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FLAVONOIDS FROM THE FLOWERS OF LIMONIUM SINUATUM MILL GROWING IN EGYPT

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From the flowers of Limonium sinuatum Mill Family Plumbaginaceae two flavonol glucosides were isolated. Their structures were established by physical, chemical and spectral methods (UV, IR, NMR and Mass) and proved to be:
5,7,4'-trihydroxyflavonol-8-o-glucopyranoside; 5,7,3',4',-tetrahydroxyflavonol-8-o-glucopyranoside.

Limonium sinuatum Mill. (=Statice sinuata L.) is a rough hairy perennial or biennial plant, about 50 - 100 ml tall and belongs to Family Plumbaginaceae[1,12].

It has been reported [6] that the leaves and petals of Limonium are rich in myricetin, quercetin and kaempferol. The only record of a flavone in the plumbaginaceae is luteolin in the flowers of L. sinuatum [6]. Aurones and Chalcons have recently been isolated from Limonium flowers [2], as well as many anthocyanins[7].

The detailed study of the plant and its flavonoid content was not completely investigated specially that grown in Egypt.

The present work was planned to study the chemical constituents of Limonium sinuatum Mill.

Experimental

Material

The flowers of Limonium sinuatum Mill., Fam. Plumbaginaceae were collected from plants grown in the Medicinal plants Experimental Station of the Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Extraction Procedure:

One kilo of the defatted powder was extracted with cold methanol. The methanolic extract was concentrated to a syrupy liquid, then added to ether, when a dark yellowish powder was precipitated (25 g.). TIC screening of the precipitated powder using cellulose adsorbent and developing system: Chloroform : Methanol : Water(30:9:1) revealed the presence of 6 flavonoidal spots (Table 1).

Column Chromatography:

10 g of the precipitated powder, was placed on the top of a 600 gm. cellulose column. Elution started initially with chloroform, then with chloroform-methanol mixture in increasing polarity till pure methanol. The column succeeded to separate in a pure form the two glycosides designated A and B.

Mild Acid Hydrolysis:

2 mg of each of the isolated glycosides were dissolved in 10 ml methanol to which 10 ml of 1 % aqueous hydrochloric acid solution were added and the mixture was refluxed on a boiling water bath for 2 hours. A sample of the hydrolysate was withdrawn with a glass capillary every 10 minutes for two hours. The samples were spotted on whatman No. 1 filter paper and the chromatogram was developed using 15 % acetic acid as solvent system. The chromatogram was visualized under UV-light(366 nm) . The two isolated glycosides were hydrolysed to their aglycones on one step.

Quantitative Acid Hydrolysis:

100 mg of each of the isolated glycosides were dissolved in 100 ml methanol to which 100 ml of 10 % aqueous hydrochloric acid solution were added and the mixture was refluxed on a boiling water bath for two hours. The hydrolysate was concentrated in order to remove methanol and the aglycone was extracted with ether. The ether was distilled off and the residue was weighed. The percentage of aglycones were 64.02 and 65.11 corresponding to the isolated glycosides A and B, respectively.

The acidic mother liquor containing the sugar moiety was concentrated and spotted alongside with authentic sugars on Whatman No. 1 filter paper using BAW (4 : 1 : 5) as the solvent system, chromatography proved that glucose was the only sugar moiety in both two glycosides.

Table 1. Chromatographic characters of flavonoids present in flowers of limonium sinuatum Mill.

h_{R_f} in	*Colour of spots in UV light*	
System 1	*Before spraying*	*After spraying with ammonia*
62	Pale orange	Dark yellow
46	Yellow	Yellow
29	Orange	Dark yellow
18	Yellow	Yellow
12	Dark brown	Lemon yellow
5	Lemon Yellow	Lemon yellow

Legend : System I = Chloroform : Methanol : water (30 : 9 : 1).

