New Anthraquinones and Anthraquinone Glycosides from *Morinda lucida*

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Dedicated to Professor Dr. F. Bohlmann on the occasion of his 60th birthday

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From the trunk heartwood of *Morinda lucida* four new anthraquinones, 3-hydroxy-anthraquinone-2-carbaldehyde, morindone-5-methyl ether, munjistin methyl ester, dannanthol-α-methyl ether and two glycosides, soranjidiol-6-β-primeroside and lucidin-3-0-β-primeroside were isolated and identified by spectroscopic and chemical methods.

*Morinda lucida* (Benth.) grows in Central- and West Africa. The various parts of the tree are reputed for their medicinal value [1, 2] and are used for dyeing purposes. As main constituents thirteen anthraquinones and two anthraquinols derived from the shikimate pathway have been isolated from wood, bark and roots [3-5]. Morindone-1,6-dihydroxy-2-methylanthraquinone and soranjidiol-1,6-dihydroxy-2-methylanthraquinone were also isolated as 6-0-β-primerosides [5]. From the leaves, oruwacin, a new iridoid furanate was obtained [6]. Since analytical thinlayer chromatography of crude extracts of the wood showed far more than the expected number of coloured bands, we decided to further investigate this plant.

Results and Discussion

Crushed and defatted heartwood of *Morinda lucida* was extracted with methanol to obtain a mixture of anthraquinone glycosides, free aglycones and other polar material, which were separated by dry column chromatography on silica gel. The fraction of anthraquinones eluted from the front part of the column was further separated by dry column chromatography and subsequently by preparative layer chromatography. In addition to the known anthraquinones, identified by mass and proton NMR spectra, four new anthraquinones, 1-4 were isolated.

The 1H NMR spectrum of the most lipophilic compound, C13H15O4, by high resolution mass spectroscopy, showed the characteristic, symmetrical multiplets (δ 7.85 and 8.35, 4H) of an anthraquinone with one aromatic ring unsubstituted. Two aromatic protons appear as singlets at δ 7.84 and δ 8.67 and place an aldehydic function (δ 10.18) and a hydroxy group (δ 11.4) in 2- and 3-position of the second ring as depicted in 1. Since 1 is an entirely new compound, we synthesized it from 3-hydroxy-2-hydroxymethylanthraquinone [7] by manganese dioxide oxidation. All spectroscopic data as well as the melting point of 259 °C of synthetic and natural material were identical. From a biogenetic point of view 1 is closely related to 3-hydroxy-2-methyl-anthraquinone found in Rubiaceae and many other plant families [3]. Interestingly the corresponding 3-hydroxy-2-hydroxymethyl and 3-hydroxy-2-carboxylic acid derivative have not been found in nature until now.

The second more polar compound, C14H22O6, gave a 1H NMR spectrum very similar to morindon; only a chelated hydroxy group is substituted by a methoxy group (δ 4.02). This is in good agreement with the structure of morindon-5-methylther (2). As expected H-8 (δ 8.17) is shifted downfield by 0.45 ppm compared to morindon due to methylation of the para hydroxy group.

Further an anthraquinone, C16H15O4, was isolated, whose 1H NMR spectrum indicated one aromatic ring to be unsubstituted, the other one to bear two chelated hydroxy groups (δ 12.6 and 14.8) and an isolated aromatic proton at δ 7.39. From this and a methoxy signal at δ 4.07 the structure of munjistin methyl ester (3) can be derived. The IR spectrum of 3 shows absorptions of a chelated and a non-chelated quinone carbonyl group (v 1625 and 1660 cm⁻¹) and a chelated ester carbonyl group (v 1670 cm⁻¹), the mass spectrum is dominated by the loss of methanol from the molecular ion. A similar cyclic fragmentation was observed in the mass spectra of α-hydroxy-benzoic acid esters however not with the isomeric α-methoxybenzoic acid as
model compounds. This eliminates the structure of munjistin-3-methyl ether for the isolated compound.

