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ISOLATION AND CHARACTERIZATION OF CHEMIAL CONSTITUENTS OF ROOT BARK OF *VENTILAGO BOMBAIENSIS Dalz*

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ABSTRACT

The powder of the root bark on extraction with acetone and isolation by chromatographic methods found to contain eight anthraquinone groups of compounds. They have been characterized by spectroscopic methods including NMR and the structures are established on comparison with authentic sample's data.

INTRODUCTION

The Traditional usage of herbs and plants in rural places is in vogue. Ventilago bombaiensis Dalz belonging to Rhamnaceae family, a climber found in these parts is in practice to cure deafness, tooth –ache, skin diseases etc. The oil obtained from the seeds find a place in household as cooking oil in some parts of rural places in Andhra prades. The skin diseases cured by reddish root bark of this plant prompted us to isolate the components of this bark and characterize them.

Experimental Section: 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 (75g) was subjected to CC on silica gel (400g). The column was eluted successively with Benzene-Hexane (1:1), Benzene with increased quantities of Ethyl acetone (9:1, 4:1 and 1:1). Fractions in 200ml were collected. The number of fractions collected with each solvent system is given in Table-I

Table-I

S.No	Fraction No.	Volume in Litres	Solvent of solution
1	01-15	3	Benzene-Hexane (1:1)
2	16-40	5	Benzene
3	41-55	3	Benzene-ethyl acetate(9:1)
4	56-65	2	Benzene-ethyl acetate(4:1)
5	66-80	3	Benzene-ethyl acetate(1:1)

Fractions exhibiting similar spot pattern on TLC plate were grouped. The grouping of these fractions is shown in Table-II

Table II

Group No	Fraction No	Colour of the eluate	No of spots	Solvent of TLC
I	1-6	Yellow	One	Benzene-Hexane(1:1)
II	7-15	Reddish Yellow	Three	Benzene
III	16-40	Brownish yellow	Three	Benzene
IV	41-55	Brown	--	Benzene-Ethylacetate (9:1)
V	56-65	Orange	One	Benzene-Ethylacetate (4:1)
VI	66-80	Pale yellow	One	Chloroform-Methanol (9:1)
VII	81-95	Brown	--	Benzene-Ethylacetate (1:1)

The solid from fractions 1-6 was found to be waxy and resisted crystallization.
The solid from fractions 7-15 was subjected to CC (Benzene-hexane 1:1) followed by TLC (Benzene) to yield three compounds A, B and C. The solid from fractions 16-40 was subjected to CC (Benzene-hexane, 2:1) followed by TLC to yield three more compounds D, E and F.
The solid from fractions 41-55 did not show any spot when examined by TLC and resisted crystallization. Hence the residue was not examined further.
The solid from fractions 56-65 crystallized repeatedly from methanol to give orange red needles, compound-G.
The solid from fractions 66-80 was subjected to CC (Benzene-Ethylacetate, 9:1) followed by crystallization from methanol to give pale yellow needles, compound-H
The R_f values and name of the compound are given in Table-III

Table-III

S.No	R_f	Solvent system	Colour of the spot	Designation of the compound	Name of the compound
1.	0.90	Benzene	Yellow	Compound-A	Islandicin
2.	0.86	Benzene	Yellow	Compound-B	Chrysophanol
3.	0.82	Benzene	Yellow	Compound-C	Physion
4.	0.44	Benzene	Violet	Compound-D	Lupeol
5.	0.30	Benzene	Yellow	Compound-E	1,1',8,8',10-pentahydroxy 3,3'-dimethyl 10',7'-bianthracene 9,9',10-trione.
6.	0.16	Benzene	Violet	Compound-F	B-sitosterol
7.	0.68	B:E(9:1)C:	Yellow	Compound-G	Emodin
8.	0.84	M(9:1	Yellow	Compound-H	Calyxanthone

Note: B=Benzene E=Ethylacetate C=Chloroform M=Methanol

The physical properties, colour, crystal structure, melting point and molecular formula of the compounds isolated are summarized in Table-IV.

