A NEW ALKALOID ANTIBIOTIC TETRAZOMINE

STRUCTURE DETERMINATION

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A new alkaloid antibiotic tetrazomine was isolated from the culture broth of *Saccharothrix mutabilis* subsp. *chichijimaensis* subsp. nov., and its structure was determined to be **I** by means of spectroscopic measurements. It has an unusual structure which consists of six rings, including piperidine, piperazine, oxazole, and pyrrolidine rings.

In the screening for new antibiotics which possess a broad-spectrum activity against bacteria,[1,2] we found a new alkaloid antibiotic, tetrazomine, in the culture broth of *Saccharothrix mutabilis* subsp. *chichijimaensis* subsp. nov. which was isolated from a soil sample collected from Ogasawara Islands, Tokyo, Japan. The structure was determined to be **I** by spectroscopic analysis, mainly relying on NMR experiments. The characteristic structural feature of tetrazomine is that it contains five heterocyclic rings. In the preceding paper[3] we described taxonomy, fermentation of the producing organism, isolation and characterization of tetrazomine. In this paper we describe the structural elucidation of tetrazomine.

Structure of Tetrazomine

The structure of tetrazomine was determined to be **I**, Fig. 1, by spectroscopic analysis, especially relying on the results of NMR analysis. ^{1}H, ^{13}C, carbon multiplicity (DEPT), COSY and heteronuclear multiple-bond correlation (HMBC) NMR spectra were recorded on tetrazomine hydrochloride in D_2O and CD_3OD. The results are summerized in Table 1. The ^{13}C NMR spectrum in CD_3OD showed 23 distinct resonances and one line at δ 56.9 ppm which was collapsed and too small to be recognized as a peak but assigned from ^{13}C-^{1}H COSY spectrum. The molecular formula $C_{24}H_{34}N_4O_5$ was determined by the HRFAB mass spectral data and the carbon number of 24 in the ^{13}C NMR spectrum. From the analysis of the COSY spectra (^{1}H-^{1}H and ^{13}C-^{1}H), five distinct spin systems were identified (**II**~**VI**) as shown in Fig. 2. The NMR spectra indicated that C-2′a is bonded with two heteroatoms. That one of them is oxgen was clearly demonstrated by its resonance at δ 92.7 ppm in the ^{13}C NMR spectrum. C-3′, C-2 and C-3 were also bonded with heteroatoms, but we could not distinguish bonding with nitrogen from bonding with oxygen by their chemical shifts in the ^{1}H and ^{13}C NMR spectra. C-13′

Fig. 1. Structure of tetrazomine (**I**).

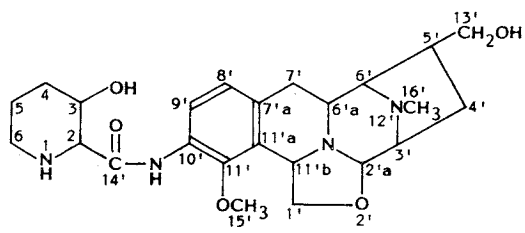
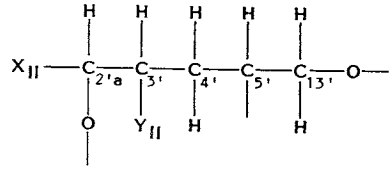


Table 1. NMR spectral data for tetrazomine.

