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Table: 1, Fig : 1

SHORT COMMUNICATION

Structure of 1 -glucosyl-piquerol A: Storage of an allelopathic monoterpene

M. JIMENEZ-ESTRADA[1*], R. REYES-CHILPA[1], R. RUIZ DE ESPARZA-VILLARREAL[1], C.K. JANKOWSKI[2] and M.R. VANCALSTEREN[3].

[1]Instituto de Química, Universidad Nacional Autónoma de México.
Circuito Exterior, Cd. Universitaria, 04510 México, D. F., México.
E. Mail: manuelj@servidor.unam.mx;

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ABSTRACT

The aerial parts of *Piqueria trinervia* Cav. (Asteraceae) afforded a new compound, 1β-glucosyl-piquerol A and its structure was established by 1D and 2D NMR, IR, UV and Mass Spectrometry. In contrast with its aglycone, 1β-glucosyl-piquerol A did not inhibit seed germination, and radicle elongation of *Amaranthus hypochondriacus* and *Echinochloa crus-galli*. Considering *P. trinervia* have medicinal properties, 1β-glucosyl piquerol A was found inactive against the human pathogenic microorganisms *Bacillus subtilis, Escherichia coli, Vibrio cholerae*, *Salmonella typhi.* and *Staphylococcus aureus*. It is proposed that synthesis of this monoterpene glycoside could be a potential mechanism to avoid autotoxicity, while hydrolysis in the soil by a microbial glycosidase could release the allelopathic compound Piquerol A. Alternatively mechanical damage of *P. trinervia* leaves, may also induce hydrolysis of 1β-glucosyl-piquerol A by an endogenous glycosidase.

Key words: Allelopathy, 1 -glucosyl-piquerol A, monoterpenes, *Piqueria trinervia*.

INTRODUCTION

Piqueria trinervia Cav. (Asteraceae) is a native herb from Mexico and Central America with perennial roots and annual stems (hemicryptophyte). It grows in temperate and tropical areas between 1000 to 3000 m altitude, in clearings of Pine-Oak forests. It is used in Mexican folk medicine since the pre-Columbian times (1) against the typhoid fever, common fever, malaria, gastrointestinal diseases and rheumatism (8,17,18). It becomes weedy on burned-over slopes, roadsides, rocky hills and cutover forests (11). In the fields, *P. trinervia* forms pure monocultures, this suggests its allelopathic potential and its main allelopathic principle, piquerol A (compound 1, Fig. 1) has been isolated (6). It is the main monoterpene constituent isolated from the water extract of the aerial parts and is also present in lower concentrations in the roots (6). Its molecular structure was determined from its spectroscopic data (15) and further confirmed by X-ray crystallographic analysis (16). It inhibits the germination and radical growth of seven

Correspondence Author; [2]Department de Chimie et Biochimie, Universite de Moncton, Moncton, NB, E1A 3E9, Canada; [3]IRSA, Agriculture Canada, 3600 Blvd Casavant , St Hyacinthe , Quebec, J25 8E3 Canada.

weeds that live in the same environment with *P. trinervia*; therefore, it may be associated with allelopathic activity (6). The piquerol A inhibits the ATP synthesis and photophosphorylation in *Pisum sativum* chloroplasts, but do not affect the basal H^+-uptake and uncoupled electron transport (12). Its derivative diacetyl piquerol A (prepared in the laboratory), also inhibits the radicle growth, microsomal H^+-ATPase of *Ipomea purpurea* (3) and photophosphorylation of *P. sativum* chloroplasts (12).

The water extract of the aerial parts was re-analyzed isolating a new compound, 1β-glucosyl-piquerol A (compound 2, Fig. 1). Its structure was elucidated by spectroscopical methods. To explore its possible phytotoxic properties, we examined its effects on germination and root growth of the weeds *Amaranthus hypochondriacus* (Dicotyledonous-Amaranthaceae) and *Echinochloa crus-galli* (Monocotyledonous-Poaceae), and tomato crop [*Lycopersicon esculentum* (Dicotyledonous-Solanaceae)]. Considering *P. trinervia* applications in Mexican traditional medicine, the possible activity of 1β-glucosyl-piquerol A, was also evaluated against the human pathogenic bacteria.

