# NEW BENZOFURANS RELATED TO EGONOL FROM IMMATURE SEEDS OF STYRAX OBASSIA

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(Revised received 1 June 1987)

Key Word Index Styrax obassia, styrocaceae, egonol, benzofuran, glucoside, 2-methylbutanoate

Abstract—Three new benzofurans have been isolated from immature seeds of St) rax obassia Their structures have been established as 5-[3-(2-methylbutanoyloxy)propyl]-7-methoxy-2-(3',4'-methylene dioxyphenyl)benzofuran (3), 5-[3-(2-methybutanoyloxy)propyl]-2-(3',4'-methylenedioxy-phenyl)benzofuran (5), and 5-[3-( $\beta$ -D-glucopyranosyl-oxy)propyl]-7-methoxy-2-(3',4'-methylenedioxyphenyl)benzofuran (6), respectively, based on their chemical and spectroscopic properties and enzymatic reaction Egonol acetate (2) were also identified

### INTRODUCTION

Egonol was first isolated in 1915 from the seed oil of Styrax japonica and the structure was determined as 5-(3-hydroxy propyl)-7-methoxy-2-(3',4'-methylenedioxyphe-nyl)benzofuran [2]. After this discovery, egonol di-glyco side from *S formosarum Matsum* [3], homoegonol from *S officinalis* [4], and demethoxy egonol from *S obassia* [5] were found as naturally occurring egonol derivatives Egonols also have been known as an effective pyrethrum synergist [6] This paper reports the isolation of three new benzofurans related to egonol and the identification of egonol acetate from immature seeds of *S obassia* 

## **RESULTS AND DISCUSSION**

The brown syrup of chloroform-methanol extracts of the fresh seeds was worked up as described in the Experimental. The compound (3) appeared as pale yellow oil, and exhibited UV maxima in ethanol at 220, 300 and 315 nm which was similar to the spectrum of egonol (1)

The spot of **3** on the SiO<sub>2</sub> TLC plate,  $(R_f 0.08)$  revealed intense fluorescence under UV light, and gave a positive reaction in the egonol-test [5] The IR (1730 cm<sup>-1</sup>) and <sup>1</sup>H NMR spectrum of the compound then suggested the presence of a fatty ester of egonol. The <sup>1</sup>H NMR spectrum contained a triplet at  $\delta 0.98$  (3H, J = 8.5 Hz) and a doublet at  $\delta 1.16$  (3H, J = 80 Hz) concerning methyl groups adjacent to methylene and to methine groups. respectively Irradiation at the frequency of the methylene ( $\delta 1$  94 bq, J = 8 5 Hz) and the methine ( $\delta 2$  34, bq, J = 8 Hz) groups collapsed the triplet at  $\delta 0.92$  to a singlet and the doublet at  $\delta 1$  16 into a singlet. Hence the fatty ester residue is 2-methylbutanoate and the structure is supported by the high resolution MS which showed M<sup>+</sup>  $m_{\ell}^{\prime}z$  410 1719 (C<sub>24</sub>H<sub>26</sub>O<sub>6</sub>) and by hydrolysis with 10% KOH-EtOH which afforded egonol (1) (identified with TLC and IR)

The second compound (5) appeared as fine colourless needles from methanol,  $C_{23}H_{24}O_5$  (by [M], FDMS), mp 55 5–56 0° and exhibited UV maxima in ethanol at 218, 302, and 319 nm which suggested the demethoxy egonol

skelton It was an ester (IR bands at  $1730 \text{ cm}^{-1}$ ) and hydrolysis with 10% KOH-EtOH afforded demethoxy egonol (4) The <sup>1</sup>H NMR spectrum again showed the pattern of a 2-methylbutanoate residue Hence this compound is the 2-methyl butanoate ester of demethoxyegonol