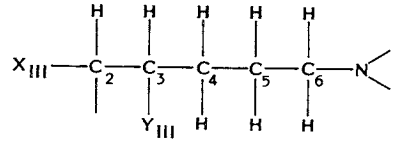
CD_3OD		D_2O		^1H-^1H Connection	Assignment
^{13}C Shift[a]	^1H Shift[a]	^{13}C Shift[a]	^1H Shift[a]		
167.2		169.9			14′
149.0		152.6			11′
134.9		138.3			7′a
130.1		129.8			10′
129.6		131.2			11′a
124.7	6.96	126.9	7.09	9′	8′
124.1	7.80	127.5	7.53	8′	9′
92.7	4.63	84.2	5.01	3′	2′a
71.6	3.80	72.7	3.89		6′
68.9	3.65, 3.42	68.7	3.80, 3.66	11′b	1′
66.7	4.13	72.1	4.01	2′a, 4′	3′
65.7	4.57	67.6	4.74	2, 4	3
64.6	3.72, 3.61	66.5	3.84, 3.73	5′	13′
62.6	4.16	63.7	4.36	3	2
61.7	3.80	64.0	3.82		15′
57.0	4.52	57.1	4.50	1′	11′b
56.9	3.49	56.9	3.54	7′	6′a
44.9	3.36, 3.07	46.5	3.54, 3.18	5	6
41.0	2.96	43.2	3.00		16′
39.0	3.01	39.6	3.03	4′, 13′	5′
32.3	2.74, 2.62	33.7	2.77, 2.71	6′a	7′
30.6	2.00, 1.89	31.4	2.11, 1.95	3, 5	4
27.4	2.44, 2.06	29.2	2.42, 2.08	3′, 5′	4′
17.7	2.14, 1.72	18.6	2.08, 1.83	4, 6	5

[a] δ ppm.

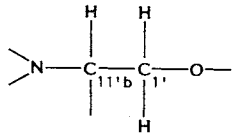
Fig. 2. Partial structures of tetrazomine revealed by COSY experiments.



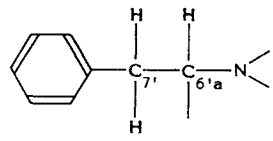
II



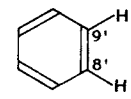
III



IV



V

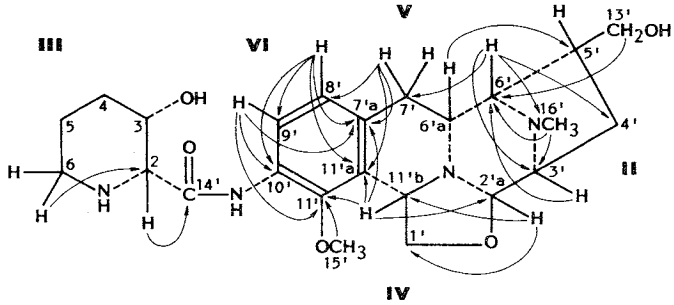


VI

X and Y mean oxygen or nitrogen.

and C-1′ are bonded with oxygen, and C-6, C-11′b and C-6′a are bonded with nitrogen as indicated by their resonances in the ^{13}C NMR spectrum. The existence of phenyl group was indicated by the chemical shifts of 8′-H and 9′-H at δ 6.96 and 7.80 ppm, respectively, in the ^1H NMR spectrum and by the coupling constant (8.3 Hz) between them. Moreover it was supported by the absorption at 1610 cm^{-1} in the IR

Fig. 3. Long range couplings observed by HMBC experiment.



The dotted lines mean the bonds undetermined before HMBC experiments.

