Phytochemistry, 1976, Vol. 15, pp. 1527–1529. Pergamon Press. Printed in England.

SACCHAROPINE AND 2-AMINOADIPIC ACID IN *RESEDA ODORATA*

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(*Revised received* 17 *December* 1975)

Key Word Index—*Reseda odorata*; Resedaceae; non-protein amino acids; L-saccharopine; 2(S),2′(S)-N^6-(2′-glutaryl)-lysine; L-pyrosaccharopine; 5(S),5′(S)-*N*-(5′-amino-5′-carboxypentyl)-2-pyrrolidone-5-carboxylic acid; L-2-amino-adipic acid; 2(S)-2-aminoadipic acid; lysine catabolism.

Abstract—2(S),2′(S)-N^6-(2′-Glutaryl)lysine (L-saccharopine) and 2(S)-2-aminoadipic acid have been isolated from *Reseda odorata*. When traditional isolation procedures are used L-pyrosaccharopine (5(S),5′(S)-*N*-(5′-amino-5′-carboxypentyl)-2-pyrrolidone-5-carboxylic acid) is formed from L-saccharopine by lactamisation. The degree of lactamisation during various isolation steps has been studied. The amino acids were identified by IR and PMR spectroscopy and the configurations established by enzymic and polarimetric analyses. The contents of saccharopine and 2-amino-adipic acid have been determined relative to the total nitrogen content at various stages in the growth cycle of *R. odorata*.

INTRODUCTION

Investigations on the contents of free amino acids in higher plants have demonstrated the presence of a large variety of compounds some of which are intermediates in primary biochemical sequences. Previous reports from our laboratory have dealt with the free amino acids and amines in *Reseda odorata* L. (mignonette) [1, 2]. These studies have been continued both to obtain information in the metabolism of some of the new compounds identified and to identify numerous unknown constituents occurring in small amounts.

The present paper describes the isolation and identification of 2(S)-2-aminoadipic acid (**1**), L-saccharopine (2(S),2′(S)-N^6-(2′-glutaryl)lysine) (**2**) and L-pyrosaccharopine (5(S),5′(S)-*N*-(5′-amino-5′-carboxypentyl)2-pyrrolidone-5-carboxylic acid) (**3**). **1** seems to be widely distributed in nature [3, 4] and it, together with **2**, is an intermediate of lysine metabolism in animals [5, 6] and fungi [7, 8 and refs. cited therein]. The biosynthesis of lysine in higher plants, as well as bacteria, has recently been shown to proceed via the 2,6-diaminopimelic acid pathway [9].

2, originally isolated from *Saccharomyces cerevisiae* where it occurs in substantial quantity [10, 11], has recently been reported as a constituent of seeds of *Fagopyrum esculentum* (buckwheat) [12]. The amounts of **2** and **3** isolated from this source (1 and 18 mg respectively from 40 kg commercial seeds) are so low that their origin from fungi contaminating the seeds cannot be excluded [9]. This assertion and the results from lysine metabolism provided an interest for further investigations of the distribution of **2** in higher plants. Since **2** can be transformed into **3** by reflux of an aqueous solution [13], the possibility has been considered that **3** is an artifact produced during the isolation. Our experimental results support this hypothesis.

RESULTS AND DISCUSSION

Compounds **1**, **2** and **3** were isolated from the inflorescence of *R. odorata* using traditional methods including ion-exchange chromatography and preparative high voltage electrophoresis (HVE) (see Experimental). From 3.5 kg of flowers (fr. wt) were obtained 16 mg of **1**, 10 mg of **2** and 18 mg of **3**, substantial losses, however, occurring in the final crystallisation steps. The identities of the isolates were established by IR and PMR spectroscopy, R_f-values from PC, and electrophoretic mobility in HVE. By comparison with authentic compounds the configurations at C-2 in **1** and **2** were established by use of an L-amino acid oxidase and the configuration at C-2 and C-2′ in **2** by determination of optical rotation.

During the isolation by traditional methods **1** may be partly transformed into 2-piperidone-6-carboxylic acid [14] and **2** partly into **3** [13]. The extent of transformation of **2** into **3** was determined under conditions similar to those used in the isolation. The results obtained are shown in Table 1 and clearly indicate that the isolation of **3** can be explained as resulting from lactamisation during the isolation. This most likely therefore also applies to the reported isolation of **3** from *F. esculentum* [12].

To avoid lactamisation, isolation was also performed without the use of reflux at any step and with all concentrations performed by lyophilization. This method was used for a semiquantitative estimate of the concentrations of **1** and **2** in relation to the total nitrogen content of the plants at various stages during growth. The results obtained are shown in Table 2 and demonstrate that both **1** and **2** are present at all stages of plant growth. The concentrations are of the same order of magnitude as those of other free amino acids [15]. No appreciable amount of **3** was found in these experiments, again corroborating its artifact origin.

