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ACIDIC AMINO ACIDS IN *RESEDA LUTEOLA*

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Key Word Index—*Reseda luteola*; Resedaceae; non-protein amino acids; 2(*S*)-4-hydroxy-4-methylglutamic acid; 2(*S*)-4-hydroxy-2-aminopimelic acid; lactones of 4-hydroxy-2-aminopimelic acid; 3-(3-carboxyphenyl)alanine; (3-carboxyphenyl)glycine; 3-(3-carboxy-4-hydroxyphenyl)alanine; (3-carboxy-4-hydroxyphenyl)glycine; γ-glutamylglutamic acid.

Abstract—3-(3-Carboxyphenyl)alanine, (3-carboxyphenyl)glycine, 3-(3-carboxy-4-hydroxyphenyl)alanine and (3-carboxy-4-hydroxyphenyl)glycine occur in all parts of *Reseda luteola*. The concentrations of the two diastereoisomers of 2(*S*)-4-hydroxy-4-methylglutamic acid undergo seasonal variation, the highest concentrations occurring in the first part of the summer. Highest concentrations are found in the inflorescences. The two diastereoisomers of 2(*S*)-4-hydroxy-2-aminopimelic acid occur in appreciable amounts in all parts of the plant. They are easily transformed into two structurally different lactones, one of which is very unstable. The structures of these amino acids have been confirmed by synthesis. Green parts of *R. luteola* also contain substantial quantities of γ-glutamylglutamic acid and glutathione.

INTRODUCTION

Seeds of *Reseda luteola* have been shown previously to contain appreciable amounts of 3-(3-carboxyphenyl)-alanine (**1**), (3-carboxyphenyl)glycine (**2**), 3-(3-carboxy-4-hydroxyphenyl)alanine (**3**) and (3-carboxy-4-hydroxyphenyl)glycine (**4**) [1]. These acidic amino acids have also been found in other Resedaceae species [2, 3]. Furthermore, it has been shown that inflorescenses of *R. odorata* L. contain substantial amounts of another acidic amino acid [2] which was originally thought to be 2(*S*),4(*R*)-4-(β-D-galactopyranosyloxy)-4-isobutylglutamic acid [4], but this compound was subsequently shown to be the stereoisomer 2(*S*),4(*S*)-4-(β-D-galactopyranosyloxy)-4-isobutylglutamic acid (**5**) [5]. The amino acid **5** is presumably biosynthetically related to the group of amino acids known as 4-substituted acidic amino acids [4, 6]. The present work is a continuation of these previous studies and is also related to studies of 4-substituted acidic amino acids in certain species of the Filicinae [6] and Leguminosae [7].

Investigations of fresh parts of *R. luteola* revealed the presence of the two diastereoisomeric forms of 2(*S*)-4-hydroxy-4-methylglutamic acid which were separated into the compound with lowest pKa_2 value (**6**) and the compound with highest pKa_2 value (**7**) [6]. Furthermore, *R. luteola* contains the two diastereoisomeric forms of 2(*S*)-4-hydroxy-2-aminopimelic acid which were separated into the compound with lowest pKa_2 value (**8**) and the compound with highest pKa_2 value (**9**) [6]. Both **8** and **9** are novel in higher plants and their synthesis is described for the first time.