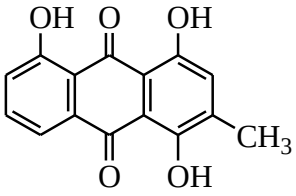
Table-IV

S.No	Name of the compound	Description	Solvent of crystallization	Melting point	Molecular formula
1.	Islandicin	Red shining crystals	Benzene-Hexane	217^0	$C_{15}H_{10}O_5$
2.	Chrysophanol	Yellow needles	Benzene-Hexane	195^0	$C_{15}H_{10}O_4$
3.	Physcion	Orange-red	Ethyl acetate-benzene	205^0	$C_{16}H_{12}O_5$
4.	Lupeol	Colourless needles	Methanol	215^0	$C_{30}H_{50}O$
5.	1,1',8,8',10-pentahy-	Orange (fluorescent)	Benzene	275^0	$C_{30}H_{20}O_8$

	droxy 3,3'-dimethyl 10',7'-bianthracene 9,9',10-trione.	cubes			
6.	β-sitosterol	Colourless needles	Methanol	138^0	$C_{29}H_{50}O$
7.	Emodin	Orange red needles	Methanol	222^0	$C_{15}H_{10}O_5$
8.	Calyxanthone	Pale yellow needles	Methanol	$>275^0$	$C_{15}H_{10}O_6$

Results and discussion: Melting points are uncorrected, Chemical shifts are given in ppm (δ- values). Samples for analysis were dried at 110^0 and 0.2mm pressure for eight hours.

Examination of Compound-A: Islandicin[1, 2]



It is sparingly soluble in hexane, moderately in benzene, methanol and fairly in chloroform

Analysis: Found: C, 66.52; H, 3.98; $C_{15}H_{10}O_5$
Requires: C, 66.67; H, 3.73%

UV-Visible data: λ_{max}^{EtOH} : 231,252,290,491,515,527 nm

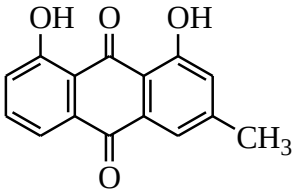
IR Data: υ_{max}^{KBr} : 3470, 1605 cm^{-1}

^{1}H NMR Data: (100 MH_z, $CDCl_3$): 2.34 (S, 3H;-CH_3), 7.12 (S, 1H;H-2), 7.27(dd, J=9.2H_z,1H,H-7), 7.64(t, J=9.9H_z,1H,H-6), 7.84(dd, J=9.2H_z,1H,H-5)

Mass spectral Data: (M^+) at m/z 270(100); other fragment ions at m/z 255(9), 242(8), 214(5), 130(3).

Colour reactions: Compound-A forms blue solution in aqueous sodium hydroxide. It produces bluish violet solution in concentrated sulphuric acid. Brownish-yellow colour is observed with ferric chloride and pin colour with methanolic magnesium acetate. Co-TLC with an authentic sample of islandicin gave the same R_f (0.90, benzene) and the mixed melting point remained undepressed.

Examination of Compound-B: Chrysophanol[3]



It is sparingly soluble in hexane, moderately in methanol, fairly in benzene, chloroform and acetone.

Analysis: Found: C, 70.98; H, 3.72; $C_{15}H_{10}O_4$
Requires: C, 70.86; H, 3.96%

UV-Visible data: λ_{max}^{EtOH} : 228,257,277,288,429nm

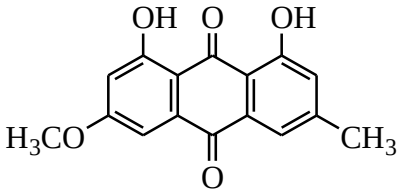
IR Data: υ_{max}^{KBr} : 1680, 1632, 1611cm^{-1}

^{1}H NMR Data: (60MH_z,$CDCl_3$): 2.40 (S,3H;-CH_3), 7.02 (d,J=1.5H_z,1H;H-2),7.2-8.0(m,4H; aromatic protons),13.55(s,1H,exchangeable with D_2O:peri –OH), 13.64 (S,1H,exchangeable with D_2O: peri –OH)

Mass spectral Data: (M^+) at m/z 254(100), other fragment ions at m/z 239(50), 226(40), 198(30), 175(13), 152(15), 141(10) and 115(18).

Colour reactions: Compound-B gives red solution with sodium dithionite in aqueous sodium hydroxide. It produces orange red solution with uranyl acetate in methanol. With magnesium acetate in methanol it gives pink solution. Co-TLC with an authentic sample of chrysophanol showed the same R_f (0.86, benzene) and the mixed melting point remained undepressed.

