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Antifeedant Xanthone from *Swertia decussata*

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Abstract

An antifeedant agent Decussatin was isolated from the ether extract of *Swertia decussate* along with Oleanolic acid and Stigmasterol. It was unambiguously characterized as its tetra methyl derivative and by UV, IR, ^{1}H NMR and MS spectroscopy**.**

Key words : *Swertia decussata*, Decussatin, Tetramethyl derivative, ^{1}H NMR and MS spectroscopy**.**

Introduction

Swertia decussata belongs to Gentianacea family. This family consists of about 80 genera and about 800 species. Swertia comprises of about 90 species of which about 34 species are found in India. The plants are used as antihelementic, antipyretic, laxatives. They are also used against Ulcer, Asthma, leucoderma, inflammation and vomiting in pregnancy[1] . The total xanthones of *Swertia* induce significant CNS stimulant actions, consistent with some Therapeutic uses of the plant extract in the Indian system of medicine.

During screening of the plants for biological activity, the ether extract of *Swertia decussata* plant was found to have antifeedant activity. Therefore systematic phytochemical examination of this plant was taken.

The flowers of *Swertia decussata*, collected from Panchgani were air dried powdered and extracted (soxhlet) with ether followed by methanol. Concentrations of the extracts under reduced pressure furnished the respective crude residues. TLC analysis of these extracts revealed the presence of several fluorescent spots.

Result and Discussion

ETHER Extract

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Concentration of the ether extract under reduced pressure afforded a brown residue. Column chromatography of this residue over silica gel using solvents of increasing polarity (petrol, petrol : EtOAc, EtOAc, MeOH) yield several fractions. These fractions were monitored by TLC and similar fractions were pooled. Repeated column chromatography and preparative TLC of these fractions resulted in the isolation of three compounds which were tentatively designed as A to C.

Compound A

It was recrystallised from methanol as color less needless, m.p 310^{o} C. It showed a violet red response to Liebermann Burchard reaction reaction and IR spectrum exhibited absorption bands at 3400 cm^{-1} (alcoholic -OH) and 1690 cm^{-1} (carboxylic >CO). The mass spectrum showed a molecular ion at m/z 456 and fragment at m/z 248 (BASE PEAK), 207, 203, 189 and 133 suggesting it to be Oleonolic acid.

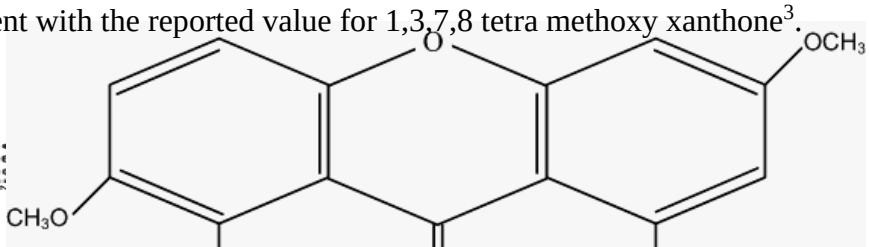
Compound B

A crystalline solid m.p. 165 - 166^{o}C. It gave positive Libermann Burchard test indicating it to be a steroid. By comparing it with authentic sample and GLC studies of its acetate derivative it was found to be stigmasterol.

Tetramethyl derivative of compund C.

The UV absorption of compound “C” showed peak at 240, 260, 326 and 378 nm suggested the chromophore may be of xanthone type[2]. The ^{1}H NMR spectrum showed the presence of three methoxyls groups in and one chelated hydroxyl group. For assigning the exact positions of methoxyl and hydroxyl groups the compound “C” was converted into its Tetramethyl derivative.

The ^{1}HNMR spectrum of tetra methyl derivative showed a pair of doublet at δ 7.26 and δ 7.12 (J = 9 Hz) suggesting ortho coupled protons and another pair of doublets at δ 6.40 and δ 6.32 (J = 2 Hz) suggesting the presence of meta coupled protons and four methoxyl groups at δ 4.02, δ 3.95, δ 3.90 and δ 3.89. The absence of C-1 and C-8 carbonyl shielded protons signals which would have occurred in the region of δ 8.0 - δ 8.5 indicated that the tetramethoxy derivative is oxygenated at C-1 and C-8. Taking these observations into consideration the tetramethyl derivative was assigned structure X *viz.* 1,3,7,8 tetra methoxy xanthone. Moreover the physical properties of the tetra methyl derivative were in agreement with the reported value for 1,3,7,8 tetra methoxy xanthone[3].



