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THREE HYDROXYMETHYLANTHRAQUINONE GLYCOSIDES FROM *RUBIA TINCTORUM*

N. A. EL-EMARY and E. Y. BACKHEET*

Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt

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Key Word Index—*Rubia tinctorum*; Rubiaceae; anthraquinones; anthraquinone glycosides; iridoids; spectroscopic analysis.

Abstract—Three hydroxymethylanthraquinone glycosides, in addition to the known compounds alizarin, lucidin-ω-ethyl ether, lucidin primeveroside and the iridoid asperuloside were isolated from the dried roots of *Rubia tinctorum*. The first three compounds were isolated for the first time from this species. The structures were established as 1-hydroxy-2-hydroxymethylanthraquinone 3-glucoside, 2-hydroxymethylanthraquinone 3-glucoside and 3,8-dihdroxy-2-hydroxymethylanthraquinone 3-glucoside. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

We wish to describe the isolation and structural elucidation of some anthraquinones and iridoids of *Rubia tinctrorum* roots cultivated in Assiut, Egypt.

RESULTS AND DISCUSSION

The 80% ethanol extract of the roots of *Rubia tinctorum* L. was fractionated by partitioning between *n*-hexane, chloroform and *n*-butanol. The hexane fraction yielded compounds **1** and **2** on chromatography over SiO_2 column. The chloroform fraction yielded compounds **3**, **4** and **5** on column chromatography over silica gel. The *n*-butanol fraction gave compounds **6** and **7** on chromatography over SiO_2 column also.

Compound **3** was obtained as yellowish-orange powder. It showed a maxima at 245 nm in the UV, suggesting an anthraquinone structure [1]. The unsubstituted ring A is indicated by aromatic protons signals at δ: 8.17, 8.25 (H-5 and H-8) and 7.81 (H-6 and H-7), other aromatic protons of ring C were displayed at δ: 7.22 (H-4) and 6.51 (H-1). Then a typical AB quartet at 4.62 for CH_2OH at C-2 of ring C was noticed which have been confirmed by a signal at δ: 50.92 in the ^{13}C NMR spectrum. The absence of hydroxyl group on C-1 was confirmed by the upfield shift of C-1 at δ: 109.06 in the ^{13}C NMR spectrum. The presence of two carbonyl functions have been demonstrated by two signals at δ: 188.45 and 184.33. The presence of a glucose unit was confirmed through the anomeric proton signal at δ: 5.00 (*d*, $J = 7.5$ Hz) and the carbon shift at 101.00 in addition to a typical glucose molecule signals. The linkage of the sugar should be β- due to the *J* value recorded (7.5 Hz). The mass spectrum *m/z* 256 and the significant fragments at *m/z* 228, 199 confirmed that **3** is 2-hydroxymethylanthraquinone-3-β-*O*-glucoside, which has been isolated from *Rubia tinctorum* for the first time.

The ^1H NMR spectral data for **4** and **6** were very similar to compound **3** except the absence of the signal corresponding to H-1 as well as the presence of the perihydroxyl group at 13.00 ppm indicated the substitution of position 1 by OH group in both compounds. This was also confirmed by the downfield shift of C-1 to 161.83 and 161.76 in ^{13}C NMR for **4** and **6** respectively.

Data for compound **6**, data when compared with **4**, revealed the presence of additional signals for the sugar xylose (the anomeric proton and carbon are at 4.81 and 103.85 respectively). Comparison of the sugar carbon resonances with published data revealed that the signal at 67.93 assigned to C-6′ of glucose was shifted downfield by ca 7.5 ppm from that of methyl-β-D-glucose [2, 3]. Thus the terminal xylose moiety is attached to the glucose at δ: C-6 through (1 → 6)-β-linkage. The mass spectrum displayed [M^+] at *m/z* 270 calculating for $C_{15}H_{10}O_5$ (the molecular formula for aglycone). Hence, **4** and **6** are 1-hydroxy-2-hydroxymethylanthraquinone-3-*O*-β-glucoside and lucidin primeveroside respectively. Compound **5**, when compared with **3**, indicated the substitution at C-8, confirmed by the absence of the signal cor-

* Author to whom correspondence should be addressed.

responding for H-8 and the presence of signal at 12.10 ppm corresponding for OH at C-8 in the ^{1}H NMR.

