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Phytochemical Investigation of *Arnebia Nobilis*

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An anthraquinone and a long chain alkane have been isolated from the *n*-hexane extract of the dried roots of *Arnebia nobilis* whereas 4-hydroxybenzoic acid and an isoquinoline alkaloid have been isolated from methanol and acidic methanol respectively.
Key Words: Phytochemical investigation, *Arnebia nobilis*.

INTRODUCTION

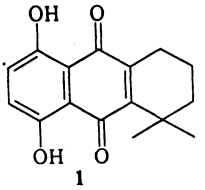
Arnebia is a genus of hispid herbs and belongs to the family *Boraginaceae*[1]. They are mostly confined to Asia, with a few species occurring in the drier parts of North Africa. Several species of *Arnebia* are known to occur in India[1, 2]. The air dried roots of *Arnebia nobilis* have been used for wound healing from ancient times[3] and have been found to be good food colorants[4]; hence the systematic isolation and characterization of natural colouring materials and other components has been reported.

The air-dried roots of *Arnebia nobilis* (Hindi: Ratanjot) were percolated with *n*-hexane for 48 h at room temperature. The solvent was removed under reduced pressure to get dark red viscous residue. The silica gel chromatographic separation of the above residue with *n*-hexane, *n*-hexane-benzene, benzene and benzene-chloroform gave seven naphthoquinone derivatives isolated earlier (arnebin-1 to arnebin-7)[2, 5–7] apart from 1,4-dihydroxy-5,5-dimethyl-5,6,7,8-tetrahydro-9,10-anthraquinone (**1**) and eicosane (**2**) which were isolated for the first time from the roots of *A. nobilis*. The structures of the isolated compounds have been confirmed by various spectroscopic techniques.

The marc after the *n*-hexane percolation was further percolated with methanol at room temperature for 24 h. The solvent was removed under reduced pressure and the residue on chromatographic separation gave three naphthoquinones (arnebin-1, arnebin-7 and arnebin-3) and a colourless pure compound which was identified as 4-hydroxybenzoic acid by m.m.p., superimposable IR and comparison with authentic sample on TLC.

The marc after methanol percolation was extracted with ethanol containing 0.5% HCl in a Soxhlet extractor for 48 h. The extract was concentrated at reduced

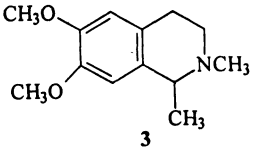
pressure and diluted with 5% HCl. The solution was filtered and extracted with ether until fresh extract was colourless, basified with ammonia solution and continuously extracted with chloroform. The chloroform layer was dried over anhydrous sodium sulphate and concentrated at reduced pressure. The crude compound was chromatographed on neutral alumina twice using petroleum ether : benzene (1 : 1) as eluent to get a thick brown liquid identified as 6,7-dimethoxy-1,2-dimethyl-1,2,3,4-tetrahydroisoquinoline (**3**) apart from a mixture of other unidentified compounds. The chromatographic separation and identification of other compounds are in progress.



1

$CH_3(CH_2)_{18}CH_3$

2



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EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IR 435 spectrometer and the ν_{max} is expressed in cm^{-1}. The electronic spectra were recorded on a Shimadzu UV-260 spectrophotometer and the λ_{max} are expressed in nanometers. The ^1H NMR spectra were recorded on Bruker Avance 300 spectrometer using TMS as internal standard (chemical shifts in ppm). All the required solvents were purchased and distilled before use.

Isolation with *n*-hexane

Air-dried, powered roots of *A. nobilis* (1 kg) were extracted by percolation with *n*-hexane (2.5 L) twice. The combined solvent was removed under reduced pressure to obtain a dark red viscous residue (10 g). The residue was chromatographed on silica gel (60–120 mesh) by using different solvents. The elution of the column with *n*-hexane gave two colourless compounds, eicosane[8] and heptacosanic acid[9]. Further, elution of the column with *n*-hexane : benzene (3 : 1) gave a red coloured 5,8-dihydroxy - 2-(1′-β,β-dimethylacryloxy - 4′-methylpent-3′-enyl)-1,4-naphthoquinone (arnebin-1) (28 mg, m.p. 116°C; lit.[5] mp. 116–117°C) and with *n*-hexane : benzene (3 : 2) gave a mixture of two compounds. The two compounds 5,8-dihydroxy-2-(4′-methylpent-3′-enyl)-1,4-naphthoquinone (arnebin-7) (24 mg, mp. 96°C; lit.[6] m.p. 95°C) and 1,4-dihydroxy-5,5-dimethyl-5,6,7,8-tetrahydro-9,10anthraquinone (12 mg; m.p. 78°C; lit.[6] m.p. 78°C; UV-Vis (C_2H_5OH) λ_{max} (log ε): 224 (3.99), 280 (4.45), 487 (2.22) and 514 (3.19) nm; ^1H NMR ($CDCl_3$): 1.53 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 2.19-2.26 (m, 4H, H-6 and H-7), 2.56 (t, 2H, H-8), 7.12 (s, 2H, H-2 and H-3), 12.38 (s, 1H, OH) and 12.54 (s, 1H, OH); MS, m/z (rel. Int.): 272 (M^+, 100), 257 (15), 229 (92),

