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Flavonoids from *Maclura tinctoria*

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Abstract

Seven flavonoids, including two new natural products, were isolated from an ethanol extract of the bark of *Maclura tinctoria* (L.) Gaud. The new compounds are steppogenin 4′-*O*-*β*-D-glucoside and orobol 5,3′-di-*O*-methyl-8-C-glucoside. Orobol, steppogenin, aromadendrin, dihydromorin and orobol 7-*O*-*β*-D-glucoside were also isolated. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Maclura tinctoria*; Moraceae; Flavonoids; Orobol; Steppogenin; Aromadendrin; Dihydromorin; Orobol 7-*O*-*β*-D-glucoside; Steppogenin 4′-*O*-*β*-D-glucoside and orobol 5; 3′-di-*O*-methyl-8-C-glucoside

1. Introduction

Maclura tinctoria (L.) Gaud. belongs to the family Moraceae, the mulberry family. The family is widely distributed in the tropics, subtropics, and some temperate regions of both hemispheres. A great deal of literature exists on the chemical composition of *Maclura pomifera* (Smith & Perino, 1981). The types of compounds isolated belong to the classes of flavonoids, xanthones, triterpenes and stilbenes. However, no work has been reported on *Maclura tinctoria*. In this study we report the isolation and characterization of seven flavonoids from the stem bark of this species.

2. Results and discussion

An ethanolic extract of the bark of *Maclura tinctoria* was chromatographed on silica gel using MeOH/$CHCl_3$ mixtures. The fractions eluted with 15–25% MeOH/$CHCl_3$ when repeatedly chromatographed on reversed-phase C18 column yielded the following flavonoids: 5,7,3′,4′-tetrahydroxyisoflavone (orobol) (**1**), 5,7,2′,4′-tetrahydroxyflavanone (steppogenin) (**2**), 3,5,7,4′-tetrahydroxyflavanonol (aromadendrin) (**3**), 3,5,7,2′,4′-pentahydroxyflavanonol (dihydromorin) (**4**), orobol 7-*O*-*β*-D-glucoside (**5**), steppogenin 4′-*O*-*β*-D-glucoside (**6**) and orobol 5,3′-di-*O*-methyl-8-C-glucoside (**7**). The chemical structures of compounds **1**–**5** were determined by comparison of their melting points and UV spectra as well as other spectroscopic data (^1H, ^{13}C NMR, MS) with literature values. Compounds **6** and **7**, on the other hand, appeared to be new and their structure identification is the subject of this study. The FABMS of **6** showed a peak at m/z 451 $[M+1]^+$ suggesting the molecular formula $C_{21}H_{22}O_{11}$. The UV spectrum showed a major absorbance at 286 nm characteristic of a flavanone skeleton (Markham & Marbry, 1975). A singlet at δ 12.14, which disappeared on deuterium exchange, indicated a C-5 hydroxyl.This functionality was further confirmed by a bathochromic shift of 24 nm in the presence of $AlCl_3$. The flavanone structure was further substantiated by ^1H and ^{13}C NMR analysis (Agrawal, 1989). The proton at C-2 appeared as a double doublet at δ 5.64 (J = 13 and 3 Hz). The equatorial proton of C-3 appeared at δ 2.64 as a double doublet (J = 17, 3 Hz). The C-3 axial proton however, was masked by the resonances of the sugar moiety and was not easily deli-

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Table 1
^{1}H and ^{13}C NMR data for compounds **2**, **6** and **7**