Results And Discussion

Concerning the separated two compounds (A and B), chromatographic, chemicel and spectral studies showed that they are flavonoidal in nature (yellow colour in visible and UV-light with both aluminium chloride and sodium hydroxide[5,11] and a characteristic peak at 1670 cm^{-1} in IR-spectra[3]

Production of rose-pink colour accomplished by magnesium and concentrated HCl, show that these compounds may be of flavonol nature. This assumption is confirmed by maxima in the range of 375-380 nm. of their methanolic solutions in UV (Table II).

They reduced Fehling's solution after being hydrolysed, gave

positive Shinoda test (in aqueous phase) [4] indicating that they are glycosides.

They gave dark green colour with ferric chloride, yellow colour with boric and citric acids [13] and orange colour with boric acid and acetic anhydride [8], indicating the presence of free hydroxyl group at C-5 .

The isolated two compounds gave deep yellow flourescence with zerkonium oxychloride and citric acid [9] indicating the presence of free hydroxyl group at C-3 .

The forementioned assumptions proved that the two compounds in hand are flavonol glycosides containing free hydroxyl groups at C-5 and C-3 .

Table II. UV-spectral data of the isolated flavonoids and their aglycones.

Compound	λ *max nm*						
	MeOH	*NaOAc*	*NaOAc* H_3BO_3	*NaOMe*	$AlCl_3$	$AlCl_3$ *HCl*	$ZrOCl_2$
Glycoside A	375	415	378	420+	435	435	450
		325		330	365	365	
	270	280	270	280	275	275	280
	255						
Aglycone A	380	dec.	390	dec.	460	440	445
						370	
	282	280	280	280	280	270	280
	265sh.						
Glycoside B	380	398	395	430sh.	465	440	450
		335		330	355sh.	375	
	270sh	275	265	280	275	265	280
	255						
Aglycone B	382	dec.	400	dec.	460	440	450
						370	
	280	280	270	290	280	270	280
	265sh.						

Legend : Sh. = Shoulder; dec. = decomposition ;
+ = increase in intensity of absorbancy.

Glycoside A :

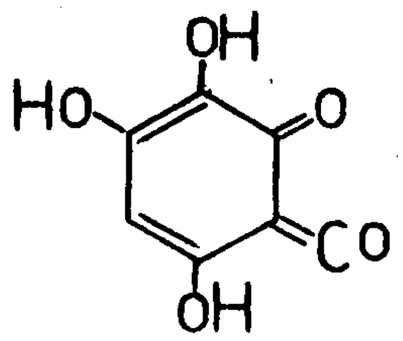
Bright yellow powder (450 mg); soluble in water, pyridine and ethanol; m.p. 212 - 214°C ; λ_{max} methanol 255, 270, 375 nm . Through its UV analysis (Table II), glycoside A gave bathochromic shifts in bands I and II with sodium acetate and sodium methoxide indicating the presence of free hydroxyl groups at C-7 and C-4' respectively. A characteristic bathochromic shift in band I with aluminium chloride (+ 60 nm) and zerkonium oxychloride[10] (+ 75 nm) indicates the presence of free OH at C-3 and C-5 respectively. UV-analysis proved that glycoside A contains four free OH at positions 3, 5, 7, 4'.

The IR-spectrum revealed the following characteristic absorption bands (cm^{1-}); 3440 - 3480 (OH groups); 1670 (γ pyrone ring); 1615 , 1560, 1510 (aromatic system); 1080, 1060 1035 (pyranose structure of the sugar); 880 (B-configuration) .

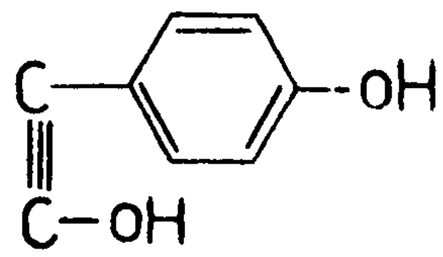
The NMR - δ DMSO : 7.9 (2H, d, J = 9Hz, H_2' H_6'); 7.0 (2H, d, J = 9Hz , H_3' , H_5'); 6.2 (1H, s, H_6); 5.1 (1H, anomeric proton of glucose); 3.4-3.68 (6H of glucose).

