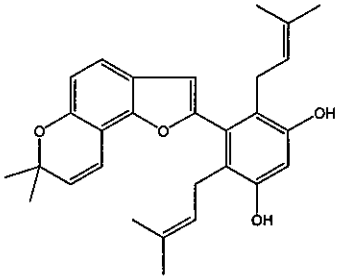


Artoindonesianin N **40**

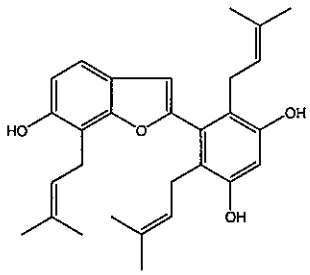


Artoindonesianin O **41**

***Artocarpus fretessi* Hassk.** is native to South Sulawesi and was also investigated for the first time in our laboratory. In addition to the flavanols afzelechin **36**, afzelechin-3-O-α-L-rhamnoside **37** and catechin **38**, *A. fretessi* also yielded two new prenylated arylbenzofuran derivatives, named artoindonesianin X **42** and artoindonesianin Y **43** [27, 28].



Artoindonesianin X **42**



Artoindonesianin Y **43**

BIOGENESIS OF PHENOLIC COMPOUNDS FROM *ARTOCARPUS*

In reviewing the essential features of the phenolic constituents so far isolated in our laboratories from several species of *Artocarpus* as described above, it was obvious that this group of compounds may be differentiated into several types of flavonoid, and modified flavonoid possessing xanthone skeleton, as well as stilbenoids and arylbenzofuran type of compounds. The flavonoids and the modified flavonoids can be divided into distinct groups according to their basic skeletons.

The majority of the numerous flavonoid derivatives known have the C_{20} basic skeleton of 3-prenylflavone possessing the same number and positions of the oxygen functions on the A and B rings of the flavone skeleton, such as artocarpin **8**, artoindonesianin Q **9** and artoindonesianin R **10**. These types of flavonoid include the basic 3-prenylflavones, which may be regarded as the immediate precursor in the diversification of the phenolic compounds produce by *Artocarpus*. Thus, the oxepinoflavone, pyranoflavone, and dihydrobenzoxanthone type of compounds, such as artoindonesianin **14**, artoindonesianin B **19** and artoindonesianin S **21**, respectively, may derive biogenetically from 3-prenylflavones, which under oxidative conditions might undergo C-O and C-C bond formations between the oxygen function at C-2' or the carbon atom at C-6' and the prenyl substituent at C-3 position. The oxepinoflavones possessed a 2'.4'-dioxygenated B ring system, and the pyranoflavones may have a 2',4'-dioxygenated or 2',4',5'-trioxygenated B ring systems, while the dihydrobenzoxanthones only have a 2',4',5'-trioxygenated functionality in ring B. This later type of compounds possessing an isopropenyl group may undergo cyclization to give the furanodihydrobenzoxanthone type of compounds, such as artoindonesianin A **24**.

The modified flavonoid type of compounds possessing xanthone skeleton might also derived biogenetically from the quinonodihydrobenzoxanthone derivatives. Artonin O **32** isolated from *A. rotundus* was the only example of this type of compounds encountered in our work. This original type of structure of the quinonodihydro-benzoxanthones, is again a branch point intermediate serving as an exclusive precursor for several different structural types of the flavonoid derived xanthones, namely cyclopentenoxanthones, xanthonolide and dihydroxanthone derivatives, such as artoindonesianin C **25**, artonol B **26** and artonol A **27**, respectively.. The quinonodihydrobenzoxanthones might undergo a Favorskii rearrangment to give the cyclopentenoxanthone type of compounds, followed by bond cleavage, decarboxylation and formation of the γ-lactone to give the xanthonolide derivatives. The dihydroxanthone type of compounds may also derive biogenetically from the quinonodihydro-benzoxanthones by retro-Diels-Alder transformation [29].

