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# NEW OBSERVATIONS ON THE SECONDARY CHEMISTRY OF WORLD *EPHEDRA* (EPHEDRACEAE)[1]

STANLEY CAVENEY,[2,6] DAVID A. CHARLET,[3] HELMUT FREITAG,[4] MARIA MAIER-STOLTE,[4] AND ALVIN N. STARRATT[5]

[2]Department of Zoology, University of Western Ontario, London, Ontario, Canada N6A 5B7;
[3]Department of Biological Sciences S2B, Community College of Southern Nevada, 3200 East Cheyenne Avenue, North Las Vegas, NV 89030;
[4]Department of Morphology and Systematics of Plants, University of Kassel, D-34132 Kassel, Germany; and
[5]Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, 1391 Sandford Street, London, Ontario, Canada N6V 4T3

For several millennia, stem extracts of *Ephedra* (Ephedraceae, Gnetales) have been used as folk medicines in both the Old and New World. Some species were used in treatments of questionable efficacy for venereal disease in North America during the last century. Many Eurasian species produce phenylethylamine alkaloids, mostly ephedrine and pseudoephedrine, that interact with adrenergic receptors in the mammalian sympathetic nervous system. Asian *Ephedra* have been used recently in the clandestine manufacture of a street drug, methamphetamine. Although ephedrine alkaloids are not detectable in New World species of *Ephedra*, together with Asian species they contain other nitrogen-containing secondary metabolites with known neuropharmacological activity. Many mesic and particularly xeric species worldwide accumulate substantial amounts of quinoline-2-carboxylic acids, or kynurenates, in their aerial parts. Many species of *Ephedra* accumulate cyclopropyl amino acid analogues of glutamate and proline in their stems and roots, and particularly in the seed endosperm. Mesic species synthesize substantial amounts of three L-2-(carboxycyclopropyl)glycine stereomers rarely seen in nature. A cyclopropyl analogue of proline with known antimicrobial activity, *cis*-3,4-methanoproline, is found in large amounts in the stems and seeds of many *Ephedra* species. The ability to synthesize cyclopropyl amino acids may be an ancestral feature in the taxon. The natural function in the taxon of these three groups of secondary compounds remains to be established.

**Key words:** *cis*-3,4-methanoproline; *Ephedra*; ephedrine alkaloids; 6-hydroxykynurenic acid; L-2-(carboxycyclopropyl)glycine; kynurenic acid; 6-methoxykynurenic acid.

*Ephedra* (family Ephedraceae, the jointfirs) is a genus of nonflowering seed plants belonging to the Gnetales, the closest living relatives of the Angiosperms (Friedman, 1996, 1998). Most of the ~50 *Ephedra* species worldwide (Stevenson, 1993; Price, 1996) are shrubs adapted to semiarid and desert conditions (Pearson, 1929) (Fig. 1). A few lianoid species are found in temperate regions along the Mediterranean coastline and humid montane sites in North Africa and southwest Asia (Freitag and Maier-Stolte, 1989). About 25 species of *Ephedra* are found in the drier regions of the Old World extending westwards from Central Asia across southwest Asia and into Mediterranean Europe and North Africa (Freitag and Maier-Stolte, 1994). The taxon is absent from Saharan Africa and Australasia. The status and distribution of some of the Old World species of *Ephedra*, particularly those of the eastern Mediterranean and southwest Asia and adjacent arid regions, have been recently revised and updated (Freitag and Maier-Stolte, 1989, 1994). In the New World, ~12 species of *Ephedra* are found ranging from the southwestern United States to the central plateau of Mexico, and ~12 more species in South America occur in an area from Ecuador to Patagonia (Hunziker, 1949; Price, 1996). While there is no recent worldwide monograph of the taxon, Price (1996) gives a synopsis.

Almost all commercial applications of *Ephedra* extracts derive from the ephedrine alkaloids found in the stems in many Eurasian species. The best-documented drug made from *Ephedra* is Ma-huang, used in Chinese medicine for >5000 yr as a treatment for fever, nasal congestion, and asthma (Zhu, 1998). Ma-huang is also an effective respiratory sedative and cough remedy. Herbal mixtures containing Ma-huang are sold in health food stores in the West as nutritional supplements under such names as Herbal Ecstasy and Escalation (Gurley, Wang, and Gardner, 1998) accompanied by dubious claims that they have energizing value or assist in dieting (White et al., 1997). Ma-huang was traditionally obtained from the dried

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[6] Author for reprint requests (e-mail: caveney@julian.uwo.ca).

