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COATLINE A AND B, TWO *C*-GLUCOSYL-α-HYDROXYDIHYDROCHALCONES FROM *EYSENHARDTIA POLYSTACHYA*

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Key Word Index—*Eysenhardtia polystachya*, Leguminosae; 3'-*C*-β-glucopyranosyl-α,2',4',4-tetrahydroxydihydrochalcone; 3'-*C*-β-glucopyranosyl-α,2',4',3,4-pentahydroxydihydrochalcone; *C*-glycosides; α-hydroxydihydrochalcones.

Abstract—The first two examples of naturally occurring *C*-glucosyl-α-hydroxydihydrochalcones have been isolated from *Eysenhardtia polystachya*, a Mexican plant with diuretic properties, and their structures have been elucidated.

INTRODUCTION

Eysenhardtia polystachya (Ort.) Sarg. (Leguminosae, Lotoideae) is a plant which grows in Mexico and has diuretic properties [1–4]. Since its chemistry has not yet been investigated, we have examined the trunk wood and here report the isolation of two dihydrochalcones.

RESULTS AND DISCUSSION

Coatline A and B were isolated from an aqueous extract of the trunk wood of *E. polystachya* as described in the Experimental, and were separated from each other on a column of cellulose by elution with water–*iso*-propanol (1 : 3): **1a** was eluted before **1b**.

Coatline B (**1b**) did not show a molecular ion in the EIMS, loss of water being too easy, but we could deduce the formula as $C_{21}H_{24}O_{11}$ from the ^{1}H NMR and ^{13}C NMR spectra as well as from the M^+ 452 observed in the FDMS. The compound has UV λ_{max}^{MeOH} nm: 328 and 285 shifted to 312 nm by addition of aluminium chloride and to 309 nm by further addition of hydrochloric acid to the aluminium chloride–methanol solution. This relatively large bathochromic shift, together with the ability of **1b** to form acid stable complexes with aluminium chloride, indicated an aromatic C=O, as similar results are observed for many flavonoids with hydroxyls in 3- and/or 5-positions of the flavone nucleus [5]. Further evidence of the C=O group was provided by the IR spectrum (absorption band at 1620 cm^{-1}) and by the ^{13}C NMR spectrum (*s* at δ 205.1 ppm). In the ^{1}H NMR spectrum of **1b** the aromatic protons were clearly separated into two groups: one typical of an AB system of two *ortho* protons ($J = 8.8$ Hz) in a 1,2,3,4-tetrasubstituted phenyl and the other of an ABX system of three protons in a 1,3,4-trisubstituted phenyl ring, one of the AB signals occurring significantly further downfield from the rest (δ 7.69). Therefore this signal was assigned to a proton *ortho* to the C=O group. However, the colourless nature and UV spectrum of **1b** preclude the second aromatic ring being conjugated with the C=O. Indeed the UV spectrum strikingly resembles those of flavanones [5], and an acyclic structure could not be excluded at this stage.

Sodium borohydride reduction of coatline B nona-acetate (**1d**) gave nona-acetyldihydrocoatline B (**2**) as a mixture of two diastereoisomers. The compounds were not separated since the signals in the ^{1}H NMR spectrum of **2** could be easily assigned by comparison with **1d**. Extensive double resonance decoupling experiments performed on the aliphatic portion of the spectrum allowed us to establish the sequence: Ar–CHOH–CHOAc–CH_2–Ar'. As proof of this structure a fine NOE effect was observed, for two of the aromatic protons forming the ABX system, by irradiation of the CH_2 group. Coatline B (**1b**) must thus possess the C=O in a C_3 open chain, with a secondary acetylable hydroxyl α to the carbonyl group and the trisubstituted aromatic ring linked to the terminal methylene.

The ^{1}H NMR spectrum of coatline B acetate could be analysed only when recorded at 68° probably because of some hindered rotations of the sugar moiety. It showed a total of 27 protons over the range δ 1.8–2.5 which could be assigned to five alcoholic and four phenolic acetyl groups [6]. A large bathochromic shift of 56 nm of the UV maximum of **1b** in the presence of sodium acetate was indicative of a

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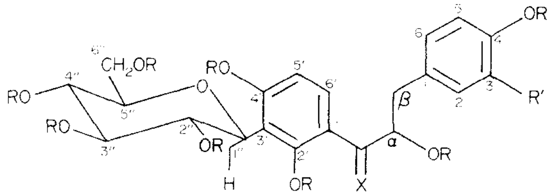
dihydrochalcone with a free 4′-hydroxyl [7]. Furthermore comparison of the ^{1}H NMR spectra of **1b** and **1d** revealed that four of the five aromatic signals, but not the doublet at δ 7.69, moved significantly downfield by acetylation and this could be explained by the known effects exerted by the hydroxyl groups on the *ortho* and *para* protons [5].

