

NOTE

Isolation of 8-Hydroxy-6-Methoxy-2-Methyl Anthraquinone-3-O- β -D-Glucopyranoside from *Raphanus sativus* and its Anti-inflammatory Activity

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The isolation of 8-hydroxy-6-methoxy-2-methyl anthraquinone-3-O- β -glucopyranoside from the leaves of *Raphanus sativus* which was confirmed by spectral data and chemical conversions. The anti-inflammatory activity of EtOH extract and isolated compound of *Raphanus sativus* was tested and the data showed a significant inhibition of carrageenan induced paw oedema in rats treated with alcoholic extract and isolated compound of *Raphanus sativus*, which is equivalent to that produced by phenylbutazone.

Raphanus sativus (family Cruciferae) has been shown to possess a number of pharmacological effects useful in liver and gall bladder troubles. Roots, leaves, flowers and pods are active against gram-positive bacteria. The juice of the fresh leaves is used as diuretic and laxative. The roots are said to be useful in urinary complaints. The seeds are said to be peptic, expectorant, diuretic and carminative¹.

The leaves of the plant *Raphanus sativus* were procured from the local market, Berhampur. Air dried leaf powdered material was extracted with EtOH under reflux on a water bath for 36 h, three times (using fresh EtOH). The EtOH (10 litres) was removed *in vacuo* yielding a viscous mass which was successively refluxed with petroleum ether and benzene. The petroleum ether fraction was concentrated and chromatographed on a neutral alumina column to give β -sitosterol (m.p., m.m.p. and co-TLC) and α -amyrin (m.p., m.m.p. and co-TLC). The benzene fraction was concentrated and passed through a silica gel column. The column when eluted with C_6H_6 : $CHCl_3$ (8 : 2) gave compound (1) as light yellow coloured needles (1.2 g).

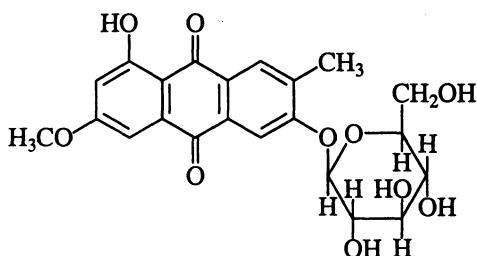
Characterisation of Compound (1): m.p. 157–158°C, UV-visible, λ_{max} : 220, 230 and 430 nm characteristic for anthraquinone skeleton. IR spectra of (1) indicated glycosidic nature (γ_{max} 825 cm^{-1}) of the compound.

Acid hydrolysis of Compound (1): A solution of (1) (200 mg) in EtOH (20 mL) was hydrolysed with 7% H_2SO_4 (40 mL) to give aglycone (2) and D-glucose (co-PC and osazone).

Characterization of Compound (2): Purified over silica gel column (C_6H_6 : $CHCl_3$) (8 : 2) and crystallized with Et_2O : $CHCl_3$ mixture as orange

coloured needles. m.p. 190–192°C (yield 500 mg). UV-Visible: 220, 228 and 430 nm; IR: 3200–3100 (OH), 2940 and 1455 (C—Me), 2860 and 1175 (MeO). 1670 and 1625 (unchelated and chelated CO), 1560, 1285, 1205, 1020 and 740 (anthraquinone skeleton); ¹H-NMR: δ 12.00 and 11.00 (2X OH), 8.00 (H-5), 7.30 (H-1), 7.00 (H-7), 4.00 (1X MeO) and 2.40 (1X Me); MS: 284 (50), 269 (80), 267 (90), 266 (65), 256 (25), 255 (22), 228 (100), 172 (85), 135 (75), 131 (40), 123 (20) and 107 (25%). (Found C: 67.58; H: 4.20; C₁₆H₁₂O₅ required C: 67.60; H: 4.22%); Acetate [100 mg (2) + 5 mL Ac₂O + 5 mL C₆H₅N], ¹H-NMR: 2.10 and 2.00 (2X OAc).

