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Infra red and Gas Chromatogram - Mass Spectral studies of Colonial Ascidian *Ecteinascidia venui* Meenakshi, 2000

[1] Sankaravadivu S*, [2] Jothibai Margret R and [3] Meenakshi VK.

[1] Department of Chemistry, V.O.Chidambaram College, Tuticorin, Tamil Nadu, India.

[2] Department of Chemistry, Pope's College, Sawyerpuram, Tuticorin, Tamil Nadu, India.

[3] Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin, Tamil Nadu, India.

*Corresponding Author: E-Mail: sankarisankaran86@gmail.com

ABSTRACT

Ascidians are rich source of bioactive compounds. *Ecteinascidia venui* is a colonial ascidian belonging to the family Perophoridae. The ethanolic extract of *Ecteinascidia venui* was subjected to Infrared (IR) spectral and Gas Chromatogram-Mass Spectral (GC-MS) studies. IR spectral studies indicate the presence of hydroxyl, carbonyl group and aromatic ring. GC-MS study showed 12 prominent peaks with retention time 10.38, 14.26, 14.34, 16.96, 18.71, 19.39, 19.46, 19.73, 21.13, 22.14, 22.52, 23.90 indicating the presence of 12 compounds 1-(2-Ethyl-3-cyclohexenyl)ethanol, (E,E)-methyl ester of 9,12-Octadecadienoic acid, (E)-methyl ester of 9-Dodecenoic acid, 9-Octadecenal, (Z)6,(Z)9-Pentadecadien-1-ol, (Z,Z)-9,12-Octadecadien-1-ol,13-methyl-Oxacyclotetradecane-2,11-dione, Eicosane, Tetra-decyloxirane, (R)-(-)-14-Methyl-8-hexadecyn-1-ol, Nonadecane, 1-Iodo-2-methylundecane.

Key words: *Ecteinascidia venui,* GC-MS studies, IR spectral studies.

1. INTRODUCTION

Ascidians are exclusively marine animals, occurring in all oceans, with more than 2800 described species [1]. They may be solitarian or constitute social groups of individuals connected by the base or be compound (colonial) with many colonial zooids embedded in a gelatinous matrix sharing the external tunic [2]. Ascidians, commonly known as "sea squirts" (Subphylum: Urochordata, Class: Ascidiacea) are the most diverse class of the phylum Tunicata. The phylum is characterized by an outer coat of cellulose-like material (the tunic) synthesized by the epidermis and exhibits striking biological activity with more than 130 natural products being isolated from them [3]. They mostly possess nitrogen-bearing metabolites, particularly aromatic heterocycles like peptides, alkaloids and amino acid derived products but also nitrogenous compounds in lesser amounts such as lactones, terpenoids or quinones [4,5]. A number of bioactive natural products have been obtained from ascidians coming from shallow as well as deep sea floors such as Palmerolide A, a group of ecdysteroids, meridianins, aplicyanins and rossinones [6,7,8&9]. Antitumour activity is exhibited by the Tetrahydro-isoquinoline alkaloid - 'Ecteinascidin 743' isolated from the ascidian *Ecteinascidia turbinata* [10]. Hence the objective of the present investigation is to identify the possible chemical constituents in the Indian colonial ascidian *Ecteinascidia venui* with the aid of IR spectral and GC-MS studies

2. MATERIALS AND METHODS

2.1. Collection of animal material

Ecteinascidia venui (Family: Perophoridae) was collected from Tuticorin coast in the month of May 2013 by SCUBA diving (Plate 1). Molluscan shell, calcrete rock fragments attached to the colony was carefully removed. They were identified using key to identification of Indian ascidians [11]. A voucher specimen AS 2247 has been submitted in the ascidian collection of museum of the Department of Zoology, A. P. C. Mahalaxmi College for Women, Tuticorin – 628002, Tamilnadu, India.

2.2. Preparation of extract

The whole colony was dried in shade and homogenized to get a coarse powder which was extracted with ethanol, concentrated in a rotary evaporator under reduced pressure. 2 µl of the extract of *Ecteinascidia venui* was employed for IR spectral studies and GC-MS studies [12].