190 (5), 108 (26) and 136 (11), were separated by preparative TLC using *n*-hexane : ethylacetate (9 : 1) as the mobile phase. The column was further eluted with *n*-hexane : benzene (1 : 1) to give 2-(1′-acetoxy-4′-methylpent-3′-enyl)-5,8-dihydroxy-1,4-naphthoquinone (arnebin-3) (18 mg, m.p. 103°C, lit[7] m.p. 104-105°C). Further elution of the column with *n*-hexane : benzene (1 : 4) gave β-sitosterol (39 mg, m.p. 135-136°C, lit.[10] mp. 136°C). The column was further eluted with benzene to give 5,8-dihydroxy-2-(1′-hydroxy-4′-methylpent-3′-enyl)-1,4-naphthoquinone (arnebin-4) (18 mg, m.p. 145°C, lit.[7] m.p. 146°C) and with benzene : chloroform (3 : 1), followed by preparative TLC (chloroform) to get 5,8-dihydroxy-2-(1′-β,β-dimethylacryloxy-4′-hydroxy-4′-methylpentyl)-1,4-naphthoquinone (Arnebin-2) (8 mg, m.p. 93°C, lit.[7] m.p. 92–94°C) and 5,8-dihydroxy-2-(4′-hydroxy-4′-methylpentyl)-1,4-naphthoquinone (arnebin-5) (13 mg, m.p. 112–113°C, lit.[5] m.p. 111–112°C). Finally, the column was eluted with benzene : chloroform (1 : 1) to give 2-(1′-acetoxy-4′-hydroxy-4′-methylpentyl)-5,8-dihydroxy-1,4-naphthoquinone (arnebin-6) (13 mg, mp. 87–88°C, lit.[5] mp. 88–90°C).

Isolation with methanol

The marc after the *n*-hexane percolation was percolated with methanol at room temperature for 24 h. The solvent was removed under reduced pressure to get dark red viscous residue. The silica gel chromatographic separation of the dark-red residue gave arnebin-1, arnebin-7 and arnebin-3 along with 4-hydroxybenzoic acid.

Isolation with acidic ethanol

The roots (500 g) were powdered and extracted with ethanol containing 0.5% HCl (1 L) in a Soxhlet extractor for 48 h. The extract was concentrated at reduced pressure and diluted with 5% HCl (100 mL). The solution was filtered and extracted with ether until fresh extract was colourless, basified with ammonia solution and continuously extracted with chloroform. The chloroform layer was dried over anhydrous sodium sulphate and concentrated at reduced pressure. The crude compound (545 mg) was chromatographed on neutral alumina twice using petroleum ether : benzene (1 : 1) as eluent to get a thick brown liquid which has been characterized as 6,7-dimethoxy-1,2-dimethyl-1,2,3,4-tetrahydroisoquinoline (**3**) (29 g, thick brown liquid (lit.[11] oil); ^{1}H NMR ($CDCl_3$): 1.30 (d, 3H, 1-CH_3), 2.90 (s, 3H, NCH_3), 3.50–3.80 (m, 4H, 3- and 4-CH_2), 3.90 (s, 6H, 2 × OCH_3), 4.50 (q, 1H, 1-H) and 6.81 (s, 2H, ArH); MS, m/z (rel. Int.): 221 (M^+, 5), 207 (15), 206 (100), 190 (10), 178 (5), 162 (7), 103 (9), 91 (8), 77 (9) and 58 (20).

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REFERENCES

1. Anonymous, The Wealth of India: Raw Materials, Vol. IA, pp. 429–30, PID, CSIR, New Delhi (1985).
2. A. Srivastava, Y.N. Shukla and S. Kumar, *J. Med. Arom. Plant Sci.*, **21**, 1131 (1999).
3. V.P. Papageorgiou, A.N. Assimoppoulou, E.A. Couladouros, D. Hcpworth and K.C. Nicolaou, *Angew. Chem. Int. Ed. Engl.*, **38**, 270 (1999).
4. B.S. Henry, *Trop. Sci.*, **21**, 206 (1979).
5. Y.N. Shukla, J.S. Tandon, D.S. Bhakuni and M.M. Dhar, *Phytochem.*, **10**, 1909 (1971).
6. Y.N. Shukla, J.S. Tandon and M.M. Dhar, *Indian J. Chem.*, **11**, 528 (1973).
7. Y.N. Shukla, J.S. Tandon, D.S. Bhakuni and M.M. Dhar, *Experientia.*, **25**, 357 (1969).
8. Dictionary of Organic Compounds, 5th Edn., Chapman Hall, London, Vol. 3, p. 3276 (1982).
9. Dictionary of Organic Compounds, 5th Edn., Chapman & Hall, London, Vol. 3, p. 2850 (1982).
10. A.M. Osman, M.E. Younes and A. Mokhtar, *Austr. J. Chem.*, **28**, 217 (1975).
11. S.D. Brown, J.E. Hodgkins, J.L. Massingill (Jr.) and M.G. Reinecke, *J. Org. Chem.*, **37**, 1825 (1972).

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