Chemical Constituents of Blepharis sindica Seeds

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Summary: Allantoin, betaine, β-sitosterol, oleanolic acid, apigenin, terniflorin, prunine-6"-O-coumarate and blepharin were isolated from the seeds of Blepharis sindica. The mass spectral fragmentations of the TMS derivatives of coumaroyl flavone glucoside are discussed.

Introduction

Blepharis sindica T.Anders (Acanthaceae) is a herb, growing widely in the Sind and Baluchistan provinces of Pakistan [1]. The seeds are used in the folklore medicine in the treatment of earache [2]. Recently 9-hydroxydodecanoic acid has been isolated [3] from the seeds oil of this plant.

Results and Discussion

The methanolic extractive of the seeds of Blepharis sindica was divided into water and ethyl acetate. The substance which remained insoluble in both solvents yielded allantoin whereas betaine could be isolated from the aqueous layer as its hydrochloride. Chromatography of the ethyl acetate soluble fraction on silica gel column yielded a mixture of straight chain hydrocarbons. β-sitosterol, oleanolic acid, apigenin, prunine-6"-O-coumarate (naringenin-7-O-(6-O"-p-coumaroyl)- β-D-glucoside 1), terniflorine (apigenin-7-O-(6"-O-p-coumaroyl)- β-D-glucoside, 2), and blepharin. The hydrocarbon mixture consists mainly of $C_{27}H_{56}$, $C_{59}H_{60}$ and $C_{31}H_{64}$. Allantoin, betaine, β-sitosterol, oleanolic acid and apigenin were identified through direct comparison with authentic compounds. Prunine -6"-O-p-coumarate was identified through UV, IR, ^{1}H-NMR and ^{13}C-NMR spectra as well as its methanolysis to naringenin, glucose and methyl p-coumarate. This compound has been isolated earlier [4] from Anacardium occidentale (Anacardiaceae). Our NMR data were identical with the published data of the compound.

Terniflorin was also identified through spectroscopic data and its methanolysis to apigenin, glucose and methyl p-coumarate. The isolation of this compound has been reported from Clematis terniflora var.robusta [5] (Ranunculaceae). Salix alba [6] (Salicaceae) and Pogostemon cablin [7] (Labiatae).

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This is the first report of the isolation of these flavone and flavanone glucosides from a plant belonging to Acanthaceae.

The pertrimethylsilyl (TMS) derivative of the 1 and 2 were analysed through high resolution mass spectroscopy. The exact mass values and corresponding element compositions are given in Tables I and II. The number of silicon atoms present in these ions was not determined from the exact mass but rather from the mass shift observed for these ions in the mass spectra of perdeuterio TMS derivatives.

The presence of a silylated hexose was indicated by a series of peak at m/z 129, 217, 243, 271 [8]. The hexose was identified as glucose through acidic hydrolysis of the glucoside, silylation of the sugar fraction and capillary gas chromatography - mass spectroscopy.

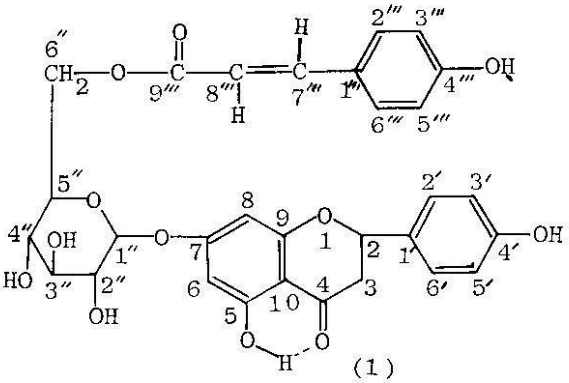
The point of glucosidation was determined to be C-7 because no bathochromic shifts of UV absorption maxima were obtained on addition of sodium acetate [9]. The p-coumaroyl group was attached to C-6" of glucose moiety as a down field shift of C-6" and an upfield shift of C-5" was observed [4].

Blepharin, which has been isolated earlier from Blepharis edulis [10], was identified through the comparison of its analytical and spectral data with the published data of the compound as well as from its hydrolysis to o-aminophenol and glucose.

