Hypoglycemic Activity of a New Carbohydrate Isolated from the Roots of *Psacalium peltatum*

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A new ulopyranose isolated from aqueous extract of roots and rhizomas of *Psacalium peltatum* has been determined to have hypoglycemic activity at doses of 100 mg/kg, comparable to that of tolbutamide and insulin in alloxan diabetic mice. The skeletal structure of the new compound was established by spectral analysis.

Key words ulopyranose; *Psacalium peltatum*; hypoglycemic effect; peltalosa

Psacalium peltatum (H.B.K.) Cass. (Syn. *Senecio peltiferus* Hemsl., Asteraceae), commonly known in Mexico as "matarique", has been used for long time in traditional medicine for the treatment of diabetes mellitus, an important endocrine ailment.[1,2] The hypoglycemic activity of the decoction prepared from roots and rhizomes of *P. peltatum* has been validated in alloxan-diabetic animals.[3—5] Recently, an aqueous extract of the plant was evaluated for its hypoglycemic activity alloxan-diabetic mice, exhibiting significant activity.[6] This work describes the structure and hypoglycemic activity of peltalosa, a new carbohydrate type ulopyranose, isolated from an aqueous extract of *P. peltatum*.

Experimental

Psacalium peltatum was acquired from the Sonora's Market in Mexico City in March of 1999. MS Abigail Aguilar from Herbarium of Instituto Mexicano del Seguro Social identified the material and a voucher specimen of the plant (11490) has been stored.

Extraction and Isolation of Peltalosa The root was dried at room temperature; ground and then 250 g were extracted with water (1 l) at 40 °C for 2 h. The aqueous extract was then filtered and freeze dried to produce dark brown solid (yield 2.0%), which was crystallized from water as light brown crystals. The purity of this compound was determined by TLC (thin layer chromatography) and HPLC (high-pressure liquid chromatography) using a MCH-5 column, the sample was eluted with MeOH–H_2O 50 : 50 mixtures with 0.6 ml/min flow rate. ELSD (evaporative light scattering detector) detector was set at 87.5 °C and 4 l/min of nitrogen flow.

Acetylation of Peltalosa A solution of 200 mg of peltalosa and 2 ml of pyridine was acetylated with 5 ml of Ac_2O and stirred at room temperature overnight. A chromatography on silica gel was made with the crude product. The acetate of peltalosa was obtained as a beige solid, its ^{1}H- and ^{13}C-MNR showed that this derivated acetate had three different acetyl groups and one free hydroxyl group.

2,4-Dinitrophenylhydrazone of Peltalosa[7] Peltalose (300 mg) was dissolved in 5 ml of ethanol and 4 ml of a solution at 10% of 2,4-dinitrophenylhydrazine in H_2SO_4 were added. The mixture was stirring at room temperature for 30 h. However no hydrazine derivative was obtained.

General Methods Melting points were determined on a Fisher Scientific apparatus and are incorrected. Infrared spectra (IR) were recorded in a Perkin Elmer FT Paragon 1000 Spectrometer, using KBr pellet. ^{1}H- and ^{13}C-NMR spectra (nuclear magnetic resonance) were recorded at room temperature for solutions in MeSO-d_6 or $CDCl_3$ on a 400 MHz Varian Mercury. Chemical shifts are expressed relative to those of MeSO-d_6 (2.49 ppm) or $CHCl_3$ (7.26 ppm). Deuterium exchanges were performed in order to confirm hydroxyl groups assignment. COSY (correlation spectroscopy), DEPT (distortionless enhancement by polarization transfer) and HMQC (heteronuclear multiple-quantum correlation) experiments were also performed on this instrument.

Optical rotation was measured in a 1-cm cell on a Perkin-Elmer Model 141 polarimeter at 20 °C. Elemental analyses were determined by USAI Facultad de Química of Universidad Nacional Autónoma de México. The molecular weight was determined by freezing point depression using camphor as solvent.

