Benzofurans from the Seeds of *Styrax obassia*

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Styrax obassia also known as 'fragrant snowbell' is a member of the Styracaceae family. It is a shrub or tree native to tropical and subtropical regions with the majority in eastern and southern Asia.[1,2] The genus *Styrax* is different from other genera of this family due to the production of resinous material, usually secreted when the barks and trunks are injured by sharp objects.[1] This resin, in the past considered as a miraculous remedy in several parts of Asia and America, has been used in traditional medicine to treat inflammatory diseases.[3] Its resin was used by Romans, Egyptians, Phoenicians and Ionians as incense and in therapeutics.[4] The pericarps are used as washing soap (skin elastic material), cough medicine and a piscicidal agent.[5] *Styrax* species contain egonol, a natural benzofuran, which is known to be an effective pyrethrum synergist.[6,7] Earlier chemical studies on several *Styrax* species have revealed them to be a rich source of arylpropanoids, triterpenoids and their glycosides[6-12] with various biological activities such as antisweet,[5] antimicrobial,[7] antiproliferative,[11] cytotoxic[12] and matrix metalloproteinase-1-inhibitor.[13] However, careful literature survey of *Styrax* species revealed that *Styrax obassia* has not been studied much so far except for a few short reports.[6,8] As a part of our on going research on chemical constituents from *S. obassia*, we isolated a hitherto unknown compound **1** along with four known compounds (**2-5**) from the seeds of *S. obassia*. This paper deals with the isolation and structure elucidation of these compounds by their comprehensive spectroscopic analysis including 2D NMR. The ^{13}C NMR data of known compound **5** is being reported here for the first time.

Compound **1** (Figure 2) was obtained as a colorless crystal and exhibited UV absorbance in $CHCl_3$ at 242 and 318 nm. The IR spectrum of compound **1** showed the bands at 2954, 1738, 1601, 1481, 1232 and 941 cm^{-1}. Compound **1** showed a molecular ion peak at *m/z* 382 ([M]$^+$, base ion) in the EIMS spectrum, its molecular formula could be determined as $C_{22}H_{22}O_6$ by HREIMS *m/z*: 382.1416 ([M]$^+$, calcd. for $C_{22}H_{22}O_6$, 382.4141). ^{1}H and ^{13}C NMR signals of compound **1** (Figure 1) were assigned by the interpretation of the DEPT, COSY, HMQC and HMBC spectra (Table 1). The ^{13}C NMR spectrum of compound **1** showed the signals for 22 carbons which were distinguished into two methyl (δ_C 9.2, 56.1), five methylene (δ_C 27.6, 30.7, 32.4, 63.6, 101.2), six methine (δ_C 100.3, 105.5, 107.4, 108.5, 112.3, 119.2) and nine quaternary (δ_C 124.6, 131.0, 136.9, 142.5, 144.7, 147.9, 148.0, 156.1, 174.5) carbons with the help of DEPT experiments. Upon integration ^{1}H NMR spectrum of compound **1** showed the presence of 22 protons. Most of the ^{1}H and ^{13}C NMR signals of compound **1** were similar to those of egonol[6] which is also isolated in this work, besides the extra ^{1}H NMR signals at δ 1.15 (3H, J = 7.5 Hz) and 2.34 (2H, q) and their corresponding ^{13}C NMR signals at δ_C 9.2 (methyl carbon), δ_C 27.6 (methylene carbon) and δ_C 174.5 (quaternary carbon) from ^{1}H-^{13}C one bond (HMQC) experiment for a propanoyl moiety. The position of propanoyl moiety was confirmed from the HMBC experiment. The proton H-3" of egonol moiety correlated with the carbon 1a of propanoyl group in the HMBC experiment, which confirmed the position of a propanoyl moiety in compound **1** (Figure 1). On the basis of these spectroscopic data compound **1** was

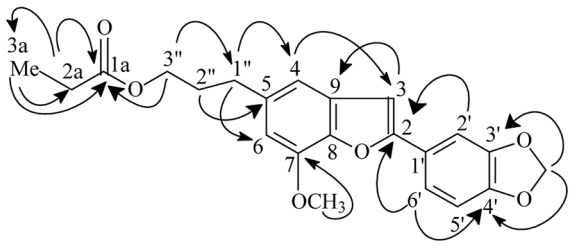
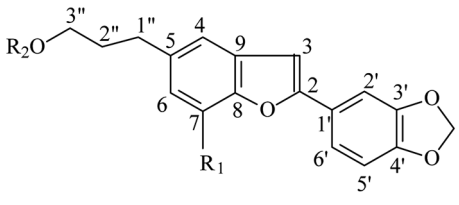


Figure 1. Key HMBC correlations of compound **1**.



