Acta Chemica Scandinavica B 37 (1983) 321–324

Absolute Configuration of 4-Hydroxy-4-methylglutamic Acids

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The absolute configurations of the naturally occurring (2*S*,4*S*)-4-hydroxy-4-methylglutamic acid and (2*S*,4*R*)-4-hydroxy-4-methylglutamic acid have been determined. L-Amino acid oxidase was used in the determination of the (2*S*)-configuration of the amino acids. The diastereoisomeric (2*S*)-4-hydroxy-4-methylglutamic acids were transformed into (*S*)(+)-citramalic acid and (*R*)(−)-citramalic acid, respectively, by oxidative deamination and decarboxylation using calcium hypochlorite. On the basis of the obtained results and the known absolute configurations of the enantiomeric citramalic acids, it was deduced that the configuration previously assigned the title compounds had to be changed. The structure of (2*S*,4*S*)-4-hydroxyglutamic acid has also been established by use of this method. The L-malic acid liberated was determined by its optical rotation and enzymatically by L-malic acid dehydrogenase.

The present work is a continuation of previous studies on a relatively large group of naturally occurring acidic amino acids which structurally are considered as 3- and/or 4-substituted derivatives of 2-aminopimelic acid, 2-aminoadipic acid and glutamic acid.[1–3] The amino acids in this group have at least two chiral centers, and several diastereoisomers very often co-exist within the same plant.[3–7] Co-occurrence of these diastereoisomers in plant extracts is not a result of the methods used for their isolation.[4,5]

Biogenetic origin of these amino acids in plants has been studied but only little definitive information is available.[8,9] It has been proposed that the reactions are of the aldolase-type in which a 3-carbon unit and a 2-keto acid yield the 2-keto-4-substituted carboxylic acids which finally are transformed into amino acids by transamination.[5–8] Biosynthesis of compounds with both (4*R*)- and (4*S*)-configuration within the same organism is known when aldolase-type reactions are involved.[10] The possibility of both *re*- and *si*-face attacks to a ketimine intermediate is thus a likely explanation of the co-occurrence of diastereoisomeric acidic amino acids within the same plant. Furthermore, it has been shown for all naturally occurring amino acids in this group, where experimental evidences are available, that the configuration at C-2 is (2*S*).[3]

The absolute configuration is established for some of the known 3- and/or 4-substituted acidic amino acids but not for all of them.[3,6,11] Methods widely used for establishing absolute configurations of compounds in this series are based on X-ray crystal structure analysis,[12,13] enzymic methods, NMR-spectroscopy,[3] determination of optical rotation, ORD and CD,[14,15] as well as synthesis of the compounds.[3,16] These methods are not easy to use for all 3- and/or 4-substituted acidic amino acids owing to easy transformation of the compounds into lactones and/or lactams [3] and difficult preparation of crystals suitable for X-ray analysis. Therefore, other methods for structure determination of these amino acids are of interest. Furthermore, recent studies on synthesis and properties of several 3- and/or 4-hydroxylated and alkylated acidic amino acids have revealed that differences in properties of these diastereoisomers are predictable from considerations of their configuration.[3] An exception is the diastereoisomeric 4-hydroxy-4-methylglutamic acids but the configuration assigned to these compounds has previously been questioned.[13]

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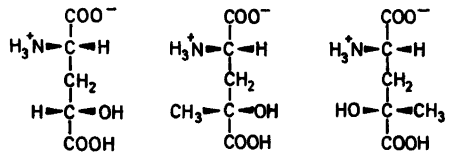

I II III

Fig. 1. Structure of the amino acids isolated from *Phlox decussata* [(2*S*,4*S*)-4-hydroxyglutamic acid, I] and from *Reseda luteola* [(2*S*,4*S*)-4-hydroxy-4-methylglutamic acid, II and (2*S*,4*R*)-4-hydroxy-4-methylglutamic acid, III].

This paper describes a simple method for the structure analysis of 4-substituted acidic amino acids based on oxidative deamination and decarboxylation in alkaline calcium hypochlorite solution. This reaction type in which carbon atom No. 2 in the amino acids is transformed into a carboxylic acid group has been used in the study of other types of compounds.[14,17-20] The carboxylic acids produced in the reaction from different acidic amino acids are easy to identify and the lactone and lactam problems are eliminated. The method is especially sensitive when used to (2*S*,4*S*)-4-hydroxyglutamic acid as the liberated L-malic acid is easy to determine quantitatively by use of malic acid dehydrogenase (EC 1.1.1.37). The method used to the natural occurring diastereoisomeric 4-hydroxy-4-methylglutamic acids appeared to be useful for determination of their absolute configuration.