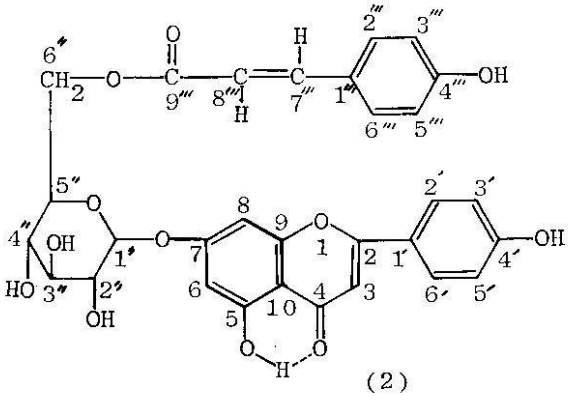
Experimental

The UV spectra were recorded on Unicam SP-800A and Shimadzu U.V-240 Graphicord. The IR spectra were obtained on Jasco IRS-1. The ^{1}H-NMR spectra were determined on Jeol PMX-60 (60 MHz) with TMS as internal standard. The ^{13}C-NMR spectra (25.01 MHz) were recorded on Bruker WP100SY spectrometer. MS spectra were recorded on varian MAT 312 instruments.

The seeds of Blepharis sindica (2 Kg) were ground to a fine powder and extracted exhaustively with boiling methanol. The extract was evaporated and the reddish viscous residue (224 g) was taken up in water and ethyl acetate.



(1)



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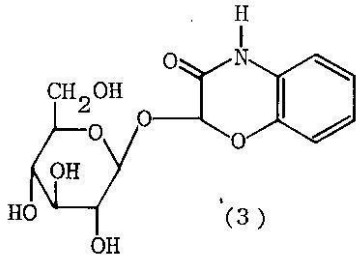
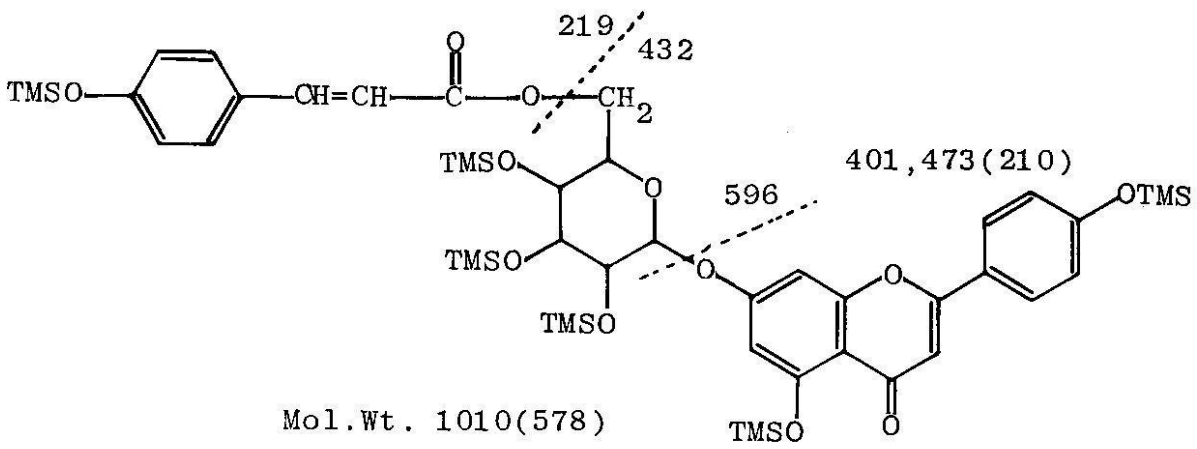


(3)



Scheme-1: Schematic representation for fragmentation of the TMS derivative of apigenin-7-*O*-β-D-(6"coummaroyl)-glucoside. Fragment ions observed in the FD mass spectrum of the free compound are given in brackets.

Allantoin

The residue (2.8 g) which remained insoluble in both solvents was crystallised from hot water whereby colourless crystals of allantoin m.p. 230° were obtained: IR ν_{max} (KBr) cm^{-1}: 3225, 3222, 1600, 1660, 1720 and 1780; MS m/z 158 (M^+) 141, 130, 115, 100,87, 70, 60. The compound showed no depression in the melting point when admixed with a synthetic sample. The IR spectra of the two samples were also superimposable.

Betaine Hydrochloride

The aqueous layer which gave a positive test with Dragendorff's reagent was evaporated completely. The residue was taken up in methanol. The insoluble material was filtered off and the filtrate was evaporated. The residue was taken up in chloroform-methanol (95:5) and filtered through a column of silica gel. The Dragendorff's positive fractions were combined together, evaporated, residue dissolved in water, acidified with HCl, filtered, evaporated again and the residue crystallised from methanol. The colourless crystals, m.p. 230° were identified as betaine hydrochloride (lit. (II) mp 227-228°) through direct comparison with an authentic sample.

Chromatography of ethyl acetate soluble fraction

The ethyl acetate layer was evaporated and the reddish gummy residue (80 g) was chromatographed on a column of silica gel (1.5 Kg).