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RESULTS AND DISCUSSION

Compounds **1**–**9** were isolated and identified by methods previously described (see Experimental). Only results concerning acidic amino acids in *R. luteola* are described here, although most of the protein amino acids together with 4-aminobutyric acid were also identified by PC and high voltage electrophoresis (HVE). Furthermore, the glucosinolates which are present in high concentration in *R. luteola*, especially 2-hydroxy-2-phenylethylglucosinolate, have been isolated from the water effluent from the strongly acidic ion-exchange resin using the previously described method [8]. The acidic amino acids **1**–**4** have previously been isolated from seeds of *R. luteola* and some other Resedaceae species [1, 2] and are included here for comparison. Saccharopine and 2-aminoadipic acid are present in *R. luteola* in concentrations similar to those found in other higher plants [9]. γ-Glutamyl peptides occur in fresh parts of *R. luteola* in very low concentrations, except for glutathione and γ-glutamylglutamic acid. These two peptides have been isolated and identified by methods previously described [10]. ^{13}C chemical shifts have also been determined for the latter; ^{1}H NMR chemical shifts and coupling constants have been determined for **1**–**4** (see Experimental). A more detailed description is given of the acidic amino acids **6** and **7** which are new to the Resedaceae, and **8** and **9** which are new to higher plants. Both **8** and **9** are easily transformed into 2-amino-4-hydroxypimelic acid-7,4-lactone [2-amino-3-(2′-oxotetrahydrofuran-5′-yl)-propionic acid] (**10**) and the less stable 2′-amino-4-hydroxypimelic acid-1,4-lactone [3-(2′-oxo-3′-aminotetrahydrofuran-5′-yl)propionic acid] (**11**). The L- or 2(*S*)-configuration for these compounds has been determined by use of L-amino acid oxidase. 2(*S*)-4-Hydroxy-2-aminopimelic acid has previously been found in some fern species ([6] and refs. cited therein), but differences in properties of the two diastereoisomers **8** and **9**, and their lactones (**10**

Table 1. R_f values and ionic mobilities of acidic amino acids isolated from *Reseda luteola*

Amino acids	R_f values in solvent*			Distance in cm obtained by HVE*		
	System 1	System 2	System 3	(1) pH 1.9	(2) pH 3.6	(3) pH 6.5
Aspartic acid	0.22	0.16	0.12	22.6	9.6	22.5
Glutamic acid	0.28	0.26	0.15	25.0	2.6	20.0
(1) 3-(3-Carboxyphenyl)alanine	0.44	0.28	0.16	19.6	3.8	15.8
(2) (3-Carboxyphenyl)glycine	0.41	0.23	0.13	13.8	6.5	16.1
(3) 3-(3-Carboxy-4-hydroxyphenyl)alanine	0.35	0.18	0.08	14.8	15.3	14.4
(4) (3-Carboxy-4-hydroxyphenyl)glycine	0.28	0.12	0.05	9.8	16.8	15.2
(6) 2(*S*),4(*R*)-4-Hydroxy-4-methylglutamic acid†	0.24	0.22	0.19	19.3	16.8	19.5
(7) 2(*S*),4(*S*)-4-Hydroxy-4-methylglutamic acid†	0.23	0.22	0.18	19.8	15.4	18.2
(8) 2(*S*)-4-Hydroxy-2-aminopimelic acid‡	0.30	0.27	0.14	22.6	0.6	17.0
(9) 2(*S*)-4-Hydroxy-2-aminopimelic acid‡	0.30	0.27	0.14	25.9	0.3	17.0
(10) 2-Amino-4-hydroxypimelic acid-7,4-lactone	0.28	0.75	0.18	25.3	−2	−2
(11) 2-Amino-4-hydroxypimelic acid-1,4-lactone	0.30	0.82	0.16	§	§	−2
γ-Glutamylglutamic acid	0.29	0.11	0.10		10.4	26.0

* For solvent and buffer systems, see Experimental.
† Discussion of the stereochemistry at C-4, see text.
‡ Configuration at C-4 is unknown.
§ HVE mobilities at this pH for 1 hr are similar to that of 4-aminobutyric acid.

and **11**), have not been described previously. The availability of synthetic reference compounds has now made this possible.