MATERIALS AND METHODS

Plant material

Piqueria trinervia Cav., was collected from Km 12 of Picacho to Ajusco Road at Distrito Federal, Mexico, in October, 2003. The aerial parts (stems, leaves and flowers, 300 g) were chopped in pieces and extracted at room temperature with distilled water for 24 h. The aqueous extract was further extracted with CH_2Cl_2 three times. The aqueous fraction was evaporated slowly on a glass pan with low heat, affording a resin residue of red-brown colour, which was extracted with methanol. The soluble part was concentrated with a rotary evaporator obtaining an oily residue (40g) very dense and amber in colour. Part of the oil (10 g) was subjected to column chromatography on silica gel 60 (Merck), eluting with EtOAc gradually enriched with MeOH. Fractions eluted with EtOAc-MeOH (9:1) afforded an uncoloured substance that was oily and dense (300 mg) which was subjected to spectroscopic (IR, ^{1}H-NMRMN, ^{13}C-NMR, HETCOR, HSQC, COSY, and DEPT) and MS analysis, for its identification. Part of this substance (100 mg) was acetylated in the usual form with anhydride acetic-pyridine. After purification, the derivative was also subjected to extensive spectroscopic analysis, for structural elucidation.

Biological Assays

The effects of compound **2** were assayed on seed germination, and radicle elongation of *Amaranthus hypochondriacus, Echinochloa crus-galli, and Lycopersicon esculentum*. The effects of compound **2** were evaluated *in vitro* at 100 ppm (100 µg/ml) using the protocol previously described (3,6,9). Possible bactericidal or bacteriostatic properties were assayed *in vitro* with the human pathogenic microorganisms [*Bacillus subtilis, Escherichia coli, Vibrio cholerae*, *Salmonella typhi.* and *Staphylococcus aureus*] with protocol previously described (2).

RESULTS AND DISCUSSION

The aqueous extract of *Piqueria trinervia* aerial parts afforded the monoterpene piquerol A (compound 1) and 1β-glucosyl-piquerol A (compound 2). Compound 2 was an oily substance and for the first time isolated from this plant. Its IR spectrum showed intense absorptions at 3380 (OH), and 2946, 2835 cm^{-1} (C-H). The FAB $MS^{(+)}$ registered in glycerol showed the protonated molecular ion at 329 (0.2%).

The ^{1}H-NMR and ^{13}C-NMR data showed the characteristic signals for piquerol A moiety (Table 1), while the presence of a sugar residue was revealed by typical glucose signals between 3 and 4 ppm and its anomeric proton at 4.52 ppm (d, 1H, J=9 Hz). The acetylation of compound 2 to pentacetate (compound 2a, Fig. 1) was the first chemical proof of the structure involved. Compound 2a was obtained as a colourless solid. Its IR spectrum showed an intense carbonyl group of acetate at 1730 cm-^{1}, while the hydroxyl band at 3380 cm-^{1} disappeared. The FAB $MS^{(+)}$ spectrum showed M^{+}+1 at m/z 539, corroborating acetylation of the five hydroxyls. In compounds 1 and 2a, signals for vinylic protons H-2 and H-3 showed better resolution, when the spectra were recorded in $CDCl_3$ (Table 1), while in compound 2 these protons appeared as singlet.

Table 1. ^{1}H, and ^{13}C NMR Data of Compounds

No.	1*	1*	2**	2**	2a***	2a***
1	4.62, sw, 1H	70.2	4.80, sw, 1H	77.0	4.70, sw, 1H	75.1
2	5.87, dd, J=11.8, 5.4, 1H	131.7	5.96, s, 2H	133.2	5.89,dd, J=8.1, 2.1, 1H	129.3
3	6.03, ddd, J=11.8, 2.7, 5.4, 1H	132.9	5.96, s, 2H	130.7	5.99, ddd, J=4.5, 1.8, 8.1, 1H,	129.5
4	4.40, st, 1H	67.8	4.30, t, 1H	69.0	5.35, m, 1H	70.2
5	3.0, d, J=2.7, 1H	52.1	2.92, sw, 1H	53.4	3.26, d, J=7, 1H	49.9
6		141.9		144.2		141.5
7	5.33, sw, 1H 5.12, sw 1H,	113.7	5.48, st, 1H 4.98, sw, 1H	114.7	5.42, sw, 1H 5.23, sw, 1H	115.6
8		142.5		144.3		141.1
9	1.85, s, 3H	23.4	1.78, s, 3H	23.9	1.75, s, 3H	22.9
10	5.38, sw, 1H; 5.06, sw, 1H	110.0	5.28, sw, 1H 5.01, sw, 1H	111.6	4.93, tw, J=1.5 & 0.9, 1H 4.86, tw, J=1.5 & 0.9, 1H	114.6
1´			4.53, d, 1H, J=9	102.4	4.72, d, J=9	98.9
2´			3.28, m, 1H	71.6	5.01, d, J=9.6, 1H	71.4
3´			3.22, m, 1H	78.0	5.21, t, J=9, 1H,	72.9
4´			3.36, m, 1H	78.2	5.07, t, J=9, 1H	68.6
5´			3.26, m, 1H	75.1	3.67, mc, 1H	71.8
6´			3.83, td, 1H; 3.64, td, 1H	62.8	4.19, td, J=7.2, 12.3, 2H	62.1
C=O						169.0-170.6, 5s
CH_3					2.01, 2.02, 2.03, 2.04, 2.09, 6s	20.5-20.9 5s

