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NOTE

Hypoglycaemic Activity of Flavonoid Isolate from Cereus Pterogonus Lemaire

V. RAGUNATHAN, K. MOHAN KUMAR and A. SANKAR*

Post Graduate and Research Department of Chemistry, Kandaswami Kandar's College, Velur-638 182, India

*Corresponding author: E-mail: sanvishnu2010@gmail.com

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The flavonoid glycoside isolated from *cereus pterogonus* Lemaire possesses hypoglycaemic activity. The aglycone is myricetin-5'-O-methyl ether and the sugars are glucose and rhamnose. All these were characterized by UV, ^{1}H NMR, ^{13}C NMR and MS studies.

Key Words: *Cereus pterogonus* Lemaire, Myricetin-5'-O-methyl ether.

In continuation of our studies on flavonoids[1a-g] as biologically active agents, we now report the isolation of flavonol glycoside *viz.*, myricetin 5'-O-methyl ether-3-O-neohesperidoside from white petals of flowers of *Cereus pterogonus* Lemaire. *Cereus pterogonus* Lemaire is a xerophyte and belongs to the Cactaceae.

Extraction and Fractionation: The fresh white petals of the flowers of *C. Pterogonus* (750 g) collected from Karur during July were extracted with 85 % 2 L analar grade ethanol under reflux and the concentrated extract was successively fractionated with 1 L petroleum ether, 750 mL benzene, 1 L peroxide free diethyl ether and 2 L ethyl acetate.

The pale yellow lustrous crystals obtained from ethyl acetate fraction gave an orange yellow precipitate with basic lead acetate, with dilute alkali it gave a yellow solution that rapidly changed to green. It did not respond Horhammer-Hansel and Molisch's tests but positive Wilson's boric acid test.

Hydrolysis of the glycoside: The glycoside (0.05 g, 0.1 mmol) was dissolved in 5 mL hot methanol (50 %) and subjected to hydrolysis with an equal volume of 10 % H_2SO_4. The aglycone was identified as myricetin-5'-O-methyl ether-from its UV spectra. The sugars were identified as glucose and rhamnose from paper chromatography by comparison with authentic samples of glucose and rhamnose. The sugar content was estimated by Folin-wu's micromethod[2] and was in agreement of a bioside. The glycoside was resistant to hydrolysis by pectinase[3] which indicated that it is neohesperidoside and not a rutinoside.

Hypoglycaemic activity: Healthy female albino rats of wistar strain (*Rattus norvegicus*) aged about 90 days and weighing approximately 100-150 g were obtained. The animals were divided into four groups, each consisting of three animals. Group 1 were normal control animals which received the vehicle (distilled water) while those in group 2 were glucose control animals which received glucose orally at a dose of 150 mg/100 g BW as 15 % glucose aqueous solution, group 3 were experimental ones which received the flavonoid glycoside solution. Group 4 were given standard drug glibenclamide at a dose of 15 mg/100 g BW as 1 % aqueous solution. After 30, 60, 120 min, glucose, flavanoid and glibenclamide administration each group of animals were sacrificed by decapitation, blood was collected, plasma separated and used for estimation of blood gluose by the method of Asatoor[4].

In the UV spectrum of the glycoside and the aglycone the band I absorption maxima are located at 362 and 374 nm, respectively which suggests that C-3 -OH may be involved in glycosylation[5]. It is also supported by the fact that the glycoside did not respond the Horhammer-Hansel test whereas the aglycone did. The bathochromic shift of 44 nm observed in the NaOMe spectrum (band I) of the glycoside indicates the presence of a free -OH at C-4[6]. An additive bathochromic shift of 36 nm observed in the $AlCl_3$ spectrum with respect to $AlCl_3$-HCl spectrum indicates the presence of *o*-dihydroxyl grouping in the B-ring[7] which is also confirmed by the bathochromic shift of 12 nm in its NaOAc-H_3BO_3 spectrum[8,9]. The presence of a free -OH at C-7 is evident from the bathochromic shift of 8 nm (band II) on the addition of NaOAc[10]. The appearance of four absorption maxima in the $AlCl_3$ spectrum, bathochromic shift of 28 nm (band I) in the $AlCl_3$-HCl spectrum with respect to methanolic spectrum[11] and the positive Wilson's boric acid test indicate the presence of a free -OH at C-5.

