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C- and *O*-Glycosyl-α-hydroxydihydrochalcones from *Eysenhardtia polystachya*

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Abstract

Three new α-hydroxydihydrochalcones, (α*R*)-α,3,4,2′,4′-pentahydroxydihydrochalcone, (α*R*)-3′-C-β-D-xylopyranosyl-α,3,4,2′,4′-pentahydroxydihydrochalcone, and (α*R*)-3′-*O*-β-D-xylopyranosyl-α,3,4,2′,4′-pentahydroxydihydrochalcone, together with the known coatline B [(α *R*)-3′-C-β-D-glucopyranosyl-α,2′,3,4′,4-pentahydroxydihydrochalcone], were isolated from the bark and trunks of *Eysenhardtia polystachya* and their structures were deduced by spectral methods. One of the isolates displayed insecticidal activity.

Keywords: *Eysenhardtia polystachya*; Leguminosae; *C*- and *O*-glycosyl α-hydroxydihydrochalcones; Insecticidal activity

1. Introduction

Eysenhardtia is a small genus of the Leguminosae (subfamily Lotoideae, subtribe Psoralieae) which comprises of three species located in North America (Standley, 1922), and all of them are used in Mexican traditional medicine (Hernández, 1959). *E. polystachya* (*lignum nefriticum*) has been the subject of several phytochemical studies (Domínguez, Franco & Díaz Viveros, 1978; Burns, Dalgarno, Gargan & Grimshaw, 1984; Beltrami, De Bernardi, Fronza, Mellerio, Vidari et al., 1982), this is due in part to it being the *Eysenhardtia* species most widely used for medicinal purposes (Hastings, 1990).

We have previously reported (Alvarez, Rios, Esquivel, Chávez, Delgado et al., 1997) the isolation of several isoflavonoids from the less polar fractions of a dichloromethane-methanol extract obtained of the bark and trunks of this plant. Here we report the structure of four α-hydroxydihydrochalcones isolated from its bark.

The acetone-soluble part of the dichloromethane-methanol extract of bark and trunks showed complex glycosidic phenolic compounds by TLC. Separation of its constituents was done by repeated column chromatography over silica gel and RP-HPLC, giving (α*R*)-α,3,4,2′,4′-pentahydroxydihydrochalcone (**1**), (α*R*)-3′-C-β-D-xylopyranosyl-α,3,4,2′,4′-pentahydroxydihydrochalcone (**2**), (α*R*)-3′-*O*-β-D-xylopyranosyl-α,3,4,2′,4′-pentahydroxydihydrochalcone (**3**) and (α*R*)-3′-C-β-D-glucopyranosyl-α,3,4,2′,4′-pentahydroxydihydrochalcone (coatline B, **4**). Coatline B had been previously isolated from this source (Beltrami et al., 1982), while compounds **1–3** are new natural products.

2. Results and discussion

The less polar compound (**1**) was obtained as a yellow oil, whose molecular formula $C_{15}H_{14}O_6$ was confirmed by EIMS ($[M]^+$ *m/z* 290) and ^{13}C and ^{13}C DEPT NMR analysis. The IR spectrum showed bands for hydroxyl groups (3398 cm^{-1}), chelated carbonyl (1620 cm^{-1}) and aromatic rings (1499 cm^{-1}).

The ^1H NMR and ^1H-^1H COSY spectra of **1** (Table 1) revealed two aromatic ABX systems; the first was located at δ 7.77 (1H, *d*, $J = 8.7$ Hz), 6.54 (1H, *dd*, $J = 8.7$, 2.1 Hz) and 6.44 (1H, *d*, $J = 2.1$ Hz); and the second, at δ 7.00 (1H, *d*, $J = 1.8$ Hz), 6.89 (1H, *d*,

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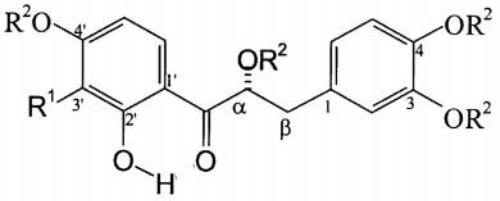
Formulae



	R^1	R^2
1	H	H
2	C-β-xylopyranosyl $(OH)_3$	H
2a	C-β-xylopyranosyl-$(OAc)_3$	Ac
3	O-β-xylopyranosyl $(OH)_3$	H
4	C-β-glucopyranosyl $(OH)_4$	H

J = 8.1 Hz) and 6.81 (1H, *dd*, 8.1, 1.8 Hz), indicating the presence of two trisubstituted aromatic rings. Aliphatic signals at δ 5.30 (1H, *dd*, J = 13.2, 3.0 Hz), 3.00 (1H, *dd*, J = 16.8, 13.2 Hz) and 2.73 (1H, *dd*, J = 16.8, 3.0 Hz), were ascribable to a third ABX system, whose chemical shifts and coupling patterns indicated the presence of an –CO–CH(OH)–CH_2– subunit. These data and the UV absorption maximum at 321, 309, 284, 218, 213 and 204 nm were indicative of dihydrochalcone structure (Mabry, Markham & Thomas, 1970).

