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NICAEENSIN, A NEW AMIDINOUREIDO COMPOUND FROM THE RED ALGA *SCHOTTERA NICAEENSIS*

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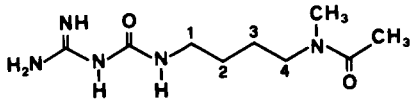
ABSTRACT.—From the basic amino acid fraction of the red alga *Schottera nicaeensis*, a previously unreported nitrogenous compound has been isolated by chromatography and its structure determined as 1-(3-amidinoureido)-4-(*N*-methylacetamido)butane (nicaeensin) [**1**] by degradation and spectroscopic measurements.

An examination of the amino acid fraction from aqueous extracts of the red alga *Schottera nicaeensis* (Duby) Schott. (Phyllophoraceae; Gigartinales) revealed the presence of a metabolite that gave an orange color with Dragendorff's reagent but did not react with ninhydrin. This unknown compound, nicaeensin, isolated by ion-exchange chromatography and further purified by partition chromatography, was optically inactive and gave a brilliant pink color with Sakaguchi's reagent and a brown color with sodium nitroprusside/potassium ferricyanide, indicating the presence in the molecule of a monosubstituted guanidino group (1). Its ir spectrum in the region 1500–1800 cm^{-1} showed bands at 1550, 1620, 1687, and 1725 cm^{-1}. The molecular formula was deduced as $C_9H_{19}N_5O_2$ from elemental analysis of the picrate, $C_{15}H_{22}N_8O_9$ (mp 171–173°), and from the fab mass spectrum (*m/z* 230 $[M+H]^+$, 252 $[M+Na]^+$, and 322 $[M+H+glycerol]^+$).

Acid hydrolysis (6 N HCl, 48 h at 110°) afforded, along with guanidine, a basic compound (E_{Arg} 1.97) whose structure was determined as *N*-methyl-1,4-butanediamine [**2**] based on its spectral properties. Important peaks in its ms spectrum were observed at *m/z* 102 $[M]^+$, 84 $[CH_2\text{-}CH_2\text{-}CH_2\text{-}CH{=}N\text{-}CH_3]^+$, 73 $[M-CH_2{=}NH]^+$, 69 $[84-Me]^+$, and 59 $[M-CH_2{=}N\text{-}CH_3]^+$. The ^{13}C-nmr spectrum dis-



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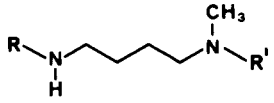

2 R=R′=H
3 R=H_2N-C(-NH)-NH-CO-, R′=H
4 R=H, R′=CH_3CO-

5 R= , R′=CH_3CO-

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played, in addition to the N-Me signal at δ 35.69, four methylene triplets at δ 51.23 (-CH_2-NH-), 41.77 (-CH_2-NH_2), 26.78, and 25.53 (-CH_2-*CH_2*-*CH_2*-CH_2-). In the ^{1}H-nmr spectrum of **2** the N-Me protons occurred as a singlet at δ 2.69, the two nitrogen-bonded methylenes appeared as partially overlapped triplets at δ 3.02 and 3.05, and the remaining two methylenes gave a four-proton multiplet at δ 1.74 (W = 54 Hz).

