

A novel naphthoquinone glycoside from *Rubia peregrina* L.

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A phytochemical study was performed on the subterranean parts (roots and rhizomes) of *Rubia peregrina*. The substances were isolated from chloroform, ethyl acetate, and water soluble parts of the methanolic extract using several chromatographic techniques. 1-Hydroxy-2-methyl-9,10-anthraquinone (**1**), 1,3-dihydroxy-2-methyl-9,10-anthraquinone (**2**), and 1,3-dihydroxy-2-methoxymethyl-9,10-anthraquinone (**3**) were isolated from the chloroform phase; and asperuloside (**4**), asperulosidic acid (**5**), rubiadin 3-*O*- β -primeveroside (**6**), lucidin 3-*O*- β -primeveroside (**7**), deacetylasperulosidic acid (**8**), liriiodendrin (**9**), and a new naphthoquinone glycoside, 2-hydroxy-3-*O*- β -primeveroside naphthalene-1,4-dione (inkumoside, **10**), from the aqueous phase. The structures of the compounds were elucidated by means of spectral analysis (¹H-NMR, ¹³C-NMR, 2D-NMR-COSY, HMBC, HMQC-, EI-MS and ESI-MS).

Key Words: *Rubia peregrina*, Rubiaceae, anthraquinones, iridoid glycosides, naphthoquinone glycoside.

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Introduction

The family Rubiaceae is represented by about 500 genera and 6000 species, mostly tropical trees and shrubs.¹ Some members of Rubiaceae contain quinonic compounds (anthraquinones, naphthoquinones, naphthohydroquinones, and their glycosides),^{2–5} iridoids,⁶ coumarins,⁷ triterpenes,⁸ and flavonoids.⁹

The genus *Rubia* comprises 60 known species in the world and is represented by 5 species in the flora of Turkey, one of which is endemic.^{1,10} *R. peregrina* grows in north-west Anatolia,¹⁰ where the underground parts have been used for dyeing woollen fibres.¹¹

This study describes the isolation and structure elucidation of the anthraquinones (**1-3**, **6**, **7**), iridoid glycosides (**4**, **5**, **8**), a lignan (liriodendrin) (**9**), and a new naphthoquinone glycoside (inkumoside) (**10**) from *R. peregrina* Figure.

Experimental

Instruments and materials

¹H-NMR spectra were recorded with a Varian Mercury plus spectrometer at 400 MHz and Bruker Avance 360 (360 MHz); ¹³C-NMR spectra were recorded with a Bruker DRx500 (125 MHz) and Varian Mercury plus spectrometer at 100 MHz. Mass spectra were recorded with Thermofinnigan Trace GC/Trace DSG/A1300, EIMS: Jeol AX505W and Micromass ZQ Mass Spectrometer (Manchester, UK). UV spectra were recorded with a HELIOS β UV-VIS Spectrophotometer. Sephadex LH-20 (Sigma-Aldrich), silica gel (Kiesel gel 60, 0.063-0.2 mm and 0.040-0.063 mm Merck and LiChroprep RP-18, 25-40 μ m, Merck 9303) for column chromatography, and silica gel 60 F₂₅₄ (Merck, 5554) for TLC were used. TLC spots were detected with a UV lamp and spraying 1% Vanillin/H₂SO₄ and heating at 120 °C for 1-2 min.

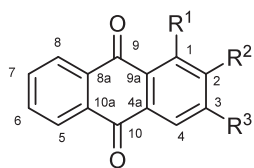
Plant material

The subterranean parts (roots and rhizomes) of *R. peregrina* were collected from İnkum (10 m) (Bartın province, Turkey) in August 2002 and identified by Dr. Ufuk Özgen. A voucher specimen was deposited in the Herbarium of Ankara University Faculty of Pharmacy (AEF 21228).

