

Chemical Examination of the Leaves of *Nerium oleander*

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Abstract: Two aristolochic acids derivatives (1 and 2) and three aristolactam derivatives (3, 4 and 5). In addition to one methylparaben (6) were isolated from the plant and identified from the leaves of *Nerium oleander*. In addition to the fatty acid, in which compound (1) was being as a major constituents of the leaves according to the percentage yield. The objective of this study is to investigate the main chemical constituents of this plant species that were not reported hearing in mind that no similar previous work on the indigenous species has been reported in the literature.

Key words: *Nerium oleander*, apocynaceae, aristolochic acid, aristolactam and methylparaben

INTRODUCTION

As may be expected in a large country like Sudan a great variety of medicinal plants is indigenous and yield a very wide range of natural products of medicinal values.

In the present study we are reporting our attempts to investigate as a major line of interest the presence of chemical constitution of the natural products of a sudanese indigenous plant *Nerium oleander*.

It is an ornamental, evergreen shrub with leathery, dark green leaves. The plant grows naturally in central, eastern and western Sudan^[1] belongs to the family Apocynaceae^[2,3] in which the latex of it consist various minerals such as calcium, iron, potassium, magnesium, chloride and phosphorus^[4]. The species is known locally by the name, warad elhamir^[5]. The species as seen naturally it grows mainly near water flows^[6].

The plant is classified as medicinal plant^[7]. The leaves used in traditional medicine against snake bites and claimed to have cardiogenic, diuretic, antibacterial and insecticidal properties^[8] and as cure for boils and guineaworm^[9,10] and is mostly used in veterinary practice for toxicity in which all plant parts are toxic if ingested and they can cause death by heart paralysis^[11-14]. The plant is known to be poisonous^[15-20]. This is the first report in the chemical constituents of *Nerium oleander*.

MATERIALS AND METHODS

General method: Melting points were uncorrected and determined by Kolfler-hot stage microscope. Infra-red spectra were taken as potassium bromide disks using Perkin-Elmer FTIR model 1330 Infra-red spectrophotometer. The absorption bands were measured

in cm^{-1} . Ultra-Violet Visible spectra were carried out on Perkin-Elmer lambda 20, UV/VIS/NIR Spectrophotometer using absolute ethanol as solvent. ¹H-NMR spectra were recorded on a JEOL JNM-GSX 400 fourier-transform spectrometer mostly in DMSO using tetramethylsilane (TMS) as the internal standard and all the signals were reported as δ values and the signals were described in terms of chemical shift, multiplicity, coupling constant(s) where applicable, number of protons and assignment^[21]. Mass spectra were measured on Finnigan MAT SSQ 710 mass spectrometers.

Plant material: *Nerium oleander* was collected from Shambat area (Khartoum-North) and was identified by Dr. Abd Elgabar Nassir. Department of Botany Faculty of Education University of Khartoum. Herbarium specimen were deposited at Faculty of Education Herbarium.

Method of extraction: The fresh organ of the plant was dried and grounded. A sample of the grounded leaves was accurately weighed and then macerated for 24 hrs using stoppered conical flask with occasional vigorous shaking, the plant was extracted with petroleum ether (60-80°C) and the insoluble defatted material was dried at room temperature for 8 hrs and then extracted with methanol ($\times 4$) and water ($\times 2$) at room temperature. The combined methanol and water extracts were concentrated in vacuo to give a brown syrup (5.87 g), which was partitioned between water and chloroform. The chloroform layer was separated and directly chromatographed on silica gel column and eluted with a gradient of chloroform and methanol to afford 12 fractions. Fractions 6 and 7 were combined and crystallized from methanol to give compound 1 (8.0 mg). The main production fraction 8 was

