www.saber.ula.ve/avancesenquimica
Avances en Química, 5(1), 63-65 (2010)
Nota

Chemical constituents of the leaves from *Trattinickia rhoifolia*

Ubaldo J. Salazar-Silvera & Carmelo Rosquete-Porcar

Laboratorio de Productos Naturales, Departamento de Química, Facultad de Ciencias, Universidad de los Andes, Mérida-5101, Venezuela.

(*) carmelor@ula.ve

Recibido: 29/01/2010 **Aceptado**: 10/03/2010

Resumen:

De las hojas frescas de *Trattinickia rhoifolia* Willd. fueron aislados los componentes mayoritarios: amentoflavona, β-sitosterol, 5α,6α- y 5β,6β-epoxi-β-sitosteroles y nonacosano. Sus estructuras fueron determinadas por métodos espectroscópicos uni- y bidimensionales y síntesis parcial.

Palabras clave: *Trattinickia rhoifolia*; amentoflavona; β-sitosterol; 5-epoxi-β-sitosterol

Abstract

Amentoflavone, β-sitosterol, 5α,6α- and 5β,6β-epoxy-β-sitosterols, and nonacosane have been isolated as major constituents from fresh leaves of *Trattinickia rhoifolia* Willd. Its structures were determined by 1D- and 2D spectroscopic methods and partial synthesis.

Keywords: *Trattinickia rhoifolia*; amentoflavone; β-sitosterol: β-sitosterol 5,6-epoxides.

Introduction

"Caraño" (*Trattinickia rhoifolia* Willd.) is a popular medicinal tree. Its resin is used for the treatment of throat diseases and vegetal material removal from skin lacerations, and its leaves (as infusion) are used as stimulant and for the treatment and prevention of tumors and leukemia. Resin was chemical and pharmacologically studied for us, and obtained results are in accordance with folk uses[1]. Dichloromethane and acetone extracts from the leaves showed moderate effect to mice leukemia cell P388. In this work we report the result obtained from the chemical study of low and media polarity extracts of *T. rhoifolia.*

Experimental

*General Experimental Procedures***.** Optical rotation was recorded on an Atago-Polax-2L Polarimeter using $CHCl_3$ as solvent. IR spectra were recorded as KBr disc on a Perkin Elmer FT-IR Spectrometer 1725X. UV spectra were recorded on an UV Varian Scan 3 using methanol as solvent. NMR spectra were run on Bruker Avance DRX 400 using $CDCl_3$ or DMSO-D_6 as solvents and TMS as internal standard. Mass spectra were recorded on a Hewlett-Packard Mass Spectrometer model 5930[a] (70 eV).

Plant material. T. rhoifolia leaves were collected at Mucujepe-Mérida State (400 m.o.s.l.) in March 1995. The plant material was identified by comparison with voucher specimens already deposited at the herbarium of Forest Sciences Faculty, Universidad de los Andes, Mérida, Venezuela (MER Nava 1-2, collected on March 1987).

Extract preparations. Fresh leaves (2.0 Kg) were extracted in Soxhlet with *n*-hexane, methylene dichloride, acetone, methanol, and water successfully.

Compounds isolation and identification. All extracts were very rich in chlorophylls which were removed using Sephadex® LH-20 column chromatography. From hexane extract (120 mg) was isolated by PTLC nonacosane (**I**) which was identified by GC/MS. Dichloromethane extract (14.16 g) afforded before Si gel CC and successful PTLC: amentoflavone (**II**, 96 mg) and β-sitosterol (**III**, 270 mg). From acetone extract (8.02 g) were obtained by Si gel CC and PTLC a mixture (67 mg, 7:3 w/w relation) of 5α,6α-epoxy-β-sitosterol (**IV**) and 5β,6β-epoxy-β-sitosterol (**V**). The mixture was quantified by ^{1}H NMR spectra and GC/MS, and **IV** was synthesized by 5,6 double-bond epoxydation of β-sitosterol with *meta*-chloroperbenzoic acid.

By chemical shift and ^{1}H,^{1}H coupling constants range in NMR-^{1}H spectrum and COSY experiment the substitution patrons of the flavones sub-unities were established for amentoflavone. ^{13}C chemical shift established the hydroxyl

substituent patron, which was confirmed by ultraviolet absorptions (in methanol and with shift reagents) and HMBC interactions. For NMR and ultraviolet data of amentoflavone see table 1 and table 2 respectively.