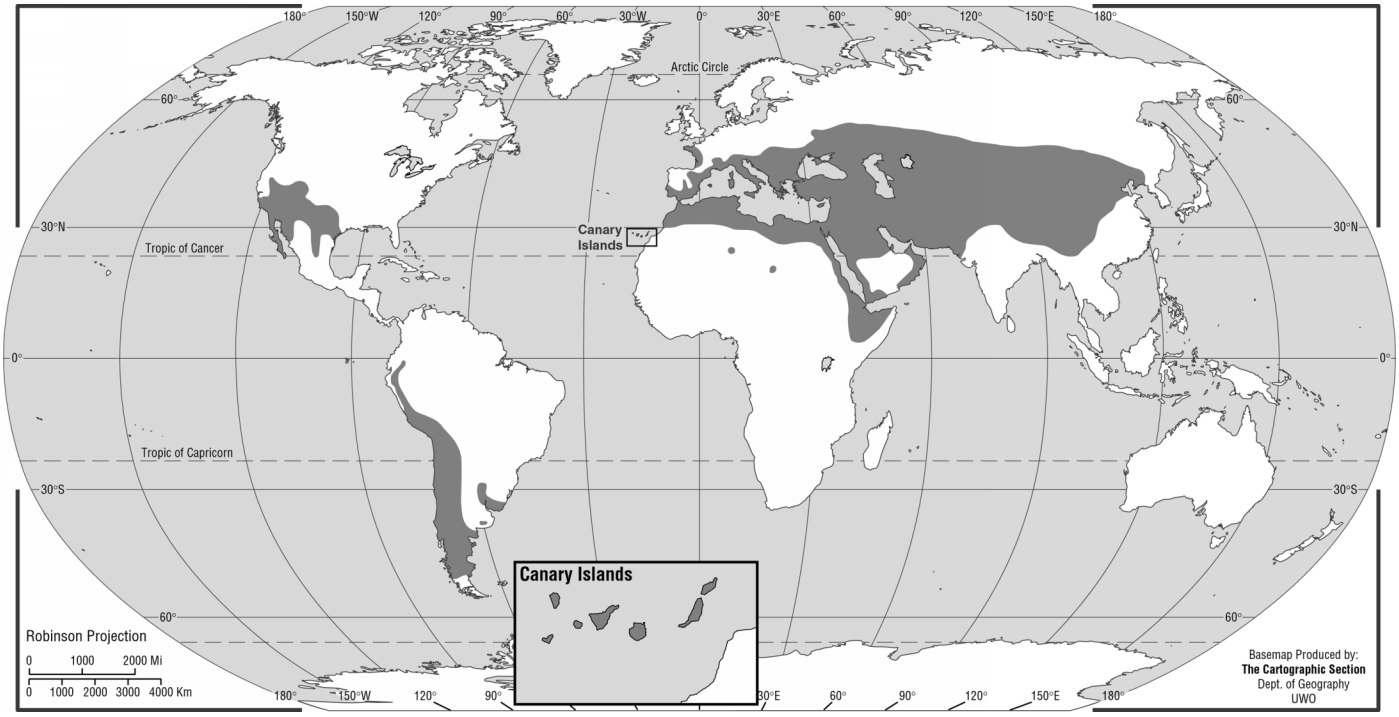


Fig. 1. Distribution of World *Ephedra*. This map was constructed mainly from data provided in Hunziker (1949), Freitag and Maier-Stolte (1989, 1993, 1994), Zhang, Tian, and Lou (1989), and Stevenson (1993). The distribution of *Ephedra* in east Asia extends north of Korea to the Sea of Japan.

stems of *E. sinica*, *E. equisetina*, and *E. intermedia* (Qazilbach, 1971; White et al., 1997; Zhu, 1998), species found in the drier regions of China, North West India, and Pakistan.

The New World species of *Ephedra* are not thought to contain significant amounts of ephedrine alkaloids (Duke, 1986; O'Dowd et al., 1998). Claims that some North American species contain pseudoephedrine (Willaman and Schubert, 1961; Max, 1991) or norpseudoephedrine (Wink, 1998) remain unconfirmed. Consequently, the phytochemical basis behind the purported stimulant and therapeutic nature of ''Mormon tea'' and other pioneer teas made from North American *Ephedra* is unresolved. Several nitrogenous secondary compounds with known neuroactivity, including nonprotein amino acids with cyclopropyl ring structures as well as quinoline-related tryptophan derivatives, have recently been identified in Eurasian species of *Ephedra* (Caveney and Starratt, 1994; Starratt and Caveney, 1995, 1996). The stems of many New World species contain these compounds also, which may explain why *Ephedra* extracts have been used so widely in traditional medicine in both the Old and New Worlds.

## MATERIALS AND METHODS

***Material***—Stem tissue was collected from wild plants, greenhouse-cultivated plants, or removed from dried herbarium material of field-collected specimens. Details of the source localities of the material, which represents 26 of the ~50 known species, are given in Tables 1–3. The material examined is listed here by geographical region. The specific epithets given are those found in Freitag and Maier-Stolte (1994) or Price (1996), where synonyms for the species names are given. Eurasian material was authenticated by H. Freitag and M. Maier-Stolte (Kassel) and American material by P. Leary and D. A. Charlet (Las Vegas). Voucher samples are housed in the institutions of the co-authors listed under ''accession no.'' in the tables.

Material was obtained from the following geographical regions: southern Europe and Mediterranean coast: *Ephedra altissima* Desf., *E. fragilis* Desf., *E. major* Host., *E. distachya* L., and *E. foemina* Forssk; North Africa and Sinai Desert: *E. alata* Dec., *E. aphylla* Forssk., *E. foliata* Boiss. ex C.A. Mey., *E. fragilis, E. major* Host. ssp. *major*, and *E. pachyclada* Boiss. ssp. *sinaica* (Reidl) Freitag and Maier-Stolte; Middle East (Israel, Jordan, Syria, Iraq): *E. aphylla, E. foemina, E. foliata*, and *E. fragilis*; Irano-Turanian region: *E. distachya, E. foliata, E. intermedia* Schrenk & C.A. Mey., and *E. major* ssp. *procera* (Fisch. et C.A. Mey.)Bornm; Indian subcontinent: *E. gerardiana* Wall. ex Stapf (member of the *E. major* complex; Freitag and Maier-Stolte, 1994), *E. foliata, E. pachyclada* Boiss. ssp. *pachyclada*, and *E. regeliana* Florin; central Asia: *E. regeliana, E. botschantzevii* Pachom., *E. intermedia, E. minuta* Florin, *E. monosperma* C.A. Meyer var. *minima* Hao, and *E. przewalskii* Stapf; southwest United States: *E. californica* S. Watson, *E. funerea* Coville & Moreton, *E. fasciculata* A. Nelson, *E. nevadensis* S. Watson, *E. torreyana* S. Watson, *E. trifurca* Torrey, and *E. viridis* Coville; South America: *E. chilensis* K. Presl and *E. tweediana* Fisch. & C.A. Meyer. In addition, seed extracts were obtained from fresh and herbarium material of *E. aphylla, E. altissima, E. distachya, E. foemina, E. foliata, E. fragilis, E. nevadensis*, and *E. viridis.*

***Isolation and identification of nonprotein amino acids***—Plant tissue (5 g fresh mass) was finely macerated and extracted four times, once by 30 min ultrasonication in 5 mL 95% ethanol and then three times by shaking overnight in 25 mL 0.1 mol/L HCl. Amino acids were isolated from the water-soluble extracted material by cation exchange chromatography over a short column of Rexyn 101 ($H^+$ form). The cyclopropyl amino acids were isolated by high performance liquid chromatography (HPLC) on a Partisil 10 SCX column and treated with (1) fluorodinitrobenzene and (2) diazomethane to afford the dinitrophenyl dimethyl ester derivatives. Comparison of the $^1H$ nuclear magnetic resonance (NMR) spectra and mass spectroscopy (MS) of the dinitrophenyl dimethyl esters of the *E. foemina* and *E. altissima* amino acids with the derivatives of the authentic compounds confirmed their identity as (2S,3S,4R)-, (2S,3R,4S)-, and (2S,3S,4S)-2-(carboxycyclopropyl)glycine (Starratt and Caveney, 1996). Amino acids were analyzed quantitatively by the Picotag® method in which phenylthiocarbamyl (PTC) derivatives of amino

acids are detected by their absorption at 254 nm after their separation on a reverse-phase HPLC column using a sodium acetate/acetonitrile gradient (Tomlin, McLean, and Caveney, 1993). Individual amino acids were identified by comparing their relative retention times ($R_t$, normalized to *hpro*) with those of commercially available common amino acids (Pierce, Rockford, Illinois, USA) and neuroactive nonprotein amino acids (Tocris-Cookson, Ballwin, Missouri, USA). The retention times of the PTC derivatives of two main *Ephedra* nonprotein amino acids corresponded exactly to those of the corresponding derivatives of L-CCGIII isolated by Fowden et al. (1969) from seeds of *Aesculus parviflora* and synthetic L-CCGIV and differed from L-CCGI. The identity of methanoproline in the seed of *E. foemina* is based on the correspondence of the retention time of its PTC-derivative ($R_t$ = 2.139) with that of the major component in seed extracts of *A. parviflora* (Fowden et al., 1969). The amounts of individual amino acids in the samples were determined by comparison with a standard amino acid mixture (Caveney et al., 1996).

