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RESEARCH ARTICLE

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QUINONES AND GLYCOSIDE FROM *VENTILAGO BOMBAIENSIS Dalz*

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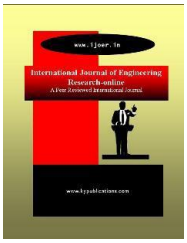


ABSTRACT
Three polar chemical constituents are isolated from the acetone extract of root bark of *ventilago bombaiensis*. Among them two are benzisochromanquinones and one glycoside; their structures have been established from their spectral data.
Key words: Ventilago bombaiensis; Rhamnaceae, Benzisochromanquinones, Glycoside.

INTRODUCTION

Ventilago bombaiensis Dalz (Family: Rhamnaceae), a large evergreen climber, is found throughout the hotter parts of India, with rambling stems upto 25cm diameter and 30m height, climbing by means of strong woody tendrils. Bark of the stem is light brown in colour, rough with deep longitudinal fissures. The sap is useful for the treatment of deafness. The tendrils of the plant are used in the treatment of tooth-ache. The oil obtained from the seeds is used for cooking purposes. The reddish root bark is considered to be useful as a cure for skin diseases. The juice of the bark and young shoots are applied to the body to banish the pains accompanied by malarial fever.

Experimental: Silica gel-G and Silica gel (100-200 mesh;Acme,India) were used for TLC and CC, respectively. Plant material was collected from Mukkali forest-Coimbatore (Tamilnadu). The species was identified with the voucher specimen preserved in the Herbarium,BSI Coimbatore.

Extraction and Purification: The bark from roots was peeled off and dried under shade. The dried bark was milled and the powder (3.5kg) of ventilago bombaiensis was extracted with acetone.

The acetone extract (125g) was dissolved in minimum volume of acetone (300 ml), impregnated on silica gel (250g) and transferred to the top of silica gel column (500g) set up with Benzene-Hexane (1:1). The column was eluted successively with Benzene-Hexane (1:1), benzene and benzene with increased quantities of Ethyl acetate (9:1, 4:1 and 1:1 and ethyl acetate. Fractions in 200ml were collected monitoring by TLC. The polarity of the solvent was increased whenever the eluate became pale in colour. Fractions exhibiting similar spot pattern on TLC plate were grouped.

The fractions eluted with benzene-ethyl acetate (1:1) showed a Violet streak on ordinary silica gel plate. This violet streak separated into two spots with R_f values 0.74 and 0.72 when examined on 3% oxalic acid impregnated silica gel plate using benzene-ethyl acetate (95:5) as developing solvent. The residue dissolved in acetone and applied on TLC plates, two coloured bands formed on the plates and these were

scrapped. The residue corresponding to the upper violet band crystallized from benzene as violet prisms (R_f value 0.74), was designated as **compound-A.** The compound corresponding to the lower orange red band crystallized from aqueous methanol as red needles (R_f value 0.72), was designated as **compound-B.**

The fractions eluted with ethyl acetate showed one yellow spot with R_f value 0.54 (Chloroform – methanol 4:1). It is further purified by column chromatography and the compound obtained is crystallized from methanol as yellow needles. It was designated as **compound-C.**

Results and Discussion

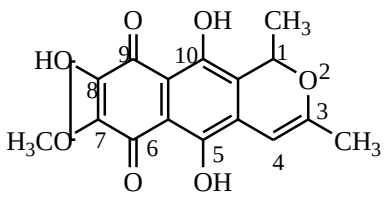
Compound-A is a naphthoquinone, its elemental analysis and high resolution mass spectrum (M^+ at m/z 318.0785, $C_{16}H_{14}O_7$ requires 318.0739) suggest the molecular formula $C_{16}H_{14}O_7$. It is optically active $[\alpha]_D^{30}618.5^o$($CHCl_3$, c 0.01). Reversible and reduction and oxidation indicated the presence of quinone system[1,2] in the molecule. The phenolic nature of this indicated by positive color reaction with ferric chloride. Compound-A gave a positive methanolic magnesium acetate test[3] suggesting it to be a hydroxylquinone. Its UV-Visible data indicated it to be a naphthazarin derivative and it is supported by the IR band at 1613 cm^{-1} [4]. The methylation and acetylation reactions indicated the presence of three hydroxyls in compound-A. This compound answers positive zirconyl nitrate test for vicinal hydroxyl system in the molecule. The IR band at 3323 cm^{-1} shows the presence of chelated hydroxyl groups. Formation of complexes with Mg^{+2}, Cu^{+2} also supports the peri-hydroxy quinonoid system in the molecule. Other spectral data also support this assignment i.e., the ^{1}H NMR spectrum shows two low field signals at 11.90 and 12.99 for two chelated hydroxyls groups. The signal at 7.05 which disappears by the addition of D_2O can be attributed to third hydroxyl group. All these data indicate the presence of three hydroxyl groups in Compound-A.

