Communications to the Editors

MAGNESIDIN A, A COMPONENT OF MARINE ANTIBIOTIC MAGNESIDIN, PRODUCED BY *Vibrio gazogenes* ATCC29988

Sir:

There are few studies about products of marine microorganisms compared with terrestrial ones[1]. Marine microorganisms live in quite different environment from terrestrial ones, and have been expected to produce compounds which possess unique structures and activities. The investigations of metabolites of marine bacteria should be one of the important fields of natural products studies. In the course of screening for new antibiotics from marine bacteria, we found that *Vibrio gazogenes* ATCC29988 produced a component of magnesidin, and named magnesidin A. In this communication, we will describe the isolation, identification by mainly NMR data, determination of unclarified stereochemistry, and tautomerism of magnesidin A.

The halophilic bacterium ATCC29988 has been isolated from marine mud[2] and classified as *Vibrio gazogenes*[3], and has been reported to produce red pigment, prodigiosin[2]. The strain was inoculated into the medium containing nutrient broth (Difco) 0.8%, glucose 0.3%, $CaCO_3$ 0.2%, and sea water 50%. The pH was adjusted to 7.4 before sterilization at 121°C for 15 minutes. It was grown at 30°C with rotary shaking for 164 hours. The culture broth exhibited the activity against Gram-positive bacteria *Bacillus subtilus* and micro alga *Prorocentrum micans*.

The fermented broth was filtered, and the filtrate (2 liters) was extracted with EtOAc. The mycelial cake was extracted with 70% aq acetone and concentrated *in vacuo*, and re-extracted with EtOAc. The extracts were combined and evaporated to dryness. The crude material was chromatographed on a flash silica-gel column with hexane - acetone to give two active fractions. Red active amorphous powder was isolated from the first fraction, and was identified as prodigiosin by the data of EI-MS. The second more active fraction obtained pale purple fine plates which were recrystallized from acetone - hexane to give colorless plates, magnesidin A (**1**: 1 mg). The mother liquid was purified by preparative reverse phase HPLC column (Shiseido capcell pak C_{18}) with MeOH - water - trifluoroacetic acid, 90: 10: 0.1, and obtained amorphous colorless powder (**2**: 10 mg). Both compounds **1** and **2** are active against *Prorocentrum micans* at the concentration of 1 μg/ml and weakly active against *Bacillus subtilus*.

The EI-MS spectrum of **2** showed the molecular ion at *m/z* 293 and fragment ion at *m/z* 251 which suggested the existence of acetyl group. The HREI-MS data indicated the molecular formula as $C_{16}H_{23}NO_4$ [Found: 293.1653 (M^+) Calcd for $C_{16}H_{23}NO_4$: 293.1627] and verified the acetyl group [Found: 251.1482 Calcd for $C_{14}H_{21}NO_3$ ($(M-Ac+H)^+$): 251.1521]. Although one proton could not be observed in ^1H NMR spectrum of it, the partial structures as shown in Fig. 1 were demonstrated by the data of ^1H, ^{13}C, ^1H-^1H COSY, HMQC, and HMBC NMR. The remaining nonprotonated sp^2 carbon signals at δ_C 102.9 (C-3) and 164.6 (C-2) were attributable to the second carbon of the enol form of 1,3-diketone system[4] and carbonyl carbon bound hetero atom, respectively. The proton signal of acetyl methyl appeared at δ_H 2.62, which attributed to the acetyl group bound to aromatic nitrogen[5].

The NMR data of derivatives of **2** confirmed the connection of a part of these remaining atoms. 5,14-Dihydro derivative (H_2/PtO_2) displayed newly occured methine proton at δ_H 4.38 and 4.64 (3: 2, tautomeric ratio) in the ^1H NMR spectrum, and

Fig. 1. Partial structures of magnesium free magnesidin A (**2**) confirmed by NMR data.

