Transtorine, a New Quinoline Alkaloid from *Ephedra transitoria*

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Transtorine (**1**), a new quinoline alkaloid, isolated from the aerial part of *Ephedra transitoria* by column chromatography, was identified as 4-quinolone-2-carboxylic acid. The structure was determined by spectroscopic methods. Transtorine exhibited growth inhibitory activity against the common bacteria, *Enterobacter cloacae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

The genus *Ephedra* L. of the family Ephedraceae, is dioecious and much branched, an erect or climbing shrub. There are five *Ephedra* species distributed through the eastern Mediterranean and Saharo-Arabian regions.[1,2] The genus *Ephedra* is known to contain ephedrine and related alkaloids,[3,4] however, studies have reported the absence of the alkaloid ephedrine in certain *Ephedra* species.[5,6] The present study was carried out to determine the constituents of *Ephedra transitoria* Riedl, an erect, multibranched shrub growing wild in the northeast desert of Jordan.[1,2]

Chromatographic separation of the alkaloid fraction prepared from the leaves of *E. transitoria* led to the isolation of an alkaloid (**1**) as a white substance. Its HREIMS established a molecular formula of $C_{10}H_7O_3N$. The UV spectrum in MeOH showed two absorption maxima at 243 and 345 nm. The latter showed a hypsochromic shift of 23 nm on addition of 1 N HCl. This shift is closely related to 4-quinolones substituted at position 2 with substituents that react with alkali.[7] The IR spectrum of **1** disclosed two carbonyl bands at 1722 and 1638 cm^{-1}, consistent with a carboxyl carbonyl and a quinolone carbonyl groups, respectively.[7,8]

The ^{1}H-NMR spectrum of **1** showed signals for five protons in the aromatic region. One of these protons (H-3) appeared as a singlet at δ 6.6, and, the remaining four protons were determined to be located at the benzenoid moiety: H-5 appeared as a doublet of doublets (δ 8.1), H-7 as a triplet of doublets (δ 7.9), H-6 as a triplet of doublets (δ 7.7), and H-8 as a doublet of doublets (δ 7.4). This was attributed to H-5 and H-7 being subjected to proximate and/or conjugate deshielding effects (mesomeric effects) of the 4-carbonyl function.[7−10] The ^{13}C NMR of **1** showed the presence of 10 carbons. The multiplicity assignments were obtained by carrying out the DEPT experiment and by using homo- and hetero-COSY experiments, which showed that **1** consists of five quaternary carbons and five CH's. The ^{13}C NMR spectrum displayed the 2- and 4-carbonyl signals at δ 163.6 and 177.5, respectively.[7,9] The LREIMS of **1** showed a fragmentation that was consistent with those reported for 4-quinolones bearing a 2-carboxylic acid moeity. The fragmentation proceeded through a loss of H_2O from the molecular ion (*m*/*z* 189, M^+), followed by the successive loss of 2 CO to give *m*/*z* 115. The spectrum also showed loss of CO_2 from the molecular ion to give *m*/*z* 145, which provided further evidence for the presence of a carboxylic function in the molecule.[7,8] From the physical and spectral data, compound **1** was confirmed to be 4-quinolone-2-carboxylic acid and was given the trivial name of transtorine.

Transtorine (**1**) showed growth inhibitory activity in vitro against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterobacter cloacae*, with MIC 0.38, 0.5, and 0.45 mg/mL, respectively. No activity was observed against *Escherichia coli*, *Proteas mirabilis*; *Shigella flexneri*, and *Bacillus subtilis*. It is worth mentioning that the alkaloids ephedrine and pseudoephedrine were not present in the plant, and this was confirmed by co-TLC with authentic samples.[11] 4-Quinolones are of rare natural occurrence in Ephedraceae, and the majority of them have been found in the family Rutaceae or in bacteria.[9] The first quinoline alkaloid from *Ephedra* species was isolated from *E. alata*.[7]

Experimental Section

General Experimental Procedures. Melting points were determined on a Stuart Scientific melting-point apparatus and are uncorrected. IR spectra were determined on a JASCO IR-810 spectrometer, and UV

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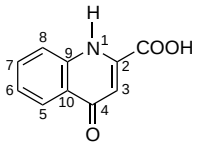