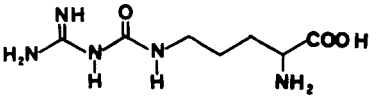
Consideration of the above findings and the molecular formula suggested that the new algal metabolite was 1-(3-amidinoureido)-4-(*N*-methylacetamido) butane (nicaeensin) [**1**]. The ^{1}H- and ^{13}C-nmr spectra were fully consistent with this structure. The ^{1}H-nmr spectrum in D_2O showed the typical signal splitting, which is frequently observed in compounds containing an amide group in the molecule, as the result of the presence in solution of two "stable" conformers. In fact, the N-Me appeared as a pair of singlets at δ 3.01 (conformer A; rel. int. 0.57) and 2.86 (conformer B; rel. int. 0.43), the MeCO protons as two singlets at δ 2.06 (A) and 2.08 (B), the C-1 methylene protons as two triplets at δ 3.10 [J = 6.0 Hz (A)] and 3.12 [J = 6.0 Hz (B)]. Two additional triplets assignable to the C-4 methylene protons at δ 3.32 [J = 7.5 Hz (A)] and 3.36 [J = 7.5 Hz (B)], and a four-proton resonance for the methylenes at C-2 and C-3 (broad signal centered at δ 1.52; W = 76.5 Hz) completed the spectrum. Assignments of the signals for each conformer were based on the relative intensities of the pertinent peaks as well as on the results of extensive spin-decoupling experiments. In the ^{13}C-nmr spectrum of nicaeensin, splitting was observed for the following signals: N-Me [quartets at δ 39.01 (A) and 36.20 (B)]; *Me*CO- [quartets at δ 23.74 (A) and 23.04 (B)]; Me*CO*- (singlets at δ 176.6 and 176.7); C-1 [triplets at δ 42.02 (A) and 42.11 (B)]; C-2 [triplets at δ 29.20 (A) and 29.08 (B)]; C-3 [triplets at δ 26.61 (A) and 27.46 (B)]; C-4 [triplets at δ 50.22 (A) and 53.60 (B)]; -C(=NH)- (singlets at δ 163.06 and 163.15); and -NH-CO-NH- (singlets at δ 166.43 and 166.71). ^{13}C-^{1}H shift correlations allowed us to assign each signal to the pertinent conformer.

The eims fully agreed with the proposed structure and contained diagnostically important peaks at *m/z* 228 $[M-H]^+$, 213 and 171 [sequential losses of Me and CH_2=CO from the ion at *m/z* 228], 141 $[228-H_2N\text{-}C(=NH)\text{-}NH\text{-}COH]^+$, 127 $[M-H_2N\text{-}C(=NH)\text{-}NH\text{-}CO\text{-}NH_2]^+$, 115 $[H_2N\text{-}C(=NH)\text{-}NH\text{-}CO\text{-}NH{=}CH_2]^+$, 100 $[NH\text{-}C(=NH)\text{-}NH\text{-}CO\text{-}NH]^+$, 86 $[H_2N\text{-}C(=NH)\text{-}NH{=}CO]^+$, and 58 $[H_2N\text{-}C(=NH)\text{-}NH_2-H]^+$.

Partial acid hydrolysis of nicaeensin (6 N HCl, 12 h at 110°) gave, in addition to guanidine and *N*-methyl-1,4-butanediamine, deacetyl nicaeensin [**3**] and *N*-methyl-*N*-acetyl-1,4-butanediamine [**4**]. Compound **3**, which had an electrophoretic mobility higher than the parent molecule (E_{Arg} 1.36), gave color reactions with Dragendorff's (orange), Sakaguchi's (pink), and sodium nitroprusside/potassium ferricyanide (brown) reagents. Compound **4** did not react with these last two chromogenic reagents but gave positive reaction with ninhydrin (violet) and Dragendorff's reagent (orange).

Splitting of signals, not seen in the ^{1}H- and ^{13}C-nmr spectra of **3** (see the Experimental section), was observed in the ^{1}H-nmr spectrum of **4**. In this spectrum the N-Me appeared as two singlets at δ 3.05 (A) and 2.90 (B), while the acetyl group gave two singlets at δ 2.10 (A) and 2.12 (B). The spectrum also contained three triplets at δ 3.38 [J = 6.3 Hz, -*CH_2*-N(Me)- (A)], 3.43 [J = 6.3 Hz, -*CH_2*-N(Me)- (B)], and 2.95 [J = 7.05 Hz, -CH_2-NH_2 (A and B)] in addition to a complex multiplet at δ 1.62 [W = 67.5 Hz, -CH_2-*CH_2*-*CH_2*-CH_2- (A and B)].

Treatment of nicaeensin with acetylacetone in alkaline medium gave the expected 1-[3-(4,6-di-methylpyrimidin-2-yl)ureido]-4-(*N*-methylacetamido)butane [**5**] (*m/z* 293, $[M]^+$). In the ^{1}H-nmr spectrum of **5** the methyls on the pyrimidine ring appeared



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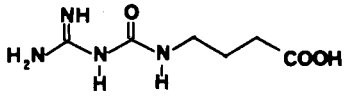

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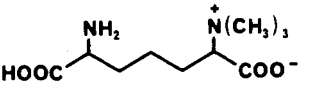

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as a singlet at δ 2.30, while the heterocyclic proton signal was a singlet at δ 6.77. The rest of the signals (see the Experimental section) showed splitting analogous to that observed in the spectrum of **1**. In the ^{13}C-nmr spectrum the signals relative to the 4,6-dimethylpyrimidine moiety were at δ 25.69 (methyls), 117.41 (methine), 158.81 (C-2), and 171.35 (C-4 and C-6).

