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Synthesis and Properties of Hydroxylated and Alkylated Acidic Amino Acids, Especially Glutamic Acids

ERIK PETER KRISTENSEN, LONE MELCHIOR LARSEN, OLE OLSEN and HILMER SØRENSEN

Chemistry Department, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Copenhagen, Denmark

4-Methylglutamic acids, 4-hydroxyglutamic acids, 4-hydroxy-4-methylglutamic acids, 4-hydroxy-2-aminoadipic acids, 5-hydroxy-2-aminoadipic acids, 4-hydroxy-2-aminopimelic acids, 2-amino-4-oxo-6-methylheptanoic acids, and 4-hydroxy-4-isobutylglutamic acids have been synthesized. The synthesized amino acids were separated into racemates of the diastereoisomers by ion-exchange chromatography and preparative high voltage electrophoresis. The separations were in accordance with the pK_{a1}- and pK_{a2}-values of the amino acids as estimated from titration curves.

Acidic amino acids with different substituents at carbon atoms Nos. 3 and 4 have been found in a number of different plants, animals, and microorganisms.[1-5] Some of these acidic amino acids co-occur in the same plants and the presence of several diastereoisomers has also been established.[4,5] Information concerning the stereochemistry of these compounds is not always available, and for some of them previous proposals of configuration are severely questioned or have to be changed.[6]

Investigations on biosynthesis and distribution of substituted acidic amino acids in plants require information on their synthesis, separation, and identification, and this is the scope of the present paper. Table 1 summarizes the structures of the compounds under discussion.

The α-L-isomer of these compounds are naturally occurring except *2a*, *14a*, *14b*, and *15ab*. No reports on the synthesis of *5a*, *5b*, *14a* and *14b* are available in the literature.

Reexamination of previously investigated plants using the described methods has revealed the presence of hitherto unidentified diastereoisomers of 3- and/or 4-substituted acidic amino acids. Therefore, the use of these methods in appropriate combinations are considered to be of great importance in biosynthetic and chemotaxonomic studies.[4,5]

RESULTS AND DISCUSSION

Most of the amino acids have been prepared by the malonic ester method.[7,8] Synthesis of the racemates *4a* and *4b* has previously been performed using another procedure.[9] The configurations assigned to these racemates[10,11] are, however, not unambiguous.[6] It is therefore of interest to compare the properties of these compounds with the corresponding properties of closely related 4-substituted acidic amino acids. Synthesis of the stereoisomeric mixture of *15ab* was performed using a previously described method.[12] For all the compounds, the last steps in the purification procedure were performed by gentle methods[13] to avoid transformation of the compounds into lactones[4,5,14] and/or lactams.[11]

The synthetic mixtures of diastereoisomers were separated into the racemates on Dowex 1 anion-exchange resins by previously described methods.[8,9,11] The racemates *2a*, *3a*, *4a*, *5a*, *14a*, and *17a* were eluted from this column before the corresponding stereoisomeric racemates named *b* in agreement with the pK_{a2}-values, Table 3 (*vide infra*). Ion-exchange chromatographic methods are thus very effective in amino acid separations: neutral and basic amino acids can be separated by cation-exchange chromatography,[15] partly according to pK_{a1}-values, whereas anion-exchange chromatography will separate acidic amino acids, partly

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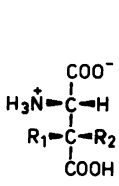
Table 1. Structures of compounds discussed in this paper. Compounds designated a number followed by a letter are synthetic, racemic mixtures except *1a* and *1b* which are the L-isomers from natural products and *15ab* which possibly is a diastereoisomeric mixture of two racemates. The letters *a* and *b* indicate the diastereoisomers with the highest and lowest pK_{a2}-values, respectively. Most of the compounds occur naturally, frequently several together in the same plant.



