

Transformation of Terpene Piquerol A to Hydroquinone and Phenolic Derivatives. Effect of These Compounds on Weeds[†]

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Piquerol A (**Ia**), an allelopathic terpene from *Piqueria trinervia* (Asteraceae) was chemically transformed into its diacetate (**Ib**), benzylic alcohol (**II**), dialkyl hydroquinone derivative (**IIIa** and **IIIb**), and phenolics (**IVa** and **V**). The structures of these derivatives were determined by spectral analysis. Piquerol derivatives were found to act as powerful radicle growth inhibitors of the weed *Amarantus hypochondriacus*. Order of inhibition was **Ib** > **IIIa** > **Ia** > **IVa** > **II**. The first three compounds were also radicle growth inhibitors of the weed *Echinocloa crusgalli*.

Keywords: *Piquerol A*; *Piqueria trinervia*; 2-methyl-3-isopropenylhydroquinone; 2-methyl-3-isopropenylphenol; 3-methyl-2-isopropenyl derivatives; weed radicle growth inhibition

INTRODUCTION

Piqueria trinervia (*P. trinervia*) Cav. (Asteraceae) is an herbaceous perennial shrub which grows in tropical and temperate zones of Mexico, Central America, and Haiti. The roots of this plant are frequently used in folk medicine as an antipyretic, antimalarial, and antirheumatic agent. Two diastereomeric monoterpenes were isolated from this plant, piquerol A and B (Romo et al., 1970). Their chemical structures were elucidated by MS, NMR, IR, and UV spectroscopies. The molecular structure of piquerol A was later confirmed by X-ray crystallographic analysis (Soriano-García et al., 1983). Piquerol A (**Ia**) and B have a tetrasubstituted cyclohexene which possesses three nonconjugated double bonds. Both piquerols share several biological properties, including inhibition of germination and plant growth. They could be responsible for the allelopathic interactions of *Piqueria trinervia* (González de la Parra et al., 1981). Molluscicidal activity and acaricidal activity (Cruz et al., 1989) against *Boophilus microplus* have also been described for piquerol A (González de la Parra et al., 1991). In previous work (Mendoza et al., 1994) we studied the mechanism of the action of piquerol A as an allelochemical compound, especially its effect on photosynthesis. We found that piquerol A inhibited ATP synthesis and phosphorylating electron rate in pea chloroplasts. H⁺-uptake and basal and uncoupled electron transport were not affected. When piquerol A was modified, as diacetylpiquerol (**Ib**), increasing lipophilicity, inhibition of photophosphorylation was enhanced (Mendoza et al., 1994). These data suggest that the active compound *in vivo* might be a more lipophilic piquerol A derivative. This derivative may also be responsible for the allelopathic interactions of *P. trinervia*.

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[†] Contribution 1453 from Instituto de Química, Universidad Nacional Autónoma de México. Part of this study was presented at the Eighth International Congress of Pesticide Chemistry, Washington, DC, July 1994.

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The diverse biological activities associated with piquerol A and its derivatives above described led the authors to transform piquerol A to aromatic derivatives (Figure 1). It is well-known that synthetic phenol and hydroquinone derivatives have herbicidal activity (Ochi et al., 1979; Bhushan-Mandava, 1985; Reynolds and Rodriguez, 1979; Harbone, 1986; Morton, 1965). The effect on the germination and radicle development of weeds was tested in order to understand which part of the structure is necessary for activity. Here we describe the conditions for the preparation, physical characterization, and the biological properties of these aromatic piquerol derivatives.

EXPERIMENTAL PROCEDURES

Apparatus. The following were used: Fisher-Johns, melting point determinations are uncorrected; Perkin-Elmer 283 IR spectrometer and Nicolet FT-IR 55X (cm⁻¹); Varian Gemini-200 and Varian VXR-300S NMR spectrometers (δ ppm); Hewlett-Packard 5985-B mass spectrometer (70 eV).

Extraction of Piquerol A. *Piqueria trinervia* Cav. plants were collected at Cerro del Ajusco, Mexico City. Piquerol A was extracted from 500 g of ground plants as previously reported (Rubio et al., 1985). A 300 mg sample of compound **Ia** (yield, 0.06%) was obtained; mp 139–140 °C (CH₂Cl₂), *R*_f 0.5 (1:1 petroleum ether–AcOEt). ¹H NMR (CDCl₃, ppm): δ 6.05 (2H, m, H₂ and H₃), 5.8 (1H, m, H₁), 5.4 (1H, d, H₄), 5.27 (1H, d, H₇), 4.45 (1H, m, H₁₀), 3.6 (1H, d, H₅), 1.8 (1H, c, H₉).

