Seasonal Variation of Kaurenoic Acid, a Hypoglycemic Diterpene Present in *Wedelia paludosa* (*Acmela brasiliensis*) (Asteraceae)

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We evaluated the variation of the concentration of kaurenoic acid (**1**), which is a bioactive diterpene, in leaves, flowers, stems and roots from *Wedelia paludosa* (*Acmela brasiliensis*) for different seasons using the HRGC/FID method. The results indicated that the concentration of **1** is higher in the roots and stems during the autumn. The pharmacological results suggested that kaurenoic acid is responsible, at least in part, for the hypoglycemic potential detected in this plant.

Key words: Wedelia paludosa, Kaurenoic Acid, Hypoglycemic Effect

Introduction

Kauranes consist of a class of diterpenes which contain a rigid tetracyclic skeleton. They are also intermediates in the biosynthesis of the gibberellins, which are plant growth hormones of several plants, some fungal metabolites and diterpene alkaloids (Ghisalberti, 1997). Antimicrobial, antiparasitic, insect antifeedant, anti-HIV and anti-inflammatory activities have been reported for different kauranes (Rezende *et al*., 2000). Ent-16-kauren-19-oic acid or kaurenoic acid (**1**) is one of the most important members of this family, exhibiting interesting biological properties, including analgesic (Block *et al*., 1998a,b), antifungal (Sartori *et al*., 2003) and smooth muscle relaxant (De Alencar Cunha *et al*., 2003) effects.

It is well-distributed in several species of plants, including those belonging to the genus *Xylopia* (Annonaceae) (Takahashi *et al*., 1995), *Mikania* (Nascimento and Oliveira, 2001), *Annona* (Oliveira *et al*., 2002) and *Wedelia* (Asteraceae) (Bresciani *et al*., 2000).

Recently, we determined the variation of **1** in the leaves, flowers, stems and roots of *W. paludosa* (Bresciani *et al*., 2000), reclassified as *Acmela brasiliensis* (Asteraceae). In this work, we present the results of the study of the concentration of kaurenoic acid (**1**) in leaves, flowers, stems and roots in relation to different seasons, using the HRGC/FID method. We also demonstrate for the first time, its hypoglycemic effect when administered to diabetic rats.

Material and Methods

Plant material

The plant was always collected at the same place, next to the Department of Chemistry/UFSC, on the following dates: 05/1998 (autumn), 08/1998 (winter), 12/1998 (spring) and 01/2001 (summer). The plant was dried at room temperature and then separated into flowers, stems, roots and leaves.

Methodology of extraction

The different parts of the plant (2.0 g each) were cut in small pieces and macerated with *n*-hexane (about 150 ml) at room temperature for 5 d. At the end of this period, the extracts were filtered and concentrated to a volume of 1–2 ml, and stored in a freezer. The samples were silylated with BSTFA [bis(trimethyl-silyl)trifluoro-acetamide] for direct HRGC analysis.

Chromatographic analysis

The chromatographic analyses were performed on a GC-14 A Shimadzu equipped with a 30 m × 0.25 mm i.d. column coated (0.3 μm film thickness)

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with cross-linked polymethylsiloxane as stationary phase (column LM-1; L&M, São Carlos, Brazil). Samples were introduced using the "splitless mode" (1 min, 1.0 μl injection volume) with a flame ionization detector (FID) temperature at 320 °C and a column temperature programming from 40 °C at 8 °C/min to 310 °C (held for 10 min). Hydrogen was used as the carrier gas. The data were processed using the Cromatografia program (Microquímica, Florianópolis, Brazil). Each determination was carried out in duplicate at least. Highly-pure kaurenoic acid (>98%) was used for calibration curves after silylation. The standard compound was previously isolated from this same plant (Block *et al*., 1998a; Bresciani *et al*., 2000).

Animals

Male Wistar rats, weighing 200–300 g, were housed in standard environmental conditions at a temperature of 23 ± 2 °C, relative humidity and a 12 h light/dark cycle. They were fed on a standard pellet diet and tap water was given *ad libitum* during the experimental period.

Biological assay

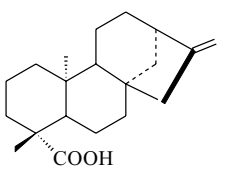
The rats with blood glucose concentrations of 90–100 mg/dl were divided into groups with 6 rats each. Prior to each study, the animals were subjected to fasting for 18 h (Alarcon-Aguilar *et al.*, 1998). Diabetes was induced in rats by single intraperitoneal administration of alloxan monohydrate (180 mg/kg) (Al-Shamaony *et al.*, 1994). After 3 d, the blood was collected (from fasting animals) and the glucose was determined by the glucose oxidase method using commercial kits. The animals with blood glucose levels above 150 mg/dl were separated for the oral glucose tolerance. All the groups received glucose (5 g/kg) after collecting the first blood sample. Blood was collected 1, 2, 4 and 6 h after the glucose ingestion. Group I was a normoglycemic control in which normal animals received saline solution (0.9% NaCl, 3 ml/kg). Groups II, III and IV were alloxan-induced diabetic rats. Group II was used as a hyperglycemic control and received saline solution (0.9% NaCl, 3 ml/kg); group III received glibenclamide (40 mg/kg) as a reference drug; group III received kaurenoic acid (**1**) (10 mg/kg) isolated from *W. paludosa* (Block *et al*., 1998a).