Examination of Compound-C: PHYSCION[4, 5]



It is sparingly soluble in hexane, moderately in methanol, fairly in chloroform and acetone

Analysis: Found: C, 67.37; H, 4.51; $C_{16}H_{12}O_5$

Requires: C, 67.60; H, 4.25%

UV-Visible data: λ_{max}^{EtOH} : 257,264,288,431nm

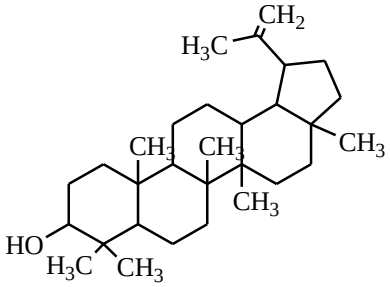
IR Data: υ_{max}^{KBr} : 1678, 1625cm^{-1}

^{1}H NMR Data: (60MH_z,$CDCl_3$); 2.46(S,3H;-CH_3), 6.64(d, J=1.5H_z, 1H;H-7), 7.36(d, J=1.5H_z, 1H,H-5), 3.92(s,3H,-OCH_3), 7.06(d, J=1.5 H_z,1H,H-2), 7.58(d, J=1.5 H_z,1H,H-4), 12.10(s,1H, exchangeable with D_2O:peri –OH), 12.30 (S,1H,exchangeable with D_2O: peri –OH).

Mass spectral Data: (M^+) at m/z 284(100), other fragment ions at m/z 269(20), 256(42), 255(50), 240(38), 228(34), 213(35), 198(35), 185(38), 149(20) and 139(30).

Colour reactions: Compound-C gives red solution with sodium dithionite in aqueous sodium hydroxide. It produces orange red solution with uranyl acetate in methanol. It produces pink colour in methanolic magnesium acetate solution. In concentrated sulphuric acid it forms dark red solution. Co-TLC of compound-C with physcion in benzene showed the same R_f (0.82) and the mixed melting point remained undepressed.

Examination of Compound-D: LUPEOL[6, 7, 8]



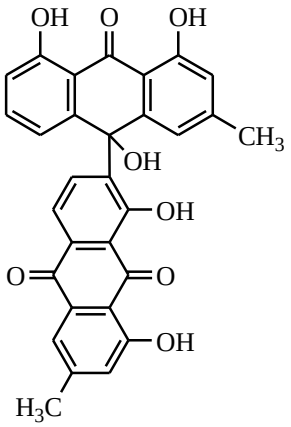
It gives positive Liebermann-Burchard test for triterpenes. It is moderately soluble in methanol and fairly in hexane, benzene, chloroform and acetone.$[\alpha]^{25}$: $+26^0$(c,o.100,$CHCl_3$)

Analysis: Found: C, 84.27; H, 11.56; $C_{30}H_{50}O$

Requires: C, 84.44; H, 11.31%

Mixed melting point with a sample of authentic lupeol remained undepressed. Co-TLC determination showed same R_f 0.44(benzene) for both the samples.

Examination of Compound-E: 1, 1', 8, 8', 10-pentahydroxy 3, 3'-dimethyl 10', 7'-bianthracene 9, 9', 10-trione.[9-14]



It is sparingly soluble in benzene, chloroform, ethyl acetate and methanol and insoluble in hexane.

Analysis: Found: C, 70.67; H, 3.78; $C_{30}H_{20}O_8$
Requires: C, 70.86; H, 3.96%

UV-Visible data : λ_{max}^{MeOH} (logε): 228(4.48), 263(4.18), 293(3.88), 390(3.90), 435(3.84) nm

λ_{max}^{MeOH} / OH^- : 255,300,380,520 nm

IR Data: υ_{max}^{KBr} : 3300, 1640, 1610cm^{-1}

^{1}H NMR Data: (360MH_z,DMSO-d_6), 2.21(s,3H,CH_3-3), 2.41 (s,3H,CH_3-3'), [6.61(br,s), 6.77(br,s)]-(2H,H-2 & H-4), 6.76(H-5),6.90(H-7), 7.14(br,s 1H,H-2') 7.49(H-6), 7.52(H-4'), 7.90(br s,1H,H-5), 8.66(br s,1H,H-6'); 12.16(exchangeable with D_2O: 2H, peri –OH), 12.24 (exchangeable with D_2O:2H peri –OH).

