Phytochemistry, 1977, Vol. 16, pp. 599–600. Pergamon Press Printed in England

SHORT REPORTS

DISTRIBUTION OF SACCHAROPINE AND 2-AMINOADIPIC ACID IN HIGHER PLANTS

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(*Received* 26 *November* 1976)

Key Word Index—Angiospermae; non-protein amino acids; saccharopine; 2(*S*),2′(*S*)-N^6-(2′-glutaryl)lysine; 2-aminoadipic acid; 2(*S*)-2-aminoadipic acid; lysine catabolism.

Abstract—Saccharopine and 2-aminoadipic acid have been identified in eleven plant species belonging to nine families. The amino acids have been isolated from green parts of the plants using ion-exchange chromatography and preparative high voltage electrophoresis, and in three cases the identification was supported by mass spectroscopy. Mild conditions were used during the isolation to avoid lactamization, and the contents of saccharopine and 2-aminoadipic acid have been determined semiquantitatively. The significance of the occurrence of the two amino acids with regard to lysine metabolism is briefly discussed.

INTRODUCTION

Previous studies have demonstrated 2(*S*)-2-aminoadipic acid (**1**) and L-saccharopine (2(*S*),2′(*S*)-N^6-(2′-glutaryl)-lysine) (**2**) as constituents of *Reseda odorata* L [1]. **2** has also been isolated from *Fagopyrum esculentum* Moench [2], and tracer experiments have shown **1** and **2** to be present in *Hordeum vulgare* L. var. Emir [3]. These results indicate that **2** possibly is a product of lysine catabolism in higher plants. In other plants and organisms, lysine is decarboxylated to cadaverine, incorporated into alkaloids or catabolized through Δ^1-piperidein-2-carboxylate, pipecolate, and Δ^1-piperidein-6-carboxylate [4–7 and refs. therein]. We have now investigated ten additional plant species from eight different families and found **1** and **2** in all of them. For comparison *R. odorata* L. is included. These results suggest that **1** and **2** occur ubiquitously in higher plants.

RESULTS AND DISCUSSION

Isolation of **1** and **2** was performed by the previously described methods [1] under mild conditions so that cyclization to the lactams was avoided. The identities of **1** and **2** were established by their elution behaviour from the ion-exchange resins, PC, and ionic mobilities by high voltage electrophoresis (HVE) at pH 3.6 and pH 6.5 [1]. Further evidence of the identity of **2** was obtained by lactamization to pyrosaccaropine (5(*S*),5′(*S*)-*N*-(5′-amino-5′-carboxypentyl)-2-pyrrolidone-5-carboxylic acid) (**3**) and subsequent identification of **3** by HVE and PC [1]. In most of the plant species investigated, an unknown compound (purple colour with ninhydrin) appeared together with **1** and **2** in the fractions from the ion-exchange columns. This unknown compound was separated from **1** and **2** by PC and HVE. R_f values in PC were 0.08 (System 1) and 0.75 (System 2) [1], and ionic mobilities in HVE were 12.0 cm (pH 6.5) and −2 cm (pH 3.6) [1]. In three cases (including *R. odorata* L.) final confirmation of the identity of **1** and **2** was obtained by mass spectroscopy (MS). Both compounds produced lactams and fragmented as expected [2]. After trimethylsilylation of **2**, a derivative of **3** with three trimethylsilyl groups was obtained, and this compound yielded a fragmentation pattern identical with that produced from authentic material (see Experimental) [8].

The species investigated and the semiquantitative determinations of **1** and **2** are presented in Table 1. The plant species contained generally 12–18 of the protein amino acids and 4-aminobutyric acid in amounts of 200–1.800 μg/10 g fr. wt plant material. Pipecolic acid was not found in any of the plant species investigated. **1** and **2** were most often present in low concentrations so that they were only detectable by PC and HVE after ion-exchange separation from the other free amino acids. The results now obtained show that **1** and **2** are widely distributed in different families of higher plants, although in relatively low concentrations, and, therefore, the catabolism of lysine in higher plants may be expected generally to proceed through **2** and **1**.

The route via Δ^1-piperidein-2-carboxylate can be distinguished from that via saccharopine since, in the

Table 1. Amounts of 2-aminoadipic acid (**1**) and saccharopine (**2**) (μg/25 g fr. weight) in green parts of different plant species

Plant species	1	2
Asparagus officinalis L. (Convallariaceae)	60*	100*
Stellaria media (L.) Vill. (Caryophyllaceae)	40	60
Rèseda odorata L. (Resedaceae)	100*	300*
Cydonia japonica Pers. (Rosaceae)	30	50
Anthriscus cerefolium (L.) Hoffm. (Umbelliferae)	60	80
Levisticum officinale Koch. (Umbelliferae)	100*	200*
Phlox paniculata L. (Polemoniaceae)	50	100
Solanum nigrum L. (Solanaceae)	80	120
Mentha crispa L. (Labiatae)	40	40
Salvia officinalis L. (Labiatae)	40	40
Lactuca sativa L. (Compositae)	80	100

* The structure was confirmed by MS; see Experimental.

former case the α-amino group and the α-hydrogen are lost, whereas in the latter saccharopine dehydrogenase and α-aminoadipic semialdehyde dehydrogenase are involved and the ε-amino group is lost. Plant enzymes concerned with the catabolism of lysine have not yet been demonstrated. Saccharopine dehydrogenase has been isolated from yeast [9] and the results now presented indicate that it might be present in higher plants.