Finally another 1,2,3-trisubstituted anthraquinone, C_{17}H_{14}O_{5}, was isolated with an aromatic proton singlet in the NMR spectrum at δ 7.68. Methoxy- and methylene signals at δ 3.48 and 4.73 indicated a 2-methoxymethyl group, whereas an aromatic methoxy group has to be placed in 1-position since there is no chelated carbonyl group observed. The resulting structure is damnacanthol-ω-methyl ether 4.

It should be mentioned that 3 and 4 might be artefacts formed from munjistin resp. damnacanthol during extraction with methanol. Both compounds contain electron donating hydroxy- and methoxy groups stabilizing intermediate acylium ions or benzyl cations, which can be trapped by nucleophiles like methanol to give the ester resp. ether. Lucidin, certainly the most reactive compound in this respect, easily forms benzylic ether in boiling methanol or ethanol [3]. This alkylating power seems to be responsible for the extraordinary high mutagenic activity of these compounds in the Ames-Test compared to 70 other anthraquinones [8].

The mixture of anthraquinone glycosides obtained in the first dry column chromatography (see above), was further separated by preparative HPLC on RP-18 silica gel with methanol, water, acetic acid as eluent. As major components, in addition to morindon 6-O-β-primveroside [5, 9], glycosides of soranjidiol 5 and lucidin 6 were isolated in pure crystalline state. Acid hydrolysis of the glycosides gave the corresponding aglycones and a mixture of glucose and xylose, which were identified by TLC. This is in good agreement with field desorption mass
spectra showing strong signals at m/e 571, 593 for 5 and 587, 603 for 6 as their sodium and potassium ion cluster. In the EI mass spectra only the molecular ions and typical fragmentations of the aglycones are observed. The complete structure of 5 and 6 as soranjidiol 6-0-/?-primveroside and lucidin 3-0-/?-primveroside can be deduced from the \( ^{1}H \) NMR spectra (see also Fig. 1). Both compounds show the expected signals for the aromatic protons and a

With 6 the signals of a hydroxy-methylgroup are observed as an ABX system at \( \delta = 4.56, 4.64 \) and 4.86. This proves that in 5 the 6-hydroxy group, in 6 the 3-hydroxy group is glycosylated as previously stated [5, 10]. The proton signals of the sugar part are almost identical in 5 and 6 and agree fairly well with that reported for cyanogenic primverosides [11]. Doublets for two anomic protons at \( \delta = 5.09 \) and 4.15 with \( J = 7.5 \) Hz indicate \( \beta \)-glycosidic linkages, doublets for all six hydroxy groups of the sugar part \( (J = 4.5-5.5 \) Hz) prove the pyranose structure for xylose and the position of the inter-sugar linkage (Fig. 1).

**Experimental**

*Material:* Trunks (wood and bark) of *Morinda lucida* Benth. were collected around Yangambi (Zaire, Africa) in November 1978. The plants were identified by Prof. Dr. M. Hans (Free University of Bruxelles, Belgium), a specimen has been deposited at the Botanical Museum of Yangambi. Analytical and preparative TLC was performed on silica gel plates (Merck, DC-Alu-Folien 0.2 mm, PSC-Fertigplatten 2 mm, KG 60 F334), which were impregnated with oxalic acid. For dry column chromatography silica gel 0.06-0.2 mm (Merck) was used; columns were 30 mm in diameter and up to 3 m long. Reversed phase chromatography was performed at 5 to 10 bar on 40 \( \times \) 420 mm columns packed with Lichrosorb RP-18 (10 \( \mu \)m, Merck) [12]. Solvent systems: (A) Ethylacetate, n-butanol, water 4:3:3, organic layer; (B) chloroform; (C) benzene, methanol 4:1; (D) methanol, water, acetic acid 40:60:1; (E) methylenechloride, methanol, acetic acid 80:20:1.

*Instruments:* \(^{1}H\) NMR spectra were run at 270 and 400 MHz on a Bruker WH 270 resp. WM 400; \(^{13}C\) NMR spectra at 20 MHz on a Varian CFT 20 (\( \delta \) in ppm, \( \delta_{PPM, \text{ internal}} = 0 \)); IR spectra on a Perkin Elmer 297, UV–VIS spectra on a Zeiss DMR 21, EI mass spectra on an AEI MS 9 (70 eV), FD mass spectra on an AEI MS 30 instrument. Optical rotations were determined with a Perkin Elmer 241 Polarimeter, melting points (uncorrected) were taken on a Kofler micro hotstage.

