

Synthesis and Properties of Some Basic Metals and Manganese Salts of *cis*-1,2-Cyclohexane dicarboxylic Acid

ÖZLEN ALTUN*, MURAT TÜRKYILMAZ, GÜLARA HÜSYİNOVA†, AYSEGÜL ÇERKEZKAYABEKİR‡ and ADILHAN FEYİZOĞLU

Department of Chemistry, Faculty of Science, Trakya University, 22030 Edirne, Turkey
E-mail: ozlenaltun@yahoo.com

In this work, sodium, potassium, ammonium and manganese salts of *cis*-1,2-cyclohexane dicarboxylic acid were synthesized. The structural characteristics of these salts were analyzed by thermo graphic and spectroscopic methods and examined on liver and wheat under the effect of their biological activities. According to these results, they can be used as a micro-fertilizer in the fields Biology and Agriculture.

Key Words: *cis*-1,2-Cyclohexane dicarboxylic acid, Biological activities, Electron microscope, Liver, Wheat, Manganese.

INTRODUCTION

Low molecular weight cyclic organic acids are liquids with disturbing odour and high viscosity, however high molecular weight cyclic ones are either liquids with light odour and low viscosity or solids. They are soluble in many organic solvents^{1,2}. Literature survey suggest that many works about *cis*-1,2-cyclohexane dicarboxylic acid can be seen in previous years^{3,4}. For example, Hunsdiecker reaction of silver salts of *cis*- and *trans*-1,2-cyclohexane dicarboxylic acid was investigated by Abell⁵. The molecular packing was affected by the shape of polycarboxylate molecules in the case of phthalate, *cis*-1,2-cyclohexane dicarboxylate and 1,1-cyclohexanediacetate. This observation has been reported by Kamino *et al.*⁶. Koster *et al.*⁷ examined hyperbranched synthetic polyesteramides were synthesized by the polycondensation of the trifunctional diisopropanolamine and dysfunctional anhydrides of succinic acid, glutaric acid, *cis*-1,2-cyclohexane dicarboxylic acid and phthalic acid.

We started this research to make potassium, sodium and ammonium salts with *cis*-1,2-cyclohexane dicarboxylic acid and then synthesized manganese salt of same acid, proved their formations by elemental analysis, IR, ¹H NMR and atomic absorption spectrophotometer and for manganese salt by TG/DTA as well. Finally we examined on liver under the effect of these salts investigated by electron microscope and investigated the effect on wheat and found that it can be used as micro-fertilizer.

†Department of Pathology, Faculty of Medicine, Trakya University, 22030 Edirne, Turkey.

‡Department of Biology, Faculty of Science, Trakya University, 22030 Edirne, Turkey.

EXPERIMENTAL

cis-1,2-Cyclohexane dicarboxylic acid (Aldrich), glutaraldehyde (Aldrich), Osmium tetroxide (Aldrich) and diethyl ether (Merck) were all organically reagent grade, MnSO₄ (Merck), NH₃ (Merck), KOH (Merck) and NaOH (Merck) were analytical reagent grade.

The ¹H NMR spectra were carried out in CDCl₃ on a Bruker, DPX-400 spectrometer, IR spectra in the range 4000-400 cm⁻¹ were determined in KBr on a IR-470 Shimadzu spectrometer. Atomic absorption measurements were recorded a Unicam model 929 atomic absorption spectrometer with air/acetylene was used for flame measurements. The yellow cathode lamp for manganese was operated at 12 mA, the wavelength for manganese was set at 279.5 nm and slits width at 0.2 nm for Mn. TG/DTA measurements were made at N₂ atm and programe rate 10 °C min on a chromel/alumel model appliance. The elementel analyses for carbon hydrogen and nitrogen were carried out by the Laboratories ODTU of Turkey. All pH measurements were obtained using a calibrated Metrohm 654 digital pH meter with a sensorex combination pH electrode recorder 120 C. The pH meter was calibrated daily before using pH of 4 and 7 Metrohm AG/CH 9100 Hersau Buffers. Liver experiments were investigated on a Zeiss EM-9 and JEM-100B Electron Microscope and wheat experiments were recorded on a JENWAY 6105 UV Spectrophotometer.

