

SYNTHESIS, THERMAL REACTIVITY, AND ANTIOXIDANT STUDIES OF AMINOGUANIDINIUM SALTS OF ASPARTIC AND GLUTAMIC ACIDS

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Received: 29 January 2020, Revised: 18 March 2020, Accepted: 19 March 2020

ABSTRACT

Objective: Our main scope and objectives are to prepare aminoguanidinium salts of amino acids and to characterize them using analytical, IR, and thermal studies, to study the mode of thermal decomposition of aminoguanidinium salts, and to characterize the antioxidants behavior of aminoguanidinium salts.

Methods: Elemental analysis for C, H, and N was performed on a Vario ELIII elemental analyzer. The IR spectra were recorded on a JASCO-4100 spectrophotometer as KBr pellets in the range of 400–4000 cm⁻¹. The simultaneous TG-DTA studies were under taken on a PerkinElmer SII thermal analyzer and the curves obtained in air using platinum cups as holders with ~ 3 mg of the samples at the heating rate of 10°C/min. The antioxidant capacities of different salts were estimated according to the literature procedure.

Results: Aspartic acid forms bis-aminoguanidinium salt, whereas glutamic acid forms both mono- and bis-aminoguanidinium salts. The IR spectral data of the aminoguanidinium salts of aforesaid acids show N-N stretching frequencies in the region 1110–1202 cm⁻¹ revealing the presence of aminoguanidinium moiety.

Conclusion: The antioxidant properties of these salts were studied using ferric reducing antioxidant power and phosphomolybdenum assay. Results showed significant ferric reducing power which indicated the hydrogen-donating ability of the extract.

Keywords: Aminoguanidine, Amino acids, Antioxidant, Thermal activity.

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INTRODUCTION

Recently, there has been a surge in research on the potential role of antioxidants in the treatment of atherosclerosis, heart failure, liver dysfunction, neurodegenerative disorders, cancer, and diabetes mellitus. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage, and DNA strand breaking. Antioxidant action involves suppressing the formation of ROS, scavenging the free radicals, and reducing agents and quenchers of singlet oxygen formation. The use of synthetic antioxidants must be under strict regulation due to potential health hazards. The search for new antioxidants as alternatives is, therefore, of great interest among researchers.

An amino acid is a type of organic acid that contains an acid functional group and an amine functional group on adjacent carbon atoms. Aspartic acid (H₂Asp) is one of two acidic amino acids. Aspartic acid plays an important role as general acids in enzyme active centers, as well as in maintaining the solubility and ionic character of proteins. Aspartic acid crystallized separately as d and l enantiomorphs under an ambient temperature [1]. Aspartic acid undergoes racemic transition at ambient conditions, which found application in chiral fields [2] parity non-conservation, chiral symmetry breaking [3], and cocrystal engineering [4]. The crystal structures of L-aspartic acid [5], DL-aspartic acid [6], DL-aspartic acid nitrate monohydrate [7], and Bis (DL-aspartic acid) sulfate [8] are well studied. Glutamic acid and its ions and salts are called glutamates. The acidic side chain of glutamic acid confers one negative charge under most conditions to proteins, in which this amino acid is found, thus increasing the water solubility of the protein.

Aminoguanidine has been one of the interesting species having versatile basicity. A great number of salts of aminoguanidine with different acids

have been studied. However, there is no report on aminoguanidinium salts of acidic amino acids in general and "glutamic and aspartic acids" in particular. Hence, our main scope and objectives are to prepare aminoguanidinium salts of amino acids and to characterize them using analytical, IR, and thermal studies, study the mode of thermal decomposition of aminoguanidinium salts, and characterize the antioxidants behavior of aminoguanidinium salts. The present research work has been taken up based on the above objectives and the results are discussed herein.

Experimental section

Preparation of aminoguanidinium aspartate and glutamate

The aminoguanidinium salts of aspartic acid and glutamic acid were prepared by mixing the respective acid with aminoguanidine bicarbonate in 1:1, 1:2, and 2:1 molar ratios and dissolved in 40 mL of distilled water. The resulting solutions were heated over water bath and pH was found to be 6.0, 6.79, and 7.03, respectively. Then, the volume is reduced to 20 mL and kept for crystallization at room temperature. After a few days, crystalline solids formed were separated out and washed with ethanol and air dried.

