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# Isolation and Structure Elucidation of Iridoide and Coumarin Derivatives from *Xeromphis nilotica* (Rubiaceae)

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**Summary.** Besides a known coumarin derivative, scopoletin, the iridoids gardenoside, $\alpha$- and $\beta$-gardiol were isolated for the first time from a *Xeromphis* species, namely *Xeromphis nilotica*. Their structures were established on the basis of their spectroscopic data.

**Keywords.** *Xeromphis nilotica*; Rubiaceae; NMR; Iridoids; Coumarins.

## Introduction

*Xeromphis nilotica* (synonyms are *Catunaregam nilotica*, *Randia nilotica*, and *Lachnosiphonium niloticum*) belongs to the family Rubiaceae [1]. The genus *Xeromphis* is represented in Sudan by one species, namely *Xeromphis nilotica* (Stapf) Keay [2], which is widespread in Central and East Africa as well as in Cameroon and Nigeria [3]. Locally it is known as Shagart-Elmarfaein [4] and is found in Rashad area in the Eastern Nuba Mountains. It grows as a medium height shrub (usually less than 3 m) with grey globose drupes, stiff spines, and deciduous leaves clustered below the spines. *Xeromphis nilotica* has a broad range of applications in the indigenous medical system. The fruits show a very strong molluscicide activity against the schistosomiasis transmitting snail *Biomphalaria glabrata* [5] and are also well known for their antispasmodic, antifertility, and antidysentric properties [6]. The bark is not only used as an anthelmintic and in connection with the treatment of jaundice and rabies but is also utilized as a fish poison [7]. Previous efforts in the isolation of bioactive substances yielded, amongst others, different saponines, coumarins, and iridoids [5–9]. The course of our search for

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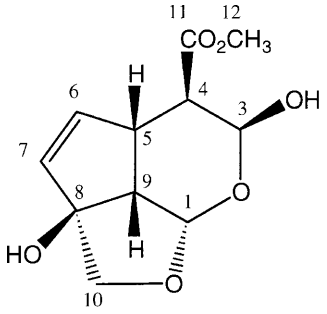
further secondary metabolites in this species led us to the isolation and characterization of three iridoids and one coumarin derivative from the leaves of this plant.

## Results and Discussion

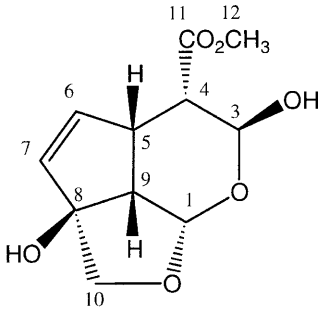
The methanolic extract of the leaves of *Xeromphis nilotica* was divided into petroleum ether, chloroform, and *n*-butanol soluble fractions. The chloroform fraction was further partitioned by Vacuum Liquid Chromatography (VLC). The elution with a gradient system ($CHCl_3$:$CH_3OH$) of increasing polarity yielded several subfractions. One of these was further subjected to repeated chromatographic separations to obtain compound **1** and **2** as an inseparable mixture. The FD mass spectrum showed only one peak ($M = 242$, $C_{11}H_{14}O_6$), therefore ruling out isomers differentiated e.g. by the nature of their functionalities or the number and sizes of rings. Consequently, both components of the mixture showed practically identical NMR spectra, presenting only subtle differences in some of the shift values. The presence of an iridoidal skeleton was established by the interpretation of HMBC data allowing to assign ring size and ring linkage unequivocally. Thus the NMR data allow to characterise compound **1** and **2** as $\alpha$-gardiol and $\beta$-gardiol [10, 11], clearly showing the presence of the two C-4 epimers. Both compounds have previously been isolated from different plants belonging to the family Rubiaceae [11–14].

Compound **3** was obtained as white crystalline solid from another subfraction of the chloroform soluble extract. From EI-MS ($M^+ = 192$, $C_{10}H_8O_4$), the characteristic UV spectrum with major peaks at 343 and 225 nm in combination with the carbonyl bands in the IR at 1705 and 1608 $cm^{-1}$, the coupled aromatic proton pair ($J = 9.5$ Hz) with resonances at 7.78 and 6.18 ppm and singuletts at 6.95 and 6.65 ppm together with a HMBC crosspeak between the methoxy group (55.2 ppm/3.90 ppm) and the CH resonance at 109.5 ppm, which is assignable as C-5 with two more HMBC crosspeaks between CH-5 and CH-4, and finally by comparison of the experimental data with a published dataset [15], compound **3** can be assigned as scopoletin, which has been already reported from *Xeromphis nilotica* [9].