Glycoside A on mild acid hydrolysis yielded the aglycone in a single step and sugar glucose. This proves that glycoside A is a monoside which was also confirmed by determining percentage of aglycone in glycoside (64.02%).

The aglycone was separated as a yellow powder; m.p. 280-284°; λ_{max} methanol 282, 380 nm. The UV-data (Table II) proved the presence of five OH at positions 3, 5, 7, 8, and 4' . The NMR : δ^{DMSO} 7.85 (2 H, d, J = 9Hz, H_2', H_6') 6.90 (2H, d, J= 9Hz , H_3', H_5'); 6.2 (1 H, s, H_6). The M.S. gave molecular ion peak at 302 and two characteristic fragments at m/e 168 (28) and 134 (19) :



m/e 168



m/e 134

Therefore, the aglycone has the following structure : 5 , 7, 8, 4', -tetrahydroxyflavonol (= 8-hydroxykaempferol).

Since glycoside A contains 4 free OH at positions 3, 5, 7, 4', while its aglycone contains 5 free OH at positions 3, 5, 7, 8, 4' . So the sugar must be attached at position 8.

Therefore, glycoside A must have the following structure : 5,7,4'-trihydroxyflavonol-8-O-B-glucopyranoside .

Glycoside B :

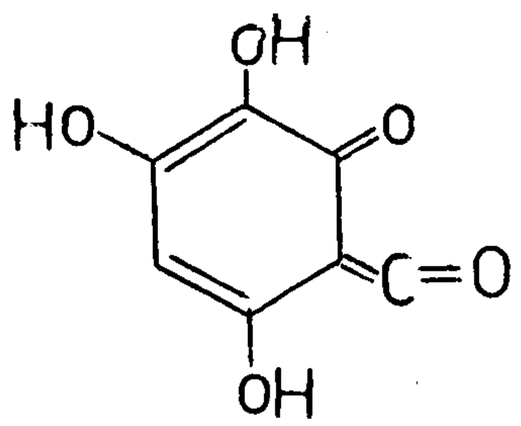
Canary yellow long needles (540 mg); soluble in water, methanol, ethanol and pyridine ; m.p. 227-229°C ; λ_{max} methanol 255, 270 sh, 380 nm. Glycoside B, as in the case of glycoside A is a monoglucoside yielding in a single step glucose and aglycone on mild acid hydrolysis and percentage of aglycone is 65.11.

The UV absorption data (Table II) indicate the presence of free hydroxyl groups at C-7,C-5 and C-3 (shifts with sodium acetate, zerkonium oxychloride and aluminium chloride respectively). Glycoside B contains orthodihydroxyl group at C-3' , C-4',this was proved by shifts with sodium acetate/boric acid as well as by aluminium chloride/hydrochloric acid. Therefore, glycoside B contains five free OH at positions 3, 5, 7 ,3', 4'' ,

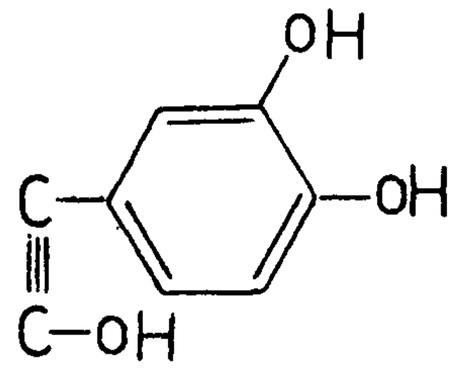
The IR-spectrum of glycoside B is closely related to that of glycoside A indicating that glucose has a pyranose form with B-configuration.

The NMR of glycoside B : δ^{DMSO} 7.50 (2H, d, J = 9Hz, H_2, H_6') 7.15 (1 H, d, J = 9Hz, H_5') ; 6.2 (1 H, s, H_6); 5.1 (1 H, anomeric proton of glucose); 3.50-3.7 (6H of glucose).