**Extraction and separation**

Air dried and coarsely powdered trunk heartwood (1 kg) was defatted with petroleum ether (60–80 °C) and exhaustively extracted with methanol in a soxhlet. The concentrated methanol extract was fractionated by dry column chromatography (solvent system A). Anthraquinones (1.0 g) were eluted with methanol from the front, glycosides (12.3 g) from the middle part of the column. Further separation of the anthraquinones was achieved by dry column chromatography (solvent C) followed by preparative TLC (solvent system B and C). The individual bands were eluted and identified by NMR and mass spectroscopy. Anthraquinone glycosides were separated by column chromatography on RP-18 silica gel (solvent system D) and then Sephadex LH-20 (Methanol). The amounts of pure compounds to be expected from 1 kg wood are 0.20 g 1, 25 mg 2, 30 mg 4, 5 mg 5 and 30 mg 6. Since large quantities of material are being lost during the separation the actual amounts present in the wood are presumably much higher.

**Identification**

3-Hydroxyanthraquinone-2-carbaldehyde (1)

Pale yellow solid, m.p. 259 °C from chloroform, \( R_f = 0.77 \) (C, MS: m/e(%) 252.0419 (100) (M+, calc. for \( C_{14}H_{12}O_4 \) 252.0423), 251(55), 234(9), 224(5), 223(7), 206(8), 196(5), 195(4), 178(4), 168(3), 167(5), 150(3), 139(16).

\(^{1}H\) NMR (CDC\(_3\)): 7.83–7.86 (m, 2H), 7.86 (s, 1H), 8.34–8.37 (m, 2H), 8.67 (s, 1H), 10.5 (s, 1H), 11.46 (s, 1H).

IR (KBr): 1670 s, 1660 m, 1650 m, 710 cm\(^{-1}\) m.

UV–VIS (MeOH): 211 (4.17), 240 Sch (4.22), 247 (4.23), 274 (4.33), 320 Sch (3.66), 331 Sch (3.63), 367 Sch (3.48) mm (lg \( e \)), after addition of a trace of NaOH 207 (4.28), 247 (4.29), 279 Sch (4.21), 309 (4.14), 385 (3.80), 460 (3.68) nm (lg \( e \)).

**Synthesis of 1**

To 20 mg of 3-hydroxy-2-hydroxymethylanthraquinone [7] in 3 ml chloroform 100 mg manganese dioxide was added. After 1 h stirring at room temperature the solution was filtered and concentrated until the product crystallized, yield 14 mg (71%), m.p. 259 °C.

1,6-Dihydroxy-5-methoxy-2-methyllanthraquinone (morindon 5-methylether) (2)

Brown-orange crystals, m.p. 223 °C after sublimation at 180 °C 10–5 mm Hg, \( R_f = 0.50 \) (C).

MS: m/e(%) 284.0679(100) (M+, calc. for \( C_{14}H_{12}O_5 \) 284.0685), 269(38), 266(42), 258(32), 255(28), 254(24), 238(62), 149(40).

\(^{1}H\) NMR (CDCl\(_3\)): 2.35 (sbr, 3H), 4.02 (s, 3H), 7.38 (H-7, d, \( J = 8.5 \) Hz), 7.52 (H-3, dbr, \( J = 7.5 \) Hz), 7.72 (H-4, d, \( J = 7.5 \) Hz), 8.17 (H-8, d, \( J = 8.5 \) Hz), 10.0 (sbr, 1H), 12.98 (s, 1H).
IR (KBr): 1650 m, 1610 cm⁻¹ s. UV–VIS (MeOH): 222 (4.54), 264 (4.49), 280 (4.33), 288 Sch (4.26), 410 (4.10) nm (lg ε), after addition of trace of NaOH 215 (4.37), 232 (4.40), 248 (4.49), 312 (4.31), 394 (3.57), 490 (4.03) nm (lg ε).