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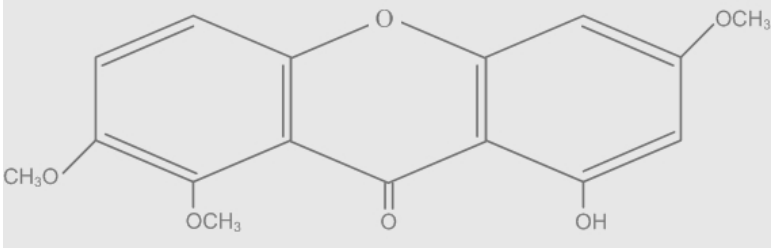
Thus it was concluded that the xanthone C, is oxygenated at 1,3,7,8 positions.

Compound "C"

Pale yellow colored compound, Melting point 157° C .

The ^{1}HNMR spectrum of compounds C, showed the presence of three methoxyls groups δ 3.89 (s), δ 3.91 (s) and δ 4.01 (s) and a presence of chelated hydroxyl group δ 13.30 (s) [D_2O exchangeable]. The spectrum also indicated the presence of a pair of ortho coupled proton δ 7.17 (d, *J* = 9 Hz) and δ 7.38 (d, *J* = 9 Hz) and a pair of doublet for meta coupled proton δ 6.26 (d, *J* = 2 Hz) and δ 6.32 (d, *J* = 2Hz).

The presence of hydroxyl group at C-1 or C-8 was suggested due to its chelated nature. Thus the substitution pattern in compound "C" was confirmed as 1-hydroxy-3, 7, 8-trimethoxy xanthone. Moreover all the spectral characteristics of the compound "C" were in perfect agreement with a previously known compound decussatin (1-hydroxy-3,7,8-trimethoxy xanthone.) isolated from *Swertia Punicea*[(4)]. Although known from different plant species, this is the first report for the isolation of this compound from *Swertia decussata.*



"C" Decussatin

Decussatin is a member of the class of xanthones that is xanthone substituted by a hydroxy group at position 1 and methoxy groups at positions 3, 7 and 8. It has been also isolated from *Caenturium erythraea* and *Gentiana verna*. It has a role as a plant metabolite. It is a member of xanthones, an aromatic ether and a member of phenols.

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IV- Experimental section

Ether extract

The ether extract (10 g) was subjected to further fractionation using column chromatography alternate fractions were concentrated using flash evaporator and monitored on TLC plate [Solvent system $CHCl_3$: MeOH (99 : 1)]. The compounds were detected under short UV and by I_2 staining. The individual compounds were purified by preparative TLC. It resulted in isolation of five compounds A, B and C.

Chromatographic System

Column chromatography : For column chromatography 100 – 200 mesh Acme grade silica gel is used. The crude reaction mixture is concentrated under reduced pressure to yield crude mass which is preadsorbed on silica gel and purified by column chromatography with increase in concentration of Ethyl acetate in Petroleum ether. The fractions having similar 'R_f' values were pooled together, concentrated and subjected for characterization using various spectroscopic techniques.

Thin layer chromatography : TLC plates were prepared using silica gel G (ACME, BOMBAY). Pet. ether: EtOAc (85 : 15) was used as the solvent system.

Radial chromatography : The circular glass plates of thickness 1 mm, were prepared by using silica gel (PF254, E. MERCK, 50 g) in cold distilled water (105 ml). For elution, gradually increasing concentrations of EtOAc in pet. ether were employed.

Experimental

Melting points were determined on a Thomas Hoover capillary melting point apparatus using digital thermometer. IR spectra were recorded on a Shimadzu FTIR Prestige model as KBr pellet. ^1H NMR spectra were recorded on a Varian 200 MHz spectrometer in $CDCl_3$. Chemical shifts were recorded in parts per million down field from tetramethyl silane. Mass spectra were recorded on a TOF MS ES mass spectrometer. Elemental analysis were carried out as a percentage on a Thermo finnigan, Flash EA 1112 series, Italy.

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Materials and Methods : Chemicals used were of a laboratory grade. The reactions were monitored by TLC on aluminium-backed silica plate visualized by UV-light.