The aglycone was separated as yellow needles;m.p. 311-314°. The UV-data of aglycone (Table II) proved the presence of six free OH at positions 3, 5, 7, 8, 3' and 4' . The NMR : δ^{DMSO} 7.45 (2H, d, J = 9 Hz, H_2, H_6') 7.10 (1 H, d, J = 9 Hz, H_5') 6.2 (1 H, s, H_6). The M.S. gave molecular ion peak at m/e 318 and two significant fragments at m/e 68 (32)and 150 (14)



m/e 168



m/e 150

From the above studies we can conclude that the aglycone has the following structure :

5,7,8,3',4'-pentahydroxyflavonol (= 8-hydroxyquercetin ; gossyptin).

Therefore, glycoside B has the following structure :

5,7,3',4'-tetrahydroxyflavonol-8-O-B-glucopyranoside (= 8-hydroxyquercetin-8-O-B-glucopyranoside).

REFERENCES

1. *Bailey L.H., "Manual of Cultivated Plants". The MacMillan Co. New York, (1963).*
2. *Asen S. and Plimmer J.R., Phytochemistry, 11, 2601 (1972).*
3. *Bellamy L. J "The infrared spectra of complex organic molecules", 2nd ed. John Wiley& Sons. Inc. N. Y. (1958).*
4. *Bryant E. T., J. Am. Chem. Soc., 39, 481 (1950).*
5. *Geissman T. A., The chemistry of flavonoid compounds, The Macmillan Company, N. Y. (1962).*
6. *Marborne J. B. Biochemistry of Phenolic Compounds, Academic Press, London (1964).*
7. *Harborne J. B., Phytochemistry, 6. 1415 (1967).*
8. *Horhammer L. and Hansel R., Archiv der pharmazie, 285/57, 9, 438-444 (1952).*
9. *Horhammer L. and Hansel R., Arch. pharm., 286/68, No.8, 425-431 (1953).*
10. *Litvinenko V. I and Maxyotina N.P., "Chemistry of Natural Prodcuts", Russian, pp.420-424 (1965).*
11. *Mabry T.J., Markham K.R.and Thomas M.B., The systematic identification of flavonoids, Springer-Verlag, Berlin, (1970).*
12. *Tackholm V., Students flora of Egypt, Anglo Egyptian Bookshop, Cairo (1974).*
13. *Wilson S.W., J. Am. Chem. Soc., 61, 2303 (1939).*

المركبات الفلافونية الموجودة فى أزهار نبات الليمونيم سنديراتم ميـــــــل الذى ينمو فى مصـــــــــر

سمير أنيس روس — دارود ونس بشاى
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من أزهار نبات الليمونيم سنديراتم ميل (ستاتيس سنيواتا) تم فصل فلافونيدان علـــــى هيئة سكاكر وذلك باستعمال كروماتوجرافيا العمود مستخدما مادة السليلوز • تم التوصـــل الى التركيب الكيمائى لهم عن طريق التحليل الطيفى بالأشعة فوق البنفسجية والأشعـــة تحت الحمراء والرنين النووى المغناطيسى وتعيين درجة الانصهار • لقد اجرت لهـــم الحلمأة الحمضية المخففة للتأكد من ان السكر احادى فى كل منهم وثبت انه سكر جلوكـــوز وكذلك تم فصل الفلافونيدات الحرة من الجزء المتحلل والتعرف عليهم بواسطة الاشعـــة فوق البنفسجية والرنين النووى المغناطيسى وايضا باستخدام مطياف الكتلة وكذلك تعيين درجة الانصهـــــاز •

تبين ان التركيب الكيمائى للمركبين المفصولين من الازهار هو :

المركب الاول : ٥ ، ٧ ، ٤َ ـ ثلاثى هيدروكسيد الفلافونول ـ ٨ ـ أ ـ جلوكوزيـــــد

المركب الثانى : ٥ ، ٧ ، ٣َ ، ٤َ ـ رباعى هيدروكسيد الفلافونول ـ ٨ ـ أ ـ جلوكوزيـــــد