Its 360 MH_z spectrum in $CDCl_3$ shows the presence of a methoxyl group (4.14, s, 3H). The chemical shift of methoxyl group is indicative of its location on the quinine ring [5]. The NMR spectrum did not show any signal attributable to the protons on quinine or benzene ring suggesting that the naphthazarin system is fully substituted. The natural abundance of ^{13}C FT- NMR spectrum shows signal at 60.56 for methoxyl carbon. It also shows signals at 180.28 and 184.01 for two carbonyl carbons. A high field methyl doublet at 1.42 (J=6.6 H_z) and one proton quartet at 5.69 (J=13.19, 6.58 H_z) deshielded by ethereal oxygen atom are coupled to each other and suggest the presence of CH_3-(CH-)-O grouping. The presence of $(M^+ - 15)$ fragment ion in its mass spectrum also supports the grouping in the molecule [6]. Further, ^{1}H NMR spectrum shows low field methyl singlet (1.99) and a proton broad singlet (5.97). ^{13}C NMR signals at 18.53, 20.73 for two methyl carbons, 69.32, 93.63 for two methane carbons and 153.43 for one quaternary carbon are compatible with the structure assignment. The heterocyclic ring can only be α-pyran on the basis of biogenetic considerations and also by chemical reactions.

Hence compound-A can be assigned the structure 5,7,10-trihydroxy-8-methoxy (or 5,8,10-trihydroxy-7-methoxy)-6,9-dihydronaphtho[2,3-c]pyran-6,9-dione (I).

This compound is reporting first time from the root bark of ventilago bombaiensis. Earlier it is reported from ventilago goughii[7].

Compound-A: It is crystallized from benzene-hexane as violet prisms. Melting point: 169^0, R_f value 0.74 (benzene-ethyl acetate 95:5), $[\alpha]_D^{30}618.5^o$ ($CHCl_3$, c 0.01).It is sparingly soluble in hexane, moderately in benzene and methanol and fairly in acetone, chloroform and ethyl acetate.



Analysis: Found: c, 60.40; H, 4.40; $C_{16}H_{14}O_7$
Requires: c, 60.38; H, 4.43%

UV-Visible Data : λ_{max}^{MeOH} (log ε): 258 (4.24), 392(3.69), 502 sh(4.03),535(4.0),576sh(3.73)nm.

λ_{max}^{MeOH} (log ε): 258 (4.59), 390(3.85), 506 sh(4.02),540(4.11),580sh(4.04)nm.

IR Data υ_{max}^{KBr}: 3323,1651,1613 (SH),1589(Very broad).

^{1}H NMR Data: (360 MH_z, CDCl3): 1.42(d,J=6.6H_z, 3H;CH_3-1), 1.99(s,3H; CH_3-3), 4.14(s,3H;-O CH_3), 5.69(q,J=13.19H_z, 6.58 H_z;1H;H-1), 5.97 (broad s, 1H;H-4), 11.90(s,1H; exchangeable with D_2O;peri-OH), 12.99 (s,1H; exchangeable with D_2O;peri-OH)

Mass Spectral Data: (M^+.) at m/z 318(48). Found 318.0785, $C_{16}H_{14}O_7$ requires 318.0739). Other fragment ions at m/z 303 (100, Found 303.0499, $C_{15}H_{11}O_7$ requires 303.0502, M^+ - CH_3), m/z at 275 (41.4), found 275.0570, $C_{14}H_{11}O_6$ requires 275.0554, M^+ -$COCH_3$), m/z at 260 (15), Found 260.0322, $C_{13}H_8O_6$ requires 260.0319), m/z at 290 (1.0), m/z at 262 (1.7).

