Phytochemistry, 1980, Vol. 19, pp. 2784 2786. © Pergamon Press Ltd. Printed in England. 0031 9422 80/1201 2784 $02.00/0

FOUR BENZOFURAN GLYCOSIDES FROM *STYRAX OFFICINALIS*

HÜSEYIN ANIL

Faculty of Chemistry, Ege University, Bornova, Izmir, Turkey

(*Revised received* 11 *April* 1980)

Key Word Index—*Styrax officinalis*; Styracaceae; egonol-β-glucoside; egonol-β-gentiobioside; 2-(3,4-dimethoxyphenyl)-5-(3-hydroxypropyl)-7-methoxybenzofuran-β-gentiobioside; egonol-β-gentiotrioside.

INTRODUCTION

Egonol is a natural benzofuran occurring widely in *Styrax* species [1]. It was initially isolated by Okada [2] as an unsaponifiable constituent of the seed-oil of *S. japonicum* Sieb et Zucc.; and its structure was determined by Kawai *et al.* [3-7] as 5-(3-hydroxypropyl)-7-methoxy-2-(3,4-methylenedioxyphenyl)benzofuran (**1**). Segal *et al.* [8] isolated another benzofuran from the glycosidic fraction of the seeds of *S. officinalis* L. by acidic hydrolysis and determined its structure as 2-(3,4-dimethoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy benzofuran (**2**). Another egonol glycoside was reported from the seeds of *S. suberifolium* Hook et Arn. by Kawai *et al.* [9]; but its structure was not determined. We now wish to report the isolation and structures of four new benzofuran glycosides from the seeds of *S. officinalis* L.

RESULTS AND DISCUSSION

TLC investigations indicated that the isolated glycoside mixture produced two aglycones and glucose as a result of acidic hydrolysis. The pure aglycones obtained by column chromatography were shown to be identical with **1** and **2** respectively by spectroscopic (IR, UV, ^{1}H NMR and ^{13}C NMR) investigation of these and their acetate derivatives and also by comparison of their mps with lit. values [3–8].

The three fractions obtained from the column chromatography of the glycoside mixture, contained the following respectively: glycoside A (**3**) (fraction a), mixture of glycoside B (**4**) and C (**5**) (fraction b) and glycoside D (**6**) (fraction c).

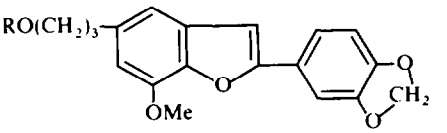
The mixture **4** and **5** was methylated and acetylated. The pure methylated glycosides B (**4a** and **5a**) and the acetylated B (**4b** and C **5b**) were obtained by column chromatography from the methylated and acetylated glycoside mixtures respectively.

The glycoside A (**3**) gave egonol (**1**) and glucose on acid hydrolysis. The mass spectrum of **3** showed characteristic peaks at *m/e* 488 (M^+), 354, 326 and its acetate derivative (**3a**) at *m/e* 656 (M^+), 354, 326, 310 and 282. The ^{1}H NMR spectrum of **3** showed the characteristic signals for egonol [8] and the spectrum of **3a** showed four singlets for the acetate groups at δ 2.01, 2.02, 2.05 and 2.06 ppm together with the characteristic signals for egonol. The configuration of the glycosidic linkage was determined as β on the basis of the chemical shift value of the anomeric carbon atom (δ 102.99 ppm) by ^{13}C-{^{1}H}-NMR spectroscopy of **3** [10, 11]. The foregoing data showed **3** to be egonol-β-glucoside.