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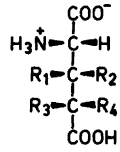
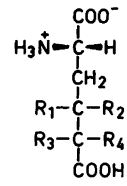


2–11



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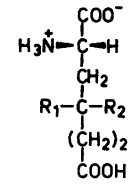
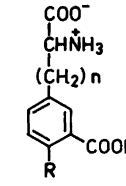


16–17



18–21

R_1	R_2	R_3	R_4	
H	H	–	–	(2*S*)-Aspartic acid (Asp)
HO	H	–	–	(2*S*,3*R*)-3-Hydroxyaspartic acid *1a*
H	HO	–	–	(2*S*,3*S*)-3-Hydroxyaspartic acid *1b*
H	H	H	H	(2*S*)-Glutamic acid (Glu)
H	H	H	CH_3	(2*S*,4*S*)-4-Methylglutamic acid (the α-L-isomer of *2a*)
H	H	CH_3	H	(2*S*,4*R*)-4-Methylglutamic acid (the α-L-isomer of *2b*)
H	H	HO	H	(2*S*,4*R*)-4-Hydroxyglutamic acid (the α-L-isomer of *3a*)
H	H	H	HO	(2*S*,4*S*)-4-Hydroxyglutamic acid (the α-L-isomer of *3b*)
H	H	CH_3	HO	(2*S*,4*S*)-4-Hydroxy-4-methylglutamic acid (the α-L-isomer of *4a*)[a]
H	H	HO	CH_3	(2*S*,4*R*)-4-Hydroxy-4-methylglutamic acid (the α-L-isomer of *4b*)[a]
H	H	HO	$(CH_3)_2CHCH_2$	(2*S*,4*R*)-4-Hydroxy-4-isobutylglutamic acid (the α-L-isomer of *5a*)
H	H	$(CH_3)_2CHCH_2$	HO	(2*S*,4*S*)-4-Hydroxy-4-isobutylglutamic acid (the α-L-isomer of *5b*)
H	H	$(CH_3)_2CHCH_2$	galactosyl	(2*S*,4*S*)-4-(β-D-Galactopyranosyloxy)-4-isobutylglutamic acid *6*
HO	H	CH_3	H	(2*S*,3*S*,4*R*)-3-Hydroxy-4-methylglutamic acid *7*
H	HO	H	CH_3	(2*S*,3*R*,4*S*)-3-Hydroxy-4-methylglutamic acid *8*
HO	H	H	CH_3	(2*S*,3*S*,4*S*)-3-Hydroxy-4-methylglutamic acid *9*
HO	H		$CH_2=$	(2*S*,3*S*)-3-Hydroxy-4-methyleneglutamic acid (*10*)
H	H		$CH_2=$	(2*S*)-4-Methyleneglutamic acid *11*
				(2*S*,2′*S*)-N^6-(2′-Glutaryl)lysine (L-saccharopine) *12*
H	H	H	H	(2*S*)-2-Aminoadipic acid *13*
H	HO	H	H	(2*S*)-4-Hydroxy-2-aminoadipic acid (*14a* or *14b*)[a]
HO	H	H	H	(2*S*)-4-Hydroxy-2-aminoadipic acid (*14a* or *14b*[a]
H	H	HO/H	H/HO	(2*S*)-5-Hydroxy-2-aminoadipic acid *15ab*[b]
H	H	–	–	(2*S*)-2-Aminopimelic acid (*16*)
H	HO	–	–	(2*S*)-4-Hydroxy-2-aminopimelic acid (*17a* or *17b*)[a]
HO	H	–	–	(2*S*)-4-Hydroxy-2-aminopimelic acid (*17a* or *17b*)[a]
			R	
		n=0	H	(3-Carboxyphenyl)glycine *18*
			HO	(3-Carboxy-4-hydroxyphenyl)glycine *19*
		n=1	H	3-(3-Carboxyphenyl)alanine *20*
			HO	3-(3-Carboxy-4-hydroxyphenyl)alanine *21*

[a] Discussion of stereochemistry, see Results and Discussion. [b] Configuration not determined possibly a mixture of four stereoisomers, see Experimental.