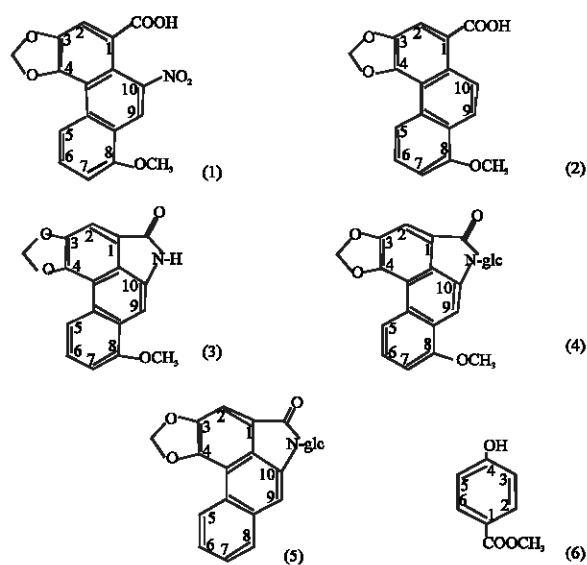


Fig. 1: The constituents isolated from the leaves of *Nerium oleander*

purified on a silica-gel column chromatography (benzene: ethylacetate = 14:1 v/v) as the eluent to afford compound 2 (0.8 mg). From fraction 9, compound 3 (1.0 mg) was isolated by preparative thin layer chromatography (silica-gel, benzene: acetone = 14:1). Compounds in fraction 12 were separated similarly to fraction 8 to obtain 4 (2.0 mg) and 5 (1.0 mg). The water layer was filtered and chromatographed on Sephadex LH-20 and eluted with a gradient of water and methanol to give 4 fractions. From fraction 2, compound 6 (5.0 mg) was obtained as a crystalline solid.

Structural determination of the isolated compounds: Six compounds as shown in (Fig. 1) including aristolochic acid (1) aristolochic acid (2) aristolactam (3) aristolactam-N-β-D-glucoside (4) aristolactam-11-N-β-D-glucoside (5) and methylparaben (6) were isolated from the leaves of *Nerium oleander*. Their structures were determined by spectral methods.

Aristolochic acid (1): Yellow needles, C₁₇H₁₁NO₇, m.p. 272-274°C. IR (cm⁻¹, KBr), 1701, 1593, 1521, 1467, 1269, 1150, 1041, 945. UV λ_{max} (EtOH, nm) 220 (a bsorbance 0.690), 250 (0.732), 315 (0.294) and 387 (0.131).

¹H-NMR (DMSO-d₆, 400 MHz): δ 8.66 (1H, d, J = 8.0 Hz, H-5), 8.59 (1H, s, H-9), 7.86 (1H, t, J = 8.0 Hz, H-6), 7.82 (1H, s, H-2), 7.38 (1H, d, J = 8.0 Hz, H-7), 6.50 (2H, s, OCH₂O), 4.07 (3H, s, OCH₃).

MS m/z (rel. int.): 342 (M⁺+1, 14), 341 (26), 295 (33), 280 (10), 263 (100), 262 (13), 177 (13), 150 (14), 131 (14), 106 (42), 78 (16), 57 (100).

Aristolochic acid (2): Pale-yellow needles, C₁₇H₁₂O₅, m.p. 290-291°C. IR (cm⁻¹, KBr), 2918, 1685, 1591, 1541, 1448, 1409, 1350, 1282, 1072, 983. UV λ_{max} (EtOH, nm) 222 (a bsorbance 0.868), 258 (0.867), 356 (0.236) and 374 (0.158).

¹H-NMR (DMSO-d₆, 400 MHz): δ 8.97 (1H, d, J = 9.8 Hz, H-10), 8.73 (1H, d, J = 8.6 Hz, H-5), 8.16 (1H, d, J = 9.8 Hz, H-9), 7.98 (1H, s, H-2), 7.61 (1H, dd, J = 8.6, 8.2 Hz, H-6), 7.21 (1H, d, J = 8.2 Hz, H-7), 6.44 (2H, s, OCH₂O), 4.05 (3H, s, OCH₃).

MS m/z (rel.int.): 297 (M⁺+1, 19), 296 (41), 219 (19), 176 (20), 149 (71), 57 (100).

Aristolactam (3): Yellow-green needles, C₁₇H₁₁NO₄, m.p. 298-300°C. IR (cm⁻¹, KBr), 3205, 1700, 1546, 1464, 1280, 1060, 938. UV λ_{max} (EtOH, nm) 230 (a bsorbance 0.765), 260 (0.491), 276 (0.245), 287 (0.300), 326 (0.324) and 392 (0.224).