In the ^{1}H NMR spectrum (270 MHz, DMSO-d_6, TMS) of the glycoside, the signals of the protons at C-2' and C-6 merge at δ 6.24 ppm. The protons at C-8 resonates at δ 6.40 ppm (d,

TABLE-1
^{13}C NMR DATA

Compund	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Glycoside (δ ppm)	158.20	134.37	178.38	161.71	99.87	164.69	95.01	157.50	105.09
Compound	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'	C of -OCH_3		
Glycoside (δ ppm)	123.07	116.33	149.10	145.22	149.10	122.34	56.80		
Compound	C-1''	C-2''	C-3''	C-4''	C-5''	C-6''	–	–	–
Glycoside (δ ppm)	102.47	74.83	77.01	70.71	76.28	61.40	–	–	–
Compound	C-1'''	C-2'''	C-3'''	C-4''	C-5'''	C-6'''	–	–	–
Glycoside (δ ppm)	101.53	71.11	69.16	72.74	69.15	18.06	–	–	–

TABLE-2
EFFECT OF FLAVONOL GLYCOSIDE ON GLUCOSE INDUCED HYPERGLYCAEMIA

Treatment	Dose mg/100 g	Glucose in 100 mL blood (mg)		
		0.5 h	1.0 h	2.0 h
Normal control	–	93.50 ± 5.61	–	–
Glucose control	150	144.58 ± 1.64	125.83.50 ± 1.05	95.83 ± 1.39
Flavonoid Glycoside	15	91.67 ± 5.6.54	89.58 ± 3.73	83.33 ± 4.46
Glibenclamide	15	114.17 ± 1.90	92.92 ± 2.30	Statistically not significant

J = 1.8 Hz). A doublet appearing at δ 6.88 ppm (J = 2Hz) corresponds to the proton at C-6'. The upfield absorption of C-2', C-6' protons indicates the presence of -OCH_3 group at C-5', the protons of which resonate at δ 3.90 ppm[12]. H-1'' of glucose resonates at δ 5.37 ppm and H-1''' of rhamnose at δ 5.15 ppm[13]. The remaining sugar protons appear in the region of δ 3.00-4.00 ppm. The methyl protons of the rhamnosyl unit can be located at δ 1.25 ppm[14].

The various signals noticed in the ^{13}C NMR spectrum of the glycoside as assigned to the different carbons are presented in Table-1. Due to glycosylation, the C-3 signal appears at δ 134.37 ppm. The methoxyl carbon resonates at δ 56.80 ppm. The appearance of C-6''' at δ 18.06 ppm and C-6'' signal at δ 61.40 ppm shows that the glycoside is a neohesperidoside[15,16].

The MS of the glycoside shows the molecular ion peak at m/z 332 $(C_{16}H_{12}O_8)^+$. The M^+ ion accounts for the aglycone (sugar units getting cleaved)[17]. The fragments at m/z 153 (RDA fragment) and 152 (RDA fragment with proton shift) give a clear picture of the substitution pattern in the A-ring of the glycoside[18] whereas the ions at 317 $(M-15)^+$ and 167 are indicative of B-ring substitution[19].

From the above chemical and spectral evidences, the glycoside from the EtOAc fraction has been characterized as myricetin 5'-methyl ether-3-O-neohesperidoside which is a new and rare glycoside to be reported from a natural source.

The flavonoid isolate has been found to cause a significant decrease ($p < 0.001$) in the fasting blood glucose level 0.5 h after glucose load administration, when the glycaemic values were the highest in the controls (Table-2). The fasting blood glucose level of animals treated with bioflavonoid has been found to be significantly different from those of the controls of the same weight and age. The flavonoid is able to lower the fasting blood glucose concentration and improve glucose tolerance.

Conclusion

The fresh white petals of flowers of *Cereus pterogonus* Lemaire have been found to contain myricetin 5'-methyl ether-3-O-neohesperidoside which has been duly characterized by chromatographic and hydrolytic studies as well as spectral techniques. The glycoside posseses considerable hypoglycaemic activity.

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