Position	**2**		**6**		**7**	
	^{1}H δ (integral, multi., J (Hz))	^{13}C	^{1}H, δ (integral, multi., J (Hz))	^{13}C	^{1}H, δ (integral, multi., J (Hz))	^{13}C
2	5.57 (1H, dd, 13, 2.6)	73.8	5.64 (1H, dd, 13, 3)	74.2	8.22 (1H, s)	151.4
3	2.57 (1H, dd, 17, 2.7) eq., 3.25 (1H, dd, 13, 17) ax.	42.3	2.64(1H, dd, 17, 3) eq., signal burried ax.	41.5	–	125.1
4	–	196.8	–	196.9	–	175.1
5	–	165.5	–	164.0	–	160.9
6	5.85 (2H, s)	95.7	5.88 (2H, s)	96.3	6.44 (1H, s)	97.8
7	–	166.6	–	167.2	–	162.0
8	5.85 (2H, s)	94.9	5.88 (2H, s)	95.4	–	108.6
9	–	163.4	–	163.7	–	158.5
10	–	101.6	–	102.1	–	105.9
1′	–	115.3	–	118.9	–	124.1
2′	–	158.6	–	156.0	7.08 (1H, d, 1.9)	114.1
3′	6.33 (1H, d, 1.9)	102.4	6.56 (2H, m)	104.4	–	147.9
4′	–	155.7	–	158.6	–	147.1
5′	6.24 (1H, dd, 8.4, 1.9)	106.4	6.56 (2H, m)	107.5	6.77 (1H, d, 8.2)	115.9
6′	7.26 (1H, d, 8.4)	128.2	7.3 (1H, d, 9.2)	128.4	6.91 (1H, dd, 1.7, 8.2)	122.4
5-OH	12.15 (1H, br, s)	–	12.14 (1H, br, s)	–	–	–
Ome	–	–	–	–	3.79 (6H, s)	56.16
Ome	–	–	–	–		56.22
1″	–	–	4.8 (1H, d, 7.5)	100.9	4.7 (1H, d, 9.5)	73.6
2″	–	–		73.7		71.2
3″	–	–	3.2 – 3.5	77.1	3.2–3.4	79.1
4″	–	–	(4H, m)	70.1	(4H, m)	70.9
5″	–	–		77.5		81.9
6″	–	–	3.7 (2H, d, 11.6)	61.1	3.7 (2H, d, 11.1)	61.6

neated. In the ^{13}C NMR spectrum, the signals for C-2 and C-3 appeared at δ 74.2 and 41.5, respectively. A two-proton signal at δ 5.88 appearing as a singlet was assigned to H-6 and H-8. A two-proton signal at δ 6.56 appearing as a multiplet was assigned to H-3′ and H-5′ and a one-proton signal at δ 7.3 (d, J = 9.2 Hz) was assigned to H-6′. These ^{1}H NMR data indicated that the A ring was hydroxylated at positions 5 and 7, while the B ring at 2′ and 4′. The ^{13}C NMR data further established the hydroxylation at C-2′ since the chemical shift of C-2 resonated at δ 74.1 (an upfield shift of ~5 ppm) indicative of the presence of a 2′-oxy substituent (Agrawal, 1989). A bathochromic shift (37 nm, band II) produced in the UV (methanol) after the addition of NaOAc indicated a free 7-OH group.

^{1}H NMR resonances at δ 3.5–3.2 and six signals in the ^{13}C NMR spectrum at 100.9–61.6 indicated the presence of an *O*-sugar moiety. A loss of 162 mass units from the molecular ion in the FABMS and a signal at δ 61.6, shown by DEPT to represent a CH_2 group suggested a hexose moiety. The carbohydrate moiety was confirmed as glucose by acid hydrolysis and subsequent TLC sugar analysis. The anomeric glucose proton appeared at δ 4.8 as a doublet with a coupling constant for H-1″/H-2″ of J = 7.5 Hz, indicating a β-linkage of the glucose unit to the aglycone. The above data in conjunction with the ^{13}C NMR spectrum (DEPT sequence) were found to be consistent with a flavanone glucoside structure. Assignments of the protonated carbons were made using HMQC. The results related to ^{1}H and ^{13}C NMR spectral interpretations are shown in Table 1 and HMBC correlations are depicted in Fig. 1. The glucose unit was placed on the C-4′ position based on the value of the proton of the anomeric carbon (δ 4.8) (Markham & Geiger, 1994a) and the HMBC correlation observed between the anomeric proton of glucose and carbon 4′. Thus, **6** was assigned as 5,7,2′-trihydroxyflavanone-4′-*O*-β-D-glucoside or steppogenin-4′-*O*-β-glucoside.

Compound **7**, exhibited in the FABMS spectrum a peak at m/z 477 [M + 1] for $C_{23}H_{24}O_{11}$. An isoflavone

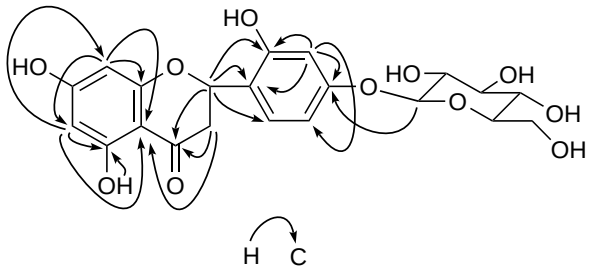


Fig. 1. HMBC correlations of **6**.

