Summary of Doctoral Thesis

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Title	Study on cytotoxic phenolic sesquiterpenes isolated from *Dysoxylum parasiticum* (Osbeck) Kosterm. [Meliaceae]

Introduction and purpose

Plants and other natural sources can provide a vast range of complex and structurally diverse compounds. Drug discovery from plants has played an important role in treating human diseases, and indeed, most current anticancer agents used in clinical practices are derived from plants. For this reason, the discovery of new drugs from plants is an exclusively important objective. Moreover, the screening method for selecting the material resources is also essential by considering the number of materials, limited cost, and limited time. One of the methods is by using primates as a screening source for plant candidates. That is rare natural resources and a unique screening method conducted by some researchers.

Based on previous research, 42 Indonesian primate-consumed plants had been utilized as anticancer. Those plants grow in the Pangandaran Nature Reserve of West Java Province, Indonesia. Several compounds were isolated from these plants, including kaempferol-3-*O*-rhamnoside isolated from *Schima wallichii*, 2′,4′-dihydroxy-6-methoxy-3,5-dimethylchalcone isolated from *Eugenia aquea*, and catechin isolated from *Garcinia celebica* with significant anticancer and antiplasmodial activities. Previously, it was discovered that some of these plants, including *Disoxylum parasiticum* (synonym of *Dysoxylum caulostachyum*), showed potential anticancer, antibacterial, and antiplasmodial activities. Therefore, the studies were focused on searching for specialized metabolites in primate-consumed plants that exhibit cytotoxic activity against cancer cells.

This thesis describes the investigation of chemical components and their cytotoxic activity from *Dysoxylum parasiticum* (Osbeck) Kosterm. belonging to Meliaceae family. This plant grows up to 36 m tall, has a trunk with a diameter of up to 60 cm, and buttress roots measuring up to 1.5 m in diameter. It has potential as a feature tree in parks or gardens in areas with subtropical or tropical climates. The leaves of this plant were collected as material research from the exact location, Pangandaran Nature Reserve area of West Java Province, Indonesia. Recently, the sesquiterpene-derivative compounds isolated from its bark exhibited cytotoxic properties against the MCF-7 breast cancer cell line. Based on this study, the chemical investigation from *D. parasiticum* leaves led to the isolation of six undescribed specialized metabolites, namely, bidysoxyphenols A–C (**3**–**5**), tridysoxyphenols A and B (**6** and **7**), and bidysoxyletine (**8**), together with four known compounds, namely dysoxyphenol (**1**), 7*R*,10*S*-3-hydroxycalamenene (**2**), 4-hydroxy-4,7-dimethyl-α-tetralone (**9**), and 3*R*,6*S*-3-hydroxy-α-ionone (**10**).

Materials and methods

The isolation of specialized metabolites from the leaves of *D. parasiticum* has been executed

utilizing several steps of the chromatography method. The structures of **3**–**7** were elucidated using nuclear magnetic resonance (NMR) spectroscopy, ultraviolet (UV) spectroscopy, infrared (IR) spectroscopy, high-resolution electrospray ionization time-of-flight mass spectrometry (HR-ESI/TOF-MS), and time-dependent density functional theory (TDDFT) calculations of the electronic circular dichroism (ECD) spectroscopic data, which were supported by their biosynthetic pathway. The structure of **8** was elucidated by analysis of NMR, UV, IR, HR-ESITOFMS, and DDFT approach using the B3LYP exchange-correlation function for ^{13}C NMR and UV spectroscopic data.

Compounds **1**–**7**, **9**, and **10** were tested for their cytotoxic activity against HL60 cells using the MTT assay, with camptothecin as a positive control. Compound **8** was excluded from the test because its red color was incompatible with this method, and it was also slightly soluble with the media.