The third compound (6) appeared as brown needles from ethanol,  $C_{25}H_{28}O_{10}$  [by (M<sup>+</sup>), FDMS], and exhibited UV maxima in ethanol at 220, 300, and 315 nm which was similar to the spectrum of egonol. The IR spectrum showed the presence of many hydroxyl groups (3600 3250 cm<sup>-1</sup>) and no ester group. The IR and <sup>1</sup>H NMR spectra of the compound suggested the egonol monoglucoside. The glucoside, on hydrolysis with emulsin, afforded egonol and D-glucose (identified with an authentic sample). In the proton decoupled <sup>13</sup>C NMR spectrum (Table 1), the location of five of 24 signals suggested the compound to be a  $\beta$ -glucoside. This was supported by the presence of a signal at  $\delta 4.21$  (d, J = 7.8 Hz, see Experimental) in the <sup>1</sup>H NMR spectrum, which could be assigned to the anomeric proton. In





addition enzymatic hydrolysis with a  $\beta$ -hydrolysing enzyme suggested the  $\beta$ -configuration for the C-3" glucose. Accordingly, the structure of **6** was elucidated as 5-[3-( $\beta$ -D-glucopyranosyloxy)-propyl]-7-methoxy-2-(3',4'-meth-ylenedioxy phenyl)benzofuran.

Egonol acetate was identified by comparing its IR, <sup>1</sup>H NMR and TLC with our synthetic compound Egonol and demethoxy egonol were also identified with authentic samples

## EXPERIMENTAL

Mps uncorr All <sup>1</sup>H NMR spectra were obtained at 200, 100 and 90 MHz with TMS as internal standard and MS were obtained using a probe TLC solvent systems used were (i) *n*hexane-Me<sub>2</sub>CO (10.2 and 10 1, v/v), (ii) *n*-hexane-EtOAc (2 1), (iii) C<sub>6</sub>H<sub>6</sub>-EtOAc (2 1) All solvent systems for TLC were used with silica gel Detection was by spraying with 18 M H<sub>2</sub>SO<sub>4</sub> followed by heating and by I<sub>2</sub> and/or by fluorescence under UV light

Table 1 <sup>13</sup>C NMR spectra of egonol glucoside (6) (<sup>13</sup>C NMR were recorded at 25.15 MHz, chemical shifts relative to TMS as int standard)

Signal No	6	Assignment
1	31 42 t	C-2"
2	31 79 t	C-1″
3	55 80 g	OMe
4	61.15 i	G-C-6
5	67 86 t	C-3′
6	70 15 d	G-4
7	73 56 d	G-C-2
8	76 79 d	G-C-3, 5
9	100 95 d	C-3
10	101 41 t	O-CH <sub>2</sub> -O
11	103 01 d	G-C-1
12	104.91 d	C-2′
13	107 88 d	C-6
14	108 73 d	C-5'
15	112.16 d	C-4
16	118 70 d	C-6′
17	124.05 s	C-1'
18	130 48 s	C-3a
19	137.82 s	C-5
20	141 67 s	C-7a
21	144 32 s	C-7
22	147 75 s	C-4′
23	147.92 s	C-3'
24	155 12 s	C-2

Isolation of compounds 1-6 Immature fresh seeds (229 g) in the fruits of Styrax obassia (914 g), collected in June 1982, in Koganei-shi, Tokyo, were crushed and extracted with CHCl<sub>3</sub>-MeOH (1 1, v/v, 3 × 1 l) After the extracts were allowed to stand for two weeks, white solids pptd The ppts were filtered off and evapn of the filtrate under red pres yielded a brown syrup (42g), which was extracted with Me<sub>2</sub>CO (35 ml) The brown oil (41g) obtained from acetone extracts of the syrup were chromatographed on a silica gel (Merck, gel 60, art 7729) column eluted with n-hexane-acetone (10 1) The partially purified compound in the fractions collected was rechromatographed on silica gel eluted with n-hexane-EtOAc (2 1) to give egonol (1) (0 5 g), demethoxy egonol (4) (0 05 g), egonol acetate 2 (0 21 g), egonol-2-methylbutanoate (3) (0 18 g) and demethoxyegonol-2-methylbutanoate (5) (0.02 g), respectively The syrup were chromatographed over silica gel eluted with EtOAc-1so-PrOH-H2O(6 2 1) and CH2Cl2 to afford egonol monoglucoside (6) Pure 6 was obtained as colourless needles (002 g) after recrystallization from 80% EtOH