2.3. Instruments and chromatographic conditions

2.3.1. Infra Red spectral studies

Infra red spectral studies were made for the dried ethanol extract. One mg of finely powdered extract was mixed with about 100 mg of dried potassium bromide (IR grade) powder. The mixture was then pressed in a special dye to yield a transparent disc. The disc was then held in the instrument beam for spectroscopic examination and the resulting IR spectrum was recorded. The following conditions were employed; Perkin Elmer Model spectrum RXI; Range 4000nm-400nm; Resolution 4; Transmittance test mode.

2.3.2. Gas Chromatogram-Mass Spectral studies

GC-MS studies were carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite -1 fused silica capillary column (30 × 0.25 mm 1D × 1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 ev; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 El was employed (split ratio of 10:1) injector temperature 250°C; ion source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 ev; a scan interval of 0.5 s and fragments from 40 to 550 Da.

2.4. Identification of compounds

Interpretation on mass spectrum of GC-MS were conducted using the data base of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown compounds was compared with the spectrum of the known stored in the NIST library. The Name, Molecular weight and Structure of the compounds of the test materials were ascertained.

3. RESULTS AND DISCUSSION

Infrared spectrum for ethanolic extract of *Ecteinascidia venui* is shown in Figure 1 which indicated a broad band at 3409.83 cm^{-1} due to the presence of moisture or hydroxyl group in the compound. The band at 1632.09 cm^{-1} is characteristic of carbonyl group and that at 1412.10 cm^{-1} shows the presence of C-H bending [13]. Presence of peaks above 3000 cm^{-1} indicates aromaticity and C-H stretching.

Plate - 1: Colony of *Ecteinascidia venui* Meenakshi, 2000

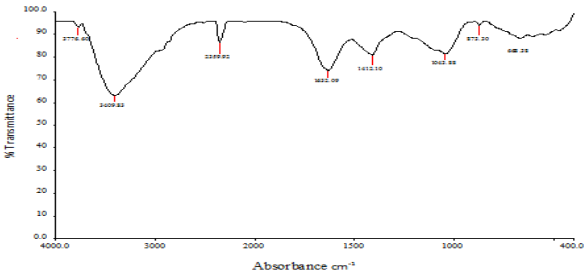

Figure - 1: IR spectrum for *Ecteinascidia venui*

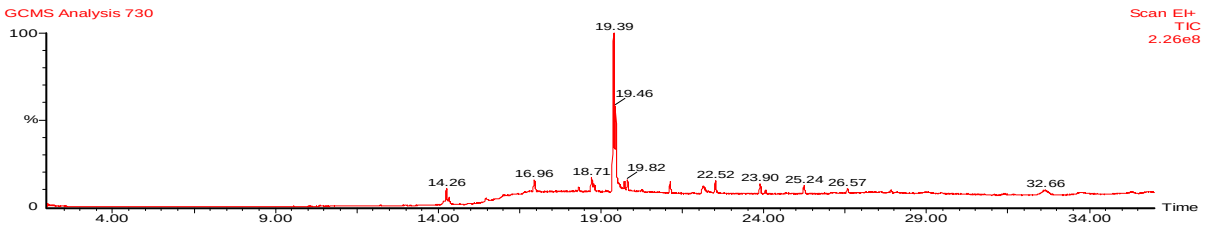

Figure - 2: GC-MS Chromatogram of the ethanolic extract of *Ecteinascidia venui*

GC-MS Chromatogram of the ethanolic extract of *Ecteinascidia venui* showed 12 prominent peaks indicating the presence of twelve compounds which is given in Table 1. The mass of these compounds are presented in Figures 2 to 14. The mass spectra of these compounds were compared with those of the compiled data for known compounds. The peak with retention 10.38 corresponds to 1-(2-Ethyl-3-cyclohexenyl)ethanol, 14.26 to (E,E)-methyl ester of 9,12-Octadecadienoic acid, 14.34 to (E)-methyl ester of 9-Dodecenoic acid, 16.96 to 9-Octadecenal, 18.71 to (Z)6,(Z)9-Pentadecadien-1-ol, 19.39 to (Z,Z)-9,12-Octadecadien-1-ol, 19.46 to 13-methyl-Oxacyclotetradecane-2,11-dione, 19.73 to Eicosane, 21.13 to Tetradecyloxirane, 22.14 to (R)-(-)-14-Methyl-8-hexadecyn-1-ol, 22.52 to

Nonadecane and 23.90 to 1-Iodo-2- methylundecane.