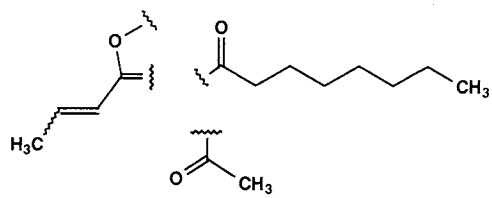
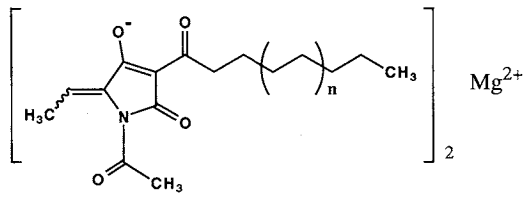
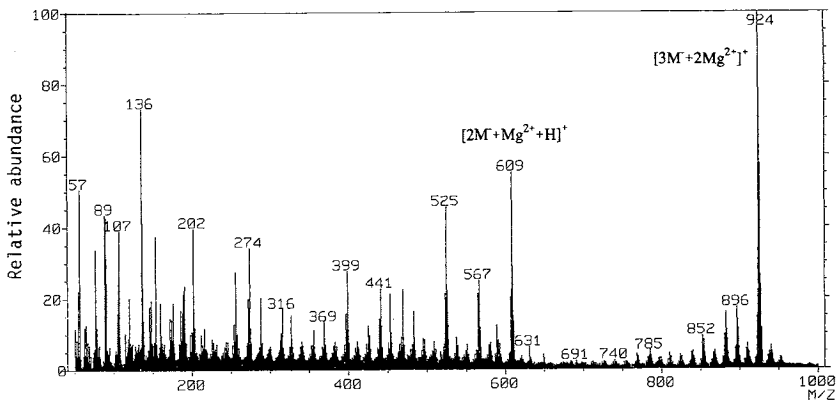


Fig. 2. Structure of reported magnesidin[6].



n=0, 1 1:1 mixture

Fig. 3. The FAB-MS spectrum of magnesidin A (**1**).



disappearance of a sp^2 signal at δ_C 129.9 (C-5) observed in the ^{13}C NMR spectrum of **2**. From the proton chemical shift of it, nitrogen was connected to C-5 carbon. The HMBC data of *N*-deacetyl compound[6] obtained by hydrolysis (2N HCl, 50°C, 2 hours) clarified that the C-3 carbon was located at the three bond position from α-proton (H-7) of alkyl carbonyl group. Thus the nitrogen containing 5-membered ring and arrangement of the side chain moiety were constructed. The determined structure of **2** was identical with that of a component of magnesidin[6] which has been reported to consist of two analogues and magnesium as shown in Fig. 2.

The colorless plate (**1**) was identified with Mg^{2+} binding compound of **2** because of the MS data. The FAB-MS spectrum of **1** indicated to contain magnesium and to be produced as a single component as shown in Fig. 3. Since trifluoroacetic acid was contained in the solvent system of final HPLC purification, **2** was considered to be magnesium free.

The stereochemistry of vinylic methyl group of magnesidin has been unknown. The NOESY spectrum of *N*-deacetyl compound showed cross peak between vinylic methyl protons (δ_H 1.82 and 1.87; 2:3 tautomer) and NH proton (δ_H 8.43 and 8.64; 3:2 tautomer). Thus, the structure including stereochemistry of magnesidin A (**1**) was determined as shown in Fig. 4. The ^1H and ^{13}C NMR total assignments of major tautomer's signals of **2** are listed in Table 1.

The tautomerism of **2** in $CDCl_3$ was confirmed by observation of cross peaks in NOESY spectrum[7] as shown in Fig. 5 and the ratio of tautomers was calculated to be 89:11 from the ^1H NMR data. The tautomerism in tetramic acids are including four enolic forms[8], but the ratio of tautomers is considered to be case by case[9]. In the case of **2**, the four tautomers are thought to be exist as shown in Fig. 6.

Fig. 4. Structure of magnesidin A (**1**).

