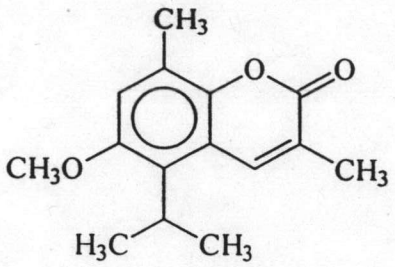
CHAPTER 4
CONCLUSION

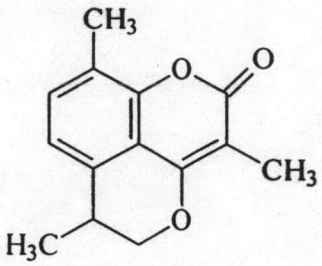
During the course of this research, the heartwoods of *Mansonia gagei* Drumm are chosen for investigation into their chemical constituents and for a search for biological active substances according to the preliminarily attractive bioassay results. By means of physical properties, chemical reactions and spectroscopic evidence, ten substances could be separated from the hexane and dichloromethane crude extracts. Nine of them were fully characterized as a long chain aliphatic ester (**1**), 3,8-dimethyl-5-isopropyl-6-methoxy coumarin (**2**), 2,3-dihydro-3,6,9-trimethyl naphtho[1,8-bc] pyran-7-oxa-8-one (**3**), 3,8-dimethyl-5-isopropyl-1,2-naphthoquinone or mansonone C (**4**), 3,8-dimethyl-5-isopropyl-6-hydroxy coumarin (**5**), a saturated long chain aliphatic ketone (**6**), a mixture of stigmasterol and β-sitosterol (**7**), 3,8-dimethyl-5-isopropyl-6-hydroxy-1,2-naphthalenedione or mansonone G (**8**) and 2,3-dihydro-4-hydroxy-3,6,9-trimethyl naphtho[1,8-bc]pyran-7,8-dione or mansonone H (**9**). Due to the limited amount of the tenth compound, its definite structure could not be concluded. However, this compound was proposed to be $C_{15}H_{14}O_4$ and should belong to the 1,2-naphthoquinone group.

Three compounds obtained, namely 3,8-dimethyl-5-isopropyl-6-methoxy coumarin (**2**), 2,3-dihydro-3,6,9-trimethyl naphtho[1,8-bc]pyran-7-oxa-8-one (**3**) and 3,8-dimethyl-5-isopropyl-6-hydroxy coumarin (**5**), were found to be new naturally occuring coumarins.

All isolated substances from the heartwoods of *Mansonia gagei* are summarized in Table 4.1.



Compound **2**



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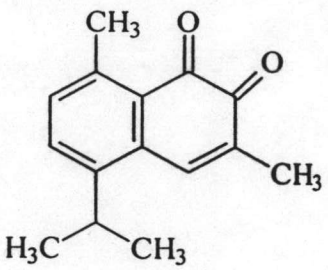

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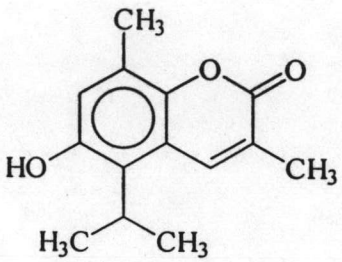
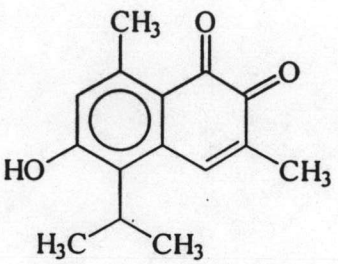
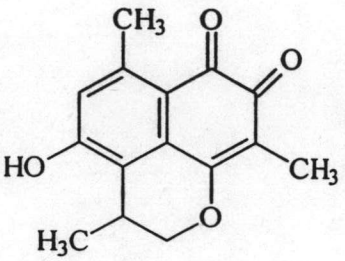