BIOLOGICAL ACTIVITY

The biological activity of some selected phenolic constituents of *Artocarpus* so far isolated in our laboratory have been evaluated for cytotoxic activity. Certain flavonoids displayed significant in vitro cytotoxic activity against murine leukemia P-388 cell lines. In Table 1 some typical compounds which are most active against the P-388 are listed. The cytotoxic activity of these compounds seemed to be well correlated with the 3-prenyl type of flavonoids as against the flavonoid derived xanthones. Study of the structure-activity relationships for several of the 3-prenylflavone derivatives seemed to indicate the structural requirements for significant activity. The requirements are principally the presence of a 3-prenyl substituent and possession of the *ortho*-oriented hydroxyl groups at C-4' and C-5' in Ring B of the flavone skeleton.

Table 1. Cytotoxic activity of phenolic compounds from *Artocarpus*

Phenolic Constituents	LC_{50} (in μg/mL)
Artocarpanone	19.3
Artonin E	0.06
Artocarpin	4.7
Artoindonesianin Q	10.3
Artoindonesianin L	0.6
Cuyclocommunol	9.0
Albanin A	2.3
Artoindonesianin (Cyclochampedol)	0.2
Chaplasin	2.0
Artoindonesianin B	1.9
Artonin O	0.9
Cycloartobiloxanthone	4.6
Artonin M	7.9
Artoindonesianin A	21.0
Artoindonesianin C	6.2
Artonol B	> 100

CONCLUSION

Artocarpus species endemic to Indonesia produce various classes of prenylated flavonoid and flavonoid derived xanthone derivatives characterized by interesting features. The unique structural features correlate very significantly with the cytotoxicity of these compounds against murine leukemia P-388 cell lines. The occurrence of 3-prenylflavones and related xanthone derivatives noted in *Artocarpus* is one of the characteristics of the genus. It may be suggested that more unique classes of compounds related to these structural types will be disclosed on further investigation of the *Artocarpus* species.

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REFERENCES

1. Nomura, T., Hano, Y. and Aida, M. (1998). *Heterocycles*. **47**(2): 1179-1205.
2. Achmad, S. A., Hakim, E. H., Makmur, L., Mujahidin, D. and Syah, Y. M. (2001). *J. Kimia Andalas*. 7(1): 6-11.
3. Verheij, E. W. M. and Coronel, R. E. (1992). (Eds.), *Plant Resources of Southeast Asia, No.2: Edible Fruit and Nuts*, PROSEA, Bogor, Indonesia, p. 79-96.
4. Heyne, K. (1987). *The Useful Indonesian Plants*, Jakarta.
5. Achmad, S. A., Hakim, E. H., Makmur, L., Mujahidin, D. and Syah, Y. M. (1999). *Keynote Speaker, Proceedings National Seminar on Chemistry of Natural Products '99*, November 16-17, Jakarta, p. 1-9.
6. Hakim, E. H., Achmad, S. A., Makmur, L. and Syah, Y. M. (2000). *Proc. 8th Internat. Symp. on Natural Products Chemistry*, January 18-22, Karachi, Pakistan.
7. Achmad, S. A., Aimi, N., Ghisalberti, E. L., Hakim, E. H., Kitajima, M., Makmur, L., Mujahidin, D., Syah. Y.M. and Takayama, H. (2000). *Proc. 10th Asian Symposium on Medicinal Plants and Spices and Other Natural Products (ASOMPS X)*, November 18-23, Dhaka, Bangladesh.
8. Achmad, S. A., Hakim, E. H., Juliawaty, L. D., Makmur, L., Aimi, N. and Ghisalberti, E. L. (1996). *J. Nat. Prod.* (USA). **59**(9): 878-879.