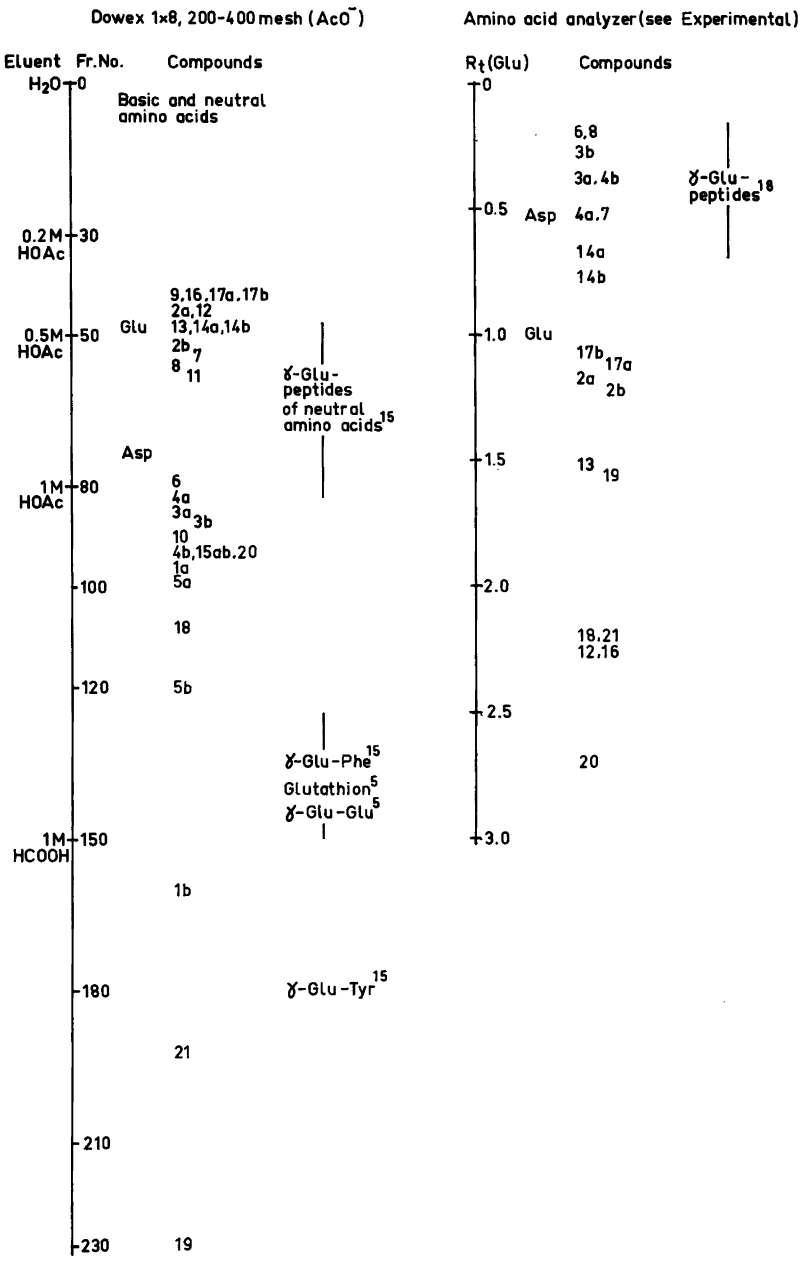


Fig. 1. Ion-exchange chromatography of acidic amino acids. The separations are obtained essentially according to pK_{a2}-values on the Dowex anion-exchange resins and according to pK_{a1}-values on the amino acid analyzer cation-exchange resins. For experimental conditions, see Experimental; for designation of compounds, see Table 1.

Table 2. R_F-values from PC and ionic mobilities by HVE of acidic amino acids and reference compounds.

Amino acid[b]	R_F-values in solvents[a]			Distances in cm obtained by HVE in buffer systems[a]		
	1	2	3	(a) pH 1.9	(b) pH 3.6	(c) pH 6.5
Asp	0.22	0.16	0.12	22.6	9.6	22.5
Glu	0.28	0.26	0.15	25.0	2.6	20.0
Gln	0.23	0.60	0.25	25.0	−2.8	−3.0
2a	0.38	0.36	0.18	24.7	2.1	18.4
2b	0.38	0.38	0.18	23.7	3.4	19.3
3a	0.16	0.12	0.10	21.4	16.1	19.4
3b	0.18	0.12	0.10	19.2	16.7	19.7
4a[5]	0.23	0.22	0.18	19.8	15.4	18.2
4b[5]	0.24	0.22	0.19	19.3	16.8	19.5
5a	0.52	0.60	0.40	16.8	14.2	16.0
5b	0.52	0.47	0.38	16.5	15.8	16.5
14a	0.24	0.22	0.14	22.4	3.4	18.7
14b	0.24	0.25	0.14	21.4	4.0	18.7
15ab	0.19	0.13	0.12	22.6	11.8	18.2
17a[5]	0.30	0.27	0.14	18.9	0.3	17.0
17b[5]	0.30	0.27	0.14	18.6	0.6	17.0
22	0.65	0.84			−2.4	−3.0