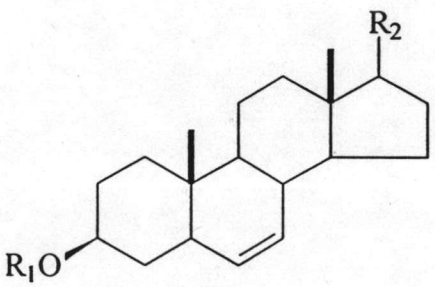
Compound **5**



Compound **8**



Compound **9**



Mixture **7** mixture of $R_1 = H$, $R_2 = C_{10}H_{23}$ and $C_{10}H_{21}$

Table 4.1 All isolated substances from the heartwoods of *M. gagei*

solvent extract	substances
hexane	a long chain aliphatic ester (**1**), 3,8-dimethyl-5-isopropyl-6-methoxy coumarin (**2**), 2,3-dihydro-3,6,9-trimethyl naphtho[1,8-bc]pyran-7-oxa-8-one (**3**)
dichloromethane	3,8-dimethyl-5-isopropyl-6-methoxy coumarin (**2**), 2,3-dihydro-3,6,9-trimethyl naphtho[1,8-bc]pyran-7-oxa-8-one (**3**), 3,8-dimethyl-5-isopropyl-1,2-naphthoquinone or mansonone C (**4**), 3,8-dimethyl-5-isopropyl-6-hydroxy coumarin (**5**), a saturated long chain aliphatic ketone (**6**), a mixture of stigmasterol and β-sitosterol (**7**), 3,8-dimethyl-5-isopropyl-6-hydroxy-1,2-naphthalenedione or mansonone G (**8**), 2,3-dihydro-4-hydroxy-3,6,9-trimethyl naphtho[1,8-bc]pyran-7,8-dione or mansonone H (**9**) and unidentified 1,2-naphthoquinone (**10**)

It can be seen from this research work that the major components in *Mansonia gegei* were 1,2-naphthoquinone and coumarin-base compounds. The presence of the former was well supported by the chemotaxonomy of the plants in *Mansonia* genus. Nevertheless, this work is the first report on the occurance of mansonones C, G and H in this particular species. In addition, to our knowledge, there has been no other of report on coumarins occurred in *Mansonia* genus before. Moreover, according to a chemical literature search, those three isolated coumarins, namely 3,8-dimethyl-5-isopropyl-6-methoxy coumarin (**2**), 2,3-dihydro-3,6,9-trimethyl naphtho[1,8-bc]pyran-7-oxa-8-one (**3**) and 3,8-dimethyl-5-isopropyl-6-hydroxy coumarin (**5**) were not reported as either synthetic or natural coumarins before. Therefore, these compounds are claimed to be new naturally occuring coumarins.

Concerning the biological activity study, it is manifest to state that the preliminary biological activity test, such as brine shrimp cytotoxicity test, was essential. This preliminary result would eventually lead to the disclosing of bioactive compounds. From this study, it could be observed that 1,2-naphthoquinone compounds such as 3,8-dimethyl-5-isopropyl-6-hydroxy-1,2-naphthalenedione or mansonone G (**8**) and 2,3-dihydro-4-hydroxy-3,6,9-trimethyl naphtho[1,8-bc]pyran-7,8-dione or mansonone H (**9**) gave promising results in the cytotoxicity test against brine shrimp. On the other hand, the coumarin compounds (Compounds **2** and **5**) did not reveal such activity. Unfortunately, some isolated compounds were obtained in such a small amount that the structural elucidation and/or biological testing were not possible. Therefore, the syntheses of those compounds may be necessary for future work.

This work, however, clearly demonstrates that the study on chemical constituents combined with the biological activity study should be fundamental work for natural product chemists. The outcome of the research work would also provide an excellent opportunity for the discovery of new therapeutic agent or other useful agents for specific purposes.

Proposal for the Future Work

Since the biological activity test results clearly showed that the methanol crude extract is another attractive fraction deserves attention, the thorough study of this fraction should be conducted. From this study, the preliminary work on cardiac glycoside testing on this fraction was performed. The results clearly confirmed the presence of cardiac glycoside in this fraction. However, attempt to separate to get a single cardiac glycoside were not successful. This may be because there were glycosides having very similar properties present. Therefore, the conventional separation tools may not be suitable. MPLC and/or HPLC are proposed to be a suitable instrument for this separation.

The biological test against various cell lines is still needed to be confirmed before being certain of these results. However, the brine shrimp cytotoxicity test was found to be helpful for the preliminary test. The cytotoxicity results were found to be of the same trend as those observed from anticell line test.

The structures of the three new coumarins could be confirmed by synthesis. Compounds related to the isolated coumarins and 1,2-naphthoquinones should be synthesized and studied for structure activity relationship. The outcome from that study would provide a clear understanding on the mechanism of activity.

Moreover, the chemical constituents and biological activity study of other parts of *Mansonia gagei* should be investigated. The results from that study would reveal the similarity or the difference and might lead to the understanding of biosynthetic pathway of some major components.