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Contributed Paper

Phytochemical Constituents of the Stems of *Styrax benzoides* Craib and Their Chemosystematic Significance

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ABSTRACT

The first chemical investigation in the dichloromethane extract from *Styrax benzoides* stems has been reported. On the basis of chromatography, five compounds including erythrodiol-3-acetate (**1**), stigmasterol (**2**), (24*R*)-24-ethylcholesta-4,22-dien-3-one (**3**), 5-(3″-hydroxy propyl)-7-methoxy-2-(3′,4′-methylenedioxyphenyl)benzofuran (**4**) and 5-(3″-hydroxy propyl)-7-methoxy-2-(3′,4′-dimethoxyphenyl)benzofuran (**5**) were successfully isolated. The chemical structures of all refined compounds were elucidated by a combination of several spectroscopic results accompanied by comparison with the literature data. All five compounds were isolated for the first time in the species *S. benzoides*. The preliminary chemosystematic analysis suggests the close taxonomic relationship between *S. benzoides* and other species in the same genus such as *S. camporum*, *S. officinalis*, and *S. ferrugineus*. Benzofurans **4** and **5** may serve as significant chemotaxonomic markers of the genus *Styrax*.

Keywords: *Styrax benzoides, Styrax*, chemotaxonomic, chemical constituents

1. INTRODUCTION

The genus *Styrax* is the largest member of *Styracaceae* family which is composed of 130 different species of trees and shrubs exposed in South America, Eastern and Southeast Asia, and Mediterranean zone [1,2]. Several plants in this genus are characterized by their ability to produce benzoin resins, resinous materials which have been used in perfumes, cosmetics and traditional medicines around the world. It is known that these resins have been utilised as treatments of inflammatory diseases in several parts of Asia and America [3,4]. Other applications of these resins are antiseptic, treatment of respiratory diseases [5], and expectorant [6]. The flower, leave, fruits and galls of some species are employed as Chinese herbal medicines [2].

S. benzoides, or 'Kam Yan' in Thai, is a species of a small slender evergreen tree

classified in the genus *Styrax*. The tree has been widely distributed in Southern Yunnan, Laos, Myanmar, Northern Thailand and Vietnam [7]. Although indigenous to the countries in South-East Asia, it is very interesting to mention that there have been no other sufficient reports regarding the phytochemical and chemotaxonomic study of *S. benzoides*. For this reason, our attention was to explore and investigate the chemical compositions of this species.

The studies of chemical compositions in many *Styrax* plants had been reported in numerous publications. Particularly, benzofurans are defined as one type of the major chemical components discovered in *Styrax* plants. They were found in many species such as *S. officinalis* [8,9], *S. macranthus* [10], *S. obassia* [11,12], and *S. perkinsiae* [13,14]. The existences of some triterpenes have been explored in *S. tonkinensis* [6,15], and *S. japonica* [4,16-19]. Novel lignan compounds were also successfully isolated from the stem bark of *S. japonica* [17,20-22] and separated from the stems of *S. camporum* [23]. Moreover, arylpropanoids from the leaves of *S. ferrugineus* [3], and sterol from the stem-bark of *S. japonica* [19], were purified and completely identified.

2. MATERIALS AND METHODS

2.1 Plant Source

The stems of *S. benzoides* were collected from Mae Sa Mai village, Chiang Mai, Thailand. The plant material (code number J.F. Maxwell 04-365) had been deposited at the herbarium of Department of Biology, Faculty of Science, Chiang Mai University, Thailand.

