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AMINO ACID COMPOSITION AND CONTENTS IN THE SEEDS OF *LYGOS RAETAM* L., *L. RAETAM* VAR. *SARCOCARPA* AND *TEPHROSIA APOLLINEA*.

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ABSTRACT

Protein and non-protein amino acids in aqueous ethanol extracts from the seeds of *Lygos raetam* (Forssk.), *L. raetam* var. *sarcocarpa* (Zho) and *Tephrosia apollinea* (Del.) were analyzed with an automatic amino acid analyzer. The amino acid composition and contents of these plants were described. Several uncommon non-protein amino acids were detected and determined quantitavely.

INTRODUCTION

Lygos raetam (Forssk) belonging to the plant family Leguminosae is known as a nutritive fodder pasture in the desert districts[1]. Chemical study on the amino acid composition and content of this genus was not described and clarified yet.

In addition, *Tephrosia* species such as *T .purpurea* and *T. villossa* are known for their different medicinal uses[2], and a rich source of essential amino acids, except methionine[3,4].

In the course of our ongoing study on the Egyptian Leguminous plants[5-7], we have investigated the characterization and biological activities of the active constituents of these species.

Obviously, Leguminous seeds are reputed for their versatile content of the uncommon non-protein amino acids; some having biological or toxic effects, while others haginv some chemotaxonomic role being spread in one subfamily, related genera or even limited to some members of a single genus[8]. Hence, the main goal of the present study was to investigate simultaneously the content of both the free amino acids of the protein- and the uncommon non-protein types.

EXPERIMENTAL

Plant materials:

Lygos raetam (Frossk.) Heywood [=*Retama raetam* (Forssk.) Weeb, *Genista raetam* (Forssk.)] and *L. raetam* var. *sarcocarpa* (Zho.) Tackh. et. Boulos, were collected at the fruiting stage in June 1989 near the Tenth of Ramadan City and Abu Mady zone (Dakahlia province), respectively. The seeds of *Tephrosia apollinea* (Del) Link. were collected in January 1989 at Gabel Elba region (South eastern region of Egypt).

All plants were identified by Prof. Nabil El-Hadidy, Professor of taxonomy, Faculty of Science, Cairo University, Egypt. Voucher specimens are deposited in the Pharmacognosy Dept., Mansoura University, Egypt.

Extraction and analysis of amino acids:

Ripe seeds of *Lygos raetam*, *L. Reatam* var. *Sarcocarpa* and *Tephrosia apollinea* (each 20 g) were powdered and individually macerated and extracted with 75% ethanol. The alcoholic extracts were filtered and evaporated in vacuo to dryness below 45°C and then dissolved in 50 ml of 0.2 N HCl (pH 1.7 - 1.8). Each single extract was analyzed by automatic amino acid analyzer (Hitachi 835-10) using Li citrate buffer system as described in Table 1 and 2[9]. The amino acids were identified by comparing their elution times with those of authentic compounds and quantitative determination of each amino acid was obtained by referring to the absolute calibration curve with regard to its measured peak area.

RESULTS AND DISCUSSION

In this study, 23 kinds of protein and non-protein amino acids in each extract of *L. raetam* and

Table 1: Standard analytical condition on a Hitachi 835-10 automatic amino acid analyzer.

Column	High pressure stainless column with peltier heating colling system, BED length 250 mm, diameter 2.6mm.
Ion exchange resin	Ultropac 8 cation exchange sodium form, particle size 8 µm.
Analytical time	250 min.
Lithium-citrate buffer system	pH 3.5-7.5
buffer flow rate	0.275 ml/min.
Ninhydrin flow rate	0.3 ml/min.
Column pressure	100-200 kg/cm^2
Ninhydrin pressure	15-53 kg/cm^2
Column temperature	33-68° (5 steps)
N_2 gas pressure	0.28 kg/cm^2
Volume injected	100 µl of each single extract

Table 2: Program on the amino acid analysis using a Hitachi 835-10 automatic amino acid analyzer.

Buffer	pH	Buffer Composition		Buffer change (min.)	Temp. (C°)
1 st	3.5	0.155	N Li-citrate	65	33-47
2 nd	3.7	0.255	N Li-citrate	65-93	47
3 rd	3.3	0.805	N Li-citrate	93-133	47-65
4 th	4.1	1.0	N Li-citrate	133-159	65
5 th	7.5	1.2	N Li-citrate	159-210	65-68

Table 3: Amino acid composition and contents in the seeds of *L. reatam* (A), *L. reatam* var. sarcocarpa (B) and *T. apollinea* (C).