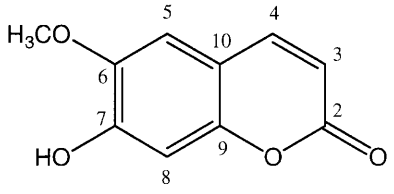
The chromatographic workup of the *n*-butanol soluble fraction yielded compound **4** as an oil. Its EI-MS gave peaks at $m/z = 427$ $[M + Na]^+$ and $m/z = 403$ $[M - H]^+$, indicating the molecular weight to be 404, corresponding to $C_{17}H_{24}O_{11}$. The IR spectrum revealed the presence of free hydroxyl groups (3398 $cm^{-1}$), olefinic moieties (1640, 1513 $cm^{-1}$), and an esterified carbonyl group (1693 $cm^{-1}$) adjacent to a double bond. The $^1H$- (with CH resonances at 4.66, 3.18, 3.26, 3.30, and 3.37 ppm, $CH_2$ resonances at 3.66 and 3.88 ppm) and $^{13}C$-NMR spectra (with CH resonances at 99.9, 74.2, 77.5, 71.1, and 77.9 ppm and a $CH_2$ signal at 62.3 ppm) clearly showed the presence of $\beta$-*D*-glucose and leaving a molecular formula of $C_{11}H_{13}O_6$ for the aglycon. The carbon skeleton connectivities of the parent compound were established by combining DQF-COSY, HSQC and HMBC data, revealing **4** to be an iridoid with double bonds in both ring systems (C-3, C-4 and C-6, C-7) and a methoxycarbonyl residue in C-4. The coupling constants of H-1 and H-9 allowed to assign the relative stereochemistry at C-1, C-5, and C-9. The relative configuration at the quaternary carbon C-8 (iridoide



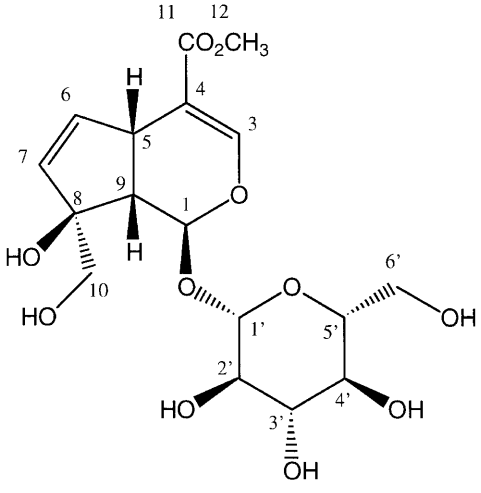

**1**




**2**




**3**




**4**

nomenclature) was determined by comparison with the published spectra [16] of the (7*R*)-isomer (monotropein-methylester, galioside, measured in $CDCl_3$, C-6 137.5 ppm, C-7 133.7 ppm, C-9 45.4 ppm) and the (7*S*)-isomer (8-epimonotropein-methylester, gardenoside, measured in $CDCl_3$, C-6 135.8 ppm, C-7 135.9 ppm, C-9 52.4 ppm) with the obtained data (measured in $CD_3OD$, C-6 135.8 ppm, C-7 135.7 ppm, C-9 51.9 ppm), allowing to identify **4** to be the latter isomer. A final comparison of the optical rotation allowed to rule out the possibility of having obtained the enantiomeric component. Gardenoside (**4**) has been already

isolated from different other Rubiaceae, e.g. from the fruits of *Rothmannia globosa* [11] and the leaves of *Gardenia jasminoides* Ellis *var. grandiflora* [17].