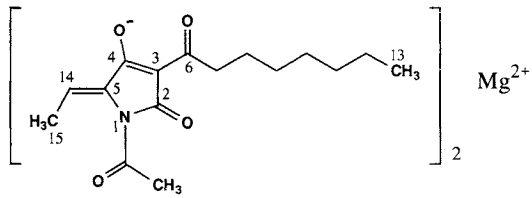


Table 1. ^1H and ^{13}C NMR chemical shifts of major tautomer's signals of magnesium free magnesidin A (**2**). (500 and 125 MHz, in $CDCl_3$, δ ppm).

Position	Proton	Carbon
1		
2		164.6
3		102.9
4		182.4
5		129.9
6		200.9
7	2.94	37.6
8	1.68	24.5
9	1.38	29.2
10	1.25~1.35	30.0
11	1.25~1.35	31.6
12	1.25~1.35	22.6
13	0.88	14.0
14	7.68	129.4
15	2.28	14.1
N-Ac C=O		171.5
Me	2.62	27.7

To identify the composition of tautomers of **2** in $CDCl_3$ solution, the deuterium shift experiment in ^{13}C NMR[10] was applied to **2**. The oxygenated sp^2

Fig. 5. The part of NOESY spectrum of magnesium free magnesidin A (**2**).

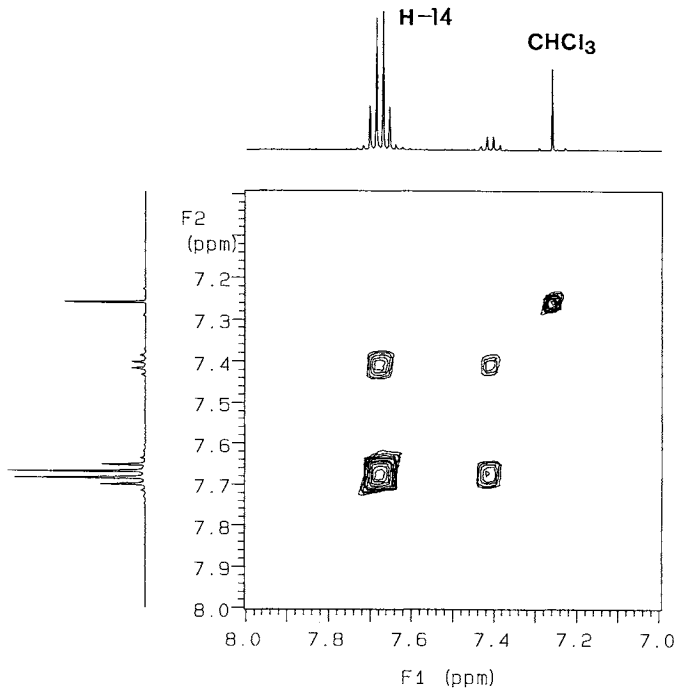
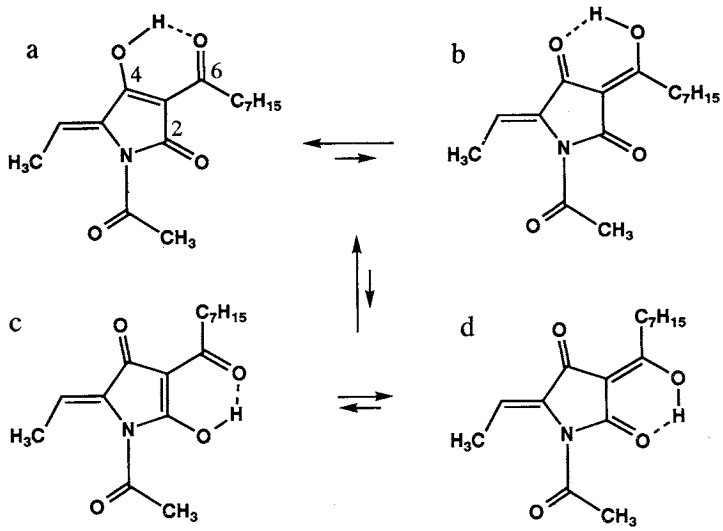