EXPERIMENTAL

Plant material. The species listed in Table 1 were grown in the Botanical Garden of the Royal Veterinary and Agricultural University, Copenhagen. Green parts of the plants, grown outdoors, were harvested in the autumn and kept at −20° until isolation was performed. The plant material (25 g) was homogenized in H_2O (200 ml) at 0–4°C and centrifuged (1.5 hr, 2°, 12000 *g*). The supernatant was taken to dryness by lyophilization, and isolation and semiquantitative determination of **1** and **2** were performed as previously described [1], except that the column of Dowex 1 (× 8, 200–400 mesh, AcO^-, 0.9 × 50 cm) was eluted with H_2O (fractions 1–30) and then with 0.2 N HOAc. **1** and **2** appeared in fractions 35–45, **2** just before **1**, glutamic acid occurred in fractions 46–50. Transformation of **2** into **3** was performed with 70% MeOH at 80°.

MS were obtained at 70 eV on an AEI MS 3074 mass spectrometer. **1** gave the following pattern: *m/e* 144, 143, 116, 99, 98 (base peak), 70, 55. The trimethylsilyl derivative of **3** produced from **2** gave the following fragmentation pattern: M^+474, *m/e* 459, 431, 387, 369, 357 (base peak), 341, 314, 285, 274, 267, 240, 218.

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Phytochemistry, 1977, Vol 16, pp 600–601 Pergamon Press Printed in England

MASS FRAGMENTOGRAPHY OF CORIOSE IN *CORIARIA* SPECIES

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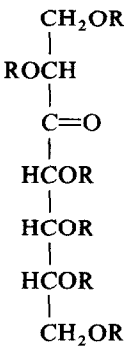
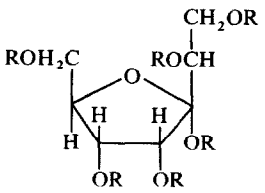
(*Received* 1 *October* 1976)

Key Word Index—*Coriaria japonica*; *C. nepalensis*; *C. intermedia*; *C. ruscifolia*; *C. thymifolia*; Coriariaceae; coriose; sedo-heptulose; *manno*-heptulose; trimethylsilyl ether; mass fragmentography.

The structure of coriose (**1**) isolated from *Coriaria japonica* has been reported [1]. Trimethylsilylation of α-coriofuranose (**1a**) gives a mixture of TMS derivatives of α-furanose (**1b**) and of the open-chain form, **1c** [2, 3].



1 R=H
1c R=TMS



1a R=H
1b R=TMS

The following plant samples were examined in the present study: (i) *C. japonica* A. Gray, collected on Mt. Hira, Shiga-ken, Japan; (ii) *C. nepalensis* Wall., collected in Bhutan between Tinlegang and Gon Chungang; (iii) *C. intermedia* Matsum., collected in Taiwan; (iv) *C. ruscifolia* L., collected in Chile, on the Pacific coast near El Mirador, Prov. Valdivia; (v) *C. thymifolia* H.B.K., collected in Peru, near the pass between Olmos and Pagua, Prov. Lam Bayaque.

A leaf (samples i–iv) or several leaves (sample v) were sufficient for the analysis. Dried leaf (samples ii–v), or both fresh and dried leaf (sample i) were extracted with boiling water, the solution was evaporated *in vacuo*, and the residue was extracted with boiling MeOH. The MeOH solution was evaporated, and the residue was trimethylsilylated with a mixture of Me_3SiCl, $Me_3SiNHSiMe_3$, and C_6H_5N (1:2:10). The mass fragmentography (MF) [4] was carried out using a 2% OV-17 GLC column and monitoring the *m/e* 437 and *m/e* 408 ions which are strongly exhibited in the mass spectra of **1b** and **1c**, respectively. MF was performed initially with *C. japonica* in which the presence of **1** had been confirmed. The gas chromatogram (detector, FID) and the MF chart in Fig. 1 represent the pattern of trimethylsilylated **1** in *Coriaria* species. Coriose was detectable when several picograms were included in an injected sample solution. All of the *Coriaria* species examined showed the presence of **1** in an analogous way as with *C. japonica*, by the MF peaks of **1b** and **1c**, which should be present in the final product of trimethylsilylation of **1** [2, 3].