The natural product **I** (200 mg) was dissolved in pyridine (2 mL) and acetic anhydride (1 mL). The reaction was heated in a steam bath for 3 h. After this time the reaction was worked up as usual to yield piquerol A diacetate **Ib** (243 mg) as an oily residue; *R*_f 0.79 (8:2 hexane–AcOEt). IR (cm⁻¹) ν_{max} : 1733 (–CO–O). ¹H NMR (CDCl₃, ppm): δ 6.2 (2H, m, H₂ and H₃), 5.8 (1H, m, H₁), 5.41 (1H, m, H₄), 5.26 (2H, dd, H₇), 4.95 (2H, m, H₁₀), 3.35 (1H, d, H₅), 2.1, 2.05 (6H, s, Me–OAc), 1.7 (3H, C, H₉). ¹³C NMR (CDCl₃, ppm) (*C*_m intensity): δ 170.48 and 170 (C₁₁ and C₁₃), 141 (C₂, 40), 140 (C₃, 44), 130 (C₆, 169), 128 (C₈, 179), 115 (C₇, 202), 114 (C₁₀, 192), 70 (C₁, 172), 69 (C₄, 169), 49 (C₅, 164), 29 (C₉, 120), 22 and 21 (C₁₂ and C₁₄, 116 and 118).

Obtention of Compound II. Compound **II** was obtained in acidic conditions by reacting piquerol A (100 mg) with concentrated HCl (0.5 mL) in water at room temperature for 12 h. The solution was extracted with CH₂Cl₂, the solvent was eliminated, and colorless oil was obtained (65 mg). IR (cm⁻¹) ν_{max} 3500. ¹H NMR (ppm): δ 7.45 (1H, m, 7.27 (m, 1H), 7.16 (m, 1H, aromatic protons), 5.23 (m, 1H, H-9), 4.90 (m, 1H, H-9), 4.70 (s, 2H, H-7), 2.08 (sbr, 3H, Me-vinyl).

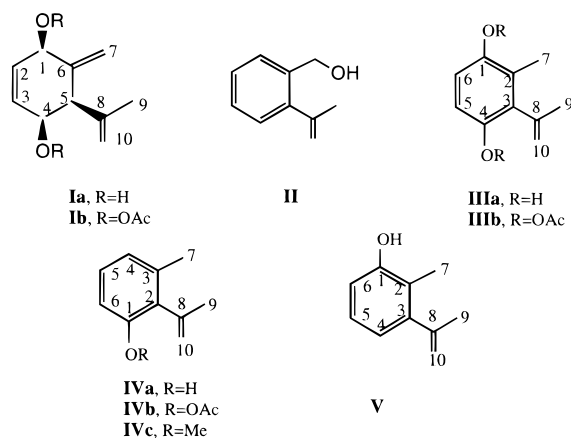


Figure 1.

Obtention of Compound IVa. A 150 mg (0.009 mol) sample of piquerol A was dissolved in ethanol (2 mL), and pieces of metallic sodium (300 mg) were added slowly until a dough was obtained. This material was dissolved in AcOEt and acidified with 10% HCl (10 mL). The resulting mixture was extracted with hexane, hexane/CH₂Cl₂, and CH₂Cl₂. The resulting organic extracts were combined and concentrated to yield an oily residue, which was purified by preparative TLC to afford **IVa** as a yellow oil (15 mg); *R_f* 0.62 (9:1 hexane–AcOEt). IR (cm⁻¹): ν_{\max} 3602 (–OH phenolic), 3080, 1578 (aromatic ring). ¹H NMR 7.0 (1H, t, H₅), 6.7 (1H, dd, *J* = 8 Hz and *J* = 2 Hz, H₄) and 6.6 (1H, dd, *J* = 8 Hz and *J* = 2 Hz, H₆), 5.16, 4.8 (2H, m, vinylic), 2.23 (3H, s, methyl aromatic), 2.0 (3H, t, Me-vinylic). MS: *m/z* (rel intens) 148 (100).

