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FOUR CYCLOPROPANE AMINO ACIDS FROM *EPHEDRA*

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Key Word Index—*Ephedra*; Ephedraceae; cyclopropane amino acids; (carboxycyclopropyl)glycines; 3,4-methanoproline.

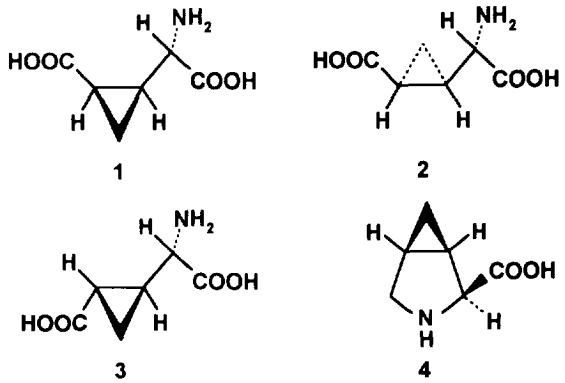
Abstract—Three diastereomers of 2-(carboxycyclopropyl)glycine and *cis*-3,4-methanoproline have been isolated from *Ephedra* species. This is the first report of the natural occurrence of (2*S*,3*R*,4*S*)-2-(carboxycyclopropyl)glycine.

INTRODUCTION

Many species of *Ephedra* are characterized by the presence of (−)-ephedrine and related alkaloids [1]. Recently, we briefly reported [2] that fresh stems of *E. foeminea* and the related species *E. altissima*, which lack the ephedrine alkaloids, contain two non-protein amino acids which were concentrated in insect epidermis by a Na^+-dependent glutamate transporter [3]. The present paper describes the isolation and characterization of these compounds, namely (2*S*,3*S*,4*R*)- and (2*S*,3*R*,4*S*)-2-(carboxycyclopropyl)glycine (**1** and **2**), as well as another diastereomer, (2*S*,3*S*,4*S*)-2-(carboxycyclopropyl)glycine (**3**), from seeds of *E. altissima*, and *cis*-3,4-methanoproline (**4**) from seeds of *E. foeminea* and *E. foliata*.

RESULTS AND DISCUSSION

Four non-protein amino acids, all featuring a cyclopropane ring, have been isolated from *Ephedra* species. Their identity was confirmed by comparison of HPLC retention times of their phenylthiocarbamyl, *N*-2,4-dinitrophenyl (DNP) and DNP methyl ester derivatives with corresponding derivatives of natural or synthetic compounds. The general stucture of the first compound isolated, (2*S*,3*S*,4*R*)-2-(carboxycyclopropyl)glycine (**1**, CCGIII), was indicated by mass and ^{1}H NMR spectra of its DNP dimethyl ester derivative. Previously, this amino acid had been reported in seeds of a few North American species of *Aesculus* (the buckeyes) [4, 5]. It and the other *cis*-diastereomer, (2*S*,3*R*,4*S*)-2-(carboxycyclopropyl)glycine (**2**, CCGIV), are major amino acid components of stem extracts of *E. foeminea* and *E. altissima*. The NMR spectra of the DNP dimethyl ester derivatives were identical to those of the corresponding derivatives of authentic **1** and **2**. This is the first report of **2** in plants. Compounds **1** and **2** differ in stereochemistry at two sites,



so it is unlikely that the mixture is an artefact of the isolation procedure. A *trans*-diastereomer, (2*S*,3*S*,4*S*)-2-(carboxycyclopropyl)glycine (**3**, CCGI), reported previously in seeds of *Blighia sapida*, *B. unijugata* and *Aesculus* species [4–6], was characterized chromatographically in seed extracts of *E. altissima*. Since diastereomers **1**, **2** and **3** can be distinguished readily by HPLC of various derivatives and by ^{1}H NMR of their DNP dimethyl ester derivatives, it is expected that the fourth isomer, CCGII, which was not available for comparison, should also exhibit unique properties leading to its elimination as a possible structure for the new compound. We believe that **1**, **2** and **3** are L-2-(carboxycyclopropyl)-glycines since they are accumulated by insect epidermis identically to the authentic L-isomers previously isolated from plants (**1** and **3**) or synthesized (**2**). *Cis*-3,4-Methanoproline (**4**), present in seeds of *A. parviflora* [4], was identified as a major component of seeds of *E. foliata* and *E. foeminea*. Based on dry tissue weight, levels of these four cyclopropane amino acids were in the range 0.2–1.0%.

The pharmacology of the CCG isomers has been extensively investigated [7] following the synthesis of the four L-CCG diastereomers [8]. In these conforma-

tionally restricted L-glutamate analogues, the cyclopropyl group fixes the glutamate chain in either an extended or folded form [8]. Although two of the 2-(carboxycyclopropyl)glycines have been isolated earlier from plants, this report of the identification of **1**, **2** and **3** in tissues of *E. altissima* is the first to describe the occurrence of more than two diastereomers in a single species. Previously, the co-existence of **1** and **3** in seeds of *Aesculus* species was reported [5]. Based on preliminary biological studies showing that **1** and **2** were concentrated by a glutamate transporter in beetle epidermis and that **1** and **3** caused a cockroach hindgut preparation to contract, it has been proposed that cyclopropane amino acids may affect insect feeding [2].