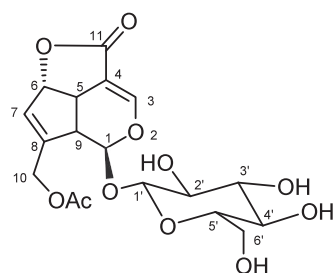
Extraction and isolation

Dried subterranean parts (500 g) of plant were extracted by refluxing with methanol (3 L \times 3) on a mantle. Methanol extract was concentrated and dried under reduced pressure to give a residue (152 g). Methanol extract (150 g) was dissolved in H₂O:MeOH (9:1) and partitioned with chloroform and then ethyl acetate. The less polar phases were concentrated and dried under reduced pressure to give 10 g and 7 g residues, respectively. The aqueous layer was freeze-dried to give 130 g residue.

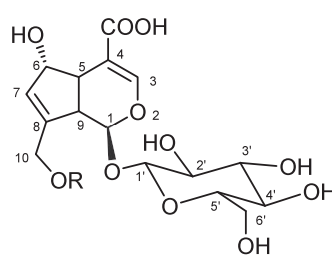
The chloroform phase (10 g) was subjected to silica gel column chromatography using *n*-hexane:EtOAc with gradient elution (9:1 \rightarrow 0:10). *n*-Hexane:EtOAc (8:2) gave compound **1** (15 mg), *n*-hexane:EtOAc (7:3) gave compound **2** (15 mg), and *n*-hexane:EtOAc (1:1) gave compound **3** (10 mg).



- 1; R¹=OH; R²=CH₃; R³=H (1-Hydroxy-2-methyl-9,10-anthraquinone)
 2; R¹=OH; R²=CH₃; R³=OH (1,3-Dihydroxy-2-methyl-9,10-anthraquinone)
 3; R¹=OH; R²=CH₂OCH₃; R³=OH (1,3-Dihydroxy-2-methoxymethyl-9,10-anthraquinone)
 6; R¹=OH; R²=CH₃; R³=O-β-primeveroside (Rubiadin 3-O-β-primeveroside)
 7; R¹=OH; R²=CH₂OH; R³=O-β-primeveroside (Lucidin 3-O-β-primeveroside)

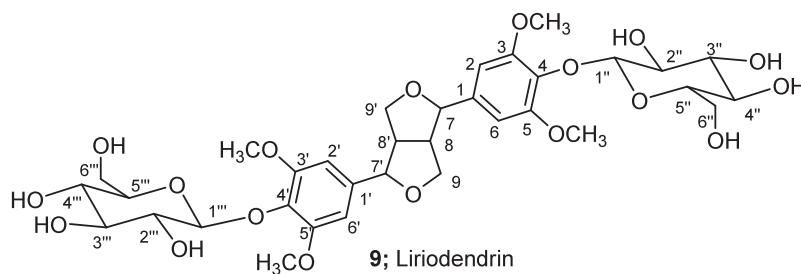


4; Asperuloside

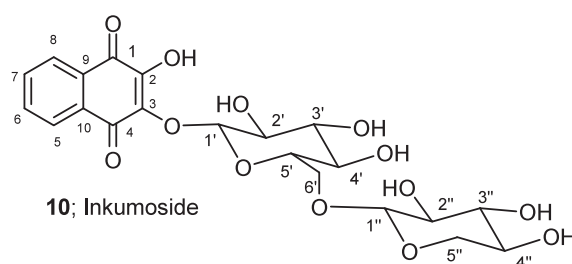


5; R=Ac (Asperulosidic acid)

8; R=H (Deacetylasperulosidic acid)



9; Liriodendrin



10; Inkumoside

Figure. Compounds isolated from *Rubia peregrina*.

There were too few compounds to isolate and identify in the EtOAc phase.

The remaining aqueous phase (100 g) was subjected to chromatography on a silica gel column eluting with CHCl₃:MeOH:H₂O (90:10:1, 80:20:2, 70:30:3, 61:32:7, 50:50:5) solvent systems, respectively. Fractions were monitored by TLC on silica gel plates and similar fractions combined to give Fraction A (Fr. 1-9, 5.2 g), Fraction B (Fr. 10-13, 24.7 g), Fraction C (Fr. 14-17, 16.8 g), Fraction D (Fr. 18-19, 4.1 g), Fraction E (Fr. 20-23, 3.8 g), Fraction F (Fr. 24-27, 14.9 g), Fraction G (Fr. 28-31, 18.7 g), and Fraction H (Fr. 32-37, 7.8 g).