In agreement with this, the ^{13}C and ^{13}C APT spectra of **1** (Table 2), showed the presence of a chelated carbonyl, twelve aromatic carbons, an aliphatic carbinol (δ 79.48) and a methylene (δ 44.0). The connectivities between each of the ^{13}C signals and the related ^1H signals were established by the HMQC spectrum, as shown in Table 2. The oxygen-bearing aromatic carbon signals at δ 164.76, 163.54, 144.98 and 144.66, the IR and mass spectra revealed that **1** possessed four aromatic hydroxyl groups. The ^1H and ^{13}C NMR data of **1** were closely comparable to those of 2′-methoxy-α,3,4′,4-tetrahydroxydihydrochalcone (**5**) (Bezuidenhout, Bezuidenhoudt & Ferreira, 1988), with the major differences being ascribable to the absence of resonances due to the 2′-methoxy group in compound **1**. Thus the planar structure of **1** was established as α,3,4,2′,4′-tetrahydroxydihydrochalcone, which is a new natural product.

The *R*-configuration of the asymmetric C-α of **1** was inferred by comparison of its CD spectrum (negative Cotton effect at 306.5 nm) with that of (α*R*)-α,2′-dihydroxy-4,4′-dimethoxydihydrochalcone (negative Cotton effect at 310 nm), isolated from *Pterocarpus angolensis*, whose absolute stereochemistry was unequivocally demonstrated by chemical correlation to (2*R*, 3*S*)-(+)-catechin 3′,4′,5,7-tetramethyl ether (Bezuidenhoudt, Brandt & Roux, 1981), and with that of (α*R*)-4-

Table 1
^1H NMR spectral data for compounds **1–4** (500 MHz, $CDCl_3$-DMSO-d_6)[a]

H	1	2	2a	3	4
α	5.30 *dd* (13.2, 3.0)	5.05 *dd* (12.5, 7.5)	5.88 *dd* (8.1, 5.7)	5.01 *s*[a]	4.93 *dd* (7.2, 5.4)
β	3.0 *dd* (16.8, 13.2,)	2.96 *dd* (14, 4.5)	3.11 *dd* (6.0, 4.5)	2.93 *dd* (14.0, 4.0)	2.84 *dd* (14.1, 5.4)
β	2.73 *dd* (16.8, 3.0)	2.77 *dd* (14, 7)		2.74 *dd* (10.0, 6.5)	2.64 *dd* (14.1, 7.5)
2	7.0 *d* (1.8)	6.65 *d* (2.0)	7.10 *m*	6.68 *d* (2.5)	6.59 *d* (2.1)
5	6.89 *d* (8.1)	6.69 *d* (8.0)	7.10 *m*	6.67 *d* (8.0)	6.57 *d* (7.8)
6	6.81 *dd* (8.1, 1.8)	6.52 *dd* (8.0, 2.0)	7.10 *m*	6.51 *dd* (8.0, 2.0)	6.43 *dd* (8.1, 2.4)
3′	6.44 *d* (2.1)				
5′	6.54 *dd* (8.7, 2.1)	6.45 *d* (9.0)	6.45 *d* (9.0)	6.45 *d* (9.5)	6.39 *d* (8.7)
6′	7.77 *d* (8.7)	7.62 *d* (9.0)	7.59 *d* (9.0)	7.66 *d* (9.0)	7.62 *d* (9.0)
1″		4.81 *d* (10.0)	5.17 *d* (9.9)	4.74 *d* (10.0)	4.63 *d* (9.9)
2″		4.06 *t* (9.0)	5.41 *t* (9.3)	4.11 *t* (9.0)	3.95 *t* (9.3)
3″		3.46 *t* (9.0)	5.27 *t* (9.9)	3.37 *t* (11.0)	3.66 *t* (9.6)
4″		3.69 *ddd* (9.25, 4.5, 4.5)	5.12 *ddd* (10.2, 10.8, 5.7)	3.63 *m*	3.45 *dd* (11.7, 4.5)
5″		3.32 *t* (10.5)	3.51 *t* (11.1)	3.27 *t* (11.0)	3.20 *t* (10.2)
5″		4.02 *dd* (11, 5.5)	4.34 *dd* (11.4, 5.7)	3.96 *dd* (11.0, 5.5)	
6″					3.8 *s*[a] 2H
2′-OH		13.0 *s*	12.79 *s*	13.22 *br s*	13.07 s
α-OH		4.68 *d* (7.5)		5.46 *d* (7.04)	4.53 *br s*
2″-OH		4.29 *br d* (3.5)			4.26 *br s*
3″-OH		4.62 *br s*			
4″-OH		4.74 *d* (4.0)			4.64 *d* (3.6)
6″-OH					4.14 *br s*