The three compounds **3–5** were isolated from *Rubia tinctorum* for the first time.

Comparative spectral data for **1**, **2**, **6** and **7** with the data cited in the literature [2–7], showed that these compounds are alizarin, lucidin-ω-ethyl ether, lucidin primeveroside and asperuloside respectively.

EXPERIMENTAL

General

Melting points were uncorrected. ^{1}H- and ^{13}C NMR were recorded by Spectrometer XL-300 (Varian) at 500 MHz and by Varian Gemini at 75.5 MHz. Silica gel GF_{254} (Merck) was used for TLC and silica gel (Merck) was used for column chromatography. The following solvent systems were used: System I: hexane–ethyl acetate (9:1). System II: hexane–ethyl acetate (8:2). System III: chloroform–methanol (9:1). System IV: chloroform–methanol (8:2). System V: chloroform–methanol (7:3).

Plant material

Roots of *Rubia tinctorum* were collected during May/June 1996 from the plants cultivated in the Experimental Station, Faculty of Pharmacy, Assiut University. The collected material was air-dried, reduced to powder No. 40 and kept for extraction.

Extraction and isolation

The air-dried powdered roots (600 g) of *Rubia tinctorum* were exhaustively extracted with ethanol 80%, concentrated under reduced pressure and the concentrate was subjected to solvent fractionation using *n*-hexane, chloroform and *n*-butanol. The obtained fractions were separately concentrated and screened by TLC for different constituents.

(*A*) *Hexane fraction*

Hexane fraction (15 g) was fractionated on silica gel column (600 g, 7 × 200 cm). Elution was started with *n*-hexane and then with hexane/ethyl acetate gradient, fractions, 150 ml each were collected, concentrated and screened by TLC (silica gel G, solvent system I and II). Similar fractions were combined, concentrated and subjected for crystallization. Two pure compounds were obtained and labeled **1** and **2**.

(*B*) *Chloroform fraction*

Chloroform fraction (8 g) was fractionated on silica gel column (320 g, 5 × 180 cm). Elution was started with chloroform followed by chloroform/methanol gradient. Fractions 150 ml each were collected, concentrated and examined by TLC (silica gel G, solvent system III and IV). Fractions eluted with chloroform–10% methanol were evaporated and purified by preparative layer chromatography gave compound **3**. While fractions eluted with chloroform–15% methanol were concentrated, passed over short silica column and eluted with chloroform/methanol gradient where compound **4** and **5** were isolated.

(*C*) n-*Butanol fraction*

n-Butanol fraction (15 g) was fractionated on silica gel column (600 g, 7 × 200 cm). Elution was started with chloroform and then with chloroform/methanol gradient. Fractions 150 ml each were collected, concentrated and examined by TLC (silica gel G, solvent systems IV and V). Fractions eluted with chloroform–20% methanol were concentrated, purified and left for crystallization where compounds **6** and **7** were separated.

Compound **1**. Red-purple crystals (15 mg), m.p. 279–283°, IR, ν_{max} (KBr), cm^{-1}: 3340, 1585, 1660 and 1625. UV λ_{max} (EtOH): 248, 263, 275, 330 (*sh*) and 430 nm. ^{1}H NMR (500 MHz, CD_3OD) at δ: 7.18 (1H, *d*, J = 8 Hz, H-3), 7.86 (2H, *dd*, J = 8.5, 1.5 Hz, H-6,7), 7.75 (1H, *d*, J = 8.5 Hz, H-4), 8.28 (2H, *dd*, J = 8.5, 1.5 Hz, H-5,8). ^{13}C NMR (75.5 MHz, CD_3OD) at δ: 150.00 (C-1), 151.09 (C-2), 121.46 (C-3), 120.41 (C-4), 120.68 (C-4a), 127.74 (C-5), 135.16 (C-6), 133.91 (C-7), 127.01 (C-8), 134.14 (C-8a), 189,53 (C-9), 116.45 (C-9a), 181.50 (C-10), 133.21 (C-10a). MS: 242 $[M+2]^+$ (100), 241 $[M+1]^+$ 214, 213, 186, 185, 139, 138, 78, 77 and 55.

