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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 a ddition 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 constituation 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 cardiotonic, 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-Elemer FTIR model 1330 Infra-red spectrophotometer. The absorption bands were measured in cm^{-1}. Ultra-Violet Visible spectra were carried out on Perkin-Elemer lambda 20, UV/VIS/NIR Spectrophotometer using absolute ethanol as solvent. ^{1}H-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 occussional 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 (×4) and water (×2) at room temperature. The combined methanol and water extracts were concentrated in vacuo to give abrown syrup (5.87 g), which was partitioned between water and chloroform. The chloroform layer was separated and directly chromatographed on asilica gel column and eluted with agradient 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

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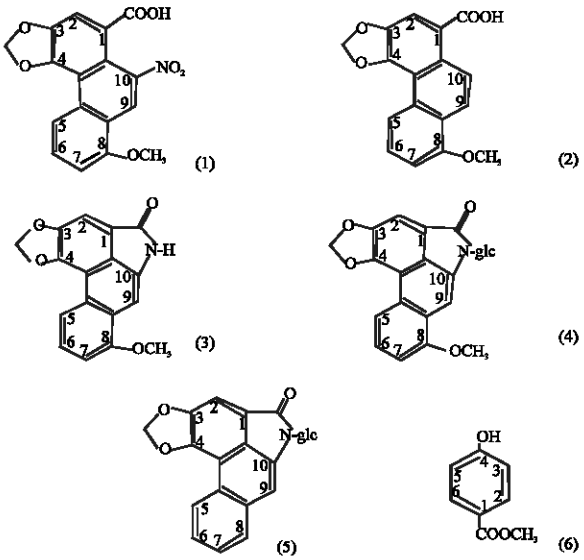

Fig. 1: The constituents isolated from the leaves of *Nerium oleander*

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.

Strutural 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, $C_{17}H_{11}NO_7$, 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).

^{1}H-NMR (DMSO-d_6, 400 MH_Z): δ 8.66 (1H, d, J = 8.0 H_Z, H-5), 8.59 (1H, s, H-9), 7.86 (1H, t, J = 8.0 H_Z, H-6), 7.82 (1H, s, H-2), 7.38 (1H, d, J = 8.0 H_Z, H-7), 6.50 (2H, s, OCH_2O), 4.07 (3H, s, OCH_3).

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, $C_{17}H_{12}O_5$, 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).

^{1}H-NMR (DMSO-d_6, 400 MH_Z): δ 8.97 (1H, d, J= 9.8 H_Z, H-10), 8.73 (1H, d, J = 8.6 H_Z, H-5), 8.16 (1H, d, J = 9.8.H_Z, H-9), 7.98 (1H, s, H-2), 7.61 (1H, dd, J = 8.6, 8.2 H_Z, H-6), 7.21 (1H, d, J= 8.2 H_Z, H-7), 6.44 (2H, s, OCH_2O), 4.05 (3H, s, OCH_3).

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

Aristolactam (3): Yellow-green needles, $C_{17}H_{11}NO_4$, 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).

^{1}H-NMR (DMSO-d_6, 400 MH_Z): δ 10.77 (1H, brs, NH), 8.14 (1H, d, J = 8.1 H_Z, H-5), 7..65 (1H, s, H-2), 7.51 (1H, t, J = 8.1 H_Z, H-6), 7.37 (1H, s, H-9), 7.20 (1H, d, J= 8.1 H_Z, H-7), 6.47 (2H, s, OCH_2O), 3.99 (3H, s, OCH_3).

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, $C_{23}H_{21}NO_9$, 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).

^{1}H-NMR (DMSO-d_6, 400 MH_Z): δ 8.20 (1H, d, J = 8.2 H_Z, H-5), 7.76 (1H, s, H-2), 7.63 (1H, s, H-9), 7.58 (1H, t, J = 8.2 H_Z, H-6), 7.23 (1H, d, J = 8.2 H_Z, H-7), 6.50 (2H, s, OCH_2O), 5.34 (1H, d, J = 9.2 H_Z, anomeric proton), 5.26 (3H, m, OH), 5.14 (1H, d, J = 5.3 H_Z, OH), 4.62 (1H, m), 4.01 (3H, s, OCH_3), 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, $C_{22}H_{19}NO_8$, 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).

^{1}H-NMR (DMSO-d_6, 400 MH_Z): 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, OCH_2O), 5.32 (1H, d, J = 9.3 H_Z H-1), 5.22 (1H, d, J = 5.3 H_Z OH), 5.10 (2H, brd, J = 8.3 H_Z OH), 4.57 (1H, brd, J = 5.9 H_Z OH), 3.73 (2H, m), 3.10-3.50 (4H, m).

Methylparaben (6): Colorless needles, $C_8H_8O_3$, 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).

^{1}H-NMR (DMSO-d_6, 400 MH_Z): δ 7.96 (2H, d, J = 8.6 H_Z H-2 and H-6), 6.86 (2H, d, J = 8.6 H_Z H-3 and H-5), 5.52 (1H, br. s, OH), 3.89 (3H, s, OCH_3).
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. ^{1}H-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 H_Z 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 peakes 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 coorsponding to the molecular formula $C_{17}H_{11}NO_7$. 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]. ^{1}H-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 H_Z. 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 H_Z, 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 $C_{17}H_{12}O_5$. Based on ^{1}H-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]. ^{1}H-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 H_Z. 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 $C_{17}H_{11}NO_4$ 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 recrstallised 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 $C_{23}H_{21}NO_9$. Strong IR absorptions were observed at 1690 cm^{-1} indicating the presence of a carbonyl group. Other intense a bsorptions were observed at 3400. The ^{1}H-NMR spectrum showed a peaks at δ 5.34 appeared as doublet for anomeric proton with coupling constant 9.2 H_Z. In addition the proton attached to carbon 5 appeared as doublet with coupling constant 8.2 H_Z 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 ^{1}H-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 $C_{22}H_{19}NO_8$. 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 ^{1}H-NMR indicated the apperance 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 H_Z. 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 $C_8H_8O_3$ indicated a benzenoid skeleton. Based on the above discussed spectroscopic data compound 6 could be identified as methylparaben.