5-[3-(2-methylbutanoyl)propyl]-7-methoxy-2-(3',4'-methylene dioxyphenyl)benzofuran (3) (egonol 2-methylbutanoate) Pale yellow oil, UV  $\lambda_{max}^{EtOH}$  nm 220, 300, 315, IR (neat) cm<sup>-1</sup>: 2950, 1730, 1600, 1480, 1240, 930 <sup>1</sup>H NMR (90 MHz, CDCl<sub>3</sub>) δ0 92 (3H, t, J = 85 Hz, Me–CH<sub>2</sub>–),  $\delta 1 16$  (3H, d, J = 80 Hz, –CH<sub>2</sub>–CH (Me)-CO-), δ1 36-1 80 (2H, m, -O-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-Ar), δ1 94 [2H, br q, J = 85 Hz, Me–CH<sub>2</sub>–CH (Me)–CO],  $\delta 234$  (1H, br q, J = 80 Hz, Et-CH(Me)-CO),  $\delta 2.70$  (2H, t, J = 7.5 Hz, -O-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-Ar),  $\delta$ 3 98 (3H, s, -OMe),  $\delta$ 4 08 (2H, t, J =72 Hz,  $-O-CH_{2}-CH_{2}-CH_{2}-Ar$ ),  $\delta 594$  (2H, s,  $-O-CH_{2}-O$ ),  $\delta 6\ 50\ (1H,\ d,\ J=1\ 5\ Hz,\ H-6),\ \delta 6\ 82\ (1H,\ d,\ J=8\ 8\ Hz,\ H-5')$  $\delta 6 90$  (1H, d, J = 1 5 Hz, H-4),  $\delta 7.28$  (1H, d, J = 1.6 Hz, H-2'),  $\delta$ 7 34 (1H, dd, J = 1.6 and 8 8 Hz, H-6') MS M<sup>+</sup> m/z 410.1719,  $C_{24}H_{26}O_6$  requires: 410 1722, m/z (rel int.): 410 (54), 396 (10), 338 (100), 282 (43), 252 (29), 178 (8), 149 (33) TLC R (0.08 (nhexane-Me<sub>2</sub>CO, 5 1)

Alkali hydrolysis of egonol 2-methylbutanoate (3) Egonol butanoate (3) (ca 20 mg) was heated with 10% KOH-EtOH (2 ml) under N<sub>2</sub> gas flow at 70° for 1 hr The reaction mixture was cooled to room temp, neutralized with 2% HCl and extracted with Et<sub>2</sub>O The reaction products were examined by TLC and separated by CC on silica gel in *n*-hexane-Me<sub>2</sub>CO (10.1) The isolated product was identical in all respects [mmp, TLC ( $R_f$ 0 23, benzene-EtOAc 2 1), IR, <sup>1</sup>H NMR] with authentic egonol