1,3-Dihydroxyanthraquinone-2-carboxylic acid methyl ether (munjistin methyl ether) (3)

Yellow crystals, m.p. 202 °C after sublimation at 180 °C/10⁻³ mm Hg. 

MS: m/e (%) 298 (M⁺, 49), 266 (100), M⁺–CH₄O, m* = 237.5, 238 (37), 266-CO, m* = 213.

1H NMR (CDCl₃): 4.07 (s, 3 H), 7.39 (s, 1 H), 7.85 (m, 2 H), 8.35 (m, 2 H), 12.8 (1 H), 14.8 (s, 1 H).

IR (KBr): 1670 s, 1660 s, 1625 m, 708 cm⁻¹ s.

180 °C/10⁻³ mm Hg, (benzene).

UV–VIS (MeOH): 224 (4.38), 246 (4.48), 310 (4.36), 398.0841, 408 (3.90) nm (lg ε), after addition of a trace of NaOH

2-Methoxybenzoic acid

MS: m/e (%) 152 (M⁺, 100), 135(36), 134(7), 133(12), 123(42), 122(5), 121(5), 107(8), 106(11), 105(96), 104(54), 103(31), (see also [13]).

3-Hydroxy-1-methoxy-2-methoxymethylanthraquinone

(damnacanthol-1-methylether) (4)

UV–VIS (MeOH): 242 (4.52), 280 (4.31), 408 (3.71) nm (lg ε), after addition of a trace of NaOH

UV–VIS (H₂O): 209, 262, 288 Sch, 328 Sch, 370 Sch, 388 Sch, 406, 428 Sch nm. [α]²⁰ = −129°(c = 0.5 mg/ml in EtOH/H₂O 1:2).

Hydrolysis of 5

1.5 mg 5 were dissolved in 1 ml 2 N HCl and methanol (1:1) and heated in a sealed tube for 3 h at 110 °C. Aglycone and sugar were separated by extraction with chloroform and identified by TLC comparison with authentic samples [5] as soranjidiol, glucose and xylose.

1-Hydroxy-2-hydroxymethyl-3-0-ß-primverosylanthraquinone (lucidin 3-0-ß-primveroside) (6)

Yellow needles, m.p. 212 °C (sint. from 160 °C) from methanol, m.p. lit. 110–112 °C [10d], Rᵢ = 0.42 (E).

FD MS: m/e 587 (M⁺ + Na⁺), 603 (M⁺ + K⁺).

1H NMR (DMSO-d₆): aglycone 13.1 (s, 1 H), 4.56 (d, J = 11, 5.5 Hz, 2-CH), 4.64 (d, J = 11, 6.5 Hz, 2-CH), 4.86 (t, J = 6 Hz, ω-OH), 7.48 (s, H-4), 7.95 (m, H-6, H-7), 8.19 (m, H-8), 8.25 (m, H-5), sugar 5.12 (d, J = 7.5 Hz, H-1′α), 3.25–3.40 (m, H-2′β, H-3′α, H-4′β), 3.60–3.70 (m, H-5′a, H-6′a), 3.94 (dbr, J = 10.0 Hz), 4.14 (d, J = 7.5 Hz, H-1′α), 2.99 (dd, J = 9.0, 7.5, 6 Hz, 2-CH), 4.64 (d, J = 4.5 Hz), 4.91 (d, J = 4.5 Hz), 4.94 (d, J = 5.0 Hz), 5.18 (d, J = 5.0 Hz), 5.24 (d, J = 4.0 Hz), 5.51 (d, J = 4.5 Hz).

13C NMR (DMSO-d₆) in part: 50.9 (2-CH₂), 104.0 (C-4), 160.1 (C-3), 161.8 (C-1), 181.4 (C-10), 187.0 (C-9); for assignment see [10d, 14].

UV–VIS (H₂O), 238 Sch, 242, 262, 324, 380 Sch, 396, 424 Sch nm.

Hydrolysis of 6

7.0 mg 6 were hydrolysed as described above. Lucindin (after oxidation to nordamnacanth with MnO₂), glucose and xylose were identified by TLC comparison with authentic samples.

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