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Translocation of isoquinoline alkaloids to the hemiparasite, *Tristerix verticillatus* from its host, *Berberis montana*

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1. Subject and source

Tristerix verticillatus (Ruiz et Pav.) Barlow et Wiens (Loranthaceae) is a stem hemiparasitic flowering species from the Andean region of South America where it grows in association with many different host-plant species (Kuijt, 1988). Translocation of alkaloids to *T. verticillatus* from its hosts was assessed by comparing alkaloid content of *T. verticillatus* hosted by *Berberis montana* Gay (Berberidaceae), an alkaloid-containing species (Fajardo et al., 1986) in a genus well known for the presence of isoquinoline alkaloids (Fajardo, 1992; Karimov, 1993; Schiff, 2000), and by alkaloid-lacking *Schinus montanus* (Philippi) (Anacardiaceae).

Representative samples of leaves of *T. verticillatus* growing on *S. montanus* (T–Sm) and on *B. montana* (T–Bm) were collected during March–April 2006 at Yerba Loca Sanctuary (33° 18′ 36″S; 70° 19′ 20″W) at altitudes between 1950 and 1970 m above sea level. Voucher specimens (T–Sm: SGO-155243 and T–Bm: SGO-155244) were deposited at the Herbarium of the National Natural History Museum, Santiago, Chile.

2. Previous work

The isoquinoline alkaloid berberine has been isolated from *B. montana* (Fajardo et al., 1986). To the best of our knowledge, no studies have been published on the chemistry of *S. montanus*.

3. Present study

Oven dried and powdered leaves of *B. montana* (1.0 kg), *S. montanus* (1.0 kg) and *T. verticillatus* from each host (0.6 kg) were sequentially extracted with MeOH at room temperature for four days (4 × 2.5 l). For each species or hemiparasite–host

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system, the combined extracts were evaporated *in vacuo*. The syrupy residue was agitated with 200 ml 10% HCl for 1 h, allowed to stand for 24 h at 10°C and filtered. The clear filtrate was washed with $CHCl_3$ (5 × 50 ml). The $CHCl_3$ washings upon evaporation yielded a brown non-alkaloidal gum which was not investigated. The aqueous phase was adjusted to pH 10 with NH_4OH and extracted with $CHCl_3$ (5 × 50 ml). Evaporation of the solvent yielded extracts potentially containing alkaloids: *B. montana*, 2.2 g, *T. verticillatus* on *B. montana*, 224 mg, *S. montanus*, 0.4 mg and *T. verticillatus* on *S. montanus*, 2.9 mg. From Dragendorff-positive extracts (only *B. montana* and *T. verticillatus* on *B. montana*), alkaloids were isolated by column chromatography on silica gel using $CHCl_3$ with increasing proportions of MeOH. The alkaloids isolated from *B. montana* were as follows: (+)-N-methylcoclaurine **1** (14 mg); amorphous solid; $[\alpha]_D^{20} = +119.0°$ (c: 0.1, $CHCl_3$); [lit. (Barton et al., 1967): amorphous solid; $[\alpha]_D = +123.0°$ (CH_3OH)], (−)-pronuciferine **2** (412 mg); amorphous solid; $[\alpha]_D^{20} = -122.7°$ (c: 2.1, $CHCl_3$); [lit. (Pérez et al., 2005): amorphous solid; $[\alpha]_D^{22} = -117.0°$ (c: 0.30, CH_3OH)], (+)-9-hydroxynuciferine **3** (19 mg); amorphous solid; $[\alpha]_D^{20} = +73.8°$ (c: 0.1, $CHCl_3$) and (+)-orientine **4** (180 mg); amorphous solid; $[\alpha]_D^{20} = +67.7°$ (c: 1.1, $CHCl_3$)]; [lit. (Guinaudeau et al., 1988): amorphous solid; $[\alpha]_D^{22} = +70.0°$ (c: 0.16, CH_3OH)]. From *T. verticillatus* on *B. montana* the following alkaloids were isolated: (−)-pronuciferine **2** (14 mg) and (+)-glaucine **5** (8 mg); amorphous solid; $[\alpha]_D^{20} = +101.0°$ (c: 0.07, CH_3OH)]; [lit. (Guinaudeau et al., 1975): $[\alpha]_D^{22} = +116.0°$ (c: 0.75, CH_3OH)]. Compound **5** was purified as its hydrochloride. Chemical structures of alkaloids are shown in Fig. 1. Identity of compounds was confirmed by NMR (400 MHz) experiments (^{1}H, ^{13}C, DEPT, HSQC, HMBC) and by comparison with spectroscopic and physical data from the literature (Barton et al., 1967; Guinaudeau et al., 1988; Asencio et al., 1993; Pérez et al., 2005). (+)-Glaucine hydrochloride was identified by comparison with a pure sample prepared by diazomethane methylation of (+)-boldine isolated from the bark of *Peumus boldus* (Monimiaceae). No alkaloids were found in *S. montanus* nor in *T. verticillatus* on *S. montanus*.

