Phytochemistry, Vol. 21, No. 1, pp. 201–203, 1982.
Printed in Great Britain.

0031-9422/82/010201-03 $03.00/0

STRUCTURE ELUCIDATION OF EUCOMMIOSIDE (2″-*O*-β-D-GLUCOPYRANOSYL EUCOMMIOL) FROM *EUCOMMIA ULMOIDES*

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(*Received* 30 *January* 1981)

Key Word Index—*Eucommia ulmoides*; Eucommiaceae; eucommiol; eucommioside; 2″-*O*-β-D-glucopyranosyl eucommiol; Birch reduction.

Abstract—The isolation of eucommioside from *Eucommia ulmoides* is described. Chemical modifications and spectral evidence identify eucommioside as the 2″-*O*-β-D-glucopyranosyl derivative of eucommiol, a known cyclopentenoid-tetrol previously isolated from the same plant.

INTRODUCTION

During a previous investigation on iridoid glycosides of *Eucommia ulmoides* [1] we isolated a new cyclopentenoid tetrol **2** named eucommiol in addition to aucubin (**1**), the main component, harpagide acetate, ajugoside, reptoside and ulmoside [2]. Eucommiol (**2**) is present in the plant only in autumn. At the same period we also isolated a rather polar compound **3** ($R_f = 0.17$) which gave the same olive-brown colour as **2** ($R_f = 0.50$) with the iridoid vanillin reagent. In the present paper we report the structure and configuration of compound **3** which we have named eucommioside.

RESULTS AND DISCUSSION

Compound **3** is a hygroscopic and viscous colourless oil, easily water soluble with neutral reaction. The UV spectrum shows an absorption at λ_{max} 208 nm (log $\varepsilon = 3.6$) indicative of an isolated C=C double bond.

The isolation and purification of compound **3** was rather difficult. Even though it appeared to be a homogeneous product by TLC analysis, its ^{1}H NMR and ^{13}C NMR spectra showed that there was contamination by an impurity which was difficult to separate. The best method for obtaining pure **3** was through repeated chromatography of its hepta-acetyl derivative **4**, which was successfully isolated as the pure compound and then hydrolysed to yield compound **3** ($C_{15}H_{28}O_9$).

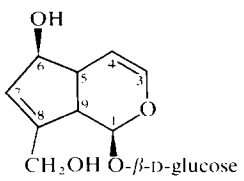
Compound **3** was stable in acids at room temperature and was hydrolysed in 2 N H_2SO_4 only at refluxing temperature for 2 hr.

The enzymatic (β-glucosidase) and acid hydrolysis of **3** afforded D-glucose (1 mol), and a stable aglycone, whose physical and spectral data were identical with those of compound **2**.

These preliminary results suggested that **3** was the β-D-monoglucoside of eucommiol (**2**). An examination of the 90 MHz ^{1}H NMR spectrum of **3** (D_2O) (see Experimental), confirmed the presence of one β-D-glucopyranosyl unit (doublet at δ 4.50, $J_{1'2'} = 7.5$ Hz, derived from the anomeric proton in the β-configuration). An important feature of this spectrum was the almost identical chemical shift values of the aglycone protons of **3** with those in the spectrum of **2** [1]. This made it difficult to identify which of the four alcoholic functions of **2** was involved in the glucosidic linkage.

A similar situation was found in the ^{1}H NMR spectrum of the hepta-acetate **4** ($CDCl_3$) which was easily prepared by reacting **3** with pyridine and Ac_2O under mild conditions. The chemical shift values for the protons of **4** were coincident with those of tetra-*O*-acetyleucommiol **5** [1] (see Experimental).

Much more useful information was obtained by a careful analysis of the ^{13}C NMR spectral data of **3** and **2** (Table 1). The assignments for the three hydroxymethyl groups of **2** were carried out in a previous paper [4]. In the



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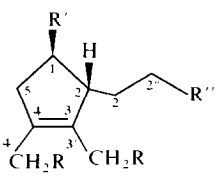

2 R = R′ = R″ = OH
3 R = R′ = OH, R″ = O-β-D-glucose
4 R = R′ = OAc, R″ = O-β-D-gluc(OAc)$_4$
5 R = R′ = R″ = OAc
6 R = H, R′ = OH, R″ = O-β-D-glucose
7 R = H, R′ = R″ = OH

Table 1. ^{13}C NMR chemical shift assignments for compounds **2**–**7**. The spectra were recorded in D_2O at 20 MHz. Chemical shifts in ppm from TMS (dioxane, 67.4 ppm, was used as int. standard)