Steroids structures (figure 1) were established by physical constants, and 1D- and 2D NMR spectra analysis (see table 3) and compared with the literature[2,3].

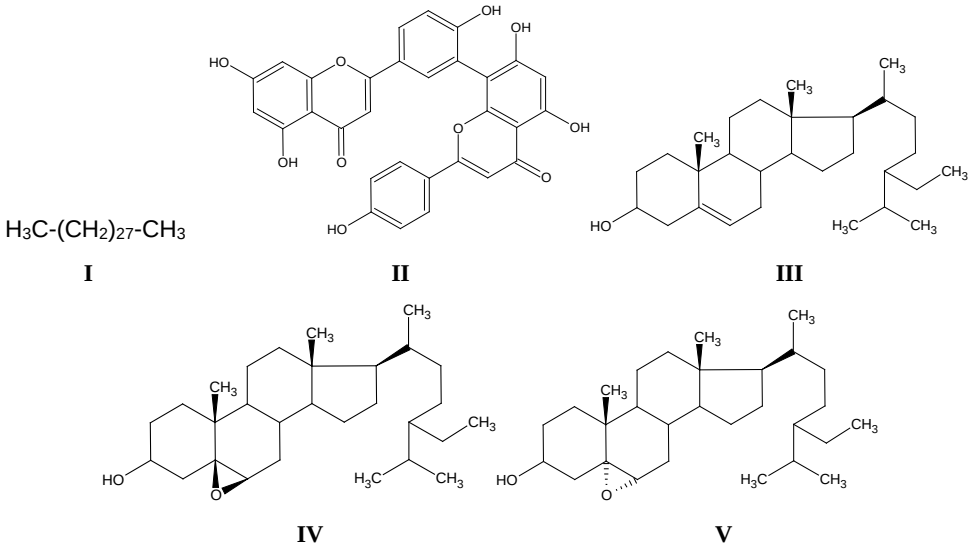

Figure 1: Compounds isolated from *T. rhoifolia* leaves.

Table 1: NMR data of amentoflavone (DMSO-D_6, 100 and 400 MHz, respectively).

C	δ_C	δ_H, multiplicity, *J*(Hz)	HMBC correlations
2	164.0	-	H-3; H-2'; H-6'
3	101.9	6.76, s	-
4	181.4	-	H-3
5	162.8	-	H-6
6	98.8	6.19, s	-
7	164.5	-	H-6; H-8
8	93.9	6.33, s	H-6
9	157.2	-	-
10	103.4	-	H-6
1'	117.9	-	H-2'; H-5'; H-6'
2'	131.3	8.24, s	H-6'
3'	123.2	-	H-2'; H-5'
4'	164.3	-	H-2'; H-5'; H-6'
5'	119.0	6.92, d (8.0)	H-6'
6'	126.4	7.87, dd (8.0, 1.8)	H-2'; H-5'
2''	164.0	-	H-3''; H-2'''; H-6'''
3''	102.3	6.69, s	-
4''	181.5	-	H-3''
5''	160.4	-	H-6''
6''	101.4	6.06, s	-
7''	164.5	-	H-6''
8''	106.7	-	H-2'; H-6''
9''	154.7	-	-
10''	101.7	-	H-6''
1'''	121.7	-	H-2''', H-6'''; H-3''', H-5'''
2''', 6'''	128.0	7.66, d (8.0)	H-3''', H-5'''
3''', 5'''	115.5	6.68, d (8.0)	H-2''', H-6'''
4'''	160.6	-	H-2''', H-6'''; H-3''', H-5'''

Table 2: UV λ_{max} (nm) absortions in methanol and shift reagents added of amentoflavone.

Shift reagent	Band II	$\Delta\lambda_{II}$	Band I	$\Delta\lambda_{I}$
MeOH	269, 291 (s)	-------	332	--------
MeOH + NaOMe	275, 295 (s)	+6,(+4)	379	47
MeOH + NaOAc	274	+5	380	48
NaOAc+ H_3BO_3	270	+1	339	+7
MeOH + $AlCl_3$	279,303	+10 (+12)	345,384	+13 (+52)
MeOH + $AlCl_3$ + HCl	279,303	+10 (+12)	345,384	+13 (+52)

Bioassays. All extracts were tested in from to P388 (mice leukemia), A549 (lung carcinoma), and HT29 (human colon carcinoma) cellular lines. Dichloromethane and acetone extracts showed moderate activity (5µg/ml) to P388 cellular line.