Fraction B was subjected to Sephadex column chromatography with MeOH and eluates monitored by TLC, similar fractions being combined. Combined fractions 10-18 (named B1) were then subjected to silica gel column chromatography and eluted with CHCl₃:MeOH:H₂O (70:30:3). Fr. 10-15 from this separation (B1a) gave compound **4** (18 mg) and Fr. 20-27 (B1b) gave compound **5** (15 mg).

Fraction C was subjected to Sephadex column chromatography with MeOH and eluates monitored by TLC, similar fractions being combined. Fr. 15-25 (C1) were chromatographed on a silica gel column with CHCl₃:MeOH:H₂O (70:30:3). Fr. 10-16 (C1a) were subjected to Sephadex LH-20 column chromatography with MeOH to give compound **6** (35 mg). Fr. 20-30 (C1b) were subjected to Sephadex LH-20 column chromatography with MeOH to give compound **7** (115 mg).

Fraction F was subjected to reversed phase silica gel column chromatography with H₂O:MeOH (9:1 → 0:10). Fr. 12-24 (F1) and Fr. 26-30 (F2) obtained from this separation underwent to Sephadex LH-20 column chromatography using MeOH as eluant. F1 gave compound **8** (12 mg) and F2 gave compound **9** (28 mg).

Fraction G was subjected to silica gel column chromatography with CHCl₃:MeOH:H₂O (61:32:7). Fr. 20-25 from this separation were purified with Sephadex LH-20 column chromatography using MeOH and compound **10** (18 mg) was obtained.

Compound 1

Yellow crystals; **EI-MS** (*m/e*) 238 [*M*⁺] (100%); **¹H-NMR** (360 MHz, CDCl₃): δ 12.98 (OH), 8.34-8.28 (*m*, 2H, H-5 and H-8), 7.83-7.78 (*m*, 2H, H-6 and H-7), 7.76 (*d*, 1H, H-3 or H-4, *J* = 7.6 Hz), 7.54 (*d*, 1H, H-3 or H-4, *J* = 7.6 Hz), 2.39 (*s*, 3H, CH₃); **¹³C-NMR** (125 MHz, CDCl₃): δ 189.4 (CO), 182.8 (CO), 161.5 (C-1), 137.7 (*d*), 135.4 (*s*), 135.0 (*d*), 134.4 (*d*), 134.2 (*s*), 133.7 (*s*), 131.8 (*s*), 127.7 (*d*), 127.3 (*d*), 119.7 (*d*), 115.7 (*s*), 16.6 (CH₃, *q*). EI-MS¹², ¹H-NMR,¹² and ¹³C-NMR data¹³ are in agreement with data given in the literature for 1-hydroxy-2-methyl-9,10-anthraquinone.

Compound 2

Orange, amorphous solid. **EI-MS** (*m/e*) 254 [*M*⁺] (100%); **¹H-NMR** (360 MHz, CDCl₃+ DMSO-*d*₆): δ 13.19 and 10.40 (OH), 8.29-8.20 (*m*, 2H, H-5 and H-8), 7.82-7.73 (*m*, 2H, H-6 and H-7), 7.38 (*s*, 1H, H-4), 2.19 (*s*, 3H, CH₃); **¹³C-NMR** (125 MHz, CDCl₃+ DMSO-*d*₆): δ 186.6 (CO), 182.6 (CO), 163.3 (C-1 or C-3, *s*), 163.0 (C-1 or C-3, *s*), 134.0 (*d*), 133.8 (*d*), 133.7 (*s*), 133.4 (*s*), 131.9 (*s*), 126.9 (*d*), 126.6 (*d*), 118.5 (*s*), 109.5 (*s*), 108.1 (*d*), 8.1 (*q*). EI-MS¹², ¹H-NMR,¹² and ¹³C-NMR¹⁴ data are in agreement with data given in the literature for 1,3-dihydroxy-2-methyl-9,10-anthraquinone (Rubiadin).