Synthesis of potassium, sodium and ammonium salts of *cis*-1,2-cyclohexane dicarboxylic acid: The *cis*-1,2-cyclohexane dicarboxylic acid in diethyl ether and KOH in water solutions were taken in the amounts according to the stoichiometric ratio. Previously it was found that pH range of the reaction was *ca.* 7, reaction temperature 35-40 °C and reaction time 0.5 h. Aqueous phase was removed and washed with diethyl ether, evaporated slowly and identified as potassium salt of *cis*-1,2-cyclohexane dicarboxylic acid (*cis*-1,2-potassium cyclohexane dicarboxylate) was obtained. Experiments were repeated with NaOH and NH₃ water solutions. All the products are white powder and they are soluble in water.

(For potassium salt of *cis*-1,2-cyclohexane dicarboxylic acid (*cis*-1,2-potassium cyclohexane dicarboxylate), Yield 92 %. Elemental analysis (%): C 38.15 (38.70), H 3.98 (4.03). ¹H NMR data in CDCl₃ at room temperature (methyl protons) δ: 1.20-1.22 (m, 4H), 1.70 (m, 2H), 1.89 (m, 2H), 2.33 (m, 2H). IR (KBr, ν_{max}, cm⁻¹): 2944 (CH), 1674-1568 (carboxyl anions), 1450 (CO).

For sodium salt of *cis*-1,2-cyclohexane dicarboxylic acid (*cis*-1,2-sodium cyclohexane dicarboxylate), Yield 90 %. Elemental analysis (%): C 43.58 (44.40), H 4.23 (4.62). ¹H NMR data in CDCl₃ at room temperature (methyl protons) δ: 1.21-1.23 (m, 4H), 1.70 (m, 2H), 1.90 (m, 2H), 2.34 (m, 2H). IR (KBr, ν_{max}, cm⁻¹): 2878 (CH), 1738-1550 (carboxyl anions), 1475 (CO).

For ammonium salt of *cis*-1,2-cyclohexane dicarboxylic acid (*cis*-1,2-ammonium cyclohexane dicarboxylate), Yield 86 %. Elemental analysis (%): C 46.33 (46.60), H 8.29 (8.74), N 12.71 (13.59). $^1\text{H NMR}$ data in CDCl_3 at room temperature (methyl protons) δ : 1.20-1.22 (m, 4H), 1.70 (m, 2H), 1.89 (m, 2H), 2.33 (m, 2H). IR (KBr, ν_{max} , cm^{-1}): 2944 (CH), 1705-1558 (carboxyl anions), 1400 (CO).

Synthesis of manganese salt of *cis*-1,2-cyclohexane dicarboxylic acid: The sodium salt *cis*-1,2-cyclohexane dicarboxylic acid in water and MnSO_4 solution in water were refluxed in the amounts according to the stoichiometric of reaction at 85-90 °C within 1 h. At the end of the reaction, solid brown manganese salt 1,2-cyclohexane dicarboxylic acid was filtered and washed with distilled water several times to remove water soluble material. Remaining solid residue kept under vacuum giving brown solid identified as manganese salt of *cis*-1,2-cyclohexane dicarboxylic acid (manganese cyclohexane dicarboxylate). ($^1\text{H NMR}$ data of manganese salt 1,2-cyclohexane dicarboxylic acid in CDCl_3 at room temperature (methyl protons) δ : 1.19-1.20 (m, 4H), 1.67 (m, 2H), 1.86 (m, 2H), 2.31 (m, 2H). IR (KBr, ν_{max} , cm^{-1}): 2944 (CH), 1593-1532 (carboxyl anions), 1436-1398 (CO). Atomic absorption (mg/mL): 11.72.

Liver experiments: Mice species called Bulb/C Albino was chosen. Samples were classified into four groups with 3 mice at each. It was estimated that tap water for the first group (control) (Fig. 1), 0.1 % potassium salt of *cis*-1,2-cyclohexane dicarboxylic acid (0.1 g potassium salt of *cis*-1,2-cyclohexane dicarboxylic acid in 100 mL water) in for the second group (Fig. 2), 0.1 % sodium salt of *cis*-1,2-cyclohexane dicarboxylic acid (0.1 g sodium salt of *cis*-1,2-cyclohexane dicarboxylic acid in 100 mL water) in for the third group (Fig. 3) and 0.1 % ammonium salt of *cis*-1,2-cyclohexane dicarboxylic acid (0.1 g ammonium salt of *cis*-1,2-cyclohexane dicarboxylic acid in 100 mL water) in for the fourth group (Fig. 4) were used to water them. The mice killed by the servical dislocassion⁸ at the end 10 days and were received liver of its. Tissue fragments were fixed with 4 % glutaraldehyde and 1 % osmium tetroxide and dehydrated. Then they were blocked Epon 812. Taken thin sections were investigated on electron microscope (Zeiss EM-9 and JEM-100B)⁹. Since manganese salt of *cis*-1,2-cyclohexane dicarboxylic acid couldn't solved in water, it wasn't included animal experiment.