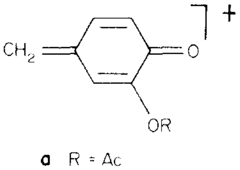
All the above data suggested the occurrence of a catechol nucleus (B ring) and a 6′-desoxyphloroglucinol unit (A ring), any structure with three vicinal hydroxyls being excluded. In accordance with these conclusions in the EIMS of **1d**, fragmentations to the ions (**a**) and (**b**) at *m/z* 164 and 122, respectively, were observed.

A $C_6H_{11}O_5$ residue, containing four alcoholic hydroxyls, remained to be accommodated on the C-3′ of the structure of **1b** to account for the molecular formula. It was therefore evident that **1b** contains a hexopyranosyl residue which was finally established to be a *C*-β-glucopyranosyl group on the basis of the NMR spectra. A detailed examination of the 300 MHz ^{1}H NMR spectrum (CD_3OD) of **1b** led us to assign all the frequencies over the range δ 3.3–5.0 to the seven protons of the glucosyl moiety which contains one CH_2O and five CHO groups. One of these, a doublet centred at δ 4.93 was assigned to the C-1″ proton, the large coupling constant ($J = 9.3$ Hz) due to a *trans* diaxial coupling with the C-2″ proton indicating a β-configuration of the glycosidic bond. ^{13}C NMR spectroscopy distinguishes flavonoid *C*- from *O*-glycosides on the basis of the chemical shift of the C-1″ which appears at *ca* δ 70 for the former and at *ca* 100 for the latter compounds [8].

The signal for C-1″ appeared at δ 75.4 in the ^{13}C NMR spectrum of **1b**, this assignment being confirmed by selective decoupling experiments of H-1″. Further evidence of the C–C bond was provided by the resistance to hydrolysis of the sugar under drastic conditions. The ^{1}H NMR spectrum of **1d** showed signals much better resolved than **1b** and the coupling constants of each sugar proton could be easily determined. Particularly, double resonance decoupling experiments showed that H-1″, H-2″, H-3″ and H-4″ all have axial configuration as occurs in a glucopyranosyl ring. Moreover one of the MeCO signals absorbed significantly further upfield (δ 1.82) from the rest and could be assigned to the C-2″ acetyl [6] as this was the most likely group to be affected by the magnetic anisotropy of the A ring. The diamagnetic shielding of the C-2″ acetyl further requires this group to be in an equatorial configuration. The spectrum therefore supported the identification of **1b** as a 3′-*C*-β-glucopyranosyl compound. Thus coatline B (**1b**) has the structure 3′-*C*-β-glucopyranosyl-α-2′, 4′, 3, 4-pentahydroxydihydrochalcone.



1a X = O; R = H; R′ = H
1b X = O; R = H; R′ = OH
1c X = O; R = Ac; R′ = H
1d X = O; R = Ac; R′ = OAc
2 X = H,OH; R = Ac; R′ = OAc



a R = Ac
b R = H

The structure of coatline A (**1a**) was easily deduced from that of **1b**, since **1a** showed physical and spectroscopic properties very similar to those for **1b**, except for changes due to the absence of one hydroxyl. Thus the ^{1}H NMR spectrum of coatline A acetate (**1c**) showed only eight MeCO groups (four of which were phenolic) and a typical AA′BB′ system for the β ring protons. This clearly indicated that coatline A is 3′-*C*-β-glucopyranosyl-α-2′,4′,4-tetrahydroxychalcone.

α-Hydroxydihydrochalcones are very rare natural products: so far only nubigenol [9] (α-2′,4′,6′,4-pentahydroxydihydrochalcone), lyonogenin [7] (4′-*O*-methylnumigenol) and lyonotin [7] (lyonogenin 2′-*O*-glucoside) have been isolated. Therefore to our knowledge coatline A and B are the first *C*-glycosyl-α-hydroxydihydrochalcones isolated from a natural source.