So far, only two amidinoureido compounds, gigartinine [**6**] (2) and gongrine [**7**] (3), have been reported as natural products, both from the red alga *Gymnogongrus flabelliformis* (Phyllophoraceae; Gigartinales); the occurrence of **1** in a seaweed belonging to the same family could be of some interest from the chemotaxonomic point of view.

Nicaeensin may be biosynthetically derived from gigartinine via decarboxylation, methylation, and acetylation. The possibility that gongrine is biogenetically related to gigartinine cannot be ruled out. Furthermore, considering that in previous work (4) *S. nicaeensis* has been shown to accumulate 6-amino-6-carboxy-2-trimethylammoniohexanoate [**8**], it is not unlikely that, in this alga, a biosynthetic relationship may exist between the latter compound and nicaeensin.

EXPERIMENTAL

PLANT MATERIAL.—Thalli of *S. nicaeensis* were collected at Castelluccio, Sicily. Voucher specimens were deposited in the University Herbarium, Institute of Botany, Catania.

GENERAL EXPERIMENTAL PROCEDURES.—Mass spectra were obtained on an AEI MS 902 instrument. ^{1}H- and ^{13}C-nmr were recorded in D_2O at 250 and 62.9 MHz, respectively, on a Bruker AC-250 instrument. Chemical shifts were measured in ppm using sodium 3-trimethylsilyl-2,2,3,3-d_4-propanoate (TSP) as internal reference. 2D ^{13}C-^{1}H shift correlations were performed using the commercially available microprogram XHCORR by polarization transfer via J_{CH}. The ir spectrum (KBr) was run on a Perkin-Elmer 684 spectrophotometer. Tlc and hptlc were carried out on glass precoated Si gel and cellulose plates (Merck), using the following solvent systems: (1) *n*-BuOH–HOAc–H_2O (12:3:5); (2) Phenol-H_2O (3:1). Spots were detected by uv light (λ = 254 mμ) and by Dragendorff's, Sakaguchi's, sodium nitroprusside/potassium ferricyanide, and ninhydrin reagents (1). Paper electrophoresis (50 V/cm) was run at pH 4.5 using a 0.05 M pyridine-HCOOH buffer. Preparative liquid chromatography (plc) was carried out on a Jobin-Yvon Mini-Prep LC instrument.

EXTRACTION AND PURIFICATION.—The total amino acid fraction of the alga (1 kg) was obtained as reported earlier (5). An aqueous solution of this fraction was applied to a column of Amberlite IRC-50 (H^+); after the resin was washed with H_2O, the basic fraction was eluted with 2 N NH_4OH and the eluate taken to dryness in vacuo. The residue was then fractionated by plc (LiChroprep Si-60; 25-40 μm; solvent 1). The separation was monitored by hptlc (Si gel, solvent 1; ninhydrin and Dragendorff's reagents; R_f 0.50). The pooled fractions containing **1** were taken to dryness, and the residue was dissolved in H_2O and applied to a column of Dowex-50W (H^+). After the resin was washed with H_2O, nicaeensin was eluted with 2 N NH_4OH; the eluate was taken to a small volume and freeze-dried to give 155 mg of an off-white highly hygroscopic powder. The purity of **1** was checked by tlc (Si gel, solvent 2, R_f 0.45; cellulose, solvent 1, R_f 0.74) and paper electrophoresis (E_{Arg} 0.76): eims (70 eV, 240°) *m/z* (%) 228 (1.26), 214 (1.48), 213 (8.30), 171 (1.18), 141 (1.26), 127 (5.55), 115 (5.26), 100 (5.33), 91 (6.29), 86 (14.07), 70 (8.88),

58 (7.40), 57 (8.88), 44 (100); ir ν max cm^{-1} 3360 and 3180 (N-H), 2910 and 2840 (CH_2), 1725, 1687, 1620, and 1550 (C=O and C=N), 1380 and 1400 (Me-N and Me-C=O).