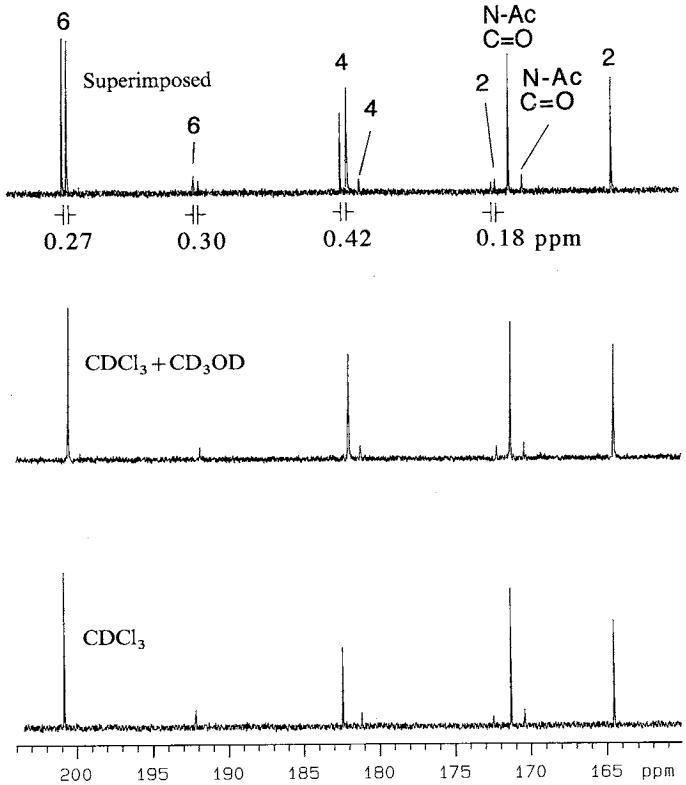
Fig. 6. The tautomerism of magnesium free magnesidin A (**2**).



region of ^{13}C NMR spectra in $CDCl_3$ and $CDCl_3$ with d_4-methanol (10 equivalent of **2**), and superimposed spectra were demonstrated in Fig. 7. The major signals of C-4 and C-6, and the minor signals of C-2 and C-6 assigned by HMBC data, were upfield shifted by deuterium addition, but others were not moved. These data clearly indicated the major observable tautomer was a pair of **a** and **b**, and minor one was a set of **c** and **d**.

The rate of exchange between **a** and **b** and between **c** and **d** was too fast to observe by NMR[9]. But the chemical shifts of major signals of C-4 and C-6 indicated the enolic form **a** was predominant between **a** and **b**. The methylation of

Fig. 7. The oxygenated sp^2 region of ^{13}C NMR spectra of magnesium free magnesidin A (**2**) in $CDCl_3$ and $CDCl_3$ with d_4-methanol, and superimposed spectra.



2 with diazomethane at 0°C gave 4-*O*-methyl derivative (**3**) identified by HMBC data as a major reaction product, which asisted the predominance of **a** in the tautomerism. The ^{13}C NMR signal of C-6 of **3** resonanced at δ_C 199.1 ppm. In comparison with **2**, up field shift of C-6 signal was too small (1.8 ppm) for cleavage of an usual hydrogen bond by 4-*O*-methylation. This behavior of ^{13}C NMR of **3** suggested some contribution of enol form **b**. The details about tautomerism of Mg^{2+}-binding compounds are under investigation.

In many cases, marine bacteria require high Mg^{2+} concentration (50 mM: at approximately sea water concentration)[11], but *Vibrio gazogenes* ATCC29988 has been reported to have no requirement of high Mg^{2+} concentration for growth[2]. The effect of Mg^{2+} concentration on production of magnesidin A were examined. The growth of the strain was equivalent at 2 to 250 mM Mg^{2+} concentration. The amount of produced magnesidin A, which were detected by HPLC, were increased with a rise of Mg^{2+} concentration to 150 mM.

The producer of magnesidin is also marine bacteria, *Pseudomonas magnesiorubra*, isolated from the surface of macro alga, *Caulerpa peltate*[12]. It is interesting that the characteristic antibiotic containing magnesium was found to be produced by different genus of marine bacteria.

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