EXPERIMENTAL

Extraction and isolation. An aq. infusion of the wood of *Eysenhardtia polystachya* (200 g in 9.5 l. H_2O) was evaporated to dryness. The dark-brown residue (35.3 g) was extracted in a Soxhlet for 4 days with *iso*-BuOH. A black H_2O-soluble residue (15.3 g) was not extracted any longer, while from the *iso*-BuOH soln a yellow solid (20 g) was obtained by evaporation. The crude yellow solid was chromatographed on a Polyamide (Woelm) column (700 g) by elution with H_2O and then with increasing percentages of *iso*-PrOH and finally with MeOH. From one of the first fractions, a solid, (5.9 g) slowly pptd and was collected. On cellulose TLC (eluent H_2O–*iso*-PrOH, 25:75) it showed two compounds: coatline A (**1a**) and coatline B (**1b**). They were separated on a cellulose column by elution with the same eluent as for TLC: 0.42 mg of **1a** and 1.1 g of **1b** were obtained in pure form.

Coatline A (**1a**). Mp 144–146°, $[\alpha]_D^{20} - 45.17°$ (MeOH), IR ν_{max}^{nujol} cm^{-1}: 3300 (OH), 1610 (C=O), 800 (aromatic absorption); UV λ_{max}^{MeOH} nm ($\log \epsilon$): 323 (3.81), 284 (4.01), (+ $AlCl_3$): 314; (+ $AlCl_3$ + HCl): 310; (+ NaOMe): 341; (+NaOAc): 341. ^{1}H NMR (100 MHz, CD_3OD, TMS): δ 2.80 (1H, *dd*, $J_{gem} = 13.5$ Hz, $J_{vic} = 7.0$ Hz, H − β), 3.03 (1H, *dd*, $J_{gem} = 13.5$ Hz, $J_{vic} = 5.0$ Hz, H − β'), 3.3–3.6 (3H, *m*, H − 5″, H − 3″, H − 4″), 3.7–3.9 (2H, *m*, H − 6″) 4.06 (1H, *br t*, $J_{2''-1''} \simeq J_{2''-3''} \simeq 9$ Hz, H − 2″), 4.91 (1H, *d*, $J_{1''-2''} = 9.7$ Hz, H − 1″), 5.12 (1H, *dd*, $J_{\alpha-\beta} = 7.0$ Hz, $J_{\alpha-\beta'} = 5.0$ Hz, H − α), 6.38 (1H, *d*, $J_{5'-6'} = 9.0$ Hz, H − 5′), 6.80 (center of an AA′BB′ system, 4H, H-2, H-3, H-5, H-6), 7.69 (1H, *d*, $J_{5'-6'} = 9.0$ Hz, H-6′). ^{13}C NMR (25.2 MHz, CD_3OD, TMS) δ: 205.4 (*s*, C=O), 165.3 (*s*, C-4′ and

C-2′), 156.9 (*s*, C-4), 133.1 (*d*, C-6′), 131.4 (*d*, C-2 and C-6), 129.1 (*s*, C-1), 116.0 (*d*, C-3 and C-5), 112.6* (*s*, C-1′), 112.0* (*s*, C-3′), 109.6 (*d*, C-5′), 82.5‡ (*d*, C-5″), 80.0‡ (*d*, C-3′), 75.4† (*d*, C-1″), 74.2† (*d*, C-α), 72.6† (*d*, C-2″), 71.6 (*d*, C-4″), 62.6 (*t*, C-6″), 42.1 (*t*, C-β). Signal multiplicity obtained by off-resonance decoupling expts. FDMS (18 mA): 436 $[M]^+$.