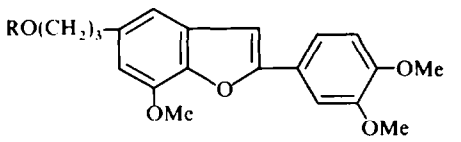
The mass spectrum of methylated glycoside B (**4a**) showed peaks at *m/e* 748 (M^+), 558, 354, 325, 310 and 282; and the acetate derivative **4b** at *m/e* 944 (M^+), 642, 354, 326, 310 and 282. The ^{1}H NMR spectrum of **4a** showed six methoxy singlets at δ 3.38, 3.49, 3.51, 3.53, 3.58 and 3.68 (2 $OC\underline{H}_3$) together with the characteristic signals of egonol (**1**). The ^{1}H NMR spectrum of **4b** also showed the signals for egonol together with the signals for seven acetyl groups as six singlets in the range δ 1.97- 2.07 ppm. These results indicated that glycoside B (**4**) contains two glucose units. Methanolysis of **4b** with 5% methanolic HCl produced two methylated glucosides, which were separated by preparative TLC and identified by mass spectroscopy [12] as Me-2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and Me-2,3,4-tri-*O*-methyl-D-glucopyranoside. On hydrolysis with 2N HCl, these methylated glucosides produced 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-glucose respectively. The identification of the above methylated monosaccharides were based on TLC and PC comparison with reference materials. The configuration of the linkages was determined as β from the ^{13}C NMR of **4a** (δ: 103.581 and 103.019 ppm). The foregoing results showed that the sugar portion of glycoside B (**4**) is gentiobiose and hence compound **4** is egonol-β-gentiobioside.

The mass spectrum of methylated glycoside C (**5a**) showed characteristic peaks at *m/e* 764 (M^+), 574, 370, 342, 326 and 298; and the acetylated glycoside C (**5b**) at *m/e* 960 (M^+), 658, 370, 342 and 298. The ^{1}H NMR spectrum of **5a** gave signals corresponding to aglycone **2** together with six singlets for seven methoxy groups at δ 3.38, 3.49, 3.53, 3.55, 3.60 and 3.63 (2 $OC\underline{H}_3$) ppm. The ^{1}H NMR spectrum of **5b** also showed signals corresponding to aglycone **2** together with seven singlets for seven acetyl groups in the range δ 1.84–2.14 ppm. Methanolysis of **5a** with 5% methanolic HCl produced the same methylated glucosides as **4a**. The configuration of the glycosidic linkages was similarly determined as β from the ^{13}C NMR of **5a** (δ 103.52 and 104.12 ppm). These findings showed that **5** is 2-(3,4-dimethoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-benzofuran-β-gentiobioside.

Glycoside D (**6**) gave egonol (**1**) and glucose on acidic hydrolysis. The mass spectrum of methylated glycoside D (**6a**) showed characteristic peaks at *m/e* 952 (M^+), 762, 558, 354, 326, 310 and 282, which indicated the presence of 3



1 R = H
3 R = Glucosyl
3a R = Tetra-*O*-acetylglucosyl
4 R = Gentiobiosyl
4a R = Hepta-*O*-methylgentiobiosyl
4b R = Hepta-*O*-acetylgentiobiosyl
6 R = Gentiotriosyl
6a R = Deca-*O*-methylgentiotriosyl



2 R = H
5 R = Gentiobiosyl
5a R = Hepta-*O*-methylgentiobiosyl
5b R = Hepta-*O*-acetylgentiobiosyl

glucose units. On methanolysis of **6a**, only Me-2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and Me-2,3,4-tri-*O*-methyl-D-glucopyranoside (2 mol) were formed, showing that **6** is egonol-β-gentiotrioside. The configuration of the glycosidic linkages was assumed to be β.

EXPERIMENTAL

The ^{1}H NMR and ^{13}C NMR spectra were recorded respectively at 90 and 20 MHz in $CDCl_3$ (except **3** in ^{12}C-DMSO-d_6) with TMS as in standard. MS were recorded at 70 eV. *S. officinalis* was collected from Marmaris (Turkey). The botanical authentication of the seed sample was carried out at the Systematical Botany Department of Ege University.

Isolation of glycosides. Seeds (200 g) were first extracted with 3 × 1.5 l. petrol to remove oils and then with 3 × 1 l. EtOH. The EtOH extract showed 3 spots on TLC in $CHCl_3$–MeOH (13:5) with R_f values 0.57, 0.30, 0.11. EtOH was evapd and the residue (8.65 g) was chromatographed on a Si gel column using the same solvent system, producing 0.13 g of **3**, 6.1 g of a mixture of **4** and **5**, and 0.14 g of **6**.