2.2 General Procedure

Unless otherwise stated, all chemicals used in this work were of analytical or reagent grade. Organic solvents were of commercial grade and were distilled prior to use for extraction and as eluents for thin layer and column chromatography. Flash column chromatography (FCC) were performed on silica gel (Merck, particle size < 45 *m*m) and sephadex™ LH-20 (Amersham Biosciences). Thin-layer chromatography (TLC) was performed on silica gel 60 PF_{254} pre-coated on an aluminum plate (Merck). Preparative thin layer chromatography (PTLC) were performed on silica gel 60 PF_{254} (Merck) by spreading an aqueous slurry on to plate size 20 × 20 cm, left standing to dry at room temperature and subsequently activated for 4 hours at 120 °C in an oven. Melting points were determined on an Electrothermal melting point apparatus and were uncorrected. The temperature was given in degree Celsius. Optical rotations of some compounds were obtained using an automatic polarimeter (ATAGO, AP-300). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 NMR spectrometer, operating at 400 and 100 MHz, respectively. Chemical shift (β) were reported in the unit of part per million with respect to the internal standard (TMS). IR spectra were recorded on a FT-IR spectrometer (Tensor 27). Spectra of the liquid samples were recorded as film technique and spectra of the solid samples were recorded as KBr pellets. High-resolution mass spectra (HRMS) were performed on a Q-TOF 2™ mass spectrometer with a Z-spray™ ES source (Micromass, Manchester, UK). Gas chromatography-mass spectrometry analysis (GC/MS) was performed on an Agilent-HP5973 mass spectrometer equipped with Alltech 15879 AT-1 MS capillary column (30 m × 0.25 mm, 0.25 μm film thicknesses). The oven temperature was programmed from 45-250 °C at the rate of 2 °C/min with final hold 12.5 min, using helium as carrier gas. Individual components were identified

by Wiley 275 and NIST database matching. Relative percentages of individual components were calculated based on GC peak areas without using correction fractions. The injector and detector temperatures were 200 °C and 230 °C, respectively. Mass spectra were taken at 70 eV with the mass range of m/z 29-550.

2.3 Isolation of the Chemical Constituents of *S. benzoides*

Dried material of *S. benzoides* stems (167.33 g) was extracted by dichloromethane (CH_2Cl_2), and the leftover solid residues were removed from its CH_2Cl_2 extract. The crude extract was evaporated to dryness under reduced pressure to give the dark-brown residue (3.40 g). The preliminary investigation of its constituents was performed by pre-coated thin layer chromatography plates (silica gel 60 PF_{254}). In this process, organic solvents, consisting of *n*-hexane, CH_2Cl_2, ethyl acetate (EtOAc) and methanol (MeOH), were used to elute the captured residue of the CH_2Cl_2 extract in a column by gradient eluting systems. At the end of the first isolation, thin layer chromatography (TLC) and I_2 reagent were utilized as the essential references to classify 12 major fractions (SB1-12).

Two crystalline substances, compounds **1** (6.77 mg, 0.0040% based on the dried material) and **2** (2.69 mg, 0.0016%), were successfully recrystallized from fraction SB7 (0.3654 g) using CH_2Cl_2. After that, the remaining residue of fraction SB7 was consequently subjected to the repeated PTLC technique using *n*-hexane:CH_2Cl_2:acetone (75:19:6, v/v/v) as mobile phase, providing compound **3** (3.17 mg, 0.0019%) as a colorless solid. The purification of fraction SB9 (0.9356 g) by column chromatography using *n*-hexane:CH_2Cl_2:acetone (64:26:10, v/v/v) as eluent under isocratic condition gave two white amorphous-powdery substances, compounds **4** (182.3 mg, 0.109%) and **5** (7.24 mg, 0.0043%).

