HORDENINE FROM THE RED ALGA *GIGARTINA STELLATA*

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Hordenine (*N*,*N*-dimethyltyramine) occurs in flowering plants such as cacti (1, 2), barley (3), and citrus (4) and has been isolated from two marine red algae, *Phyllophora nervosa* (5) and *Ahnfeltia paradoxa* (6). In addition to hordenine, other *N*-methylated derivatives of tyramine occur naturally in plants. These are *N*-methyltyramine (2, 4, 7) and candicine (*N*,*N*,*N*-trimethyltyramine) (8). Both candicine (9) and candicine-*O*-sulfate (10) have been isolated from *Ahnfeltia paradoxa*. In this communication we report the occurrence of hordenine in the marine red alga *Gigartina stellata*.

MATERIAL AND METHODS

ALGAE INVESTIGATED—
CHLOROPHYCEAE: *Ulva lactuca* L.
PHAEOPHYCEAE: *Laminaria saccharina* (L.) Lamour., *Fucus serratus* L., *F. spiralis* L. and *Sargassum muticum* (Yendo) Fensholt.
FLORIDEOPHYCEAE: *Furcellaria lumbricalis* (Huds.) Lamour., *Gracilaria verrucosa* (Huds.) Papenf., *Ahnfeltia plicata* (Huds.) Fries, *Gymnogongrus crenulatus* (Turn.) J. Ag., *G. griffithsiae* (Turn.) Mart., *Phyllophora pseudoceranoides* (S. G. Gmel.) Newr. et A. R. A. Taylor, *Chondrus crispus* Stackh., *Gigartina pistillata* (S. G. Gmel.) Stackh., *G. stellata* (Stackh. in With.) Batt., *Grateloupia doryphora* (Mont.) Howe, *Palmaria palmata* (L.) O. Kuntze, *Griffithsia flosculosa* (Ellis) Batt., *Halurus equisefolius* (Lightf.) Kütz., *Chondria dasyphylla* (Woodw.) C. Ag., *Laurencia hybrida* (DC) Lenorm. ex Duby, *L. obtusa* (Huds.) Lamour. and *L. pinnatifida* (Huds.) Lamour.

BANGIOPHYCEAE: *Porphyra umbilicalis* (L.) J. Ag.

F. lumbricalis was collected at Bembridge, Isle of Wight, in June 1978, *P. pseudoceranoides* in November 1980 and *C. dasyphylla* and *L. obtusa* in August 1978. *A. plicata* and *G. griffithsiae* were collected at Ladram Bay, Devon, in November 1980, *G. crenulatus* at Lulworth Cove, Dorset, in August 1980 and *G. pistillata* at Mewstone, Devon, in August 1979. Other algae were collected at Southsea, Hampshire, in October 1978. Algal material was freed from macroscopic epiphytes. Algae from Southsea were frozen within 2 hr and stored at −20°. At other locations algae were immersed in methanol containing 5% v/v hydrochloric acid and stored in this form.

EXTRACTION OF ALGAE.—Algae stored in acid-methanol were extracted by refluxing for 15 min. Each extract was evaporated *in vacuo* at 50° and the residue extracted with 2 ml of water per g algae processed. Algae stored frozen were extracted by heating at 90° for 15 min with 3 ml of 0.5N hydrochloric acid per g algae, followed by homogenizing with a Thomas homogenizer.

TLC OF ALGAL EXTRACTS.—Cellulose layers of 500 μm wet thickness, dried at 50° for 4 hr, were developed with propan-1-ol-chloroform-ammonia solution (S.G. 0.88)-water (12:5:2:2) (system I) and butan-1-ol-acetic acid-water (12:3:5) (system II). Air-dried silica gel G layers of 250 μm wet thickness were developed with chloroform-methanol-ammonia solution (S.G. 0.88) (80:20:1) (system III) (1). After drying, chromatograms were sprayed with diazotized sulfanilic acid solution followed by sodium carbonate solution (11). Compounds giving a positive reaction with this locating reagent are termed diazo-positive in the text.