Table 1. Transformation of saccharopine into pyrosaccharopine

Reaction time	% Pyrosaccharopine in the reaction mixture (determined after separation from 2 by HVE)		
	70% MeOH at 80°	N pyridine at 60°	N HOAc at 60°
30 min	35	5	15
60 min	50	8	25
120 min	70	8	35

1 is widely distributed in nature [3, 4], and its presence in *R. odorata* is therefore not surprising. However, the present study clearly establishes **2** as a constituent of higher plants. Therefore, even if **2** isolated from seeds of buckwheat might originate from contaminating fungi, its presence in additional plant species, as for example buckwheat, is likely.

The present results together with other studies from our laboratory [16] indicate that **2** and **1** are widely occurring catabolic products of lysine in higher plants. Lactam formation may be the reason for the lack of previous identification of **2** in plant material although **3** also reacts with ninhydrin. Since the metabolism of L- and D-lysine shows differences in plants ([17] and references cited therein), the establishment of the L-configuration for both **1** and **2** is also of importance.

EXPERIMENTAL

Plant material. Reseda odorata "grandiflora" purchased from J. E. Ohlsens Enke, Copenhagen.

General methods and instrumentation. IR spectra were determined in KBr pellets. Optical rotations were determined on a photoelectric polarimeter in 1 dm tubes. PMR spectra were determined on a 60 MHz instrument. PC was performed in *n*-BuOH–HOAc–H_2O (12:3:5) (solvent 1), PhOH–H_2O–conc.NH_3 (120:30:1) (w/v/v) (solvent 2) by the descending technique on Whatman No. 1 paper. HVE was carried out on Whatman 3 MM paper using a flat-plate unit in the following systems (1) Buffer pH 3.6 (Py–HOAc–H_2O) (1:10:200), 1 hr at 3 kV, and 90 mA; (2) Buffer pH 6.5 (Py–HOAc–H_2O) (25:1:500), 50 min at 5 kV, and 90 mA. PC R_f-values and electrophoretic mobilities for **1** and **2** are listed in Table 3.

Isolation of 2-aminoadipic acid (**1**), *saccharopine* (**2**) *and pyrosaccharopine* (**3**). Freshly harvested flowers from plants 4 months old (3.5 kg) were homogenized in CCl_4 and defatted twice with CCl_4 by refluxing (2.5 hr), cooling, and filtration. After air drying, the residue (1.3 kg) was extracted 2× with MeOH–H_2O (7:3, 3 l. each time) by refluxing (3 hr), cooling, and filtration. The combined filtrates were concentrated to leave a dark syrup (300 g). The residue was suspended in H_2O (1.5 l.), filtered and applied to a column of Amberlite IR 120 (H^+, 5 × 90 cm). After washing with H_2O (15 l.) the amino acids were eluted with 2 N NH_3 (5 l.). The eluate was evaporated to dryness (60 g), dissolved in H_2O and applied to a column of Dowex 1 (×4, 20–50 mesh, AcO^-, 2.5 × 90 cm). 15 ml fractions were collected at 150 ml/hr. After washing with H_2O (3 l.), the column was eluted with N HOAc producing 3 fractions of acidic amino acids, (1), 6.17 g, (fractions 26–85), (2), 4.44 g, (fractions 86–110) and (3), 1.28 g, (fractions 161–205). These fractions were further separated on columns of Dowex 1 (×8, 200–400 mesh, AcO^-, 2.5 × 90 cm). 20 ml fractions were collected at 80 ml/hr. After washing with H_2O (fractions 1–30), the columns were eluted with 0.5 N HOAc. (1) afforded **2** and **1** in (1.2), 1.83 g, (fractions 64–78), glutamic acid in (1.3), 0.7 g, (fractions 79–94), aspartic acid and **3** in (1.6), 64 mg, (fractions 141–163). (2) afforded only traces of **2** and **1** in fractions before glutamic acid, but substantial amounts of **3** were found together with aspartic acid in (2.6), 0.9 g, (fractions 130–165). (3) afforded only traces of **2** and **3**. (1.2) dissolved in H_2O was passed through a carbon column (Darco G 60, deactivated with stearic acid 1 × 3 cm) and subsequently applied to Dowex 1 resin as above. After washing with H_2O (fractions 1–30), the column was eluted with 0.2 N HOAc. **2** appeared in fractions 62–74 (1.2.5.), 210 mg, **1** appeared in fractions 75–85 (1.2.6.), 110 mg, glutamic acid appeared in fractions 101–115 (1.2.8), 18 mg.

Preparative HVE in buffer at pH 6.5 of (1.2.5.) followed by chromatography on Dowex 50 w (×8, 200–400 mesh, H^+, 0.7 × 10 cm) afforded chromatographically pure **2** (39 mg) from the Py eluate. Recrystallization from H_2O yielded a colourless sample (10 mg). $[\alpha]_D^{23}$ +34.4° (*c* 0.27, 0.5 N HCl). Lit. value for L-saccharopine $[\alpha]_D^{23}$ +33.6° (*c* 1, 0.5 N HCl) [10]. IR and PMR spectra were identical with those obtained from an authentic sample and as reported in the lit. [11, 13].