METHODS

All reagents and chemicals were of A. R grade and used without purification. Double-distilled water was used as a solvent for the synthesis and analysis throughout the experiment. Elemental analysis for C, H, and N was performed on a Vario-ELIII elemental analyzer. The IR spectra were recorded on a JASCO-4100 spectrophotometer as KBr pellets in the range of 400–4000 cm⁻¹. The simultaneous TG-DTA studies were under taken on a PerkinElmer SII thermal analyzer and the curves obtained in air using platinum cups as holders with ~ 3 mg of the samples at the heating rate of 10°C/min.

Antioxidant assays

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacities of different salts were estimated according to the procedure described by Pulido *et al.* [9]. FRAP reagent (2700 μL), prepared freshly and incubated at 37°C, was mixed with appropriate concentration of test sample whose total volume was 360 μL . A test tube with 360 μL of distilled water in place of the sample served as the blank. All the test tubes were incubated at 37°C for 30 min in a water bath. The FRAP reagent was prepared by mixing 2.5 mL of 20 mM TPTZ in 40 mM HCl, 2.5 mL of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and 25 mL of 0.3 M acetate buffer (pH-3.6). At the end of incubation, the absorbance of the blue color developed was read immediately at 593 nm against the reagent blank.

Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto *et al.* [10]. Initially, an aliquot of 300 μL of samples was taken into a series of test tubes. About 300 μL of distilled water taken in a test tube was considered as the blank. All the test tubes were added with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and vortexed well to mix the contents. The mouth of the test tubes was covered with foil and incubated in a water bath at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the mixture was measured at 695 nm against the reagent blank. Ascorbic acid (AA) was used as the reference standard and the results were expressed as milligrams of AA equivalents per gram salts.

RESULTS AND DISCUSSION

The compositions of the salts were fixed by estimating hydrazine moiety in aminoguanidine and the structural abstract is depicted

in Scheme 1. The analytical data of the prepared compounds are presented in Table 1 and are in good agreements with the proposed formula. Aspartic acid does not yield the dicationic salt, results viscous solution.