[a] The assignments were established by COSY, NOESY, HETCOR (for compound **3**), HMQC and HMBC analyses

Table 2
^{13}C NMR spectral data for compounds **1**, **2**, **4** (125 MHz) and **3** (75 MHz) on $CDCl_3$-DMSO-d_6

C	1	2	2a	3	4
C=O	190.85	203.25	198.82	204.76	204.08
α	79.48	72.34	70.38	72.89	73.11
β	44.00	40.57	37.26	40.74	40.47
1	130.65	127.65	127.28	128.62	128.29
2	113.51	114.57	123.50	115.23	116.53
3	144.66	143.76	142.01	144.75	144.43
4	144.98	142.79	141.21	143.59	143.37
5	115.20	115.88	110.21	116.87	115.13
6	118.14	119.78	124.45	120.02	120.05
1′	113.89	110.75	108.74	112.23	110.41
2′	163.54	163.30	163.11	164.58	163.50
3′	103.03	109.74	134.60	110.27	111.73
4′	164.76	163.10	164.04	163.92	163.73
5′	110.73	107.92	108.74	107.97	108.40
6′	128.68	130.71	131.77	131.91	131.36
1″		73.98	70.66	114.82	73.55
2″		70.47	74.20	73.97	71.10
3″		78.44	74.44	79.23	78.38
4″		69.20	69.09	70.28	69.77
5″		69.65	67.58	69.96	80.57
6″					60.83

These assignments were established by COSY, NOESY, HETCOR, HMQC and HMBC analyses

methoxy-α,2′,4′-trihydroxydihydrochalcone (negative Cotton effect at 283 nm) obtained from *Pericopsis elata* (Bezuidenhoudt, Swanepoel, Augustyn & Ferreira, 1987) and *Virgilis oroboides* (Malan & Swinney, 1990). Thus, compound **1** is assigned the structure (α*R*)-α,3,4,2′,4′-pentahydroxydihydrochalcone.

The 200 MHz ^1H NMR spectra of **2–4** were almost identical, but high resolution (500 MHz) ^1H NMR analysis permitted the differentiation of these compounds.

Compound **2** was obtained as pale yellow amorphous powder and its molecular formula was ascertained to be $C_{20}H_{22}O_{10}$ based on the number of protons and carbon signals observed in the ^1H and ^{13}C NMR spectra respectively. This was supported by its EI mass spectrum which showed the $[M-H_2O]^+$ at *m*/*z* 404. The IR spectrum suggested the presence of hydroxyl groups (3400, 3150 cm^{-1}), chelated carbonyl (1650 cm^{-1}) and aromatic rings (1590, 1540 cm^{-1}).

The ^1H NMR spectral data (Table 1) showed a hydrogen-bonded hydroxyl group at δ 13.00 (1H, *s*). The appearance of two aromatic one-proton doublets at δ 7.62 (1H, *d*, *J* = 9.0 Hz) and 6.45 (1H, *d*, *J* = 9.0 Hz) indicated the occurrence of a 1,2,3,4-tetrasubstituted benzene ring, while the aromatic ABC type resonances at δ 6.69 (1H, *d*, *J* = 8 Hz), 6.65 (1H, *d*, *J* = 2.0 Hz) and 6.52 (1H, *dd*, *J* = 8.0, 2.0 Hz), the chemical shifts and coupling patterns clearly showed the presence of a 1,3,4-trisubstituted benzene ring. Examination of its homonuclear correlation spectra led to the identification of an aliphatic ABX system which, in conjunction with the observed chemical shifts, led to the assignment of the Ar–CH_2–CH(OH)–C=O subunit. In the same way, we found all the correlations of the aliphatic proton signals which support the presence of a β-xylopyranose moiety (Table 1) (Agrawal, 1992). These data, along with the UV maximum at 321.5, 284.5, 253.5, 218.0, 213.5 and 204.0 nm suggested that **2** was a glycosyldihydrochalcone.

In agreement with this, the ^{13}C NMR spectrum (Table 2) exhibited the signals of a carbonyl carbon (δ 203.2), along with 12 aromatic carbons, four of which have an oxygen function, the aliphatic carbons C-α (δ 72.34) and C-β (δ 40.57), together with five aliphatic oxygen-bearing methines corresponding to the pentose moiety at δ 73.9, 70.47, 78.44, 69.20, and 69.65. The observation that **2** was resistant to hydrolysis with HCl and that the signal of the anomeric carbon was not observed in the δ 110–118 region of the spectra (Pawan, Agrawal & Mahesh, 1989), provided evidence that there was a C-glycoside linkage in the molecule.