Erythrodiol-3-acetate (**1**): White crystalline solid, mp 229.8-232.5 °C (Lit. 228-230 °C) [24]; R_f 0.17 (10% EtOAc in *n*-hexane), IR (KBr) ($\nu_{max,}$ cm^{-1}): 3490, 2945, 1708, 1640, 1464, 1369, 1267, 1007; ^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), J/Hz): 0.83 (1H, *brd*, J = 2.96 Hz, 5-C<u>H</u>), 0.86 (3H, *s*, 24-C<u>H</u>$_3$), 0.87 (3H, *s*, 23-C<u>H</u>$_3$), 0.87 (3H, *s*, 30-C<u>H</u>$_3$), 0.93 (3H, *s*, 26-C<u>H</u>$_3$), 0.95 (3H, *s*, 25-C<u>H</u>$_3$), 1.01 and 1.71 (2H, *m*, 15-C<u>H</u>$_2$), 1.08 and 1.74 (2H, *m*, 19-C<u>H</u>$_2$), 1.08 and 1.63 (2H, *m*, 1-C<u>H</u>$_2$), 1.16 (3H, *s*, 27-C<u>H</u>$_3$), 1.16 and 1.30 (2H, *m*, 7-C<u>H</u>$_2$), 1.18 and 1.89 (2H, *m*, 21-C<u>H</u>$_2$), 1.33 and 1.55 (2H, *m*, 16-C<u>H</u>$_2$), 1.35 and 1.52 (2H, *m*, 22-C<u>H</u>$_2$), 1.41 and 1.56 (2H, *m*, 6-C<u>H</u>$_2$), 1.57 (1H, *m*, 9-C<u>H</u>), 1.63 and 1.85 (2H, *m*, 11-C<u>H</u>$_2$), 1.69 (2H, *m*, 2-C<u>H</u>$_2$), 1.99 (1H, *m*, 18-C<u>H</u>), 2.05 (3H, *s*, OCOC<u>H</u>$_3$), 3.21 and 3.54 (2H, *dd*, J = 10.96, 10.94 Hz, 28-C<u>H</u>$_2$), 4.49 (1H, *dd*, J = 7.98, 6.07 Hz, 3-C<u>H</u>OCOCH$_3$), 5.18 (1H, *t*, J = 3.54 Hz, 12-C<u>H</u>=), ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 15.56 (C-25), 16.67 (C-24), 16.70 (C-26), 18.21 (C-6), 21.32 (<u>C</u>H$_3$COO), 21.95 (C-21), 23.51 (C-11), 23.53 (C-2), 23.56 (C-30), 25.50 (C-15), 25.88 (C-27), 28.00 (C-23), 30.94 (C-20), 31.02 (C-16), 32.47 (C-22), 33.17 (C-29), 34.05 (C-7), 36.79 (C-10), 36.92 (C-17), 37.81 (C-4), 38.24 (C-1), 39.77 (C-8), 41.68 (C-14), 42.31 (C-18), 46.39 (C-19), 47.47 (C-9), 55.21 (C-5), 69.70 (C-28), 80.88 (C-3), 122.26 (C-12), 144.19 (C-13), 171.02 (C=O), HRMS (ESI) m/z: 485.3995, calcd 485.3995 for $C_{32}H_{53}O_3$ $[M+H]^+$; $[\alpha]_D^{21}$ +49.99 (c 0.1, $CHCl_3$), (Lit $[\alpha]_D^{20}$ +47.57, c 1.0, $CHCl_3$) [24].

Stigmasterol (**2**): Colorless crystalline solid, mp 164.5-165.8 °C (Lit. 165-167 °C) [11]; R_f 0.10 (10% EtOAc in *n*-hexane),