Coatline B (**1b**). Mp 205–206°, $[\alpha]_D^{20} - 50.74°$ (MeOH). $CD[\theta]_{328} - 10560$ (MeOH); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1620 (C=O), 792 and 780 (aromatic absorptions), UV λ_{max}^{MeOH} nm (log ϵ): 328 (3.91), 285 (4.13); (+ $AlCl_3$): 312; (+ $AlCl_3$ + HCl): 309; (+NaOMe): 341 shifting to 431 after a while; (+NaOAc): 342 shifting more slowly to 431. ^1H NMR (300 MHz, CD_3OD, TMS): δ 2.78 (1H, *dd*, $J_{gem} = 13.6$ Hz, $J_{vic} = 7.4$ Hz, H-β), 2.96 (1H, *dd*, $J_{gem} = 13.6$ Hz, $J_{vic} = 5.0$ Hz, H − β'), 3.35–3.45 (1H, *m*, H-5″), 3.45 − 3.52 (2H, *m*, H-3″ and H-4″), 3.73 (1H, *dd*, $J_{gem} = 12.1$ Hz, $J_{vic} = 4.8$ Hz, H-$6''_A$), 3.86 (1H, *dd*, $J_{gem} = 12.1$ Hz, $J_{vic} = 1.8$ Hz, H-$6''_B$), 4.11 (1H, *t*, $J_{2''-1''} \simeq J_{2''-3''} \simeq 9$ Hz, H-2″), 4.93 (1H, *d*, $J_{1''-2''} = 9.3$ Hz, H-1″), 5.15 (1H, *dd*, $J_{\alpha-\beta} = 7.4$ Hz, $J_{\alpha-\beta'} = 5.0$ Hz, H-α), 6.42, 1H, *d*, $J_{5'-6'} = 8.8$ Hz, H-5′), 6.53 (1H, *dd*, $J_{6-5} = 8.1$ Hz, $J_{6-2} = 1.8$ Hz, H-6), 6.64 (1H, *d*, $J_{2-6} = 1.8$ Hz, H-2), 6.65 (1H, *d*, $J_{5-6} = 8.1$ Hz, H-5), 7.69 (1H, *d*, $J_{5'-6'} = 8.8$ Hz, H-6′). ^{13}C NMR (25.2 MHz, CD_3OD, TMS): δ 205.1 (*s*, C=O), 165.2 (*s*, C-4′ and C-2′), 145.8* (*s*, C-3), 144.8* (*s*, C-4), 133.0† (*d*, C-6′), 129.8 (*s*, C-1), 121.9 (*d*, C-6), 117.6‡ (*d*, C-2), 116.3‡ (*d*, C-5), 112.6§ (*s*, C-1′), 112.0§ (*s*, C-3′), 109.5† (*d*, C-5′), 82.4‖ (*d*, C-5‡), 79.9‖ (*d*, C-3″), 75.4† (*d*, C-1″), 74.2† (*d*, C-α), 72.7† (*d*, C-2″), 71.6 (*d*, C-4″), 62.6 (*t*, C-6″), 42.3 (*t*, C-β). Signal multiplicity obtained by off resonance decoupling experiments. FDMS (26 mA): 452 $[M^+]$.

Octa-acetyl coatline A (**1c**). ^1H NMR (100 MHz, $CDCl_3$, TMS, 60°): δ 1.74 (3H, *s*, MeCO), 1.98 (3H, *s*, MeCO), 2.00 (3H, *s*, MeCO), 2.01 (3H, *s*, MeCO), 2.02 (3H, *s*, MeCO), 2.22 (3H, *s*, MeCO), 2.35 (3H, *s*, MeCO), 2.36 (3H, *s*, MeCO), 2.88 (1H, *dd*, $J_{gem} = 15.0$ Hz, $J_{vic} = 8.5$ Hz, H-β), 3.11 (1H, *dd*, $J_{gem} = 15.0$ Hz, $J_{vic} = 4.3$ Hz, H-β'), 3.75 (1H, *ddd*, $J_{5''-4''} = 9.5$ Hz, $J_{5''-6_A''} = 2.3$ Hz, $J_{5''-6_B''} = 4.5$ Hz, H-5″), 4.00 (1H, *dd*, $J_{gem} = 12.5$ Hz, $J_{vic} = 2.3$ Hz, H-$6_A''$), 4.36 (1H, *dd*, $J_{gem} = 12.5$ Hz, $J_{vic} = 4.5$ Hz, H-$6_B''$), 4.78 (1H, *d*, $J_{1''-2''} = 10.0$ Hz, H-1″), 5.03–5.39¶) (2H, *m*, H-3″ and H-4″), 5.71 (1H, *t*, $J_{2''-1''} \simeq J_{2''-3''} \simeq 9.5$ Hz, H-2″), 5.93 (1H, *dd*, $J_{\alpha-\beta} = 8.5$ Hz, $J_{\alpha-\beta'} = 4.3$ Hz, H-α), 7.10 (4H, center of an AA′BB′ system, H-2, H-3, H-5, H-6), 7.09 (1H, *d*, $J_{5'-6'} = 8.8$ Hz, H-5′), 7.65 (1H, *d*, $J_{6'-5'} = 8.8$ Hz, H-6′). FDMS 25 mA: 772 $[M]^+$.