NICAEENSIN PICRATE.—Treatment of **1** with an aqueous saturated solution of picric acid gave nicaeensin picrate as a yellow precipitate, which was recrystallized from H_2O/EtOH: mp 171–173°. *Anal.* calcd for $C_{15}H_{22}N_8O_9$, C 39.29, H 4.84, N 24.45%, found C 39.22, H 4.89, N 24.41%. The ^{1}H-nmr spectrum contained resonances at δ 1.52 [m, W = 78.0 Hz; -CH_2-CH_2-CH_2-CH_2-], 2.06 [s, MeCO- (conformer A)], 2.08 [s, MeCO- (conformer B)], 2.86 [s, N-Me (B)], 3.01 [s, N-Me (A)], 3.17 [t, J = 6.0 Hz, -NH-CH_2- (A)], 3.19 [t, J = 6.0 Hz, -NH-CH_2- (B)], 3.32 [t, J = 6.6 Hz, -CH_2-N(Me)- (A)], 3.37 [t, J = 7.0 Hz, -CH_2-N(Me)- (B)], 8.94 (s, aromatic protons).

ACID HYDROLYSIS OF NICAEENSIN.—Total hydrolysis of nicaeensin was carried out in a sealed vial at 110° with 6 N HCl for 48 h. For partial hydrolysis the reaction time was 12 h. In both cases, the products were isolated by preparative tlc (Si gel, solvent 1) and then converted into the corresponding free bases by ion-exchange chromatography on Amberlite IRC-50 (H^+) column. Total hydrolysis of **1** (30 mg) afforded guanidine (6.7 mg) and *N*-methyl-1,4-butanediamine (12.5); partial hydrolysis of **1** (50 mg) gave guanidine (2.8 mg), *N*-methyl-1,4-butanediamine (1.9 mg), deacetyl nicaeensin (15 mg), and *N*-methyl-*N*-acetyl-1,4-butanediamine (4.8 mg).

Guanidine was identified by comparison of its chromatographic and electrophoretic properties with those of an authentic sample (Si gel, solvent 1, R_f 0.58; solvent 2, R_f 0.40; cellulose, solvent 1, R_f 0.68; E_{Arg} 1.82).

N-Methyl-1,4-butanediamine [**2**].—Compound **2** (Si gel, solvent 1, R_f 0.06; cellulose, solvent 1, R_f 0.22): eims (70 eV) *m/z* (%) 102 (4.3), 100 (5.4), 84 (10.3), 73 (8.8), 70 (15.5), 69 (19.5), 59 (100), 57 (23.0).

*1-(3-Amidinoureido)-4-(*N*-methylamino)butane (deacetyl nicaeensin)* [**3**].—Compound **3** (Si gel and cellulose, solvent 1, R_f 0.16 and 0.53, respectively; E_{Arg} 1.36): ^{1}H nmr δ 1.59 (m, W/2 = 21 Hz, -CO-NH-CH_2-CH_2-), 1.72 [m, W/2 = 19.5 Hz, -CH_2-CH_2-N(Me)-], 2.72 (s, N-Me), 3.06 [t, J = 6.3 Hz, -CH_2-N(Me)-], 3.18 (t, J = 7.5 Hz, -CO-NH-CH_2-); ^{13}C nmr δ 25.82 [t, -CH_2-CH_2-N(Me)-], 29.12 (t, -CO-NH-CH_2-CH_2), 35.60 (q, N-Me), 41.66 (t, -CO-NH-CH_2-), 51.75 [t, -CH_2-N(Me)-], 158.00 [s, -C(=NH)-], 158.57 (s, -NH-CO-NH-); eims (70 eV) *m/z* (%) 128 $[M-59]^+$ (94.80), 98 $[128-CH_2{=}NH_2]^+$ (51.95), 86 $[NH_2\text{-}C(=NH)\text{-}NH\text{-}CO]^+$ (29.87), 84 $[128-CH_2{=}NHMe]^+$ (41.56), 69 $[86-NH_3]^+$ (86.23), 59 $[NH_2\text{-}C(=NH)\text{-}NH_2]^+$ (67.01), 58 (38.96), 57 (100).