¹H-NMR (DMSO-d₆, 400 MHz): δ 10.77 (1H, brs, NH), 8.14 (1H, d, J = 8.1 Hz, H-5), 7.65 (1H, s, H-2), 7.51 (1H, t, J = 8.1 Hz, H-6), 7.37 (1H, s, H-9), 7.20 (1H, d, J = 8.1 Hz, H-7), 6.47 (2H, s, OCH₂O), 3.99 (3H, s, OCH₃).

MS m/z (rel.int.): 293 (M⁺, 42), 278 (34), 263 (100), 262 (13), 177 (13), 150 (14), 131 (14), 106 (42), 78 (14), 57 (10).

Aristolactam-N-β-D-glucoside (4): Yellow needles, C₂₃H₂₁NO₉, m.p. 297-298°C. IR (cm⁻¹, KBr), 3400, 1690, 1610, 1430, 1363. UV λ_{max} (EtOH, nm) 239 (a bsorbance 0.765), 243 (0.491), 249 (0.245), 259 (0.300), 275 (0.324), 289 (0.245), 330 (0.542), 342 (0.224) and 394 (0.221).

¹H-NMR (DMSO-d₆, 400 MHz): δ 8.20 (1H, d, J = 8.2 Hz, H-5), 7.76 (1H, s, H-2), 7.63 (1H, s, H-9), 7.58 (1H, t, J = 8.2 Hz, H-6), 7.23 (1H, d, J = 8.2 Hz, H-7), 6.50 (2H, s, OCH₂O), 5.34 (1H, d, J = 9.2 Hz, anomeric proton), 5.26 (3H, m, OH), 5.14 (1H, d, J = 5.3 Hz, OH), 4.62 (1H, m), 4.01 (3H, s, OCH₃), 3.75 (2H, m), 3.30-3.40 (3H, m).

MS m/z (rel.int.): 456 (M⁺+1, 1).

Aristolactam-11-N-β-D-glucoside (5): Yellow needles, C₂₂H₁₉NO₉, m.p. 280-281°C. IR (cm⁻¹, KBr), 3442, 1679, 1616, 1419, 1369, 1197, 1058, 885. UV λ_{max} (EtOH, nm) 211 (a bsorbance 0.765), 232 (0.491), 264 (0.245), 277 (0.300), 288 (0.324), 328 (0.245), 342 (0.542), 374 (0.224) and 393 (0.221).

¹H-NMR (DMSO-d₆, 400 MHz): δ 8.57 (1H, m, H-5), 8.04 (1H, m, H-8), 7.64 (1H, s, H-2), 7.57 (2H, m, H-6 and H-7), 7.45 (1H, s, H-9), 6.51 (2H, s, OCH₂O), 5.32 (1H, d, J = 9.3 Hz, H-1), 5.22 (1H, d, J = 5.3 Hz, OH), 5.10 (2H, brd, J = 8.3 Hz, OH), 4.57 (1H, brd, J = 5.9 Hz, OH), 3.73 (2H, m), 3.10-3.50 (4H, m).

Methylparaben (6): Colorless needles, C₈H₈O₃, m.p. 129-131°C. IR (cm⁻¹, KBr), 3400, 1690, 1610, 1595, 1518. UV λ_{max} (EtOH, nm) 257 (a bsorbance 0.765), 310 (0.244).

¹H-NMR (DMSO-d₆, 400 MHz): δ 7.96 (2H, d, *J* = 8.6 Hz H-2 and H-6), 6.86 (2H, d, *J* = 8.6 Hz H-3 and H-5), 5.52 (1H, br. s, OH), 3.89 (3H, s, OCH₃).
MS *m/z* (rel.int.): 152 (M⁺, 44), 121 (100), 65 (24).

RESULTS AND DISCUSSION

The air-dried powdered leaves of *Nerium oleander* was extracted with methanol and water, each extract was concentrated and chromatographed on silica gel column to afforded six pure compounds which were identified by direct comparison of melting point, co-chromatography and spectral analysis with authentic samples.

Compound 1 was obtained from the methanol extract as yellow needles with melting point 272-274°C. ¹H-NMR revealed the presence of one methoxyl groups (singlets) at δ 4.07, two doublets at δ 8.66 and 7.38 with coupling constants 8.0 Hz corresponds to a proton attached to carbon 5 and carbon 7, the proton attached to carbon 6 appeared as triplet at δ 7.86. In addition to three singlets peaks at δ 8.59, 7.82 and 6.50 for proton at carbon 9 and carbon 2 the latest will be for methylene dioxide. The mass spectrum showed molecular ion peak at *m/z* 341 corresponding to the molecular formula C₁₇H₁₁NO₇. Based on the spectral data and comparison with previous study^[22,23] compound 1 was found to be aristolochic acid.