Nona-acetyl coatline B(**1d**). Mp 95–96°; IR ν_{max}^{KBr} cm^{-1}: 1775, 1755, 1735 (acetate C = O), 1605, 1505 (aromatic absorptions), 1220, 1180 (C–O). ^1H NMR (100 MHz, $CDCl_3$, TMS, 68°): δ 1.82 (3H, *s*, MeCO), 2.07 (3H, *s*, MeCO), 2.09 (3H, *s*, MeCO), 2.11 (3H, *s*, MeCO), 2.14 (3H, *s*, MeCO), 2.32 (6H, *s*, 2 × MeCO), 2.44 (6H, *s*, 2 × MeCO), 2.98 (1H, *dd*, $J_{gem} = 15.0$ Hz, $J_{vic} = 8.5$ Hz, H-β), 3.20 (1H, *dd*, $J_{gem} = 15.0$ Hz, $J_{vic} = 4.3$ Hz, H-β'), 3.86 (1H, *ddd*, $J_{5''-4''} = 9.5$ Hz, $J_{5''-6_A''} = 2.3$ Hz, $J_{5''-6_B''} = 4.5$ Hz, H-5″), 4.10 (1H, *dd*, $J_{gem} = 12.5$ Hz, $J_{vic} = 2.3$ Hz, H-$6_A''$), 4.49 (1H, *dd*, $J_{gem} = 12.5$ Hz, $J_{vic} = 4.5$ Hz, H-$6_B''$), 4.91 (1H, *d*, $J_{1''-2''} = 10.0$ Hz, H-1″), 5.16–5.50¶ (2H, *m*, H-3″ and H-4″), 5.79 (1H, *t*, $J_{2''-1''} \simeq J_{2''-3''} \simeq 10.0$ Hz, H-2″), 6.00 (1H, *dd*, $J_{\alpha-\beta} = 8.5$ Hz, $J_{\alpha-\beta} = 4.3$ Hz, H-α) 7.06–7.2 (4H, *m*, H-5′, H-2, H-5 and H-6), 7.69 (1H, *d*, $J_{6'-5'} = 8.8$ Hz, H-6′). FDMS (26 mA): 830 $[M]^+$; EIMS (probe) 75 eV, *m/z* (rel. int.): 788 $[M - 42]^+$, (1), 746 $[M - 2 \times 42]^+$, (7), 728 $[M - 2 \times 42 - 18]^+$, (2), 686 $[M - 2 \times 42 - 60]^+$, (3), 644 $[M - 3 \times 42 - 60]^+$, (9), 602 $[M - 4 \times 42 - 60]^+$, (2), 567 $[M - 2 \times 42 - 2 \times 60 - 59]^+$, (1), 551 $[M - 42 - 60 - 3 \times 59]^+$, (12), 509 $[M - 2 \times 42 - 60 - 3 \times 59]^+$, (14), 467 $[M - 3 \times 42 - 60 - 3 \times 59]^+$, (15), 244 (4), 202 (3), 164 (ion **a**, 6), 148 (10), 122 (ion **b**, 20), 97 (18), 96 (14), 83 (24), 69 (22), 60 (35), 57 (25), 55 (36), 45 (43), 43 (100).

Nona-acetyldihydrocoatline B (**2**). 17 mg nona-acetylcoatline B (**1d**) were reduced with 2 mg $NaBH_4$ in MeOH at 0–5°. The reaction was quenched with AcOH and the solvent was evaporated to dryness *in vacuo*. Prep. TLC (GF-254, 0.25 mm, $CHCl_3$–Me_2CO, 4 : 1) gave 7 mg of nonacetyldihydrocoatline B as a mixture of two epimers at CHOH. ^1H NMR (100 MHz, $CDCl_3$, TMS): δ 6.09 (1H, *d*, $J_{vic} = 7.0$ Hz, one $\underline{CH}$OH stereoisomer), 6.40 (1H, *d*, $J_{vic} = 3.0$ Hz, the second $\underline{CH}$OH stereoisomer).

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*,‡,§,‖The assignments of signals with the same sign may be reversed.

†The assignments of these signals were confirmed by selective proton decoupling experiments.

¶In the 270 MHz spectrum of **1c** and **1d** at 60° H-4″ and H-3″ appear as two broad triplets ($J_{vic} \simeq 9.5$ Hz) at δ 5.18 and 5.29.