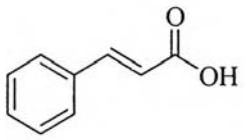
CHAPTER IV

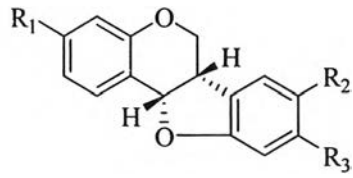
CONCLUSION

4.1 Biological Constituents from the Heartwoods of *Dalbergia oliveri*

Three substances, long chain saturated ketone, a mixture of triterpenoid ester and stigmasterol were isolated from the hexane extract of the *D. oliveri* heartwoods. Eight pure compounds (**D1-D8**) were isolated from CH_2Cl_2 extract. Concerning with the EtOAc extract, its chemical constituents contained **D3**, **D5**, **D6** and **D8** which were the same as those found in CH_2Cl_2 extract. Isolated substances were further elucidated their structures by means of their physical properties and spectroscopic evidences, and explored biological activity including scavenging effect on DPPH radical, antiplantpathogenic fungal activity, antimicrobial activity and cytotoxicity activity. The structures of isolated substances are depicted as shown in Figure 4.1. The results of their biological activity are summarized as presented in Table 4.1.

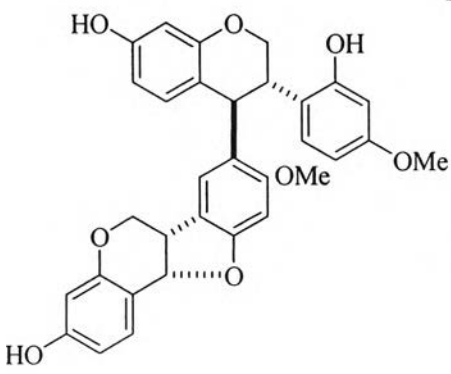


D1: *trans*-cinnamic acid

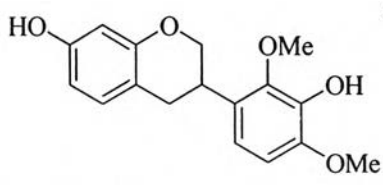


D2: (+) (*6aS*, *11aS*)-pterocarpin, R_1 = OMe, R_2R_3 = -OCH_2O-

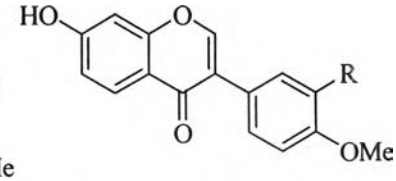
D3: (+) (*6aS*, *11aS*)-medicarpin, R_1 = OH, R_2 = H, R_3 = OMe



D4: (+)-isoflavan-pterocarpan

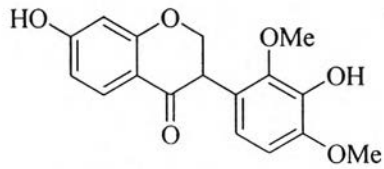


D5: (±)-mucronulatol



D6: formononetin, R = H

D8: calycosin, R = OH



D7: (±)-violanone

Figure 4.1 Structures of isolated substances

Table 4.1 Results of biological activity of isolated substances from CH_2Cl_2 extract from *D. oliveri* heartwoods

