LIGNANS FROM *IRYANTHERA JURUENSIS* WARB

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ABSTRACT

Four lignans Otobaphenol (**1**), Cagayanin (**2**), Otobain (**3**) and Hydroxyotobain (**4**), together with a tocopherol like compound identified as (5*S*)-3a-acetyl-2,3,5-trimethyl-7a-hydroxy-5-(4,8,12-trimethyltridecanyl) -1,3a,5,6,7,7a-hexahydro-4-oxainden-1-one (**5**) and a very long unbranched alcane (**6**) reported here as natural product for the first time were found in the hexane and dichloromethane extract from the leaves of *Iryanthera Juruensis* warb, a plant frequently used as a medicinal plant in the amazons rain forest, the structure elucidation was performed by NMR spectroscopy and mass spectrometry.

RESUMEN

Cuatro Lignanos Otobafenol (**1**), Cagayanin (**2**), Otobain (**3**) y Hydroxiotobain (**4**) se encontraron junto con un compuesto similar al tocoferol identificado como: (5*S*)-3a-acetil-2,3,5-trimetil-7a-hidroxy-5- (4,8,12-trimethyltridecanil) -1,3a,5,6,7,7a-hexahydro-4-oxainden-1-ona (**5**) y un alcano lineal muy largo (**6**), reportado aquí como producto natural por primera vez, se encontraron en los extractos hexanicos y diclorometanicos de las hojas de *Iryanthera Juruensis* warb, usada frecuentemente como planta medicinal in la selva del amazonas, la elucidación estructural se realizo mediante espectroscopia de resonancia magnetica nuclear y espectrometria de masas.

INTRODUCTION

Iryanthera Juruensis Warb. Myristicaceae is also called "Sangre de Toro" (bull's blood in English), because its bark exude an intense red latex when wounded. It is a forestall tree and its wood has a value on the high quality furniture market and the seeds are esteemed in Brazil for their high yields of fatty acids used in pharmaceutical and cosmetic industries (Silva et al 2001). Leaves and latex of this particular tree, have been used traditionally to heal seriously infected wounds and cuts as well as to treat stomach infections with a mixture of the latex and warm water (Schultes and Holmstedt, 1991).

Vieira et al in 1975 isolated the γ-lactones juruenolide and juruenolide B. Later studies showed that the fruits of *Iryanthera juruensis* contain lipophilic antioxidants, in particular the two tocotrienols sargachromenol and 7-methyl-sargachromenol as well as the lignans epi-guaiacin, guaiacin, verrucosin and nectandrin (Silva 2001). In the same investigation sargaquinoic acid, 3-methyl-sargaquinoic acid and three ω-arylalkanoic acids which didn't show any antioxidant activity were also identified. As the leaves of *Iryanthera Juruensis* is a by-product in the exploitation of the wood, we decided to investigate their chemical contents in an attempt to discover new and/or potentially valuable secondary metabolites and thereby increase the economic value of the plant.

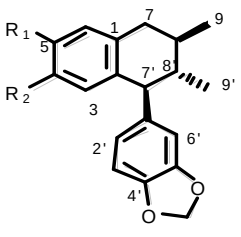
RESULTS AND DISCUSSION

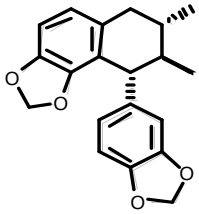
The investigation concerns the analysis of the hexane and dichlorometane extracts of *Iryanthera Juruensis*. The hexane extract was submitted to straight phase chromatography which eventually yielded β-sitosterol, an long unbranched alcane **6** reported as a natural product for the first time, triacontanol, a polyprenol identified as Betulaprenol 11, Phytol, Nerolidol, a tocopherol related compound (5*S*)-3a-acetyl-2,3,5-trimethyl-7a-hydroxy-5- (4,8,12-trimethyltridecanyl) -1,3a,5,6,7,7a-hexahydro-4-oxainden-1-one (**5**) , Ethyl oleate, Ethyl linolenate, Oleic acid and Linolenic acid. Identification was carried out by

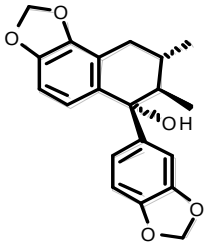
HRESIMS+, NMR and comparison with reported data.

The dichloromethane extract gave lignans **1** to **4**, whose structure were elucidated by Mass spectroscopy and NMR means, the four compounds showed to have similar squeleton. Compound **1** was identified as otobaphenol, by extensive analysis of 1D and 2D NMR spectra. Final confirmation of the structure was carried out by HRMS. The data found is in very close agreement with the reported literature. Lemeshko in 2003 studied the antioxidant activity of otobaphenol, showing that otobaphenol is a powerful antioxidant and substantially protects mitocondria against permeability transition induced by t-BOOH and by Ca^{2+} .