Colour Reactions

Compound-A: Compound-A produces pink color in concentrated sulphuric acid. With aqueous sodium hydroxide it gives blue coloration and the color gets bleached by the addition of sodium dithionite. It produces violet color with ferric chloride and pink color with methanolic magnesium acetate. In acetic acid it forms violet solution. On addition of zinc, the solution becomes colorless. The solution regains its original violet colour within few minutes after removal of zinc by filtration.

Compound-B: Compound-B gave positive methanolic magnesium acetate test indicating the presence of hydroxyquinone nature. Formation of blue colored solutions with aqueous sodium hydroxide clearly indicated it to be a naphthazarin. It is also evinced by UV-visible and IR data. ^{1}H NMR spectra showed that the presence of two peri hydroxyls, a methoxyl and [2,3-c] dimethylpyran system. The presence of third hydroxyl group was indicated by the solubility of compound-B in aqueous sodium bicarbonate solution. From the comparison of R_f values, spectral data and melting point it appeared to be ventiloquinone-C (II). Compound-B is obtained by catalytic hydrogenation (10% palladium on charcoal) of compound-A, was found to be identical with ventiloquinone(c) isolated from ventilago maderaspatana[8] and also later in Ventilago goughii[7]. Hence structural discussion of compound-B is not necessary and is almost similar with that of compound-A.

This compound is not reported earlier and not published in any journal from the root bark of ventilago bombiensis.

Examination of Compound-B: Compound-B crystallizes from aqueous methanol as red needles. Melting point: 137^{0}C. R_f value 0.72 (benzene-ethyl acetate 95:5). It appears as a red streak on silica gel plate. $[\alpha]_D^{25}648.5^o$ ($CHCl_3$, c 0.12). It is highly soluble in benzene, chloroform, acetone and ethyl acetate but sparingly soluble in hexane.

Analysis: Found: C, 59.84; H, 4.98; OCH_3 $C_{16}H_{16}O_7$
Requires: C, 60.00; H, 5.04; 1- OCH_3, 9.69%

UV-Visible Data:

λ_{max}^{EtOH} (log ε): 250 (4.10), 308(3.68), 461(3.65), 488(3.71), 526(3.57) and 565(3.14)nm.

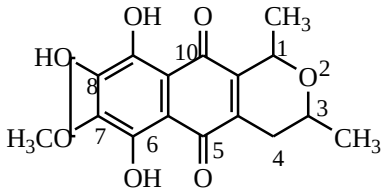
IR Data υ_{max}^{Nujol}: 3300(broad) and 1590 cm^{-1}.

^{1}H NMR Data: (220 MH_z ,$CDCl_3$): 1.37(d, J=6H_z,3H;CH_3-3), 1.64(d, J=6H_z ,3H; CH_3-1), 2.42(dd, J=18,11,3.5H_z, 1H;H_a ,-4), 2.90(dt, J=18.19,3.3H_z,1 H; H_e-4),3.65 (m, 1H;H-3), 4.20(s,3H;-OCH_3), 5.0(m,1H;H-1), 11.97-12.23(1H; exchangeable with D_2O), 12.90-13.17(1H, exchangeable with D_2O.

Mass Spectral Data: (M^+) at m/z 320.0896(98) $C_{16}H_{14}O_7$ requires 320.0893; other fragment ions at m/z 305 (100), 302(60), 292 (44), 291(76), 290(81), 277(89), 276(97), 275(66), 264(43), 263(70).

Colour Reactions

Compound–B produces pink color in sulphuric acid. With aqueous sodium hydroxide it gives violet coloration and the color gets bleached by the addition of sodium dithionite. It produces violet color with ferric chloride and pink color with methanolic magnesium acetate. In acetic acid it gives red solution but when zinc is added to the acetic acid solution, it becomes colorless. When zinc is removed by filteration the solution regains the red color. It forms red-violet solution with zirconyl nitrate in the nitric acid.



Compound-C: (Emodin-1-O-α-L-Rhamnopyranoside): Compound-C analysed for $C_{21}H_{20}O_9$. The colour reactions and UV-visible data indicate it to be an anthraqinone. The hydroxyquinone nature of compound-C was indicated by the positive colour reaction with methanolic magnesium acetate test[3]. It gave positive Molisch test. It reduced neither Fehling's solution for Tollen's reagent: No colour was observed with aniline hydrogen phthalate indicating its non-reducing nature and suggesting it to be a glycoside. This compound-C can be an anthraquinone glycoside.