Substance	Antioxidant IC_{50} (mM)	Antifungal				Antimicrobial[b] (µg/ml)			Cytotoxicity				
		TLC Assay		Minimum amount required[a] (µg)		*E.*[f]	*S.*[g]	*S.*[h]	HepG2	Diamonback moth		Mosquito larvae	
		F.[c]	*C.*[d]	*F.*[c]	*A.*[e]				IC_{50} (µg/mL)	LC_{50} (ppm)	LC_{95} (ppm)	LC_{50} (ppm)	LC_{95} (ppm)
D1	-	+	+	1.0	1.0	>100	>100	>100	10.5	172	3192	210	3906
D2	-	-	-			>100	>100	>100	2.3				
D3	-	+	+	0.5	0.5	>100	>100	>100	2.3	99	1142	200	1374
D4	>1.00	-	-			>100	>100	>100	11.1				
D5	0.32	+	+	0.5	>10	>100	>100	>100	3.7				
D6	-	-	-			>100	>100	>100	10.5	102	1652	162	1482
D7	0.48	+	+	1.0	>10	>100	>100	>100	4.7	92	794	121	1048
D8	0.54	-	-			>100	>100	>100	11.1				
BHA	0.12												
Iprodion				0.1	0.1								
Captan				0.1	0.1								
Streptomycin						6-12	6-12	-					
Econazole						-	-	<3					

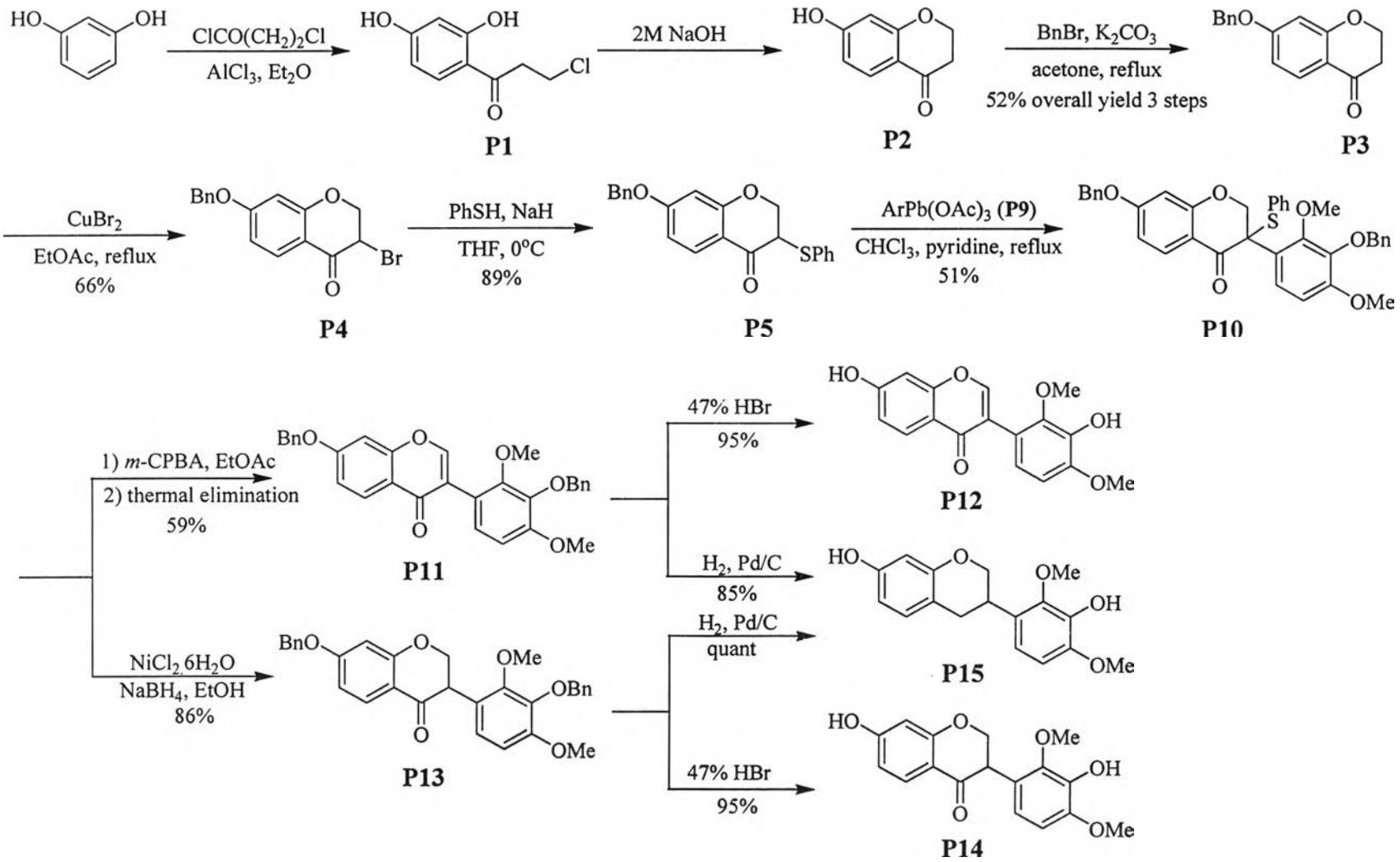
Note [a]minimum amount required for the inhibition of fungi growth on TLC plates, [b]The minimum inhibitory concentration was evaluated as the microbial agent concentration inhibiting 80% of absorbance regarding to the reference (no agent added), *F.*[c]: *Fusarium oxysporum*, *C.*[d]: *Colletotrichum gloeosporioides*, *A.*[e]: *Alternaria brassicicola*, *E.*[f]: *Escherichia coli*, *S.*[g]: *Staphylococcus aureus*, *S.*[h]: *Saccharomyces cerevisiae*, +: positive, - : negative