N-Methyl-N-acetyl-1,4-butanediamine [**4**].—Compound **4** (Si gel, solvent 1, R_f 0.28; cellulose, solvent 1, R_f 0.61; E_{Arg} 1.10): ^{1}H nmr see text.

1-[3-(4,6-DIMETHYLPYRIMIDIN-2-YL)UREIDO]-4-(*N*-METHYLACETAMIDO)BUTANE [**5**].—Synthesis of **5** was carried out according to the procedure of Leclercq *et al.* (6). Compound **1** (0.05 mM) was dissolved in EtOH (10 ml), and 10% $NaHCO_3$ (5 ml) and $MeCOCH_2COMe$ (10 ml) were added; the mixture was kept at 100° for 4 h. After addition of HOAc to pH 6 the reaction mixture was extracted with Et_2O and the aqueous phase dried in vacuo. The residue, dissolved in H_2O, was applied to a column of Dowex-50W (H^+); the resin was washed with H_2O and then eluted with 2 N NH_4OH. The eluate was taken to dryness to give 13 mg of pure **5** (Si gel, solvent 1, R_f 0.69). The ^{1}H-nmr spectrum of **5** displayed signals at δ 1.56 [m, W = 78.5 Hz, -CH_2-CH_2-CH_2-CH_2- (conformers A and B)], 2.05 [s, MeCO- (A)], 2.06 [s, MeCO- (B)], 2.30 [s, methyls on the pyrimidine ring], 2.85 [s, N-Me (B)], 3.00 [s, N-Me (A)], 3.25 [t, J = 6.0 Hz, -NH-CH_2- (B)], 3.28 [t, J = 6.6 Hz, -NH-CH_2- (A)], 3.34 [t, J = 6.7 Hz, -CH_2-N(Me)- (A)], 3.38 [t, J = 6.9 Hz, -CH_2-N(Me)- (B)], 6.77 [s, heterocyclic proton]. The ^{13}C-nmr spectrum contained resonances at δ 23.04 [q, *Me*CO- (B)], 23.72 [q, *Me*CO- (A)], 25.69 [q, methyls on the pyrimidine ring], 26.97 [t, -CH_2-CH_2-N(Me)- (A)], 27.77 [t, -CH_2-CH_2-N(Me)- (B)], 28.81 [t, -NH-CH_2-CH_2- (A)], 28.98 [t, -NH-CH_2-CH_2- (B)], 36.26 [q, N-Me (B)], 39.05 [q, N-Me (A)], 42.17 [t, -NH-CH_2- (A)], 42.26 [t, -NH-CH_2- (B)], 50.17 [t, -CH_2-N(Me)- (A)], 53.51 [t, -CH_2-N(Me)- (B)], 117.41 [d, heterocyclic methine], 158.81 [s, C-2 of the heterocyclic ring], 159.14 [s, -NH-CO-NH- (B)], 159.52 [s, -NH-CO-NH- (A)], 171.35 [s, C-4 and C-6 of the heterocyclic ring], 176.53 [s, Me*C*O- (B)], 176.64 [s, Me*C*O- (A)]. Fabms showed peaks at *m/z* 294 $[M+H]^+$, 316 $[M+Na]^+$. Eims (70 eV, 45°) displayed peaks at *m/z* (%) 294 (1.95), $[M]^+$ 293 (3.74), $[M-MeCO-CH_2{=}NH_2]^+$ 220 (4.00), 219 (3.74), $[M-MeCO-CH_2{=}NMe]^+$ 207 (6.17), $[220-CH{\equiv}CH]^+$ 194 (12.30), 180 (4.08), 179 (4.78), [*N*-(4,6-dimethylpyrimidin-2-yl)carbamoyl moiety]$^+$ 150 (60.86), $[150-H]^+$ 149 (97.40), [2-amino-4,6-dimethylpyrimidine]$^+$ 123 (100), $[149-HOCN]^+$ 106 (19.30), 86 (25.82), 70 (24.52), 44 (76.52); the transition *m/z* 149↦106 was supported by a meta-stable peak at *m/z* 75.41.

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