***Identification and analysis of kynurenic acid, 6-hydroxykynurenic acid and 6-methoxykynurenic acid***—Kynurenic acid, 6-hydroxykynurenic acid, and 6-methoxykynurenic acids in extracts of selected *Ephedra* species were analyzed by HPLC with photodiode array detection at 254 nm on a μ-Bondapak $C_{18}$ column (3.9 × 300 mm) using 0.05 mol/L $NaH_2PO_4$-acetonitrile or 20 mmol/L ammonium acetate-methanol gradients. The spectra of the peak compounds in the extracts were compared with spectra of chromatographed standards. Chromatographically pure 6-hydroxykynurenic acid from *E. foemina* and *E. foliata* and 6-methoxykynurenic acid from *E. pachyclada* were further characterized by their physical (mp), chromatographic (HPLC, thin layer chromatography [TLC]) and spectroscopic (ultraviolet [UV], infrared [IR], and mass spectroscopy [MS]) properties and their identities confirmed by direct comparison with synthetic material (Starratt and Caveney, 1996). Heating either the natural or the synthetic acid to the melting point (290°C) yielded 4,6-dihydroxyquinoline as the main product (HPLC retention time and UV evidence).

***Isolation and identification of ephedrine and pseudoephedrine***—Acidic substances including 6-hydroxykynurenic acid were removed by application of aliquots of plant extracts (generally prepared by steeping 0.5 g fresh tissue in 2.5 mL ethanol) to Accell Plus QMA Sep-Pak cartridges (Waters) after dilution with an equal volume of water. Ephedrine and pseudoephedrine levels in the aqueous eluent were determined by HPLC using a method based on that described by Barkan, Weber, and Smith (1981). Samples were chromatographed on a μBondapak $C_{18}$ column (3.9 × 300 mm) using 1% acetonitrile in 0.05 mol/L monobasic sodium phosphate at 1 mL/min. Substances responsible for peaks detected at 210 nm were identified by comparison of retention times and UV spectra (Shimadzu Model SPD-M6A photodiode array detector, Columbia, Maryland, USA) with those of authentic compounds. Retention times: ephedrine, 11.2 min; pseudoephedrine, 13.5 min.

***Measurement of pseudoephedrine in extracts containing interfering peaks***—In some cases, preparations derived from the North American *Ephedra* species, analyzed as described above, exhibited HPLC peaks at the retention time of pseudoephedrine but did not show the correct spectrum. The absence of detectable amounts of pseudoephedrine in these species was demonstrated by derivatization of the sample with fluorodinitrobenzene and chromatography of the product on a μBondapak $C_{18}$ column (3.9 × 300 mm) using a 10 mmol/L trifluoroacetic acid-acetonitrile gradient and detection at 390 nm. Pseudoephedrine added at a level of 0.01%, based on wet tissue mass, to an *Ephedra* sample that did not exhibit a peak at the retention time for this alkaloid resulted in a peak for the dinitrophenyl derivative of pseudoephedrine indicating that amounts above this level should be detected.

***Assay for presence of tannins***—The condensed tannin content of the stems was graded on the basis of the rate and extent of the color reaction that developed on the addition of three drops each of 5% acetic acid and 5% sodium nitrite to 0.5 mL acid-alcohol stem extract. The color reaction that developed provided a crude estimate of the polyphenol content of the tissue (Reeve, 1951).

## RESULTS

Most of the 13 000 nitrogen-containing secondary metabolites found in plants are derived from the 20 common protein amino acids (Southon and Buckingham, 1989). Many N-containing plant metabolites in *Ephedra* are structural analogues of amino acids such as L-glutamate and L-proline important in cell metabolism or are products of amino acid metabolism (kynurenates as catabolic products of tryptophan and its derivatives [Mullins, 1985]; ephedrine alkaloids as products of phenylalanine metabolism [Grue-Sorensen and Spenser, 1993]). The many flavonoid and related compounds described from *Ephedra* species (Hegnauer, 1986) are not considered here. The condensed tannin content of the species analyzed is included because the tannin and nonprotein amino acid content in *Ephedra* species is often inversely related. This may have ethnopharmacological significance (S. Caveney and D. A. Charlet, unpublished data).

***Cyclopropyl amino acids***—*Ephedra* tissues contain substantial amounts of several cyclopropyl analogues of L-glutamate and methanoproline, a cyclopropyl analogue of L-proline, in addition to common amino acids such as L-glutamate, L-glutamine, L-serine, and L-proline. Several L-2-(carboxycyclopropyl)glycines and methanoproline are present in the young stems (Tables 1 and 2; also Caveney et al., 1996), seeds (Table 3), and fruit pulp (not shown) of *Ephedra* and may appear in elevated amounts in the stems of greenhouse-grown plants (Tables 1 and 2). *Ephedra* seeds (Table 3) are a particularly rich source of these compounds, even in species where the compounds are absent from the stems, such as *E. viridis* and *E. altissima.*

The compound (2S,3R,4S)-3,4-methanoproline (*cis*-3,4-methanoproline) occurs widely in both stems and seeds (Tables 1 and 2) as well as the roots (S. Caveney, unpublished data). This proline analogue is toxic to the bacteria *Escherichia coli* and *Salmonella typhimurium* through its feedback inhibition of proline synthesis (Rowland and Tristram, 1971). It is also a high affinity competitive inhibitor of proline uptake in these organisms (Rowland and Tristram, 1971, 1975). Although not incorporated into nascent protein, it also disrupts protein synthesis in bacteria (Fowden et al., 1969). While it is feasible that the purported antisyphilitic efficacy of certain species in the southwestern United States is due to their methanoproline content, this remains to be demonstrated.