spectrum. Five partial structures comprised 20 out of 24 carbons in the molecule. The remaining carbons are two methyls (N-CH_3 and O-CH_3), one amide carbonyl which was observed at δ 167.2 ppm in the ^{13}C NMR spectrum and at 1680 cm^{-1} in the IR spectrum and one methine whose couplings were so weak that we could not determined the direct correlations with it. The partial structures could be combined to give the structure **I** by means of HMBC spectra in D_2O and CD_3OD. The results of HMBC spectra are summarized in Fig. 3. The details of the connections of the partial structures are as follows. As for the partial structure **II**, 2'a-H is coupled with C-1', so the connection between C-2'a and C-1' through oxygen was indicated. Mutual couplings between 2'a-H and C-11'b and between 11'b-H and C-2'a indicated that X_{II} is nitrogen and that C-2'a and C-11'b are connected through nitrogen, giving an oxazole ring. Thus the partial structure **IV** was connected with **II**. 16'-Hs are coupled with C-3' and C-6', so that Y_{II} is nitrogen, and that C-6' is bonded with C-3' through a methylated nitrogen was indicated. This connection was supported by the couplings for 6'-H and C-3' and C-16'. 6'-H is furthermore coupled with C-4', indicating that C-6' is directly connected with C-5', giving a pyrrolidine ring. This connection was supported by the coupling between 13'-Hs and C-6'. 6'a-H is coupled with C-5', and 6'-H is coupled with C-7', indicating that C-6'a is bonded directly with C-6'. Consequently the partial structure **V** was connected with **II**. 7'-Hs are coupled with C-7'a, C-8' and C-11'a. On the other hand, 11'b-H is coupled with C-7'a, C-11' and C-11'a, indicating that C-11'b is bonded with C-11'a which is *ortho* to C-7'a. Furtheremore 8'-H is coupled with C-7'a, C-9', C-10' and C-11'a, so the carbon sequence of C-10'-C-9'-C-8'-C-7'a-C-11'a-C-11' was established. 9'-H is coupled with C-7'a, C-10' and C-11', therefore the benzene ring was confirmed. Bonding of the methoxyl group to C-11' was indicated by its resonance at δ 3.80 ppm in the ^1H NMR spectrum and by the coupling between 15'-Hs and C-11'. Moreover tetrazomine did not show a characteristic UV shift in alkaline solution. As for the partial structure **III**, 6-Hs are coupled with C-2, indicating that X_{III} is nitrogen and that C-6 is bonded with C-2 through nitrogen to form a piperidine ring. Therefore Y_{III} was shown to be oxygen by the number of oxygens in the molecule. The only remaining part of the structure is an amide group in which C-14' is coupled with 2-H but not with 9'-H. Absoption at 244 nm in the UV spectrum also indicated that the benzene ring is not bonded directly with a carbonyl group.[4] It was thus determined that C-2 is bonded with the carbonyl carbon and C-10' is bonded with the nitrogen of the amide group. The 3-hydroxypiperidine ring was further confirmed by the strong fragment at *m/z* 100 in the mass spectrum. Finally it was easily determined by consideration of the number of nitrogens that the nitrogen which is bonded with C-6'a is the same one as that in the oxazole ring. C-13' is a hydroxymethyl group because there was no counterpart to bond with.

Discussion

Structurally, tetrazomine is unusual and belongs to a small group of antibiotics that includes quinocarcin,[5,6] saframycins[7,8] and naphthyridinomycin A.[9] Though tetrazomine shares partial structures with these antibiotics, the tetrazomine-producing strain did not produce detectable levels of any of them. In the ^1H-^1H COSY spectrum, we could not detect the coupling between 5′-H and neighboring protons. According to a molecular model, the dihedral angle between 5′-H and 6′-H is nearly 90° in two conformations, and that between 6′-H and 6′a-H could be about 70° in one of two conformations. That may be the main reason for our observation. As for the stereochemistry of tetrazomine, we are investigating this with NMR spectroscopy, for example by comparing the coupling constants of tetrazomine with those of quinocarcine and saframycins and by measuring NOEs.

Tetrazomine is active against Gram-positive and Gram-negative bacteria with MICs of $1.56 \sim 25\,\mu g/ml$ and $3.13 \sim 50\,\mu g/ml$, respectively. Compared with the structurally most closely related antibiotic, quinocarcine, tetrazomine exhibits stronger activity, especially against Gram-negative bacteria. For example, MIC values of tetrazomine against *Escherichia coli* and *Klebsiella pneumoniae* are 1.56 and $3.13\,\mu g/ml$, but those of qunocarcin are >100 and 25 mg/ml, respectively. One of the reasons for the activity difference may be a difference in cell wall permeability between the two compounds. We are interested in making more active compounds by structural modification.

Acknowledgments

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