[a] For experimental conditions, see Ref. 5. [b] For designation of compounds, see Table 1.

according to pK_{a2}-values. Factors other than pK_a-values have influence on the elution behaviour; amino acids containing bulky groups[16] or aromatic rings[5] appear in fractions later than expected from consideration of pK_a-values and mobilities in electrophoresis *(vide infra)*.

Fig. 1. shows results from anion-exchange chromatography and cation-exchange chromatography on an amino acid analyzer. Some of the substituted acidic amino acids are easily separated from each other and in some cases diastereoisomeric mixtures may be separated into racemates. These separations are easily obtained for the 3-hydroxy substituted acidic amino acids (*1a*, *1b*, 7−10) and the 4-substituted glutamic acids (*2a*−*11*) but not for the larger homologs (*14a*, *14b*, *17a*, *17b*) and the 5-hydroxy-2-aminoadipic acid (*15ab*). The 3- and/or 4-substituted acidic amino acids,[5] *12*, and 13,[17] the aromatic acidic amino acids (*18*−*21*),[5] and some of the numerous γ-glutamyl peptides[15,18] often appear in nearly the same fractions from the ion-exchange columns. Differences in the relative concentrations of the amino acids often lead to improper separations and other methods have to be used.

Paper chromatography (PC) and high voltage electrophoresis (HVE) are effective methods to distinguish between substituted acidic amino acids and γ-glutamyl peptides (unpublished results) and for preparative purposes. Table 2 reveals that these procedures in combination with ion-exchange chromatography are effective tools in screenings of plant material for acidic amino acids. Closely related compounds require use of reference compounds, e.g. Asp, Glu, Gln, on both sides of the paper for standardization purposes, since the results are rather dependent on experimental conditions affecting the partition between mobile and stationary phases.

Table 3. pK_a-values for acidic amino acids.[a] Designation of compounds, see Table 1.

	Asp	Glu	*2a*	*2b*	*3a*	*3b*	*4a*	*4b*	*5b*
pK_{a1}	2.05	2.10	2.12	2.12	1.92	1.92	1.85	1.92	1.70
pK_{a2}	3.70	4.10	4.12	4.08	3.28	3.23	3.28	3.22	3.30
pK_{a3}	9.60	9.45	9.42	9.40	9.40	9.40	9.58	9.58	9.70

[a] Determined by petentiometric titration. For experimental conditions, see Experimental.

The pK_a-values for some of the synthesized amino acids have been estimated from titration curves, Table 3. No attempt has been made to correct the obtained pK_a-values or to determine the microscopic dissociation constants, although the differences between pK_{a1} and pK_{a2} indicate that they are certainly not independent of each other. Therefore, not surprisingly, the pK_{a2}-values for *3b*,[19] *4a* and *4b*[9] determined under different conditions are deviating from the values in Table 3. The standardization of the experimental conditions permits the specification to two decimals.

Most of the pK_a-values are in agreement with the results presented in Fig. 1 and Table 2. Similar results have been obtained for *7* and *8*.[20] The elution behaviour from anion-exchange resins and the HVE mobility at pH 3.6 are both in agreement with the pK_{a2}-values, *i.e.* the compounds with the lowest pK_{a2}-values (for diastereoisomers the racemates *b*) are normally the latest eluted from the column and have the highest mobilities. The HVE mobilities also reflect, to a lesser extent, the connection between negative net charge and molecular weight (size). The elution behaviour from strongly acidic cation-exchange resins[16,21,22] and the HVE mobility at pH 1.9 are, correspondingly, both in agreement with the pK_{a1}-values, *i.e.* the compounds with the lowest pK_{a1}-values are normally the first eluted from the column and have the lowest mobilities.[5] The HVE mobilities reflect as listed above the connection between positive net charge and molecular weight (size).