ISOLATION OF HORDENINE.—*Gigartina stellata* (100 g), collected at Southsea, October 1979, was heated with 500 ml 1N hydrochloric acid for 30 min at 90°. The extract was made alkaline with sodium hydroxide, centrifuged and the supernatant extracted with 5 x 200 ml portions of diethyl ether, which were bulked and evaporated *in vacuo*. The residue was dissolved in ethanol, acidified with hydrochloric acid and then precipitated by the addition of diethyl ether. This procedure was repeated until the supernatant was colorless, after which the precipitate was dissolved in and recrystallized from ethanol. This yielded 14 mg of crystals, mp[1] of 176.5–177.5°, which was not depressed by admixture with hordenine hydrochloride. Also the ir[2] and nmr[3] spectra were identical to those of hordenine hydrochloride.

ASSAY OF HORDENINE.—Hordenine was extracted from aqueous extracts into diethyl

[1]Mp were determined on a Kofler Block and are uncorrected.

[2]Infrared spectra were measured in potassium bromide discs on a Perkin Elmer 377 Grating Infrared Spectrophotometer.

[3]Nmr spectra were determined in D_2O on a Bruker 270 MHz machine.

ether and back into 0.2N hydrochloric acid (12). The hordenine in the 0.2N hydrochloric acid phase was assayed colorimetrically. To do this, the volume was brought to 1 ml, then 0.2 ml of 20% sodium carbonate solution was added followed by 0.05 ml diazotized sulfanilic acid (11) after which the absorption was measured at 490 nm.

RESULTS AND DISCUSSION

Analysis by tlc of an extract of *G. stellata* from Southsea revealed a diazo-positive component with characteristics similar to those of hordenine. The compound was isolated and identified as hordenine from tlc, melting point, and ir and nmr spectroscopic data. To determine whether hordenine is a normal constituent of *G. stellata*, extracts of the alga from England and Ireland were examined by tlc, which indicated the compound to be present in each sample. The amount of hordenine present was assayed colorimetrically with a modified diazotized sulfanilic acid reagent (11). This was feasible since examination by tlc systems I, II and III showed that hordenine was the only diazo-positive component of the 0.2N hydrochloric acid phase used for the colorimetric assay. Table 1 shows that the hordenine content of the samples ranged from about 1 mg to 5 mg per g dry weight.

TABLE 1. Hordenine content of *Gigartina stellata* from different sites.

Collection site[a]	mg hordenine/g dry weight[b]
Chapman's Pool, Dorset, England	4.12±0.08
River Yealm, Devon, England	2.44±0.04
River Erme, Devon, England	1.20±0.08
Finavarra, Co. Clare, Ireland	4.97±0.60
Fanore, Co. Clare, Ireland	4.66±0.06

[a]Algae were collected in July 1979.
[b]The values for the hordenine content are given as the mean ± standard deviation of three determinations carried out on a single sample of algae.

Following the identification of hordenine in *G. stellata*, some other algae of the same family, Gigartinaceae, were examined for the presence of this compound. These were *C. crispus*, samples of which were collected at the same locations as those shown in table 1, and one sample of *G. pistillata*. Also, since hordenine has been reported to occur in two members of the Phyllophoraceae (5, 6), some British members of this family were examined: *A. plicata*, *G. crenulatus*, *G. griffithsiae* and *P. pseudoceranoides*. In addition, locally available species of the Chlorophyceae, Phaeophyceae, Florideophyceae, and Bangiophyceae were investigated. The algal extracts were examined by tlc under conditions where the lower detection limit was 10 μg hordenine per g dry weight. Hordenine was not detected in any of these algae.

The results indicate that hordenine is a consistent component of *G. stellata* and that it occurs in high amounts. Hordenine is an indirectly acting sympathomimetic agent (13). Therefore, the consumption of the whole alga or foods prepared with the whole alga could produce undesirable effects in persons such as those being treated with some antidepressant drugs. In this respect, it is of interest that several samples of *C. crispus*, which is commonly used to prepare an edible gel (14), did not contain detectable amounts of hordenine.

Of the algae examined hordenine was detectable only in *G. stellata*, which indicates that the compound may be of limited occurrence in British marine algae. However, in the case of most algae, only a single sample was examined, and it has been reported that hordenine was not detectable at certain times of the year in *A. paradoxa*, an alga from which hordenine has been previously isolated (9). Therefore, the absence

of the compound from one sample does not preclude the possibility that it may be present at another time of year or another stage of the alga's development.

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