Glycoside A (**3**). Mp 166–167° (from MeOH): $[\alpha]_D^{22}$ − 15.2° (*c* 0.6, MeOH). MS (*m/e*): 488 (M^+, 34), 354 (23), 326 (17), 308 (18) and 282 (100). (Found: C, 61.67; H, 5.92. Calc. for $C_{25}H_{28}O_{10}$: C, 61.47; H, 5.78.) **3** (50 mg) was acetylated in the usual manner using 4 ml pyridine and 2 ml Ac_2O. The acetate derivative (**3a**) was crystallized from MeOH as colourless needles (44 mg). Mp 135–136°; $[\alpha]_D^{22}$ − 13.5° (*c* 0.4, $CHCl_3$). MS *m/e* (rel. int.): 656 (M^+, 69), 354 (17), 326 (11), 310 (32) and 282 (100). (Observed: 656.211, MS, h.r.; Calc. for $C_{33}H_{36}O_{14}$: 656.261.)

Hydrolysis of glycoside A (**3**). Glycoside (50 mg) was hydrolysed by refluxing with 10 ml 2 N HCl–EtOH mixture (1:1) at 90° for 12 hr. The cooled hydrolysate was poured into ice-H_2O and the pptd aglycone (egonol) was filtered off (18 mg). The filtrate was neutralized with Ag_2CO_3 and the presence of glucose was determined by PC using EtOAc–pyridine-H_2O (3.6:1:1.15) [13] as solvent.

Methylation of mixture of glycoside B (**4**) *and C* (**5**). Glycoside mixture (2.6 g) was methylated in the usual manner using 20.8 ml DMF, 5.2 g Ag_2O and 4 ml of MeI. The resulting methylated mixture was chromatographed on a Si gel column, eluting with petrol–EtOAc Me_2CO (5:3:1); this produced 1.73 g of **4a** and 0.18 g of **5a**.

Methylated glycoside B (**4a**). Mp 109 110° (from petrol EtOAc): $[\alpha]_D^{22}$ − 27° (*c* 1.3, $CHCl_3$). MS (*m/e*): 748 (M^+, 81), 558 (22), 354 (96), 326 (10), 325 (18), 310 (100), 282 (60) and 187 (21). (Found: C, 60.97; H, 6.98. Calc. for $C_{38}H_{52}O_{15}$: C, 60.95; H, 6.99.)

Methylated glycoside C (**5a**). Mp 132–134° (from petrol–EtOAc: $[\alpha]_D^{22}$ − 30.5° (*c* 0.6, $CHCl_3$). MS *m/e* (rel. int.): 764 (M^+, 100), 574 (15), 370 (69), 342 (21), 326 (61), 298 (46) and 187 (29). (Found: C, 60.95; H, 7.41. Calc. for $C_{39}H_{56}O_{15}$: C, 61.21; H, 7.38) (Observed: 764.362, MS, h.r.: Calc. for $C_{39}H_{56}O_{15}$: 764.370).

Glycoside D (**6**) was obtained as a white powder from MeOH; mp 196–199°. Glycoside (30 mg) was hydrolysed by refluxing with 12 ml 2 N HCl–EtOH mixture (1:1) at 90° for 12 hr; aglycone (egonol) and glucose were formed. **6** (80 mg) was methylated using 6 ml DMF, 160 mg Ag_2O and 1 ml of MeI. The product **6a** was purified by prep. TLC eluting with petrol–EtOAc–Me_2CO (5:3:1) and was obtained as a syrup $[\alpha]_D^{22}$ − 34.6° (*c* 0.35, $CHCl_3$). MS *m/e* (rel. int.): 952 (M^+, 24), 762 (8), 558 (21), 354 (68), 326 (19), 310 (75), 282 (37) and 187 (100). $C_{47}H_{68}O_{20}$ (952.440), M^+ *m/e* 952.

Methanolysis of **4a**, **5a** *and* **6a**. **4a** (220 mg) was refluxed at 90° for 6 hr in 16 ml of dry 5% methanolic HCl. The cooled reaction mixture was neutralized with Ag_2CO_3 and poured into ice-H_2O. The pptd aglycone was filtered off and the remaining methylated glucosides were separated by prep. TLC using C_6H_6–Me_2CO (1:1).