Results discussion

Gutmann and coworkers[4] proposed what amentoflavone is implicated in the inhibitory effect of *Hypericum* extracts on adenosine, GABA A, GABA B, 5HT1 (5-hydroxy-tryptamide), central benzodiazepinic, forskolinic, and inositol triphosphate receptors, and MAO A and MAO B enzymes; which can explain the anti-depressive activity attributed to the plant leaves. Amentoflavone also showed phospholipase Cγ1[5], and cAMP phosphodiesterase inhibitory activity accelerating tissues regeneration process in cellulites treatment[6]. Additionally, amentoflavone showed antioxidant and anti-fungal[7], anti-inflammatory[8], and anti-HIV activities[9].

In a review on β-sitosterol pharmacological activities[10] suggest to consume a dairy dose of: a) 300 mg up to 5g for reduction of high blood cholesterol. b) Between 60 and 130 mg for prostatic hyperplasia-related symptoms. c) 300 mg supplement is recommended as dietary health supplements.

Conclusions

The main applications found for isolated majority products of *T. rhoifolia* explains the popular use that occurs to this tree, since it is used in the prevention of the cancer and regulation of prostate diseases. Biflavone amentoflavone is a metabolite for which antirust activities are reported (what it would explain its preventive effect of the cancer) and anti-depressing. As far as β-sitosterol, pharmacological activities of great interest are attributed to him in the field of the medicine, this one presents/displays a regulating activity for the sugar and in addition it shows to be a controller of the cholesterol levels in the blood. Some other applications found in the popular medicine can have their elucidation in extracts of greater polarity.

Acknowledgments

To CDCHT by financial support through C-1214-04-08-B project.

References

1. C Rosquete, E Del Olmo, J López, A San Feliciano. Eudesmanolides and other terpenoids from the resin of *Trattinickia rhoifolia*. *Submitted* to **Avances en Química**.
2. C Rosquete. Estudio químico de la *Ageratina stevioides* (Steyermark) R.M. King & H. Robinson (Compositae). Tesis de Maestría. Universidad de Los Andes. Mérida-Venezuela (1985).
3. W Meyer, H Jungnikel, D Konrad, M Jandre, G Spiteller. On the cytotoxicity of oxidized phytosterols isolated from photo-autotrophic cell cultures of *Chenopodium rubrum* tested on meal–worms *Tenebrio molitor*. **Phytochemistry, 47**, 789-797 (1998).
4. H Gutmann, R Bruggisser, W Schaffner, K Bugman, A Botomino, J Drewe. Transport of amentoflavone across the blood-brain barrier *in vitro*. **Planta Medica, 68**, 804-807 (2002).
5. H Lee, W Oh, B Kim, S Ahn, D Kang, D Shin, J Kim,T Mheen, J Ahn. Inhibition of phospholipase $C_\gamma 1$ activity by amentoflavone isolated from *Selaginella tamariscina*. **Planta Medica, 62**, 293 (1996).
6. R Saponara, E Bosisio. Inhibition of rat adipocyte cAMP phosphodiesterase by biflavones of *Ginkgo biloba* L. **J. Nat. Prod., 61**, 1098-1101 (1998).
7. M Krauze-Baranowska, M Wiwart. Antifungal activity of biflavones from *Taxus baccata* and *Ginkgo biloba*. **J. Biosc., 58**, 65-69 (2003).
8. T Banerjee, G Valacchi, Z Ziboh, A van der Vliet. Inhibition of TNFa-induced cyclooxygenase-2 expression by amento-flavone through suppression of NF-kB activation in A549 cells. **Mol. Cell. Biochem., 238**, 105-110 (2002).
9. Y Lin, H Anderson, M Flavin, Y Pai, E Greenwood, T Pengsuparp, J Pezzuto, R Schinazi, S Hughes, F Chen. *In vitro* anti-HIV activity of biflavonoids isolated from *Rhus succedanea* and *Garcinia multiflora*. **J. Nat. Prod., 60**, 884-888 (1997).
10. M Lam. Beta-sitosterol. http://www.drlam.com/opinion/betasitosterol.cfm. Visited on January, 28th, 2010. 8:25 h.