Experimental Animals Male mice, CD1 strain, weighing from 20 to 30 g were used. The handling of the laboratory animals was performed in agrees with the statutes of the CICUAL (Institutional Committee for the Care and Use of the Animals) and with the Official Mexican Rule.[8] The animals were kept in an air-conditioned animal room with a 12 h light–12 h dark cycle; they were given a commercial feed prepared by Purina and allowed tap water *ad libitum*. Prior to each study, the animals were subjected to fasting for 12 h.

Induction of Experimental Diabetes A freshly prepared solution of alloxan in normal saline (ISS) was injected three times into the caudal vein (50 mg/kg body wt.) at intervals of 48 h.[9] Blood glucose levels were determined in animals with 12 h of fasting 7 d after the last alloxan administration. Only animals with blood sugar ranging between 190 to 340 mg/dl were included.

Hypoglycemic Effect in Mild Alloxan-Diabetic Mice Mice with glycemic levels in fasting between 190—299 mg/dl (mild alloxan diabetic mice) were divided into 5 groups: The first group was administered with ISS as control (4 ml/kg), the second one received tolbutamide (50 mg/kg), third and fourth groups received 50 or 100 mg/kg body wt. of peltalose, and the last one received insulin (0.1 U.I./kg).

Hypoglycemic Effect in Severe Alloxan-Diabetic Mice Mice with fasting glycemic levels higher than 300 mg/dl (severe alloxan diabetic mice) were divided into four groups: The first group was administered with ISS as control (4 ml/kg), the second one received tolbutamide (100 mg/kg), the third group received 100 mg/kg body wt. of peltalosa, and the last group received insulin (0.1 U.I./kg).

The substances were intraperitoneally (i.p.) injected. Peltalosa and tolbutamide were dissolved in ISS. Blood samples were obtained from tail vein. Glucose level was determined by the glucose-oxidase-peroxidase method with an Accutrend Sensor Confort apparatus using reactive strips (Roche).[10]

Statistical Analysis Results were expressed as mean±S.E.M. (standard error media). The significance of the differences among the means of the tests and control studies was established by one way analysis of variance (ANOVA) followed by Tukey–Kramer Multiple-Comparison Test, using NCSS computer package, and p values <0.05 were considered significant.

Results

Aqueous extract gave a light brown solid (peltalosa), mp 187—189 °C, $[\alpha]_D^{20} = -310°$ (c=0.01, H_2O). *Anal.* Calcd $C_{10}H_{18}O_{10}$: C, 40.27; H, 6.04. Found: C, 39.93; H, 5.80. Molecular weight 298. IR ν_{max} 3388, 2923, 1434, 1385, 1034 and 935.

FAB mass spectrum was not possible to obtain because peltalosa decomposed under these conditions.

^{1}H-NMR: δ 3.59 (d, 2H×2, J=15 Hz), 3.72 (m, 1H×2), 3.97 (d, 1H×2, J=11.5 Hz), 4.13 (m, 1H×2), 4.68 (s, 1H×2), 4.84 (s, 1H×2), 5.30 (s, 1H×2), 5.31 (s, 1H×2).

^{13}C-NMR: δ 61.8, 74.4, 77.1, 81.7, 103.4.

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Table 1. Effect of Peltalosa on the Blood Glucose Level in Mild Alloxan Diabetic Mice

Group	0 h	2 h	4 h
Control	255±12.5	252±10.3	248±4.0
Tolbutamide (50 mg/kg)	258±7.5	230±8.0	217.2±6.5*
Compound I (50 mg/kg)	253±10.0	248±8.5	250±6.0
Compound I (100 mg/kg)	198.7±3.7	161.9±6.3*	62.5±5.5*
Insulin (0.1 U.I./kg)	268±8.5	197.4±8.0*	168±6.5*

The results are expressed in mg/dl of glucose in blood (mean±S.E.M., $n=10$). Significant differences: $*p<0.05$.