(1.2.6.) was purified by preparative HVE in buffer at pH 6.5 followed by ion-exchange chromatography as described above. Recrystallization from H_2O yielded **1** (16 mg). IR and PMR spectra were identical with those obtained from an authentic sample.

3 (2.6) was separated from aspartic acid by preparative PC in solvent 2 affording 96 mg of essentially chromatographically homogenous material. Final purifications were performed by preparative HVE in buffer pH 6.5 and chromatography on Dowex 1 (×8, 200–400 mesh, AcO^-, 0.9 × 50 cm) 6 ml fractions were collected at 40 ml/hr. After washing with H_2O (fraction 1–30), the column was eluted with 0.2 N HOAc. **2** appeared in fraction 39–40 (2 mg) and **3** in fractions 72–80.

Table 2. Amount of saccharopine and 2-aminoadipic acid in *Reseda odorata* at various stages (mmol amino acid/mol of total nitrogen)

	Part of plant			
	Seeds	25 days old seedlings	Inflorescence from 90 days old plants	Inflorescence from 120 days old plants
(**2**) L-Saccharopine	0.2	0.1	0.2	0.3
(**1**) 2-Aminoadipic acid	0.2	0.05	0.05	0.1

Table 3. R_f-values and ionic mobilities of acidic amino acids

Amino acids	R_f-values in solvent		Distance in cm obtained by HVE in buffer system	
	System 1*	System 2	1, pH 3.6*	2, pH 6.5
Aspartic acid	0.22	0.16	4.8	22.5
Glutamic acid	0.28	0.26	1.3	20.0
(1) 2-Aminoadipic acid	0.31	0.30	0.4	17.0
(2) Saccharopine	0.14	0.60	1.1	13.0
(3) Pyrosaccharopine	0.32	0.64	7.5	14.0

* For solvent and buffer systems see Experimental.

Chromatographically pure **3** (39 mg) was recrystallized from H_2O to give 18 mg of colourless crystals. IR and PMR spectra were identical with those obtained from an authentic sample of L-pyrosaccharopine, and as reported in the lit. [12, 13].

Isolation and semiquantitative determination of **1** *and* **2** *under mild conditions.* All fractions were taken to dryness by lyophilization. The residues were dissolved in H_2O before application to ion-exchange resins or preparative HVE. Plant material (25 g) was homogenized in H_2O (100 ml) at 0–4° and centrifuged. After lyophilization of the supernatant the residue was dissolved in H_2O and applied to a column of Amberlite IR 120 (H^+, 1.6 × 35 cm). 18 ml fractions were collected at 80 ml/hr. After washing with H_2O (fractions 1–20), the column was eluted with N Py. Acid and neutral amino acids usually appeared in the fractions 25–45 (1). (1) was applied to a column of Dowex 1 (×8, 200–400 mesh, AcO^-, 0.9 × 50 cm). 6 ml fractions were collected at 40 ml/hr. The column was eluted with H_2O (fractions 1–30) and then with 0.5 N HOAc. **1** and **2** appeared in fractions 31–37 (1.2), glutamic acid in fractions 38–45 (1.3), and aspartic acid in fractions 55–65 (1.6). Final purification was accomplished by preparative HVE in buffer at pH 6.5 followed by chromatography on Dowex 50 W (×8, 200–400 mesh, H^+, 0.7 × 10 cm). After washing with H_2O the amino acids were eluted from the column with N Py. The purity of the samples were controlled by R_f-values and electrophoretic mobilities (see Table 3). The amount of the amino acids in the samples was established by quantitative ninhydrin determination [18, 19]. Total N content of the plant material was determined by the micro-Kjeldahl method.

Transformation of **2** *into* **3**. Lactam formation of **2** was investigated under the following conditions (a) with 70% MeOH at 80°; (b) N Py at 60°; (c) N HOAc at 60°. **2** (1.3 mg) was applied to the preheated solvent (0.5 ml) and samples were removed at intervals and separated by HVE in buffer pH 3.6. The amounts of **2** and **3** were found by quantitative nihydrin determination (see Table 1).

L-*amino acid oxidase* (EC. 1.4.3.2) from *Crotalus terr. terr.* venom was used to establish the configuration at the α-center in isolated **1** and **2**. These investigations are part of unpublished results. The oxidation rates of commercial L-2-aminoadipic acid and L-saccharopine isolated from *Saccharomyces* species [10] were identical with those obtained for the amino acids of *R. odorata*.

Acknowledgements—The author wishes to thank Professor P. O. Larsen for valuable discussions during the present work. Thanks are due to T. Hjortkaer, Department of Plant Culture, Royal Veterinary and Agricultural University, Copenhagen for assistance in growing plants.

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