Compound **2** showed similar features as **1**, two ^{13}C NMR signals at δ 100.7 ppm and 100.5 ppm indicates the presence of $O\text{-}CH_2\text{-}O$ groups each one located in a different aromatic ring. Comparison of NMR data with published data confirms the compound is Cagayanin. Confirmation was achieved by HRESIMS+. Lopes et al, in 1999 studied the antifungal properties of Cagayanin, were it showed to be inactive against *Cladosporium cladosporioides.*



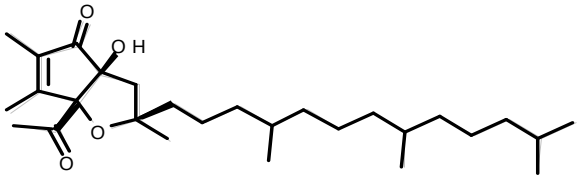




1 : R1 = OCH3 R2 = OH
2 : R1 + R2 = -O-CH2-O-

3

4



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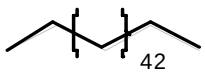

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Illustration 1 Lignans and other compounds found in *Iryantehra juruensis* warb

For the compound **3** identified as Otobain the same structure analysis made for **2** was made. Otobain is known since the decade of the 60, and several reports indicate its presence in several plants associated with medicinal uses (Yankep 1999) , but no specific studies concerning biological activity were found. The compound **4** shows a trisubstituted and a tetrasubstituted aromatic ring defined by the protons at δ 6.70 (d, J=8.0Hz) δ 6.62 (d, J=8.0Hz). The proton from C-7' at δ 3.39 in the compound **1** doesn't appear and the carbon signal corresponding to this proton was moved downfield from δ 54.2 to 75.8 ppm. Confirming the replacement of the proton for and hydroxyl whose hydrogen appear at δ 2.25 ppm we conclude that compound **4** is hydroxyotobain. Ruge in 1998 reported preliminary studies of the antimicrobial activity of the ethanolic extract of *Virola calophylla* were hydroxyotobain was found, unfortunately no further studies were found.

The compound **5**, was elucidated by HRESIMS to identify the molecular formula [M+H] 463.3745 for C29H51O4 calculated 463.3787, confirmation of the structure was done by comparison of the ^{1}H and ^{13}C NMR spectrum reported by Ukiya 2003, the same report showed that **5** has a potent inhibitory effect (100 % at $1x10^{3}$/TPA) on Epstein-Barr Virus.

Compound **6** was identified by H NMR and C NMR analysis of the spectra gives unequivocal description for an unbranched alcane, final confirmation of the structure was carried out by EI+ Mass spectroscopy. This is the first report of this compound found in a natural product.

Myristicaceae species are representative from the tropical rain forest, and the seeds are a rich source of lignans Danelutte 2000, other studies showed that lignans are also found in the bark Yankep 1999 and leaves Martinez 1994. The powerful antioxidant activity of otobaphenol, the lipophylic antioxidants found by Silva, and the antiviral properties of compound 5, could be related to the medicinal properties attributed to this plant, in similar way this plant is a very attractive source of lipophylic antioxidants.

EXPERIMENTAL

General

^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded at room temperature with a Bruker DRX500 spectrometer with an inverse multinuclear 5 mm probehead equipped with a shielded gradient coil. The spectra were recorded in $CDCl_3$, and the solvent signals (7.26 and 77.0 ppm, respectively) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constants (*J*) in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^{1}J_{CH}$ = 145 Hz and $^{n}J_{CH}$ = 10 Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker XWIN-NMR software (rev. 010101). High-resolution mass spectra were recorded on a Waters Micromass-Q-Tof micro spectrometer.

Plant Material

Leaves of *Irianthera Juruensis* Warb (Myristicaceae) were collected in the Valle de Sacta part of the Carrasco National park in Cochabamaba, Bolivia, during January 2003. The identification was carried out by the National herbarium Martin Cardenas Cochabamba, were the voucher specimen RB-772 of the plant is deposited.

Extraction and isolation.

Air dried powdered leaves were macerated with 96% ethanol three times during 24 Hrs each time at room temperature, after the solvent was removed under reduced pressure to obtain a crude extract. The crude extract was dissolved in aqueous methanol (10 % Water) and partitioned with hexane and later with Dichloromethane. Each fraction was further purified by Flash Chromatography and sephadex LH-20.

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