Compound 3

Orange, amorphous solid. **EI-MS** (*m/e*) 284 [*M*⁺] (9%), 252 (100%), 196 (22%), 168 (13%); **¹H-NMR** (360 MHz, CDCl₃): δ 13.30 (*s*, OH), 9.39 (*s*, OH), 8.31-8.24 (*m*, 2H, H-5 and H-8), 7.81-7.74 (*m*, 2H, H-6 and

H-7), 7.30 (*s*, 1H, H-4), 4.94 (*s*, 2H, CH₂O), 3.58 (*s*, 3H, OCH₃); ¹³C-NMR (125 MHz, CDCl₃): δ 186.9 (CO), 182.2 (CO), 164.1 (C-1 or C-3, *s*), 161.9 (C-1 or C-3, *s*), 134.1 (*d*, 2C), 134.1 (*s*), 133.5 (*s*, 2C), 127.4 (*d*), 126.7 (*d*), 114.4 (*s*), 109.8 (*d*), 109.7 (*s*), 69.0 (CH₂O), 59.4 (OCH₃). EI-MS, ¹H-NMR, and ¹³C-NMR data are in agreement with data given in the literature for 1,3-dihydroxy-2-methoxymethyl-9,10-anthraquinone.¹⁵

Compound 4

White crystals. ¹H-NMR (400 MHz, CD₃OD): δ 7.30 (*d*, 1H, H-3, *J* = 1.8 Hz), 5.96 (*d*, 1H, H-1, *J* = 1.1 Hz), 5.73 (*m*, 1H, H-7), 5.56 (*dm*, 1H, H-6, *J* = 6.6 Hz), 4.77 (*dm*, 1H, H-10a, *J* = 14.2 Hz), 4.68 (*d*, 1H, H-1', *J* = 8.1 Hz), 4.65 (*dm*, 1H, H-10b, *J* = 14.2 Hz), 3.92 (*dd*, 1H, H-6'a, *J* = 12.1 Hz, *J* = 2.2 Hz), 3.70-3.64 (overlapped H-6'b and H-5), 3.39-3.16 (*m*, 5H, H-9 and 4 x sugar protons), 2.07 (*s*, 3H, CH₃). ¹³C-NMR (100 MHz, CD₃OD): δ 171.1 (2C, 2xCO), 149.1 (C-3), 143.1 (C-8), 127.8 (C-7), 105.0 (C-4), 98.8 (C-1'), 92.1 (C-1), 85.1 (C-6), 77.2 (C-3'), 76.7 (C-5'), 73.5 (C-2'), 70.4 (C-4'), 61.6 (C-6'), 60.7 (C-10), 44.1 (C-9), 36.3 (C-5), 19.4 (CH₃CO). ¹³C-NMR data are in agreement with data given in the literature for asperuloside.¹⁶

Compound 5

White, amorphous solid. ¹H-NMR (400 MHz, CD₃OD): δ 7.65 (*d*, 1H, H-3, *J*_{3,5} = 1.5 Hz), 6.01 (*d*, 1H, H-7, *J*_{6,7} = 1.8 Hz), 5.04 (*d*, 1H, H-1, *J*_{1,9} = 9.2 Hz), 4.94 (*d*, 1H, H_a-10, *J* = 15.2 Hz), 4.83 (*dd*, 1H, H-6, *J*_{5,6} = 8.0 Hz, *J*_{6,7} = 1.8 Hz), 4.80 (*d*, 1H, H_b-10, *J* = 15.2 Hz), 4.73 (*d*, 1H, H-1', *J* = 8.1 Hz), 3.85 (*dd*, 1H, H_a-6', *J*_{gem} = 12.0 Hz, *J*_{5',6'a} = 1.5 Hz), 3.62 (*dd*, 1H, H_b-6', *J*_{gem} = 12.0 Hz, *J*_{5',6'b} = 5.5 Hz), 3.41 (*t*, 1H, H-3', *J*_{2',3'} = *J*_{3',4'} = 9.0 Hz), 3.32-3.22 (*m*, 3H, overlapped, H-2', H-3', H-4'), 3.01 (*bt*, 1H, H-5, *J* = 6.6 Hz), 2.63 (*bt*, 1H, H-9, *J* = 8.4 Hz), 2.10 (*s*, 3H, CH₃); ¹³C-NMR (100 MHz, CD₃OD): δ 171.5 (CO), 170.0 (CO), 154.2 (C-3), 146.8 (C-8), 130.7 (C-7), 107.5 (C-4), 100.1 (C-1), 99.3 (C-1'), 77.3 (C-3'), 76.6 (C-5'), 74.3 (C-6), 73.7 (C-2'), 70.3 (C-4'), 62.7 (C-10), 61.8 (C-6'), 45.1 (C-9), 41.3 (C-5), 19.7 (CH₃). ¹H-NMR data and ¹³C-NMR data are in agreement with data given in the literature for asperulosidic acid.¹⁷