## Experimental

### *General*

Melting points were measured on a Büchi/Tottoli melting point apparatus and are uncorrected. IR spectra were scanned on a Perkin-Elmer 2000 FT-IR-Spectrometer. UV/VIS spectra were recorded on a Shimadzu UV-160A spectrometer. Optical Rotations were measured on a Perkin-Elmer 241 MC Polarimeter. The EI-MS (70 eV) were recorded on a platform-LCZ (Micromass). The FD-MS spectrum was recorded on a Finnigan MAT 900S. NMR spectroscopy was performed on a Varian Unity Inova 600 and a Varian Unity Inova 400 NMR spectrometer ($^{13}C$ spectra) for components **3** and **4** and on a Bruker DRX400 WB for components **1** and **2**. All instruments were equipped with either tuneable broadband inverse probes tuned to $^{13}C$ for the inverse detected gradient selected 2D experiments or dual $^{1}H/^{13}C$ probes for the 1D experiments. The measurements were carried out in $CD_3OD$ or $CDCl_3$ provided by Uetikon/Switzerland and the spectra were referenced to internal *TMS*. The sample temperatures were kept at 308 K (**3**), 303 K (**4**), and 300 K (**1**, **2**). Standard pulse sequence programs provided by the spectrometer manufacturer were used. The HMBC experiments were optimised for a long range coupling constant of 8 Hz.

### *Plant material*

The plant material used in this study was collected in November 1999 from the Rashad area in the Eastern Nuba Mountains, Kordofan State. It was authenticated by taxonomists at the Department of Botany, Faculty of Science, University of Khartoum and the Medicinal and Aromatic Herb Research Institute, National Council for Research. Voucher specimens were deposited at the Department of Botany, University of Khartoum and at the Institute of Pharmaceutical Chemistry and Pharmaceutical Technology, Karl-Franzens University, Graz.

### *Extraction and isolation*

The coarsely powdered air-dried leaves of *Xeromphis nilotica* were extracted with methanol and defatted with petroleum ether. The extract was subsequently fractioned with $CHCl_3$ and *n*-butanol. The $CHCl_3$ soluble fraction was subjected to Vacuum Liquid Chromatography (VLC) over silica gel and eluted with $CHCl_3$/MeOH mixtures of gradual increasing polarities, yielding subfractions A to F. Subfraction B was further subjected to column chromatography (CC) using again $CHCl_3$ and MeOH mixtures with increasing polarities followed by a gel-filtration on Sephadex LH-20 (MeOH as eluent) to give two major fractions $B_1$ and $B_2$. Fraction $B_1$ was re-chromatographied over silica and RP-18 to obtain compound **1** and **2** as a mixture. Fraction $B_2$ yielded after another purification step on silica gel compound **3** by crystallization from methanol. The *n*-butanol fraction was subjected to VLC and was eluted with increasing polarities of a $CHCl_3$/MeOH mixture to obtain subfractions A to C. Chromatography of subfraction C over silica and furthermore over RP-18 afforded compound **4** as an oil.

### *(2aR,4aR,5R,6R,7aR,7bR)-(+)-2a,4a,5,6,7a,7b-Hexahydro-2a,6-dihydroxy-2H-1,7-dioxacyclopent[cd]indene-5-carboxylic acid-methyl ester, α-Gardiol* (**1**) *and (2aR,4aR,5S,6R,7aR,7bR)-rel-(+)-2a,4a,5,6,7a,7b-Hexahydro-2a,6-dihydroxy-2H-1,7-dioxacyclopent[cd]indene-5-carboxylic acid-methyl ester, β-Gardiol* (**2**)

α-Gardiol (**1**) and β-gardiol (**2**) were isolated as inseparable oil. UV ($CH_3OH$) $\lambda_{max}$ nm ($\log \varepsilon$) = 209 (1.53); IR (KBr) $\nu_{max} = 3453$, 1710, 1081 $cm^{-1}$; EI-MS $m/z = 241$ (51, $M^+$) and 225 (100,