All protonated carbons were assigned by CH correlation spectroscopy (HETCOR). The oxygen-bearing aromatic carbon signals at δ 163.30, 163.10, 143.76 and 142.79, and the IR and mass spectra revealed that **2** possessed four aromatic hydroxyl groups. The number of hydroxyl groups in **2** was established by acetylation with acetic anhydride/pyridine to afford a heptacetate **2a**, in which it remains the chelated proton at δ 12.79.

The HETCOR and HMBC spectra were used to assemble the segments of the partial structures of **2**, followed by assignment of each carbon and proton signals. The HMBC spectrum of **2** (Fig. 1), showed correlations between one of the protons (δ 7.62) on the 1,2,3,4-tetrasubstituted benzene ring and the carbonyl carbon (δ 203.25), which indicated that the proton was located at a *peri*-position to the carbonyl group. The same proton showed cross peaks with the aromatic oxygenated carbons at δ 163.30 and 163.10. Furthermore, the HMBC spectrum exhibited correlations between the chelated hydroxyl proton (δ 13.00) and three quaternary carbons (δ 109.74, 110.75 and 163.30. The carbon signal at δ 110.75 was assigned to C-1′ as it presented a cross-peak with H-α (δ 5.05). The β-xylopyranosyl moiety was determined to be linked at the C-3′, since cross peaks between xylopyranosyl H-1″ (δ 4.81) and C-2′ (δ 163.30) and C-4′ (163.10) were observed. Therefore, the plausible partial structure of **2** was considered to be **A** in Fig. 1. On the other hand, long-range correlations of the only methylene of the molecule allowed us to link the two aromatic parts through an α-hydroxydihydrochalcone framework, already suggested by the UV data. The methylene group at δ 2.96 and 2.97 showed corre-

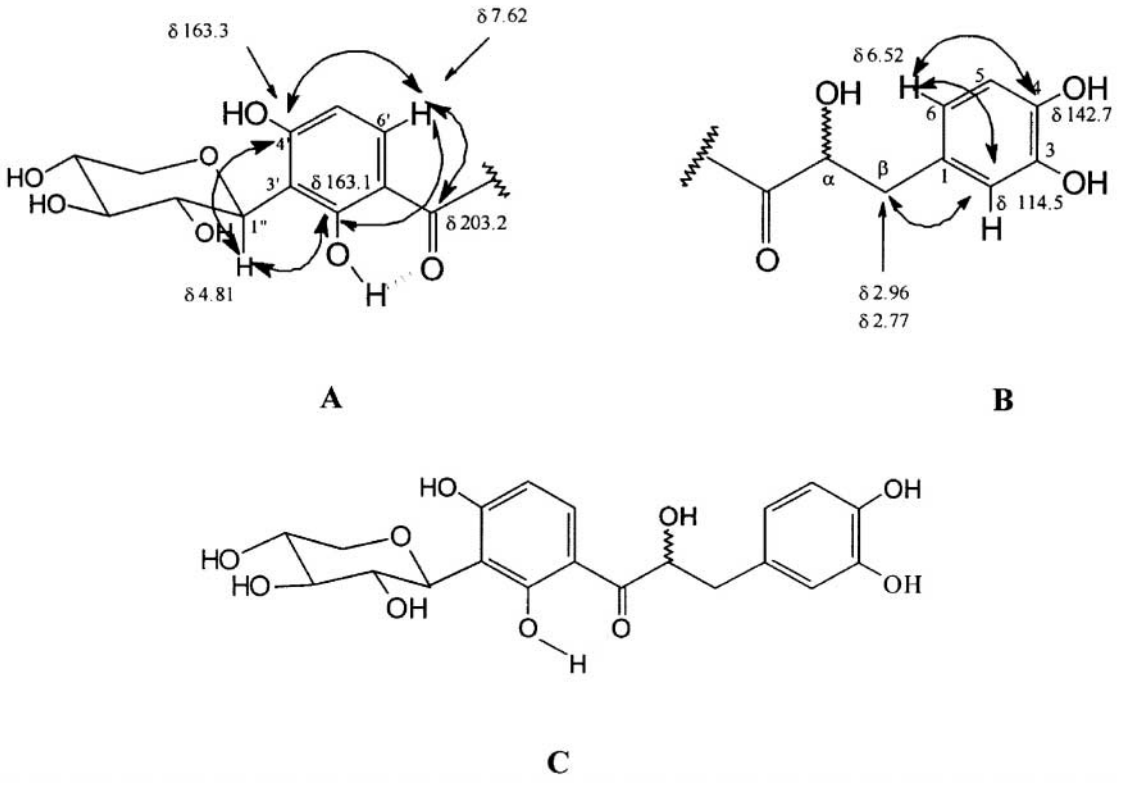


Fig. 1. Partial structures (**A**, **B** and **C**) of **2**.