IR (KBr) ($\nu_{max,}$ cm^{-1}): 3453, 1636, 1343, 968; (400 MHz, $CDCl_3$, δ (ppm), J/Hz): 0.70 (3H, *s*, 18-C<u>H</u>$_3$), 0.79 (3H, *d*, J = 6.97 Hz, 27-C<u>H</u>$_3$), 0.80 (3H, *t*, J = 7.20 Hz, 29-C<u>H</u>$_3$), 0.85 (3H, *d*, J = 6.36 Hz, 26-C<u>H</u>$_3$), 0.95 (1H, *m*, 9-α-C<u>H</u>), 1.02 (1H, *s*, 14-α-C<u>H</u>), 1.02 (3H, *s*, 19-C<u>H</u>$_3$), 1.02 (3H, *d*, J = 7.33 Hz, 21-C<u>H</u>$_3$), 1.04 and 1.65 (2H, *m*, 15-$\alpha\beta$-C<u>H</u>$_2$), 1.08 and 1.86 (2H, *m*, 1-$\alpha\beta$-C<u>H</u>$_2$), 1.15 (1H, *m*, 17-α-C<u>H</u>), 1.19 and 1.44 (2H, *m*, 28-$\alpha\beta$-C<u>H</u>$_2$), 1.19 and 2.00 (2H, *m*, 12-$\alpha\beta$-C<u>H</u>$_2$), 1.24 and 1.72 (2H, *m*, 16-$\alpha\beta$-C<u>H</u>$_2$), 1.46 (1H, *m*, 8-β-C<u>H</u>), 1.46 (2H, *m*, 11-$\alpha\beta$-C<u>H</u>$_2$), 1.51 and 1.97 (2H, *m*, 7-$\alpha\beta$-C<u>H</u>$_2$), 1.51 (1H, *m*, 25-C<u>H</u>), 1.54 (1H, *m*, 24-C<u>H</u>), 1.55 and 1.85 (2H, *m*, 2-$\alpha\beta$-C<u>H</u>$_2$), 2.00 (1H, *m*, 20-C<u>H</u>), 2.25 and 2.33 (2H, *m*, 4-$\alpha\beta$-C<u>H</u>$_2$-), 3.52 (1H, *m*, 3-α-C<u>H</u>OH), 5.02 (1H, *dd*, J = 15.16, 8.69 Hz, 23-C<u>H</u>=), 5.16 (1H, *dd*, J = 15.16, 8.58 Hz, 22-C<u>H</u>=), 5.35 (1H, *brd*, J = 5.10 Hz, 6-C<u>H</u>=), ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 12.04 (C-29), 12.24 (C-18), 18.97 (C-27), 19.39 (C-19), 21.07 (C-11), 21.07 (C-26), 21.21 (C-21), 24.35 (C-15), 25.40 (C-28), 28.91 (C-16), 31.65 (C-7), 31.65 (C-2), 31.89 (C-8), 31.89 (C-25), 36.51 (C-10), 37.25 (C-1), 39.67 (C-12), 40.49 (C-20), 42.20 (C-13), 42.30 (C-4), 50.15 (C-9), 51.23 (C-24), 55.94 (C-17), 56.86 (C-14), 71.80 (C-3), 121.70 (C-6), 129.27 (C-23), 138.30 (C-22), 140.74 (C-5), HRMS (ESI) m/z: 435.3603, calcd 435.3603 for $C_{29}H_{48}ONa$ $[M+Na]^+$; $[\alpha]_D^{21}$ -68.23 (*c* 0.2, $CHCl_3$).

(24*R*)-24-Ethylcholesta-4,22-dien-3-one (**3**): Colorless crystals, mp 101.9-103.7 °C; R_f 0.19 (10% EtOAc in *n*-hexane), IR (KBr) ($\nu_{max,}$ cm^{-1}): 2927, 1686,1625, 1462, 1377,1230, 971; ^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), J/Hz): 0.73 (3H, *s*, 18-C<u>H</u>$_3$), 0.79 (3H, *d*, J = 6.78 Hz, 27-C<u>H</u>$_3$), 0.80 (3H, *t*, J = 6.78 Hz, 29-C<u>H</u>$_3$), 0.83 and 1.30 (2H, *m*, 16-$\alpha\beta$-C<u>H</u>$_2$), 0.85 (3H, *d*, J = 6.27 Hz, 26-C<u>H</u>$_3$), 0.88 and 1.56 (2H, *m*, 15-$\alpha\beta$-C<u>H</u>$_2$), 0.93 (1H, *m*, 9-α-C<u>H</u>), 1.01 (1H, *m*, 14-α-C<u>H</u>), 1.02 (3H, *d*, J = 6.65 Hz, 21- C<u>H</u>$_3$), 1.03 and 1.82 (2H, *m*, 7-$\alpha\beta$-C<u>H</u>$_2$), 1.16 (1H, *m*, 17-α-C<u>H</u>), 1.17 (3H, *s*, 19- C<u>H</u>$_3$), 1.19 and 2.00 (2H, *m*, 12-$\alpha\beta$-C<u>H</u>$_2$), 1.31 (2H, *m*, 22-C<u>H</u>$_2$), 1.43 and 1.54 (2H, *m*, 11-$\alpha\beta$-C<u>H</u>$_2$), 1.52 (1H, *m*, 8-β-C<u>H</u>), 1.53 (1H, *m*, 24-C<u>H</u>), 1.70 and 2.02 (2H, *m*, 1-$\alpha\beta$-C<u>H</u>$_2$), 1.83 (1H, *m*, 25-C<u>H</u>), 2.02 (1H, *m*, 20-C<u>H</u>), 2.24 and 2.33 (2H, *m*, 2-$\alpha\beta$-C<u>H</u>$_2$), 2.26 and 2.41 (2H, *m*, 6-$\alpha\beta$-C<u>H</u>$_2$-), 5.02 (1H, *dd*, J = 15.17, 8.66 Hz, 23-C<u>H</u>=), 5.15 (1H, *dd*, J = 15.15, 8.56 Hz, 22-C<u>H</u>=), 5.73 (1H, *s*, 4-C<u>H</u>=), ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 12.13 (C-29), 12.24 (C-18), 17.37 (C-19), 18.97 (C-27), 21.00 (C-11), 21.09 (C-26), 21.15 (C-21), 24.24 (C-15), 25.39 (C-28), 28.86 (C-16), 31.86 (C-25), 32.02 (C-7), 32.94 (C-6), 33.97 (C-2), 35.60 (C-8), 35.67 (C-1), 38.61 (C-10), 39.50 (C-12), 40.46 (C-20), 42.26 (C-13), 51.22 (C-24), 53.82 (C-9), 55.88 (C-17), 55.97 (C-14), 123.73 (C-4), 129.43 (C-23), 138.12 (C-22), 171.72 (C-5), 199.68 (C-3), HRMS (ESI) m/z: 411.3627, calcd 411.3627 for $C_{29}H_{47}O$ $[M+H]^+$; $[\alpha]_D^{21}$ -57.62 (*c* 0.06, $CHCl_3$).