Wheat experiments: Wheat species called Bezostia are chosen. Samples are classified in five groups each with 25 seeds. For the 1st group (control), water, for the other groups (samples), our solutions were used to wet them. They are kept in a sterilizer for 9 days at 25 °C. The size of the wheat was measured at the end 9 days. Since wheat gave green leaves after 9 days of growth, we performed measurements just after 9 days is completed to test the germination progress^{10,11}. α -Amylase activity values for the germination of the wheat were determined according to the dextrogenic method. This method reported by Pfueller *et al.*¹² is based on the reaction of iodine with starch. Activity measurements were realized spectrophotometrically (JENWAY 6105 UV), monitoring the colour change in the enzyme

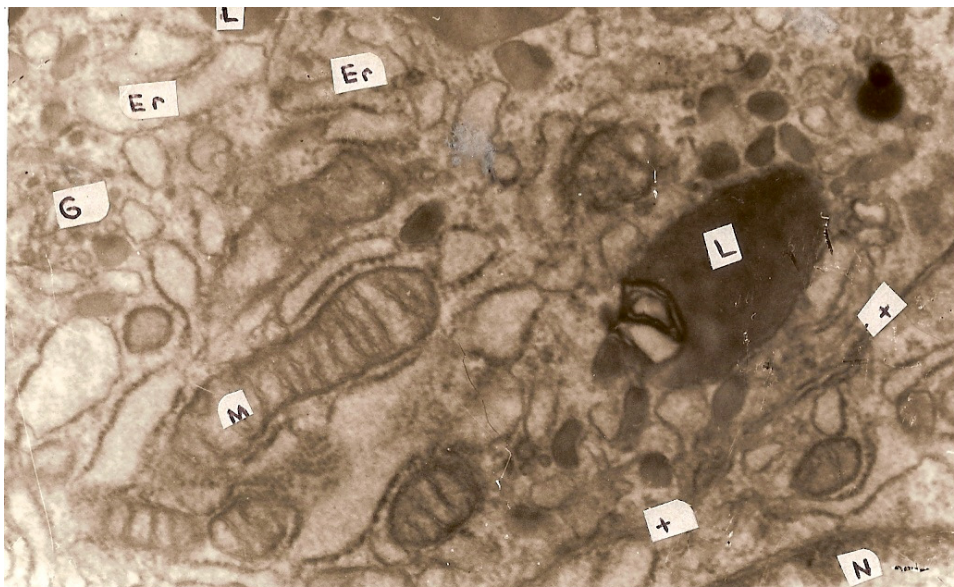


Fig. 1. Effect of control group; The parts are seen of two liver cell nucleus (N) is regular and outside membranes of liver cell (+) are normal. There are mitochondria (M), endoplasmic reticulum (Er), free ribosomes (R) and glycogen (G) in the cytoplasm