Pure hexane eluted a crystalline compound (mp 60°) from the column which showed, in the mass spectrum, three molecular peaks at m/z 380 ($C_{27}H_{56}$) 408 ($C_{29}H_{60}$) and 436 ($C_{31}H_{64}$) and the characteristic fragmentation pattern of straight chain saturated hydrocarbons. This fraction was not pursued any further.

β-Sitosterol

The fractions eluted with hexane-benzene (90:10) yielded a crystalline compound which after recrystalli-

Table-1 Selected mass spectral data for the trimethylsilyl derivative of the flavanone glucoside naringenin-7-O-(6"-O-p-coumaroyl)- -D-glucoside.

Measured	Intensity	2H Shift	Elemental Composition	Mass Error (mmu)
219.0845	100	9	$C_{12}H_{15}O_2Si$	0.4
401.1299	10	15	$C_{20}H_{25}O_5Si_2$	0.9
437.1611	4	24	$C_{23}H_{33}O_5Si_2$	2.5
596.2465	4	36	$C_{27}H_{48}O_7Si_4$	1.2
1012.3977	2	54	$C_{48}H_{76}O_{12}Si_6$	2.7

Table-2: Selected mass spectral data for the trimethylsilyl derivative of the flavone glucoside apigenin-7-O-p-D-(6" coumaroyl)-glucoside.

Measured	Intensity	2H Shift	Elemental Composition	Mass Error (mmu)
219.0831	100	9	$C_{12}H_{15}O_2Si$	1.0
399.1075	66	15	$C_{20}H_{23}O_5Si_2$	0.8
471.1468	5	24	$C_{23}H_{31}O_5Si_3$	1.0
596.2476	3	36	$C_{27}H_{48}O_7Si_4$	0.1
1010.3806	14	54	$C_{48}H_{74}O_2Si_6$	1.0

zation from methanol melted at 138°; MS m/z 414 (M^+) 399, 396, 381, 329, 303, 255, 231. It was identified as β sitosterol through co-TLC mixed melting point, superimposable IR and ^{1}H-NMR spectra with an authentic sample.

The benzene-ethyl acetate (95:5) eluted an oil from the column.

Oleanolic Acid

Benzene-ethyl acetate (80:20) eluted colourless crystals of oleanolic acid which melted at 310° after recrystallisation from methanol; MS m/z 456 (M^+), 248 (100%),207, 203. A portion of the substance was acetylated with acetic anhydride/ pyridine and the product esterified

with diazomethane. The acetyl oleanolic acid methyl ester (mp 218°) identical NMR spectrum as reported in literature [12].

Apigenin

The fractions eluted with benzene ethyl acetate mixture (70:30 to 50:50 v/v) were mixed and evaporated. The residue was crystallised from methanol when light yellow coloured crystals of apigenin mp 320°C were obtained; UV λ_{max} methanol nm: 269, 296 (sh), 335; UV λ_{max} MeOH-$AlCl_3$ nm: 276, 301, 348, 384. MS m/z 270 (M^+) 242, 153, 152, 124, 121, 118. High resolution MS 270. 0530 ($C_{15}H_{10}O_5$ requires 270.0528).

The identity of the sample with apigenin was confirmed through direct comparison, co-TLC, superimposable IR spectra, with an authentic sample purchased from Sigma Chemical Co., Mo., U.S.A.

Prunine-6"-O-p-coumarate (1)

The substance which was eluted with pure ethyl acetate was initially obtained as a brownish yellow gum. It was purified by filtration through a short column silica gel eluting the column with chloroform-methanol (95:5 v/v). After recrystallisation from methanol, colourless, crystals m.p. 170° were obtained.

UV; λ_{max} MeOH nm (log): 224 (4.53), 285 (4.48) and 313 (4.35); UV; λ_{max} MeOH-NaOAc nm: (no bathchromic shift); IR ν_{max} Nujol cm^{-1}: 3380, 1600, 1640, 1685; ^{1}H-NMR (DMSO-d_6 100 MHz TMS as int. standard): δ 7.56 (d, J = 16 Hz, H-7"'), 7.50 (2H, d, J = 8 Hz, H-2"' and H-6"'), 7.32 (2H, d, J = 8 Hz, H-2' and H-6'), 6.38 (d, J = 16 Hz, H-8"'), 6.38 (2H, d, J = 9 Hz, H-3"' and H-5"'), 6.81 (2H, d, J = 8 Hz, H-3' and H-5'), 6.19 and 6.22 (br, s, H-6 and H-8), 5.5 (H-2), 4.0-4.6 (m, sugar protons), 3.57 (br, s, sugar proton), 2.6-3.25 (2H, H-3). ^{13}C-NMR (DMSO-d_6)-identical with published spectra (4). MS (F.D) m/z 581 (M + 1), 580 (M^+).