Table 1 shows the results obtained by PC and HVE of the amino acids isolated from *R. luteola*, some of which have been reported elsewhere [1, 11, 12]. Using 2D-PC in solvent systems 1–2 or 1–3, it is possible to separate most of the structurally different acidic amino acids isolated from fresh parts of the plant including γ-glutamyl peptides [10], 2-aminoadipic acid and saccharopine [13], but these PC systems do not separate **8** and **9** from glutamic acid or the diastereoisomers **6**–**7** and **8**–**9** from each other. However, the easy transformation of **8** and **9** into **10** and **11** (*vide infra*) makes it possible to obtain indirect evidence for the presence of **8** and/or **9** by 2D-PC. The mobilities obtained by HVE at pH 1.9 are mainly determined by the pKa_1 values of the amino acids as are the results obtained by use of the amino acid analyser, but, due to adsorption [2], the aromatic amino acids are eluted later from the amino acid analyser [14] than expected from consideration of their pKa values [15]. By use of HVE, all of the compounds listed in Table 1 could be separated using one or more of the three buffer systems. At pH 3.6, the net charge of the compounds is mainly determined by their pKa_2 values and it is possible to separate the diastereoisomers at this pH as well as at pH 1.9. At pH 6.5, all of the compounds listed in Table 1 have a net charge of −1, except for **10**, **11** and γ-glutamylglutamic acid, therefore, their mobilities at this pH are mainly determined by the size of the molecule [11].

The structures of the natural products **6**–**9** were supported by evidence obtained from ^1H NMR spectra. The spectrum obtained for **6** was identical with that previously described for 2(*S*),4(*R*)-4-hydroxy-4-methylglutamic acid [16] and quite different from that obtained for **7**. The spectrum of **7** was identical with that previously described for 2(*S*),4(*S*)-4-hydroxy-4-methylglutamic acid [16], but more recent work [5] has raised doubt concerning the absolute configurations assigned to **6** and **7**. The structures of **8** and **9** were confirmed by synthesis using standard methods, as described in Experimental. R_f values, mobilities by HVE, as well as ^1H and ^{13}C NMR spectra of the racemic mixtures of synthetic **8** and **9**, were identical with those of the natural products. The difference in chemical shifts obtained for the protons at C-2 in **8** and **9** has been confirmed by recording spectra of a mixture of **8** and **9**; this difference is appreciable in the 270 MHz spectra but not in the 60 MHz spectra. The ^{13}C NMR spectra of **8** and **9** show appreciable differences between the diastereoisomers in the chemical shifts of the C-2, C-3 and C-4. However, the ^{13}C chemical shift of these atoms showed a marked pH dependence with sizeable downfield shifts caused by deprotonation of the carboxylic acid and ammonium group. Deprotonation of the ammonium group in **9** caused shifts from 51.8, 37.0 and 63.5 to 55.3, 41.9, and 70.8 ppm of the C-2, C-3 and C-4, respectively. The real differences in ^{13}C chemical shifts of these atoms in **8** and **9** have been confirmed by recording the spectra of mixtures of these compounds. These shift values are thus useful in identification of **8** and **9**.

The absolute configuration of **8** and **9** is unknown and obviously the assignment of configuration to C-4 in the two diastereoisomers based on NMR data is uncertain [5]. In M HCl at room temperature, **8** and **9** are readily transformed into the structurally different lactones **10** and **11**, each of which represents two diastereoisomers with unknown configuration at C-4 and L- or (*S*)-configuration at C-2. After 2 hr in M HCl, only a few percent of **8** or **9** are unchanged in the solution as observed from HVE. In alkaline solution the free amino acids **8** and **9** are stable at room temperature for some days. The diastereoisomers of **10** and **11** were indistinguishable in all the PC and HVE systems employed; however, **10** was easily separated from **11** by HVE at pH 3.6. The HVE mobilities (Table 1) show that **10** is the 7,4-lactone and **11** is the 1,4-lactone. These lactones are artifacts produced during isolation of **8** and **9**.