The colours produced by the reaction of ninhydrin with the 4-substituted amino acids are often of diagnostic value. The compounds containing a double bond at C(4), *10*, *11*, and 2-amino-4-oxo-6-methylheptanoic acids (*22*), yield a yellow colour, and this is also the case for *6*.[2] The hydroxy substituted compounds often yield specific colours deviating from the "normal" purple.[23]

^1H and ^{13}C NMR spectroscopy yield further structural information. Small differences between the racemates of the diastereoisomers are observed in the spectra and they are confirmed by spectra obtained from mixtures of the diastereoisomers. ^{13}C chemical shifts values show a marked pH dependence.[5] The values reported in this paper correspond to amino acids with deprotonated forms of carboxylic acid and ammonium groups. The ^{13}C NMR spectra recorded under identical conditions with respect to solvent, pH, and concentration are very useful in the identification of substituted acidic amino acids. Variations in shift values as a function of changes in pH are useful identification tools as well.[5]

The ^1H NMR spectrum of the racemate *2a* is different from that of *2b*. The latter is in agreement with the spectrum shown in the literature[24] for the plant product (2*S*,4*R*)-4-methylglutamic acid.[25] The spectrum of the racemate *3b* is identical with the spectrum of (2*S*,4*S*)-4-hydroxyglutamic acid[8,14,25] found in plants.[1] The L-isomers of *4a* and *4b* have both been isolated from plants.[4,5,10,11] The ^1H NMR spectrum of *4b* is similar to that of *5b* concerning both chemical shifts and coupling constants for the protons at C(2) and C(3), whereas significant deviations are found for the corresponding protons in the spectra of *4a* and *5a*. A tentative (2*S*,4*R*)-configuration previously assigned to the L-isomer of *5b* based on ^1H NMR spectroscopic comparison with *4a* and *4b*[2,10] has to be changed,[6] the amino acid in *Reseda odorata* being (2*S*,4*S*)-4-(β-D-galactopyranosyloxy)-4-isobutylglutamic acid (*6*).

The configurations attributed *4a* and *4b*[10,11] are also not in accordance with the relation between configuration and pK_a-values found for the other acidic amino acids. The amino acids with the lowest pK_{a2}-values (designated *b*) have *erythro* configuration at the C(2)-amino and C(4)-alkyl group (*2b*, *5b*) and *threo* configuration at the C(2)-amino and C(4)-hydroxy group (*3b*, *5b*) or C(3)-hydroxy group (*1b*). These relations between configuration and pK_a-values are reflected in the results presented in Fig. 1 and Table 2. The same relations are found for the compounds *7*, *8* and *9* (Fig. 1 and Ref. 3). The opposite relations are apparently found between the configuration assigned to *4a* and *4b* (Table 1) and the experimental results in Fig. 1. Tables 2 and 3, and the NMR spectra. The stereochemistry of *14a*, *14b*, *15ab*, *17a*, and *17b* is unknown.[5]

In summary, the results now reported show that the isolation and separation of substituted acidic amino acids can be achieved by combinations of cation- and anion-exchange chromatography, HVE, and PC. The results are to some extent explainable from the pK_a-values and the molecular weight (size) for the amino acids. The identification, including the stereochemistry, is mainly based on the same techniques in combination with observations on stability during acidic and basic conditions and several spectroscopic and/or enzymatic techniques.[4,5]

EXPERIMENTAL

General methods. IR spectra were determined in KBr pellets. ^{1}H NMR spectra were recorded on a JEOL C-60 HL instrument, amino acids were dissolved in 1 M NaOD. Chemical shifts are in ppm downfield from sodium 2,2,3,3,-tetradeuterio-3-(trimethylsilyl)propionate; coupling constants are in Hz. The symbols s, d, t, m, dd, and qq represent singlet, doublet, triplet, multiplet, two sets of doublets and two sets of quartets, respectively. The ^{13}C NMR spectra were recorded in the same solution as the ^{1}H NMR spectra and as previously described.[5] Chemical shifts are in ppm downfield from TMS; dioxane was used as internal standard [δ(TMS) = δ(dioxane) + 67.4 ppm]. PC, preparative PC, and HVE were carried out as previously described.[5]