Amino acid	Elution time (min.)	Micromoles of amino acids per gram of seeds		
		A	B	C
Taurine+	7	0.13	0.12	0.09
Aspartic acid	20	0.66	0.44	1.25
Threonine*	26	0.21	0.17	0.35
Serine	28	0.83	0.41	0.56
Asparagine	31	4.41	354	6.91
Glutamic acid	33	3.31	2.58	1.72
α-Aminoadipic acid+	47	--	0.05	--
Proline	54	0.94	0.62	1.31
Glycine	58	0.44	0.35	1.43
Alanine	63	2.11	1.33	4.24
Citrulline+	68	0.26	0.18	0.47
Valine*	83	0.88	0.38	0.41
Cysteine	91	0.17	0.37	0.75
Methionine*	95	--	--	0.06
Isoleucine*	99	0.38	0.17	0.12
Leucine*	102	0.41	0.16	0.12
Tyrosine	106	0.04	0.11	--
Phenylalanine*	112	0.02	--	0.06
β-Alanine	117	0.29	0.29	--
γ-Amino butyric acid+	127	0.56	0.41	0.43
Ethanolamine	132	1.42	--	1.50
Ornithine	153	0.04	0.05	--
Lysine*	158	0.13	0.12	0.32
Histidine*	163	0.12	0.32	0.39
Arginine*	190	0.82	1.47	2.19
Homoarginine+	212	--	--	0.47
Total		18.40	13.64	25.15

* Essential amino acid. + Non-protein amino acid.

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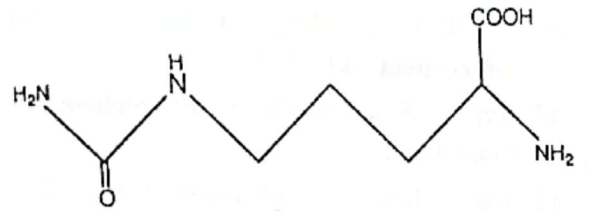
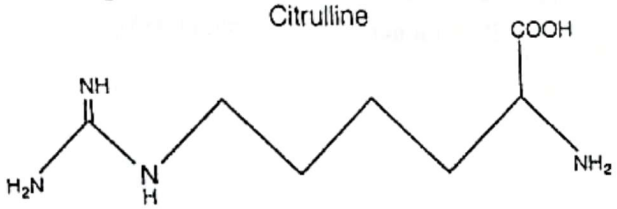
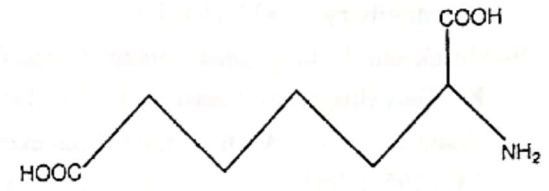


Citrulline



Homoarginine



α-amino adipic acid

GABA

Taurine

L. raetam var. *sarcocarpa* could be detected (Table 3). Their essential amino acid composition and contents in ethanol extracts were almost the same, including threonine, valine, isoleucine, leucine, lysine, histidine and arginine. However, *L. reatam* contained one more essential amino acid, phenylalanine than *L. raetam* var. *sarcocarpa*. Both plants contained γ-aminobutyric acid (GABA): 0.56 and 0.41 μmol/g dry wt., respectively, while tryptophan could not be detected in both *Lygos* plants.

On the other hand, 22 kinds of protein and non-protein amino acid could be detected in the extract of *T . apollinea* (Table 3). The essential amino acid composition and contents of *T. apollinea* was almost the same as that of L. reatam, but it contained one more, essential amino acid, methionine. *T. apollinea* furnished a large amount of asparagine and arginine and also it's total amino acid contents per gram weight was more larger than those of other two *Lygos* plants. This study revealed the presence of sulphur containing amino acids such as taurine, methionine and cysteine in *T. apollinea* in contrary to that reported in the literature about *Tephrosia* specie[3,4]. Moreover, *T. apollinea* contained some non-proteine, ninhydrin positive amino acids and amine such as γ-aminobutyric acid, ethanolamine and homoarginine, which are potentially effective compounds[10,11].

The γ-aminobutyric acid is viewed as temporary nitrogen storage compound for protein amino acids in plants especially under stress condition[12]. Also, this compound serves as an intermediate in the catabolism of arginine, ornithine and polyamines, spermidine and spermine[13-15].

Homoarginine has a dual role, not only as a nitrogen storage compound but also, as a chemical defendant against beetles and bacterial growth[16]. Its presence in *Tephrosia apollinea* classified this species under the leguminous homoarginine -containing plants[8].

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كمية ومحتوى الأحماض الأمينية لبذور الليجوس رتم والليجوس رتم صنف الساركوكاربا وكذلك التفروزيا زبوللينا

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فى هذا البحث تم تحليل الأحماض الأمينية الحرة لبذور الليجوس رتم والليجوس رتم صنف الساركوكاربا وكذلك التفروزيا أبو للينا الموجودة فى خلاصة الكحول الايثلى وذلك باستخدام جهاز تحليل الأحماض الأمينية الذاتى.

ومن ثم تم معرفة محتوى الأحماض الأمينية البروتينية الشائعة وكذلك الأحماض الأمينية الغير بروتينية والغير شائعة (نوعا وكما) فى كل عينة تحت الفحص وألقى الضوء على أهمية وجود الأخيرة حيويا وتقسيميا.