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Figure 1.

spectra, on a Unicam-810 Kontron spectrophotometer. ^{1}H-NMR spectra were determined at 300.13 MHz and ^{13}C-NMR spectra at 75.46 MHz using a Bruker DPX-300 spectrometer and TMS as internal standard. LRE-IMS were recorded on a quadrupole instrument Finnigan Mat 112, 70 eV. The HREIMS was obtained on the same instrument model CH-5 spectrometer. Si gel (Kieselgel 60-Merck) was used for column chromatography, while Si gel (Kieselgel 60-F_{254}, Merck) was used for TLC. Anhydrous Na_2SO_4 was routinely used for drying organic solvents, and all solvents were evaporated under reduced pressure at 40 °C.

Plant Material. Plant material of *E. transitoria* was collected from the Safawi area, 65 km northeast of Amman, Jordan, in May 1994, and identified by one of the authors (D. El-Eisawi). A herbarium specimen was deposited at the herbarium of the Department of Biological Sciences, Faculty of Science, University of Jordan, Amman, Jordan.

Extraction and Purification of Transtorine (1). Powdered, dried aerial part of *E. transitoria* (4.65 kg) was extracted by percolation with 96% EtOH (28 L), and the solvent was evaporated to leave a syrupy residue (184.2 g). The residue was stirred with tartaric acid 5% (1 L, 3 ×) and filtered. The insoluble residue was treated with Et_2O (1 L, 3 ×), filtered, and the filtrate was partitioned with tartaric acid 5% (0.5 L, 3 ×). The tartaric acid solutions were combined, basified with NH_4OH to pH 8−9, and extracted with $CHCl_3$ (1 L, 3 ×) to afford a dark oily residue (178.3 mg; fraction A). Fraction A was adsorbed onto Si gel (35 g) and chromatographed over a Si gel column (320 g) in $CHCl_3$. Elution with $CHCl_3$−MeOH mixtures afforded various fractions that were collected (250 mL) and combined according to TLC analysis.

Transtorine (1). Elution of the column with $CHCl_3$-MeOH (97:3 and 95:5) (1.5 L) afforded a white solid substance (112.6 mg), which on treatment with MeOH gave compound **1** (87.3 mg): mp 123 °C, UV (MeOH) λ_{max} (log ϵ) 243 (4.23), 326 sh (3.80), 345 (3.84) nm; + HCl 243 (4.27), 322 (3.75); IR (KBr) ν_{max} 3200, 1722, 1638, 1603, 1478, 1325, 1160, 1120, 1040, 795, 740 cm^{-1}; ^{1}H NMR (DMSO-d_6)δ 6.6 (1H, s, H-3), 7.4 (1H, dd, J = 7.79, 1.47 Hz, H-8), 7.7 (1H, td, J = 7.79, 1.47 Hz, H-6), 7.9 (1H, td, J = 7.79, 1.47 Hz, H-7), 8.1 (1H, dd, J = 7.79, 1.47 Hz, H-5); ^{13}C NMR (DMSO-d_6) δ 139.0 (C-2), 109.7 (C-3), 177.5 (C-4), 125.6 (C-5), 124.5 (C-6), 132.4 (C-7), 119.5 (C-8), 139.9 (C-9), 123.8 (C-10), 163.6 (C-2′, C═O carboxylic); EIMS m/z 189 [M$^+$] (12.7) 145 (100), 146 (10), 115 (37.3), 105 (7.5), 89 (23), 63 (15.7), 44 (32); HREIMS m/z 189.17 (calcd for $C_{10}H_7O_3N$ 189.17).

Antimicrobial Activity. The antimicrobial activity was tested on seven clinical isolates taken from patients in two local hospitals (Ibn Alhaitham and Albasheer Hospitals) (Gram negative: *P. aeruginosa*, *E. cloacae*, *E. coli*, *P. mirabilis*, *S. flexneri*; Gram positive: *S. aureus* and *B. subtilis*, by disk diffusion method at concentration of 2.5 mg/disk as described previously.[11] The determination of MIC was carried out by measuring the inhibition zone (20−25 mm), using ciprofloxacin as a positive control with MIC 0.02 mg/mL.[13]

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