Results

Based on the 1-dimensional (1D) and 2-dimentional (2D) NMR data of **3** implied that **3** was a sesquiterpene phenol dimer comprising two 8-isopropyl-1,5-dimethyl-5,6,7,8-tetrahydronaphthalen-2-ol structures, units A and B, which their planar structures were the same as those in dysoxyphenol (**1**). Meanwhile, the ^{1}H and ^{13}C NMR data of **4** and **5** were similar to those of **3**, with the difference in the benzene ring of unit B, which the planar structures corresponded to that 5-isopropyl-3,8-dimethyl-5,6,7,8-tetrahydronaphthalen-2-ol (**2**) in **4** and 8-isopropyl-3,5-dimethyl-5,6,7,8-tetrahydronaphthalen-2-ol (chiloscyphenol A) in **5**. The two signals of **3** at δ_C 141.9 (C-3) and 152.4 (C-4′), corresponding to oxygen-bearing benzenoid carbons, as well as the nuclear Overhauser effect (NOE) correlation of H-2/H-3′ suggested that the two monomeric units A and B were connected by a 3-O-4′ (ether) linkage. In addition, the two oxygen-bearing benzenoid carbons of **4**, which showed signals at δ_C 141.5 (C-3) and 152.1 (C-3′), as well as the NOE correlation of H-2/H-2′, suggested that the two monomeric units A and B were connected by a 3-O-3′ linkage. Moreover, **5** showed signals at δ_C 141.7 (C-3) and 152.0 (C-4′), corresponding to the two oxygen-bearing benzenoid carbons, as well as NOE correlation of H-2/H-5′, suggesting that the two monomeric units A and B were connected by a 3-O-4′ linkage. The relative configurations of both units A and B in **3**–**5** were determined on the basis of the NOE correlation. The NOE data of **3**–**5** revealed the trans relationship of both 10-CH_3 and 7-isopropyl groups in unit A and both 10′-CH_3 and 7′-isopropyl groups in unit B. The ECD data of **3** were compared with that calculated data of **3** using TDDFT at the B3LYP/6–311G (d) level. The calculated spectral data of **3** was in good agreement with the experimental spectrum. This comparison suggested that the C-7, C-10, C7′, and C-10′ in **3** were 7*R*, 10S, 7′*R*, and 10′*S*. In addition, the proposed biosynthetic pathway of dimeric compound **3**, from **1** aided the determination of the absolute configuration of compound **3**. Therefore, the absolute configuration of **3** was established, and the compound was named bidysoxyphenol A. Moreover, The ECD spectral data of **4** and **5** were compared with those of **3**. Those comparations showed that the experimental ECD curves of **4** and **5** were similar to that of **3**, suggesting good accordance for assigning the absolute configurations of **4** and **5** (7*R*,10*S*,7′*R*,10′*S*). In addition, the proposed biosynthetic pathways of dimeric compounds **4** and **5** from **1**, **2**, and chiloscyphenol A aided in the determination of the absolute configurations of **4** and **5**. Therefore, the structures of **4** and **5** were established as shown in and were referred to as bidysoxyphenols B and C, respectively.

The NMR data of the remaining 45 carbons in **6** indicated that they were assigned to the same three sesquiterpene phenol moieties, designated as units A, B, and C in the structure. In addition, the characteristic signals of four oxygen-bearing benzenoid carbons at δ_C 142.9 (C-3), 141.7 (C-4′), 146.3 (C-3′), and 153.2 (C-4″), as well as rotating-frame Overhauser enhancement spectroscopy (ROESY) correlations of H-2/H_3-15′ and H-2′/H-3″ indicated that compound **6** might be a trimer of **1**. Moreover, the two monomeric units, A and B, were connected by a 3-O-4′ linkage, whereas units B and C were connected by a 3′-O-4″ linkage. The ROESY data of **6** suggested that the 10-CH_3 and 7-isopropyl groups in unit A, the 10′-CH_3 and 7′-isopropyl groups in unit B, and the 10″-CH_3 and 7″-isopropyl groups in unit C are in trans configuration. Thus, the ROESY data suggest that the relative configurations of units A, B, and C in **6** were similar to those in **1.** To determine the absolute configuration of **6**, its ECD spectrum was calculated using the TDDFT calculations. The calculated ECD spectrum of **6** at the B3LYP/6–311G (d) level was in good agreement with the experimental ECD spectrum, which established the absolute configuration of **6**. In addition, we proposed that the trimeric compound **6** was biosynthetically produced via radical addition of a dimeric sesquiterpene phenol, bidysoxyphenol A (**3**), by its monomer, dysoxyphenol (**1**). Therefore, the absolute configuration of **6** was established and the compound was named tridysoxyphenol A.

A comparison of the ^{1}H and ^{13}C NMR data of **7** revealed that compounds **7** and **6** were closely related and shared the same trimeric sesquiterpene phenol skeleton, except for the benzene ring of unit C. In addition, the planar structure of unit C in **7** is the same as that in **2**. The ^{1}H, ^{13}C NMR, and HMBC data of the unit C structure in **7** also revealed that they are similar to those of the unit B structure in **4**. These results, as well as the nuclear Overhauser effect spectroscopy (NOESY) correlation of H-2′/H-2″, suggested that monomeric units B and C in **7** were connected by the 3′-O-3″ linkage. Based on the NOESY data for **7**, the relative configurations of each unit in **7** were similar to those assigned in **6** at C-7, C-10, C-7′, C-10′, C-7″, and C-10″. The absolute configurations of **7** was established by comparing its ECD spectrum with that of **6**, which showed good agreement for assigning the absolute configuration of **7**. In addition, the proposed biosynthetic pathway of trimeric compound **7** via the radical addition of **3** by compound **2** aided in the determination of the absolute configuration of compound **7**. Based on the previously described spectral data and the proposed biosynthetic pathway, the absolute configuration of **7** was determined and the compound was named tridysoxyphenol B.