*300 MHz, $CDCl_3$;** 500 MHz, MeOD; ***300MHz, $CDCl_3$-MeOD. S, singlet; d, doublet; t, triplet; m, multiplet; w, wide, j, coupling constant.

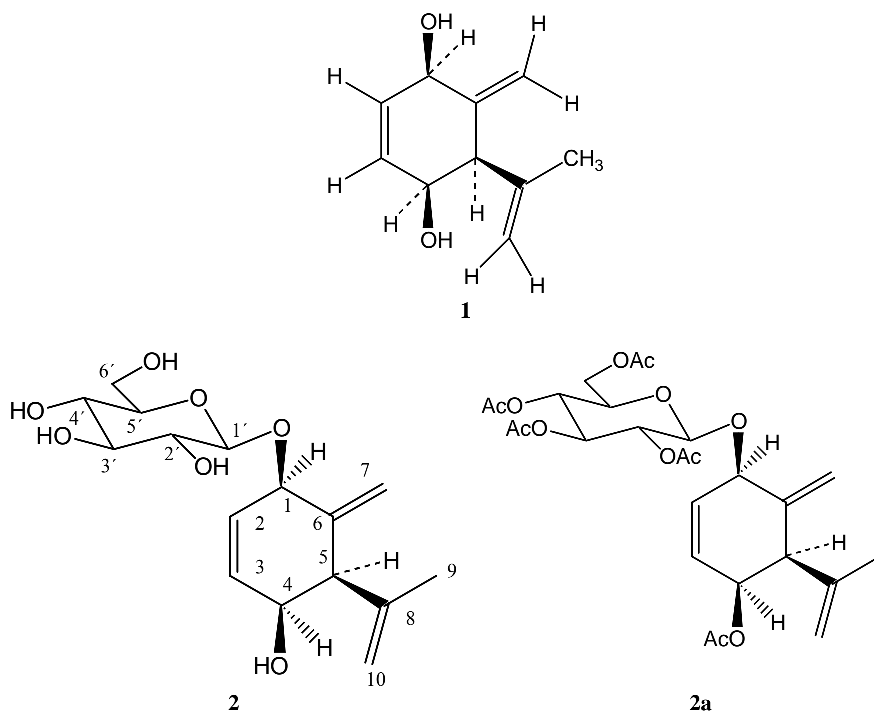

Figure 1. Natural compounds (**1**,**2**), and transformed (**2a**) compounds of *Piquería trinervia*.
1: Piquerol A. **2**: 1 -glucosyl-piquerol A. **2a**: penta-acetylated 1 -glucosyl-piquerol A.

Full assignation of carbons and the sugar configuration were solved by 1D (COSY, DEPT), 2D (HETCOR, HSQC, HMBC, and NOESY) NMR experiments. To elucidate the insertion position of glucose on piquerol A skeleton, chemical shifts of both H-4 and H-1 were examined. The ^{1}H-NMR spectrum of compound 2a showed that signal for H-4 shifted to lower field (5.35 ppm) than original compound 2 (4.33 ppm); on the other hand, signal for H-1 shifted only -0.10 ppm as compared with resonance for this proton for compound 2a (Table 1). An HMBC experiment clearly showed coupling of H-1 with anomeric carbon (C-1´). A NOESY experiment also revealed the coupling of H-1 and H-1´ through space. These data clearly indicated that glucose is attached to the oxygen on C-1.

Compound 2 was subjected to acidic hydrolysis (HCl-MeOH) and the products were analysed by TLC. Presence of piquerol (compound 1) and glucose was determined by comparing with authentic samples. In summary, the detailed analysis of the NMR, IR, and mass spectroscopic data enabled identification of compound 2 as 1β-glucosyl-piquerol A, and its penta-acetylated derivative (compound 2a).