Statistical analysis

The data are expressed as the mean ± S. D. The significance of the difference between the mean values for the test and control studies was established by analysis of variance (ANOVA) followed by the Dunnett test.

Results and Discussion

Several analytical methods can be used for the quality control of plants, however, gas chromatography is the technique which has been most used and with greater success for the extracts and phytotherapeutic standardization (Vilegas *et al*., 1995; Bauer and Tittel, 1996; Cechinel Filho and Yunes, 1998).



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The seasonal variation of kaurenoic acid (**1**) can be seen in Table I, which indicates the profile of its concentration at different parts of the plant for different seasons. It can be noted that the variation of **1** in the different seasons (autumn, winter, spring and summer) was fairly accentuated. The amounts of **1** are higher in the roots and stems during the autumn. This fact suggests that kaurenoic acid (**1**) may be the precursor for other groups of compounds with biological function of growth, such as gibberellins (Ghisalberti, 1997).

Our research group previously showed that the hydroalcoholic extract and, specially hexane fraction, exhibit pronounced hypoglycemic properties indicating that non-polar components could be acting on the glycemia (Novaes *et al*., 2001; Dutra *et al*., 2001). For this reason, we have now eval-

Table I. Seasonal variation of kaurenoic acid (**1**) present in different parts of *W. paludosa*.

Parts of plant	Mass fraction of kaurenoic acid [mg/g dry plant]			
	Autumn	Winter	Spring	Summer
Roots	6.65	0.0049	1.35	0.101
Stems	4.96	0.0735	0.037	0.0142
Leaves	1.06	0.052	0.114	0.288
Flowers	1.04	0.856	0.301	0.0255

Table II. Effect of kaurenoic acid (**1**), saline (control), or glibenclamide on blood glucose levels in alloxan-induced diabetic rats.

	Basal value	1 h	2 h	4 h	6 h
Normal control (n = 9)	64.02 ± 13.58	126.92 ± 28.43**	113.29 ± 13.21**	113.28 ± 23.92**	78.15 ± 14.02
Hyperglycemic control (n = 6)	325.33 ± 80.9	448.11 ± 91.8	502.59 ± 124.4*	539.58 ± 122.28*	577.4 ± 139.03**
Glibenclamide (40 mg/kg) (n = 6)	256.56 ± 38.59	470.52 ± 100.75**	434.3 ± 99.08**	365.53 ± 53.95	332.03 ± 69.93
Kaurenoic acid (10 mg/kg) (n = 5)	316.92 ± 130.05	587.58 ± 78.27**	413.26 ± 131.33	421.92 ± 112.99	296.75 ± 78.09

* $p < 0.05$; ** $p < 0.01$ (Dunnett test).

uated the hypoglycemic potential of **1**. As shown in Table II, in alloxan-induced diabetic rats compound **1** at 10 mg/kg presented significant and considerable effects on glucose levels in relation to the control group, being more efficacious than the hydroalcoholic extract and hexane fraction, studied previously (Novaes *et al*., 2001; Dutra *et al*., 2001). It also lowered the glucose blood levels more rapidly than glibenclamide, used here as a reference drug, in the period analyzed. This suggests that **1** is contributing to the hypoglycemic activity of *W. paludosa*. Although preliminary, these results are of particular interest, considering the increasing search by the pharmaceutical industries to discover new and effective clinical agents for the treatment of diabetes mellitus, which is a chronic disease characterized by high blood glucose levels caused by inadequate insulin secretion or impaired insulin action (Mandrup-Poulsen, 1998; Venkatesh *et al*., 2003). Another important fact is the high yield of compound **1** in *W. paludosa*, which may be used as start material to obtain more potent derivatives. Thus, considering the previous biological properties described for *Wedelia paludosa* (*Acmela brasiliensis*), in some case directly related to the presence of kaurenoic acid (**1**), the determination of the best season for its production is suitable for standardization of the extracts of this plant and their phytopreparations. The determination of **1** as the main active principle is a potential help in future pre-clinical and clinical investigation. However, other studies are required because it is well-known that the secondary metabolites are influenced, either qualitatively or quantitatively, by the age of the plant, variety, soil type, apprenticeship of development of the plant and climatic conditions (Cechinel Filho and Yunes, 1998; Hook *et al.*, 1999).

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