Titration curves were registered on a Metrohm Herisau Potentiograf E 436, the amino acids (0.1 mmol) were dissolved in 300 μl 1 M NaOH and titrated with 1 M HCl. The results are presented in Table 3. The pK_a-values of each amino acid are the result of at least two titrations. The experimental conditions: Volume, concentration, temperature and ionic strength changes were identical for all compounds, and, furthermore, relatively high concentrations of amino acids were used to minimize problems of the buffering capacity of the water. Ion-exchange chromatography on Dowex 1 × 8, 200–400 mesh, AcO^-, as presented in Fig. 1, was performed on a 0.9 × 60 cm column, 6 ml fractions were collected at 40 ml/h. Mixtures containing 0.03 mmol of each amino acid were applied to the column and subsequently eluted with H_2O (fractions 1–30), 0.2 M HOAc (fractions 31–50), 0.5 M HOAc (fractions 51–80), and 1 M HOAc (fractions 81–150); more acidic amino acids were then eluted with 1 M HOOCH. Amino acid analysis was performed on a Beckman Model 120 C instrument, the elution (Fig. 1) is shown as R_t(Glu) = elution time of amino acid/elution time of glutamic acid.[16]

Synthesis. The stereoisomeric mixture of *4-methylglutamic acids* (*2a*, *2b*) was prepared as described in the literature.[7] The racemic amino acids were isolated from the pyridine eluate from Dowex 50 w × 8, 50–100 mesh, H^+, 2.5 × 100 cm, and finally purified on a column containing Dowex 1 × 8, AcO^-, as described later. Yield 8.5 g (53 %).

The stereoisomeric mixture of *4-hydroxyglutamic acids* (*3a*, *3b*) was prepared as described in the literature.[8] The racemic amino acids were isolated on ion-exchange resins as described for *2a* and *2b*. Yield 14.7 g (40 %).

The stereoisomeric mixture of *4-hydroxy-4-methylglutamic* acids (*4a*, *4b*) was synthesized[8] and purified by ion-exchange chromatography as described for *2a* and *2b*. From 28.5 g (177 mmol) 2-acetoxy-3-chloro-2-methylpropionitrile[26,27,28] a yield of 3.0 g (9.7 %) was obtained.

Separations of 2a from 2b, 3a from 3b, and 4a from 4b were performed on Dowex 1 × 8, 200–400 mesh, AcO^-, 2.5 × 100 cm, 20 ml fractions, 80 ml/h. Portions of 500 mg synthetic material dissolved in 7 ml 1 M NH_3 were applied to the column. After washing with H_2O (fractions 1–30), the column was eluted with 0.2 M HOAc for the separation of *2a* from *2b*. For the separation of *3a* from *3b* and *4a* from *4b*, elution was performed with 0.5 M HOAc. The racemic amino acids appeared in fractions Nos. 77—83 (*2a*), 90–100 (*2b*), 230–248 (*3a*), 260–288 (*3b*), 225–240 (*4a*), and 265–290 (*4b*). Recrystallizations from H_2O yielded colourless crystalline *2a*, *2b*, *3a*, *3b*, *4a*, and *4b*. The identity of the compounds was established by titration curves (Table 3), PC and HVE (Table 2), ^{13}C and ^{1}H NMR spectroscopy. The ^{13}C spectra exhibited signals at the following values; for *2a*: 184.4 (C-1), 55.4 (C-2), 40.9 (C-3), 40.1 (C-4), 187.0 (C-5), 17.7 (C-1′); for *2b*: 184.0 (C-1), 55.7 (C-2), 40.7 (C-3), 40.2 (C-4), 186.7 (C-5), 18.7 (C-1′); for *3a*: 182.1 (C-1), 55.1 (C-2), 40.8 (C-3), 71.9 (C-4), 183.4 (C-5); for *3b*: 182.3 (C-1), 54.2 (C-2), 40.3 (C-3), 70.9 (C-4), 183.3 (C-5); for *4a*: 183.8 (C-1), 53.9 (C-2), 45.8 (C-3), 75.9 (C-4), 184.1 (C-5), 26.8 (C-1′); for *4b*: 182.5 (C-1), 55.3 (C-2), 43.8 (C-3), 77.9 (C-4), 183.7 (C-5), 27.4 (C-1′). The ^{1}H NMR spectra exhibited the following signals; for *2a*: 3.75 (1H, t), 2.00 (2H, t), 2.50 (1H, m), 1.25 (3H, d); for *2b*: 3.80 (1H, dd), 2.05 (2H, m), 2.60 (1H, m), 1.25 (3H, d); for *3a*: 3.43 (1H, dd), 2.11–1.67 (2H, m), 4.09 (1H, dd); for *3b*: 3.41 (1H, dd), 1.98–1.77 (2H, m), 4.14 (1H, dd); for *4a*: 3.36 (1H, dd), 2.09–1.80 (2H, qq), 1.35 (3H, s); for *4b*: 3.29 (1H, dd), 2.20–1.57 (2H, qq), 1.35 (3H, s). The IR spectrum of *4b* was identical with the spectrum of (2*S*, 4*R*)-4-hydroxy-4-methylglutamic acid isolated from *Ledenbergia roseo-aenea*[9] and similar identity was found between the IR spectra of *4a* and the amino acid isolated from *Pandanus veitchii* (the (2*S*, 4*S*)-isomer).[9]