The compound (2S,3S,4S)-2-(carboxycyclopropyl)glycine (CCGI), first isolated from the akee (*Blighia sapida,* family Sapindaceae; Fowden et al., 1969), is present in the seeds of several species in the taxon, including the Mediterranean species *Ephedra altissima* (Caveney and Starratt, 1994; Starratt and Caveney, 1996) and the North American species *E. viridis* (Table 3). Considerable amounts (0.5% dry mass) of CCGI are present in the stems of *E. antisyphilitica* (Table 2), and lower levels were found in the stems of some greenhouse material (Tables 1 and 2). The compound CCGI is a potent selective agonist of two subtypes of metabotropic glutamate receptors in the mammalian central nervous system, namely MGluR2 and MGluR3. These subtypes are most closely related to the metabotropic glutamate receptor of *Drosophila* (Conn and Pin, 1997).

The compound (2S,3S,4R)-2-(carboxycyclopropyl)glycine (CCGIII) is common in the seeds and stems of *Ephedra* spp. but has a more limited distribution in the stems (Tables 1 and

TABLE 1. Chemical composition of the stems of Eurasian *Ephedra*. See MATERIALS AND METHODS for analytical details.

| Species | Source | Country[a] | L-CCGI | L-CCGIII | L-CCGIV | Methano-proline | 6-OH KYNA | KYNA | 6-MeO KYNA | Ephedrine | Pseudo-ephedrine | Tannin | Accession no. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. alata* | Sinai | EG | − | − | − | − | + | − | − | ++ | ++++ | +++ | Freitag 19.957 |
| *E. altissima* | Hyeres | FR | − | − | − | − | − | − | − | − | − | − | Caveney 520 |
| *E. aphylla* | Edom | JO | − | − | − | − | +++ | − | − | | | + | Baierle J24 |
| | Sinai | EG | − | − | − | − | ++ | ++++ | − | − | − | ++ | Freitag 19.962 |
| *E. distachya* | Valencia | ES | − | − | − | − | ++ | ++ | − | − | +++ | +++ | Caveney 399N |
| | Schlanders | IT | − | − | − | − | + | − | − | − | ++ | +++ | Freitag 26.678 |
| | greenhouse | (CA) | + | + | − | +++ | +++ | +++ | − | | | | Caveney 399 |
| *E. botschantzevii* | Chimkent | KZ | − | − | − | − | +++ | − | − | ++ | +++ | +++ | Freitag 26.412 |
| *E. foemina* | Delphi | GR | − | − | − | − | +++ | − | − | | | − | Freitag 19.801 |
| | Caesarea | IL | − | ++ | − | − | − | ++ | − | | | − | Caveney 515 |
| | Jerusalem | IL | − | + | − | − | + | + | − | − | − | − | Caveney 517 |
| | Herceg Novi | BA | − | ++ | + | − | +++ | − | − | | | + | Caveney 608 |
| | greenhouse | (CA) | − | ++++ | +++ | ++ | +++ | − | − | − | − | − | Caveney 131 |
| | greenhouse | (GE) | − | ++++ | +++ | ++ | +++ | − | − | | | − | grown from seeds of Freitag 19.801 |
| *E. foliata* | Karachi | PK | − | − | − | − | +++ | − | − | − | − | − | Freitag 18.178 |
| | greenhouse | (CA) | − | − | − | ++ | +++ | − | − | − | − | − | Caveney 271 |
| *E. fragilis* | Alicante | ES | − | ++ | − | − | ++ | + | − | +++ | − | ++++ | Caveney 387A |
| | Edom | JO | − | ++ | − | − | + | ++++ | − | + | − | +++ | Baierle 86-163 |
| *E. gerardiana* | Hindukush | PK | − | − | − | − | + | − | − | ++ | + | + | Miehe 6889 |
| | Karakoram | PK | − | − | − | − | + | − | − | | | + | Miehe 3201 |
| *E. intermedia* | Kalkan | KZ | − | − | − | − | + | − | − | | | ++++ | Freitag 26.187 |
| | Karakoram | PK | − | − | − | − | + | +++ | − | ++ | +++ | ++++ | Miehe 5958 |
| | Fergana | KY | − | − | − | − | +++ | − | − | ++ | ++++ | +++ | Freitag 26.662 |
| *E. major* | | | | | | | | | | | | | |
| ssp. *procera* | Kalat | PK | − | − | − | − | + | + | − | +++ | ++ | +++ | Freitag 18.875 |
| ssp. *major* | Monegros | ES | − | − | − | − | + | +++ | − | | | +++ | Caveney 605 |
| *E. minima* | greenhouse | (US) | + | − | − | + | − | − | − | ++ | ++ | − | T. Lemieux unnumbered[b] |
| *E. monosperma* | greenhouse | (CA) | − | − | − | + | − | − | − | + | − | ++ | Caveney 476 |
| *E. pachyclada* | | | | | | | | | | | | | |
| ssp. *pachyclada* | Kalat | PK | − | − | − | − | + | ++++ | +++ | − | ++++ | +++ | Freitag 18.866 |
| ssp. *sinaica* | Sinai | EG | − | − | − | − | ++ | ++++ | +++ | +++ | +++ | +++ | Freitag 19.959 |
| *E. przewalskii* | Xinjiang | CN | − | − | − | − | − | − | − | − | − | ++++ | Miehe 5403 |
| *E. regeliana* | Karakoram | PK | − | − | − | − | + | − | − | +++ | ++ | ++ | Miehe 5419 |

*Note:* Amounts of nitrogenous compounds are expressed as percentage of dry mass: (+), <0.05%; (++), 0.05–0.19%; (+++), 0.2–0.5%; (++++), >0.5%. Tannin content is scored according to intensity of color reaction.

[a] Countries of origin are indicated by Internet extension codes. Brackets indicate origin of greenhouse material.

[b] *E. minima* was obtained from Tom Lemieux of the University of Colorado, Boulder, Colorado, USA.