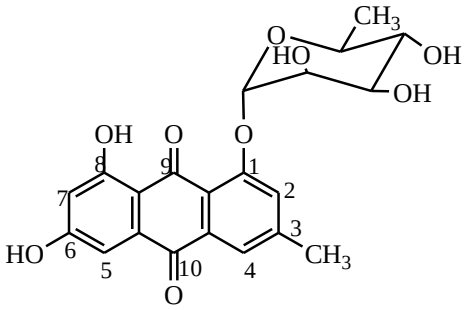
Acid hydrolysis of compound-c with 6% aqueous hydrochloric acid afforded anaglycone ($C_{15}H_{10}O_5$), m.p.255^{0}C. The sugar part was identified with L-rhamnose by ascending paper chromatography using BAW (n-BuOH-AcOH-H_2O 4:1:5) as developing solvent. These observations and characteristic C-CH_3 signal at δ 1.2 integrating for three protons [9, 10] further established the sugar moiety to be L-rhamnose.

The color reactions and physical properties of the aglycone of compound-C showed close resemblance to those of emodin. Direct comparison (Co-TLC and m.m.p)of compound-C with an authentic sample of emodin-1-o- α-L-rhamnoside established their identity.

The glycoside was completely hydrolysed by the enzyme taka-diastase, thereby, showing the linkage to be α. From the spectral and hydrolytic findings the structure of compound-c was established as 6,8-dihydroxy-3-methyl anthraquinone-1-o- α-L-rhamnopyranoside (III)

To the best of our knowledge, it is to be reported first time from plant ventilago bombaiensis root bark. Earlier it is reported by J.U.M.Rao et al[11] from ventilago calyculata.

Examination of compound-C: **Emodin-1-O-α-L-Rhamnopyranoside:**



Compound-C is an anthraquinone glycoside. It is crystallized from methanol yellow needles. Melting point: 184^0. R_f 0.54 (chloroform-methanol 4:1). It is sparingly soluble in methanol but insoluble in hexane, benzene, chloroform, ethyl acetate and acetone

Analysis: Found: C, 60.32; H, 4.71 $C_{21}H_{20}O_9$
Requires: C, 60.58; H, 4.84%

UV-Visible Data: λ_{max}^{MeOH} (log ε): 225 (4.32), 290(4.38)and 420 93.96)nm.

IR Data υ_{max}^{KBr} : 3400, 2900, 1630 and 825 cm^{-1}

Colour Reactions: Compound-C forms yellowish brown solution in concentrated Sulphuric acid. In aqueous sodium hydroxide, it produces bluish pink solution. With magnesium acetate, it forms pink solution. But it does not answer the alkaline zirconium nitrate test.

References:

[1]. R.H.Thomson , "Naturally occurring quinones" (1971)p.40, Academic press, London.
[2]. P.S.Steyn and P.L. Wessels, Tetrahedron,(1979) 35,1551
[3]. S.Shibata, M.Takido, and O.Tamaka, J.Am.Chem.Soc.(1950) 72,2789
[4]. M.L.Josian, N.Fuson,J.M.Lebas and T.M.Gregory,J.Chem.Phys.,(1953)21,331.
[5]. R.E.Moore and P.J.Scheuer,J.Org.Chem.,(1966)31,3272
[6]. H.Tanaka,Y.Koyama and T.Nagai, J.Antibiotics, (1975) 28,868
[7]. Subba Rao Jammula et al.,Phytochemistry,(1991)30,3744
[8]. T.Hanumaiah, David S.Marshall, B.K.Rao, C.P.Rao, G.S.R.Rao, J.U.M.Rao, K.V.J.Rao and Ronald H.Thomson, Phytochemistry,(1985) 24,2373.
[9]. M.Viscontini, D.Hochand and P.Karrer,Helv.Chim.Acta,(1955) 38,642
[10]. T.J.Mabry, K.R.Markham and M.B.Thomas, "The systematic identification of flavonoids",(1970)p.269.springer-verlag Berlin.
[11]. J.U.M.Rao,T.Hanumaiah ,B.K.Rao, and K.V.J.Rao,J.Nat.Products,(1986) 49,2,343.