Table 2. Effect of Peltalosa on the Blood Glucose Level in Severe Alloxan Diabetic Mice

Group	0 h	2 h	4 h
Control	310.0±2.3	301.1±3.7	304.4±4.2
Tolbutamide (100 mg/kg)	317.0±7.5	285.3±3.0*	263.0±3.5*
Compound I (100 mg/kg)	328.0±12.5	298.0±4.5	262.1±6.0*
Insulin (0.1 U.I./kg)	323.2±11.0	243.2±4.0*	202.0±6.5*

The results are expressed in mg/dl of glucose in blood (mean±S.E.M., $n=10$). Significant differences: $*p<0.05$.

Acetate of peltalosa, mp 73—75 °C, $[\alpha]_D^{20}=-69°$. *Anal.* Calcd $C_{22}H_{30}O_{16}$: C, 48.00; H, 5.45. Found: C, 47.92; H, 5.21. IR ν_{max} cm^{-1} 3332, 2928, 1738, 1460, 1371, 1249 and 1049.

^{1}H-NMR: δ 2.08 (s, 3H×2) 2.09 (s, 3H×2), 2.1 (s, 3H×2), 3.72 (d, 2H×2, J=8.8 Hz), 3.84 (d, 1H×2, J=9.2 Hz), 4.19 (m, 1H×2), 4.38 (m, 1H×2), 5.4 (s, 1H×2).

^{13}C-NMR: δ 29.4, 29.6, 29.7, 63.8, 75.5, 75.8, 77.2, 103.7, 169.8, 170.1 and 170.7.

The acetate was prepared in order to determine the molecular weight, however, as well as peltatose, FAB-MS (fast atom bombardment mass spectrometry) was not possible to obtain because the acetate also decomposed under the used conditions.

The results obtained when peltalosa was i.p. administered in doses of 50 and 100 mg/kg to mice with mild diabetes are shown in Table 1. Basal glycemia oscillated among 198±3.7 and 268 mg/dl. Control group glycemia lightly changed from 255±12.5 to 248±4.0 mg/dl at 4 h ($p<0.05$). In this model, tolbutamide and insulin caused significant reductions of glycemia at 4 h ($p<0.05$). However, the most prominent hypoglycemic effect was produced with the administration of 100 mg/kg body wt. of peltalosa (from 198.7±3.7 to 62.5±5.5 mg/dl), being statistically significant ($p<0.01$). Finally, glycemia in severe alloxan diabetic mice in fast ranged from 310.0±2.3 to 328.0±12.5 mg/dl (Table 2). Glycemia did not show changes in control group, but equivalent doses (100 mg/kg) of tolbutamide and peltalosa caused significant reductions, almost similarly as that produced by insulin at 4 h ($p<0.05$).

Discussion

The aim of this research was to determine the hypoglycemic activity of a new type ulopyranose compound (peltalosa), isolated from roots and rhizomes of *Psacalium peltatum*, in alloxan diabetic mice.

Peltalosa was identified as 2,6-anhydro-5-ulopyranose (Fig. 1). This compound contained hydroxyl groups

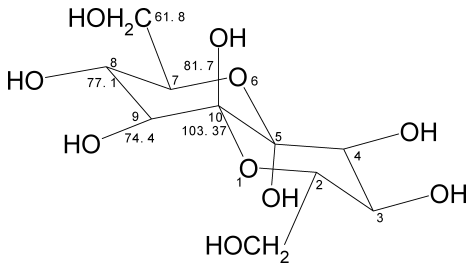

Fig. 1. Structure of Petalosa

(3388 cm^{-1}) and an ether group (1034 cm^{-1}). The molecular formula was determined to be $C_{10}H_{18}O_{10}$ by elemental analysis.

