Acta Pharmaceutica Sciencia
49: 127-132 (2007)

Hordenine amount in *Phyllophora nervosa* (D.C.Grev) (Marine Alga) collected from Şile (the Black Sea) and Dardanelle

Phyllophora nervosa (D.C. Grev) (deniz algi) Şile (Karadeniz) ve Çanakkale boğazı örneğinde hordenin miktarı

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Abstract

In our earlier work, hordenine, an alkaloid was first time extracted from *Phyllophora nervosa* collected from Şile in 1969. In this work, this alkaloid was extracted in the same alga collected from Şile and Dardanelle and the amount of hordenine was determined by GC/MS analysis. The hordenine content was 39.66 µg/g for Şile and 1.15 mg/g for Dardanelle sample.

Key words: *Phyllophora nervosa,* hordenine, Şile, Dardanelles

Introduction

Hordenine (anhaline) is N, N dimethyltryamine. It is an alkaloid phenylalkyl group. It was found in terrestrial plants and also marine algae. It was isolated first from *Phyllophora nervosa* (D.C. Grev) (Gigartinales, Phyllophoraceae) [new name *Phyllophora crispa* (Hudson) P.S. Dixon] collected from Şile (Black Sea coast of Turkey) by Guven et al. (1969,1970), later in *Ahnfeltia paradoxa* (Gigartinales, Phyllophoraceae) (Kawauchi and Sasaki, 1978) and in *Gigartina stellata* (Gigartinales, Gigartinaceae) (Barwel and Blunden, 1981). Later the same result was published by Barwel et al. (2006) in the name of *Mastocarpus stellatus* (synonym of *Gigartina stellata*) (Levring, Hoppe and Schmid, 1969).

Hordenine is diuretic and a remedy for diarrhea and dysentery. Its extraction is important in the diagnosis of phaeochromocytoma. Hordenine as a positive inotropic effect upon the heart, increases systolic and diastolic blood pressure, peripheral blood volume, inhibits gut movement. All effects are short and only possible after high doses.

In this paper is reported the hordenine amount in *Phyllophora nervosa* collected in the Black Sea and Dardanelle.

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Material and Methods

The algae were collected from Şile in November 2006 and from Soğandere, Dardanelle in April 2007. The sampling stations are shown in figure 1.

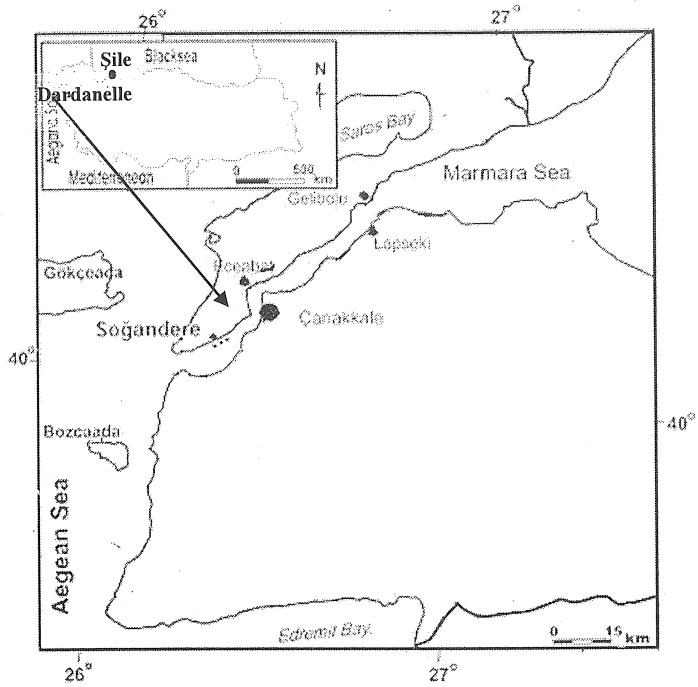

Figure 1. The map of collection area of *Phyllophora nervosa* Şile, the Black Sea (a) and Soğandere, Dardanelle (b).

The algae were dried in air, grounded and extracted firstly with ethanol (90%), and then acetone in reflux for 4 h.

The extracts were filtered, combined and distilled in rotary evaporator at 40°C. The residue was extracted with dichloromethane (DCM). The aqueous part was alkalinized with ammonia and then extracted 2 times with DCM and the extract was distilled at 36°C. The residue was dissolved in ethylacetate-methanol (1:1, v:v) and applied to GC/MS analysis.