Table 2 shows the content of acidic amino acids in fresh parts of *R. luteola*. In the green parts of the plant, the concentrations of **7** and **9** far exceed those of **1**–**4**. A trace amount of **5** has been detected in extracts from the inflorescences. The content of amino acids in seeds of this plant has been reported previously, but without information about **5**–**9** [1]. The amino acids **6** and **7** are present

Table 2. Acidic amino acids in *Reseda luteola*

Compounds	Plants harvested in October				Inflorescence harvested in		
	Leaves	Stems	Roots	Inflorescence	August	July	Seeds
Aspartic acid	+++	+++	+++	++	++	+++	†
Glutamic acid	++	++++	++++	+++	+++	++++	†
(1) 3-(3-Carboxylphenyl)alanine	+	(+)	(+)	+	(+)	(+)	†
(2) (3-Carboxyphenyl)glycine	(−)	(+)	(+)	(+)	(+)	(+)	†
(3) 3-(3-Carboxy-4-hydroxyphenyl)alanine	+	+	+	+	+	++	†
(4) (3-Carboxy-4-hydroxyphenyl)glycine	(+)	(+)	(+)	(+)	(+)	+	†
(6) 2(*S*),4(*R*)-4-Hydroxy-4-methylglutamic acid*	(+)	(+)	(+)	+	++	++	
(7) 2(*S*),4(*S*)-4-Hydroxy-4-methylglutamic acid*	+	+	+	++	++++	++++	
(8) 2(*S*)-4-Hydroxy-2-aminopimelic acid*	+	+	+	++	++	++	
(9) 2(*S*)-4-Hydroxy-2-aminopimelic acid*	+++	+++	++	+++	++++	++++	

The table shows relative amounts of the amino acids as observed from the intensity of the ninhydrin spots after PC and HVE or from the peaks by use of the amino acid analyser; regarding the amount isolated from inflorescences harvested in July, see Experimental. (+) = Not detectable before concentration by ion-exchange chromatography; + = weak; ++ = medium; +++ = strong; ++++ = very strong.

* For discussion of the stereochemistry, see text.

† For investigation of amino acids in seeds, see ref. [1].

in higher concentrations in the inflorescences than in other parts of the plant and at the highest concentrations when the plants are harvested in the summer [6]. Correspondingly, no marked seasonal changes were observed for **1**–**4**, **8** and **9**. The concentrations of **7** and **9** exceed those of **6** and **8**; the opposite is found in some other plants [6]. A common biogenetic origin has been proposed for the numerous 3- and/or 4-substituted glutamic acid derivatives occurring in plants [7, 17]. The co-occurrence of **6** and **7**, which are new in the Resedaceae, together with **5**, which is the major acidic amino acid in *R. odorata* [4] and with **8** and **9**, which are new to higher plants but not to ferns [6], suggests that **8** and **9** belong biogenetically to the group of 3- and/or 4-substituted acidic amino acids. Possible precursors of the 2-keto acids corresponding to **8** and **9** are a 3-carbon unit and succinic acid semialdehyde which in an aldolase-type reaction may yield these acids.

EXPERIMENTAL

Plant material. Inflorescences (2.85 kg) of *Reseda luteola* L. were collected in July from plants growing in their natural habitat at Faxe, Denmark. Different parts of *R. luteola* were collected in the Botanical Garden of the University of Copenhagen at different times of the plants' growth cycle. The plant material was freeze-dried and stored at −20° until extractions were carried out.

General methods. ^{1}H NMR spectra were determined in M DO^-Na^+, D_2O soln at 60 and 270 MHz. The chemical shifts are in ppm downfield from Na 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate used as internal standard; coupling constants are in Hz. The ^{13}C NMR spectra were recorded in the same soln as the ^{1}H NMR spectra at 67.889 MHz on a Bruker 270 instrument using the pulse technique with Fourier transformation. The chemical shifts are in ppm downfield from TMS; dioxane was used as internal standard [δ (TMS) = δ (dioxane) + 67.4 ppm]. PC was performed in *n*-BuOH–HOAc–H_2O (12:3:5) (solvent 1), PhOH–H_2O–12 M NH_3 (120:30:1) (w/v/v) (solvent 2) and *i*-PrOH–H_2O–12 M NH_3 (8:1:1) (solvent 3) by the descending technique on Whatman No. 1 paper; HVE was carried out on Whatman 3 MM paper using a flat plate unit at 4° in the following systems: (1) buffer pH 1.9 (HOAc–HCOOH–H_2O) (4:1:45), 2 hr at 3.2 kV and 90 mA; (2) buffer pH 3.6 (Py–HOAc–H_2O) (1:10:200), 2 hr at 3 kV and 90 mA; (3) buffer pH 6.5 (Py–HOAc–H_2O) (25:1:500), 50 min at 5 kV and 90 mA. Prep. PC and prep. HVE were carried out on Whatman 3 MM paper. Amino acid analysis was performed on a Beckman Model 120 C instrument, and the elution of the amino acids is shown as R_i(Glu) = elution time of the amino acid/elution time of glutamic acid [14].