Obtention of Compounds IIIa and IVa with Pd/C. Piquerol A (**Ia**) 200 mg (0.001 mol) was mixed with 200 mg Pd/C (5%) in EtOAc (20 mL) and refluxed for 3 h. The mixture was filtered and concentrated and distilled *in vacuo*. An oily compound was obtained (198 mg), which was purified by preparative TLC to yield a crystalline compound (20 mg) which was identified as **IIIa**; mp 89–91 °C, *R_f* 0.33 (8:2 petroleum ether–AcOEt). IR (cm⁻¹): ν_{\max} 3605, 3533 (OH, phenol), 1276 (C–O). ¹H NMR (ppm): δ 6.63 (2H, s, H₅, H₆), 5.51, 5.0 (2H, m, vinylics), 2.65 (3H, s, aromatic-Me), 2.0 (3H, c, vinylic-Me). EM: M⁺, *m/z* = 164 (100%). ¹³C NMR (CDCl₃, ppm (C_m, intensity)): δ 147 (C₁, 39), 145 (C₄, 33), 141 (C₈, 44), 130 (C₆, 16), 121 (C₅, 40), 118 (C₃, 239), 114 (C₂, 204), 112 (C₁₀, 189), 23 (C₉, 141), 13 (C₇, 100). In addition, 63 mg of **IVa**, *R_f* 0.6 (9:1 petroleum ether–EtOAc), was obtained as a volatile colorless oil. IR (cm⁻¹): ν_{\max} 3522 (–OH phenol), 1276 (C–O). ¹H NMR (ppm): δ 7.06 (1H, t, H₅), 6.7 (1H, dd, *J* = 8 Hz and *J* = 2 Hz, H₄) and 6.6 (1H, dd, *J* = 8 Hz and *J* = 2 Hz, H₆), 5.5, 5.05 (2H, m, vinylics), 2.2 (3H, s, aromatic-Me), 2.0 (3H, t, vinylic-Me).

Compounds **IIIa** (18 mg) and **IVa** (15 mg) were separated, dissolved in pyridine (0.5 mL) and acetic anhydride (0.5 mL). The mixtures were heated in a steam bath for 1 h and worked up as usual to obtain **IIIb** and **IVb**, respectively. Compound **IIIb** was oily; *R_f* 0.5 (8:2 petroleum ether–EtOAc). IR (cm⁻¹): ν_{\max} 1758 (C=O) and 1164 (C–O). ¹H NMR (CDCl₃, ppm): δ 6.91 (2H, s, H₅, H₆), 5.23, 4.78 (2H, dd, vinylics), 2.3, 2.25 (6H, s, methyl acetate), 2.1 (3H, s, aromatic-Me), 1.9 (3H, s, vinylic-Me). Compound **IVb** (15 mg) was also an oil; *R_f* 0.30 (9.5:0.5 petroleum ether–EtOAc). IR (cm⁻¹): ν_{\max} 1760 (C=O), 1189 (C–O). ¹H NMR (CDCl₃, ppm): δ 7.06 (1H, t, H₅), 6.91 (1H, dd, *J* = 8 Hz and *J* = 1.9 Hz, H₄), 6.85 (1H, dd, *J* = 8 Hz and *J* = 1.9 Hz, H₆), 5.25, 4.8 (2H, m, vinylics), 2.23 (3H, s, methyl acetate), 2.26 (3H, s, aromatic-Me), 1.98 (3H, c, vinylic-Me).

Obtention of Compound IVc. A 40 mg (0.002 mol) sample of **IVa** was dissolved in THF (3 mL) and flushed with argon. The mixture was stirred for 10 min, and then sodium hydride (138 mg) and CH₃I (1 mL) were added slowly at room temperature during 2 h. The mixture was saturated with NaCl and extracted with CHCl₃ to yield **IVc** as an oil (13 mg); *R_f* 0.58 (9.5:0.5 petroleum ether–EtOAc). IR (cm⁻¹): ν_{\max} 2854 (–OMe), 1256 (=COC). ¹H NMR (CDCl₃, ppm): δ 7.06 (1H, t, H₅), 6.85 (1H, dd, *J* = 8 Hz and *J* = 2 Hz, H₄) and 6.75 (1H,