GC/MS analysis: GC (HP 6890) coupled to mass spectrometer HP 5972 A. A split/splitless injector was used, injection; 2 µl, split time: 1 min^{-1}, flow 45 ml min^{-1}. Column; HP-PONA: 50 m x 0.2 mm x 0.5 µm, The injector temperature was maintained at 280°C. The GC temperature programme was: from 40°C to 280 °C at 8 °C min^{-1}. The carrier gas was helium, flow rate 1 ml min^{-1}.

Reference compound: Hordenine sulphate was purchased from Merck. All solvents and other chemicals are also Merck products.

Hordenine base was obtained from hordenine sulphate. It was dissolved in distilled water and alkalinized with ammonia solution (5:95, v:v), then extracted with DCM dried over anhydrous sodium sulphate, filtered and distilled at 36^0C. The residue was dissolved in ethylacetate-methanol (1:1, v:v) and applied to GC/MS for purity control.

Quantitative analysis of hordenine:

Calibration curve of hordenine was plotted in a concentration of 50, 100, 150, 200, 250 µg/g in ethyl acetate/methanol 1:1 (v/v). The equation of calibration curve was calculated from the peaks area on GC/MS chromatograms.

Results and Discussion

Calibration curve of hordenine and its equation are shown in Fig. 2.

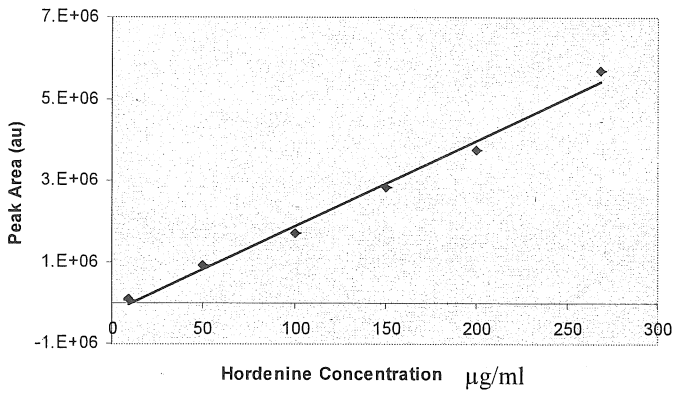

Figure 2. Calibration curve of hordenine.

The linear relationship could be fitted with the following equation:

$A = 21119 \times C - 237095$ $r^2 = 0.9911$ (Eq. 1)

A: area, C: concentration (µg/ml)

Rt of hordenine is 19.07 min.

The chromatogram of hordenine reference compound and obtained from *Phyllophora nervosa* are shown in Figures 3.1, 3.2 and 3.3 respectively.

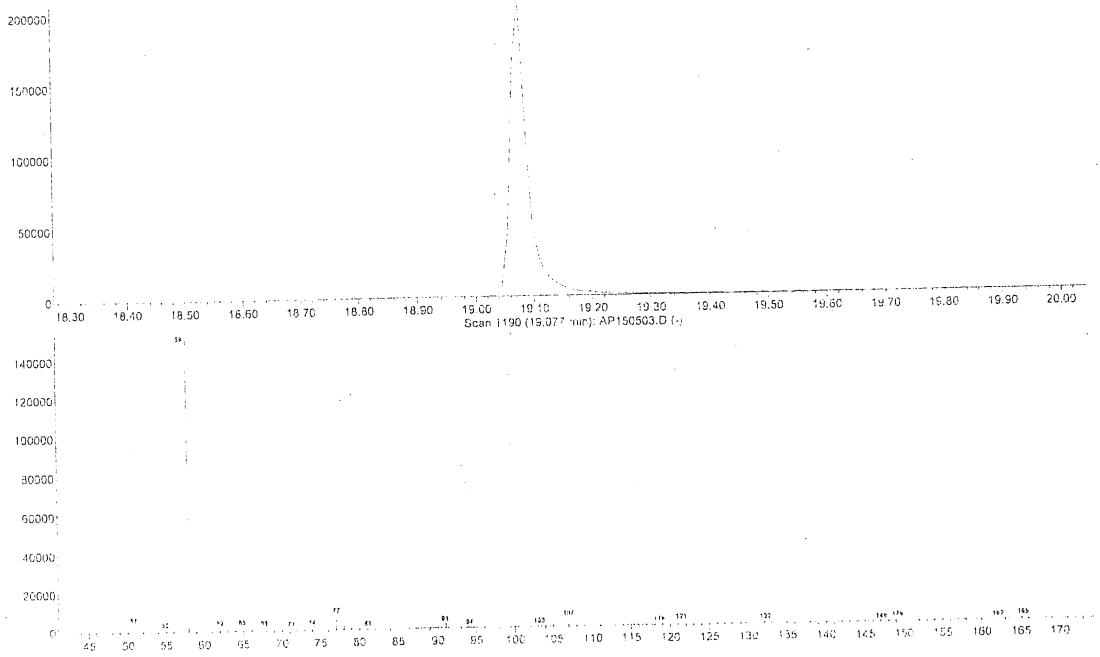

Figure 3.1. The chromatogram and spectrum of hordenine reference compound.