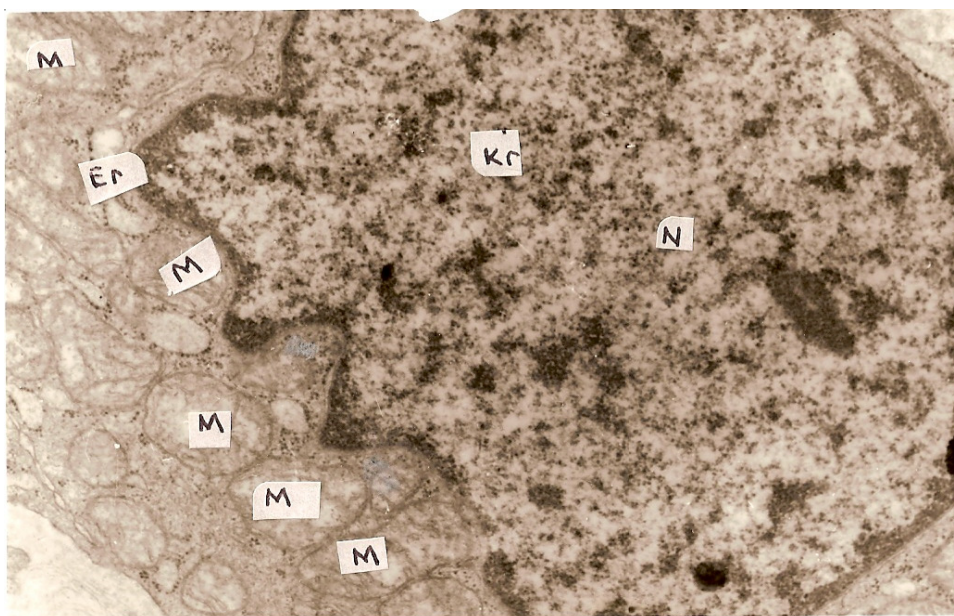


Fig. 2. Effect of *cis*-1,2-potassium cyclohexane dicarboxylic acid; Hypertrophic increase and hyperplastic swelling are seen in the cytoplasm of hepatocytes in liver. Projections are obtained in nucleus (N). Thin chromatin is seen in nucleoplasm

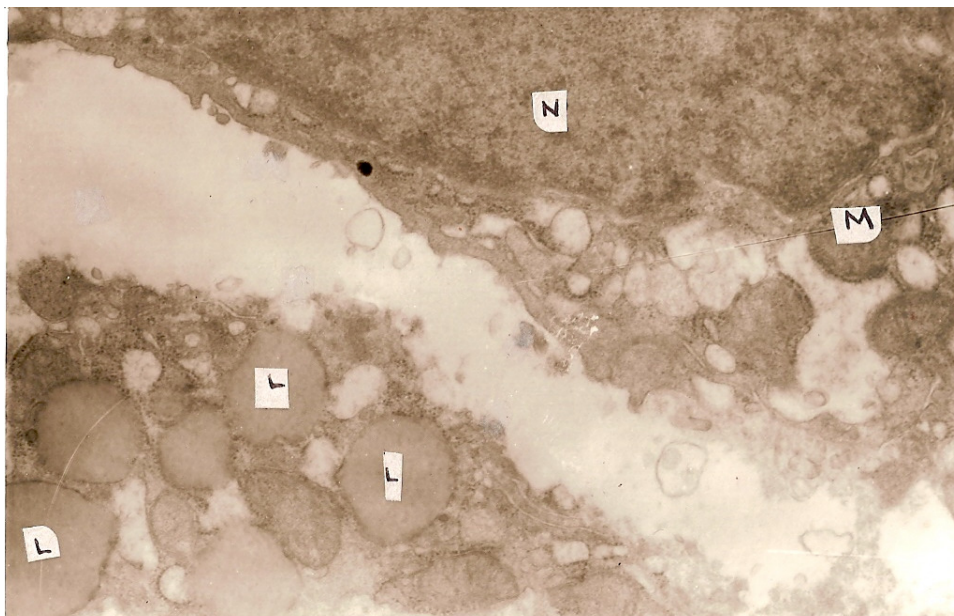


Fig. 3. Effect of *cis*-1,2-sodium cyclohexane dicarboxylic acid; The parts are seen of two liver cell Nucleus (N) are become impolite. Endoplasmic reticulum (Er) is broadened in cytoplasm. Lipids (L) are increased. Defects are obtained outside membrane of liver cell