Carbon No.	2	3	4*	5*	6	7
C-1	75.3 *d*	75.2 *d*	76.8 *d*	77.1 *d*	75.5 *d*	75.6 *d*
C-2	52.9 *d*	53.0 *d*	50.7 *d*	51.2 *d*	56.1 *d*	56.0 *d*
C-3	137.1 s	137.3 s	134.6 s	135.2 s	130.3 s	130.0 s
C-4	139.0 *s*	138.9 *s*	136.1 *s*	135.6 *s*	132.4 *s*	132.6 *s*
C-5	42.2 *t*	42.2 *t*	40.3 *t*	40.4 *t*	45.7 *t*	45.7 *t*
C-2′	33.1 *t*	30.7 *t*	30.5 *t*	29.6 *t*	30.8 *t*	33.3 *t*
C-2″	60.8 *t*	69.5 *t*	67.4 *t*	62.3 *t*	69.7 *t*	61.1 *t*
C-3′	56.2 *t*	56.3 *t*	58.5 *t*	58.5 *t*	12.0 *q*	12.1 *q*
C-4′	57.9 *t*	57.9 *t*	59.9 *t*	59.9 *t*	13.8 *q*	13.8 *q*
c-1′		103.1 *d*	100.9 *d*		103. 1*d*	
c-2′		74.0 *d*	71.5 *d*		74.0 *d*	
c-3′		76.7 *d*	72.9 *d*		76.7 *d*	
c-4′		70.5 *d*	68.8 *d*		70.5 *d*	
c-5′		76.7 *d*	71.9 *d*		76.7 *d*	
c-6′		61.6 *t*	62.0 *t*		61.6 *t*	

*Additional signals from acetoxy groups.

spectrum of **3**, besides the signals of the 6 carbons of the β-D-glucopyranose moiety which were easily assigned on the basis of lit. data [3], the following diagnostic correlations between the aglycone signals of **3** and of eucommiol **2** were observed: (a) the strong deshielding (8.7 ppm, α-effect) of C-2″ of **3** (69.5 ppm) with respect to **2** (60.8 ppm); (b) the concomitant upfield shift observed for the C-2′ carbon resonance in the same couple **2** → **3** (33.1 → 30.7 ppm, $\Delta\delta = 2.4$, β-effect); (c) the very similar chemical shift values of C-3′ and C-4′ in the couple **2**–**3** (C-3′, 56.2 and 56.3 ppm; C-4′, 57.9 and 57.9 ppm, respectively), excluding any glucosidation effect on both allylic alcoholic functions linked to C-3 and C-4 carbons.

The significant shift differences observed in the spectra of **2** and **3** for C-2′ and C-2″ resonances, in agreement with the general rule for carbohydrates by which the glycosidation of a hydroxylgroup causes a down-field shift (8–10 ppm) of the resonance of the α-carbon [5] and an up-field shift (0–4 ppm) of the β-carbons [6–8], allowed the locations of the *O*-β-D-glucopyranose unit at C-2″ of **3**.

The further comparison of the ^{13}C NMR spectra of hepta-*O*-acetyleucommioside (**4**) and tetra-*O*-acetyleucommiol (**5**) corroborated the above suggestion. The exact coincidence of the chemical shift values observed for C-3′ (58.5 ppm in **4** and **5**) and for C-4′ (59.9 ppm in **4** and in **5**) eliminated the presence of the *O*-β-D-glucopyranosyl unit at these carbons while the glucosidation effect on C-2″ (α-effect) decreased to 5.1 ppm and that on C-2′ became of opposite sign (down-field shift of 0.9 ppm).

The linkage position of the *O*-β-D-glucopyranosyl unit at C-2″ was definitively confirmed by selective hydrogenolysis of both free allylic CH_2OH groups in **3**.

Thus hepta-acetyleucommioside (**5**) was transformed by Birch reduction [9] at −33° into 3′,4′-bisdeoxyeucommioside (**6**). Its ^1H NMR spectrum (D_2O) (see Experimental) showed as the main feature, with respect to **3**, the appearance of both expected vinylic methyl groups (singlet at δ 1.57, 6H) and the disappearance of the corresponding CH_2OH resonance at 4.24 (4H); the remaining signals did not exhibit notable modifications. A comparison of the ^{13}C NMR spectrum of **6** with that of **3**, revealed the appearance of two allylic methyl carbons (12.0 and 13.8 ppm) instead of the corresponding carbons relative to CH_2OH functions. The chemical shift values of the remaining aglycone carbons of **6** were as expected, nearly the same as those of 3′,4′-bisdeoxyeucommiol (**7**), apart from the down-field shift of C-2″ (8.6 ppm) and the up-field shift of C-2′ (2.5 ppm) attributable to α- and β-glucosidation effects, respectively. The preparation of **6** therefore demonstrates unequivocally the structure 2″-*O*-β-D-glucopyranosyl eucommiol for compound **3**.