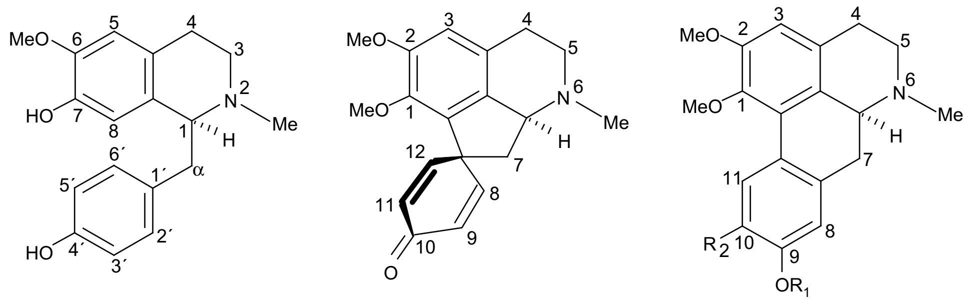
4. Chemotaxonomic and ecological significance

B. montana was shown to contain four monomeric isoquinoline alkaloids: the benzylisoquinoline (+)-N-methylcoclaurine **1**, the proaporphine (−)-pronuciferine **2**, and the aporphines (+)-9-hydroxynuciferine **3** and (+)-orientine **4**. Other Chilean *Berberis* species studied have been reported to contain dimeric structures: bisbenzylisoquinolines, proaporphine-benzylisoquinolines and aporphine-benzylisoquinolines and proaporphine-benzylisoquinoline oxides respectively (Fajardo et al., 1986; Fajardo, 1992). The occurrence of only monomeric isoquinoline alkaloids in *B. montana* suggests a lack of enzymatic capacity for dimerization, representing a distinctive chemical feature of this species among others of the same genus in Chile (Stadler et al., 1988). On the other hand, berberine was absent from the extracts analysed most likely on account of its preferential occurrence in roots, rhizomes and stem bark.

The principal alkaloid isolated from *B. montana*, (−)-pronuciferine **2**, was also identified in *T. verticillatus* growing on *B. montana*. No alkaloids were found in *T. verticillatus* on *S. montanus*, suggesting the incapacity of *T. verticillatus* to biosynthesize alkaloids, and reinforcing the hypothesis of translocation of isoquinoline alkaloids from *B. montana* to *T. verticillatus*.

The aporphine alkaloid (+)-glaucine **5** was isolated from *T. verticillatus* on *B. montana* but not from the host. Biotransformation in the hemiparasite of translocated alkaloids from the host is considered very unlikely because enzymes responsible for isoquinoline alkaloid biosynthesis are highly specific and restricted to plant families containing these compounds (Frenzel and Zenk, 1990; Pauli and Kutchan, 1998; Sato et al., 2007). Since (+)-glaucine **5** has been found in other species of *Berberis* (Karimov et al., 1995; Khamidov et al., 1997), a more plausible explanation for its presence in *T. verticillatus* is its occurrence in *B. montana*, albeit in undetected amounts.

The translocation of secondary metabolites from hosts to aerial hemiparasites has been well documented for quinolizidine alkaloids (Stermitz and Harris, 1987; Arslanian et al., 1990; Schneider and Stermitz, 1990; Stermitz and Pomeroy, 1992; Martín-Cordero et al., 1993; Bäumel et al., 1994; Adler and Wink, 2001; Woldemichael and Wink, 2002), pyrrolizidine



1 **2** **3** $R_1 = R_2 = H$
4 $R_1 = Me$ $R_2 = H$
5 $R_1 = Me$ $R_2 = OMe$

Fig. 1. Alkaloids of *B. montana* (**1–4**) and *T. verticillatus* hosted by *B. montana* (**2,5**).

alkaloids (Stermitz and Harris, 1987; Woldemichael and Wink, 2002), bipiperidyl alkaloids (Martín-Cordero et al., 1993), norditerpenoid alkaloids (Marko and Stermitz, 1997) and piperidinyl alkaloids (Schneider and Stermitz, 1990), the hemiparasite species belonging to the families Scrophulariaceae, Santalaceae, Convolvulaceae and Viscaceae. To the best of our knowledge, this is the first report of alkaloid translocation to a Loranthaceae.

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