Table - 1: Chemical compounds identified in the ethanolic extract of *Ecteinascidia venui*

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	10.38	1-(2-Ethyl-3-cyclohexenyl)ethanol	$C_{10}H_{18}O$	154	0.21
2	14.26	Methyl ester of (E,E)-9,12-Octadecadienoic acid	$C_{19}H_{34}O_2$	294	5.35
3	14.34	Methyl ester of (E)-9-Dodecenoic acid	$C_{13}H_{24}O_2$	212	1.94
4	16.96	9-Octadecenal	$C_{18}H_{34}O$	266	14.74
5	18.71	(Z)6,(Z)9-Pentadecadien-1-ol	$C_{15}H_{28}O$	224	5.01
6	19.39	(Z,Z)-9,12-Octadecadien-1-ol	$C_{18}H_{34}O$	266	37.07
7	19.46	13-methyl-Oxacyclotetradecane-2,11-dione	$C_{14}H_{24}O_3$	240	21.36
8	19.73	Eicosane	$C_{20}H_{42}$	282	1.73
9	21.13	Tetradecyloxirane	$C_{16}H_{32}O$	240	2.53
10	22.14	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	252	4.76
11	22.52	Nonadecane	$C_{19}H_{40}$	268	2.78
12	23.90	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296	2.53

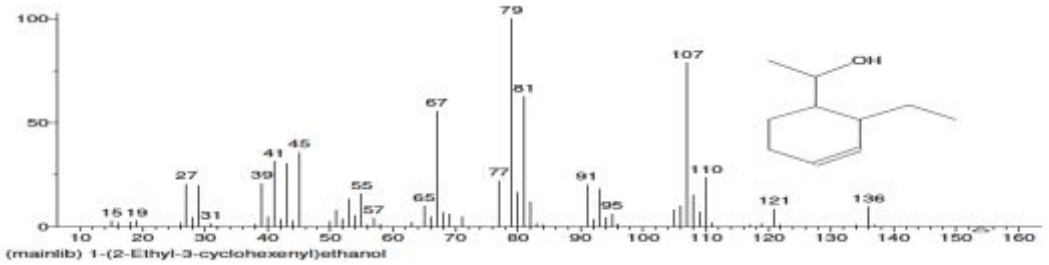

Figure - 3: Mass spectrum of 1-(2-Ethyl-3-cyclohexenyl) ethanol (RT :10.38)

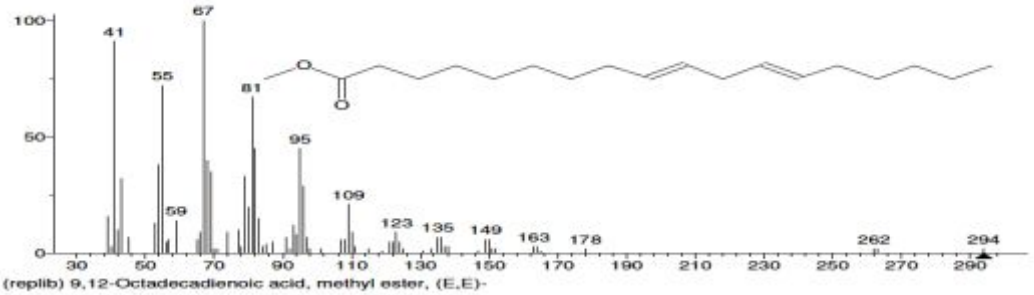

Figure - 4: Mass spectrum of (E,E)-methyl ester of 9,12-Octadecadienoic acid (RT:14.26)