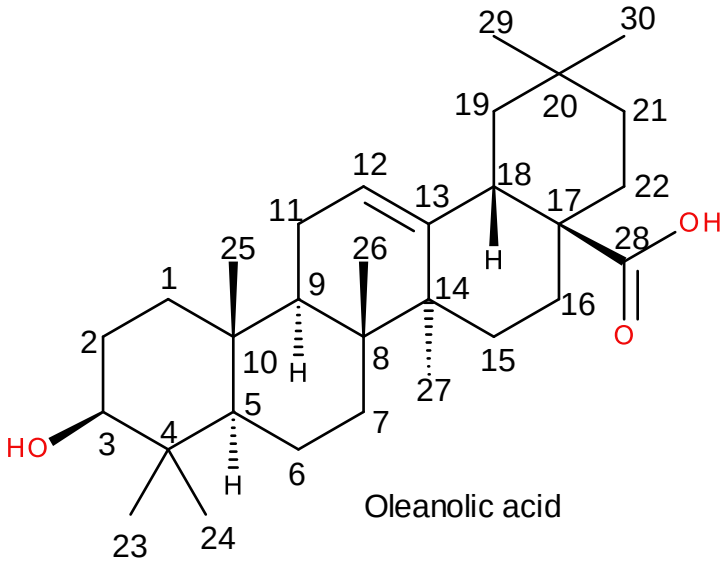
Compound A (Oleonolic acid)

It was crystallized from methanol as colorless needles.

Molecular formula : $C_{30}H_{48}O_3$

m.p : 310^0 C

IR (KBr) cm^{-1} : 3408 cm^{-1} (alcoholic -OH), 2800 – 2500 (-OH stretching of –COOH group), 1689 cm^{-1} (>C=O), 1460, 1382, 1107 (C-O), 1081.



Oleanolic acid

^1H and ^{13}C NMR data of Oleanolic Acid :- Structural characterizations were carried out using a combination of 1D ^1H, ^{13}C and various 2D experiments for its unambiguous assignments.

C	δ_C	δ_H
1	39.0	α 1.02
		β 1.57
2	28.1	α 1.82
		β 1.82
3	78.2	α 3.44 dd
4	39.4	----------

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5	55.9	α 0.88 d
6	18.8	α 1.58
		β 1.39
7	33.4	α 1.53
		β 1.36
8	39.8	----------
9	48.2	α 1.71 triplet
10	37.4	----------
11	23.8	α 1.96
		β 1.96
12	122.6	α 5.49 triplet
13	144.8	----------
14	42.2	----------
15	28.4	α 1.22
		β 2.19
16	23.8	α 2.12 triplet
		β 1.96
17	46.7	----------
18	42.1	β 3.30 dd
19	46.6	α 1.83
		β 1.32
20	31.0	----------
21	34.3	α 1.46
		β 1.23
22	33.2	α 1.82
		β 2.04
23	28.8	α 1.24 s
24	16.5	β 1.02 s
25	15.6	β 0.93 s
26	17.5	β 1.04 s

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27	26.2	α 1.30 s
28	180.0	----------
29	33.4	α 0.97 s
30	23.8	β 1.02 s

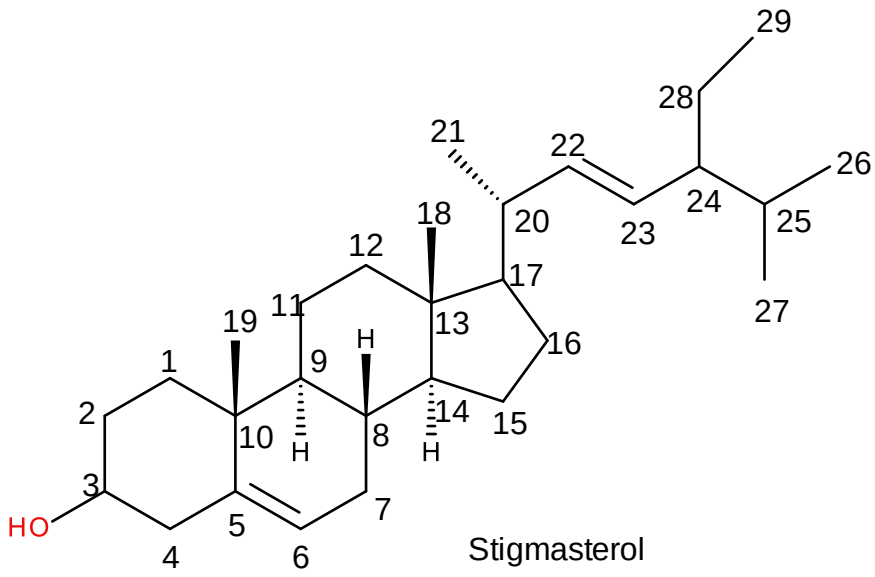
Compound B (Stigmasterol) :

It gave positive Libermann Bruchard test indicating its steroidal nature. It was identified as stigmasterol by comparison of its spectroscopic data mixed mp and sperimposable IR spectrum with authentic sample.