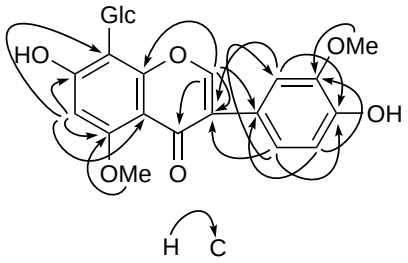


Fig. 2. HMBC correlations of **7**.

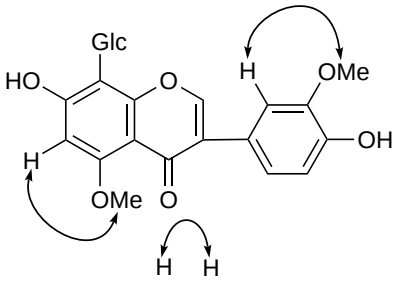
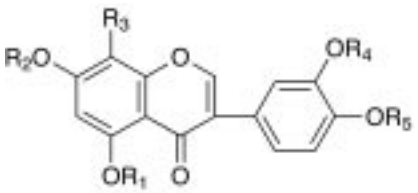
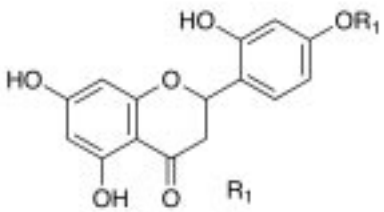


Fig. 3. Long-range COSY connectivities observed for **7**.

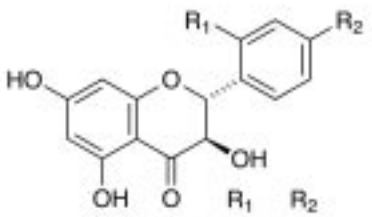
skeleton was suggested by a major absorbance in the UV at 259 nm and the appearance in the proton ^{1}H NMR spectrum of a one proton singlet at δ 8.22. The UV value of band II (ring A) at δ 259 nm was about 6 nm nearer in the UV than the equivalent 5-hydroxyisoflavone glycoside (**1a**) (Nunes, Haag, & Bestmann, 1989). The absence of a bathochromic shift in the UV (methanol) spectrum after the addition of $AlCl_3$ was evidence of the absence of a free 5-OH group. The ^{13}C NMR spectrum provided additional confirmation of the lack of a free 5-OH group as the carbonyl signal of **7** resonated at δ 175.1 instead of 180.0 as shown in the spectrum of compound **1a** (Nunes et al., 1989). The 7-hydroxyl group was free as indicated by a bathochromic shift in band II (ring A) of 11 nm upon the addition of sodium acetate. The ^{1}H NMR spectrum showed a one proton singlet at δ 6.44 indicating that ring A was trisubstituted. From HMBC data this proton showed correlations with carbons 5 and 8 instead of 6 and 9, therefore it was assigned to position 6. Ring B showed a pattern of three one-proton signals at δ 7.08 (1H, d, J = 1.7 Hz), δ 6.77 (1H, d, J = 8.2 Hz) and δ 6.91 (1H, dd, J = 1.7, 8.2 Hz), the mutiplicity of which showed one proton coupled to the remaining two which were, in turn, not coupled to each other. The size of the coupling constants (1.7 and 8.2 Hz) is characteristic of *meta* and *ortho* couplings as found in a 3′,4′-oxygenated flavonoids. In addition, signals in the ^{13} CNMR spectrum at δ 147.9 and δ 147.1 indicated that the oxygenated carbons are adjacent due to the shielding effect ($>\delta$10) each oxygen exerts on its neighboring *ortho* carbons. The presence of two methoxyl groups in the molecule was indicated by a peak in the ^{1}H NMR spectrum at δ 3.79 appearing as a singlet and integrating for 6 protons and in the ^{13}C NMR spectrum as two signals at δ 56.16 and δ 56.22. These two methoxyl groups were placed on carbons 5 and 3′ (δ 160.9 and δ 147.9) based on HMBC correlations of the methoxyl group with the above mentioned carbons (Fig. 2). In addition, from long-range COSY connectivities (Fig. 2) the positions of the methoxyl group on ring A and ring B were confirmed since cross-peaks from H-6 to OMe were found as well as from H-2′ to OMe. The ^{13}C NMR spectrum showed signals at δ 73.6, 71.2, 79.1, 70.9, 81.9 and 61.6 characteristic of a C-glucoside (Nunes et al., 1989). The glucose residue was assigned to position 8 based on HMBC correlations (Fig. 3). The ^{1}H and ^{13}C NMR data are presented in Table 1. The conformation of the anomeric proton was deduced to be β based on the signal at δ 4.7 appearing as a doublet with a coupling constant of 9.5 Hz (Nunes et al., 1989). The structure of **7** was thus established as orobol 5,3′-di-*O*-methyl-8-C-glucoside.