TABLE 2. Chemical composition of the stems of North and South American species of *Ephedra*.

| | Collection site | State | L-CCGI | L-CCGIII | L-CCGIV | Methano-proline | 6-OH KYNA | KYNA | 6-MeO KYNA | Ephed-rine | Pseudo-ephedrine | Tannin | Accession no. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **North American species** | | | | | | | | | | | | | |
| *E. antisyphilitica* | Austin | TX | +++ | − | − | ++ | +++ | − | − | − | − | + | Caveney 645-6 |
| *E. californica* | San Bernardino | CA | − | − | − | − | + | − | + | − | d | +++ | Leary 1035 |
| *E. fasciculata* | Newberry Mtns | NV | − | − | − | − | + | − | + | | | +++ | Charlet 2400-1 |
| | Dante's View DV | CA | − | − | − | − | + | ++++ | − | − | − | + | Caveney 550 |
| | Titus Canyon DV | CA | − | − | − | + | + | ++++ | − | − | − | + | Caveney 555 |
| *E. funerea* | Ryan DV | CA | ++ | − | − | ++ | + | ++++ | − | − | − | + | Caveney 551 |
| | Ash Meadows | NV | − | − | | ++ | + | ++++ | − | | | + | Caveney 590-6 |
| | Salsberry Pass DV | CA | + | − | − | ++ | + | +++ | − | | | + | Caveney 599-602 |
| | Dante's View DV | CA | | | | | + | ++++ | − | | | + | Holland 702 |
| | Dante's View DV | CA | − | − | − | + | + | ++++ | − | | | + | Caveney 548-9 |
| *E. nevadensis* | Lake Mead | NV | − | − | − | − | +++ | ++++ | − | − | − | +++ | Charlet 2397-8 |
| | Lovell Canyon | NV | − | ++ | − | − | ++++ | − | − | − | − | +++ | Caveney 546 |
| | Wellington | NV | − | ++ | − | − | +++ | − | − | | | +++ | Caveney 556 |
| | Great Smokey Valley | NV | − | − | − | + | +++ | − | − | | | ++ | Caveney 557 |
| | Daylight Pass DV | CA | − | − | − | − | ++ | − | − | | | ++ | Caveney 553-4 |
| | Mercury | NV | − | − | − | − | ++ | + | − | | | +++ | T. Charlet 1 |
| | greenhouse[a] | NM | ++ | − | − | ++ | +++ | − | − | − | − | + | Nursery stock |
| *E. torreyana* | Little Colorado Rvr | AZ | − | − | − | + | ++ | − | − | | | +++ | Caveney 360 |
| | Ash Meadows | NV | − | − | − | − | ++var | − | − | | | +++ | Charlet 2353-56 |
| | Ash Meadows | NV | + | − | − | − | ++ | − | + | − | − | ++ | Caveney 581-9 |
| *E. trifurca* | Las Cruces | NM | +++ | − | − | − | + | − | ++ | − | − | +++ | Caveney 634 |
| | El Paso | TX | +++ | − | − | − | | | | − | − | +++ | Caveney 635 |
| *E. viridis* | Meteor Crater | AZ | − | − | − | − | ++ | − | − | | | +++ | Caveney 358 |
| | Mountain Springs | NV | − | − | − | − | ++ | + | − | − | − | +++ | Caveney 537-45 |
| | Great Smokey Valley | NV | − | − | − | − | ++ | + | − | | | ++ | Caveney 558 |
| | Carson Range | NV | − | − | − | − | ++++ | + | − | | | +++ | Charlet 2358 |
| | Daylight Pass DV | CA | − | − | − | − | ++++ | − | − | | | +++ | Caveney 552 |
| **South American species** | | | | | | | | | | | | | |
| *E. chilensis* | greenhouse[a] | (CL) | + | − | − | ++++ | ++ | − | − | − | − | − | UColorado 94.082 |
| *E. tweediana* | San Isidro | AG | +++ | − | − | + | +++ | − | − | − | − | − | Caveney 609 |
| | greenhouse[a] | (AG) | + | − | − | ++++ | ++ | − | − | − | − | − | UColorado 94.081 |

*Note:* Amounts are expressed as percentage of dry mass: (+), <0.05%; (++), 0.05–0.19%; (+++), 0.2–0.5%; (++++), >0.5%; (d), detected at <0.01% wet mass. Tannin content is scored according to intensity of color reaction.

[a] Greenhouse material supplied by the University of Colorado, Boulder, Colorado, USA.

TABLE 3. Chemical composition of *Ephedra* seeds.

| Species | Source | Country | L-CCGI | L-CCGIII | L-CCGIV | Methano-proline | 6-OH KYNA | KYNA | 6-Meo KYNA | Accession no. |
|---|---|---|---|---|---|---|---|---|---|---|
| *E. foemina* | Delphi | GR | − | ++++ | +++ | ++++ | ++ | − | − | Freitag 19.801 |
| | Kephallonia | GR | − | ++++ | ++ | ++++ | ++ | − | − | Freitag 19.807 |
| | Jerusalem | IL | − | ++++ | ++ | ++++ | + | − | − | Caveney 517 |
| | Herceg Novi | BA | − | ++++ | +++ | ++++ | + | − | − | Caveney 608 |
| *E. altissima* | Gharyan | LY | +++ | − | − | − | ++ | − | − | Davis 49755 |
| | Taroudanti | MA | − | − | − | − | ++ | − | − | Bramwell 480 |
| | Biougra | MA | ++ | − | − | − | ++ | − | − | BM Exped. 277 |
| | Hyeres | FR | ++ | + | − | − | + | + | − | Caveney 520 |
| *E. foliata* | Karachi | PK | − | − | − | +++ | + | − | − | Freitag 18.178 |
| | Punjab | PK | − | − | − | ++++ | + | − | − | Freitag 21.506 |
| | Koh-i-Elburz | AF | ++ | − | − | + | ++ | − | − | Furse 7776 |
| | Al-Batin | SA | + | − | − | ++++ | ++ | − | − | Mandaville 3104 |
| | Tibesti | CD | + | − | − | − | ++ | − | − | Scholz 250 |
| *E. aphylla* | Burg-el-Arab | EG | − | + | − | + | − | − | − | Freitag 19.697 |
| | Cyrenaica | LY | − | − | − | + | − | − | − | Pampanini 118 |
| | Wadi Hof | EG | − | + | − | + | − | − | − | Freitag 19.604 |
| *E. fragilis* | Malaga | ES | − | ++ | − | − | + | − | − | Caveney 387 |
| | Sidi Faruch | DZ | − | + | − | − | − | − | − | Davis 59547 |
| | Ksebi | MA | − | − | − | − | + | − | − | Lewalle 8282 |
| | Monegros | ES | + | ++++ | ++ | + | + | − | − | Caveney 574 |
| *E. distachya* | Schlanders | IT | − | − | − | − | − | − | − | Freitag 26.678 |
| | Monegros | ES | + | − | − | ++++ | + | − | − | Caveney 572 |
| *E. californica* | San Bernardino | US | ++ | − | − | ++++ | − | − | − | Leary, UNLV 6241 |
| *E. funerea* | Primm | US | +++ | − | − | ++++ | − | − | − | Landau 2 |
| *E. nevadensis* | comm. supplier | US | ++ | − | − | + | − | − | − | Caveney 611 |
| *E. trifurca* | Las Cruces | US | ++ | − | − | − | + | − | − | Caveney 634 |
| *E. viridis* | comm. supplier | US | ++ | − | − | + | + | − | − | Caveney 535 |

*Note:* Amounts are expressed as percent dry mass; (+), <0.05%; (++), 0.05–0.19%; (+++), 0.2–0.5%; (++++), >0.5%. Seed extracts were made from plant specimens kept in the collection of the herbarium at the University of Kassel, Germany, and in other European herbaria, with the exception of the Caveney seed accessions, which are in the first author's possession. The seeds of *E. nevadensis* and *E. viridis* were supplied by Richters (Goodwood, Ontario, Canada L0C 1O1), a commercial seed house.