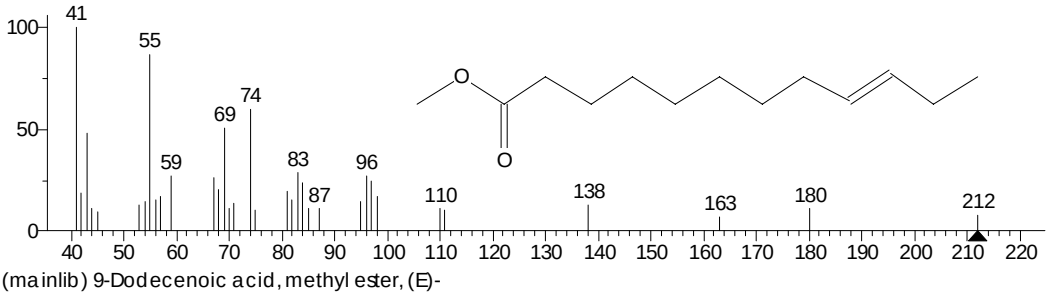

Figure - 5: Mass spectrum of (E)-methyl ester of 9-Dodecenoic acid (RT:14.34)

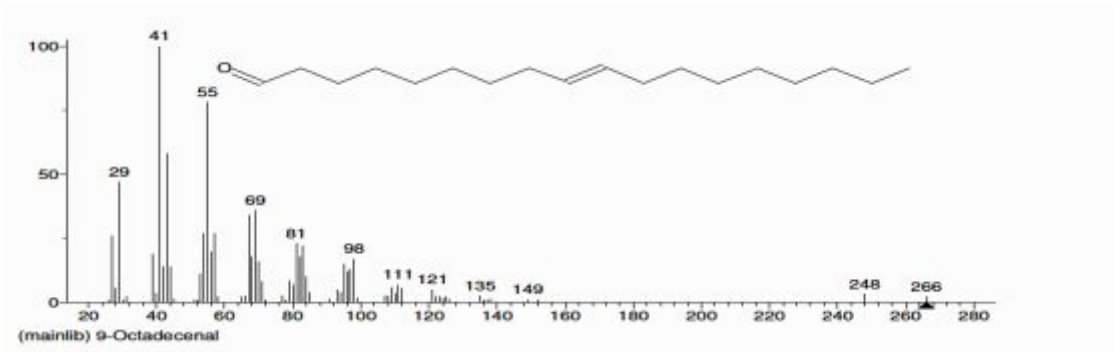

Figure - 6: Mass spectrum of (E)-methyl ester of 9-Octadecenal (RT:16.96)

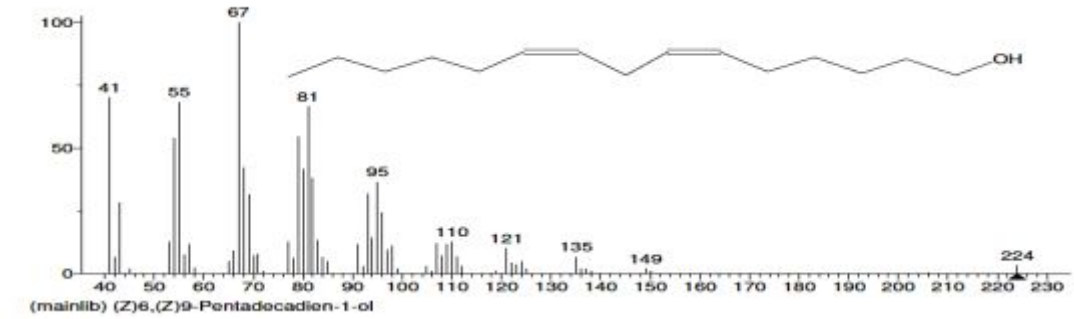

Figure - 7: Mass spectrum of Z(6),Z(9)-Pentadecadien-1-ol (RT:18.71)