The probable biogenetic correlation [1] between eucommiol (**2**) and the aglycone of aucubin (**1**), which is present in the plant as the major iridoid constituent throughout the year, does not seem to be excluded by the location of the *O*-β-D-glucosyl moiety at C-2″ of **3**.

EXPERIMENTAL

CC was on cellulose CF 11 (Whatman) or Si gel 70–230 mesh. TLC used Si gel SIF_{254} (Erba) and cellulose (Merck) plates. PC was on Schleicher & Schüll No 2043 Mgl paper. Spray reagents: 2 N H_2SO_4, heating at 120° (Si gel plates), vanillin (vanillin 1 g, conc HCl 2 ml, MeOH 100 ml) and 3,5-dinitrosalicylic acid (3,5-dinitrosalicylic acid 0.5 g, NaOH 4 g, H_2O 100 ml), heating at 100° (cellulose TLC and PC). All evapns of volatile material were performed under red. pres. ^1H NMR spectra were recorded at 90 MHz. Chemical shifts are given in δ values and coupling constants in Hz. HDO was used as int. standard at 4.70 ppm for D_2O solns and TMS for $CDCl_3$.

Isolation of iridoid fraction. Eucommia ulmoides (4.8 kg of fresh leaves collected in the autumn) was roughly chopped and extrd 2 × with EtOH (10 l. × 2) at room temp. for 24 hr. The combined EtOH solns were evapd *in vacuo* at 50° to an aq. suspension (0.5 l.) which was extrd with 1 l. of petrol (30–50°) on a continuous extraction apparatus, and then diluted to 1 l. Decolourizing charcoal (C.Erba, 0.4 kg) was added and the aq. suspension stratified in a Gooch funnel (ϕ 13 cm). Mono- and disaccharides were removed by elution with H_2O (15 l.) and H_2O–EtOH (95:5; 5 l.). At this point benzidine and resorcine tests for sugars were negative, and the elution of compounds with

positive vanillin reaction was carried out with H_2O–EtOH (1:1; 20 l.). This fraction, evapd *in vacuo*, gave 80 g of an amorphous residue.

Eucommioside (**3**). The residue (80 g) was chromatographed on cellulose powder (350 g). Elution with BuOH satd with H_2O (BW) gave reptoside 0.6 g ($R_f = 0.55$), eucommiol (**2**) 9 g ($R_f = 0.51$), ajugoside 0.5 g ($R_f = 0.48$), harpagide acetate 0.8 g ($R_f = 0.37$), aucubin (**1**) 8.8 g ($R_f = 0.28$), eucommioside (**3**) 7.5 g ($R_f = 0.17$) and finally ulmoside 1.2 g ($R_f = 0.03$). The crude eucommioside (1.2 g) was further chromatographed on cellulose powder (80 g) in BW, giving eucommioside (0.6 g) as a colourless, viscous oil but still containing impurities.

^{1}H NMR spectrum of **3** (D_2O, 90 MHz): δ 4.50 (*d*, $J_{1',2'}$ = 7.5 Hz, anomeric H-1'), 4.24 (*br s*, H-1, 2 H-3', 2 H-4'), 3.9–3.6 (2 H-2''), 2.82 (*m*, H-2), 2.90 and 2.32 (*br dd*, *br d*, J_{AB} = 18 Hz, 2 H-5), 2.2–1.2 (*cm*, 2 H-2').

^{1}H NMR spectrum of **2** (D_2O, 90 MHz): δ 4.24 (*br s*, H-1, 2 H-3', 2 H-4'), 3.71 (*t*, J = 7.0 Hz, 2 H-2''), 2.72 (*m*, H-2) 2.90 and 2.32 (*br dd*, *br d*, J_{AB} = 18 Hz, 2 H-5), 2.1–1.2 (*cm*, 2 H-2').

Hepta-O-acetyleucommioside (**4**). Compound **3** (0.25 g) was dissolved in dry pyridine (1.8 ml) and Ac_2O (3.6 ml) and allowed to stand at room temp. for 1 hr. After addition of MeOH (5 ml) the soln was concd under red. pres. The residue (0.3 g) was chromatographed several times on Si gel, eluting with C_6H_6–Et_2O (1:1) to obtain finally pure **4** (0.15 g) as a colourless, viscous oil. (Found: C, 53.58; H, 6.60. $C_{29}H_{42}O_{16}$ requires: C, 53.86; H, 6.65%).