lations to C-1 (δ 127.65), the carbonyl carbon (δ 203.25) and to two aromatic protonated carbons at δ 119.78 and 114.57 assignable to C-6 and C-2 respectively. The former carbon was further correlated to an aromatic proton δ 6.52 (H-6) which also correlated to the aromatic carbons at 114.57 (C-2) and 142,79 (C-4). As the doublet signals at δ 6.69 (H-5) and δ 6.65 (H-2) in **2** shifted both to δ 7.10 in **2a**, the presence of hydroxyl groups at C-3 and C-4 was clarified.

In the HMBC spectrum, the H-5 signal (δ 6.69) was correlated to the aromatic carbons at δ 127.65 (C-1) and 143.76 (C-3), showing that the partial structure of **2** was characterized as B in Fig. 1. Other correlations observed in the HMBC spectrum supported the partial structure B.

Thus, the planar structure of **2** was established as 3′-C-xylopyranosyl-α,3,4,2′,4′-pentahydroxydihydrochalcone (structure **C** in Fig. 1).

This structure was confirmed by NOESY experiment, which further allowed the establishment of the preferred conformation as shown in Fig. 2.

The CD curve of **2** shows sequential positive and negative Cotton effects at 290 nm (π, π^* transition) and 327 nm (n, π^* transition) respectively indicating an αR configuration (Augustyn, Bezuidenhoudt, Swanepoel & Ferreira, 1990).

Compound **3** was obtained as an amorphous powder, whose molecular formula was confirmed to be $C_{20}H_{22}O_{11}$ by mass spectrometry and analysis of its ^{13}C and ^{13}C DEPT NMR spectra. UV and IR absorptions suggested that **3** also had a dihydrochalcone skeleton. The ^{1}H and ^{13}C NMR spectral data of **3** were similar to those of **2** except for the presence of an anomeric carbon signal at δ 128.6 indicating that **3** is a *O*-glycosyldihydrochalcone. The ^{13}C NMR revealed 20 carbon signals which were sorted by DEPT ^{13}C NMR into one carbonyl group, five aromatic methines, seven aromatic quaternary carbons, five aliphatic methines

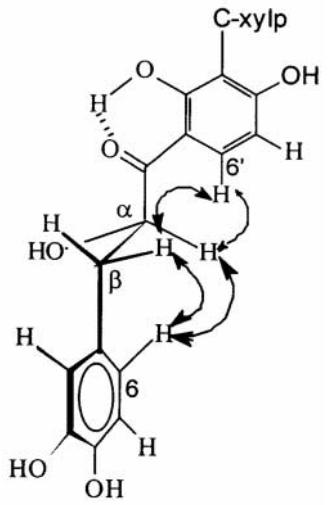


Fig. 2. NOE interactions and preferred conformation of **2**.

and two aliphatic methylenes. The ^{1}H NMR spectrum (Table 1) also displayed a hydrogen-bonded hydroxyl group at δ 13.22; one aromatic AB system due to the protons at H-6′ and H-5′; the aromatic ABX system ascribable to the H-2, H-5 and H-6 of the trisubstituted A ring. The ^{1}H NMR spectrum also displayed an aliphatic ABX system assigned to the CO–CH(OH)–CH_2–Ar subunit; as well as the due to a β-xylopyranose moiety. The 2D NMR spectrum of **3** showed close resemblance to those of **2**, confirming the identical connectivities in these two isolates.

It was demonstrated that changes in the functionality of C-α and C-2′ hydroxyl groups cause great influence on the conformation of α-hydroxydihydrochalcones and consequently on their CD curves (Augustyn et al., 1990). The CD curve of **3** shows a negative Cotton effect at 327 nm, but did not show a positive Cotton effect due to the π, π^* transition (ca 290 nm), owing probably to the presence of the *O*-xylopyronasyl moiety in C-3; even though, the shape of the curve is very similar to that of **2** in that region, further demonstrating their similarity in stereochemistry. Therefore, the structure for this compound was assigned as (α*R*)-3′-*O*-β-D-xylopyranosyl-α,3,4,2′,4′-pentahydroxydihydrochalcone (**3**), a novel α-hydroxydihydrochalcone of *E. polystachya*.