5a (70 mg) in 6 ml of 5% HCl–MeOH and **6a** (40 mg) in 4 ml of the same reagent were methanolysed in the same manner and the products separated. The separated methylated glucosides were identified as Me-2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and 2,3,4-tri-*O*-methyl-D-glucopyranoside by MS [8].

The above methylated glucosides on hydrolysis with 2 N HCl produced 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-glucose. The identities of these compounds were established by TLC comparison with reference materials using petrol-$CHCl_3$-Me_2CO (2:1:1) and $CHCl_3$–MeOH (7:1) as the solvent systems and by PC in EtOH–*n*BuOH-H_2O–conc NH_3 (10:40:49:1, upper phase) and C_6H_6 EtOH–H_2O–conc NH_3 (200:47:14:1, upper phase) as solvent systems.

Acetylation of mixture of glycoside B (**4**) *and* **5**. A mixture of **4** and **5** (1.64 g) were acetylated in the usual way using 20 ml pyridine and 20 ml Ac_2O. The acetylated glycoside mixture was separated by prep. TLC in petrol EtOAc–Me_2CO (5:3.2:1.2) to produce 474 mg of **4b** and 47 mg of **5b**.

Acetylated glycoside B (**4b**). Mp 147-149° (from petrol–EtOAc): $[\alpha]_D^{22}$ − 21.7° (*c* 1.2, $CHCl_3$). MS *m/e* (rel. int.): 944 (M^+, 51), 902 (3), 642 (7), 354 (21), 326 (15), 310 (51), 282 (100) and 169 (49). (Found: C, 57.15; H, 5.46; Calc. for $C_{45}H_{52}O_{22}$: C, 57.20; H, 5.55).

Acetylated glycoside C (**5b**). Mp 131–133° (from petrol-EtOAc: $[\alpha]_D^{22}$ − 14.2° (*c* 1.1, $CHCl_3$). MS *m/e* (rel. int.): 960 (M^+, 100), 918

(18). 658 (14), 370 (42), 342 (14), 298 (29) and 169 (19). $C_{46}H_{56}O_{22}$ (960.334), M^+ *m/e* 960.

Acknowledgements—The spectroscopic investigations used in this work were carried out at the Organic and Biochemistry Institute of Bonn University. I therefore extend my thanks to Prof. Dr. R. Tschesche. I also thank TUBITAK for their financial support of this work.

REFERENCES

1. Hegnauer, R. (1973) *Chemotaxanomie der Pflanzen*, Bd. 4, p. 473. Birkhäuser, Basel.
2. Okada, H. (1915) *J. Pharm. Soc. Jpn.* 657.
3. Kawai, S. and Miyoshi, T. (1938) *Ber.* **71B**, 1457.
4. Kawai, S. and Suga, M. (1938) *Ber.* **71B**, 2071.
5. Kawai, S. and Yoshimuru, F. (1938) *Ber.* **71B**, 2415.
6. Kawai, S. and Sugiyama, N. (1938) *Ber.* **71B**, 2421.
7. Kawai, S. and Yamagami, K. (1938) *Ber.* **71B**, 2438.
8. Segal, R., Goldzweig, I. M., Sokoloff, S. and Zaitschek, D. V. (1967) *J. Chem. Soc.* 2402.
9. Kawai, S. and Sugimota, K. (1940) *Ber.* **73**, 774.
10. Breitmaier, E. and Bauer, G. (1977) ^{13}C-*NMR-Spektroskopie*, p. 385. Georg-Thieme, Stuttgart.
11. Gorin, P. A. J. and Mazurex, M. (1975) *Can. J. Chem.* **53**, 1212.
12. Heyns, K., Sperling, K. R. and Grützmacher, H. F. (1969) *Carbohydr. Res.* **9**, 79.
13. Colombo, P., Corbetta, D., Pirotta, A., Ruffini, G. and Sartori, A. (1965) *J. Chromatogr.* **3**, 343.