In contrast with piquerol A, which inhibits *in vitro* germination and radicle growth of *Amaranthus hypochondriacus* and *Echinochloa crus-galli* weeds (9), its

derivative 1β-glucosyl-piquerol A was found inactive at 100 ppm with these species and tomato (*Lycopersicon esculentum*). None of the three tested species grows in the same habitat of *P. trinervia*, but were assayed, because they are good models to determine the phytotoxicity of natural compounds against mono- and dicotyledonous species. Therefore, phytotoxicity *in vitro* suggests, but not prove, its allelopathic role in natural conditions. Considering *P. trinervia* applications in Mexican traditional medicine, 1β-glucosyl-piquerol A was also tested *in vitro* against several human pathogenic bacteria, but no activity was detected.

Compound 2 could be vacuolated or stored in the plant cell, and possibly act in auto-defence mechanism. That is, a protection against the phytotoxicity of piquerol A, in a similar way to the glycosylated secondary metabolites which have been documented in the literature as plant antimicrobial compounds. Many plants contain non-toxic glycosylated secondary metabolites (usually glucosides) that release highly toxic labile compounds at the wound site as a defence against the fungal and bacterial phytopathogens. Formation of the toxic product depends upon the action of a glycosidase, which is spatially separated from substrates in the same cell. For example, arbutin, a phenolic glucoside in pear trees is active against *Erwinia amylovora* and associated with high glucosidase content (7). The enzyme catalyzes the hydrolysis of arbutin to yield hydroquinone, which is toxic to the pathogen. In some instances, the pathogens provide the glucosidase instead of host. This is the case with *Ascophyta imperfects* upon infecting *Medicago sativa* leaves, as the flavonoid glycosides are converted to fungitoxic aglycones at the infection site (14).

Hydroquinone is very phytotoxic (50 ppm w/v) to the root growth of *Euphorbia esula* weed, while, arbutin was moderately phytotoxic (300 ppm w/v). Both compounds were isolated from the ether extract of *Antennaria microphylla*. The observed phytotoxicity of hydroquinone and the high-yield natural occurrence of arbutin, a water soluble and easily hydrolyzed monoglucoside of hydroquinone, is consistent with the participation of these two compounds in the observed *Antennaria microphylla* allelopathy in the fields against *Euphorbia esula* (10)

Besides avoiding autotoxicity, the production and storage of 1β-glucosyl-piquerol A, could also be advantageous for transportation. The leaves of *Piqueria trinervia* contain this stable glycoside that can be released by mechanical damage or excretion and then washed by rainwater to the soil. The phytotoxic compounds syringin (a glycosilated phenylpropanoid) and (–)-lariciresinol (lignan) are washed from the leaves of *Prosopis juliflora* to the rhizosphere and play a role in the allelopathy of this bush (13).

The piquerol A may be released from compound 2 due to the action of a microbial, or an endogenous glycosidase. Nearly half of the total monoterpenes are present in some tissues as glucosides, for instance in the petals of fully opened rose flowers. Production of the monoterpenes geraniol, nerol and citronellol, and their glucosides, in rose flowers is function of flower maturity (5). Exogenous enzymatic hydrolysis of monoterpene glycosides occurs in passion fruit and mango with a β-glucosidase from yeast (4). Alternatively, the hydrolytic breakdown of monoterpene glycosides, presumably by an endogenous glycosidase, is responsible for the production of monoterpenes linalol and geraniol in disrupted tea shoots (19). The production of linalol and geraniol is inhibited by Hg^{2+} and glucono-1,4-lactone, a specific inhibitor of glucosidase; contrarily, addition of exogenous glucosidase to steamed tea homogenate, releases the linalol and geraniol (19).

Piquerol A may also undergo further chemical transformations that could influence its potential allelopathic activity. Structural activity relationships of piquerol A, and their hydroquinone and phenolic derivatives prepared in laboratory, suggest that inhibition of radicle growth is related to the presence of two oxygenated groups: 1,4-dihydroxyallylic functionality of piquerol A, or a para-hydroquinone moiety (9). Therefore, it can be hypothesized that in nature piquerol A could undergo aromatization to form an intermediate compound, which can also act as an allelopathic molecule.

CONCLUSIONS

It was concluded that 1β-glucosyl-piquerol A was not phytotoxic and probably is an autoprotection device against the herbicidal activity of piquerol A, in a similar way to other glycosilated plant chemicals involved in allelopathic interactions and defence against fungi and bacteria.

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