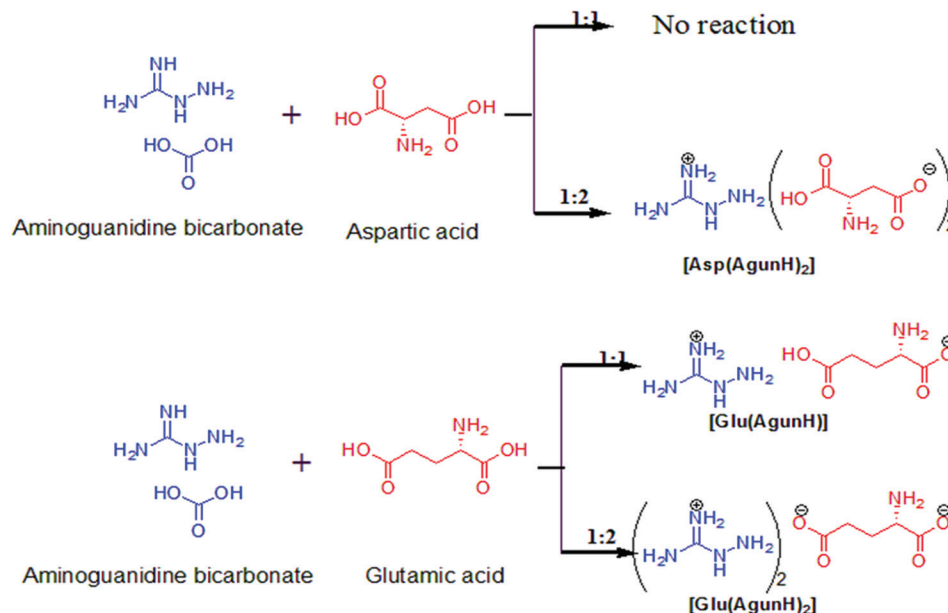
FT-IR spectra

The important IR bands of the acid, base, and their salts are listed in Table 2 including their band assignments. The FT-IR spectra of free acid, base (Figs. S1-S3), and their salts (Figs. 1-3) are given. The IR spectra show broad band in the region of 3450 cm^{-1} , due to O-H stretching. The peaks around 3300 and 1680 cm^{-1} are assigned to N-H and C=N (imines) stretching vibrations [11]. The bands in the region of 1590–1600 cm^{-1} and 1390–1350 cm^{-1} are assigned for asymmetric and symmetric stretching frequencies of the carboxylate ions, respectively [12]. The N-N stretching frequency has been observed around 1100 cm^{-1} which confirms the presence of aminoguanidine moiety.

Thermal studies

The thermal data of the compounds are listed in Table 3 and the thermograms are shown in Figs. 4-6. The composition of the intermediates and final product is those, which best fit with observed mass loss in the TG curve. The thermogravimetric results are in the good agreement with the DTA data. The thermogram of $[\text{Asp}(\text{AgunH})_2]$ shows two distinct endotherms at 136 and 175°C due to the continuous loss of carbon dioxide and two aminoguanidine itself. These results were best fit with observed weight loss. In the preceding step, the amino acid intermediate starts to decompose.

In $[\text{Glu}(\text{AgunH})]$, broad endotherm around 170°C resulting decarboxylation with the loss of aminoguanidine moiety forming corresponding amino acid, which further decomposes to give gaseous products. The diamminoguanidinium salts of glutamic acid show similar decomposition pattern as that of $[\text{Asp}(\text{AgunH})_2]$ around 178°C in



Scheme 1: Synthesis of salts

Table 1: Analytical data

Compound	Color	Solubility in water	Melting point (°C)	% of hydrazine	
				Observed	Calculated
$[\text{Asp}(\text{AgunH})_2]$	Orange crystals	Hot water	157	22.97	22.85
$[\text{Glu}(\text{AgunH})]$	White crystals	Cold water	171	14.74	14.48
$[\text{Glu}(\text{AgunH})_2]$	White crystals	Hot water	164	21.23	21.69

Table 2: FT-IR spectral data (in cm^{-1})

Compounds	$\nu_{\text{O-H}}$	$\nu_{\text{N-H}}$	$\nu_{\text{C=N}}$ (iminidine)	ν_{OCO} asym	ν_{OCO} sym	$\nu_{\text{N-N}}$
(AgunH)HCO ₃	-	3295	1660	-	-	1113
Aspartic acid	3410	3283	-	1706	-	-
[Asp(AgunH) ₂]	3440	3230	1680	1634	1352	1114
Glutamic acid	3270	3200	-	1700	-	-
[Glu(AgunH)]	3470	3250	1673	1590	1395	1146
[Glu(AgunH) ₂]	3450	3335	1670	1630	1353	1113