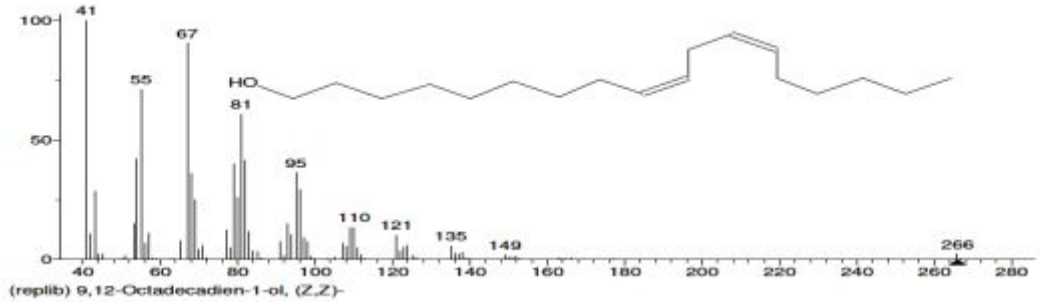

Figure - 8: Mass Spectrum of (Z,Z)-9,12-Octadecadien-ol (RT:19.39)

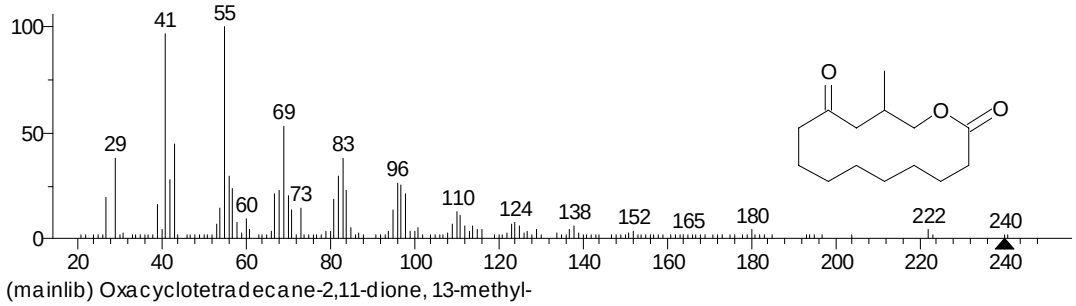

Figure - 9: Mass spectrum of 13-methyl-Oxacyclotetradecane-2,11-dione (RT:19.46)

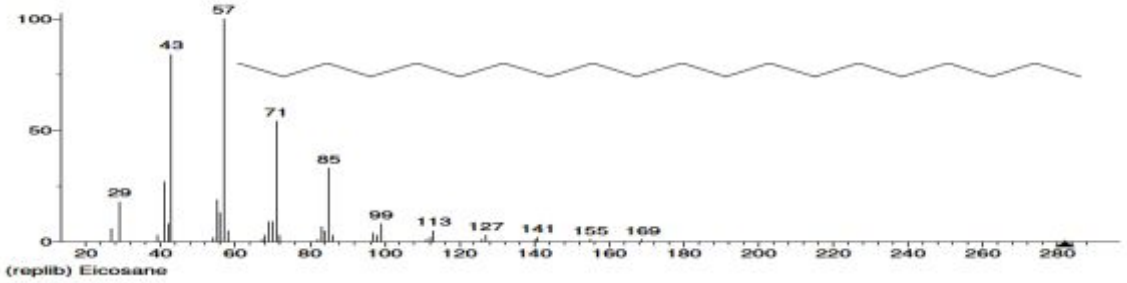

Figure - 10: Mass spectrum of Eicosane (RT:19.73)

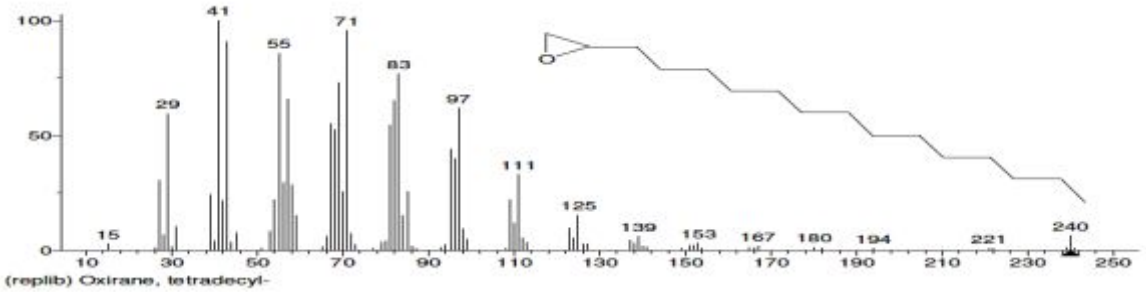

Figure - 11: Mass spectrum of Tetradecyloxirane (RT:21.13)

