Department of Chemistry[1] University of Dhaka, Bangladesh

Neolignan and megastigmane glycosides from the leaves of *Pterospermum semisagittatum*

M. S. H. KHAN[1], N. NAHAR[1], M. MOSIHUZZAMAN[1], M. A. RASHID[2]

Received December 17, 2003, accepted April 8, 2004

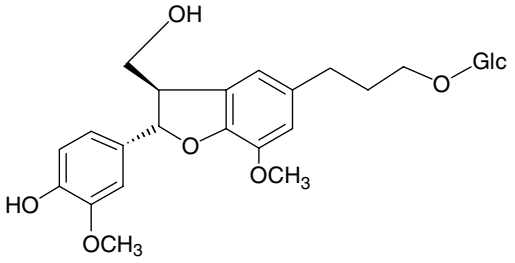
Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka-1000, Bangladesh
rashidma@aitlbd.net

Pharmazie 60: 72–74 (2005)

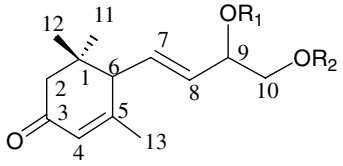
Three compounds namely, (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol-9′-*O*-β-D-glucopyranoside (**1**), 10-hydroxy-4,7-megastigmadien-3-one-9-*O*-β-D-glucopyranoside (**2**) and 9-hydroxy-4,7-megastigmadien-3-one-10-*O*-β-D-glucopyranoside (**3**) were isolated from the methanol extract of *Pterospermum semisagittatum*. The structures of these compounds were elucidated by spectroscopic methods. While glycosides **2** and **3** appear to be new, this is the third report of isolation of compound **1** from any natural source.

1. Introduction

Pterospermum semisagittatum Buch.-Ham. ex Roxb. (Sterculiaceae), known as "Laona asswar" (Indian name "Asswar") is a medium to large tree, distributed in the Indian subcontinent (The Wealth of India 1969). Flavonoids, terpenes, sterols and their glycosides, cycloprenoid and fatty acids have been reported from this plant (Dan and Dan 1988). As part of our continuing investigations on the *Pterospermum* species, we published the antihyperglycemic effect of the methanol soluble extractives (Mamun et al. 2001; Khan et al. 2003) of the leaves of *P. semisagittatum*. This paper describes the isolation and structure elucidation of three compounds, (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol-9′-*O*-β-D-glucopyranoside (**1**), 10-hydroxy-4,7-megastigmadien-3-one-9-*O*-β-D-glucopyranoside (**2**) and 9-hydroxy-4,7-megastigmadien-3-one-10-*O*-β-D-glucopyranoside (**3**) from the methanol extract of leaves of this plant.



1



2 :R_1 = β-D-glucopyranosyl; R_2=H
3 :R_1 = H; R_2 = β-D-glucopyranosyl
4 :R_1 = Tetraacetyl-β-D-glucopyranosyl; R_2 = Ac
5 :R_1 = Ac; R_2 = Tetraacetyl-β-glucopyranosyl
6 :R_1 = H; R_2 = H

2. Investigations, results and discussion

Repeated chromatographic separation and purification of the methanolic extract of leaves of *P. semisagittatum* provided three compounds (**1**–**3**). The molecular formula of compound **1** was deduced as $C_{26}H_{34}O_{11}$ from its FABMS data. The ^{13}C NMR spectrum of **1** confirmed the presence of 26 carbon atoms, while a HSQC experiment indicated that out of the 26 carbons 19 were attached to protons. The multiplicities of the carbon signals were determined by DEPT experiments, which revealed the presence of 2 methyls, 5 methylenes, 12 methines and 7 quaternary carbon atoms in compound **1**. The ^1H and ^{13}C NMR spectral data of **1** were identical to those of (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol-9′-*O*-β-D-glucopyranoside. Thus compound **1** was identified as (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol-9′-*O*-β-D-glucopyranoside, a neolignan glucoside previously reported from *Glochidion obovatum* (Euphorbiaceae) (Takeda et al. 1998) and *Glochidion zeylanicum* (Otsuka et al. 2000). Thus this is the third report of the isolation of substance **1** from any natural sources.
The FABMS of compound **4**, the acetyl derivative of **2**, showed a pseudomolecular ion peak at m/z 597 ($[M+H]^+$) which established its molecular formula as $C_{29}H_{40}O_{13}$. The ^1H NMR spectral data of compound **4** (Table) were, in part, identical to that of 9,10-dihydroxy-4,7-megastigmadien-3-one (**6**) previously reported from *Aglaia* species (Greger et al. 2001). This revealed a close structural similarity between these two compounds, **4** and **6**. However, the ^1H NMR spectrum of **4** displayed five acetyl group resonances at δ 1.96, 1.99, 2.03 (6H) and 2.04. In addition the spectrum also showed a one proton