2). Particularly high levels may be present in the seeds of *E. foemina* and *E. fragilis* (Table 3). CCGIII is reported elsewhere only in the bottlebrush buckeye (*Aesculus parviflora*, family Hippocastanaceae) and a few other North American species of *Aesculus* (Fowden et al., 1969; Fowden, Anderson, and Smith, 1970). The compound CCGIII is a potent blocker of high-affinity $Na^+$-dependent glutamate transport in the mammalian central nervous system (Kawai et al., 1992) and insect tissues (Caveney et al., 1996) and has been used as a male sterilant in wheat (Machackova and Zmrhal, 1983).

The compound (2S,3R,4S)-2-(carboxycyclopropyl)glycine (CCGIV) was first reported in the stems (Starratt and Caveney, 1996; Table 1) and seeds (Table 3) of *Ephedra foemina*. This species remains its sole natural source, with one possible exception, a specimen of the related species *E. fragilis* collected in Spain (Table 3). The compound CCGIV accumulates in larger amounts in the stems of greenhouse-grown *E. foemina* (Table 1) and is a potent and selective agonist acting at the glutamate-binding site of the NMDA receptor in the mammalian central nervous system (Shinozaki et al., 1989; Kawai et al., 1992).

Cyclopropyl amino acids are also present in the swollen and fleshy fruit bracts of *Ephedra* species placed in the section Pseudobaccatae (Price, 1996). The bright-red berries of many Eurasian species have a pleasant taste and are edible (Bamber, 1976; Freitag and Maier-Stolte, 1994). Presumably the seeds are dispersed by fruit-eating birds, which would explain the disjunct distribution of some species along routes of bird migration (Bianco et al., 1988; Freitag and Maier-Stolte, 1989). The fruit pulp is rich in many amino acids (Vishin and Razdan, 1962; S. Caveney, personal observation) and is used to make jams. In Israel, however, birds apparently avoid the red berries of *E. foemina* (M. Avishai, The Hebrew University of Jerusalem, personal communication). The fruit pulp of this species contains high levels of CCGIII and methanoproline (as well as phenylalanine); that of *E. altissima* from Hyeres (France) contains traces of CCGI and CCGIII (S. Caveney, unpublished data). Overall, it would appear that the fruit pulp of most *Ephedra* species is not distasteful or toxic to birds or rodents (R. R. Askew, Tarporley, UK, personal communication).

***Kynurenates***—Kynurenic acid and several derivatives, namely 6-hydroxykynurenic acid, 6-methoxykynurenic acid, and 7-methoxykynurenic acid, are present in the photosynthetic stem tissue of many *Ephedra* species (Nawwar et al., 1985; Starratt and Caveney, 1996; Al-Khalil et al., 1998). Relatively low levels of 6-hydroxykynurenate are found in the seeds (Fig. 3). Kynurenates are absent from the roots (S. Caveney, unpublished data). Xanthurenic acid (8-hydroxykynurenic acid) has not been found in *Ephedra*. Many indolylalkylamine derivatives of the amino acid tryptophan are found in fungi and higher plants and may act as chemical defenses against herbivory (Smith, 1977). In general, indolylalkylamines have strong pharmacological actions on mammalian tissues and consequently have received much attention. Kynurenates are products of tryptophan catabolism. Kynurenic acid is synthesized via the intermediate kynurenine and was first detected as an excretory product in the urine of mammals. A similar pathway is proposed for plants (MacNicol, 1968; Schennen and Hoelzl, 1986) and insects (Mullins, 1985). Outside the

taxon, kynurenic acid is present in the leaves of *Ginkgo biloba* (K. Drieu, Institut Henri Balfour, Paris, personal communication).

The compound 4-hydroxyquinoline-2-carboxylic acid (kynurenic acid) is found in *Ephedra* stems in amounts up to ~1% dry mass in at least two species of the southwest United States, *E. funerea* and *E. fasciculata* (Table 2). In most species, it occurs in trace amounts only. For instance, a recent paper (Al-Khalil et al., 1998) reported the isolation from *E. transitoria* of small amounts (<0.01% dry mass) of ''transtorine,'' a compound for which the structure of kynurenic acid is assigned.

The compound 6-hydroxykynurenic acid is widely distributed in both Eurasian and American species of *Ephedra* (Tables 1 and 2). This compound occurs in trace amounts in many other plants (MacNicol, 1968) but in large quantities only in the senescing leaves of *Ginkgo biloba* (Schennen and Hoelzl, 1986; Matile, 1994). The 6-hydroxykynurenate levels in individual plants sampled in a southern Nevada community of *E. viridis* can be quite variable (S. Caveney, personal observation). Seeds typically have a lower 6-hydroxykynurenate content than the stems of the same species (e.g., *E. nevadensis* and *E. viridis*).

The compound 6-methoxykynurenic acid is present typically in low amounts in some U.S. species of arid-adapted *Ephedra* and in large amounts (1% dry mass) in *E. pachyclada* from Asia (Starratt and Caveney, 1996) (Tables 1 and 2). This compound has not been isolated from any other plant group.

The compound 7-methoxykynurenic acid (ephedralone) was the first kynurenate reported from an *Ephedra* species, *E. alata (*Nawwar et al., 1985). It has recently been isolated from *E. aphylla* (Hussein et al., 1997). We have been unable to confirm the identity of this compound in stem samples of these species.

***Ephedrine alkaloids***—A wide variety of plant phenylalkylamines are derived through the decarboxylation of the amino acids phenylalanine and tyrosine, which donate a $C_6$ ring skeleton and/or a $C_2$ to $C_3$ carbon side-chain containing a nitrogen atom (Wink, 1997). The biosynthesis of the ephedrine alkaloids, more strictly aromatic amines (Wink, 1997), from phenylalanine (Grue-Sorensen and Spenser, 1993) follows this route. The ephedrine alkaloids are a group of plant phenylethylamines (Smith, 1977) capable of mimicking the actions of catecholamine neurotransmitters in the mammalian sympathetic nervous system.