Compound **4**, $C_{21}H_{24}O_{11}$ was obtained as pale yellow needles. The IR and UV spectra closely resembled that of **2** and **3**, indicating that **4** is also an α-hydroxydihydrochalcone. Spectral analysis of **4**, in particular the ^{13}C and HMBC spectra, showed that the structure of **4** was identical to that of coatline B, previously isolated from this source (Beltrami et al., 1982). In our present study, the complete assignment of ^{13}C NMR was achieved with the aid of HMQC and HMBC spectra, the results of which are shown in Table 2. Furthermore, the CD curve of this compound was comparable with that of compounds **2** and **3**, indicating that the absolute configuration in C-α is (*R*).

None of the isolated compounds (**2–4**) showed any antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Candida albicans*, at concentrations up to 400 μg/ml. These compounds did not show any toxic effect against UISO, HCT-15, OVCAR and KB tumor cell lines in culture (ED_{50} values above 4 μg/ml). Compounds **2–4** were also tested for their insecticidal activity against *Epilachna varivestis* (Conchuela del frijol) larvae. The results of these experiments demonstrate that compounds **2** and **4** were not toxic (LC_{50} > 1000 ng/cm^2); while (**3**) displayed toxicity (LC_{100} < 100 ng/cm^2).

The occurrence of *C*- and *O*-glycosyl-α-hydroxydihydrochalcones (**2–4**) in *E. polystachya* is of chemotaxonomic interest, since so far only coatline A (3′-C-β-glucopyranosyl-α,4,2′,4′-tetrahydroxydihydrochalcone) (Beltrami et al., 1982; Bezuidenhoudt, Brandt & Ferreira, 1987), and coatline B (3′-C-β-glucopyranosyl-α,3,4,2′,4′-pentahydroxydihydrochalcone, **4**) (Beltrami et al., 1982) had been isolated as natural products. Therefore, *C* - glycosyl - α - hydroxydihydrochalcones could be considered as chemotaxonomic markers for the genus (Hegnauer & Grayer-Barkmeijer, 1993).

3. Experimental

Mps uncorr. IR: KBr Nicolet Magna IR TM 750 and Perkin Elmer 283B instruments; UV and CD spectra were obtained in MeOH; ^{1}H and ^{13}C NMR: Varian VXR-300 and Varian Unity Plus-500 instruments. The chemical shift values are reported in ppm units relative to TMS and chloroform signals and the coupling constants are in Hz. Samples for NOE experiments were degassed and sealed under Argon. Standard pulse sequences were used for COSY, NOESY, DEPT, HETCOR, HMQC and HMBC experiments; IEMS: 70 eV on a JEOL JMS-AX; CC: Merck silica gel 60 (70–230 mesh); TLC and prep. TLC: Merck silica gel 60 F254 plates (0.25 mm and 2 mm respectively), spots and bands were detected by UV irradiation (254 and 365 nm); Vacuum liquid chromatography (VLC): Merck silica gel 60 (0.040–0.063 mm).

HPLC was performed using a Waters 600 programmable pump with UV detection. Semipreparative HPLC purifications employed a Waters microporasil C-18 column (5 μm particles, 300 × 10 mm) at a flow rate of 6.0 ml/min with UV detection at 250 nm; whereas for analytical HPLC, a 300 × 3.9 mm microporasil C-18 column was used.

3.1. Plant material

Voucher specimens (CHR 739) of *Eysenhardtia polystachya* are deposited at the National Herbarium (MEXU), Instituto de Biología, UNAM.

3.2. Extraction and isolation of compounds

Extraction of plant material, and the first fractionation has been described previously (Alvarez et al., 1997). The acetone soluble fraction (56 g) was eluted from a vacuum-liquid chromatography column (280 g Si/gel) with $CHCl_3$–MeOH mixtures (100% $CHCl_3$ to 100% MeOH). The material eluted with $CHCl_3$–MeOH (9:1, 265 mg) was further chromatographed by preparative TLC (CH_2Cl_2–acetone, 85:15) to afford **1** (8.3 mg).

Fractions eluted with $CHCl_3$-MeOH (21:4, 230 mg) were loaded on a silica cartridge (Sep-pak vac Silica, Waters), previously equilibrated with 12 ml MeOH,

followed by 15 ml CH_2Cl_2 and attached to a 5 ml syringe; the elution system used was a gradient of CH_2Cl_2–MeOH; compound **2** (62 mg) eluted with 25 ml of a mixture of CH_2Cl_2–MeOH (93:7) and comp. **3** (8 mg) was obtained after elution with 20 ml of CH_2Cl_2–MeOH (22:3). Several fractions of the main VLC column crystallized. This solid (6.32 g) contained mixtures of glycosyl-α-hydroxydihydrochalcones, as determined by ^1H and ^{13}C NMR analyses. An aliquot (3.12 mg/100 μl) on H_2O–CH_3CN, was applied to a microporasil C-18 column (3.9×300 mm, 5 μm, Waters) and eluted with a 30 min linear gradient of H_2O–CH_3CN. Elution details: solvent ratio H_2O–CH_3CN initial 85:15; final 5:95; flow rate 1.5 ml/min and eluent monitoring at $\lambda = 250$ nm. For semipreparative purposes, aliquots (7.8 mg/250 μl) were applied to a microporasil C-18 column as before, using a flow rate of 6 ml/min. The first major peak ($R_t = 2.0$, 29.4 mg) eluted was coatline B (**4**). The second major peak ($R_t = 3.0$ min, 12.6 mg) was still a mixture. Purification on a silica cartridge as above, yielded additional amounts of 9.8 mg of **2**, and 2.8 mg of 3.