UV-visible and IR spectra of compound (1) indicated the presence of anthraquinone glycoside which was further confirmed by positive Borntrager reaction. Acid hydrolysis of (1) gave an aglycone (2) and D-glucose (co-PC and osazone). The aglycone was found to be hydroxyanthraquinone by its usual colour tests, IR and UV-visible spectra and gave 2-methylanthracene on Zn-dust distillation. The ¹H-NMR spectrum of (2) showed 2X OH groups which was also confirmed by its diacetate (3) (¹H-NMR: 2.10 and 2.00). (2) formed a complex with EtOH-CuSO₄⁴ and gave orange-red colour with 5% MeOH-(AcO)₂Mg and also pink colour with zirconium nitrate solution confirming the OH groups at positions C-3⁵ and C-8⁶. CrO₃ oxidation of methyl ether of one of the oxidation products indicated the OMe at C-6 in (2). Thus (2) was assigned as 3,8-dihydroxy-6-methoxy-2-methylanthraquinone which was confirmed by its mass spectrum. (1) could not be methylated with CH₂N₂. (1) did not give the colour as in reference 6 but the same was given by (2), confirming the presence of D-glucose at 3-OH in (2). Almond emulsin hydrolysis and HIO₄ oxidation showed that D-glucose was in β-linked pyranose form and assigned the structure (1).



8-Hydroxy-6-methoxy-2-methyl anthraquinone-3-O-β-D-glucopyranoside

Compound (1)

Pharmacological Screening: The anti-inflammatory activity of EtOH extract and compound (1) (dried and dissolved in H₂O) of *Raphanus sativus* was tested in albino rats each weighing 200–225 g (fasted overnight) by the method of Winter *et al.*² The percentage inhibition of inflammation after 5 h was calculated by the method of Newbould³ using the following formula:

$$\text{Percentage inhibition} = 100 \left(1 - \frac{(a - x)}{(b - y)} \right)$$

where 'x' and 'a' are the mean foot volumes of the rats before and after the

administration of carrageenan injection respectively in the test of standard group. 'y' and 'b' are the mean foot volumes of rats before and after the administration of carrageenan respectively in control group.

The data (shown in Table-1) indicate a significant inhibition (69.76% and 67.44%) of carrageenan induced paw oedema in rats treated with extract and compound (1) of *Raphanus sativus* (1000 mg/kg), which is equivalent to that produced by 100 mg/kg of phenylbutazone (72.09%).

TABLE-1
EFFECT OF *Raphanus sativus* ON CARRAGEENAN INDUCED PAW OEDEMA IN RATS

Volumes of paw (mL) after carrageenan administration (mean \pm S.E.) in different groups

Time (h)	Control group	Rats with 1000 mg/kg per os dose of EtOH extract of <i>Raphanus sativus</i>	Rats with 1000 mg/kg per os dose of compound (1)	Rats with 100 mg/kg per os dose of phenylbutazone
0	0.62 \pm 0.06	0.72 \pm 0.05	0.69 \pm 0.04	0.70 \pm 0.05
1	0.70 \pm 0.03	0.72 \pm 0.02	0.70 \pm 0.03	0.72 \pm 0.02
2	0.83 \pm 0.05	0.74 \pm 0.03	0.72 \pm 0.06	0.74 \pm 0.03
3	0.92 \pm 0.07	0.76 \pm 0.06	0.76 \pm 0.05	0.75 \pm 0.02
4	0.99 \pm 0.03	0.81 \pm 0.04	0.78 \pm 0.03	0.79 \pm 0.04
5	1.05 \pm 0.02	0.83 \pm 0.05	0.83 \pm 0.04	0.82 \pm 0.02
Total increase in paw volume after 5 h	0.43 \pm 0.03	0.13 \pm 0.05	0.14 \pm 0.03	0.12 \pm 0.02
Percentage inhibition (%)		69.76	67.44	72.09

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