5-[3-(2-methylbutanoyloxy)propyl]-2-(3',4'-methylene dioxvphenyl)benzofuran (5) (demethoxyegonolbutanoate). Colourless needles, recrystallized from MeOH mp 55.5-56° UV  $\lambda_{max}^{EiOH}$  nm 218, 302, 319 IR(KBr) cm<sup>-1</sup> 2960, 1730, 1610, 1490, 1470, 1440, 1360, 1290, 1270, 1240, 930  $^1{\rm H}\,{\rm NMR}$  (90 MHz, CDCl<sub>3</sub>).  $\delta 0$  94 (3H, t, J = 8.2 Hz,  $-CH_2-Me$ ),  $\delta 1.16$  (3H, d, J = 8.0 Hz, = CH-<u>Me</u>),  $\delta 1$  40-1 80 (2H, m, -O-CH<sub>2</sub>-<u>CH<sub>2</sub></u>-CH<sub>2</sub>-Ar),  $\delta 1$  98 (2H, br q, J = 82 Hz, Me-CH<sub>2</sub>-CH=),  $\delta 2$  34 (1H, br q J = 80,  $-CH_2-CH_2-(Me)-CO_2$ ,  $\delta 2.76$  (2H, t, J=7.5 Hz,  $Et-CH_2$ ),  $\delta 4.10$  (2H, t, J = 7.2 Hz,  $-O-CH_2-CH_2-CH_2-Ar$ ),  $\delta 6.00$  (2H, s,  $-O-CH_2-O-$ ),  $\delta 6.75$  (H, s, H-3),  $\delta 6.84$  (1H, d, J = 8.2 Hz, H-5'),  $\delta$ 7.05 (1H, dd, J = 8 2 and 1 6 Hz, H-6), 7.26 (1H, d, J = 1 5 Hz, H-2'),  $\delta$ 7 28 (1H, d, J = 1 6 Hz, H-4),  $\delta$ 7 35 (1H, dd, J = 8 2 Hz, J = 1 5 Hz, H-6'),  $\delta$ 7 38 (1H, d, J = 8 2 Hz, H-7), MS<sup>•</sup> M<sup>+</sup> 380 1632, C23H24O5 requires: 380 1624, m/z (rel. int) 380 (100), 278 (36), 251 (40), 221 (2), 179 (2), 149 (8).

TLC  $R_f$  0.16 (n-hexane-Me<sub>2</sub>CO, 10 2)

Alkalı hydrolysis of demethoxy egonol butanoate (5). Demethoxy egonol butanoate (5) (ca 30 mg) was heated with 10% KOH-EtOH (15 ml) under  $N_2$  gas flow at 70° for 1 hr The reaction mixture was cooled to room temp, neutralized with 2% HCl and extracted with Et<sub>2</sub>O The reaction products were examined by TLC and separated by CC on silica gel in *n*-hexane-acetone (10.1).

The isolated product was identical in all respects [mmp TLC  $R_f$  0.29 (C<sub>6</sub>H<sub>6</sub>-EtOAc, 2 1), IR, <sup>1</sup>H NMR] with authentic demethoxy egonol

5-(3-Acetoxypropyl)-2-(3',4'-methylenedioxyphenyl)-benzo-furan (2) (egonol acetate) Colourless needles, recrystallized from MeOH, mp 104–105°, UV  $\lambda_{max}^{E1OH}$  nm 222, 302, 317, IR (KBr) cm<sup>-1</sup>, 2950, 1720, 1620, 1600, 1500, 1470, 1245, 1224, 1040 <sup>1</sup>H NMR (100 Mz, CDCl<sub>3</sub>), δ2 05 (3H, s, AcO-), δ1 80-2 20 (2H,  $AcO-CH_2-CH_2-Ar$ ),  $\delta 2\,75$  (2H, t,  $J = 7\,5$  Hz, m. AcO-CH2-CH2-CH2-Ar), 64 00 (3H, s, Ar-OMe), 64 10 (2H, t,  $AcO - CH_2 - CH_2 - CH_2 - Ar$ ),  $\delta 590$ J = 7.2 Hz,(2H, S.  $-O-CH_2-O-$ ),  $\delta 6$  55 (1H, d, J = 1 5 Hz, H-6),  $\delta 6$  72 (1H, s, H-3),  $\delta 6.80 (1H, d, J = 8.8 Hz, H-5')$ ,  $\delta 6.90 (1H, d, J = 1.5 Hz, H-4)$ ,  $\delta$ 7 28 (1H, d, J = 1 6 Hz, H-2'),  $\delta$ 7 35 (1H, dd, J = 1 6 and 8 8 Hz, H-6'), MS m/z (rel int) M<sup>+</sup> 368 (100), 295 (14), 283 (93), 282 (41) The isolated egonol acetate was identical in all respects (mmp. TLC  $R_f$  0.05, *n*-hexane acetone 2.1, IR, <sup>1</sup>H NMR) with an authentic sample