9. Hakim, E. H., Fahriyati, A., Kau, M. S., Achmad, S. A., Makmur, L., Ghisalberti, E. L. and Nomura, T. (1999). *J. Nat. Prod.* (USA). **62**(4): 613-615.
10. Makmur, L., Syamsurizal, Tukiran, Achmad, S. A., Aimi, N., Hakim, E. H., Kitajima, M. and Takayama, H. (2000). *J. Nat. Prod.* (USA). **63**(2): 243-244.
11. Parenti, P., Pizzigoni, A., Hanozet, G., Hakim, E. H., Makmur, L., Achmad, S. A. and Giordana, B. (1998). *Biochem. Biophys. Res. Comm.*, p. 244, 445-448.
12. Syah, Y. M., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H., Makmur, L. and Mujahidin, D. (2001). *Fitoterapia* (In print).
13. Achmad, S. A., Hakim, E. H., Makmur, L., Mujahidin, D., Juliawaty, L. D. and Syah, Y. M., *In Biomolecular Aspects of Biodiversity and Innovative Utilization* Sener, B. (Editor), Kluwer Academic/Plenum Publishers (2002), p. 91-99.
14. Hakim, E. H., Marlina, E. E., Mujahidin, D., Achmad, S. A., Ghisalberti, E. L. and Makmur, L (1998). *Proc. ITB.* **30**(1): 31-36.
15. Hakim, E. H., Aripin, A., Achmad, S. A., Aimi, N., Kitajima, M., Makmur, L., Mujahidin, D., Syah, Y. M. and Takayama, H. (2001). *Proc. ITB.* **33**(3): 69-73.
16. Syah, Y. M., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H., Makmur, L. and Mujahidin, D. (2002). *Phytochemistry* **61**: 949-953.
17. Ferlinahayati, Hakim, E. H., Achmad, S. A., Aimi, N. Kitajima, M. and Makmur, L. (1999). *Proceedings National Seminar on the Chemistry of Natural Products '99*, Jakarta, November 16-17, p. 162-167.
18. Armin, A. N., Hakim, E. H., Achmad, S. A., Ghisalberti, E. L., Makmur, L. and Syah, Y. M. (1999). *Proceedings National Seminar on the Chemistry of Natural Products '99,* November 16-17, Jakarta, p. 140-147.
19. Makmur, L., Syamsurizal, Tukiran, Achmad, S.A., Aimi, N., Hakim, E. H., Kitajima, M. and Takayama, H. (2000). *J. Nat. Prod.* **63**: 243-244.
20. Suhartati, T., Achmad, S. A., Aimi, N. and Hakim, E. H. (1999). *J. Mat. Sci.* **4**(2): 178-184.
21. Suhartati, T., Achmad, S. A., Auimi, N., Hakim, E. H., Kitajima, M., Takayama, H. and Takeya, K. (2001). *Fitoterapia*, **72**: 912-918.
22. Ersam, T., Achmad, S. A., Ghisalberti, E. L. and Hakim, E. H. (1999). *J. Mat. Sci.* **4**(2): 172-177.
23. Ersam, T., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H., Makmur, L. and Syah, Y. M. (2002). *J. Chem. Res.* (*S*), p. 186-187.
24. Achmad, S. A., Murniana, Udjiana, S. S., Aimi, N., Hakim, E. H. and Makmur, L. (1998). Proceedings ITB. **30**(2): 1-7.
25. Hakim, E. H., Ulinnuha, U. Z., Syah, Y. M. and Ghisalberti, E. L. (2002). *Fitoterapia*. **73**: 597-603.
26. Kurniadewi, F. and Hakim, E. H. (2003). *Bull. Soc. Nat. Prod.* **3**(2): 78-83.
27. Soekamto, N. H., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H. and Syah, Y. M. (2002). *Bull. Soc. Nat. Prod. Chem.* **2**(2):45-50.
28. Soekamto, N. S., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H. and Syah, Y. M. *Phytochemistry* (In print).
29. Aida, M., Yamaguchi, N., Hano, Y. and Nomura, T. (1997). *Heterocycles*. **45**(1): 163-175.