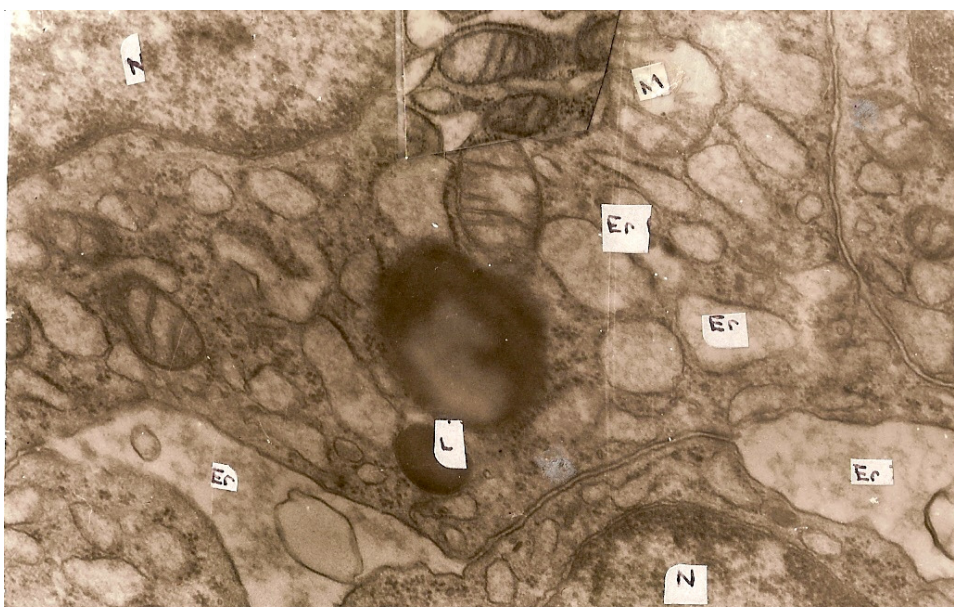


Fig. 4. Effect of *cis*-1,2-ammonium cyclohexane dicarboxylic acid; Endoplasmic reticulum (Er) is broadened in cytoplasm. Mitochondria (M) are spoiled and outside membranes are dried

substrate mixture. Heights and enzyme activities of wheat in the presence of sodium, potassium and ammonium cyclohexane dicarboxylate were demonstrated in Table-1. The maximum heights was seen at 0.027 % concentration. Heights and enzyme activities of wheat in the presence of sodium, potassium and ammonium cyclohexane dicarboxylate at 0.027 % concentration were shown in Table-2 and in Table-3, high and enzyme activities of wheat in the presence of manganese cyclohexane dicarboxylate are demonstrated.

TABLE-1
HEIGHT OF WHEAT IN THE PRESENCE OF SODIUM, POTASSIUM AND
AMMONIUM *cis*-1,2- CYCLOHEXANE DICARBOXYLIC ACID

Samples	High (cm)			
	Control	<i>cis</i> -1,2-Sodium cyclohexane dicarboxylate	<i>cis</i> -1,2-Potassium cyclohexane dicarboxylate	<i>cis</i> -1,2-Ammonium cyclohexane dicarboxylate
Control	9.2			
0.003 %		10.3	10.0	10.0
0.009 %		11.0	10.2	10.0
0.015 %		11.2	10.2	10.1
0.021 %		11.4	10.3	10.2
0.027 %		12.0	10.6	10.4

TABLE-2
HEIGHT AND ENZYME ACTIVITIES OF WHEAT IN THE PRESENCE OF SODIUM,
POTASSIUM AND AMMONIUM *cis*-1,2-CYCLOHEXANE DICARBOXYLIC
ACID AT 0.027 % CONCENTRATION

Samples	Height (cm)	Enzyme activity (v/mL)
Control (seed + water)	9.2	5.36
<i>cis</i> -1,2-Sodium cyclohexane dicarboxylate	12.0	5.66
<i>cis</i> -1,2-Potassium cyclohexane dicarboxylate	10.6	5.37
<i>cis</i> -1,2-Ammonium cyclohexane dicarboxylate	10.4	5.40

TABLE-3
HEIGHT AND ENZYME ACTIVITIES OF WHEAT IN THE PRESENCE OF
MANGANESE *cis*-1,2-CYCLOHEXANE DICARBOXYLIC ACID

Samples	Height (cm)	Enzyme activity (v/mL)
Control (seed + water)	9.0	5.29
Manganese cyclohexane dicarboxylate	10.4	5.40

RESULTS AND DISCUSSION

In this study, the synthesis and the properties of salts of *cis*-1,2-cyclohexane dicarboxylic acid with potassium, sodium, ammonium and manganese were studied. Spectroscopic methods NMR and IR showed the possible structure of these salts.

For the manganese salt synthesis, two methods were employed, % yields in both methods were found fairly close. The localization of the central cation in the complex molecule was determined by thermogravimetric analysis.