5-(3″-Hydroxypropyl)-7-methoxy-2-(3′,4′-methylenedioxyphenyl)benzofuran (**4**): white amorphous powder, mp 118.1-119.4 °C, (Lit. 116-117 °C) [25]; R_f 0.09 (*n*-hexane:CH_2Cl_2:acetone; 68:23:9), IR (KBr) ($\nu_{max,}$ cm^{-1}): 3357, 2940, 1599, 1478, 1366, 1231, 930, 871; ^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), J/Hz): 1.94 (2H, *m*, 2″-C<u>H</u>$_2$), 2.77 (2H, *t*, J = 7.65 Hz, 1″-C<u>H</u>$_2$), 3.71 (2H, *t*, J = 6.38 Hz, 3″-C<u>H</u>$_2$), 4.03 (3H, *s*, -OC<u>H</u>$_3$), 6.00 (2H, *s*, O-C<u>H</u>$_2$-O), 6.63 (1H, *brd*, J = 1.30 Hz, 6-C<u>H</u>), 6.79 (1H, *s*, 3-C<u>H</u>), 6.87 (1H, *d*, J = 8.14 Hz, 5′-C<u>H</u>), 6.97 (1H, *brd*, J = 1.13 Hz, 4-C<u>H</u>), 7.33 (1H, *d*, J = 1.66 Hz, 2′-C<u>H</u>), 7.40 (1H, *dd*, J = 8.14, 1.66 Hz, 6'-C<u>H</u>), ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 32.40

(C-1″), 34.64 (C-2″), 56.13 (O$\underline{C}H_3$), 62.27 (C-3″), 100.33 (C-3), 101.28 (O-$\underline{C}H_2$-O), 105.51 (C-2′), 107.38 (C-6), 108.59 (C-5′), 112.29 (C-4), 119. 18 (C-6′), 124.67 (C-1′), 131.01 (C-3a), 137.49 (C-5), 142.42 (C-7a), 144.75 (C-7), 147.94 (C-4′), 148.01 (C-3′), 156.06 (C-2), HRMS (ESI) *m*/*z*: 349.1054, calcd 349.1052 for $C_{19}H_{18}O_5Na$ $[M+Na]^+$.