From the above results, **D2** ((+)-pterocarpin) revealed significant cytotoxicity toward HepG2 with IC_{50} value of 2.3 μg/mL. **D3** ((+)-medicarpin) displayed significant antifungal activity against both *F. oxysporum* and *A. brassicicola* and cytotoxicity toward human hepatocellular carcinoma (HepG2) cells and medium and low cytotoxicity against diamondback moth and mosquito larvae with LC_{50} values of 99 and 200 ppm, respectively. **D5** ((±)-mucronulatol) exhibited the highest scavenging activity toward DPPH radical as well as the highest antifungal activity against *F. oxysporum* and the medium cytotoxicity against HepG2 cells whereas the minimum amount of this compound required to inhibit growth of the fungus *A. brassicicola* was determined as >10 μg. **D7** ((±)-violanone) showed high cytotoxicity against diamondback moth and mosquito larvae with LC_{50} values of 92 and 121 ppm, respectively, medium antifungal activity against *F. oxysporum*, scavenging activity toward DPPH radical and cytotoxicity against HepG2 cells and low antifungal activity against *A. brassicicola.* Concerning with bioactivities of the other isolated substances, **D1** (*trans*-cinnamic acid) showed moderated antifungal activity against both *F. oxysporum* and *A. brassicicola* and low cytotoxicity against HepG2, diamondback moth and mosquito larvae. **D4** ((+)-isoflavan-ptercarpan) exposed low cytotoxicity against HepG2 with IC_{50} value of 11.1 μg/mL. **D6** (formononetin) displayed moderate cytotoxicity against diamondback moth and mosquito larvae with LC_{50} values of 102 and 162 ppm, respectively and low cytotoxicity against HepG2 with IC_{50} value of 10.5 μg/mL. **D8** (calycosin) disclosed moderate scavenging activity toward DPPH radical with IC_{50} value of 0.54 mM and low cytotoxicity against HepG2 with IC_{50} value of 11.1 μg/mL. Concerning with antimicrobial activity against *E. coli*, *S. aureus* and *S. cerevisiae*, all isolated substances revealed insignificant result.

4.2 Synthesis of Selected Isoflavonoids

In this research, 3',7-dihydroxy-2',4'-dimethoxy-isoflavone, -isoflvanone and -isoflavan were selected to synthesize. These isoflavonoids could be synthesized using arylation of α-phenylthiochroman-4-one with aryllead (IV) triacetate. Isoflavone could be synthesized by two routes. The first route was arylation of α-phenylthiochroman-4-one with lead (IV) tetraacetate; alternatively, using the ring closure of deoxybenzoin.