The distribution of ephedrine alkaloids in *Ephedra* species has been extensively researched and reviewed (Hegnauer, 1986; O'Dowd et al., 1998; Zhu, 1998). Many Eurasian species of *Ephedra* living in xeric habitats contain substantial amounts of ephedrine alkaloids in their young aerial parts (Hegnauer, 1962, 1986; O'Dowd et al., 1998). We feel justified in supplementing these data because many species have been misidentified or overlooked in the past. Ephedrine and pseudoephedrine are the dominant and most pharmacologically useful alkaloids isolated from the stems of *Ephedra* species, although the related alkaloids such as norephedrine and norpseudoephedrine (Grue-Sorensen and Spenser, 1989), methylephedrine (Sagara, Oshima, and Misaki, 1983; White et al., 1997) and normethylpseudoephedrine (Qazilbach, 1971) are also present. The ephedrine precursor cathinone (norpseudoephedrine) may be present in the stems of these species (Grue-Sorensen and Spenser, 1993) as well as an oxazolidone derivative, ephedroxane (Konno et al., 1979).

Generally, the distribution of ephedrine alkaloids in the taxon follows recognized natural groupings. The Distachyae group of Eurasian species (Freitag and Maier-Stolte, 1994), which includes the widespread species *Ephedra major* and *E. distachya*, contains the richest natural source of ephedrine and pseudoephedrine. Some species contain considerably more of one alkaloid than the other (Table 1). The *E. major* (syn. *E. distachya*) complex in the group is rich in both ephedrine and pseudoephedrine (Qazilbach, 1971). Total alkaloid content may reach 2.5% dry mass in *E. equisetina* and *E. monosperma* stems (Zhang, Tian, and Lou, 1989) and between 1 and 2% in *E. gerardiana* stems (Grue-Sorensen and Spenser, 1989; Zhang, Tian, and Lou, 1989), *E. major* ssp. *major* and ssp. *procera* (sub *E. nebrodensis* and *E. procera* in Hegnauer, 1986; Qazilbach, 1971), and in *E. botschantzevii*. Pseudoephedrine, with less ephedrine, is present in *E. pachyclada* from Pakistan (Qazilbach, 1971), although considerable amounts of both alkaloids are present in *E. pachyclada* from the Sinai (Table 1). The *E. distachya* complex within the Distachyae group includes *E. regeliana*, *E. intermedia*, and several other Asian species closely related to *E. distachya*, such as *E. sinica*, a traditional source of Ma-huang. *Ephedra distachya* and *E. sinica* are rich in ephedrine (Hegnauer, 1986; Zhang, Tian, and Lou, 1989), whereas European *E. distachya* appears to be richer in pseudoephedrine (O'Dowd et al., 1998; Caveney and Starratt, 1994) (Table 1). The six ephedrine alkaloids listed above have recently been detected in all 12 Chinese species of *Ephedra* examined by Zhang's group (Zhang, Tian, and Lou, 1989; Cui, Niu, and Zhang, 1991). Both ephedrine and pseudoephedrine occur in high levels in *E. monosperma* (Zhang, Tian, and Lou, 1989). *Ephedra lepidosperma* contains low or undetectable amounts of ephedrine alkaloids (Zhang, Tian, and Lou, 1989; Gurley, Wang, and Gardner, 1998).

Of the Eurasian members in the Alatae group (Price, 1996), ephedrine alkaloids, especially pseudoephedrine, are present in *Ephedra alata* (Smith, 1977; Table 1), but reportedly low or absent in *E. przewalski* (Zhang, Tian, and Lou, 1989) and *E. strobilacea* (Hegnauer, 1986). In the group Sarcocarpae, *E. lomatolepis* contains both alkaloids (Zhang, Tian, and Lou, 1989), but *E. transitoria* has little of either (Hegnauer, 1986). Ephedrine alkaloids are absent from most species in the group Fragilis (tribe Scandentes of Stapf [1896]; see Price, 1996) of Eurasian species. Ephedrine and pseudoephedrine were not detected in *E. foemina* (syn *E. campylopoda*) and *E. altissima*, despite claims to the contrary (Kawatani et al., 1959, other references in O'Dowd et al., 1998). *Ephedra foliata* in India and Pakistan has no alkaloids (Table 1; also Chaudhri, 1957; O'Dowd et al., 1998). *Ephedra fragilis* may be the exception in the Fragilis group in that it has a high ephedrine content (Caveney and Starratt, 1994; O'Dowd et al., 1998); it may also be distinguished from other Eurasian members of group by its high tannin content (Table 1).

In sharp contrast, all North American and South American *Ephedra* we have examined lack ephedrine completely. Pseudoephedrine, if present at all in the North American species analyzed, is synthesized at barely detectable levels (<0.01% wet mass) (Table 2). We are unable to state categorically that pseudoephedrine is absent, because our chromatographic identification was hampered by a compound that comigrated with authentic pseudoephedrine (see also Max, 1991). Claims that some North American species contain ephedrine (Duke, 1986; O'Dowd et al., 1998) need to be validated.

***Proanthocyanidins***—Condensed tannins occur in large amounts in the stems of many Eurasian (Khushbaktova et al., 1989; Zhang and Hu, 1997) and American species of *Ephedra* (Gurni and Wagner, 1984), yet are lacking in others (Tables 2 and 3). Ephedra Herbae, purportedly from Iranian *E. distachya* (although this species is rare in Iran), contains a complex mixture of condensed tannins that decreases the effects of uremic toxicity after kidney failure in rats (Yokozawa et al., 1995). The tannin deposits often turn the stem pith a brown color, and this may assist in species identification (e.g., *E. fragilis*; Freitag and Maier-Stolte, 1989, 1993). The stems of greenhouse-grown plants of these species may have a reduced tannin content (Table 1). The stems and the fleshy ''fruits'' (swollen bracts) of field-collected material of European species may lack tannin altogether (Table 1). The ''body'' and the appellation ''tea'' given to the infusions made from the stems of species in the southwest United States is likely a result of their proanthocyanidin content. The hydrolysable tannins (gallotannins and ellagitannins) found in many woody angiosperms appear to be absent from the Gnetales.