5-(3″-Hydroxypropyl)-7-methoxy-2-(3′,4′-dimethoxyphenyl)benzofuran (**5**): white amorphous powder, mp 123.1-125.2 °C, R_f 0.06 (*n*-hexane:CH_2Cl_2:acetone; 68:23:9), IR (KBr) ($\nu_{max,}$ cm^{-1}): 3364, 2936, 1600, 1512, 1483, 1335, 1249, 857; ^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J*/Hz): 1.95 (2H, *m*, 2″-C$\underline{H}_2$), 2.78 (2H, *t*, *J* = 7.65 Hz, 1″-C$\underline{H}_2$), 3.73 (2H, *t*, *J* = 6.37 Hz, 3″-C$\underline{H}_2$), 3.93 (3H, *s*, 3′-OC$\underline{H}_3$), 3.99 (3H, *s*, 4′-OC$\underline{H}_3$), 4.04 (3H, *s*, 7-OC$\underline{H}_3$), 6.64 (1H, *brd*, *J* = 1.00 Hz, 6-C$\underline{H}$), 6.84 (1H, *s*, 3-C$\underline{H}$=), 6.93 (1H, *d*, *J* = 8.42 Hz, 5′-C$\underline{H}$), 6.98 (1H, *brd*, *J* = 0.66 Hz, 4-C$\underline{H}$), 7.37 (1H, *d*, *J* = 1.91 Hz, 2′-C$\underline{H}$), 7.46 (1H, *dd*, *J* = 8.36, 1.95 Hz, 6′-C$\underline{H}$), ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 32.45 (C-1″), 34.69 (C-2″), 55.98 (3′-O$\underline{C}H_3$), 56.07 (4′-O$\underline{C}H_3$), 56.09 (7-O$\underline{C}H_3$), 62.33 (C-3″), 100.27 (C-3), 107.22 (C-2′), 108.15 (C-6), 111.26 (C-5′), 112.28 (C-4), 118.07 (C-6′), 123.51 (C-1′), 131.14 (C-3a), 137.49 (C-5), 142.51 (C-7a), 144.78 (C-7), 149.12 (C-4′), 149.53 (C-3′), 156.36 (C-2), HRMS (ESI) *m*/*z*: 365.1365, calcd 365.1365 for $C_{20}H_{22}O_5Na$ $[M+Na]^+$.

3. RESULTS AND DISCUSSION

3.1 Structural Elucidation of Compounds 1-5

The study on the dichloromethane extract of *S. benzoides* led to the successful isolation and identification of five compounds in total, two cholesterols (**1**, **3**), one steroid (**2**), and two benzofurans (**4**, **5**). Their chemical structures were characterized by a combination of several NMR experiments (e.g. ^{1}H, ^{13}C, DEPTS, COSY, HMQC, and HMBC), FT-IR, high-resolution mass spectrometry, physical properties (e.g. melting points, R_f, and optical rotation), and comparison with literature data. All isolated compounds were identified as erythrodiol-3-acetate (**1**) [24], stigmasterol (**2**) [26], (24*R*)-24-ethylcholesta-4,22-dien-3-one (or stigmasta-4,22-dien-3-one) (**3**), 5-(3″-hydroxypropyl)-7-methoxy-2-(3′,4′-methylenedioxyphenyl)-benzofuran (**4**) [25], 5-(3″-hydroxypropyl)-7-methoxy-2-(3′,4′-dimethoxyphenyl)-benzofuran (**5**) [23] as depicted in Figure 1.

3.2 Chemotaxonomic Significance

To the best of our knowledge, this is the first time that (24*R*)-24-ethylcholesta-4,22-dien-3-one (**3**) has been reported the genus *Styrax*. Nevertheless, compound **3** has been previously isolated and characterized from many plants in other genera such as *Solidago* [24], *Michelia* [27], and *Hedychium* [28]. Therefore, the results from this study suggest that the formation of cholesterol **3** in *S. benzoides* might be considerably unique compared to other species of the genus *Styrax*.

Compound **1**, a pentacyclic triterpenoid, had been characterized as erythrodiol-3-acetate which was previously isolated from the stem bark of *S. japonica* [17]. Additionally, stigmasterol (**2**), a common phytosterol in many plants, was also found in the stem bark extract of *S. japonica* [19].

Benzofurans are a group of plant ingredients generally distributed in the genus *Styrax* and considered to be potential chemotaxonomic markers for several species in this genus. Two benzofuran derivatives, **4** [25], and **5** were purified from the seed extracts of *S. officinalis* [8] and the leaves extract of *S. ferrugineus* [3]. Both compounds possess

good biological activity against human leukaemic cells [29], in addition to their antibacterial and antifungal activity [3]. Recently, the quantification of these two compounds using high-performance liquid chromatography [30] in several *Styrax* species such as *S. camporum*, *S. pohlii*, and *S. ferrugineus* demonstrated the presence of significant amounts of both compounds **4** and **5**. Therefore, the discovery of both benzofurans **4** and **5** in *S. benzoides* in this study as the major phytochemical components certainly supported its close taxonomical relationship to other species in the tribe of *Styrax*. This finding definitely strengthened the possibility of using benzofurans as chemotaxonomic species to differentiate plants in the genus *Styrax* from other genera.

