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QUINOLINE-2-CARBOXYLIC ACIDS FROM *EPHEDRA* SPECIES

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Key Word Index—*Ephedra*; Ephedraceae; quinoline-2-carboxylic acids; 6-methoxykynurenic acid; structural elucidation.

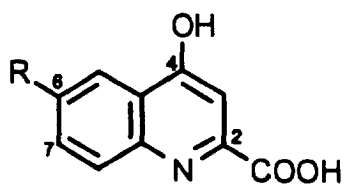
Abstract—A new quinoline-2-carboxylic acid, 4-hydroxy-6-methoxyquinoline-2-carboxylic acid (6-methoxykynurenic acid), has been isolated from *Ephedra pachyclada* ssp. *sinaica*. Kynurenic and 6-hydroxykynurenic acids, previously reported from plants, were also isolated from *Ephedra*.

INTRODUCTION

Most studies of *Ephedra* species. have concerned the ephedrine alkaloids [1], although a number of other natural products, including 4-hydroxy-7-methoxyquinoline-2-carboxylic acid (ephedralone) [2], have been isolated. In the course of an investigation of non-protein amino acids in *Ephedra* [3], UV-absorbing substances were observed, which did not yield phenylthiocarbamyl derivatives when treated with phenyl isothiocyanate. These have been identified as the quinoline-2-carboxylic acids: kynurenic acid (**1**), 6-hydroxykynurenic acid (**2**), and a new member of this family of naturally occurring compounds, 6-methoxykynurenic acid (**3**).

RESULTS AND DISCUSSION

A crystalline substance occurring as a major component in fresh stem extracts of *E. foeminea* and *E. foliata* was identified on the basis of its chromatographic and spectroscopic properties as 6-hydroxykynurenic acid (**2**). This substance, whose occurrence in these and other *Ephedra* species [4] was recently reported without any substantiating information, has been isolated previously from several unrelated plants [5–8]. Our further investigations have led to the identification of two related quinoline-2-carboxylic acids, kynurenic acid (**1**) and 6-methoxykynurenic acid (**3**), in extracts of *E. pachyclada* ssp. *sinaica*, in addition to **2**. Compound **1**, which has been mentioned as a constituent of *Ginkgo biloba* leaves [9], and compound **2** were identified by direct comparison (HPLC, TLC and UV) with authentic samples and by conversion into 4-hydroxyquinoline and 4,6-dihydroxyquinoline, respectively, upon heating to their mps [10, 11]. The third compound, $C_{11}H_9NO_4$, showed UV and ^1H NMR spectra and chromatographic properties identical to authentic **3** and yielded the same methylation product, 4,6-dimethoxyquinoline-2-carboxylic acid methyl ester, when both were methylated as described [12] for xanthurenic acid (8-hydroxykynurenic acid). The same three compounds were found in extracts of *E. pachyclada* ssp. *sinaica* collected form another location. This is the first report of **3** in plants. The closely related 7-methoxykynurenic acid, clearly differentiated from **3** by its UV spectrum, has been reported from *E. alata* [2]. Although overlooked until recently, it appears that **1** and related quinoline-2-carboxylic acids, which can exist as both 4-keto and 4-hydroxy tautomers [13], are distributed quite widely within the genus *Ephedra*. Compound **1** and its derivatives are currently receiving considerable attention due to their antagonistic actions on glutamate receptors in mammalian brain [13].



1 R = H

2 R = OH

3 R = OCH_3

EXPERIMENTAL

General. Mps: uncorr. EIMS: 70 eV. TLC: Macherey–Nagel Polygram Sil G/UV_{254} plates (0.25 mm);

spots visualized by viewing under UV light. HPLC: Waters μBondapak C_{18} columns; photodiode array detector used to monitor sepns and record spectra.

Plant material. Fresh stems of greenhouse-grown *E. foeminea* and *E. foliata* were supplied by J. Stein (Department of Biology, Erindale College, Toronto) and D. Boyce (Department of Plant Sciences, University of Western Ontario, London, ON). Dried *E. pachyclada* ssp. *sinaica* stems were supplied by Prof. H. Freitag (Department of Morphology and Plant Systematics, University of Kassel, Germany), who also identified all plant material. Voucher specimens are deposited in the Plant Sciences Herbarium, University of Western Ontario, or in the Plant Systematics Herbarium, University of Kassel.

Extraction and isolation. Finely chopped fresh stems of *E. foeminea* (7.5 g) were extracted with EtOH and EtOH–0.1 M HCl (1:1). The H_2O-soluble portion of the extract was applied to a cation exchange column (Rexyn 101, H^+ form) and the amino acids and other basic substances were eluted with 1 M pyridine. The residue was fractionated by HPLC (150 × 19 mm column), using a 30 min linear gradient of 0–40% CH_3CN–H_2O containing 10 mM TFA, yielding **2** (4.8 mg), recrystallized from 2 M HOAc, mp 289–291° (dec.) (lit. 283° [5], 298–300° [11]); UV λ_{max} 214, 257, 351 nm; blue spot, R_f 0.12, when TLC (EtOAc–*iso*-PrOH–28% NH_3, 9:6:4) was sprayed with a mixt. of $FeCl_3$ and $K_3Fe(CN)_6$ [14]. A similar extract of fresh *E. foliata* (7.2 g) was applied to the cation exchange column and eluted with a large vol. of H_2O, as described for **2** and related compounds [15], yielding the same compound (5.5 mg). Finely ground stems of *E. pachyclada* ssp. *sinaica* (0.5 g) were extracted with EtOH, and the extract was analysed and then sepd by HPLC (300 × 3.9 mm column) using a 15 min gradient of 0–20% CH_3CN–0.05 M NaH_2PO_4 to yield, in order to elution, **2** (0.8 mg), **1** (3.9 mg; UV λ_{max} 216, 244, 334, 345(sh) nm) and **3** (1.7 mg; λ_{max} 216, 258, 350 nm). For further characterization, **3** acid was isolated from another stem extract by chromatography over a μBondapak C_{18} PrepPak cartridge (100 × 25 mm) using MeOH–20 mM NH_4OAc (1:4).

6-Methoxykynurenic acid (**3**). Crystals from MeOH, mp 298–300° (dec.) (lit. 294–295° (dec.) [16]); ^1H NMR (200 MHz, DMSO-d_6): δ 3.83 (3H, *s*, OMe), 6.62 (1H, *s*, H-3), 7.35 (1H, *dd*, J = 2.9, 9.1 Hz, H-7), 7.46 (1H, *d*, J = 2.9 Hz, H-5), 7.91 (1H, *d*, J = 9.1 Hz, H-8); HR-EIMS, *m/z* $[M]^+$ 219.0523 (calc. for $C_{11}H_9NO_4$: 219.0531); EIMS, *m/z* (rel. int.): 219 (50), 175 (100), 173 (36), 160 (10), 158 (14), 145 (10), 135 (25), 132 (28), 119 (13), 105 (18). ^1H NMR, UV, HPLC, TLC identical to authentic sample supplied by D. Mullins. Methylation of this substance with CH_3I/K_2CO_3 in DMF at room temp. [12] yielded a product identical (HPLC, UV λ_{max} 255, 319 nm) to 4,6-dimethoxyquinoline-2-carboxylic acid methyl ester [mp 133–134°; HR-EIMS, *m/z* 247.0838 $[M]^+$ (calc. for $C_{13}H_{13}NO_4$: 247.0844)] prep by similar methylation of authentic **3**.

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