## DISCUSSION

The stems and seeds of the ~50 species of world *Ephedra* differ considerably in the amount of stored nitrogenous secondary phytochemicals such as cyclopropyl amino acids, kynurenate derivatives of tryptophan, and ephedrine-alkaloid derivatives of phenylalanine. The ability to synthesize cyclopropyl amino acids appears to be an ancestral (plesiomorphic) feature in the Ephedraceae. Elsewhere in plants cyclopropyl amino acids have very limited distribution. They are reported in two related angiosperm families, the Hippocastanaceae and the Sapindaceae (Fowden et al., 1969; Fowden, Anderson, and Smith, 1970). Cyclopropenoic fatty acids are found in the seeds of the related gnetalean species, *Welwitschia mirabilis* (Aitzenmuller and Vosmann, 1998). The neurotoxicity of cyclopropyl amino acids suggests that they may function as a defense against vertebrate herbivores as well as insect stem and seed predators. However, the pathways involved in their synthesis may have evolved originally as a response to microbial and fungal attack.

Kynurenic acid, first detected as an excretory product in the urine of mammals, is synthesized via the intermediate kynurenine. A similar pathway is proposed for plants (MacNicol, 1968; Schennen and Hoelzl, 1986) and insects (Mullins, 1985). Kynurenates are products of tryptophan catabolism with no known pharmacological activity. Other indolylalkylamine derivatives of tryptophan found in fungi and higher plants, however, act as strong chemical defenses against herbivory (Smith, 1977; Wink and Schimmer, 1999). These indolylalkylamines have strong pharmacological actions on mammalian tissues and consequently have received much attention. Kynurenic acid is also present in the leaves of *Ginkgo biloba* (K. Drieu, Institut Henri Balfour, Paris, personal communication), a popular herbal treatment for memory loss. Plant kynurenates remain compounds in search of a physiological function.

The same can be said of the ephedrine alkaloids, which are restricted to the stems of Old World *Ephedra.* Ephedroxane, an anti-inflammatory drug related in structure to ephedrine, is found only in certain Eurasian species of *Ephedra* (Konno et al., 1979). Although the roots of Chinese *Ephedra* are low in ephedrine-alkaloid content, they contain maokonine and several ephedradines, macrocyclic spermidines reported to have a hypotensive effect (so-called Ma-huang Gen or Radix Ephedrae; reviewed in Zhu, 1998). The synthesis and/or accumulation of ephedrine-related compounds appears to be a derived (apomorphic) feature in the taxon. Although this peculiar geographical distribution of the ephedrine alkaloids and their derivatives has not been adequately explained, it suggests that the taxon speciated for a time after the continents separated. Ephedrine alkaloids, however, are synthesized by plants in many families outside the Ephedraceae (Smith, 1977; Chaudhuri and Thakur, 1991), the most notable being *Catha edulis* (fam. Celastraceae) (Kalix, 1991; Max, 1991). The leaves of *C. edulis* form the basis of qat (or khat), a popular stimulant chewed by the Yemenis on the Arabian Peninsula and the Somalis on the horn of Africa. It contains both norephedrine and norpseudoephedrine (Grue-Sorensen and Spenser, 1989; Kalix, 1991).

The similarity in the tissue distribution of ephedrine alkaloids and kynurenates in *Ephedra* suggests that these compounds have the same natural role in the stems. Certain kynurenates function as optical brighteners, that is, they absorb UV-B light and emit light in the blue portion of the visible spectrum. The compound 6-hydroxykynurenic acid, for example, strongly fluoresces in leaves in situ (Matile, Flach, and Eller, 1992). Consequently these compounds could protect plants that store them in their stem tissue from DNA-damaging UV radiation. The well-documented build-up in ephedrine content in the stems of Eurasian species towards the end of the growing season suggests that they may have a similar protective function to the plant. *Ephedra* does not shed its photosynthetic (i.e., stem) tissue during periods of dormancy. Ephedrine is known to be toxic to weevils (Janzen, Juster, and Bell, 1997). From the human perspective, of course, the ephedrine alkaloids are commercially important sympathomimetic drugs. Ephedrine has agonistic activity on $\alpha_1$, $\beta_1$, and $\beta_2$ adrenoreceptors (White et al., 1997), increasing blood pressure through vasoconstriction, raising the heart rate, and stimulating the central nervous system (Zhu, 1998). Pseudoephedrine causes bronchodilation through its $\beta_2$ agonist activity and relieves the symptoms of nasal congestion through its $\alpha$-agonist effects (White et al., 1997). A detailed pharmacological account of the activity of ephedrine alkaloids can be found elsewhere (Kalix, 1991; Max, 1991).

The kynurenates in *Ephedra* stems may have antimicrobial activity. Kynurenates are related to the 4′-quinolones (quinoline-3-carboxylic acids), a group of compounds that are potent inhibitors of bacterial DNA gyrase, an enzyme involved in the incorporation of nascent DNA strands into the bacterial chromosome (Andriole, 1988). Ciprofloxacin, for example, is a substituted fluoropiperazine-quinolone approved for use as a broad spectrum antibiotic in the treatment of many diseases caused by gram-negative bacteria, including *N. gonorrhoeae* and urinary tract infections, for which it is an extremely effective clinical treatment (Andriole, 1988; Bosker, 1997). Kynurenic acid has reported activity against several Gram-positive and Gram-negative bacteria (Al-Khalil et al., 1998), although considerably less than ciprofloxacin. A related antibiotic compound, 5,8-dihydroxykynurenic acid, has been isolated from a sponge (Molinski and Faulkner, 1988). Kynurenic acid is an endogenous antagonist at the glycine binding site of the NMDA-type glutamate receptor in the mammalian central nervous system. Substituted kynurenates are potent inhibitors of glutamatergic neurotransmission (Leeson et al., 1992). Kynu-

renate analogues such as 6-hydroxykynurenate have been shown to protect against ischemia-induced glutamate neurotoxicity (Leeson et al., 1992; Ghribi et al., 1994) and reverse the neurotoxic effects of lathyrism (Meldrum and Garthwaite, 1990).

The chemical diversity of *Ephedra* may explain why the taxon has been exploited in so many different ways by native peoples across, and in some instances beyond, its natural distribution range in the Old and New Worlds. In recent years, ephedrine's stimulatory action on the central nervous system and heart has raised concerns about its inclusion in herbal mixtures sold as nutritional supplements (Gurley, Wang, and Gardner, 1998). The recreational use of methamphetamine (''speed''), an illegally manufactured derivative of ephedrine sold as a street drug, has been widely publicized in the popular press and condemned in the scientific literature (Andrews, 1995; Gurney, Wang, and Gardner, 1998). What is clear, however, is that the complex phytochemistry of *Ephedra* needs further analysis before its myriad ethnopharmacological uses can be fully comprehended.

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