

Short Communication

N-methylpyrrolidone: Isolation and characterization of the compound from the marine sponge *Clathria frondifera* (class:Demospongiae)

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An investigation of the chemical composition of the marine sponge *Clathria frondifera* resulted in the isolation of the compound N-methylpyrrolidone which is widely used in the petrochemical, microelectronics fabrication, paint and pharmaceutical industry. This is the first report on the isolation and structural characterization of N-methylpyrrolidone from a marine source. The compound was purified by column chromatography and analyzed by TLC. The functional groups were analyzed by FTIR spectrum. The structure of the compound was established by spectroscopic analysis (UV, ^1H and ^{13}C NMR) and elucidated with the aid of COSY, HMQC, and HMBC experiments.

[**Key words:** *Clathria frondifera*, N-methylpyrrolidone, NMR, sponge]

Since last two decades, research into marine natural products has expanded rapidly. The oceans are a source of a large group of structurally unique natural products that are mainly found from invertebrates such as sponges, tunicates, bryozoans, and molluscs¹. Among all marine organisms screened, marine sponges produce the largest number of structurally diverse natural products, and in fact represent the single best source of marine bioactive compounds^{2,3}. These compounds are secondary metabolites produced by the sponges. The roles that these metabolites play in sponge biology remain largely unknown⁴. Secondary metabolites of sponges may act to inhibit biofouling and overgrowth^{5,6}, inhibit the growth of microorganisms⁷, prevent damage caused by UV radiation⁸, or act as allelopathic agents⁹⁻¹¹. Compounds from marine sponges have been identified for their anti-cancer¹², anti-bacterial¹³, anti-fungal¹⁴, anti-viral¹⁵ and anti-inflammatory¹⁶ properties. In this report N-methylpyrrolidone is described for the first time from a marine sponge *Clathria frondifera* (family Microcionidae).

The sponge *Clathria frondifera* (order, Poecilosclerida; family, Microcionidae) was

collected at a depth of 30 m, off Nagapattinam along the east coast of India on 30 June. The specimen was stored in MeOH and transported to laboratory for extraction. The sponge (60 g) was homogenized and re-extracted twice with MeOH (600 ml) at room temperature and filtered using Whatman #1 filter paper. The MeOH extract was concentrated under vacuum (60°C; 2 hours) to yield a residue of 6 g dry weight.

The residue 6 g dry weight was subjected to successive fractionation and purification by column chromatography (Silica gel 60-120) using CHCl_3 , CHCl_3 -MeOH and MeOH. The fractions eluting with 90 % CHCl_3 /MeOH were pooled and analyzed by TLC (Silica gel 60 F₂₅₄; 20X20 cm; 0.25 mm thick; CHCl_3 /MeOH solvent system). Commercially available N-methylpyrrolidone (NMP), obtained from Merck®, was used as the standard. Comparative TLC (CHCl_3 /MeOH solvent system) of the crude extract (5 µg), purified fraction from the sponge (1 µg) and NMP (0.5 µg) was performed.

The purified fraction was scanned in the range of 190-1100 nm with a spectrophotometer (UNICAM UV 300). To analyze the functional groups of the compound, FTIR spectrum was recorded with a resolution of 4 cm^{-1} , auto gain and an average of 40 scans in the frequency range of 4,000–500 cm^{-1} (Shimadzu FTIR-820 IPC). A dried purified sample

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of 2 mg was used for FTIR measurement. The purified fraction was dried and 15 mg was dissolved in CDCl_3 and analyzed by ^1H NMR and ^{13}C NMR spectroscopy (Bruker Avance 300 spectrometer). Chemical shifts are expressed in δ -values (ppm) using the solvent as internal reference. The structure was determined using COSY, HMBC and HMQC.

Marine bacteria isolated from biofilms on data buoys deployed along the Indian coast were used to study the antibacterial activity of the compound. The taxonomic identification of the bacterial isolates was confirmed using the Microbial Identification System (Hewlett-Packard, Palo Alto, Calif.). Anti-bacterial studies were carried out by disc diffusion in agar-plated petri dishes¹⁷ against the four strains *Pseudomonas fluorescens*, *Flexibacter* sp., *Cytophagus* sp., and *Flavobacterium* sp.. Whatman #1 filter paper discs were loaded with solutions of the purified compound in MeOH to give a concentration of 500 μg per disc. Commercial NMP was also loaded to filter paper discs and used for comparison with purified compound. The discs were placed on Zobell marine agar (pH 7.3) plates seeded with different strains of bacteria selected for the study. Plates were incubated for 24 h at room temperature ($28^\circ\text{C} \pm 1^\circ\text{C}$) and monitored for inhibition zones. The assay was performed in triplicate. Solvents alone were used as negative controls.

Cytotoxicity screening of the compound was done using brine shrimp (*Artemia salina*) lethality assay

method^{18,19}. *Artemia salina* larvae were hatched from cysts, by incubation in filtered seawater for 24 h at 28°C . The purified compound was dissolved in seawater to give different extract solutions with concentrations of 2, 1, 0.5, 0.25 and 0.125 mg/ml. Extracts and hatched *Artemia salina* larvae were transferred to multiwell plates (10 larvae/well). Each assay was carried out in triplicate. Plates were incubated for 24 h at 28°C . The number of dead and living larvae were recorded for each concentration and percentage of mortality was calculated.