RESULTS AND DISCUSSION

Methods for isolation and separation of diastereoisomeric 4-substituted glutamic acid derivatives have been described elsewhere.[3] (2*S*,4*S*)-4-Hydroxyglutamic acid was isolated from *Phlox decussata* L.,[7] the two diastereoisomeric (2*S*)-4-hydroxy-4-methylglutamic acids were isolated from *Reseda luteola* L. and their (2*S*)-configuration was determined by use of L-amino acid oxidase.[5] Structures of the *Phlox* amino acid with well-established configuration at both C-2 and C-4 [15] and the *R. luteola* amino acids are shown in Fig. 1.

The amino acids I–III and glutamic acid have been treated with an alkaline solution of calcium hypochlorite. Under the reaction conditions used (see Experimental) the oxidative decarboxylation

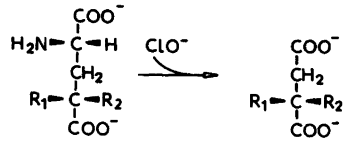

Fig. 2. Oxidation of amino acids in alkaline hypochlorite solution.

	R_1	R_2
Amino acids		
Glutamic acid	H	H
I	H	OH
II	CH_3	OH
III	OH	CH_3
Carboxylic acids		
Succinic acid	H	H
L-Malic acid	H	OH
(*S*)(+)-Citramalic acid	CH_3	OH
(*R*)(−)-Citramalic acid	OH	CH_3

and deamination proceeded in high yield (Fig. 2) and with retention of the configuration at C-4 in the amino acids – *i.e.* C-2 in the carboxylic acids – as shown by transformation of I into (2*S*)- or L-malate. The reactions have also been performed with chloramine-T as described elsewhere,[14] but the reaction modified with respect to use of calcium hypochlorite instead of chloramine-T was preferred owing to an easier isolation procedure for the carboxylic acids.

The carboxylic acids were isolated from the reaction mixtures in a procedure including ion-exchange chromatography (see Experimental). Identity and purity of the isolated carboxylic acids were confirmed by comparison of HVE, PC, TLC and ^{1}H NMR properties of the compounds (Table 1) with corresponding results obtained with authentic reference compounds. Also GLC of trimethylsilyl derivatives of the carboxylic acids were used and the optical rotations were determined for L-malic acid, (*S*)(+)- and (*R*)(−)-citramalic acids. L-Malic acid produced from I was furthermore determined by use of malate dehydrogenase (EC 1.1.37) in a coupled reaction with glutamate-oxaloacetate transaminase (EC 2.6.1.1.) as described for the commercially available test-combination kit.

The results obtained with the two diastereoisomeric (2*S*)-4-hydroxy-4-methylgluta-

Table 1. R_f-values from PC and TLC and ionic mobilities by HVE of some carboxylic acids together with R_t-values by GLC of their per-trimethylsilyl derivatives.

Compound	GLC R_t (min)	R_f on PC in solvent system [a]			HVE mobility (cm) in buffer system [a]		R_f on TLC in solvent [a]	
		(1)	(2)	(3)	pH 3.6	pH 6.5	(4)	(5)
Malonic acid	12.7	0.55	0.18	0.14	34.7	38.7	0.08	0.08
Succinic acid	15.6	0.73	0.24	0.16	7.2	35.8	0.34	0.67
Glutaric acid	17.8	0.72	0.32	0.18	5.5	33.1	0.55	0.75
Malic acid	19.2	0.38	0.12	0.08	19.4	34.4	0.02	0.09
Citramalic acid	18.5	0.42	0.17	0.06	18.2	24.5	0.04	0.14
Aspartic acid [b]	–	0.22	0.16	0.12	9.6	22.5	–	–
Glutamic acid [b]	–	0.28	0.26	0.15	2.6	20.0	–	–

[a] Experimental conditions, see Experimental, the HVE buffer systems were: pH 3.6 (pyridine–HOAc–H_2O) (1:10:200); pH 6.5 (pyridine–HOAc–H_2O) (25:1:500). TLC was performed on Si-gel plates, chromatographic systems were: (1) n-BuOH–HOAc–H_2O (12:3:5); (2) PhOH–H_2O–13 M NH_4OH (120:30:1) (w/v/v): (3) iso-PrOH–H_2O–13 M NH_4OH (8:1:1): (4) $CHCl_3$-*tert*-amyl alcohol–HCOOH–H_2O (136:24:27:83): (5) C_6H_6–HOAc–H_2O (125:73:2). [b] For comparison with properties of 3- and 4-substituted acidic amino acids, see Ref. 3.

mic acids showed that the diastereoisomer with highest pK_{a_2}-value was transformed into (*R*)(−)-citramalic acid (Fig. 2) and (*S*)(+)-citramalic acid was produced from the amino acid with the lowest pK_{a_2}-value. The absolute configuration for the enantiomeric citramalic acids is known,[21–24] and with the established (2*S*)-configuration for both of the diastereoisomeric 4-hydroxy-4-methylglutamic acids, it is deduced that the amino acid III (Fig. 1) has higher pK_{a_2}-value than the isomer II. The absolute configuration now determined for the two chiral atoms in II and III implicates that the previously assigned configuration for carbon atom No. 4 (Ref. 13 and references cited therein) has to be changed. Thereby, the discrepancy no longer exists between the structure and properties of II and III, and there is now full agreement between the stereochemistry of the hitherto examined 3- and/or 4-substituted acidic amino acids and their chemical and spectroscopical properties.[3] CD and optical rotary dispersion curves of several 2-substituted succinic acids previously described [25–27] may also be of value in combination with the method now described for determination of the stereochemistry of 4-substituted glutamic acid derivatives.