3.2.1. (α-*R*)-α,3,4,2′,4′-pentahydroxydihydrochalcone (*1*)

Oil: $C_{15}H_{14}O_6$ (found: C 62.13, H 4.75; requires: C 62.06, H 4.86); IR ν_{max}(film) cm^{-1}: 3398, 1620, 1499; CD: $\Delta\epsilon_{306.5}-1.337$, $\Delta\epsilon_{330.5}$ 0.1310, $\Delta\epsilon_{380}-0.1612$; UV λ_{max} nm (log ϵ): 321 (3.31), 284 (3.47), 213 (4.07); EIMS *m*/*z* (rel. int): 290 $[M]^+$ (1); ^1H and ^{13}C NMR see Tables 1 and 2.

3.2.2. (αR)-3′-C-β-D-xylopyranosyl-α,3,4,2′,4′-pentahydroxydihydrochalcone (*2*)

Amorphous powder, $C_{20}H_{22}O_{10}$ (found: C 56.88, H 5.27; requires: C 56.86, H 5.25); mp 153–155°C; IR ν_{max} (KBr) cm^{-1}: 3412, 1624, 1503, 1255; UV λ_{max} nm (log ϵ): 321.0 (3.19), 309.0 (3.16), 285.0 (3.44), 253.0 (2.68), 204.0 (3.76); CD: $\Delta\epsilon_{203}-2.81$, $\Delta\epsilon_{212}$ 1.8463, $\Delta\epsilon_{239}-16.7231$, $\Delta\epsilon_{256}$ 1.3783, $\Delta\epsilon_{275.5}-3.9135$, $\Delta\epsilon_{290}$ 0.5665, $\Delta\epsilon$ 327.5–18.3713; EIMS *m*/*z* (rel. int.): 404 $[M-H_2O]^+$ (5), 386 (33), 368 (10), 282 (77), 264 (52), 251 (53), 233 (100), 149 (56), 123 (75); ^1H and ^{13}C NMR see Tables 1 and 2.

3.2.3. Acetylation of *2*

Compound **2** (43 mg) was acetylated with Ac_2O (1.0 ml) and pyridine (0.3 ml) to afford **2a** (38 mg) as a yellow syrup. **2a**: IR ν_{max} (film) cm^{-1}: 3352, 1735, 1625, 1519, 1488, 1252; ^1H and ^{13}C NMR see Tables 1 and 2.

3.2.4. (αR)-3′-O-β-D-xylopyranosyl-α,3,4,2′,4′-pentahydroxydihydrochalcone (*3*)

$C_{20}H_{22}O_{11}$. Amorphous powder, mp 146–149°C; IR ν_{max} (KBr) cm^{-1}: 3399, 1621, 1517, 1499, 1255; UV λ_{max} nm (log ϵ): 322.5 (3.85), 309 (3.81), 284.5 (4.10), 253.0 (3.29), 218.5 (4.27), 213.0 (4.26), 203.5 (4.46); CD: $\Delta\epsilon_{207}$ 6.60, $\Delta\epsilon_{239.5}-5.5709$, $\Delta\epsilon_{327}-5.2836$; EIMS *m*/*z* (rel. int.): 402 $[M-H_2O-H_2O]^+$ (0.8), 386 (29), 282 (85), 251 (61), 233 (100), 179 (51), 149 (67), 123 (73), 107 (70), 57 (50); ^1H and ^{13}C NMR see Tables 1 and 2.

3.2.5. (αR)-3′-C-β-D-glucopyranosyl-α,3,4,2′,4′-pentahydroxydihydrochalcone (*4*)

$C_{21}H_{24}O_{11}$. Pale yellow needles, mp 202–205°C; IR ν_{max} (KBr) cm^{-1}: 3398, 1621, 1518, 1499, 1255, 1114, 1082; UV λ_{max} nm (log ϵ): 321.5 (3.85), 309.0 (3.82), 284.5 (4.10), 253.5 (3.34), 218.0 (4.25), 213.5 (4.23), 204.0 (4.42); CD: $\Delta\epsilon_{239}$-3.2015; EIMS *m*/*z* (rel. int.): 452 $[M]^+$ (1.2), 405 (7), 386 (32), 368 (8), 313 (6), 282 (62), 251 (43), 233 (70), 149 (55), 123 (100), 55 (21); ^1H and ^{13}C NMR see Tables 1 and 2.