Thermogravimetric analysis of manganese salt of *cis*-1,2-cyclohexane dicarboxylic acid indicated, two steps for decomposition and also the amount of water in the composition of the molecule. Experimental thermolysis of it was given in Table-4 and Fig. 5.

TABLE-4
THERMOGRAVIMETRIC ANALYSIS DATA OF
MANGANESE-CYCLOHEXANE DICARBOXYLATE

Compound	First step decomposition		Second step decomposition		MnO ₂ (%)
	T _b -T _c (°C)	Weight loss (%)	T _b -T _c (°C)	Weight loss (%)	
Manganese cyclohexane dicarboxylate	0-181	4.0	306-724	56.5	39.5

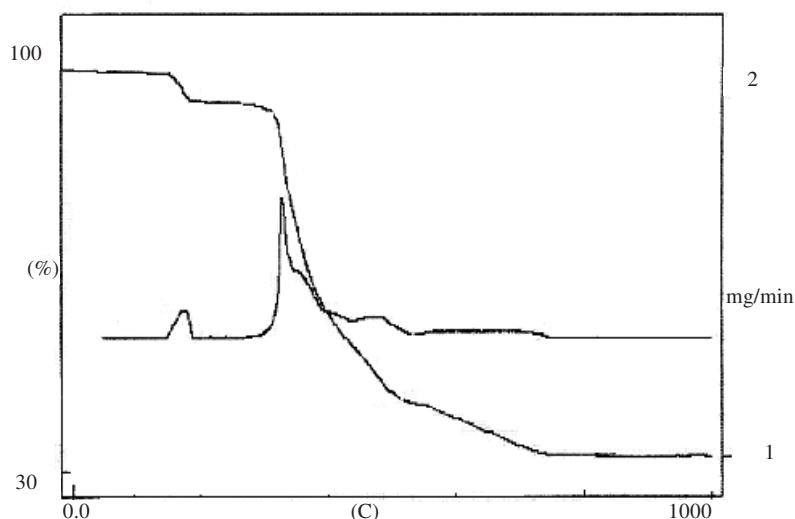
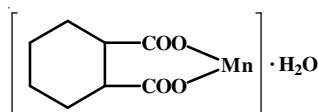


Fig. 5. TG/DTA spectrum of manganese cyclohexane dicarboxylic acid

The decomposition of two carboxyl groups can be monitored from the thermogram. Up to 181 °C, a water molecule leaves the compound and then between 306-724 °C, organic component was decomposed. The remaining compound over 724 °C was determined as MnO₂. After these observations we concluded that the structure of manganese salt was [Mn(C₈H₁₀O₄)]·H₂O. The structural formula of the compound is shown below:



According to the results of animal experiments, in control group there wasn't any changing in liver but ultra structural and functional activities were observed by the effect of potassium salt of *cis*-1, 2-cyclohexane dicarboxylic acid and dystrophic changes were occurred in hepatocytes and functional activities of liver were decreased by the effect of sodium and ammonium salts of *cis*-1,2-cyclohexane dicarboxylic acid.

According to the results of wheat experiments, sodium, potassium, ammonium and manganese salts of *cis*-1,2-cyclohexane dicarboxylic acid, it was found that compounds were effective on the growth of the plant as a biologically active reagent and they had no toxicologic effect on the wheat and activity of α -amylase increased. As a result of this activity increase, the height of the plant also increased.

Conclusion

In summary, we obtained sodium, potassium, ammonium and manganese salts of *cis*-1,2-cyclohexane dicarboxylic acid and determined the physical and chemical properties and structural characteristics for these carboxylates.

As a result of this work, when we used sodium, potassium and ammonium salts of *cis*-1,2-cyclohexane dicarboxylic acid on the wheat, it is shown that wheat growing was increasing because of the average of α -amylase activitie values and also the other advantage of their non-toxic properties. On the other hand, we investigated on liver of white mice under the effect of these salts by using Zeiss EM-9 and JEM-100B Electron Microscope and found that ultra structural and functional activities were followed in liver by the effect of potassium salt of *cis*-1,2-cyclohexane dicarboxylic acid and functional activities were decreased in liver by the effect of sodium and ammonium salts of *cis*-1,2-cyclohexane dicarboxylic acid.

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