Table: ^{1}H NMR spectral data for compounds 4–6†

Position	**4** ^{1}H, mult (J in Hz)	**5** ^{1}H, mult (J in Hz)	**6** ^{1}H, mult (J in Hz)
H-2a	2.41, d (17.0)	2.38, d (17.0)	2.33, d (16.4)
H-2b	2.05‡	2.06‡	2.09, d (16.4)
H-4	5.89, br. s	5.89, br. s	5.91, s
H-6	2.80, d (9.5)	2.69, d (8.5)	2.55, d (9.0)
H-7	5.81, dd (15.0, 9.0)	5.76, dd (15.0, 8.5)	5.71, dd (15.2, 9.0)
H-8	5.63, dd (15.0, 6.0)	5.60, dd (15.0, 7.0)	5.62, dd (15.2, 5.5)
H-9	4.41, m	5.37, m	4.30, m
OAc-9		2.05*, s	
H_2-10	4.06, m	3.86, m	3.50, dd (11.0, 7.4) 3.69, dd (11.0, 3.5)
OAc-10	1.96*, s		
H_3-11	0.99, s	0.94, s	0.96, s
H_3-12	1.03, s	1.01, s	1.03, s
H_3-13	1.92, br. s	1.90, br. s	1.90, d (1.6)
H-1′	4.10, d (8.0)	4.72, d (8.0)	
H-2′	4.89, dd (9.5, 8.0)	4.85, m	
H-3′	5.01, t (9.5)	5.00, t (9.5)	
H-4′	5.25, t (9.5)	5.23, t (9.5)	
H-5′	3.83, m	3.70, m	
H-6′$_a$	4.13, m	4.13, br.d (12.5)	
H-6′$_b$	4.23, dd (12.0, 4.0)	4.26, dd (12.5, 4.0)	
OAc	1.99*, s	1.95*, s	
OAc	2.03*, s	2.00*, s	
OAc	2.03*, s	2.05*, s	
OAc	2.04*, s	2.00*, s	

† Acquired at 500 MHz in CD_3OD for compounds **4** and **5** and at 400 MHz in $CDCl_3$ for compound **6** [6]; *values within the same column are interchangeable;
‡ overlapped signal

doublet (J = 8.0 Hz) at δ 4.10 having coupling with another proton at δ 4.89 (dd, J = 9.5, 8.0 Hz), which in turn was coupled to a one proton triplet (J = 9.5 Hz) at δ 5.01. The latter proton demonstrated an additional coupling with a signal at δ 5.25 (1 H, t, J = 9.5 Hz). The ^{1}H NMR spectrum of compound **4** displayed further resonances at δ 3.83 (1 H, m), 4.13 (1 H, m) and 4.23 (1 H, dd, J = 12.0, 4.0 Hz). These spectral features suggested the presence of a tetra-*O*-acetyl-β-D-glucopyranosyl moiety, which was subsequently confirmed by comparison of these ^{1}H spectral data with that of the acetylated β-D-glucopyranose in plumeride (Hasan et al. 1994) and a benzoic acid-derived glucopyranoside (Rashid et al. 1995). The position of the sugar moiety and the fifth acetyl group in **4** were established by careful comparison of the ^{1}H NMR data of this compound with 9,10-dihydroxy-4,7-megastigmadien-3-one (**6**) (Greger et al. 2001). In compound **4** the C-9 methine proton appeared at δ 4.41, as compared to δ 4.30 in compound **6** whereas the C-10 methylene protons in these compounds appeared at δ 4.06 (2 H) versus 3.50 (1 H) and 3.69 (1 H), respectively. These spectral data allowed placement of the tetra-*O*-acetyl-β-D-glucopyranosyl moiety in compound **4** at C-9 and the fifth acetyl group to C-10. On this basis compound **4** was characterized as 10-*O*-acetyl-4,7-megastigmadien-3-one-9-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl)-β-D-glucopyranoside, which appears to be new.

The FABMS of compound **5** established its molecular formula as $C_{29}H_{40}O_{13}$. This revealed that compound **5** was isomeric to compound **4**. The ^{1}H NMR spectral data of compound **5** (Table) was almost identical to that of compound **4**, thus confirming a close structural similarity between these two compounds. However, the C-9 methine and C-10 methylene protons in **5** appeared at δ 5.37 and 3.86 (2 H) as compared to 4.41 and 4.06 (2 H), respectively in compound **4**. This allowed to assign the acetyl group at C-9 and the sugar residue at C-10 in compound **5**. Thus, compound **5** was identified as 9-*O*-acetyl-4,7-megastigmadien-3-one-10-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl)-β-D-glucopyranoside, which also appears to be new.