Table 1. Effect of Piquerol A (**Ia**) and Compounds **Ib**, **II**, **IIIa**, and **IVa** on the Radicle Growth of *Amaranthus hypochondriacus* and *Echinochloa crusgalli*

compd	amount, ppm	radicle growth, %	
		<i>A. hypochondriacus</i>	<i>E. crusgalli</i>
Ia	10	123.0 ^a	82.0
	30	70.1 ^a	43.8 ^a
	100	0.0 ^a	17.1 ^a
Ib	10	45.8 ^a	85.0 ^a
	30	35.3 ^a	62.0 ^a
	100	0.0 ^a	25.0 ^a
II	10	71.8 ^a	111.8
	30	72.8 ^a	96.0
	100	49.7 ^a	90.7
IIIa	10	50.5 ^a	110
	30	28.5 ^a	78.6 ^a
	100	0.0 ^a	59.5 ^a
IVa	10	71.8 ^a	92.0
	30	71.3 ^a	92.3
	100	35.0 ^a	81.5

^a *P* = 0.001 (significance). Control growth = 100%.

dd, *J* = 8 Hz and *J* = 2 Hz, H₆), 5.29, 4.80 (2H, m, vinylics), 3.81 (3H, s, O-Me), 2.6 (3H, s, aromatic-Me), 1.98 (3H, c, vinylic-Me).

Preparation of Compound V. A 241 mg (0.009 mol) sample of piquerol A diacetate (**Ib**) was dissolved in THF (10 mL), and NaH (400 mg) was added slowly at room temperature for 4 h. The mixture was then heated (steam bath) for 10 min. The reaction mixture was poured slowly in water (20 mL) and saturated with NaCl. From this mixture an oily volatile compound **V** (18 mg) was obtained by extraction (CH₂Cl₂); *R_f* 0.65 (8:2 petroleum ether–EtOAc). IR (cm⁻¹): ν_{\max} 3600, 3343 (OH). ¹H NMR (ppm): δ 7.02 (1H, t, H₅), 6.71 (2H, m, H₄, H₆), 5.18, 4.83 (1H, m, vinylics), 2.19 (3H, s, aromatic-Me), 2.01 (3H, c, vinylic-Me). EM: *m/z* = 148 (11%). ¹³C NMR (ppm (C_m, intensity)): δ 143 (C₁, 145), 112–126 (C₂–C₆ and C₁₀), 24 (C₉, 54), 12 (C₇, 42).

Bioassays with Seeds. Bioassay experiments were designed to evaluate the effect of piquerol A and aromatic derivatives at 10, 30, and 100 μ M on the radicle growth of *Amaranthus hypochondriacus* (*A. hypochondriacus*) (Amaranthaceae) and *Echinochloa crusgalli* (*E. crusgalli*) (Poaceae). A completely randomized block design with four repetitions for each target species was used. Ten seeds of each species were sown on Whatman No. 4 qualitative filter paper in petri dishes. Distilled water (1.5 mL) was added to each dish. The dishes were incubated in darkness at 27 °C. The radicle length was measured after 24 h in the case of *A. hypochondriacus* and 48 h in the case of *E. crusgalli*. The data were analyzed by ANOVA (Table 1).

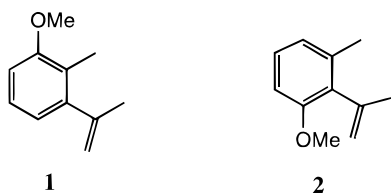
RESULTS AND DISCUSSION

In this study the natural product piquerol A (**Ia**) was transformed into aromatic derivatives. Firstly, piquerol A was transformed into benzylic alcohol **II** by reacting piquerol A with HCl in water.

Secondly, piquerol A was aromatized by treatment with Pd/C 5% in EtOAc. As a result of this procedure two derivatives were obtained. The more polar derivative was purified by preparative TLC and was characterized as 2,3-dialkylhydroquinone **IIIa** by IR, ¹H NMR, and MS techniques. Compound **IIIa** was diacetylated with pyridine/acetic anhydride and gave **IIIb**, thus confirming the structure of 2,3-dialkylhydroquinone.

The less polar compound obtained by TLC was a colorless volatile oil. The structure **IVa** was deduced by IR, ¹H NMR, and MS data. The acetyl derivative **IVb** was obtained. Compound **IVa** was also methylated with MeI/NaH and gave **IVc** (Flores, 1992). In this piquerol A aromatization, two other products were obtained with a very low yield and it was not possible to determine their structural characteristics.

