

Chemical Constituents of the Red Alga *Acanthophora spicifera*

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Abstract

Analysis of the petroleum-ether and chloroform extracts of the marine red alga *Acanthophora spicifera* led to the isolation of a sterol, cholesterol, fatty acids, stearic, palmitic, behenic (C₂₂) and arachidic acids (C₂₀) and a fatty ester, methyl palmitate, identified on the basis of their chemical and spectroscopic characteristics.

Introduction

Naqvi *et al.* (1980) reported that petroleum ether and chloroform extracts of the red alga, *Acanthophora spicifera* (Vahl) Børgesen exhibit promising (100%) antifertility activity. Herein we report the isolation of fatty acids, a steroid and a fatty ester from these active fractions.

This red alga is also known to possess *in vitro* anti microbial activity against *S. aureus*, *C. albicans* and *Mycobacterium smegmatis* (Burkholder *et al.* 1960).

Material and Methods

Algal material collected from the Goa coast was dried and powdered. About 3 kg of this powder was extracted thrice with 90% aqueous methanol at room temperature. The extract was concentrated at reduced pressure and then fractionated into petroleum-ether, chloroform, n-butanol and water soluble fractions. The petroleum-ether soluble fraction, after chromatography over silica gel and elution with petroleum ether, ether and acetic acid successively, gave three major components designated as A, B and C, one from each of the eluents. Similarly, the chloroform fraction after chromatography over silica gel and elution with petroleum ether : ethyl acetate mixture (9 : 1) yielded two compounds designated as D and E. All these compounds were purified by repeated

chromatography over silica gel with eluents of increasing polarity.

Antifertility testing

Adult female and male albino rats maintained in air-conditioned quarters under standard husbandary conditions were used. The animals were mated by the mass mating technique and vaginal smears of the female rats were taken every morning. The days on which spermatozoa were seen in the vaginal smears were designated as day 1 of pregnancy. The algal extracts were macerated with equal quantities of gum acacia and suspended in distilled water to achieve the desired dose. The suspension was introduced into the lower part of the oesophagus by a feeding needle. The control rats received a mixture of gum acacia and distilled water in a similar manner. The extract was administered daily for 7 days from day 1 to 7 post-coitum. The rats were laparotomized on day 10 of pregnancy and the number of corpora lutea and implantations was recorded. The crude methanolic extract prevented implantation in 100% of the animals at a dose of 200 mg/kg body weight and the petroleum-ether and chloroform fractions showed 60% anti-implantation activity at a dose of 50 mg/kg of body weight whereas the remaining n-butanol and water soluble fractions were found inactive at the same dose. None of the compounds isolated so far from the petroleum-ether fractions seems to exhibit the antifertility activity.

Results

Compound A

It has been identified as an aliphatic ester (0.001% of dry wt of seaweed), methyl palmitate. It was obtained as a liquid, IR-spectra of which showed bands at 1730, 1000, 1110, 1160, 1180 cm^{-1} . ^1H NMR spectra at 200 MHz (CDCl_3) showed a triplet at 0.9 δ (3H, $-\text{CH}_3$ group of aliphatic chain), multiplet centered at 1.3 δ (24H, 12 $-\text{CH}_2$ -groups of the aliphatic chain) multiplet centered at 1.6 δ (2H, β protons in the acid portion of the molecule) triplet centered at 2.3 δ (2H, α -protons in the acid portion of the molecule) and a singlet at 3.68 δ (3H, $-\text{CH}_3$ group in the alcohol portion of the molecule). Mass spectrum showed molecular ion peak at m/e 270 corresponding to the molecular formula $\text{C}_{17}\text{H}_{34}\text{O}_2$, a base peak at m/e 74, characteristic of straight chain methyl esters, which arises by Mc Lafferty rearrangements. Besides, there were also fragments of the general formula $[(\text{CH}_2)_n \text{COOCH}_3]^+$ indicated by m/e 87, 143, 199, 185, 213 and 227 ($M^+ - 43$).

Compound B

It is a steroid cholesterol (0.0012% of dry wt of seaweed) obtained as colourless platelets having a m. p. 146 $^\circ\text{C}$ and answered tests characteristic of sterols. It formed an acetyl derivative with a m. p. 113 $^\circ\text{C}$. The sterol was identified as cholesterol. IR spectra showed bands at 3430, 2952, 2870, 1475, 1380 and 1070 cm^{-1} . ^1H NMR spectra bands at 0.7 δ , 0.88 δ , 1.0 δ , 1.25 δ , 2.32 δ , 3.45 δ , 5.35 δ and 7.76 δ . Mass spectrum showed the molecular ion peak at m/e 386. Its identity was confirmed by determination of the mixed m. p. with an authentic sample.

Compound C

It is an aliphatic acid m. p. 69 $^\circ\text{C}$ stearic acid, characterised as its methyl ester (0.1% of dry wt of seaweed), obtained as a colourless solid to which was assigned the molecular formula $\text{C}_{18}\text{H}_{36}\text{O}_2$, based on the mass spectra of the acid ($M^+ - 284$) and its methyl ester ($M^+ - 298$). The mass spectrum showed base peak at m/e 74 formed by Mc Lafferty rearrangement. The mass spectrum also showed the separation of most of

the fragments by 14 mass units. IR spectra displayed characteristic peaks found for saturated long chain acids. The ^1H NMR spectra (60 MHz) of its ester provided confirmatory evidence regarding its paraffinic nature. The $-\text{CH}_2$ -methylene protons appeared as a singlet at 1.2 δ (28 H), $-\text{CH}_3$ group of aliphatic chain appeared at 0.84 δ multiplet centered at 2.2 δ (4 H) was assigned to the α and β protons in the acid portion of the molecule and singlet at 3.4 δ corresponding to the $-\text{CH}_3$ group in the alcohol portion of the molecule.

Compound D

It is also an aliphatic acid, palmitic acid, m. p. 63 $^\circ\text{C}$ (0.003% of dry wt of seaweed). The molecular wt determination by mass spectrometry gave the molecular formula as $\text{C}_{16}\text{H}_{32}\text{O}_2$ ($M^+ 256$). IR spectra showed bands at 3420, 2875, 2858, 2835, 1700, 1325, 1295, 1270, 1248, 1230, 1210 and 1185 cm^{-1} . ^1H NMR spectra of its methyl ester displayed signals at the same position as in the spectra of methyl stearate the only exception being that the singlet at 1.2 δ corresponded to (24 H) as compared to the (28 H) in the ^1H NMR spectra of methyl stearate.

Compound E

This substance was obtained as colourless platelets the mass spectrum of which showed that the sample contains a peak at m/e 312 (arachidic acid), m/e 340 (behenic acid) in addition to the peak at 256 due to the molecular ion of palmitic acid. However, these substances seem to be present only in trace amounts.

To our knowledge this is the first report on the chemical constituents of this alga.

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References

- Burkholder, P. R., M. L. Burkholder and L. R. Almodovar. 1960. Antibiotic activity of some marine algae of Puerto Rico. *Bot. Mar.* 2: 149-156.
- Naqvi, S. W. A., Solimabi, S. Y. Kamat, L. Fernandes, C. V. G. Reddy, D. S. Bhakuni and B. N. Dhawan. 1980. Screening of some marine plants from the Indian coast for biological activity. *Bot. Mar.* 24: 51-55.