1-Chloro-4-methyl-2-pentanone (23) was prepared by the Arndt–Eistert reaction without Wolff rearrangement. A solution of diazomethane (120 mmol) in 600 ml diethyl ether was obtained from *N*-methyl-*N*-nitroso-*p*-toluenesulfone amide (43 g, 200 mmol) and KOH (12 g).[29] This solution, cooled to 5 °C, was transferred to 7.2 g isovaleroyl chloride (60 mmol). After 3 h, dry HCl was gently bubbled through the solution for 30 min. The reaction mixture was left at room temperature for 20 h[30] and then concentrated to dryness. The product (8 g) was purified by distallation at 11 mmHg, b.p. 56–57 °C (lit.: b.p. 57.5 °C at 10.5 mmHg[30]). Yield 5.41 g (68 %). Identity of the compound was established by ^{1}H NMR spectroscopy.

The racemic mixture of *2-amino-4-oxo-6-meth-*

ylheptanoic acids (*22*) was synthesized[8] and purified on a strongly acidic ion-exchange resin as described for *2a* and *2b*. From 7.0 g *23* (52 mmol) a pyridine eluate containing *22* and glycine (6.4 g) was obtained. These amino acids were separated on a column (2.5 × 100 cm) containing silica gel by elution with solvent 1 (see PC). Fractions (20 ml) were collected at 80 ml/h. Fractions 28–35 were taken to dryness yielding 2.54 g of colourless semicrystalline *22* (28 %). Identity of the compouns was established by PC and HVE (Table 2) and ^{1}H NMR spectroscopy: 4.45 (1H, t), 3.42 (2H, d), 2.53 (2H, d), 2.7–1.9 (1H, m), 0.96 (6H, dd).

Methyl 2-acetamido-4-oxo-6-methylheptanoate (*24*). Compound *22* (2.50 g, 1.45 mmol) was dissolved in 50 ml 3 M HCl in MeOH and left at room temperature for 18 h. The reaction mixture was concentrated to dryness, dissolved in pyridine (40 ml), cooled to 0–2 °C, and supplied with 1.74 g $(AcO)_2O$ (18 mmol) in the course of 3 h. After concentration to dryness, the residue was dissolved in 20 ml $CHCl_3$. This solution was washed with 5 % $NaHCO_3-H_2O$, dried with Na_2SO_4 and concentrated to dryness. Yield 3.05 g *24* (86 %). Identity of the compound was established by ^{1}H NMR spectroscopy.

Methyl 2-acetamido-4-acetoxy-4-cyano-6-methylheptanoate (*25*). To a bi-phasic system of 3.05 g *24* in 25 ml benzene and 3.09 g KCN in 15 ml H_2O was transferred 13.1 ml 40 % H_2SO_4. After 6 h at room temperature, the benzene phase was transferred to 20 ml benzene, cooled to 7 °C, mixed with 40 µl conc. H_2SO_4 and 2.52 g $(AcO)_2O$, and left for 2 h at 7 °C. The reaction mixture was washed with 4 × 10 ml H_2O (0–2 °C), dried with Na_2SO_4, and concentrated to dryness leaving 2.22 g *25*. The identity was established by ^{1}H NMR spectroscopy.