	R_1	R_2	R_3	R_4	R_5
1	H	H	H	H	H
1a	H	H	Glc	H	H
5	H	Glc	H	H	H
7	Me	H	Glc	Me	H



2	H
6	Glc



3	H	OH
4	OH	OH

3. Experimental

3.1. Plant material

The plant material (stem bark) used in this study was collected in October, 1994 in Venezuela and identified by Robert F. Smith. Voucher specimens are on deposit at the National Center for the Development of Natural Products (Voucher # 13002).

3.2. General experimental procedures

^{1}H and ^{13} C NMR, DEPT,HMQC, HMBC and long-range COSY spectra were recorded in DMSO-d6 on a Bruker Avance DPX 300 operating at 300 and 75 MHz, respectively, and using the solvent signals at δ 2.49 (^{1}H) and δ 39.5 (^{13}C) as reference. Mass spectra were obtained on a ZAB HS mass spectrometer (VG Analytical, Manchester, UK) equipped with an 11/250 J data system. Fast atom bombardment (FAB) experiments were performed using a Xenon gun operated at 8 keV energy and 0.8 mA emission. A sample in DMSO was added to glycerol as the matrix. UV spectra were recorded on a Hewlett Packard 8453 equipped with a Biochemical Analysis System following established procedures for the identification of flavonoids (Mabry, Markham, & Thomas, 1970a). Optical rotations were measured on a Jasco DIP 370 Digital Polarimeter. Circular dichroism were obtained on a Jasco J-715 spectropolarimeter. TLC was performed on precoated polyester backing silica gel G UV_{254} plates (Whatman) using 7% MeOH/$CHCl_3$ (solvent A) and benzene/EtOAc/MeOH (65:26:34, solvent B); or reversed-phase C18 (E. Merck) using MeOH/H_2O (68:32, solvent C).

3.3. Extracton and isolation

The dried powdered bark (500 g) was extracted several times with 95% EtOH. The combined extracts were evaporated to dryness under red. pres. (34.5 g) and then a portion of the extract (19 g) was chromatographed on silica gel 60 column (480 g) using MeOH/$CHCl_3$ mixtures. Based on TLC similarities, 19 combined fractions were obtained. Fractions from this column were separated further on reversed-phase C18 column (Bakerbond 40 μm, J.T. Baker) using MeOH/H_2O (1:1) and increasing amounts of MeOH to afford flavonoids **1**–**7**. Isolated compounds were identified on the basis of UV, ^{1}H NMR, ^{13}C NMR and MS.

3.4. 5,7,3′,4′-Tetrahydroxyisoflavone (orobol) (**1**)

Amorphous solid, m.p. 275–278°C (acetone/$CHCl_3$) (lit. (Van Heerden, Brandt, & Roux, 1980), 270°), R_f 0.43 (reversed-phase C18, solvent C), 0.46 (silica gel, solvent A); identified by comparison (UV, ^{1}H, ^{13}C NMR) with literature data (Mabry et al., 1970a; Van Heerden et al., 1980; Markham & Geiger, 1994).

3.5. 5,7,2′,4′-Tetrahydroxyflavanone (steppogenin) (**2**)

Colorless fine needles, m.p. 248–253°C (acetone/$CHCl_3$) (lit (Sotnikova, Chagovets, & Litvinenko, 1968), 254–257°), R_f 0.47 (reversed-phase C18, solvent C), 0.36 (silica gel, Solvent A); identified by comparison of UV data (Sotnikova et al., 1968). ^{1}H and ^{13}C NMR spectra are listed in Table 1 and reported for the first time. CD (*c*, 0.0135%, MeOH):$[\theta]_{299}+5145$(max); $[\theta]_{292}$ +2857 (sh); $[\theta]_{236}$ −3696 (max); $[\theta]_{227}$ −1826 (max); indicating a 2R configuration (Gaffield, 1970).