Isolation of acidic amino acids. Inflorescences of *R. luteola* (2.85 kg) were freeze-dried, homogenized in boiling MeOH (70%, 15 l.), boiled for 3 min, cooled and filtered. The residue was washed twice with MeOH (70%, 10 l.) and the combined filtrates were concd to *ca* 200 ml, filtered and transferred to an Amberlite IR 120 (H^+, 10 × 70 cm) column. After flushing with H_2O (6 l.), neutral and acidic amino acids were eluted with M Py (fractions of 1 l.). From the H_2O effluent, 26.2 g of glucosinolates were isolated by the ion-exchange methods described elsewhere [8]. Fractions 9–15 containing the amino acids were pooled and concd to a semisolid residue (13 g) which was dissolved in H_2O (200 ml) and applied to a column of Dowex 1 (×8, 200–400 mesh, AcO^-, 2.5 × 90 cm). Fractions (21 ml) were collected at 150 ml/hr. After flushing with H_2O (fractions 1–90), the column was eluted with 0.5 M HOAc (fractions 91–200), 2 M HOAc (fractions 201–320) and M HCOOH (fractions 321–600). The various fractions were subjected to 2D-PC. Fractions 8–70 (2.9 g) contained neutral amino acids, the lactones of 4-hydroxy-4-methylglutamic acid and of 4-hydroxy-2-aminopimelic acid (**10** and **11**); fractions 126–133 (1.16 g) contained **8** and **9**; fractions 134–155 (20.5 g) contained glutamic acid; fractions 156–185 (0.71 g) contained γ-glutamyl peptides of neutral amino acids; fractions 186–200 (0.43 g) contained aspartic acid; fractions 201–229 (1.39 g) contained **6** and **7**; fractions 230–242 (0.89 g) containing **1**; fractions 243–260 (0.29 g) contained **2**; fractions 261–320 (0.20 g) contained γ-glutamylglutamic acid; fractions 321–475 (1.23 g) contained glutathione and some unknown acidic amino acids; fractions 476–525 (0.55 g) contained **3** and fractions 526–600 (0.43 g) contained **4**.

Further purification of the residue from fractions 526–600 on a column of Dowex 1 (×8, 200–400 mesh, $HCOO^-$, 0.9 × 50 cm) resulted in crystalline **4** (40 mg) as previously described [1]. The compound was pure according to PC and HVE, and with R_f values and HVE mobilities as shown in Table 1. UV

