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Flavonoids and a Stilbene from *Triadenum fraseri* (Spach) Gleason

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1. Subject and source

Triadenum fraseri (Spach) Gleason (Family, Clusiaceae), commonly known as Fraser's marsh St. John's Wort, is a perennial native to North American marshes (United States Department of Agriculture Natural Resources Conservation Services Plants Database). Aerial parts of *T. fraseri* were collected in Forest County, Pennsylvania, USA in September 2005 and identified by Mr. Joseph A. Isaac. A voucher specimen (18935) has been deposited at the Carnegie Museum Herbarium in Pittsburgh, PA, USA.

2. Previous work

To date, only one other member of the *Triadenum* genus, *T. japonicum*, has been the subject of previous phytochemical investigation (Krasovskya et al., 1986). Flavonoids were the main chemical constituents reported from *T. japonicum* and included quercetin, hyperin (quercetin-3-galactoside), avicularin (quercetin-3-arabinofuranoside) and (+)-catechin.

3. Present study

Air-dried leaves of *T. fraseri* (495 g) were sequentially extracted with hexanes, acetone, and methanol to yield 11.3 g, 21.4 g, and 141.4 g of dried extracts, respectively. A portion of the methanol extract (30 g) was selected for further purification by repeated silica gel and Sephadex-LH20 column chromatography followed by C18 semi-preparative HPLC. First, the extract was chromatographed on 45 x 3 cm silica gel column eluted with a CHCl₃-CH₃OH gradient system (5:1 to 1:2, v/v) to afford thirteen fractions, A1-A13. Fr. A5 was re-chromatographed on a 37 x 5.5 cm silica gel column eluted with a CHCl₃-CH₃OH gradient system (4:1 to 0:1, v/v) to afford eight subfractions, B1-B8. Subfraction B5 (1.15 g) was further purified over a Sephadex LH-20

column with an isocratic solvent system of CH₃OH-H₂O (7:3, v/v) to afford six subfractions, C1-C6. Subsequent semi-preparative HPLC of subfraction C6 (80 mg) using a Waters Sunfire C18 column (250 × 10 mm i.d., 5 μm, flow = 2 mL/min) with a gradient elution system of CH₃OH-H₂O (0.1% trifluoroacetic acid) afforded compound **1** (60 mg; myricitrin) (Shen et al., 2009). Similarly, semi-prep HPLC purifications of subfraction B2 (50 mg) yielded an inseparable mixture of compounds **2** & **4** (15 mg; quercetrin and nicotiflorin, respectively) (Tastis et al., 2007; Eldahshan et al., 2008) and that of fraction A6 (1.08 g) yielded compound **3** (11 mg; rutin) (Li et al., 2009). Fr. A1 (0.31 g) was chromatographed on a silica gel column (37 × 5.5 cm) with a gradient solvent system of Me₂CO: hexanes (1:10 to 1:1, v/v) to afford twelve subfractions, D1-D12. Subfraction D1 (80.0 mg) was purified by semi-preparative HPLC and yielded compound **5** (3 mg; myricetin-3-*O*-glucoside) (Scharbert et al., 2004). Fr. A9 (7.30 g) was purified on a Sephadex LH20 column with an isocratic solvent system of CH₃OH-H₂O (7:3, v/v) followed by semi-preparative HLPC to afford compound **6** (10 mg; 3-*p*-coumaroylquinic acid) (Norbaek et al., 2002). Finally, semi-preparative HPLC of subfraction B3 yielded compound **7** (3 mg; piceatannol-4'-*O*-β-D-glucopyranoside) (Vastano et al., 2000).

The structures of **1-7** were elucidated by a combination of spectroscopic data (ESI-MS, ¹H and/or ¹³C NMR) and by comparisons of these with published literature and also by direct HPLC comparison with an authentic sample (for compound **3**).

4. Chemotaxonomic significance

This is the first report of the isolation and identification of flavonoids (**1-5**), a cinnamate derivative (**6**), and a stilbene (**7**) from *T. fraseri*. The presence of flavonoids as the major class of secondary metabolites in this species is in agreement with the previous report from *T.*

japonicum (Krasovskaya et al., 1986). Remarkably, this is the first report of a stilbenoid in any of the estimated thirty-seven genera of the Clusiaceae family (Gustafsson et al., 2002), which is a significant chemotaxonomic finding. It is possible that stilbenes may be regarded as a chemotaxonomic marker of this genus but more research is needed to confirm this. Arguably, St. John's Wort (*Hypericum perforatum* L.) is the most widely known and studied species in the Clusiaceae family. This is due in large part to its extensive use as an herbal supplement for treating depression (Shelton, 2009). Thus, from a chemotaxonomic perspective, the occurrence of a stilbene in Fraser's marsh St. John's Wort, and its absence in St. John's Wort, is interesting considering the similarities in common names assigned to these plants.

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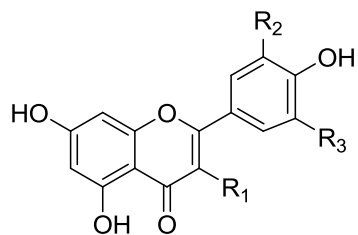
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Appendix. Supporting information

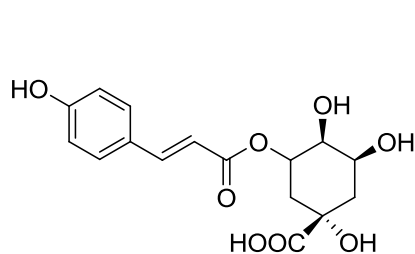
Supporting information associated with this article is provided.

Fig. 1. Structures of compounds **1-7** isolated from aerial parts of *Triadenum fraseri* (Spach)

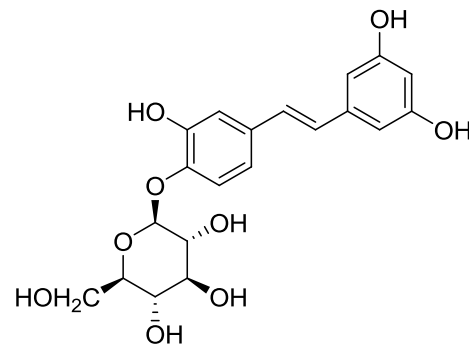
Gleason.



- 1 R₁=O-rha; R₂=OH; R₃=OH
- 2 R₁=O-rha; R₂=H; R₃=OH
- 3 R₁=O-glu-rha; R₂=H; R₃=OH
- 4 R₁=O-glu-rha; R₂=H; R₃=H
- 5 R₁=O-glu; R₂=OH; R₃=OH



6



7

