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.12 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 glycosides⁶⁻¹² with various biological activities such as antisweet, antimicrobial, 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 ¹³C 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 CHCl₃ at 242 and 318 nm. The IR spectrum of compound 1 showed the bands at 2954,

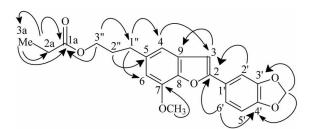


Figure 1. Key HMBC correlations of compound 1.

1738, 1601, 1481, 1232 and 941 cm⁻¹. Compound 1 showed a molecular ion peak at m/z 382 ([M]¹, 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]⁴, calcd. for C22H22O6, 382.4141). 1H and 13C NMR signals of compound 1 (Figure 1) were assigned by the interpretation of the DEPT, COSY, HMQC and HMBC spectra (Table 1). The ¹³C NMR spectrum of compound 1 showed the signals for 22 carbons which were distinguished into two methyl ($\delta_{\rm C}$ 9.2, 56.1), five methylene (δ_C 27.6, 30.7, 32.4, 63.6, 101.2), six methine ($\delta_{\rm C}$ 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 ¹H NMR spectrum of compound 1 showed the presence of 22 protons. Most of the ¹H and ¹³C NMR signals of compound 1 were similar to those of egonol⁶ which is also isolated in this work, besides the extra 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 $\delta_{\rm C}$ 9.2 (methyl carbon), $\delta_{\rm C}$ 27.6 (methylene carbon) and $\delta_{\rm C}$ 174.5 (quaternary carbon) from ¹H-¹³C 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 propancyl 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

1. $R_1 = OMe$, $R_2 = \overset{3a}{C}\overset{2a}{H}_3\overset{2a}{C}\overset{1a}{H}_2\overset{1}{C}O$

2. R₁=OMe, R₂=H

3. $R_1 = OMe$, $R_2 = {}^{2a}_{CH_3}{}^{1a}_{CO}$

4. R₁=OMe, R₂= ${}^{4a}_{CH_3}{}^{3a}_{CH_2}{}^{5a}_{CH_3}{}^{2a}_{CHCO}$

5. R₁=H, R₂=CH₃CH₂(CH₃)CHCO

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

Table 1. 1D" and 2D NMR data in CDCl₃ for compound 1

C/H	DEPT	$ec{\delta}_{\mathbb{C}}$	$\delta_{ extsf{II}}$	J(Hz)	COSY	$HMBC (H \rightarrow C)$
2	С	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 ^r	CH	105.5	7.31, d	1.5	_	C-2/C-3'/C-4'
3 ^r	C	148.0	_	_	_	_
4 ¹	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

^{a1}H and ¹³C 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), ¹³C NMR (125 MHz), DEPT, COSY, HMQC, and HMBC spectra were obtained with a Varian Unity-Inova 500 spectrophotometer. The NMR samples were prepared in CDCl₃/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 F₂₅₄ (0.2 mm, Merck, Germany) plates. Preparative thin layer chromatography was carried out on pre-coated Silica gel 60 F_{254} (20 × 20 cm², 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 μ m, 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-hexanetethyl 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 nhexane: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 \rightarrow 5:2:1 \rightarrow 3:2:1 \rightarrow 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. [α]_D^{20.4} +4.7° (c = 0.22, CHCl₃). UV (CHCl₃) 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 C₂₂H₂₂O₆, 382.4141). ¹H NMR (CDCl₃, 500 MHz), ¹³C NMR (CDCl₃, 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 ¹³C 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 ¹³C 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 ¹³C NMR data are in agreement with litrature.^{6.8.9}

7-Demethoxyegonol-2-methylbutanoate (5): Colourless needles. m.p. 54-55 °C (lit. 55.5-56 °C). EIMS *m/z*: 380 ([M]⁺). UV, IR, ¹H NMR data are in agreement with litrature. Algorithm 125 MHz, CDCl₃): δ 11.6q (C-4a), 16.6q (C-5a), 26.8t (C-3a), 30.9t (C-2"), 32.1t (C-1"), 41.1d (C-2a), 63.4t (C-3"), 100.0d (C-3), 101.3t (-O-CH₂-O-), 105.4d (C-2'), 108.6d (C-5'), 110.7d (C-7), 119.1d (C-6'), 120.0d (C-4), 124.5d (C-6), 124.8s (C-1'), 129.5s (C-9), 135.9s (C-5), 148.0s (C-4'), 148.1s (C-3'), 153.4s (C-8), 156.0s (C-2), 176.8s, (C-1a).

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