The aromatization of piquerol A to yield compound **IVa** was performed by treatment with metallic sodium.

**Figure 2.**

The structure of compound **IVa** was proposed by spectral and spectrometric analysis (see Experimental Procedures).

Bolhmann et al. (1978) isolated a compound from *P. trinervia*, and structure **1** was proposed. However, later the structure was confirmed to be **2** by Sangaiah and Krisna (1981). These authors synthesized compounds **1** and **2**, thus confirming unequivocally that the natural product isolated by Bolhmann et al. has structure **2** (see Figure 2). Our NMR results indicated that compound **IVc** has structure **2**, according to Sangaiah and Krisna data.

Formation of Compounds IIIa and IVa. Palladium reaches compound **Ia** from the less steric hindered side, and the hydrogen atoms withdraw from C₄ and C₅. According to Rubio et al. (1985), these carbons have a lower electronic density than C₁, and therefore the hydrogens on C₄ and C₅ are more acidic. The calculated bond energy and the Mulliken index for the C₄–C₅ bond indicates that this bond is easily broken, giving a dienic intermediate, which is aromatized to give compound **IIIa**.

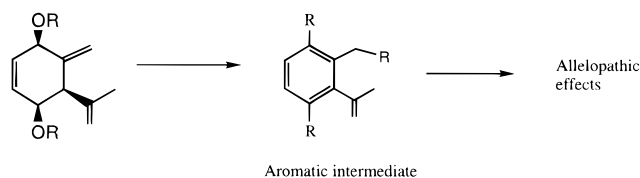
It is postulated that in the formation of compound **IVa**, the palladium withdraws first the hydrogen from C₅ and then that from C₄. This intermediate loses water and is then aromatized to the 2,3-disubstituted phenol.

In order to know if the hydrogen from C₅ is more acidic than those from C₄ and C₁, the diacetate derivative of piquerol A (**Ib**) was prepared. Thereafter it was treated with NaH/THF and finally with water. After these treatments, a highly volatile oily compound **V** was obtained in very low yield by preparative TLC of the reaction mixture.

Bioassay of Compounds Ia, Ib, II, IIIa, and IVa. Table 1 shows the effect of piquerol A and its derivatives on the radicle growth of the weeds *A. hypocondriacus* (Amaranthaceae) and *E. crusgalli*. In the case of *A. hypocondriacus*, piquerol A (**Ia**) stimulated the growth by 23% at 10 ppm and thereafter inhibited the radicle growth in 30% (30 ppm) and 100% (100 ppm). Piquerol derivatives were also inhibitory in the following order: **Ib** > **IIIa** > **Ia** > **IVa** > **II**. The first three compounds also significantly inhibited the growth of *E. crusgalli*, but in this case the most active compound was **Ia** followed by **Ib** > **IIIa**. Compounds **II** and **IVa** did not show any inhibitory effect on this species.

Structural–activity relationships were not straightforwardly evidenced. At the present time, radicle growth inhibitory properties seem to be related to the presence of two oxygenated functionalities, for instance 1,4-dihydroxyallylic (**Ia**) or *p*-hydroquinone (**IIIa**). On the other hand, the presence of one hydroxyl, phenolic (**IVa**), or benzylic (**II**) renders reduced or lack of growth inhibition. Compounds **V** differ from **IV** in hydroxyl position, but unfortunately the former was not tested due to its low yield.

To our best knowledge this is the first time that piquerol aromatic derivatives have been prepared and tested as phytogrowth inhibitors. According to our results, the aromatic derivative **IIIa** has allelopathic activity. Considering this property, it can be hypoth-

**Figure 3.**

esized that in nature piquerol A could suffer aromatization into an intermediate compound and then act as an allelopathic molecule (Figure 3). Circumstantial evidence of this hypothesis could be the presence of compound **2** (or **IVc**) as natural product in *P. trinervia*.

ACKNOWLEDGMENT

We thank R. Villena, R. Gaviño, L. Velasco, and R. Patiño for technical assistance.

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Received for review November 23, 1994. Revised received July 24, 1995. Accepted June 24, 1996.® We thank PUMA for Grant 102/93.

JF9406626

® Abstract published in *Advance ACS Abstracts*, August 15, 1996.