5-[3-(\beta-ducosyloxy)propyl]-7-methoxy-2-(3',4'-methylenedioxy phenyl)benzofuran (6) (egonol glucoside) Brown needles, recrystallized from EtOH mp 169-170°, IR (KBr) cm<sup>-1</sup> 3600-3250, 2950, 1600, 1480, 930<sup>-1</sup>H NMR (200 Mz, DMSO-d<sub>6</sub>)  $\delta$ 192 (2H, m, G-O-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-Ar),  $\delta$ 276 (2H, t, J = 7 3 Hz,  $G-O-CH_2-CH_2-CH_2-Ar$ ),  $\delta$  3 09 (1H, ddd, J = 7 8, 83, and 46 Hz, G-2),  $\delta$ 3 10-3 30 (3H, m, G-3,4.5),  $\delta$ 3 40-3 60  $(2H, m, G-O-CH_2-CH_2-CH_2-Ar), \delta 3 76 (2H, dd, J = 11 2 and$ 5.6 Hz, G-6),  $\delta 4.00$  (3H, s, QMe),  $\delta 4.21$  (1H, d, I = 7.8 Hz, G-6-OH),  $\partial 497$  (1H, b, G-3-OH),  $\partial 502$  (1H, d, J = 39 Hz, G-4-OH),  $\delta 5 11$  (1H, d, J = 46 Hz, G-2-OH),  $\delta 6 12$  (2H, s,  $-O-CH_2-O-$ ),  $\delta 6 18$  (1H, d, J=0.9 Hz, H-6),  $\delta 7 01$  (1H, d, J = 0 9 Hz, H-4),  $\delta$ 7 04 (1H, d, J = 7 1 Hz, H-5'),  $\delta$ 7 18 (1H, s, H-3),  $\delta$ 7 42 (1H, dd, I = 71 and 1.7 Hz, H-6'),  $\delta$ 7 44 (1H, d, I = 1.7 Hz, H-2'), MS [M]<sup>+</sup> 488 1689, C<sub>25</sub>H<sub>28</sub>O<sub>10</sub> requires 488 1682, TLC,  $R_f = 0.56$  (EtOAc-iso-PrOH-H<sub>2</sub>O, 6.2.1)

Enzymatic hydrolysis of egonol glucoside (6)  $\beta$ -glucosidase The glucoside (6) (3 mg) was emulsified with emulsin (4 mg) in NaOAc-HOAc buffer (pH 5) The reaction mixture was workedup in the usual way for 24 hr to give egonol as the only aglucone The products were identified by PPC, TLC, and IR comparison with authentic egonol and D-glucose respectively, and gave the same colour responses after spraying with 18 MH<sub>2</sub>SO<sub>4</sub> followed by heating (egonol), naphthoresorcinol (glucose) and anisalde-hydec (glucose) reagent The latter gave a positive test for D-glucose with D-glucose oxidase

Preparation of authentic egonol acetate Prepared by the acetylation of egonol (100 mg) with  $Ac_2O$  and pyridine (0.5 ml) at 60° The acetate was recrystallized from MeOH Mp 105 (bt 103-105°)

Acknowledgements---The authors thank Dr S Kondo, Dr Y Okami, Dr H Naganawa and Dr S Gomi (Institute of Microbial Chemistry, Shinagawa, Tokyo) for measurements of NMR, and MS Thanks are due to Keiogijuku Academic Fund for financial support

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