$M^+$–$CH_3$); FD-MS $m/z = 242$ ($MH^+$); $^1$H-NMR ($CD_3OD$, 400 MHz): **1**: $\delta = 5.55$ (d, 1H, $J = 6$ Hz, H-1), 5.18 (d, 1H, $J = 8$ Hz, H-3), 2.60 or 2.75 (m, 1H, H-4), 3.56 (m, 1H, H-5), 5.80 (s, 2H, H-6, H-7), 2.60 and 2.75 (m, 1H, H-9), 3.80 or 3.95 (dd, 2H, $J = 8$ Hz, H-10), 3.75 (s, 3H, H-12) ppm; **2**: $\delta = 5.51$ (d, 1H, $J = 6$ Hz, H-1), 5.38 (d, $J = 8$ Hz, 1H, H-3), 2.60 or 2.75 (m, 1H, H-4), 3.52 (dd, 1H, $J = 2$ Hz, H-5), 5.92 (dd, 1H, $J = 2$ Hz, H-6), 5.74 (dd, 1H, $J = 2$ Hz, H-7), 2.60 or 2.75 (m, 1H, H-9), 3.55 and 3.79 (dd, 2H, $J = 9$ Hz, H-10), 3.73 (s, 3H, H-12) ppm; $^{13}$C-NMR ($CD_3OD$, 100 MHz): **1**: $\delta = 101.8$ (d, C-1), 91.0 (d, C-3), 49.0 (d, C-4), 40.6 (d, C-5), 138.0 (d, C-6), 135.3 (d, C-7), 93.7 (s, C-8), 52.3 (d, C-9), 76.7 (t, C-10), 52.4 (q, C-12) ppm; **2**: $\delta = 101.4$ (d, C-1), 90.5 (d, C-3), 49.6 (d, C-4), 40.5 (d, C-5), 138.0 (d, C-6), 135.3 (d, C-7), 94.0 (s, C-8), 49.8 (d, C-9), 74.9 (t, C-10), 173.1 (s, C-11), 52.4 (q, C-12) ppm. The obtained experimental data were in good agreement with Refs. [10, 11].

*7-Hydroxy-6-methoxy-2H-1-benzopyran-2-one, Scopoletin* (**3**)

Scopoletin (**3**) was obtained from methanol as white crystalline solid. Physical and spectroscopic data were in good agreement with the literature [15].

*(1S,4aS,7S,7aS)-1-(β-D-Glucopyranosyloxy)-1,4a,7,7a-tetrahydro-7-hydroxy-7-(hydroxymethyl)-cyclopenta[c]pyran-4-carboxylic acid-methyl ester, Gardenoside* (**4**)

Gardenoside (**4**) was isolated as a yellowish oil with an optical rotation of $[\alpha]_D^{23} = -84.6°$ ($c = 0.52$, $CH_3OH$); UV ($CH_3OH$) $\lambda_{max}$ nm ($\log \varepsilon$) = 233.5 (1.82); IR (KBr) $\nu_{max} = 3399$, 1693, 1640, 1513 $cm^{-1}$; EI-MS $m/z = 427$ (56, $M^+$), 207 (36), 115 (100), 93 (48); $^1$H-NMR ($CD_3OD$, 400 MHz): $\delta = 5.74$ (d, 1H, H-1), 7.37 (s, 1H, H-3), 3.67 (m, 1H, H-5), 6.18 (dd, 1H, H-6), 5.74 (d, 1H, H-7), 2.61 (dd, 1H, H-9), 3.55 and 3.65 (d, 2H, H-10), 3.60 (s, 3H, H-12), 4.66 (d, 1H, H-1′), 3.18 (t, 1H, H-2′), 3.37 (t, 1H, H-3′), 3.26 (t, 1H, H-4′), 3.30 (m, 1H, H-5), 3.66 and 3.88 (m, 2H, H-6′) ppm; $^{13}$C-NMR ($CD_3OD$, 100 MHz): $\delta = 93.9$ (d, C-1), 151.6 (d, C-3), 111.1 (s, C-4), 38.4 (d, C-5), 135.8 (d, C-6), 135.7 (d, C-7), 85.8 (s, C-8), 51.9 (d, C-9), 66.6 (t, C-10), 168.5 (s, C-11), 52.3 (q, C-12), 99.9 (d, C-1′), 74.2 (d, C2′), 77.5 (d, C-3′), 71.1 (d, C-4′), 77.9 (d, C-5′), 62.3 (t, C-6′) ppm. The NMR data were in agreement with Ref. [16].

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