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MICROWAVE-ASSISTED DERIVATIZATION AND GC-MS ANALYSES OF AMINO ACIDS FROM *Ipomoea cairica* AQUEOUS EXTRACT

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Due to the complexity of the several matrices, different procedures have been proposed, in general based on the use of chromatographic methods to obtain amino acid profiles. Such techniques frequently need a previous derivatization step in order to either increase analyte volatility in gas chromatography (GC) or improve analysis sensitivity in high-performance liquid chromatography (HPLC) [1].

Amino acids have been studied by GC due to the low cost, speed, high sensitivity, and high resolution of this technique. To increase GC performance, silylation is the most versatile derivatization method because this reaction is carried out in only one step. This procedure uses the trimethylsilyl (TMS) function to obtain volatile amino acids, which are appropriate for GC analysis [2, 3]. Usually, silylation is made with the reagents *bis*-trimethylsilyl-trifluoroacetamide (BSTFA) or *N*-methyl-*N*-*tert*-butyldimethylsilyl-trifluoro-acetamide (MBDSTFA) under dry conditions and heating from 1 to 3 h. Alkyl-alkoxycarbonyl esters are used when the reaction is made in aqueous solution because they react with amino acids in the place of sugars [2].

Recently, the protein composition of the cell walls of *Panax notoginseng* roots was estimated by determination of the amino acid profile [4]. The amino acid profile has also been proposed as a marker to identify the botanical origin of honey [5].

In this work, we describe the employment of GC-MS in the determination of amino acids in plant materials by the use of a domestic microwave oven to reduce the trimethylsilylation reaction time of 1 hour in the classic method to several minutes. To this end, the aerial parts of *Ipomoea cairica* (L.) Sweet (corda-de-viola), a climbing shrub that grows in tropical regions of the world, were used. This species, along with others of great economic and ethnopharmacological importance such as *I. batatas* (sweet potato), belongs to the Convolvulaceae family and is frequently used in the treatment of several diseases in Brazilian popular medicine [6, 7]. The number of studies on *I. cairica* in the last ten year shows its great relevance in the chemical and pharmacological context [8–17].

The *I. cairica* extract was obtained totally free of interfering compounds such as sugars, polyphenols, and biogenic amines, demonstrating the optimal applicability of the method to obtain fractions rich in amino acids from plant tissues. The best reaction condition to obtain amino acid TMS derivatives was performed in 3 min instead of 1 h in the classic method with the use of a domestic microwave operating at medium power (180 W). The reaction product was directly injected into the chromatographer without further purification.

We detected 18 out of the 20 protein amino acids simultaneously with excellent resolution for alanine, glycine, valine, leucine, serine, threonine, methionine, lysine, histidine, tyrosine, and tryptophan. The co-elution problem of proline-isoleucine, aspartic acid-hydroxyproline, and phenylalanine-glutamic acid was solved by the extraction of the characteristic ions of each amino acid in the ion-monitoring mode (Table 1).

Amino acids arginine and cystine did not produce detectable derivatives, considering the method sensitivity. Cysteine had a low yield of the *N,S*-substituted derivative.

Mass spectral analysis and comparison with the spectrum database (Wiley) showed that all the amino acids have the hydrogen of the carboxyl group substituted and that most produced only N-1TMS-type derivatives. Other substitutions occurred in hydroxyls (e.g., hydroxyproline) and thiol (cysteine). This resulted in a single derivative for each amino acid, which simplified analysis significantly. Only glycine presented two derivatives (N-1TMS and N-2TMS) at ratios of 5.5 and 4.5, respectively.

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TABLE 1. Amino Acid Contents of *Ipomoea cairica* Aerial Parts

Amino acids TMS	RT, min.	$[M]^+$	$[M-117]^+$	%	μg/g of plant
Ala 2TMS	10.14	233	116	1.74	207
Gly 2T MS	10.63	219	102	0.54	64
Val 2 TMS	13.39	261	144	2.74	326
Leu 2T MS	14.99	275	158	1.10	131
Pro 2T MS	15.53	259	142	2.97	354
Ile 2T MS	15.58	275	158	0.99	118
Gly 3T MS	15.82	291	174	0.44	53
Ser 3T MS	17.47	321	204	0.83	99
Thr 3T MS	18.17	335	218	1.05	125
Met 2T MS	21.69	293	176	t	t
Asp 3T MS	22.16	349	232	7.51	894
Hyp 3TMS	22.26	347	230	t	t
Cis 3T MS	23.18	337	220	t	t
Phe 2T MS	25.72	309	192	1.03	123
Glu 3T MS	25.86	363	246	1.40	167
Lys 3T MS	30.00	290	174	t	t
His 2T MS	30.44	299	179	0.46	55
Tyr 3T MS	31.18	485	368	t	t
Trp 2T Ms	33.92	348	231	1.84	219
Total	-	-	-	24.64	2935

t: not detected.

The molecular ion $[M]^+$ is rarely detected because amino acids lose the carboxyl group too easily in the mass spectrometer. In the case of the TMS derivative, this loss involves the *m/z* 117 $[(CH_3)_3SiCO_2.]$ group. In this way, the characteristic peak of each amino acid is represented by the ion *m/z* $[M–117]^+$ (Table 1). Furthermore, the spectra present some peculiar peaks relative to trimethylsilylated derivatives. The *m/z* 73 $[(CH_3)_3Si]^+$ peak is the most important representative, which is always very intense (from 80 to 100%); *m/z* 147, 204, and 218 are also always observed [2].

It was determined that aspartic acid is the most abundant amino acid in the *Ipomoea cairica* tissue, followed by proline and valine. The total amino acid content was 2.94 mg/g of dry plant material (Table 1). This amount includes free and protein-hydrolyzed amino acids, which are usually present due to the use of the aqueous extracts in traditional medicine.

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