^{1}H-NMR δ 4.68, 4.84, 5.30, 5.31 signals must be assigned to 4 hydroxyl groups, because the signals at δ 4.68, 4.48 and 5.30 disappeared when pentalose is acetylated. Following connectivity from COSY and HMQC spectra, the doublet at δ 3.97 (J=11.5 Hz) was assigned to H-4, and it was correlated to C-4 (74.4 ppm). The multiplets signals at δ 3.72 and 4.13 were designated to H-2 and H-3 and they were connected to C-2 (81.7 ppm) and C-3 (77.09 ppm) respectively. Each of these signals integrates for one proton. The doublet signal at δ 3.59 (J=15 Hz), integrated for two protons, exhibits correlation to the C-6 (61.8 ppm) of hydroxymethylen group.

^{13}C (DEPT)-NMR spectrum indicated that peltalosa contains three CH at δ 74.4, 77.1 and 81.7, one CH_2 at δ 61.8, and one quaternary carbon (δ 103.4 ppm).

^{1}H-NMR spectrum of peltalosa acetate showed a signal at δ 5.4 that disappeared with D_2O, so it can be assigned to a tertiary hydroxyl group given the fact that these groups can not be acetylated.[11] ^{13}C-NMR spectrum showed three signals at δ 169.8, 170.1 and 170.7; they were assigned to three acetate carbonyl groups. Following connectivity from COSY and HMQC spectra, the three signals at δ 2.08, 2.09 and 2.11 were correlated to methyl groups of acetate at δ 29.4, 29.6 and 29.7 respectively. The multiplet signals at δ 4.19 and 4.38 were assigned to H-2 and H-3 respectively, and they were connected to C-2 (77.2 ppm), and C-3 (75.8 ppm). The doublet signal at δ 3.84 was designated to H-4 and δ 3.72 for hydroxymethylen group protons which correlated to C-4 (75.5 ppm) and C-6 (63.8 ppm) respectively.

Peltalosa is a carbohydrate, however did not give 2,4-dinitrophenylhydrazone, this fact suggests that this compound did not have a free anomeric carbon. These results are in agreement with the structure shown in Fig. 1, which takes into account this fact.

In relation to the hypoglycemic activity of peltalosa, results show that when peltalosa was administered to mild diabetic mice (with blood sugar levels below to 300 mg/dl of blood) at doses of 100 mg/kg, there was a definitive reduction at 4.0 h (68.5%). However, at doses of 50 mg/kg there was not blood sugar decrease (Table 1). In addition, when the blood glucose level is higher than 300 mg/100 ml the decrease is lower (20%). These results indicate that peltalosa has a hypoglycemic activity on mice with mild diabetes, but this activity diminished on mice with severe diabetes (Table 2). It is likely that the hypoglycemic effect is similar to tolbutamide, and possibly due to an enhanced secretion of insulin from the islets of Langerhans or an increased utilization of

glucose by peripheral tissues.[12)]

The herein reported hypoglycemic activity in *P. peltatum* agrees with previously reported results,[3—6)] in which the traditional preparation (aqueous decoction) of this plant showed significant hypoglycemic effect in temporarily and alloxan hyperglycemic rabbits, as well as in healthy and alloxan diabetic mice. We recently also studied the hypoglycemic activity of four extracts obtained from the roots and rhizomes of *P. peltatum* (hexane, chloroform, methanol, and water extracts), concluding that the two most polar extracts exhibited hypoglycemic activity in healthy mice.[6)] Many attempts have been made to isolate hypoglycemic principles submitting methanol extract through different separation processes and various fractions and subfractions have been obtained, however hypoglycemic activity have not been detected in performed biological trial. In some cases the hypoglycemic activity of these fractions is lost through the pharmacological screening.[6,13)] In the present investigation an aqueous extract was also submitted to separation process obtaining peltalosa, a new hypoglycemic ulopyranose type carbohydrate.

It is necessary to mention that various hypoglycemic carbohydrates have been proposed as an alternative in the diabetes mellitus control.[14)] However, the lack of clinical studies with these principles is because there are no toxicological studies. Therefore, it is important to start toxicological and clinical studies with these hypoglycemic substances for using as an alternative for the control in type 2 diabetic patients.

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