Compound 6

Yellow crystal; EI-MS (m/e) 254.0 [MH-sugar]+H; ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.22 (*m*, 2H, H-5 and H-8), 7.82 (*m*, 2H, H-6 and H-7), 7.47 (*s*, 1H, H-4), 5.08 (*d*, 1H, H-1 glucose, *J* = 7.0 Hz), 4.12 (*d*, 1H, H-1 xylose, *J* = 7.0 Hz), 3.96 (*d*, 1H, *J* = 9.5 Hz, sugar proton), 3.73-3.58 (*m*, 3H, sugar protons), 3.40-3.30 (*m*, 4H, sugar protons), 3.12-2.97 (*m*, 3H, sugar protons), 2.16 (*s*, 3H, CH₃); ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 187.8 (C-9, CO), 182.3 (C-10, CO), 162.1 (C-1 or C-3), 161.9 (C-1 or C-3), 135.5 (C-6 or C-7), 135.3 (C-6 or C-7), 133.7 (C-4a or C-8a or C-10a), 133.5 (C-4a or C-8a or C-10a), 132.7 (C-4a or C-8a or C-10a), 127.6 (C-5 or C-8), 127.2 (C-5 or C-8), 121.4 (C-2), 111.7 (C-9a), 106.6 (C-4), 104.7 (C-1, xyl), 101.0 (C-1, glc), 77.1 (C-3 xyl or C-3 glc), 76.8 (C-3 xyl or C-3 glc), 76.4 (C-4 xyl or C-4 glc or C-5 glc), 74.0 (C-2 xyl), 73.9 (C-2 glc), 70.2 and 69.8 (C-4 xyl or C-4 glc or C-5 glc), 68.7 (C-6 glc), 66.3 (C-5 xyl), 9.2 (CH₃). ¹H-NMR and ¹³C-NMR data are in agreement with data given in the literature for rubiadin 3-*O*-β-primeveroside.¹⁴

Compound 7

Yellow powder; ¹H-NMR (400 MHz, D₂O): δ 8.23-8.16 (*m*, 2H, H-5 and H-8), 7.87-7.83 (*m*, 2H, H-6 and H-7), 7.50 (*s*, 1H, H-4), 5.07 (*d*, 1H, H-1 glucose, *J* = 7.3 Hz), 4.70 (A part of AB system, *d*, 1H, H_a-6, *J* = 11.4 Hz), 4.59 (B part of AB system, *d*, 1H, H-1, H_b-6, *J* = 11.4 Hz), 4.13 (*d*, 1H, H-1 xylose, *J* =

7.3 Hz), 3.96 (*d*, 1H, $J = 9.5$ Hz, sugar proton), 3.73-3.58 (*m*, 3H, sugar protons), 3.44-3.30 (*m*, 4H, sugar protons), 3.12-2.98 (*m*, 3H, sugar protons); $^{13}\text{C-NMR}$ (100 MHz, D_2O): δ 186.6 (CO), 182.3 (CO), 161.7 (C-3), 160.9 (C-1), 135.3 (C-7), 135.2 (C-6), 133.4 (C-10a), 131.9 (C-4a), 131.7 (C-8a), 127.0 (C-8), 126.5 (C-5), 122.2 (C-2), 111.2 (C-9a), 106.1 (C-4), 103.7 (C-1 of xylose), 99.6 (C-1 of glucose), 75.9 (C-3 xyl or C-3 glc), 75.6 (C-3 glc), 75.4 (C-5 glc), 73.2 and 72.9 (C-2 xyl and C-2 glc), 69.5 (C-4 xyl), 69.1 (C-4 glc), 68.3 (C-6 glc), 65.4 (C-5 xyl), 51.5 (CH_2OH). $^1\text{H-NMR}$ data and $^{13}\text{C-NMR}$ data agree with data given in the literature for lucidin 3-*O*- β -primeveroside.¹⁴