Compound 2 it was obtained as pale yellow needles with melting point 290-291°C^[24]. ¹H-NMR indicated the presence of methoxy group appeared as singlet at δ 4.05 indicating the presence of three protons. The other singlet appeared at δ 7.98 for the proton at carbon 2 where as the proton at carbon 6 appeared as doublet of doublet at δ 7.61 with coupling constants 8.6 and 8.2 Hz. The proton at carbon 10, carbon 5, carbon 9 and carbon 7 appeared as doublets at δ 8.97, 8.73, 8.16 and 7.21 with coupling constants 9.8, 8.6, 9.8 and 8.2 Hz, respectively. The mass spectrum of the investigated compound showed molecular ion peak at *m/z* 296 with a base peak at *m/z* 57 which confirmed the molecular formula C₁₇H₁₂O₅. Based on ¹H-NMR, infrared and mass spectral features, aristolochic acid was assigned for compound 2.

Compound 3 was isolated as yellow-green needles with melting point 298-300°C^[25,26]. ¹H-NMR revealed the presence of NH group appeared as a broad at δ 10.77, the singlet protons at δ 7.65 and δ 7.37 indicated the presence of two protons at position 2 and 9, the two equivalent protons at carbon 5 and carbon 7 present as doublet at δ 8.14 and δ 7.20 with coupling constant 8.1 Hz. The methoxy group at carbon 8 will appeared as singlet having three protons at δ 3.99. The molecular formula of the compound was found to be C₁₇H₁₁NO₄ which was confirmed by the fragment at 293 with a base peak at the

fragment 263. According to the above mention data comparing with the previous studies^[27,28] compound 3 was estimated to be aristolactam.

Compound 4 was recrystallised from methanol as yellow needles with a melting point 297-298°C. Mass spectral data showed a molecular ion peak at *m/z* 455 expected for C₂₃H₂₁NO₉. Strong IR absorptions were observed at 1690 cm⁻¹ indicating the presence of a carbonyl group. Other intense absorptions were observed at 3400. The ¹H-NMR spectrum showed a peaks at δ 5.34 appeared as doublet for anomeric proton with coupling constant 9.2 Hz. In addition the proton attached to carbon 5 appeared as doublet with coupling constant 8.2 Hz at δ 8.20. The singlet peaks at δ 7.76 and δ 7.63 attributed to the two protons at carbon 2 and carbon 9, respectively. The structural assignment of compound 4 was assigned to be aristolactam-N-β-D-glucoside.

Compound 5 was isolated as yellow needles with melting point 280-281°C. The IR spectrum indicated the presence of hydroxyl group at 3442 cm⁻¹ which occurred as a strong and broad band. The presence of carbonyl functionality and CO stretching were indicated by the absorptions at 1679 cm⁻¹. The UV absorptions maxima at 393 nm suspected the presence of aristolactam nucleus. The ¹H-NMR spectrum of compound 5 gave doublet at δ 5.32 was assigned to the H-1. The two protons at carbon 4 and carbon 5 occurred as singlets at δ 6.51 indicated the presence of methylene dioxide. Mass spectrum gave a molecular ion peak at *m/z* 425 which is consistent with the molecular formula C₂₂H₁₉NO₈. Based on these spectral data this aristolactam was found to be identical with aristolactam-11-N-β-D-glucoside.

Compound 6 was found to be as colorless needles with melting point 129-131°C. The infrared spectrum indicated the presence of hydroxy group at 3400 cm⁻¹. The ¹H-NMR indicated the appearance of four protons at carbon 2, carbon 6, carbon 3 and carbon 5 appeared as doublet at δ 7.96 and δ 6.86 with coupling constant 8.6 Hz. Where as the hydroxy group at carbon 4 will indicated by the broad singlet peak at δ 5.52. The mass fragment at *m/z* 152 of composition C₈H₆O₃ indicated a benzenoid skeleton. Based on the above discussed spectroscopic data compound 6 could be identified as methylparaben.

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