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REFERENCES

1. Thonner, R.F., 1988. Flowering plants of Africa. Dulauand Company Ltd. 37 Soho. Square, London, pp: 176.
2. Omino, E.A. and L.G. Maesen, 1996. The plant family Apocynaceae in East Africa. The biodiversity of African plans. Proceedings of the 14th AETFAT Congress Netherlands, pp: 504-506.
3. Andrews, F.W., 1985. Flowering plants of the Anglo-Egyption Sudan, T. Runcle and company Ltd., Arbroathscotland, pp: 1-21.
4. Jayabalan, M., K. Rajarathinam, G. Augustus, T. Sekar and S. Veerasamy, 1995. Analysis of minerals by EDAX in the latex of Apocynaceae. J. ecotoxicol. and environmental monitoring, 5: 45-49.
5. Hassan, M.H., 1974. An illustrated Guide to the Plants of Erkoutt, Khartoum University Press, pp: 13.
6. Chandra, D.I. and K.N. Rao, 1996. PANS, Pest Articles and New Summaries, 29: 223-229.
7. Mulas, M., B. Perinu, A. Francesconi, C. Johnson and C. Franz, 2002. Evaluation of spontaneous *Nerium oleander* and *Nerium indicum* as a medicinal plant. J. Herbs Species and Med. Plants, 9: 121-125.
8. Sushma, S., D. Singh and S. Singh, 1997. Molluscicidal activity of *Nerium indicum* leaf. Fitoterapia, 68: 545-546.
9. Irvine, R.N., 1994. Glossary of Indian medicinal plant (CSIR), New Delhi, pp: 24.
10. Sofowoya, A.M., 1982. Medicinal plants and traditional medicine in Africa, John Wiley and Sons Ltd., England, pp: 196-197.
11. Taklitajan, A., 1998. Woody Plants of Ghana and Africa. Oxford University Press. London, pp: 37.
12. Elsalm, E.B., 1998. Veternary and Human Toxicol., pp: 29-133.
13. Haeba, M., A. Mohamed, A. Mehdi and G. Nair, 2002. Toxicity of *Nerium oleander* leaf extract in mice. J. Environmental Biology, 23: 231-237.
14. Adam, S., M. Alyahya and A. Alfarhan, 2002. Toxicity of *Nerium oleander* in sheep. American J. Chiness Med., 30: 255-262.
15. Hughes, K., A. Dart and D. Hodgson, 2002. Suspected *Nerium oleander* poisoning in a horse. Australian Vet. J., 80: 412-415.
16. Priestap, H.A., 2001. Phytochemistry, pp: 26-519.
17. Watt, J.M. and M.G. Breyer, 1986. Poisonous plants of Southern and Eastern Africa, 2nd Edn., E. and S. Living Stone Ltd., London, pp: 118.
18. Karunanidhi, P., N. Sunder, J. Reddy and P. Choudhuri, 1997. Nerium poisoning in bovines., Indian Vet. J., 74: 977-978.
19. Monzani, V., A. Rovellini, G. Schinco and E. Rampoldi, 1997. Acute oleander poisoning after a self prepared tisane. J. Toxicology. Clinical Toxicology, 35: 667-668.
20. Galey, F., D. Holstege, K. Plumlee, E. Tor, B. Johnson, M. Anderson, P. Blanchard and F. Brown, 1996. Diagnosis of oleander poisoning in livestock. J. vet. Diagnostic Investigation, 8: 358-364.
21. Beat, B., A.J. Clemens, D. Wright, T. Rali and S. Otto, 1990. An antimicrobial alkaloid from Ficus septica. Phytochemistry, 29: 3327-3330.
22. Breit, B. and W. Voelter, 1974. ^{1}H-NMR spectroscopy methods and application. Bergstr. Verlagchemie Gmb H, pp: 102.
23. Yamauchi, T., K. Minato and F. Abel, 1996. Presence of alkaloids and ursolic acid from oleander leaves. Phytochemistry, 42: 45-49.
24. Buckingham, J., 1994. Dictionary of natural products 2: Chapman and Hall. London, pp: 1623.
25. Naherstedt, A., P. Prokch and E. Conn, 1987. Aristoloctam and aristoloctam glycosides from *Chamaebatia* sp. Phytochemistry, 26: 1546-1547.
26. Munasingle, V., F. Pattendan, C. Rhodes and D. Roberts, 1974. Dictionary of Natural Products 4: Chapman and Hall. London, pp: 4889.
27. Ding, K., J. Fang, T. Dong and W. Tasim, 2003. Characterisation of the compounds isolated from *Nerium oleander* and their activities on PC12 pheochromocytoma cells. J. natural prod., 66: 7-10.
28. Ikhlas, A.K., T. Rali and O. Sticher, 1993. Alkaloids from *Ficus pachyrhachis*. Planta Medica, pp: 59-286.