^{1}H NMR spectrum of **4** ($CDCl_3$, 90 MHz): δ 5.2–4.9 (H-1), 4.72 (*br s*, 2 H-3', 2 H-4'), 3.9–3.4 (2 H-2''), 2.92 and 2.36 (*br dd*, *br d*, J_{AB} = 18 Hz, 2 H-5), 2.9–2.7 (H-2), 2.2–1.2 (2 H-2').

^{1}H NMR spectrum of **5** ($CDCl_3$, 90 MHz): δ 5.08 (*se*, H-1), 4.72 (*br s*, 2 H-3', 2 H-4'), 4.13 (*t*, J = 7.0 Hz, 2 H-2''), 2.88 (*m*, H-2) 2.92 and 2.39 (*br dd*, *br d*, J_{AB} = 18 Hz, 2 H-5), 2.1–1.2 (*cm*, 2 H-2').

Hydrolysis of **4** *to eucommioside* (**3**). Compound **4** (100 mg), dissolved in MeOH (5 ml), was treated with satd $Ba(OH)_2$ (2 ml) and left overnight at room temp. The soln was acidified with dil. HCl and stirred with decolourizing charcoal (500 mg). The suspension was then stratified on a Gooch funnel (ϕ 2 cm) and eluted with H_2O to remove salts. The final elution with MeOH afforded **3** (45 mg) as a colourless, viscous oil, pure by TLC.

Enzymatic hydrolysis of **3**. Eucommioside (**3**, 100 mg) was completely hydrolysed in 1 hr at 35° with β-glucosidase (EC 3.2.1.21, Fluka, 20 mg) in 0.1 M citrate buffer (3 ml, pH 6). The aq. soln was extrd with EtOAc (7 ml, 8 ×) and the residue of the organic phase (46 mg) was chromatographed on Si gel (6 g). Elution with $CHCl_3$–MeOH (4:1) afforded the pure aglycone (30 mg) whose physical data were identical with those of an authentic sample of **2**.

Bisdeoxyeucommioside (**6**). Liquid NH_3 (150 ml) was added to hepta-acetate **4** (0.3 g) dissolved in abs. EtOH (4 ml), and then, with stirring, Li (0.15 g) was added in small pieces over a period of 1 hr, keeping the temp. at −33°. The blue final soln was decolourized with abs. EtOH (1 ml) and left overnight to allow the NH_3 to evaporate. After removal of volatile liquids at red. pres., the residue was dissolved in H_2O and neutralized with cold 6 N HCl. To the neutral soln decolourizing charcoal was added (3 g), and the suspension, stratified on a Gooch funnel (ϕ 10 mm), and washed with H_2O to eliminate the salts. Elution with MeOH (0.3 l.) afforded a residue (0.1 g) which was chromatographed on Si gel (8 g) in BW giving pure **6** (60 mg) as a viscous, colourless oil. (Found: C, 56.16; H, 8.85. $C_{15}H_{28}O_7$ requires C, 56.23; H, 8.81%).

^{1}H NMR spectrum of **6** (D_2O, 90 MHz): δ 4.40 (*d*, $J_{1',2'}$ = 7.5 Hz, anomeric H-1'), 4.10 (*se*, H-1), 3.9–3.6 (2 H-2''), 2.40 (*br d*, H-2), 2.66 and 2.06 (*br dd*, *br d*, J_{AB} = 18 Hz, 2 H-5), 2.0–1.2 (*cm*, 2 H-2'), 1.57 (*br s*, 3 H-3', 3 H-4').

REFERENCES

1. Bianco, A., Iavarone, C. and Trogolo, C. (1974) *Tetrahedron* **30**, 4117.
2. Bianco, A., Bonini, C. C., Guiso, M., Iavarone, C. and Trogolo, C. (1978) *Gazz. Chim. Ital.* **108**, 17.
3. Breitmaier, E. and Voelter, W. (1974) ^{13}C NMR *Spectroscopy*. Verlag Chemie, Weinheim.
4. Bianco, A., Bonini, C. C., Guiso, M., Iavarone, C. and Trogolo, C. (1981) *Tetrahedron* **37**, 1773.
5. Usui, T., Yamaaka, N., Matsuda, K., Tuzimura, K., Sugiyama, H. and Seto, S. (1973) *J. Chem. Soc. Perkin Trans.* 1, 2425.
6. Cox, D. D., Matzner, E. K., Cary, L. W. and Reist, E. J. (1978) *Carbohydr. Res.* **23**, 67.
7. Lemieux, R. U. and Driguez, H. (1975) *J. Am. Chem. Soc.* **97**, 4069.
8. Beier, R. C., Mundy, B. P. and Strobel, G. A. (1980) *Can. J. Chem.* **58**, 2800.
9. Smith, M. (1968) *Reduction* (Augustine, R. L., ed.) pp. 38–111. Dekker, New York.