The total synthesis of three target molecules using arylation of the chromanone moiety is presented in Scheme 4.1.

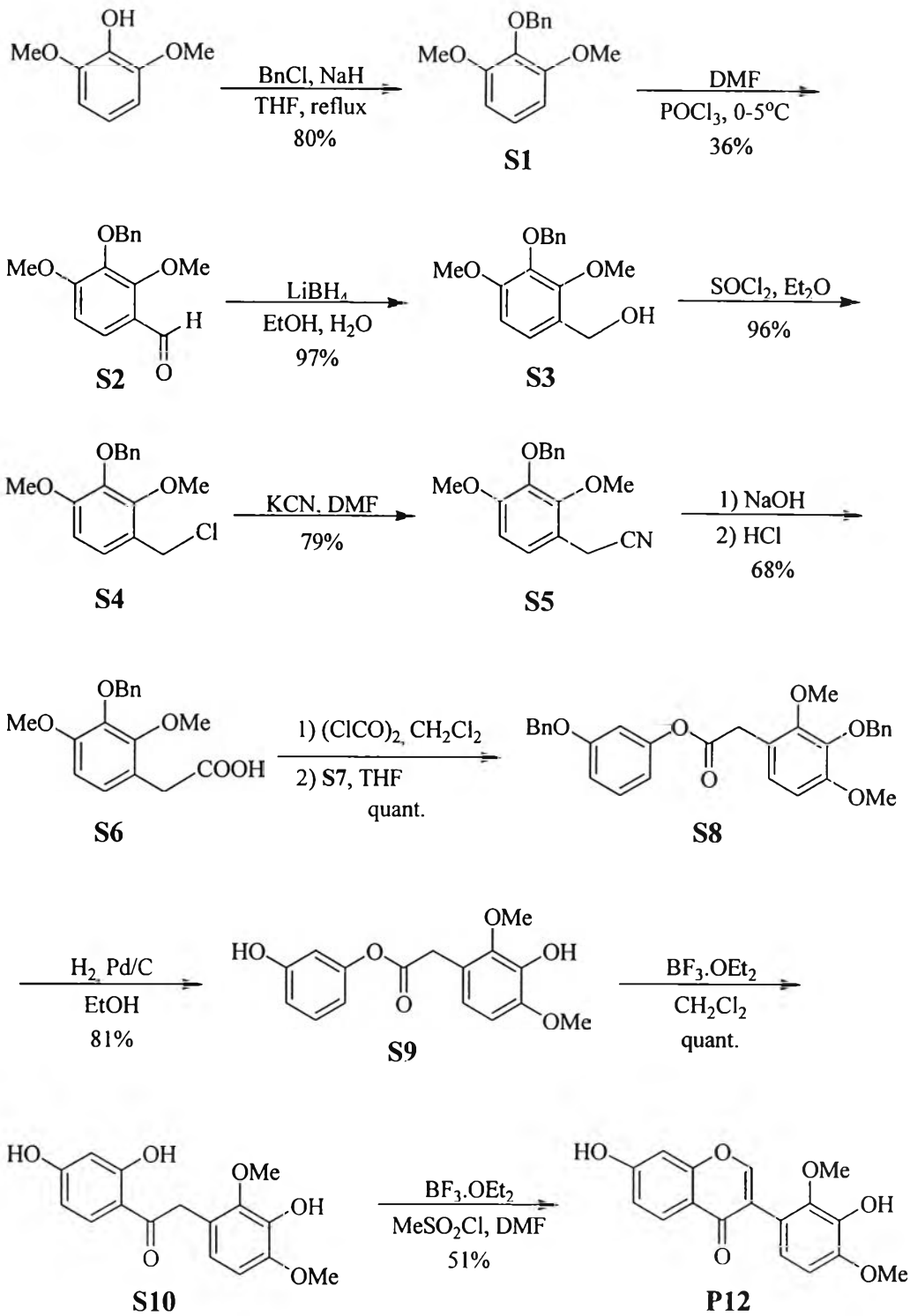


Scheme 4.1 Total synthesis of three target molecule using arylation of chromanone moiety