1. R_1=OMe, R_2=$\overset{3a}{C}H_3\overset{2a}{C}H_2\overset{1a}{C}O$
2. R_1=OMe, R_2=H
3. R_1=OMe, R_2=$\overset{2a}{C}H_3\overset{1a}{C}O$
4. R_1=OMe, R_2=$\overset{4a}{C}H_3\overset{3a}{C}H_2(\overset{5a}{C}H_3)\overset{2a}{C}H\overset{1a}{C}O$
5. R_1=H, R_2=$\overset{4a}{C}H_3\overset{3a}{C}H_2(\overset{5a}{C}H_3)\overset{2a}{C}H\overset{1a}{C}O$

Figure 2. Chemical structures of the isolated compounds from the seeds of *S. obassia*.

Table 1. 1D[a] and 2D NMR data in $CDCl_3$ for compound **1**

C/H	DEPT	δ_C	δ_H	J (Hz)	COSY	HMBC (H → C)
2	C	156.1	–	–	–	–
3	CH	100.3	6.78, *s*	–	–	C-2/C-8/C-9
4	CH	112.3	6.95, *s*	–	–	C-3/C-6/C-8
5	C	136.9	–	–	–	–
6	CH	107.4	6.60, *s*	–	–	C-7/C-8
7	C	144.7	–	–	–	–
8	C	142.5	–	–	–	–
9	C	131.0	–	–	–	–
1'	C	124.6	–	–	–	–
2'	CH	105.5	7.31, *d*	1.5	–	C-2/C-3'/C-4'
3'	C	148.0	–	–	–	–
4'	C	147.9	–	–	–	–
5'	CH	108.5	6.87, *d*	8.5	H-6'	C-1'/C-3'/C-4'
6'	CH	119.2	7.40, *dd*	1.5, 8.5	H-5'	C-2/C-4'
OCH_2O	CH_2	101.2	6.00, *s*	–	–	C-3'/C-4'
1"	CH_2	32.4	2.74, *t*	7.5	H-2"	C-4/C-5/C-6/C-2"/C-3"
2"	CH_2	30.7	2.00, *m*	–	H-1", H-3"	C-5/C-1"/C-3"
3"	CH_2	63.6	4.13, *t*	6.5	H-2"	C-1"/C-1a
OMe	CH_3	56.1	4.03, *s*	–	–	C-7
1a	C	174.5	–	–	–	–
2a	CH_2	27.6	2.34, *q*	7.5	H-3a	C-1a/C-3a
3a	CH_3	9.2	1.15, *t*	7.5	H-2a	C-1a/C-2a

[a] ^1H and ^{13}C NMR recorded at 500 and 125 MHz, respectively.

elucidated as egonol propanoate.

Known compounds (**2-5**) (Figure 2) were identified by comparison of their spectral data with literature values as follow: egonol[6,8,9] (**2**), egonolacetate[6,8,9] (**3**), egonol-2-methylbutanoate[6,8,9] (**4**) and 7-demethoxyegonol-2-methylbutanoate[6,8] (**5**).

Experimental Section

General Methods. Melting points were determined on an Electrothermal IA-9200 melting point apparatus (Electrothermal Engg. Ltd. U.K.) and are uncorrected. Optical rotations were taken on a Jasco P1020 polarimeter. UV spectra were recorded on a Hewlett Packard 8452A Diode Array Spectrophotometer. IR spectra were recorded in KBr with a NEXUS FT-IR spectrophotometer. EIMS and HREIMS were obtained with a JEOL JMS-SX102A spectrophotometer. ^1H NMR (500 MHz), ^{13}C NMR (125 MHz), DEPT, COSY, HMQC, and HMBC spectra were obtained with a Varian Unity-Inova 500 spectrophotometer. The NMR samples were prepared in $CDCl_3$/DMSO with tetramethylsilane (TMS) as an internal standard. The chemical shifts and coupling constants (J) were expressed in δ and Hz, respectively. Thin layer chromatography (TLC) was carried out on pre-coated Silica gel 60 F_{254} (0.2 mm, Merck, Germany) plates. Preparative thin layer chromatography was carried out on pre-coated Silica gel 60 F_{254} (20 × 20 cm^2, 2.0 mm, Merck, Germany) plates. TLC plates were developed with solvent system A (toluene/ethyl formate/formic acid = 20:2:1, v/v/v) and B (*n*-hexane/ethyl acetate/toluene = 8:1:1, v/v/v). Developed TLC plates were visualized under UV light at 254 and 365 nm. Silica gel 60 (40-100 μm, Kanto Chemical Co. Japan) was used for the column chromatography. An ADVENTEC SF-1600 was used as the automated fraction collector in the column chromatography.

Plant Material. The fruits of *S. obassia* were collected from Jiri mountain (Hadong-kun) in Kyungnam, Korea in September, 2004 and identified by Dr. Y. H. Kwon (Korea National Arboretum, Pocheon, Korea). A voucher specimen has deposited at the Korea Forest Research Institute, Seoul, Korea.

Extraction and Isolation. 8.0 Kg of air-dried and powdered seeds of *S. obassia* were extracted three times with MeOH at room temperature for 72 hrs each. The combined MeOH extracts were concentrated under vacuum at 40 °C until MeOH was completely removed. The concentrated MeOH extract was dissolved in distilled water and successively partitioned with *n*-hexane, dichloromethane and ethyl acetate.