data were as given in ref. [1]. The ^{1}H NMR spectrum in M NaOD/D_2O exhibited signals from the benzylic proton at 4.26 ppm (1H, *s*) and from the aromatic protons at 6.67 (1H, *d*, J = 8 Hz), 7.16 (1H, *dd*) and 7.35 (1H, *d*, J = 2 Hz). By use of the same purification procedure as applied to **4**, the residue from fractions 476–525 yielded crystalline **3** (160 mg), pure according to PC and HVE, and with R_f values and HVE mobilities as shown in Table 1. UV data were as given in ref. [15]. The ^{1}H NMR spectrum in M NaOD/D_2O exhibited signals from the benzylic protons at 2.75 ppm (2H, *m*), from the C-1 proton at 3.40 (1H, *dd*) and from the aromatic protons at 6.57 (1H, *d*, J = 8 Hz), at 7.02 (1H, *dd*) and at 7.14 (1H, *d*, J = 2 Hz). Purification of the residue from fractions 243–260 on a Dowex 1 (×8, 200–400 mesh, AcO^-, 0.9 × 50 cm), followed by prep. PC and chromatography on Dowex 50 w (×8, 200–400 mesh, H^+, 0.7 × 10 cm) resulted in crystalline **2** (33 mg), pure according to PC and HVE, and with R_f values and HVE mobilities as shown in Table 1. UV data were as given in ref. [15]. The ^{1}H NMR spectrum in M NaOD/D_2O exhibited signals from the benzylic proton at 4.42 ppm (1H, *s*) and from the aromatic protons at 7.47 (2H, *m*) and 7.77 (2H, *m*). The same procedure applied to the residue from fractions 230–242 resulted in crystalline **1** (130 mg), pure according to PC and HVE, and with R_f values and HVE mobilities as shown in Table 1. The ^{1}H NMR spectrum in M NaOD/D_2O exhibited signals from the benzylic protons at 2.98 ppm (2H, *m*), from the C-1 proton at 3.35 (1H, *dd*), and from the aromatic protons at 7.42 (2H, *m*) and 7.74 (2H, *m*).

γ-Glutamylglutamic acid was isolated from fractions 261–320 (66 mg) and glutathione was isolated from fractions 321–475 (8 mg) by the method used for **1** and **2**. Hydrolysis, PC behaviour and the ^{1}H NMR spectra of these two peptides were as previously described [18]. The ^{13}C NMR spectrum of γ-glutamylglutamic acid in D_2O exhibited signals at 26.4, 26.5, 30.9, 31.8, 52.9, 53.9, 172.6, 175.2, 175.8 and 177.7 ppm. Using off resonance decoupling, the signals at 26.4, 26.5, 30.9 and 31.8 appeared as triplets and the signals at 52.9 and 53.9 appeared as doublets. For PC and HVE behaviour of γ-glutamylglutamic acid, see Table 1.

The amino acids **6** and **7** were obtained in purified form from fractions 201–229 by use of ion-exchange chromatography as described for **1** and **2**, resulting in crystalline **6** and **7** (830 mg), pure according to PC and HVE. Separation of **6** and **7** and the use of L-amino acid oxidase to determine the 2(*S*)-configuration have been described elsewhere [6]. The PC and HVE properties of **6** and **7** are shown in Table 1, the NMR data are described in the Results. R_t(Glu) 0.35 for **6** and 0.43 for **7** were obtained by use of the amino acid analyser.

Compounds **8** and **9** were purified from fractions 126–133 by the methods previously described for the purification of 2-aminoadipic acid and saccharopine [13], resulting in **8** and **9** (640 mg) contaminated with <5% of the lactones **10** and **11**, as revealed from PC and HVE. Separation of **8** from **9** and the use of L-amino acid oxidase to determine the 2(*S*)-configuration have been described elsewhere [6]. For PC and HVE behaviours of **8**–**11**, see Table 1. R_t(Glu) 1.28 for **8** and 1.32 for **9** were obtained by use of the amino acid analyser. The ^{1}H NMR spectrum of **8** in M NaOD/D_2O exhibited signals from the C-5 and C-6 protons at 1.6–2.0 ppm (4H, *m*), from the C-3 protons at 2.2–2.4 (2H, *m*), from the C-2 proton at 3.6 (1H, *dd*) and from the C-4 proton at 3.8 (1H, *m*). In the corresponding spectrum of **9**, the signals from the C-5 and C-6 protons appeared at 1.6–2.0 ppm (4H, *m*), from the C-3 protons at 2.2–2.4, from the C-2 proton at 3.5 (1H, *dd*) and from the C-4 proton at 3.8 (1H, *m*). The ^{13}C NMR spectrum of **8** in M NaOD/D_2O exhibited signals at 34.1 (C-6), 34.5 (C-5), 41.9 (C-3), 54.2 (C-2), 69.6 (C-4), 183.0 (C-1) and 183.9 (C-7) ppm: the corresponding ^{13}C chemical shifts for **9** are 34.1 (C-6), 34.5 (C-5), 41.9 (C-3), 55.3 (C-2), 70.8 (C-4), 183.0 (C-1) and 183.9 (C-7).