3.3. Biological activity

3.3.1. Antimicrobial activity

Screening studies were carried out by the conventional disc assay procedure (Vanden & Vlietnick, 1991; Paxton, 1991) for activity against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimorium* (ATCC 065922) and *Candida albicans* (ATCC 10231). Nystatin (5–160 μg/mL) and gentamicin (2–128 μg/mL) were included as controls.

3.3.2. Cytotoxic evaluation

Studies were performed in UISO (uterin-cervix cancer), HCT-15 (colon carcinoma), OVCAR (ovarian cancer) and KB (nasopharingeal carcinoma) cell cultures according to Geran and Greenberg's screening protocols (Geran, Greenberg, MacDonald, Schumacher & Abbott, 1972).

3.3.3. Insecticidal activity

Epilachna varivestis (Coleoptera: occinellidae) larvae were used for this bioassay by ingestion of solutions of compounds **2–4** by target insect following published protocol (Kubo, 1991). The concentration ranged from 100 to 1000 ng per square centimeter of surface of artificial diet on which larvae were reared (10 larvae for each concentration). The experiments were run in triplicate.

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References

Agrawal, P. K. (1992). *Phytochemistry, 31,* 3307.

Alvarez, L., Rios, M. Y., Esquivel, C., Chávez, M. I., Delgado, G., Aguilar, I., Villarreal, M. L., & Navarro, V. (1997). *J. Nat. Prod.*, in press.

Augustyn, J. A. N., Bezuidenhoudt, B. C. B., Swanepoel, A., & Ferreira, D. (1990). *Tetrahedron, 46,* 4429.

Beltrami, E., De Bernardi, M., Fronza, G., Mellerio, G., Vidari, G., & Vita-Finzi, P. (1982). *Phytochemistry, 21,* 2931.

Bezuidenhout, S. C., Bezuidenhoudt, B. C. B., & Ferreira, D. (1988). *Phytochemistry, 27,* 2329.

Bezuidenhoudt, B. C. B., Brandt, E. V., & Roux, D. G. (1981). *J. Chem. Soc., Perkin Trans I*, 263.

Bezuidenhoudt, B. C. B., Brandt, E. V., & Ferreira, D. (1987). *Phytochemistry, 26,* 531.

Bezuidenhoudt, B. C. B., Swanepoel, A., Augustyn, J. A. N., & Ferreira, D. (1987). *Tetrahedron Letters, 28,* 4857.

Burns, D. T., Dalgarno, B. G., Gargan, P. E., & Grimshaw, J. (1984). *Phytochemistry, 23,* 167.

Domínguez, X. A., Franco, R., & Díaz Viveros, Y. (1978). *Revista Latinoamer. Quím., 9,* 209.

Geran, R. I., Greenberg, N. H., MacDonald, M. N., Schumacher, A. M., & Abbott, B. J. (1972). *Cancer Chemother. Rep., 3,* 1.

Hastings, R. B. (1990). *Economic Botany, 44,* 336.

Hegnauer, R., & Grayer-Barkmeijer, R. (1993). *Phytochemistry, 34,* 3.

Hernández, F. (1959). Historia Natural de la Nueva España. In: *Obras Completas de F. Hernández, vol. II*. (p. 173). Mexico: UNAM.

Kubo, I. (1991). In: P. M. Dey, M. J. B. Harborne, & K. Hostettmann, *Methods in plant biochemistry*, *vol. 6* (p. 179). New York: Academic Press.

Mabry, T. J., Markham, K. R., & Thomas, M. B. (1970). In: *The systematic identification of flavonoids* (p. 25). Berlin: Springer.

Malan, E., & Swinny, E. (1990). *Phytochemistry, 29,* 3307.

Pawan, K., Agrawal, P. K., & Mahesh, C. B. (1989). In P. K. Agrawal, *Carbon-13 NMR of flavonoids* (p. 285). Amsterdam: Elsevier.

Paxton, J. D. (1991). In: P. M. Dey, M. J. B. Harborne, & K. Hostettmann, *Methods in plant biochemistry*, *vol. 6* (p. 33). New York: Academic Press.

Standley, P. C. (1922). Trees and shrubs of Mexico. *Contr. U.S. Natl. Herb., 23*(2), 429.

Vanden, D. A., & Vlietnick, A. J. (1991). In: P. M. Dey, M. J. B. Harborne, & K. Hostettmann, *Methods in plant biochemistry*, *vol. 6* (p. 47). New York: Academic Press.