Column chromatography of an oily mass from *n*-hexane soluble fraction on silica column gave 93 fractions (250 mL each) in benzene:ethyl acetate (20:1, v/v). On the basis of TLC profiles, these fractions are divided into four groups. Group one (46.6 g) was chromatographed on silica gel column using *n*-hexane:ethyl acetate (17:1, v/v) as an eluent to collect nine fractions (100 mL each), and then column was washed with MeOH to give an oily mass (43.7 g). Fraction 2 formed some precipitate which was washed with MeOH to give a pure compound **3** (3.7 g). At the same time, fraction 7 was purified by preparative TLC in *n*-hexane:

ethyl acetate (5:1, v/v) to give a pure compound **4** (40 mg). The oily mass (43.7 g) upon silica gel column chromatography in *n*-hexane:ethyl acetate (15:1, v/v) gave 85 fractions (250 mL each). TLC profiles of these fractions led them to divide into four groups. Group one (10.0 g) was chromatographed on silica column in *n*-hexane:chloroform: ethyl acetate (23:1:1, v/v/v) to give three fractions. Rechromatography of fraction 2 (3.5 g) on silica column in chloroform:toluene:ethyl acetate (17:1:1, v/v/v) gave pure compound **5** (88.8 mg). On the other hand, group four (1.36 g) was chromatographed on silica gel column using *n*-hexane:benzene:ethyl acetate (8:1:1, v/v/v) as an eluent to yield 80 fractions (4.0 g each by a fraction collector). On the basis of TLC profiles these fractions are divided into three parts. Part two (255 mg) was finally chromatographed using chloroform:toluene:ethyl acetate (8:1:1, v/v/v) as an eluent on silica to give a pure compound **1** (42.7 mg).

The ethyl acetate solubles (122.7 g) from MeOH extract was chromatographed on silica column with increasing polarity of *n*-hexane:ethyl acetate:acetone (9:2:1 → 5:2:1 → 3:2:1 → 1:2:2, v/v/v) to collect five fractions. Fraction 3 was concentrated to produce a powdery mass which was washed with toluene, benzene and finally with ethyl acetate. The ethyl acetate soluble part produced pure compound **2** (1.08 g).

5-(3"-Propanoyloxypropyl)-7-methoxy-2-(3',4'-methylenedioxyphenyl)-benzofuran (1): Colourless crystal. m.p. 86-87 °C. $[\alpha]_D^{20.4}$ +4.7° (c = 0.22, $CHCl_3$). UV ($CHCl_3$) λ_{max} nm (log ε): 242 (3.9), 318 (4.3). IR (KBr) ν_{max}: 2954, 1738, 1601, 1481, 1371, 1232, 1190, 1038, 941 and 812 cm^{-1}. EIMS *m/z*: 382 ($[M]^+$, base ion), 308, 282, 267 and 251. HREIMS *m/z*: 382.1416 ($[M]^+$, calcd. for $C_{22}H_{22}O_6$, 382.4141). ^1H NMR ($CDCl_3$, 500 MHz), ^{13}C NMR ($CDCl_3$, 125 MHz), COSY and HMBC see Table 1.

Egonol (**2**): White powder. m.p. 112-113 °C (lit.[8] 113-115 °C). EIMS *m/z*: 326 ($[M]^+$). UV, IR, ^1H and ^{13}C NMR data are in agreement with litrature.[6,8,9]

Egonolacetate (**3**): Yellowish powder. m.p. 104-105 °C (lit.[8] 103-105 °C). EIMS *m/z*: 368 ($[M]^+$). UV, IR, ^1H and ^{13}C NMR data are in agreement with litrature.[6,8,9]

Egonol-2-methylbutanoate (**4**): Pale yellow oil. EIMS *m/z*: 410 ($[M]^+$). UV, IR, ^1H and ^{13}C NMR data are in agreement with litrature.[6,8,9]

7-Demethoxyegonol-2-methylbutanoate (5): Colourless needles. m.p. 54-55 °C (lit.[6] 55.5-56 °C). EIMS *m/z*: 380 ($[M]^+$). UV, IR, ^1H NMR data are in agreement with litrature.[6,8] ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.6*q* (C-4a), 16.6*q* (C-5a), 26.8*t* (C-3a), 30.9*t* (C-2"), 32.1*t* (C-1"), 41.1*d* (C-2a), 63.4*t* (C-3"), 100.0*d* (C-3), 101.3*t* (-O-CH_2-O-), 105.4*d* (C-2'), 108.6*d* (C-5'), 110.7*d* (C-7), 119.1*d* (C-6'), 120.0*d* (C-4), 124.5*d* (C-6), 124.8*s* (C-1'), 129.5*s* (C-9), 135.9*s* (C-5), 148.0*s* (C-4'), 148.1*s* (C-3'), 153.4*s* (C-8), 156.0*s* (C-2), 176.8*s*, (C-1a).

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