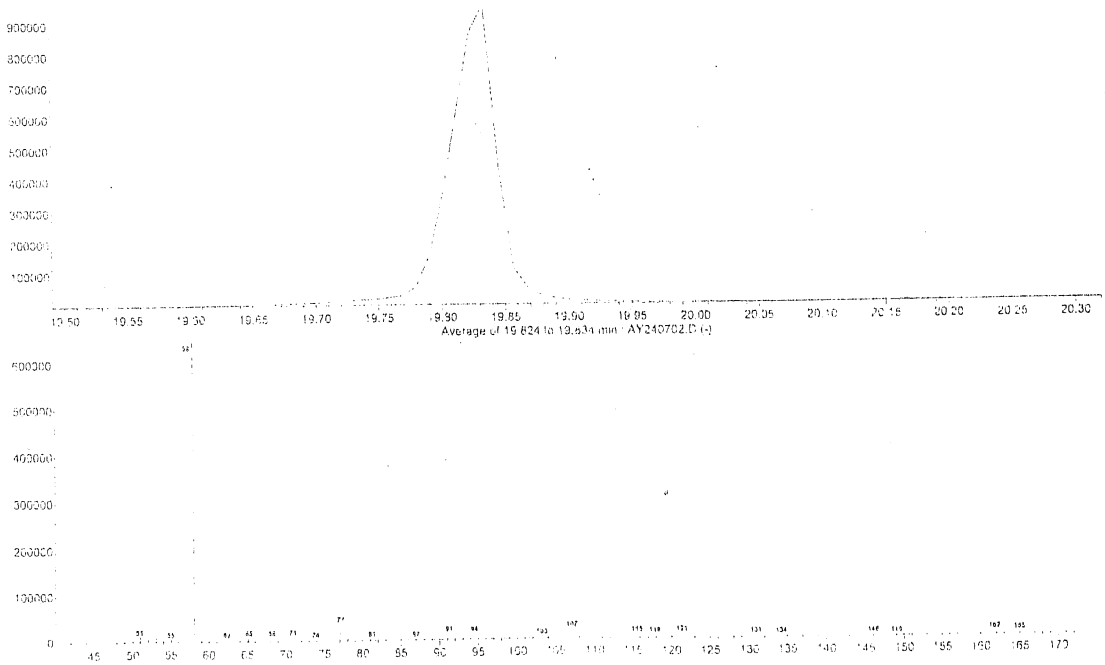

Figure 3.2. The chromatogram and spectrum of hordenine obtained from *P. nervosa* collected from Dardanelles.

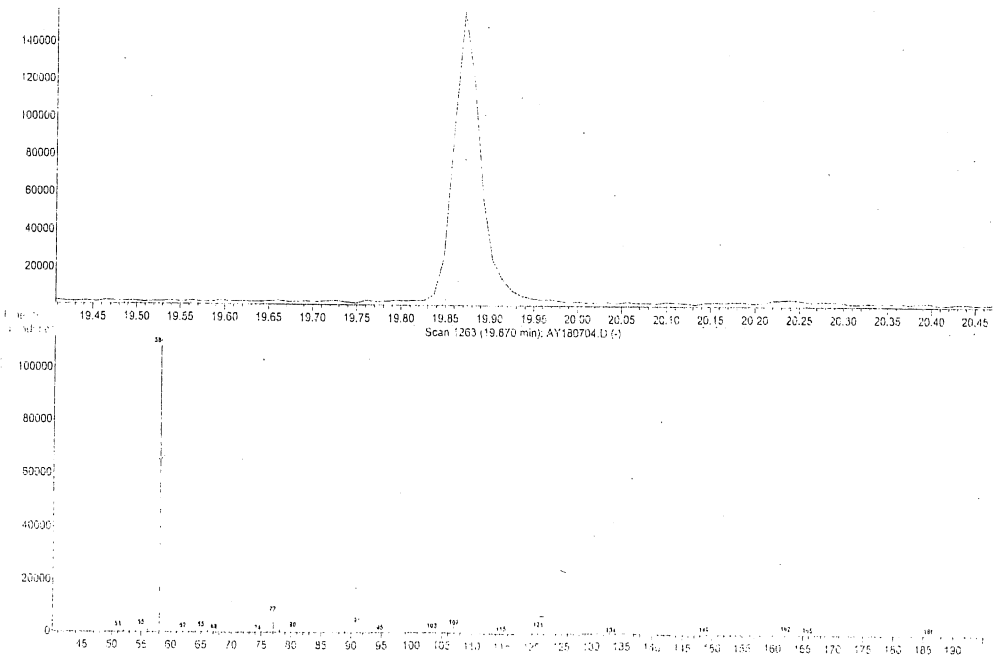

Figure 3.3. The chromatogram and spectrum of hordenine obtained from *P. nervosa* collected from Şile.