Investigation of the content of acidic amino acids in different parts of *R. luteola* (25 g portions) was performed by the method described previously [6, 13] and the results are presented in Table 2.

Synthesis of 4-pentenonitrile. 4-Pentenonitrile has been synthesized by adaption of the method described for synthesis of 5-hexenonitrile [19]. 4-Bromo-1-butene (0.311 mol), KCN (0.384 mol) and 125 ml ethylene glycol were stirred at 100° for 2 hr. The light brown soln was diluted with 125 ml H_2O and the nitrile was extracted with Et_2O (3 × 250 ml). The residue from the washed and dried Et_2O soln was distilled at atmos. pres.: yield 23 g (0.284 mol, 91%); bp 144–145° (lit. bp 144–147°) [20]. The ^{1}H NMR spectrum exhibited signals at 2.2–2.7 ppm (4H, *m*), 5.05–5.45 (2H, *m*) and 5.55–6.25 (1H, *m*) [20]. The product exhibited conspicuous IR bands (neat liquid) at ν_{max} cm^{-1}: 3110 (s), 3030 (m), 3010 (s), 2960 (s), 2880 (m), 2280 (s), 1660 (s), 1460 (s), 1442 (s), 1432 (s), 1015 (s), 935 (s).

Synthesis of 4-acetoxy-5-chloropentanonitrile. A solution of monochlorourea (100 ml, 0.284 mol) prepared as previously described [21], 13.9 ml HOAc and 23 g (0.284 mol) 4-pentenonitrile was left at room temp. for 18 hr. The reaction mixture was neutralized with $NaHCO_3$ and extracted with Et_2O (4 × 250 ml). The Et_2O extracts were combined, dried, evapd and acetylated as previously described [22], leaving a light brown liquid (27.5 g) which by GLC was separated into 3 peaks. Prep. GLC of 11.6 g at 200° on a metal column (0.8 i.d. × 560 cm, packed with 20% SE30; 40 ml He/min: injection port 240°, detector 240°) resulted in fraction (a) = 2.01 g, fraction (b) = 2.00 g and fraction (c) = 4.10 g (0.028 mol, 24% yield) corresponding to 4-acetoxy-5-chloropentanonitrile. The ^{1}H NMR spectrum exhibited signals at 2.1–2.8 ppm (4H, *m*), 2.12 (3H, *s*), 3.6–3.9 (2H, *m*) and 4.9–5.3 (1H, *m*).

Synthesis of 4-hydroxy-2-aminopimelic acid. The amino acids have been synthesized by adaption of the method described previously [23]; 6.52 g (0.03 mol) diethyl acetaminomalonate in 100 ml EtOH containing 0.8 g (0.034 mol) Na and 4.10 g (0.028 mol) 4-acetoxy-5-chloropentanonitrile. The amino acids were isolated by ion-exchange chromatography on Amberlite IR 120 (H^+, 2.5 × 90 cm) succeeded by Dowex 1 (×8, 200–400 mesh, AcO^-, 2.5 × 90 cm) columns as described above for **8** and **9**, resulting in 331 mg (0.0017 mol, 6%) 4-hydroxy-2-aminopimelic acids which were separated into the enantiomers corresponding to **8** and **9** by prep. HVE as described above. The synthetic compounds were identical with the natural products by co-chromatography, co-electrophoresis (Table 1), ^{1}H and ^{13}C NMR spectra, as well as retention times from the automatic amino acid analyser.

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