Mass spectral Data: (M^+) at m/z 508.1168 $C_{30}H_{20}O_8$ requires 508.1158).other fragment ions at m/z 490(100), 476(26).

Colour reactions: Compound-E produces pink colouration with methanolic solution of magnesium acetate and imparts red colour to sulphuric acid. It forms pink solution in sodium hydroxide solution and the pink colour is changed to orange red by the addition of sodium dithionite. It does not answer zirnonyl nitrate test.

Acetylation of Compound-E: To compound-E (8 mg) dissolved in pyridine (1ml), acetic anhydride (1ml) was added and heated on a boiling water bath for 1 hour. The reaction mixture was poured into ice cold water and extracted with chloroform (3x10ml). The chloroform layer was washed with water (3x10ml), dried over anhydrous sodium sulphate and the solvent evaporated. The anhydrous sodium sulphate and the solvent evaporated. The yellow solid crystallized from ethanol as pale yellow needles(5 mg), Melting point 190^0, $[\alpha]^{20}$ = -20^0(c,o.3,$CHCl_3$)

Analysis: Found: C, 66.19; H, 5.19; $C_{40}H_{40}O_{13}$
Requires: C, 66.02; H, 5.29%

UV-Visible data: λ_{max}^{MeOH} (logε): 263(4.75), 345(3.75) nm

IR Data: υ_{max}^{KBr} : 1780, 1680, 1610cm^{-1}

$\upsilon_{max}^{CHCl_3}$: 1780,1680,1610cm^{-1}

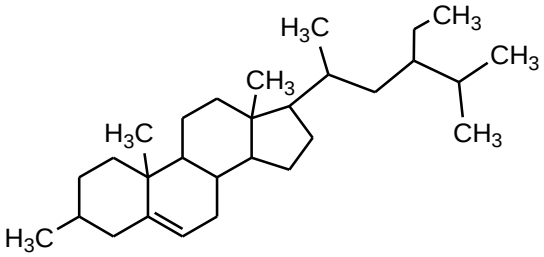
^{1}H NMR Data: 2.31(s,3H;CH_3-3), 2.49(s,3H;CH_3-3'), 6.76(H-5), 6.89(br s,1H,H-2), 7.08(H-&), 7.12(br s,1H;H-2'), 7.23(br s,1H,H-4), 7.41(H-6),8.00(br s,1H,H-4'), 8.38(d,J=8H_z,1H,H-6'), 8.80(d,J=8H_z,1H,H-5'), 1.96(s, 3H,Ac), 2.06(s,3H, Ac), 2.30(s,6H, CH_3 and Ac),2.50 (s,9H, CH_3 and 2 x Ac)

Mass spectral Data: (M^+) at m/z 718. It gives fragment ions at m/z 676(M^+ -Ac), 634 (M^+ -2xAc), 592(M^+ -3xAc), 550(M^+ -4xAc), 508(M^+ -5xAc)

Reductive Cleavage of Compound-E with Alkaline Sodium Dithionite: To a solution of compound-E(15 mg) dissolved in (1 ml) 2N alkali, sodium dithionite(50 mg) was added. The mixture was heated on a boiling water

bath for 30 minutes. The solution was acidified with dilute hydrochloric acid and extracted with ethyl acetate (3x10 ml). The ethyl acetate extract was washed with water (3x10 ml) and dried over anhydrous sodium sulphate. It was filtered and the solvent evaporated. The yellow solid was dissolved in minimum volume of acetone (1 ml) and applied on the preparative plates (15 plates, 20 x 20 cm) using benzene as the developing solvent. The yellow coloured band formed on the plates was scrapped, extracted with acetone, and acetone evaporated. The yellow solid crystallized from benzene-hexane as yellow needles (2 mg). Mixed melting point of the product of the product above with an authentic sample of chrysophanol remained undepressed. On Co-TLC, these two showed the same R_f value (0.86, benzene).

Examination of Compound-F: β-sitosterol



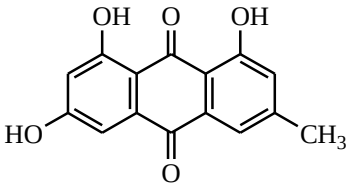
It is moderately soluble in methanol, fairly in benzene and chloroform. It gives positive Liebermann-Burchard test for sterols. Its optical rotation, $[\alpha]^{25} = -38^0$(c, o.09 in $CHCl_3$)

Analysis: Found: C, 83.76; H, 12.30; $C_{29}H_{50}O$

Requires: C, 83.99; H, 12.15%

Mixed melting point with β-sitosterol remained undepressed. Co-chromatography showed that both were having the same R_f value, 0.16(benzene).