Sponge *Clathria frondifera* was extracted with MeOH and 6 g of residue collected after extraction was chromatographed onto a silica column to yield 15 mg of N-methylpyrrolidone. Comparative TLC of the purified fraction with commercially available NMP confirmed the presence of the compound N-methylpyrrolidone in the sponge extract (Figs. 1,2). The R_f values of NMP and the compound were found to be 0.625. The UV spectrum showed maximum absorption at 206 nm.

^1H -NMR (CDCl_3 , 300 Mhz) data revealed two doublets at $\delta = 2.30$ and 3.30 ppm, a triplet at $\delta = 1.95$ ppm and a singlet at $\delta = 2.77$ ppm (Table 1). ^{13}C -NMR (CDCl_3 , 75 MHz) revealed 5 signals, one at $\delta = 175.1$ ppm, three at $\delta = 17.5$ ppm, $\delta = 30.5$ ppm, $\delta = 49.3$ ppm and one at $\delta = 29.2$ ppm indicating presence of one carbonyl, three $-\text{CH}_2-$ and a $-\text{CH}_3$ carbons. ^1H - ^1H COSY spectrum showed coupling of the signals at $\delta = 1.95$ and $2.3, 3.3$ and 2.77 ppm.

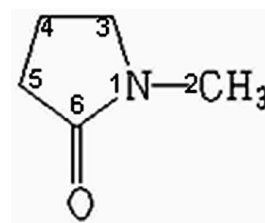


Fig. 2 — Structure of N-methylpyrrolidone

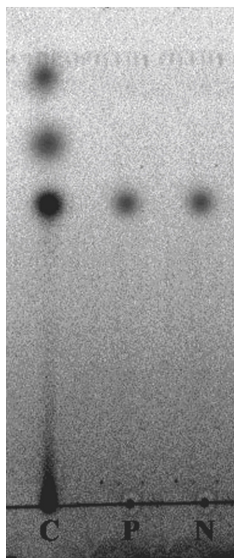


Fig. 1—Comparative TLC. C—Crude extract; P—N-methylpyrrolidone from the sponge; N— Commercial NMP

Table 1— ^1H (CDCl_3 , 300 Mhz) and ^{13}C (CDCl_3 , 75 Mhz) NMR data for N methylpyrrolidone

$^{13}\text{C}^a$ ppm	^1H (δ , m, J Hz)	COSY	Position
175.1			6
49.3	3.30, d	H-5	5
30.5	2.30, d	H-4, H-5	4
29.2	2.77, s	H-2, H- 3	3
17.5	1.95, t	H-2, H-3, H-4	2

^a ^{13}C NMR assignments supported by HMQC, DEPT 135° NMR experiments

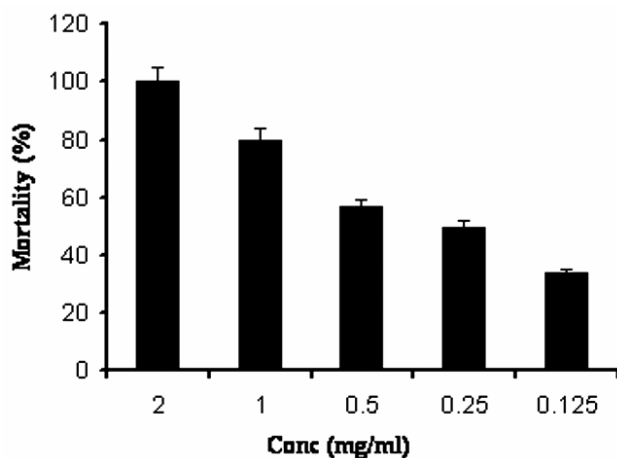


Fig. 3—Cytotoxic activity of N-methylpyrrolidone from the sponge in different concentrations against brine shrimp (*Artemia salina*)

Analysis of HMQC spectrum revealed the pairing of H and C atoms. C-H correlations were investigated by the HMBC spectrum. The presence of methyl alkane and carbonyl group was confirmed by characteristics absorptions (2925.44 and 1667.62 cm^{-1}) in the IR spectra. Moreover NMR of the commercially available and the purified compounds were compared which also confirms that the isolated compound was N-methylpyrrolidone (data not shown).

The purified compound N-methylpyrrolidone was evaluated for its anti-bacterial activity at different concentrations against marine bacterial isolates. The compound did not exhibit any significant antibacterial activity. Commercial NMP also did not exhibit any antibacterial activity. Cytotoxicity of the compound to brine shrimp larvae was observed at concentrations as low as 0.125 mg/ml with 50% mortality at 0.25 mg/ml (Fig. 3).

While many pharmacologically active substances have been characterized from sponges belonging to the genus *Clathria*²⁰⁻²⁴, this is the first report on the isolation of NMP in the sponge, *Clathria frondifera*. Commercially synthesized NMP is used as a solvent for many coatings, including polyurethanes, printing inks, epoxy resins, polyamidimide-based wire enamels, and water-based coatings. The role of the compound in the sponge is yet to be explored. However, the present study is the first report of N-methylpyrrolidone (Fig. 2) from a natural source. Sterol was isolated from *Clathria lissosclera* of the related species²⁵. The study also indicates that the sponge *Clathria frondifera* can be further studied in search for bioactive and unusual molecules.

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