Compound **2**. Yellow crystalline needles (7 mg), m.p. 168–171°. IR, ν_{max} (KBr), cm^{-1}: 3210, 1675, 1630, 1590 and 1280. UV λ_{max} (EtOH): 246, 281 and 418 nm. ^{1}H NMR (500 MHz, $CDCl_3$) at δ: 13.28 (1H, *s*, OH-1), 1.35 (3H, *t*, J = 7.0 Hz, CH_3), 3.75 (2H, *q*, J = 7.0 Hz, CH_2-O-*CH_2*-CH_3), 4.97 (2H, *s*, *CH_2*-O-CH_2-CH_3), 9.62 (1H, *br s*, OH-3), 7.26 (1H, *s*, H-4), 7.77 (2H, *m*, H-6,7) and 8.27 (2H, *m*, H-5,8). ^{13}C NMR (75.5 MHz, $CDCl_3$) at δ: 161.99 (C-1), 114.84 (C-2), 164.34 (C-3), 109.77 (C-4), 133.73 (C-4a), 127.52 (C-5), 134.28 (C-6), 134.16 (C-7), 126.89 (C-8), 133.73 (C-8a), 187.07 (C-9), 109.97 (C-9a), 182.43 (C-10), 133.73 (C-10a), 67.79 (*CH_2*-O-CH_2-Me), 67.15 (CH_2-O-*CH_2*-Me) and 15.15 (CH_2-*O*-CH_2-*Me*). MS, *m/z*, 252 (100) $[M-CH_3CH_2OH]^+$, other fragments: 253, 254, 199, 196, 77 and 55.

Compound **3**. Yellowish-orange powder (7 mg). IR, ν_{max} (KBr), cm^{-1}: 3510, 1670, 1620, 1580, 1470, 1360 and 1285. UV λ_{max} (MeOH): 245, 265 and 412 nm. ^{1}H NMR (500 MHz, CD_3OD) at δ: 8.17, 8.25 (2H, *dd*, J = 8.0, 2.0 Hz, H-5,8), 7.81 (2H, *dd*, J = 7.5, 1.5 Hz, H-6,7), 7.22 (1H, *s*, H-4), 6.61 (1H, *s*, H-1), 4.62 (2H, *s*, *CH_2*OH), 5.35 (1H, *m*, CH_2*OH*), 5.00 (1H, *d*, J = 7.5 Hz, H-1′) and 3.00–4.60 (sugar protons). ^{13}C NMR (75.5 MHz, CD_3OD) at δ: 109.06 (C-1), 120.06 (C-2), 164.89 (C-3), 107.50 (C-4), 134.13 (C-4a), 128.26 (C-5), 135.71 (C-6), 135.62 (C-7), 128.26 (C-8), 135.61 (C-8a), 188.45 (C-9), 109.06 (C-9a), 184.33 (C-10), 135.38

(C-10a), 50.86 (CH_2OH at C-2), 101.00 (C-1′), 73.20 (C-2′), 77.06 (C-3′), 67.89 (C-4′), 72.45 (C-5′) and 58.00 (C-6′). MS, *m/z*, [M (aglycone + 2)]$^+$: 256, 255, 228, 199 and 168.

Compound **4**. Yellowish powder (50 mg), m.p. 244–245°. IR ν_{max} (KBr), cm^{-1}, 3415, 1655, 1621, 1578, 1474, 1362 and 1287. UV λ_{max} (MeOH): 244, 266, 333 and 404 nm. ^{1}H NMR (d_6-DMSO, 300 MHz) at δ: 13.00 (1H, s, OH-1), 8.18, 8.25 (2H, *dd*, J = 8.0, 2.0 Hz, H-5,8), 7.94 (2H, *dd*, J = 7.5, 1.5 Hz, H-6,7), 7.49 (1H, *s*, H-4), 5.43 (1H, *m*, CH_2OH), 5.10 (2H, *s*, CH_2OH), 5.00 (1H, *d*, J = 7.5 Hz, H-1′) and 3.00–4.70 (sugar protons). ^{13}C NMR (300 MHz, d_6-DMSO) at δ: 161.83 (C-1), 123.51 (C-2), 161.53 (C-3), 106.20 (C-4), 132.61 (C-4a), 126.61 (C-5), 134.57 (C-6), 134.42 (C-7), 126.28 (C-8), 133.52 (C-8a), 186.72 (C-9), 111.21 (C-9a), 181.05 (C-10), 132.69 (C-10a), 50.92 (CH_2OH at C-2), 100.82 (C-1′), 75.91 (C-2′), 77.24 (C-3′), 69.43 (C-4′), 73.81 (C-5′) and 60.41 (C-6′). MS, *m/z*,: 270 [M (aglycone)]$^+$, 254, 253, 252 (100), 224, 196, 168 and 139.