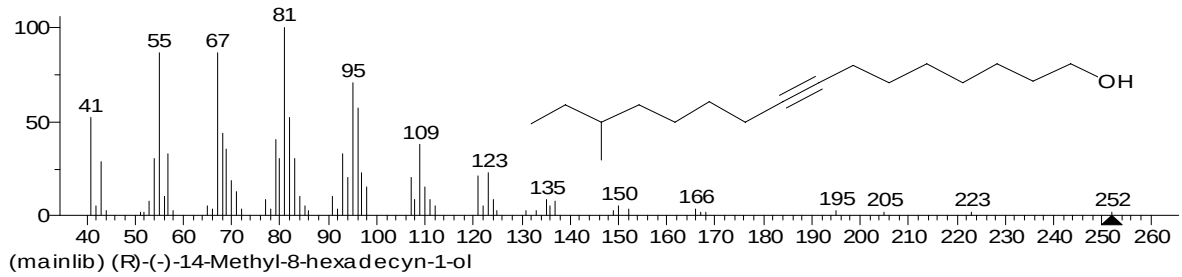

Figure - 12: Mass spectrum of (R)-(-)-14-Methyl-8-hexadecyn-1-ol (RT:22.14)

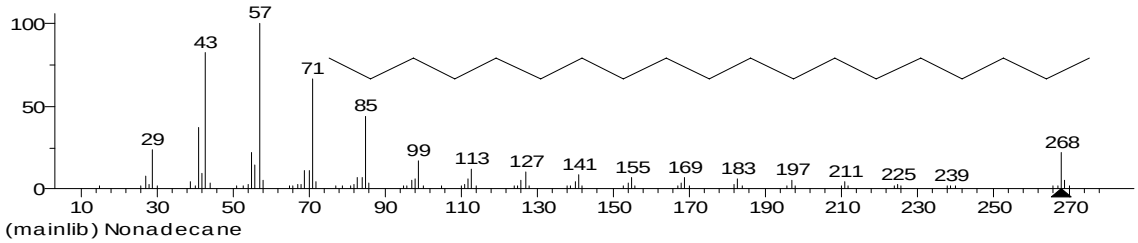

Figure - 13: Mass spectrum of Nonadecane (RT:22.52)

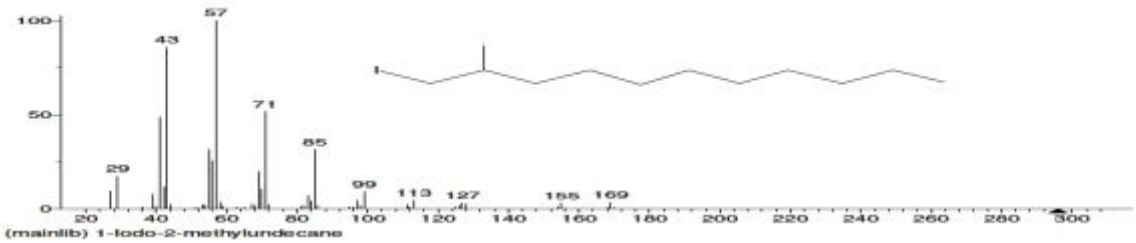

Figure - 14: Mass spectrum of 1-Iodo-2-methylundecane

4. CONCLUSION

The study clearly indicates that the ethanolic extract of *Ecteinascidia venui (remove bold)*is rich in many biochemical compounds. Infrared and Gas chromatogram mass spectral (GC-MS) studies are highly effective and best method for pharmaceutical research. It can be stated that these 12 chemical constituents have not been reported elsewhere from ascidians especially from *Ecteinascidia venui* and hence can be considered as first report to the world.

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