Compound 8

White, amorphous solid. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 7.17 (*bs*, 1H, H-3), 5.79 (*bs*, 1H, H-7), 4.81 (*d*, 1H, H-1, $J = 8.0$ Hz), 4.68 (*bd*, 1H, H-6, $J = 6.6$ Hz), 4.52 (*d*, 1H, H-1', $J = 7.7$ Hz), 4.23 (*d*, 1H, H_b-10, $J = 16.1$ Hz), 3.94 (*d*, 1H, H_a-10, $J = 16.1$ Hz), 3.65-3.05 (sugar protons, 5H, H-3', H-4', H-5', 2xH-6'), 2.96 (*t*, 1H, H-2', $J = 8.4$ Hz), 2.82 (*bt*, 1H, H-5, $J = 7.0$ Hz), 2.35 (*t*, 1H, H-9, $J = 8.1$ Hz). $^1\text{H-NMR}$ data are in agreement with data given in the literature for deacetylasperulosidic acid.¹⁷

Compound 9

White crystals. $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ 6.64 (*s*, 4H, H-2, H-2', H-6, H-6'), 4.96 (*d*, 2H, 2xOH, $J = 2.6$ Hz), 4.95 (*d*, 2H, 2xOH, $J = 2.6$ Hz), 4.89 (*d*, 2H, 2xOH, $J = 5.1$ Hz), 4.86 (*d*, 2H, H-1'', $J = 7.7$ Hz), 4.65 (*d*, 2H, H-7, H-7', $J = 4.0$ Hz), 4.29 (*t*, 2H, H-6''-OH, H-6'''-OH, $J = 5.4$ Hz), 4.18 (*dd*, 2H, H-9, H-9', $J = 9.0$ Hz, $J = 6.6$ Hz), 3.81 (*dd*, 2H, H-9, H-9', $J = 9.0$ Hz, $J = 3.7$ Hz), 3.74 (*s*, 12H, 4xOCH₃), 3.57 (*ddd*, 2H, H-6'', H-6''', $J = 11.3$ Hz, $J = 4.8$ Hz, $J = 1.8$ Hz), 3.41-3.36 (*m*, 2H, sugar protons), 3.20-3.07 (10H, 8xsugar protons, H-8 and H-8'); $^{13}\text{C-NMR}$ (100 MHz, CD_3OD): δ 153.3 (C-3, C-3', C-5, C-5'), 137.8 (C-4, C-4'), 134.4 (C-1, C-1'), 104.9 (C-2, C-2', C-6, C-6'), 103.3 (C-1'', C-1'''), 85.7 (C-7, C-7'), 77.9 (C-5'', C-5'''), 77.2 (C-3'', C-3'''), 74.8 (C-2'', C-2'''), 72.0 (C-9, C-9'), 70.6 (C-4'', C-4'''), 61.6 (C-6'', C-6'''), 57.1 (4xOCH₃), 54.3 (C-8, C-8'). $^1\text{H-NMR}$ data and $^{13}\text{C-NMR}$ data agree with data given in the literature for liriodendrin.¹⁸

Compound 10

Orange amorphous solid. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$), $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$) (Table); **Positive ESI-MS**, ($\text{C}_{21}\text{H}_{24}\text{O}_{13}$), *m/e*: 551 [M-2H+3Na]⁺, 335 [Primeverose+Na]⁺; 203 [Glucose+Na]⁺, **UV** λ_{max} (H_2O) nm: 253, 287, 431. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): See Table, $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): See Table.