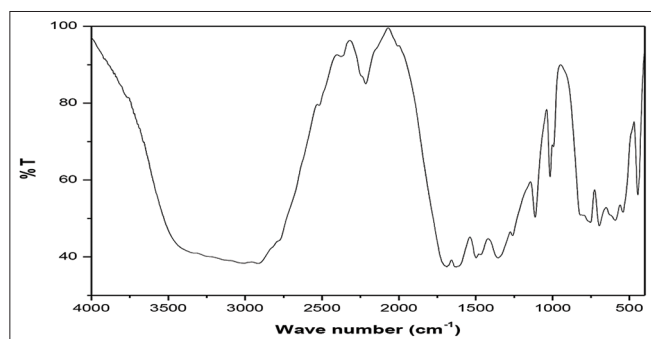


Fig. S1: IR Spectrum of Aminoguanidine bicarbonate

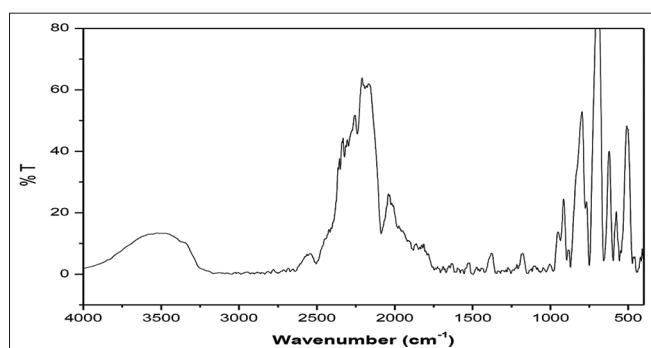


Fig. S2: IR Spectrum of Aspartic acid

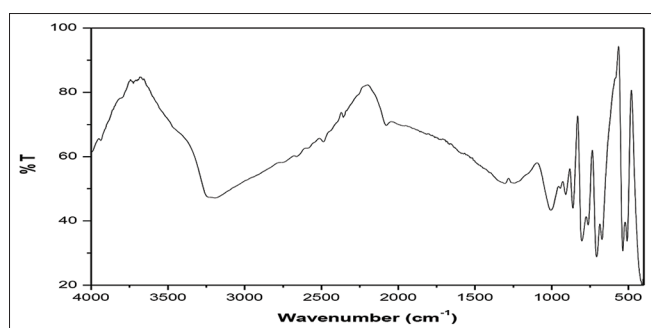


Fig. S3: IR Spectrum of Glutamic acid

DTA. Then, the remaining 35% of mass loss occur exothermally in the temperature range of 550–700°C.

Antioxidant studies

There are many *in vitro* methods to assess the antioxidant activity and depend on various generation of free radicals acting through different mechanisms to cover all aspects of antioxidant efficacy. Free radical scavenging activity and reducing capacities of newly synthesized

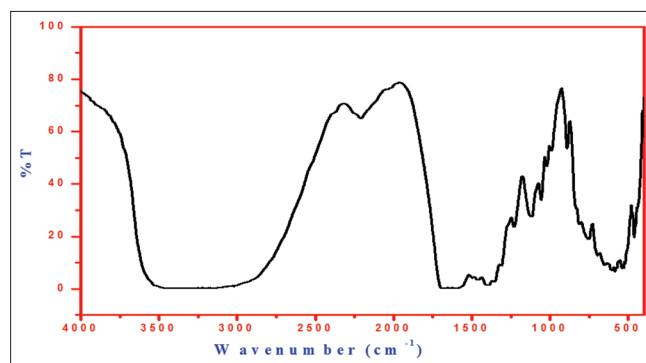
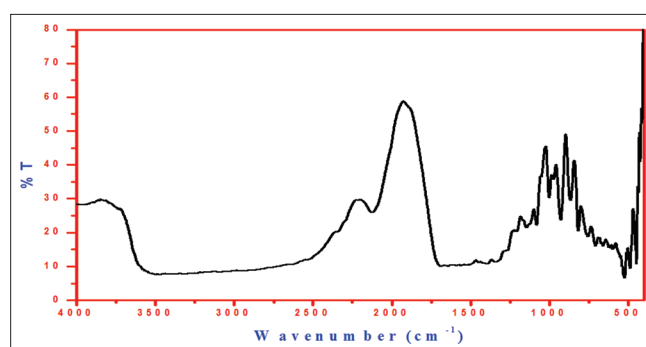
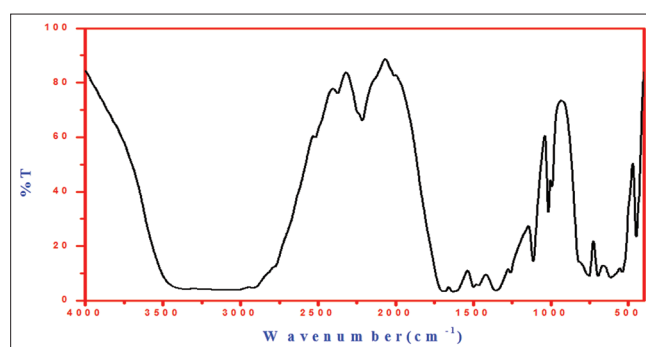
Fig. 1: FT-IR spectrum of [Asp(AgunH)₂]

Fig. 2: FT-IR spectrum of [Glu(AgunH)]

Fig. 3: FT-IR spectrum of [Glu(AgunH)₂]

compounds were confirmed by FRAP and phosphomolybdenum assay. The salts exhibit good antioxidant property is due to the presence of nitrogen content [13].