Molecular formula : $C_{29}H_{48}O$

m.p : $160^0 – 164^0$ C

IR (KBr) cm^{-1} : 3410 cm^{-1} (alcoholic -OH), 3000 – 2800 (methyls, methylenes and methines), 1634 cm^{-1} (olefinic), 1461, 1443, 1331, 1243, 1089, 987.



Stigmasterol

^1H and ^{13}C NMR data of Stigmasterol :- Structural characterizations were carried out using a combination of 1D ^1H, ^{13}C and various 2D experiments for its unambiguous assignments.

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C	Type (DEPT)	^{13}C NMR	^{1}H NMR (multiplicity)
1	-CH_2-	37.28	1.46 (m)
2	-CH_2-	31.69	1.56 (m)
3	>CH(-OH)	71.82	3.54 (m)
4	-CH_2-	42.33	2.32 (m)
5	>C<	140.77	----------
6	=CH-	121.73	5.37 (overlapping triplet)
7	-CH_2-	31.93	2.04 (m)
8	>CH-	31.93	1.69 (m)
9	>CH-	50.16	1.55 (m)
10	>C<	36.51	----------
11	-CH_2-	21.11	1.52 (m)
12	-CH_2-	39.80	1.51 (m)
13	>C<	42.34	----------
14	>CH-	56.79	1.50 (m)
15	-CH_2-	24.33	1.58 (m)
16	-CH_2-	28.27	1.85 (m)
17	>CH-	56.08	1.45 (m)
18	-CH_3	11.89	0.73 (s)
19	-CH_3	19.42	1.03 (s)
20	>CH-	36.17	1.60 (m)
21	-CH_3	18.84	0.94 (overlapping doublet)
22	-CH_2-	33.98	0.93 (m)
23	-CH2-	26.11	1.15 (m)
24	>CH-	45.86	1.38 (m)
25	>CH-	29.19	1.57 (m)
26	-CH_3	19.84	0.84 (overlapping doublet)
27	-CH_3	19.06	0.86 (d)
28	-CH_2-	23.10	1.10 (m)

MS[M^+] : 412

Methyl ether of C, (Tetra methyl ether)

Molecular formula : $C_{17}H_{16}O_6$

m.p. : 167^0 C

IR (KBr) cm^{-1} : 1685 (>C=O), 1630, 1595 (Aromatic), 1572, 1490, 1280, 1220.

^{1}H NMR (200 MHz $CDCI_3$) δ ppm : 3.94 (s, 6H, Ar x 2 –OCH_3), 3.99 (s, 3H, Ar x –OCH_3), 4.08 (s, 3H, Ar x –OCH_3), 6.32 (d, J = 2 Hz, ArH, H-2, meta coupling), 6.41 (d,

J = 2 Hz, ArH, H-2, meta coupling), 7.11 (d, J = 9 Hz, ArH, H-5, ortho coupling), 7.27 (d, J = 9 Hz, ArH, H-5, ortho coupling).

MS[M^+] : 316

Compound 'C' (Decussatin)

Yellow crystalline compound.

Molecular formula : $C_{16}H_{14}O_6$

m.p. : 157^0 C

IR (KBr) cm^{-1} : 1690 (>C=O), 1630, 1600 (Aromatic), 1572, 1490, 1280, 1220.

^{1}HNMR (200 MHz $CDCI_3$) δ ppm : 3.89 (s, 3H, Ar x $–OCH_3$), 3.91 (s, 3H, Ar x $–OCH_3$), 4.01 (s, 3H, Ar x $–OCH_3$), 6.26 (d, J = 2 Hz, ArH, H-2, meta coupling), 6.32 (d, J = 2 Hz, ArH, H-2, meta coupling), 7.17 (d, J = 9 Hz, ArH, H-5, ortho coupling), 7.38 (d, J = 9 Hz, ArH, H-5, ortho coupling), 13.30 (brs, 1H, -OH, D_2O exchangeable). Confirmed by co-tlc and melting point.

MS[M^+] : 302

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