Examination of Compound-G: Emodin[15, 16]



It is sparingly soluble in hexane, moderately in benzene, chloroform, and methanol and fairly in acetone.

Analysis: Found: C, 66.88; H, 3.68; $C_{15}H_{10}O_5$

Requires: C, 66.67; H, 3.73%

UV-Visible data: λ_{max}^{EtOH} : 253,267,289,435nm

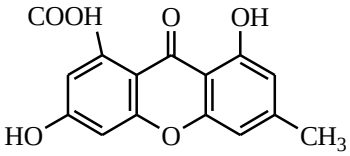
IR Data: υ_{max}^{KBr} : 3390, 1675 and1630cm^{-1}

^{1}H NMR Data: (90 MH_z, acetone-d_6): 2.41(s,3H:-CH_3), 6.66(d,J=2H_z,1H:H-7), 7.12(broad,1H:H-2), 7.23(d,J=2H_z,1H:H-5), 7.53(br,1H,H-4), 12.12(s,1H,exchangeable with D_2O,peri –OH), 12.20 (s,1H,exchangeable with D_2O, peri –OH).

Colour reactions: Compound-G produces pinkish red solution in concentrated sulphuric acid. In aqueous sodium hydroxide it produces pinkish red solution. With sodium dithionate in alkali it forms red solution. With methanolic magnesium acetate it forms light pink solution.

Mixed melting point determination of compound-G with an authentic sample of emodin remained undepressed. On Co-TLC, these two showed the same R_f value 0.68(benzene-ethyl acetate 4:1).

Examination of Compound-H: Calyxanthone [17, 18]



It is sparingly soluble in methanol but insoluble in hexane, benzene, chloroform, ethyl acetate and acetone.

Analysis: Found: C, 62.66; H, 3.39; $C_{15}H_{10}O_6$

Requires: C, 62.94; H, 3.62%

UV-Visible data: λ_{max}^{MeOH} (logε): 236(4.36), 304(3.80) and 348(4.18) nm

IR Data: υ_{max}^{KBr} : 3070, 1680,1640,1235,1210 and 1165cm^{-1}

^{1}H NMR Data: 2.38 (3H,d, J=0.58H_z,-CH_3), 6.77(1H,d, J=2.32H_z,H-5), 6.88(1H,d, J=2.29H_z,H-7), 6.64(1H,dd, J=1.40,0.72H_z,H-2), 6.86(1H,dd, J=2.29,1.31,0.70H_z,H-4), 12.42 (1H,s, exchangeable with D_2O, peri –OH).

Mass spectral Data: (M^+) at m/z 286.0487 ($C_{15}H_{10}O_5$ requires 286.0477, 88.8); other fragment ions at m/z 268.0395($C_{15}H_8O_5$ requires 268.0371, 44.4): 242.0575 ($C_{14}H_{10}O_4$ requires 242.0579, 100) and 213.0549 ($C_{13}H_9O_3$ requires 213.0552, 16.6).

Colour reactions: Compound H forms violet-red coloured solution with magnesium-concentrated hydrochloric acid. With ferric chloride, an ethanolic solution of compound-H turns green.

Acetylation of Compound-H:

To compound –H (10 mg) in pyridine (1 ml), acetic anhydride (1 ml) was added and left at room temperature for 48 hours. The reaction mixture was poured into ice-cold water and the separated solid was filtered, washed with water and dried in a desiccators. Crystallization of the solid from benzene-hexane gave colourless needles (8 mg). Melting point 224°, R_f value 0.62 (chloroform-methanol 95:5).

Analysis: Found: C, 61.76; H, 3.62; $C_{19}H_{14}O_8$

Requires: C, 61.63; H, 3.81%

Mixed melting point with calyxanthone remained undepressed. Co-chromatography showed that both compound-H and calyxanthone having the R_f value 0.84 (chloroform-methanol 9:1).

These eight compounds were also found among seventeen compounds isolated from the acetone extract of the stem bark of ventilago bombaiensis[19].

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