Compounds **4** and **5** are the acetylated analogs of the mixture of compounds **2** and **3** (see section 3.4) and as the ^{1}H NMR spectrum of the mixture of **2** and **3** did not show any acetyl proton resonances, the structures of compound **2** and **3** were elucidated as 10-hydroxy-4,7-megastigmadien-3-one-9-*O*-β-D-glucopyranoside and 9-hydroxy-4,7-megastigmadien-3-one-10-*O*-β-D-glucopyranoside, respectively. An extensive literature search revealed that both the parent glycosides (**2**, **3**) and their corresponding pentaacetyl derivatives (**4**, **5**), appear to be new although the aglycones of these glycosides have previously been reported from Fijian *Aglaia* species (Greger et al. 2001).

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra including DEPT, ^{1}H-^{1}H COSY and HMBC spectra were recorded on an AMX 500 MHz NMR instrument. The chemical shifts are in reported ppm with respect to residual non-deuterated solvent signals. The FAB mass spectra were recorded on a JEOL SX 102 mass spectrometer (resolving power = 10,000) using m-nitrobenzyl alcohol (NBA) or polyethylene glycol as matrix.

3.2. Plant material

Leaves of *P. semisagittatum* were collected from the Chunotia Forest near Cox's Bazar (Bangladesh). The plant was identified by Dr. Khairul Alam at Bangladesh Forest Research Institute (BFRI), where the voucher specimen (No. 7004) has been deposited. The collected materials were cleaned, air dried and finally dried at 40 °C in an oven. The dried leaves were ground to a coarse powder with a Cyclotec grinder.

3.3. Extraction

The powdered leaves (2.35 kg) of *P. semisagittatum* were successively extracted at room temperature with $CHCl_3$ (10 L × 6; 24 h) followed by MeOH (10 L × 6; 24 h). The extracts were filtered off and the filtrates were evaporated to dryness at 40 °C with a rotary evaporator and finally freeze-dried to afford 257 g of $CHCl_3$ and 207 g of MeOH extracts. The total MeOH extract was suspended in water (1 L) and partitioned

with EtOAc (1 L × 3) followed by 1-butanol (1 L × 3). The EtOAc and 1-butanol soluble parts were evaporated to dryness to get EtOAc (52.82 g) and 1-butanol (86.0 g) soluble extractives.

3.4. Isolation of compounds

A portion of the butanol soluble part (75 g) was fractionated by column chromatography (RP-18) using a gradient elution of water and methanol with 10% increment and six fractions ($1F_1$–$1F_6$; each 70 mL) were collected. Fraction $1F_5$ (2.64 g) was refractionated by a reversed phase C_{18} column using the same mobile phase and five fractions ($2F_1$–$2F_5$; each 40 mL) were collected. Fraction $2F_4$ (1.86 g) was passed through a polyamide column using MeOH to remove tannins and the tannin-free materials were separated into two fractions ($3F_1$ and $3F_2$) by HPLC [column – RP-18 (250 mm × 4.6 mm i.d.), mobile phase 85% aqueous acetonitrile, flow rate 1.3 mL/min, UV detection at 286 nm, oven temperature 40 °C]. Fraction $3F_2$ (40 mg) was again purified by HPLC to get compound **1** (12 mg) using the same column, [mobile phase-79% aqueous acetonitrile, flow rate 1 mL/min, UV detection at 267 nm]. Purification of fraction $1F_2$ (3.88 g) by a silica gel (60G) column using EtOAc and a mixture EtOAc and MeOH with 5% increment as eluents afforded six fractions ($4F_1$–$4F_6$; each 50 mL). Fraction $4F_3$ (1 g) of this column was again separated by a RP-18 column using 95% aqueous acetonitrile as mobile phase and the composition of the mobile phase was gradually changed by adding acetonitrile and 18 fractions ($5F_1$–$5F_{18}$; each 20 mL) were collected. The purity of all fractions was monitored by HPLC or ^{1}H NMR spectroscopy. Fraction $5F_2$ (350 mg) showed a mixture of two compounds (**2**, **3**) having very close retention times, which could not be separated. It was acetylated with acetic anhydride-pyridine (1 mL, 1 : 1, 120 °C, 20 min) and the acetylated product was purified over a RP-18 column using 50% aqueous methanol followed by 5% increment of methanol and three fractions ($6F_1$–$6F_3$; each 30 mL) were collected. Fraction $6F_2$ (19 mg) was separated by HPLC [60% aqueous acetonitrile, flow rate – 1 mL/min, UV detection at 265 nm] to yield compounds **4** (3.0 mg) and **5** (3.9 mg).