The proposed biosynthetic pathway of **1**–**7** is presented in this thesis. Compound **3** is proposed to be originated from **1** via a biosynthetic pathway, and produced by the coupling of the two phenolic systems of **1** in a process that could be readily rationalized by radical reactions. Meanwhile, compounds **4** and **5** are produced by coupling the two phenolic systems of **1**/**2** and **1**/chiloscyphenol A, respectively. Moreover, I proposed that compounds **6** and **7** are thought to arise from **3** via radical addition of **1** and **2**, respectively, catalized by an H_2O_2-dependent peroxidase enzyme, and followed by dehydrogenation. Interestingly, compound **1** and chiloscyphenol A have the basic skeleton isomeric to those hydroxycalamenenes, in which the Me-15 were located at C-5 for **1** and C-3 for chiloscyphenol A, instead of at C-4. Compound **1** and chiloscyphenol A have been isolated for the first time from *D. densiflorum* and *Chiloscyphus polyanthus*, respectively. Both compounds′ formations were thought to arise from an irregular farnesane, via various oxidation and reductive cyclization processes. This irregular farnesane, namely tanacetene, has been isolated for the first time from *Tanacetum longifolium*. Tanacetene is a rare, irregular, non-head-to-tail sesquiterpene derived from a natural product that does not follow

the biogenetic isoprene rule.

The simplicity of the NMR spectra, the high molecular weight, the deshielding chemical shift of hydroxyl and aromatic protons at 3-OH and H-2, the shielding chemical shift of ester carbonyl at C-7, and the UV absorption maximum at 425 nm of **8** suggested that the compound has a dimeric structure. Moreover, the ^{1}H and ^{13}C NMR data of **8** were similar to those castanaguyone, a biisocoumarin derivative isolated from *Zanthoxylum fagara*. The two differences were the positions of hydroxyls and geranyls on two aromatic groups. According to the 1D and 2D NMR data, four possibilities of compound **8** were revealed. The discrimination between these four possibilities (**8a**–**8d**) was achieved using theoretical NMR calculations. To reduce a vast number of conformations and make this analysis feasible, the structures were set to replace the two geranyl fragments with methyl groups. The experimental carbon resonances of **8** matched with the calculated data for **8b** with the most significant correlation coefficient, R^2=0.9896. These results indicated that 3,3′-dihydroxyl-5,5′-dimethyldibenzonaphthyrone-type (**8b**) could be the structure of **8**. In support of these theoretical NMR calculation data, the experimental UV spectrum of **8** in MeOH and the spectrum calculated for **8a**–**8d** at the TD-DFT B3LYP/6–311G (d)//B3LYP/6–31G (d) and the TD-DFT mPW1PW91/6–311G (d)//B3LYP/6–31G (d) levels, including the PCM solvent model for MeOH, were compared and confirmed that the structure of **8** was the same as **8b**. In conclusion, the structure of **8** was identified as a bicoumarin derivative and named bidysoxyletine.

Based on the cytotoxicity assay, the results showed that compound **1** exhibited medium inhibitory activity, with an IC_{50} value of 18.25 ± 1.52 μM, while compound **3** showed low inhibitory activity, with an IC_{50} value of 39.04 ± 3.12 μM. Moreover, the results showed that compounds **2** and **4** were not cytotoxic at low concentrations, which showed the IC_{50} values more than 50 μM, but they suppressed the viability of cell at 119.85 ± 10.03 and 137.41 ± 24.18 μM, respectively. The IC_{50} values of compounds **5**–**7**, **9**, and **10** were higher than 150 μM, and thus, these compounds were considered less active or inactive. These implied that the activity of the sesquiterpene phenol derivatives decreased with increasing molecular weight. Referring to their IC_{50} values, compound **1** showed higher cytotoxicity as compared to compound **2**. This might be due to the –OH group position at C-4 and the methyl position at C-5 in the benzene ring affecting the cytotoxic properties. Compounds **3** and **4** were dimeric structures of **1** and **2**, respectively, and the cytotoxicity of compound **3** was more potent than that of compound **4**. These findings indicate that the monomeric structure **1** has an important role in exhibiting cytotoxicity against the HL60 cell line and suggest that compounds **1** and **3**, as sesquiterpene phenol derivatives, are potential candidates for developing new anticancer drugs. However, further studies involving the analysis of more analogs of monomeric and oligomeric compounds with hydroxyl and methyl substituent groups are required to support this preliminary conclusion. Furthermore, understanding these compounds′ molecular mechanisms of target cytotoxicity may provide valuable information for their possible applications in cancer management.

Conclusion and consideration

In conclusion, three undescribed sesquiterpene phenol dimers (compounds **3**–**5**), two undescribed sesquiterpene phenol trimers (compounds **6** and **7**), an undescribed bicoumarin derivative (**8**), along with four known compounds (**1**, **2**, **9**, and **10**), were isolated from the leaves of *D. parasiticum*. Compounds **1** and **3**, as sesquiterpene phenol derivatives, displayed potential cytotoxicity against the HL60 cell line. Our results suggested that these compounds may be utilized

as lead compounds for further development of anticancer agents.

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