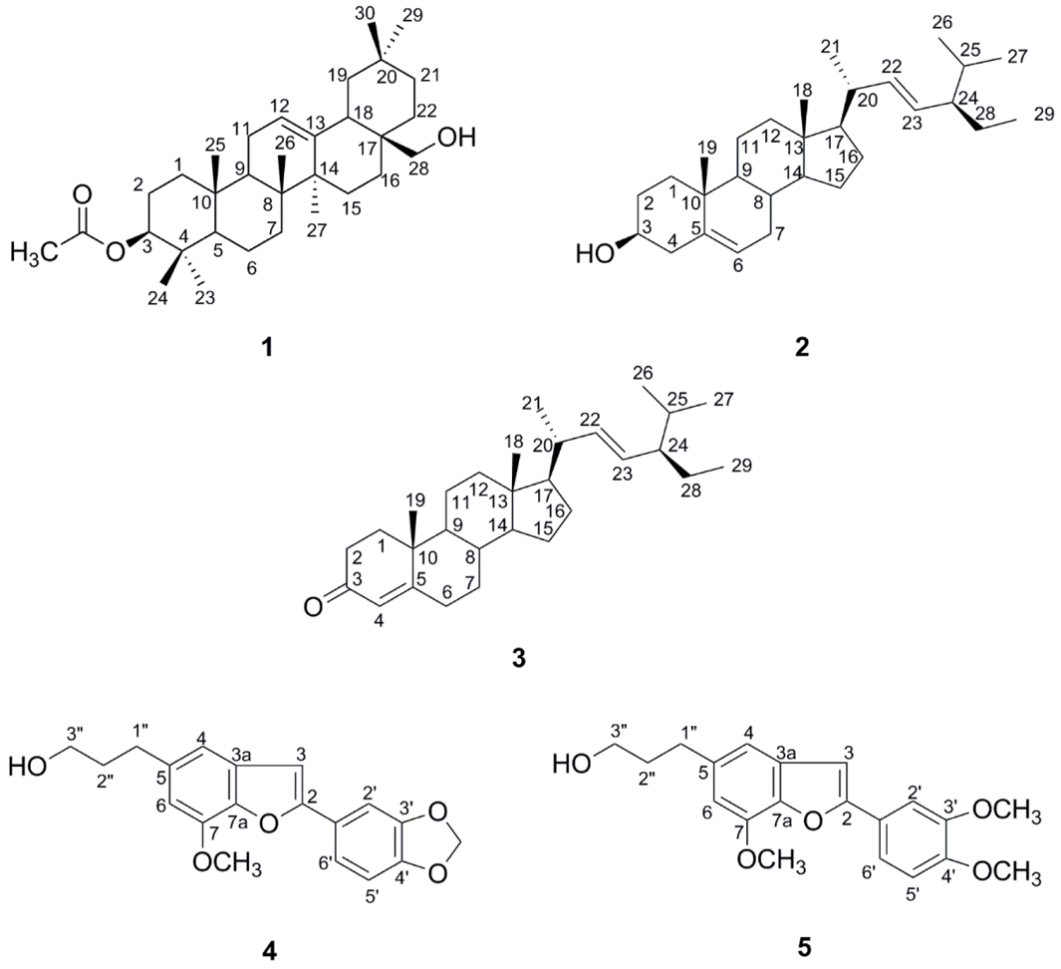

Figure 1. Structures of compounds **1-5** isolated from the stems of *S. benzoides* Craib.

4. CONCLUSION

In summary, this research provides profound details regarding the chemical compositions and evidences for the possible chemotaxonomy of *S. benzoides*. The isolation of cholesterol **3** was firstly discovered in the genus *Styrax* and this compound could possibly be produced in *S. benzoides* with an exception compared to other plants of the same genus. More importantly, the presence

of benzofurans **4** and **5** certainly shows the close chemotaxonomic relationship among the different species of *Styrax* and might serve as potential chemotaxonomic markers for this group of plants.

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