FRAP assay

FRAP assay is an inexpensive procedure which is simple, reproducible, and rapid that measures the ability of antioxidant compound to reduce the ferric ion Fe^{3+} to ferrous ion Fe^{2+} , as a measure of total antioxidant capacity. The scavenging activities are expressed as 50% inhibitory concentration (IC_{50}) values, which represent the concentrations of the compound used to scavenge 50% free radicals. The IC_{50} values for compounds and standard (natural AA) against free radicals are tabulated in Table 4.

Among the three salts, [Glu(AgunH)₂] has the highest antioxidant property comparable with that of standard AA. Our results showed significant ferric reducing power which indicated the hydrogen-donating ability of the extract.

Table 3: Thermal data

Compounds	DTA Peak temp (°C)	Thermogravimetry		Intermediates/end product	
		Temp. range (°C)	Mass loss %		
			Observed		Calculated
(AgunH)HCO ₃	(+)135 (+)175 (-)320	90-180 180-600	68.50 100.00	69.00 100.00	NH=C=NH Complete decomposition
[Asp(AgunH) ₂]	(+)136 (+)175 (-)634	100-200 200-700	70.00 100	69.28 100	Elimination of 2Agun and CO ₂ Complete decomposition
[Glu(AgunH)]	(+)174 (-)611	120-231 231-700	54.00 100	53.39 100	Elimination of Agun and CO ₂ Complete decomposition
[Glu(AgunH) ₂]	(+)178 (-)610	110.6-236 236-700	65.4 100	65.76 100	Elimination of 2Agun and CO ₂ Complete decomposition

(+): Endo, (-): Exo

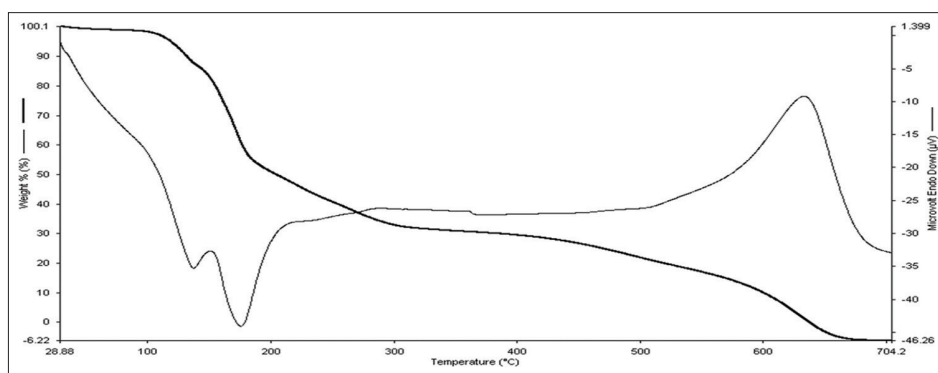


Fig. 4: Simultaneous TG - DTA of [Asp(AgunH)₂]

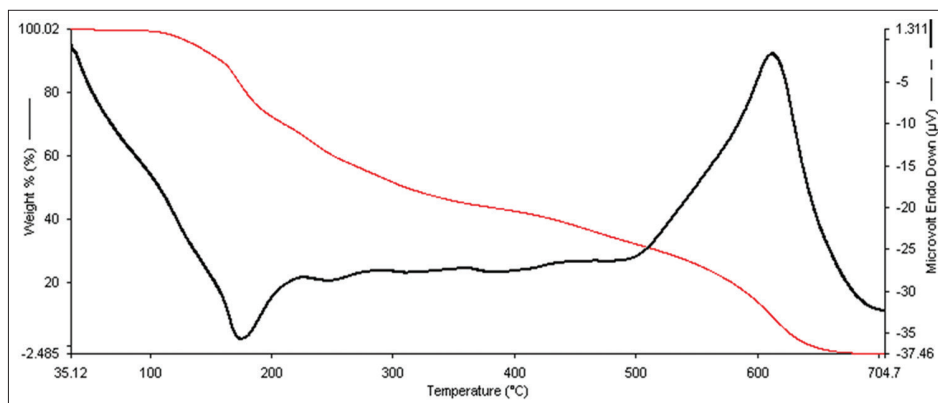


Fig. 5: Simultaneous TG - DTA of [Glu(AgunH)]