3.4.1. (7S 8R)-Dihydrodehydrodiconiferyl alcohol-9′-O-β-D-glucopyranoside (**1**)

Yellowish gum; FABMS *m/z* 545 $[M + Na]^+$, appropriate for $C_{26}H_{34}O_{11}$ + Na; ^{13}C NMR (CD_3OD) data were observed at δ 89.0 (C-2), 55.5 (C-3), 118.0 (C-4), 136.9 (C-5), 114.3 (C-6), 145.2 (C-7), 56.4 (7-OCH_3), 147.5 (C-8), 129.9 (C-9), 134.8 ((C-1′), 110.6 (C-2′), 149.1 (C-3′), 56.4 (3′-OCH_3), 147.5 (C-4′), 116.1 (C-5′), 119.7 (C-6′), 70.0 (C-1″), 32.9 (C-2″), 33.0 (C-3″), 104.5 (C-1‴), 75.2 (2‴), 78.2 (C-3‴), 71.7 (C-4‴), 77.9 (C-5‴), 62.8 (C-6‴). ^{1}H and ^{13}C NMR data were compatible to previously reported values (Takeda et al., 1998).

*3.4.2. 10-O-Acetyl-4,7-megastigmadien-3-one-9-O-(2′,3′.4′,6′-tetra-O-acetyl)-β-*D*-glucopyranoside* (**4**)

Light yellowish gum; FABMS m/z 597 $[M + H]^+$ appropriate for $C_{29}H_{40}O_{13} + H$; ^{1}H NMR (CD_3OD) data in the Table.

*3.4.3. 9-O-Acetyl-4,7-megastigmadien-3-one-10-O-(2′,3′.4′,6′-tetra-O-acetyl)-β-*D*-glucopyranoside* (**5**)

Light yellowish gum; FABMS m/z 597 $[M + H]^+$ appropriate for $C_{29}H_{40}O_{13} + H$; ^{1}H NMR (500 MHz, CD_3OD) data in the Table.

Acknowledgement: The authors wish to thank the International Program in the Chemical Sciences (IPICS) for financial support. The authors also wish to thank the Natural Product Chemistry Section of the Laboratory of the Drug Discovery Research and Development, Frederic Cancer Research and Development Center, National Cancer Institute (NCI), Frederic, Maryland 21702, USA for assisting with the NMR studies and National Institute of Diabetic Disorders and Kidney Diseases (NIDDK), Bethesda, Maryland, USA for mass spectral measurement.

References

Dan S, Dan SS (1988) Chemical investigation in the family Sterculiaceae. Fitoterapia 59: 348–349.

Greger H, Pacher T, Brem B, Bacher M, Hofer O (2001) Insecticidal flavaglines and other compounds from Fijian *Aglaia* species. Phytochemistry 57: 57–64.

Hasan CM, Rashid MA, Haider SS (1994) Plumeride: ^{1}H NMR assignment of its acetyl derivatives. Fitoterapia 65: 175–176.

Khan SH, Mosihuzzaman M, Nahar N, Rashid MA, Rokeya B, Ali L, Khan AKA (2003) Three megastigmane glycosides from the leaves of *Pterospermum semisagittatum*. Pharm Biol 41: 512–515.

Mamun MIR, Rokeya B, Chowdhury NS, Muniruzzaman M, Nahar N, Ahmed MU, Mosihuzzaman M, Ali L, Khan AKA, Khan SH (2001) Anti-hyperglycemic effect of *Pterospermum acerifolium* Willd. and *Pterospermum semisagittatum*. Diabetes Res 35: 163–170.

Otsuka H,, Hirata E, Shinzato T, Takeda Y (2000) Isolation of lignan glucosides and neolignan sulphate from the leaves of *Glochidion zeylanicum*. Chem Pharm Bull 48: 1084–1086.

Rashid MA, Gustafsan KR, Cardellina II JH, Boyd MR (1995) A benzoic acid glycoside from *Geniostoma anthertricum*. Phytochemistry 41: 1205–1207.

Takeda Y, Mima C, Masuda T, Hirata E, Takushi A, Otsuka H (1998) Glochidioboside, a glucoside of (7*S*, 8*R*)-dihydrodehydrodiconiferyl alcohol from leaves of *Glochidion obovatum*. Phytochemistry 49: 2137–2139.

The Wealth of India (1969) Raw Materials, C.S.I.R., New Delhi, Vol. 8. p. 310.