Results and discussion

Compounds **1-9** had been isolated from other sources, including other *Rubia* species. However, no compound could be found whose data matched those for compound **10**. The UV spectra showed maximum absorbances at 253, 287, and 431 nm, characteristic for 1,4-naphthoquinones.²¹

$^1\text{H-NMR}$ spectra showed a typical unsymmetrical naphthalene-1,4-dione structure in which H-5 and H-8 resonate as a double doublet and H-6 and H-7 as a double triplet (Table). Sugar protons were observed between

Table. NMR Spectroscopic data for **10** (^1H : 400 MHz, ^{13}C : 100 MHz).

	10 (DMSO- d_6)			
C/H atom	δC	DEPT	δH ppm, J (Hz)	HMBC (H \rightarrow C)
1	184.6 ^a	C	-	
2	^b	C	-	
3	^b	C	-	
4	180.8 ^a	C	-	
5	126.6 ^c	CH	8.00 <i>dd</i> (8.2, 1.1)	C-4, C-6 ^f , C-7 ^f
6	134.0 ^d	CH	7.76 <i>dt</i> (7.3, 1.5)	C-5 ^g , C-8 ^g , C-9 ^h /10 ^h
7	133.2 ^d	CH	7.71 <i>dt</i> (7.3, 1.5)	C-5 ^g , C-8 ^g , C-9 ^h /10 ^h
8	126.3 ^c	CH	7.96 <i>dd</i> (8.1, 1.3)	C-1, C-6 ^f , C-7 ^f
9	135.1 ^e	C		
10	135.2 ^e	C		
Glucose				
1'	103.1	CH	4.86 <i>d</i> (7.7)	
2'	74.8	CH	3.32*	
3'	76.8	CH	3.15*	
4'	70.2	CH	3.14*	
5'	76.5	CH	2.90 <i>t</i> (8.8)	
6'	68.9	CH ₂	3.72 <i>d</i> (11.0); 3.36*	C-1''
Xylose				
1''	104.2	CH	3.85 <i>d</i> (7.4)	
2''	73.7	CH	2.84	
3''	76.4	CH	3.15*	
4''	70.0	CH	3.14*	
5''	66.0	CH ₂	2.80; 3.50*	

^{a,c,d,e,f,g,h}) Interchangeable^b) Disappeared

*) Signal patterns are not clear due to overlapping

δ 4.86 ppm and δ 2.80 ppm and integrated for 2 sugars, especially since 2 anomeric protons were seen at 4.86 (*d*, $J = 7.7$ Hz) and 3.85 (*d*, $J = 7.4$ Hz) corresponding to typical shifts for glucose and xylose respectively, and so it is likely that the sugar is primeveroside. J values show that the sugars have β configurations. ^{13}C -NMR spectra showed 2 carbonyl and 6 aromatic carbon signals between 184.6 and 126.3 ppm, further confirming a naphthalene structure, although 2 more C signals are needed to complete all those that should be observed. Despite very careful and repeated studies the 2 signals belonging to C-2 and C-3 could not be determined. This was attributed to the quaternary status of C-2/3 and a possible keto-enol tautomerisation. The chemical shifts were in good agreement with data given in the literature for 2-hydroxy-1,4-naphthoquinone glycosides.²² Eleven carbon signals between 104.2 and 66.0 ppm confirm the primeveroside structure. DEPT, HETCOR, and HMBC spectra confirm the suggested structure, as did the ESI-MS, because of the positive molecular ion peak at m/e

551 [M-2H+3Na]⁺. **10** was therefore designated as 2-hydroxy-3-*O*-β-primeveroside naphthalene-1,4-dione and given the trivial name inkumoside. **10** has been isolated from nature for the first time in this study.

In addition to this novel compound, known anthraquinones, anthraquinone glycosides, iridoid glycosides, and a lignan liriodendrin were isolated from the underground parts of *Rubia peregrina*.

It is known that *Rubia* species are rich in quinonic compounds and iridoid glycosides, while naphthoquinones are rarely found in this genus.^{19,20}

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