Acidic hydrolysis of (1)

The flavanone glycoside was dissolved in methanol (10 ml), conc. HCl (0.5 ml) was added to the solution and refluxed till the hydrolysis was complete as judged by thin layer chromatography. The solvent was evaporated and the residue washed with water, dried, evaporated and the residue was taken up in ethyl acetate and water. The ethyl acetate layer was washed with water, dried, evaporated and the residue subjected to preparative layer chromatography whereby two substances could be isolated from it.

a) p-coumaric acid methyl ester, mp 130° MS m/z : 178 (M^+), 163, 147, 119, 91, 65.

The identity of the compound was confirmed through co-TLC superimposable IR spectra and mixed melting point with an authentic sample.

b) Naringenin, mp 250°: MS m/z 272 (M^+), 225, 254 229, 201, 179, 166, 153 (100), 120. The identity of this compound was also confirmed through co-TLC, superimposable UV, IR spectra with a purchased (Sigma Chemical Co., Mo. U.S.A.) sample of naringenin.

Paper chromatography of the water layer in solvent system BuOH: AcOH:H_2O (4:1:5, upper layer) showed the presence of glucose in it.

Terniflorin (2)

The fractions eluted with ethyl acetate-chloroform mixture (80:20 v/v) were combined, evaporated and crystallised from large quantity of hot methanol or ethyl acetate yielding cream coloured crystals m.p. 260°. UV λ_{max}^{MeOH} nm (log ε): 268 (4.92), 296 (sh), 317 (5.08), UV $\lambda_{max}^{MeOH-AlCl_3}$ nm: 277, 299, 318, 382. IR ν_{max} KBr cm^{-1}: 3400, 1685, 1660, 1605. ^{1}H-NMR (DMSO d_6-TMS as int.standard) δ 7.93 (d, 2H, J = 8.5 Hz, H-2' and H-6'), 7.50 (d, J = 15 Hz, H-7"'), 7.36 (d, 2H, J = 8.5 Hz, H-2"' and H-6"'), 6.92 (d, 2H, J = 8.5 Hz, H-3' and H-5'), 6.82 (br, s, H-3 and H-6), 6.67 (d, 2H, J = 8.5 Hz, H-3"' and H-5"'), 6.48 (d, J = 2 Hz, H-8), 6.3 (d, J = 15 Hz, H-8 "'), 3.7-4.7 (m, 4.9-6.0 (m) (sugar protons).

^{13}C-NMR (DMSO-d_6-TMS): (C-2) 164.14s, (3) 103.14d, (4) 181.82s, (5) 161.245, (6) 99.46d, (7) 162.61s,(8) 94.74d, (9) 156.75s, (10) 105.31s (1') 120.89s, (2') 128.50d, (3') 116.51d, (4') 161.01s, (5') 116.51d, (6') 128.50d, (1") 99.46d, (2") 77.92, (3") 76.21d, (4") 69.96d (5") 73.82d, (6") 63.42t, (1"') 124.81s, (2"') 129.92d, (3"') 115.80d, (4"') 159.65s (5"') 115.80d, (6"') 129.92d (7"') 144.82, (8"') 113.90d, (9"') 166.30s. MS (FD), m/z 601 $(M+Na)^+$, 579 $(M+1)^+$, 578 (M^+).

Acidic hydrolysis of the flavone glucoside (2)

The hydrolysis was carried out as described above for the flavanone glycoside. The products isolated through PLC of the ethyl acetate portion included apigenin mp 230°, and methyl p-coumarate mp 130°. Both substances were identified through direct comparison with an authentic samples.

Blepharin (3)

The fractions eluted with ethyl acetate-chloroform (40:60) yielded after further purification colourless crystals of blepharin mp 226° (lit [10] mp 226-27°). IR ν_{max} cm^{-1} 3360 (br) 1690 (sh), 1680, 1610, 1500, 750. MS m/z 327, 165, 149, 136 (100%), 120, 109, 85, 73, 57.

Hydrolysis of Blepharin

20 mg of blepharin were dissolved in methanol (20 ml), conc. HCl added (0.2 ml) and refluxed on the water bath. The solvent was evaporated. The residue taken up in water basified with ammonia and extracted with ether. The ethereal layer yielded after purification through PLC a small amount of o-aminophenol, mp 172°, which was identified through superimposable IR spectra with an authentic sample.

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