3.6. 3, 5, 7, 4′-Tetrahydroxyflavanonol (aromadendrin) (**3**)

Colorless needles, m.p. 229–231°C (acetone/$CHCl_3$). R_f 0.54 (reversed-phase C18, solvent C), 0.52 (silica gel, solvent A); identified by comparison (UV, ^{1}H ^{13}C NMR) with literature data (Mabry, Markham, & Thomas, 1970b; Shen & Theander, 1985). CD (*c*, 0.0059%, MeOH): $[\theta]_{330}$ +7744 (max); $[\theta]_{303}$ −11997 (max); $[\theta]_{288}$ −38432 (max); $[\theta]_{254}$ +5056 (max); $[\theta]_{230}$ −3556 (max); indicating a 2*R*,3*R* configuration (Gaffield, 1970).

3.7. 3,5,7,2′,4′-Pentahydroxyflavanonol (dihydromorin) (**4**)

Colorless needles, m.p. 232–235°C ($CHCl_3$/MeOH) (lit. (Deshpande, Srinivasan & Rama Rao, 1975), 228°), R_f 0.68 (reversed-phase C18, Solvent C), 0.24 (silica gel, solvent A); identified by comparison (UV, MS, ^{1}H NMR) with literature data (Deshpande, Srinivasan, & Rama Rao, 1975). CD (*c*, 0.0195%, MeOH): $[\theta]_{330}$ +4255 (max); $[\theta]_{309}$ −1587 (max); $[\theta]_{274}$ +2743 (max); $[\theta]_{234}$ +2013 (max); indicating a 2*R*,3*R* configuration (Gaffield, 1970).

3.8. Orobol 7-*O*-β-*D*-glucoside (**5**)

Cream amorphous solid, m.p. 269–271°C (MeOH/H_2O) (lit.(Anhut et al., 1984), 275–276), R_f 0.74 (reversed-phase C18, solvent C), 0.45 (silica gel, solvent B); identified by comparison (UV, ^{1}H, ^{13}C NMR) with literature data (Markham & Geiger, 1994b; Anhut et al., 1984).

3.9. Steppogenin 4′-*O*-β-*D*-glucoside (**6**)

Colorless amorphous solid, m.p. 259–263° C(MeOH/H_2O); R_f 0.62 (reversed-phase C18, solvent

C), 0.53 (silica gel, solvent B); $[\alpha]_D^{25}$ −65.5° (MeOH, *c*, 1.01); UV λ_{max}^{MeOH} nm: 332 (sh), 286; + NaOMe: 320, 243 (sh); + $AlCl_3$: 362 (sh), 310; + $AlCl_3$–HCl: 362 (sh), 308; + NaOAc: 323, 280 (sh). FABMS, *m/z* (rel. int.): 451 [M + 1] (35), 154 [A_1 + 1] (100), 137 [B_3^+·] (90). ^{1}H NMR and ^{13}C NMR (see Table 1). CD (*c*, 0.010%, MeOH): $[\theta]_{327}$ −605 (max); $[\theta]_{285}$ +5871 (max); $[\theta]_{237}$ −1285 (max); $[\theta]_{229}$ −5946 (max); indicating a 2*R* configuration (Gaffield, 1970).

3.10. Orobol 5,3′-di-O-methyl-8-C-glucoside (7)

Fine pale yellow needles, m.p. 233–237°C (MeOH/H_2O); R_f 0.79 (reversed-phase C18, solvent C), 0.33 (silica gel, solvent B); UV λ_{max}^{MeOH} nm: 292 (sh), 259; + NaOAc: 332 (sh), 270; + $AlCl_3$: 292 (sh), 260; + $AlCl_3$/HCl: 292 (sh), 260; + NaOMe: 308 (sh), 275. FABMS, *m/z* (rel. int.): 477 [M + 1](85).). ^{1}H and ^{13}C NMR (see Table 1).

3.11. Acid hydrolysis of **6**

Compound **6** (5 mg) was refluxed for 75 min with 4 N HCl in MeOH (8 ml). The HCl hydrolysate was concentrated, extracted with EtOAc and examined by TLC on silica gel in 7 % MeOH/$CHCl_3$ for the liberated aglycone, which was identified by direct comparison with the isolated naturally occurring **2** (steppogenin). The acidic mother liquor was neutralized with Na_2CO_3, filtered and evaporated to dryness for examination of the sugar moiety, which proved to be glucose by detection on TLC (EtOAc–isopropanol–H_2O (65:23:12) and sprayed with *p*. anisaldehyde–H_2SO_4 reagent followed by heating (Stahl, 1962).

Acknowledgements

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