In conclusion, three target molecules were accomplishly synthesized in 8 step using arylation of common α-phenylthiochroman-4-one with aryllead (IV) triacetate reagent as a key step. The reaction of 3-benzyloxy-2,4-dimethoxyphenyllead (IV) tetraacetate (**P9**) with 7-benzyloxy-3-phenylthio-chroman-4-one (**P5**) was carried out in the presence of pyridine to afford moderate yield of the corresponding 3-aryl-3-phenylthiochroman-4-one (**P10**). Removal of the phenylthio group by oxidation with *m*-CPBA led to the corresponding isoflavone while reduction of **P10** with a large excess nickel boride led to the corresponding isoflavanone. After deprotection, the target isoflavone and isoflavanone were received. Hydrogenation either of the corresponding isoflavone or isoflavanone obtained the desired isoflavan.

The total synthesis of 3',7-dihydroxy-2',4'-dimethoxyisoflavone *via* deoxybenzoin is depicted in Scheme 4.2.

3',7-Dihydroxy-2',4'-dimethoxyisoflavone could also be synthesized using ring closure of deoxybenzoin in 10 steps with 6% overall yield. This procedure concerned with benzylation reaction of the appropriate phenol followed by reduction of aldehyde to alcohol, chlorination, cyanation and hydrolysis to obtain the corresponding arylacetic acid (**S6**). After chlorination using oxalylchloride and followed by condensation with 3-benzyloxyphenol gave the corresponding ester (**S8**). After deprotection and followed by Fries rearrangement, the corresponding deoxybenzoin (**S10**) was attained. Finally, condensation with DMF in the presence of $MeSO_2Cl$ and $BF_3.Et_2O$ afforded the ring closure isoflavone by insertion of one carbon into C-2 and followed by cyclization.



Scheme 4.2 Total synthesis of 3',7-dihydroxy-2',4'-dimethoxyisoflavone *via* deoxybenzoin

Three synthetic compounds were further examined for cytotoxicity against human epithelial mammary HBL100 cells. 3',7-Dihydroxy-2',4'-dimethoxyisoflavan displayed high cytotoxicity against HBL100 cells with IC_{50} value of 5.21 μM whereas the other synthetic compounds were inactive (IC_{50} > 50 μM).

4.3 Proposal for the Future Work

The incidence of (+)-medicarpin as a major constituent from CH_2Cl_2 extract of the heartwoods of *Dalbergia oliveri* revealed an influence on the antifungal activity against both *F. oxysporum* and *A. brassicicola*. This work provided the possibility to use this plant as a raw material for antifungal agent. The possible future work of medicarpin derivatives and other biological activities may provide an opportunity to understand the structure activity relationship of this class of compound. Another aspect that would make this dissertation fulfill is the chemical constituents and biological activity investigations of MeOH extracts and other parts of *D. oliveri*. This would provide informative data for the chemotaxonomy of this plant.

Moreover, from results of preliminarily biological screening test as shown in Section 1.1.2, it was found that EtOAc extract of the leave and both CH_2Cl_2 and EtOAc extracts of the seed of *P. lathyroides* disclosed good insecticidal activity against *S. litura* (≥ 70% mortality). Therefore, chemical constituents and biological activities of *P. lathyroides* might be worthwhile to explore.

Since the synthesis of isoflavonoids using arylation reaction between chromanone moiety and aryllead (IV) triacetate is effective. It would offer a good chance for manipulating a series of isoflavonoids with different substituents at 3-position. Therefore, the possible future work is the synthesis of a series of isoflavonoids containing different substituents at 3-position to study structure activity relationship (SAR). Other possible future work is to synthesize other biological isoflavonoids employing this methodology.