EXPERIMENTAL

General methods ^1H NMR spectroscopy, GLC, PC, TLC and HVE were performed as previously described.[5,28,29]

The sodium salts of (*R*)(−)-citramalic acid and (*S*)(+)-citramalic acid as well as L- and D-malic acids were purchased from Sigma Chemical Company.

Isolation and identification of the amino acids I–III are described elsewhere,[3,5,7] as is the use of L-amino acid oxidase [4] to establish their (2*S*)-configuration.

Hypochlorite oxidation of the amino acids. The amino acids (1.35 m mol) were dissolved in 1.5 ml 2 M NaOH and mixed with a solution of $Ca(OCl)_2$ (71.5 mg, 0.5 m mol) in H_2O (1.5 ml). After 2 h at 60 °C the reaction mixture was acidified with HCl (3.5 ml, 1 M) and 10 ml of an H_2O solution containing Br_2 (0.8 ml Br_2 in 50 ml H_2O) were added. This mixture was left at room temperature for 48 h after which excess of Br_2 was destroyed with $Na_2S_2O_3$ (0.1 M in H_2O, *ca.* 8 ml). After concentration almost to dryness, the residue was treated three times with ethyl acetate (5 ml each time) and the extracts were concentrated to dryness. The residue was purified on a strongly acidic ion-exchange resin (Dowex 50 W×8, 200–400 mesh, H^+, 0.7×10 cm). The column was washed with H_2O (30 ml) and the effluent was concentrated to dryness yielding the carboxylic acids: succinic acid from glutamic acid (52 %); (*S*)(+)-citramalic acid from the most acidic of the diastereoisomeric (2*S*)-4-hydroxy-4-

methylglutamic acids from *R. luteola* (48 %); (*R*)(−)-citramalic acid from the *R. luteola* amino acid (2*S*)-4-hydroxy-4-methylglutamic acid with highest pK_{a_2}-value (43 %). Identity of the compounds was established by PC, TLC, and HVE as well as by GLC of the per-trimethylsilylated carboxylic acids [28] (Table 1). ^{1}H NMR spectra of the isolated carboxylic acids were identical with those obtained from authentic carboxylic acids.[23] Optical rotation was determined for: (L)(−)-malic acid, $[\alpha]_D^{23}$ −19.0°, $[\alpha]_{578}^{23}$ −20.8°, $[\alpha]_{546}^{23}$ −21.9°, $[\alpha]_{436}^{23}$ −20.2°, (c 0.35, 0.1 M NaOH); $[\alpha]_D^{23}$ −4.2°, $[\alpha]_{578}^{23}$ −4.6°, $[\alpha]_{546}^{23}$ −5.8°, $[\alpha]_{436}^{23}$ −5.0° (c 0.28, 1 M HCl); (*R*)(−)-citramalic acid, $[\alpha]_D^{23}$ −27.7°, $[\alpha]_{578}^{23}$ −31.0°, $[\alpha]_{546}^{23}$ −34.6°, $[\alpha]_{436}^{23}$ −56.4° (c 0.31, 0.1 M NaOH); $[\alpha]_D^{23}$ −2.6°, $[\alpha]_{578}^{23}$ −9.8°, $[\alpha]_{546}^{23}$ −12.7°, $[\alpha]_{436}^{23}$ −19.2° (c 0.23, 1 M HCl); (*S*)(+)-citramalic acid, $[\alpha]_D^{23}$ +25.5°, $[\alpha]_{578}^{23}$ +27.8°, $[\alpha]_{546}^{23}$ +28.7°, $[\alpha]_{436}^{23}$ +52.0°(c 0.31, 0.1 M NaOH; $[\alpha]_D^{23}$ +10.5°, $[\alpha]_{578}^{23}$ +11.5°, $[\alpha]_{546}^{23}$ +12.4°, $[\alpha]_{436}^{23}$ +16.0° (c 0.23, 1 M HCl). Lit. value [22] $[\alpha]_D^{25}$ +23.6° (c 3.0, H_2O).

Malate dehydrogenase (L-malate: NAD oxidoreductase, EC 1.1.1.37) was used in conjunction with glutamate-oxaloacetate transaminase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1.) for both qualitative and quantitative determination of L-malate in an assay as described in the information sheet from Boehringer Mannheim GMBH.

Acknowledgement. Support from DANIDA, the Danish Council of Development Research is gratefully acknowledged.

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Received September 14, 1982.