EXPERIMENTAL

General. ^{1}H NMR, 200 MHz. TLC: silica gel. HPLC: μBondapak C_{18} analytical column (300 × 3.9 mm) and other columns described in text; photodiode array detector used to monitor separations. Plant amino acids were analysed by the Pico-Tag method as reported previously [9] and cyclopropane amino acids were identified by comparing the retention times of their derivatives with those of phenylthiocarbamyl derivatives of 2-(carboxycyclopropyl)glycines from Tocris-Neuramin and Sir Leslie Fowden, or methanoproline isolated from seeds of *A. parviflora.*

Plant material. Fresh stems of *E. foeminea* and *E. altissima* were collected from plants grown in the greenhouse. Seeds of *A. parviflora* were obtained from a shrub on the campus of the University of Western Ontario. Seeds of *E. altissima, E. foeminea* and *E. foliata* were supplied by Prof. H. Freitag, University of Kassel. Voucher specimens are deposited in the Plant Sciences Herbarium, University of Western Ontario, or the Plant Systematics Herbarium, University of Kassel.

Extraction and isolation. (a) Finely chopped stems (5 g) of *E. foeminea* and *E. altissima* were exhaustively extracted with EtOH and EtOH–0.1 M HCl (1:1) at room temp. The water soluble portion of the extract was applied to a cation exchange column (Rexyn 101, H^+ form) and the amino acids eluted with 1 M NH_4OH. Two major components, subsequently shown to be cyclopropane amino acids **1** and **2** were separated from less polar components by HPLC over a μBondapak C_{18} column (150 × 19 mm) using a linear gradient of 0–40% CH_3CN in 10 mM TFA at 6 ml min^{-1} over 30 min. The early eluting amino acids were further separated on a Partisil 10 Magnum 9 SCX column (250 × 9.4 mm) with 2.5 mM ammonium formate, pH 3.1, at 3 ml min^{-1}. Amino acid analyses indicated that peaks eluting at 6.6 and 9.7 min were rich in **1** and **2**. Treatment with fluorodinitrobenzene yielded the DNP derivatives, which were purified by HPLC. Chromatography on the μBondapak C_{18} analytical column using 25% CH_3CN in 10 mM TEA at 1 ml min^{-1} yielded peaks with the retention times (7.8 and 15.0 min, respectively) and UV spectra (λ_{max} 349 nm) identical to that found for derivatives of synthetic **1** and **2**. Amino acid analyses indicated that extracts also contained **4**.

(b) Seeds (40 mg) of *E. altissima, E. foliata and E. foeminea* were extracted with EtOH–0.1 M HCl (1:1) and the extracts in 10 mM TFA were filtered through Sep-Pak C_{18} cartridges. Treatment of the filtrates with fluorodinitrobenzene gave DNP derivatives, which were purified as above by HPLC. The retention time (12.2 min) and UV spectrum (λ_{max} 349 nm) of the derivative of the major component in the extract of *E. altissima* was identical to that of the derivative of **3** and the retention time (20.6 min) and spectrum (λ_{max} 370 nm) of the main peak in the sample from *E. foliata* corresponded to the derivative of *cis*-3,4-methanoproline (**4**) isolated similarly from seeds of *A. parviflora.* Three major peaks in the sample from *E. foeminea* seeds were identified (HPLC) as DNP derivatives of cyclopropane amino acids **1**, **2** and **4**.

N-(2,4-*Dinitrophenyl*) *dimethyl ester derivatives of cyclopropane amino acids.* The DNP derivatives of the cyclopropane amino acids in MeOH were methylated with CH_2N_2/Et_2O and the products purified by TLC ($CHCl_3$–MeOH, 49:1) or HPLC (μBondapak C_{18}, 35% CH_3CN in 10 mM TFA). The identities of the derivatives of **1** and **2** from the stems were confirmed by comparison (NMR, TLC, HPLC, UV) with authentic samples. HPLC properties and UV spectra of the DNP dimethyl ester derivatives of the cyclopropane amino acids from the seeds were also identical to those of standards.

DNP dimethyl ester of **1**. Yellow needles, m.p. 128–130° (uncorr.); HR-EIMS 70 eV, *m/z* [M]$^+$ 353.0855 (calcd for $C_{14}H_{15}N_3O_8$, 353.0859); ^{1}H NMR ($CDCl_3$: δ1.26–2.07 (4H, *m*, H-cyclopropyl), 3.57, 3.84 (3H each, *s*, –OMe), 4.62 (1H, *dd*, *J* = 8.0, 10.0 Hz, N-CH), 6.82 (1H, *d*, *J* = 9.5 Hz, H-6′), 8.26 (1H, *dd*, *J* = 2.7, 9.5 Hz, H-5′), 8.77 (1H, *br d*, *J* = 8.0 Hz, NH), 9.15 (1H, *d*, *J* = 2.7 Hz, H-3′).

DNP dimethyl ester of **2**. ^{1}H NMR ($CDCl_3$): δ1.26–2.09 (4H, *m*, H-cyclopropyl), 3.80, 3.82 (3H each, *s*, –OMe), 4.70 (1H, *dd*, *J* = 7.9, 10.0 Hz, N-CH), 6.93 (1H, *d*, *J* = 9.5 Hz, H-6′), 8.28 (1H, *dd*, *J* = 2.7, 9.5 Hz, H-5′), 9.03 (1H, *br d*, *J* = 7.9 Hz, NH), 9.18 (1H, *d*, *J* = 2.7 Hz, H-3′).

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