Compound **5**. Yellowish powder (5 mg). IR ν_{max} (KBr), cm^{-1}: 3410, 1650, 1622, 1575, 1474, 1362 and 1287. UV λ_{max} (MeOH): 245, 265, 322 and 404 nm. ^{1}H NMR (500 MHz, CD_3OD) at δ: 12.10 (1H, *b*, *s*, OH-8), 8.35 (1H, *dd*, J = 9.0, 1.5, H-5), 7.91 (2H, *m*, H-6,7), 7.47 (1H, *s*, H-4), 6.61 (1H, *s*, H-1), 4.62 (2H, *s*, CH_2OH), 3.00–4.80 (sugar protons) and 5.00 (1H, *d*, J = 7.0 Hz, H-1′). MS, *m/z*,: 272 [M (aglycone) + 2]$^+$, 254, 225, 198, 169, 168, 139, 138.

Compound **6**. Yellowish powder (200 mg), m.p. 227–228°. IR ν_{max} (KBr), cm^{-1}: 3390, 1665, 1622, 1585, 1478, 1366 and 1286. UV λ_{max} (MeOH): 244, 266, 333 and 404 nm. ^{1}H NMR (300 MHz, d_6-DMSO) at δ: 13.00 (1H, *s*, OH-1), 8.17, 8.25 (2H, *dd*, J = 7.5, 1.5 Hz, H-5,8), 7.92 (2H, *dd*, J = 7.5, 1.5 Hz, H-6,7), 7.47 (1H, *s*, H-4), 5.10 (2H, *s*, CH_2OH), 5.46 (1H, *m*, CH_2OH), 4.86 (1H, *d*, J = 7.5 Hz, H-1′), 4.81 (1H, *d*, J = 7.2 Hz, H-1″) and 3.00–4.70 (sugar protons). ^{13}C NMR (300 MHz, d_6-DMSO) at δ: 161.76 (C-1), 123.54 (C-2), 161.56, C-3), 106.31 (C-4), 133.53 (C-4a), 126.67 (C-5), 134.56 (C-6), 134.48 (C-7), 126,27 (C-8), 132.54 (C-8a), 186.73 (C-9), 111.22 (C-9a), 181.10 (C-10), 132.68 (C-10a), 50.92 (CH_2OH at C-2), 100.76 (C-1′), 73.13 (C-2′), 75.65 (C-3′), 69.16 (C-4′), 75.63 (C-5′), 67.93 (C-6′), 103.85 (C-1″), 73.13 (C-2″), 76.24 (C-3″), 69.38 (C-4″) and 65.48 (C-5″). MS, *m/z*, [M (aglycone) + 2]$^+$: 254 (100), 252, 253, 225 and 197.

Compound **7**. Colourless needles (30 mg), m.p. 173–175°. IR ν_{max} (KBr), cm^{-1}: 3600–3000, 1732, 1691, 1653. UV λ_{max} (MeOH): 236, 280, 318 and 335 nm. ^{1}H NMR (d_6-DMSO) at δ: 2.05 (3H, *s*, COO*Me*), 2.90–3.70 (sugar protons), 4.50 (1H, *d*, J = 7.5 Hz, H-1), 4.55 (1H, *s*, H-9), 4.65 (1H, *s*, H-5), 4.95 (2H, *br s*, CH_2-10), 5.55 (1H, *dd*, J = 7.0, 2.0 Hz, H-6), 5.700 (1H, *s*, H-7), 5.85 (1H, *s*, H-1) and 7.40 (1H, *s*, H-3). MS: *m/z*, 431 (glycoside), 253 [M (aglycone) + 1]$^+$, 235, 217 and 193.

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