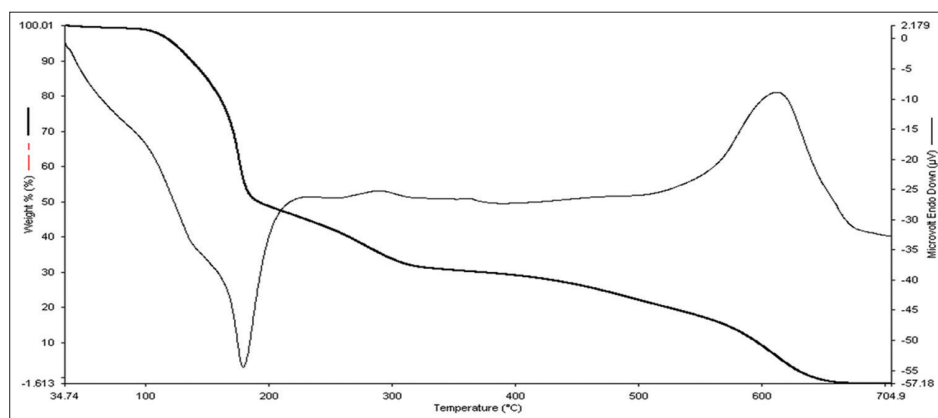


Fig. 6: Simultaneous TG - DTA of [Glu(AgunH)₂]

Table 4: Antioxidant activities of compounds and Vitamin C against radicals

Compounds	IC ₅₀ (μM)	
	Ferric reducing antioxidant power	Phosphomolybdenum
[Asp(AgunH) ₂]	43.98	108.89
[Glu(AgunH)]	58.01	90.18
[Glu(AgunH) ₂]	50.50	73.10
Vitamin C	52.45	75.59

Phosphomolybdenum assay

Phosphomolybdenum method is alternative to the methods available for the evaluation of antioxidant capacities due to its simplicity. This method is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by sodium sulfide. The obtained complex is oxidized by addition of nitrite and this causes a reduction in intensity.

CONCLUSION

The new aminoguanidinium salts of aspartic acid and glutamic acid have been prepared by the reaction of aqueous solution containing aminoguanidine bicarbonate with acidic amino acids. Aspartic acid forms bis-aminoguanidinium salt, whereas glutamic acid forms both mono- and bis-aminoguanidinium salts. The IR spectral data of the aminoguanidinium salts of aforesaid acids show N-N stretching frequencies in the region of 1202–1110 cm⁻¹ revealing the presence of aminoguanidinium moiety. The asymmetric and symmetric stretching frequencies of carboxylates are seen in the region of 1680–1670 and 1395–1352 cm⁻¹. The thermal studies of the salts revealed that all of them decompose in endo, followed by exothermic fashion, giving gaseous end products. The antioxidant properties of these salts were studied using FRAP and phosphomolybdenum assay. Results showed significant ferric reducing power which indicated the hydrogen-donating ability of the extract.

ACKNOWLEDGMENTS/FUNDING

The author is gratefully acknowledges the financial assistance received from the Department of Science and Technology, New Delhi, India (Grant No. SR/WOS[A]/CS-42/2013).

AUTHORS' CONTRIBUTIONS

The study was designed and executed by S. Parveen and Dr. S. Govindarajan. All the experimental section and analysis section were

performed by them also. Editing and reviewing were done by Dr. D. Manikandan and Dr. P. A. Periasamy.

CONFLICTS OF INTEREST

There are no conflicts to declare.

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