The stereoisomeric mixture of *4-hydroxy-4-isobutylglutamic acids* (*5a, 5b*) was synthesized by dissolving *25* (2.2 g) in 20 ml 1 M NaOH containing 1.3 g KCN and leaving the solution at 65 °C for 48 h before concentration to dryness. The residue was purified on a strongly acidic ion-exchange resin as described for *2a* and *2b*. From the pyridine eluate was obtained 128 mg (*5a, 5b*) (yield 4.7 %). The identity of the compounds was established by ^{1}H NMR spectroscopy.

Separation of 5a *from* 5b was performed by preparative PC using solvents 1 and 2 (Table 2). The racemic mixtures of the amino acids were eluted from the paper with H_2O and the solutions were concentrated by lyophilization. Recrystallizations from H_2O yielded 3.6 mg colourless semicrystalline *5a* and 28.2 mg colourless crystalline *5b*. The identity of the compounds was established by titration curves for *5b* (Table 3), PC and HVE (Table 2), and ^{1}H NMR spectroscopy; for *5a*: 3.55 (1H, dd), 2.00 (2H, dd), 1.9–1.2 (3H, m), 0.95 (6H, dd), for *5b*: 3.27 (1H, dd), 2.2–1.6 (2H, qq), 2.0–1.3 (3H, m), 0.93 (6H, dd). IR spectra of *5b* and 4-hydroxy-4-isobutylglutamic acid isolated from a hydrolysis mixture of *6*, present in *Reseda odorata*,[2] were identical.

The stereoisomeric mixture of *4-hydroxy-2-aminoadipic acids* (*14a, 14b*) was synthesized[8] and purified by ion-exchange chromatography as described for *2a* and *2b*. From 12.91 g (79.9 mmol) 3-acetoxy-4-chlorobutyronitrile a yield of 0.92 g (7 %) was obtained.

Separation of 14a *from* 14b was performed by preparative HVE at pH 3.6, 3 h. The identity of the compounds was established by PC and HVE (Table 2) and ^{1}H and ^{13}C NMR spectroscopy. The ^{13}C spectra exhibited signals at the following values: for *14a*: 187.0 (C-1), 61.4 (C-2), 40.4 (C-3), 67.3 (C-4), 47.0 (C-5), 187.0 (C-6); for *14b*: 187.0 (C-1), 62.6 (C-2), 42.1 (C-3), 67.3 (C-4), 47.3 (C-5), 187.0 (C-6). The ^{1}H NMR spectra exhibited the following signals: for *14a*: 3.4 (1H, dd), 1.9–2.7 (2H, m), 3.9 (1H, m), 1.9–2.7 (2H, m); for *14b*: 3.4 (1H, dd), 1.9–2.7 (2H, m), 3.9 (1H, m), 1.9–2.7 (2H, m).

The stereoisomeric mixture of *5-hydroxy-2-aminoadipic acids* (*15ab*) was prepared from 60 mmol (18 g) 2-hydroxy-5,5-dicarbethoxy-5-acetamidopentanonitrile according to the previously described method.[12] After purification as described for *2a* and *2b* a yield of 8.2 g (77 %) was obtained. Identity of the amino acids, expected to be present in the diastereoisomeric mixture, was established by PC and HVE (Table 2) and ^{1}H and ^{13}C NMR spectroscopy. The ^{13}C spectrum exhibited signals at the following values 181.2 (C-1), 56.3 (C-2), 31.1 (C-3), 30.3 (C-4), 72.7 (C-5), 181.9 (C-6). The ^{1}H NMR spectrum exhibited the following signals: 3.6 (1H, dd), 1.6–2.1 (4H, m) 4.05 (1H, dd). Attempt to resolve the diastereoisomeric mixture on ion-exchange columns and by HVE was not successful.

The stereoisomeric mixture of *4-hydroxy-2-aminopimelic acids* (*17a, 17b*) was synthesized and separated into racemates by preparative HVE as described elsewhere.[5]

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