The peaks superimposed on GC/MS chromatogram of hordenine reference compound and hordenine obtained from *Phyllophora nervosa* are shown in Figure 4.

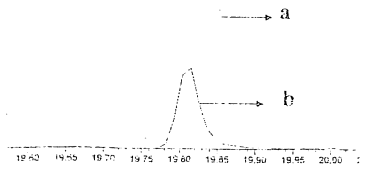

Figure 4. The peaks superimposed on GC/MS chromatogram of hordenine reference compound (a) and hordenine obtained from *Phyllophora nervosa* (b).

Mass spectrum of hordenine:

Mass spectrum: reference compound :

m/z: 165, 121, 107, 77 , 58.

Mass spectrum: obtained from the algae

m/z: 165, 121 ($-C_2H_6N$), 107 ($-C_3H_8N$), 77 ($C_6H_5^+$), 58 (C_3H_8N).

Mass spectrum of reference compound and hordenine from *Phyllophora nervosa* analyzed by GC/MS are similar.

Thus the comparison of the results confirmed that the compound obtained from the alga is hordenine.

The quantitative analysis of hordenine obtained from *Phyllophora nervosa* collected from two regions are as follow:

Phyllophora nervosa from Şile 39.66 µg/g.

Phyllophora nervosa from Dardanelles 1.15 mg/g.

The hordenine content was determined in *Mastocarpus stellatus* (Gigartina stellatus) by Barwel et al. (2006) and they found that the alga contains 1.5-5.5 mg/g collected in November. It was found that the hordenine content of alga depends on the production of carrageen and can reduce the hordenine content by 4 % to 80 %. The low amount of hordenine determined in *Phyllophora nervosa* from Şile could be attributed to collection time and sea condition as temperature and salinity.

Özet

Daha önceki çalışmamızda Şile'den toplanan *Phyllophora nervosa*'dan hordenin izole edilmişti. Bu çalışmada Karadeniz Şile'de ve buna ek olarak Çanakkale'de Soğandere bölgesinden toplanan *Phyllophora nervosa* (D.C.Grev) [Yeni adı *Phyllophora crispa* (Hudson) P.S. Dixon] içerdiği hordenin miktarı tayin edildi. Bu şekilde aynı algin Şile ve Çanakkale Boğazı örneğindeki miktarları karşılaştırıldı. Bu tayin için hordenin sülfat (Merck)'dan hordenin baz elde edildi ve miktar tayini GC/MS'de yapıldı. Buna ait standart eğrisi çizildi ve areadan bu denklemin eğrisi hesaplandı. Bu denklemden Şile örneğinde 39.66 µg/g ve Çanakkele örneğinde 1.15 µg/g hordenin bulunduğu saptandı. Hordenin'in ispatlanması GC/MS analizine ait spektrumdan ve alge ait kromatogramda tespit edilen hordenin pikine referans hordenin yüklemesi yapılarak tespit edildi. Aynı algin farklı bölgelerinden toplanan örneklerindeki hordenin miktarındaki farklılık toplandığı mevsim ve bölgedeki tuzluluk ile ilgilidir.

References

Barwell, C. and Blunden, G. (1981). Hordenine from the red alga *Gigartina stellata*. *J. Natural Products* 44: 500-502.

Barwell, C.J., Canham, C.A. and Guiry, M.D. (2006). Hordenine content of the marine alga *Mastocarpus stellatus* and the algal food product carrageen. *Phytotherapy Research* 3:67-69.

Güven, K.C., Bora, A. and Sunam, G. (1969). Alkaloid content of marine algae. I. Hordenine from *Phyllophora nervosa*. *Eczacılık Bul.* 11: 177-184.

Güven, K.C., Bora, A. and Sunam, G. (1970). Hordenine from the alga *Phyllophora nervosa*. *Phytochemistry* 9: 1893.

Kawauchi, H. and Sasaki, T. (1978). Isolation and identification of herdenine, p-(2-dimethylamino)ethylphenol from *Ahnfeltia paradoxa*. *Bull. Jap. Soc. Sci. Fish.* 44: 135-137.

Levring, T., Hoppe, H.A. and Schmid, O.J. (1969). Marine Algae. Cram, De Gruyter & CO., Hamburg.

Received: 10.03.2007

Accepted: 03.04.2007