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THE STRUCTURE OF AN ANTIBIOTIC, DITYROMYCIN

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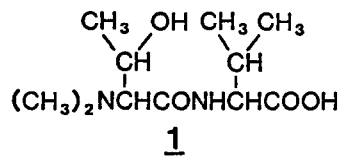
Summary: Dityromycin is a peptide antibiotic isolated from the culture broth of the soil microorganism, Streptomyces sp. strain No. AM-2504, showing activities against Bacillus, Corynebacterium, and Clostridium.[1] Dityromycin was clarified to be composed of N-MeVal, Pro, Val, Phenylglycine, CH_3NH_2, N,N-Me_2Thr and di-α-amino acid containing diphenylether moiety. The peptide sequence except one labile amino acid residue was determined by partial hydrolysis, chemical cleavage, and the Edman degradation. The unknown amino acid was finally deduced to be the dehydroamino acid containing epoxide, resulting in an elucidation of whole structure of the antibiotic.

Dityromycin was obtained from the culture broth of the soil microorganism, Streptomyces sp. strain No. AM-2504 by Ōmura et al. Antibacterial activities were recognized against Gram positive bacteria such as Bacillus, Corynebacterium, and Clostridium.[1] A molecular weight of dityromycin was determined to be 1288 from FAB-MS spectrometry. No N- and C-terminal amino acids could be detected by dansyl method and hydrazinolysis respectively. An acid hydrolysis of dityromycin (6M HCl, 140°C, 30h) gave N-methylvaline[2](N-MeVal)(2), Pro(2), Val(1), phenylglycine (Pgl)(1), and CH_3NH_2(1) (Table 1), whereas weaker hydrolysis conditions (6M HCl, 110°C, 20h) afforded a dipeptide, N,N-Me_2-L-Thr-L-Val-OH (1) in place of Val. The structure of peptide 1 was determined by NMR and FAB-MS (M+H: 247) and finally confirmed by synthesis.[3]

Table 1. Amino Acid Composition of Dityromycin

Pretreatment	–	1M NaOH/EtOH	–
Hydrolysis Conditions	6M HCl 140°C 30 h	6M HCl 140°C 30 h	57% HI/P 110°C 24 h
N-MeVal	2.01(2)	2.23(2)	1.65(2)
Pro	2.00(2)	2.19(2)	2.02(2)
Gly	– (0)	0.71(1)	– (0)
Val	0.79(1)	1.04(1)	0.61(1)
Pgl	1.01(1)	1.03(1)	1.00(1)
NH_3	2.41	1.48	0.55
CH_3NH_2	1.00(1)	1.00(1)	– (0)



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One mole of Gly was newly detected by acid hydrolysis of the original peptide after treatment with sodium hydroxide. Alkaline treatment followed by hydrogenation and acid hydrolysis gave an aromatic amino acid 2 whose structure was deduced by NMR and FAB-MS (M+H: 316). Further alkaline hydrolysis of 2 gave another product 2', whose structure was con-

firmed by synthesis.[4] Moreover, a reductive hydrolysis of dityromycin with 57% HI-P (110°C, 24h)[5] gave di-α-amino acid 3 in place of of CH_3NH_2. The amino acids, 2 and 3, may be derived from the same amino acid component as shown in Fig 1. In addition, γ-hydroxyisoleucine lactone (4) was also obtained by reductive hydrolysis of the antibiotic with 57% HI-P.[6]

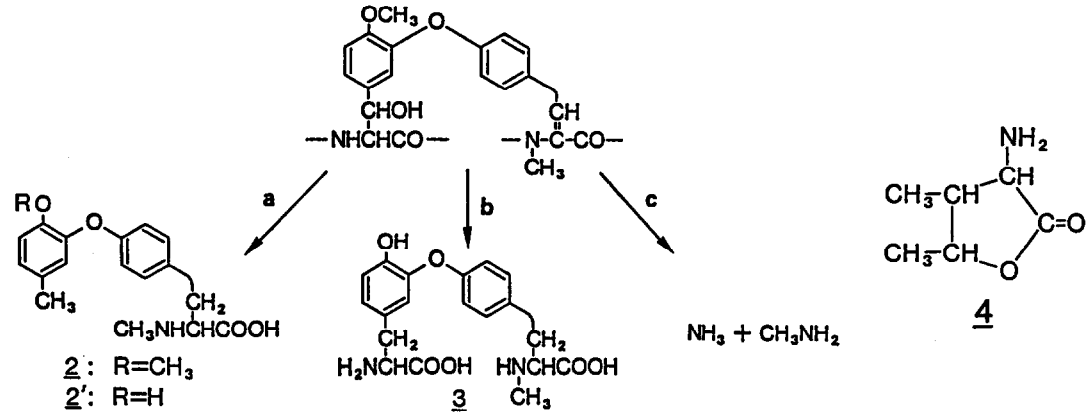

Fig. 1. a: 1)NaOH, 2)H_2/Pd, 3)6M HCl. b: 57% HI-P. c: 6M HCl

In order to elucidate the amino acid sequence, dityromycin was treated with 1M NaOH (r.t. 3h) to give peptide fragments 5 and 6. The peptide 5 of a molecular weight of 589 (FAB-MS, M+H: 590) is composed of N-MeVal(1), Pro(1), Pgl(1), and N,N-Me_2Thr-Val(1) (Table 2). Partial hydrolysis of 5 gave 5-1 and 5-2 losing N-MeVal and Pro successively from 5. Furthermore, N-MeVal was not detected in the hydrolyzate of reduction product (5-3) which was obtained from 5 by methyl esterification followed by reduction with $NaBH_4$, indicating that C-terminal amino acid should be N-MeVal. From these results, the sequence of 5 was determined to be a pentapeptide as shown in Fig. 2, which was supported by fragmentation pattern of FAB-MS. Another peptide fragment 6 giving two peaks on HPLC assumed to be an equilibrium mixture since two peptides separated by HPLC reproduced again a mixture of two peaks on HPLC. The

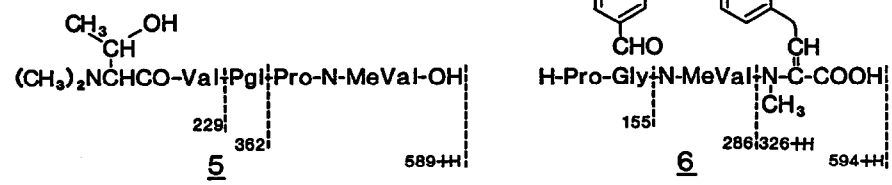

Fig. 2. Structures and FAB-MS Fragmentations of 5 and 6

Table 2. Amino Acid Composition of Peptide Fragments Obtained by Alkaline Treatment

	5	5-1	5-2	5-3	6
N-MeVal	1.04(1)	- (0)	- (0)	- (0)	1.08(1)
Pro	1.00(1)	1.02(1)	- (0)	1.03(1)	1.00(1)
Gly	- (0)	- (0)	- (0)	- (0)	0.81(1)
Pgl	0.93(1)	1.00(1)	1.00(1)	1.00(1)	- (0)
NH_3	0.30	0.68	0.57	1.47	0.55
CH_3NH_2	- (0)	- (0)	- (0)	- (0)	0.98(1)
N,N-Me_2Thr-Val*	1.15(1)	1.14(1)	1.20(1)	1.09(1)	- (0)

Hydrolysis conditions: 6M HCl, 110°C, 24h.
* Detected by HPLC.

molecular weight of 6 was determined to be 594 by FAB-MS. The amino acids N-MeVal(1), Pro(1), Gly(1), and CH_3NH_2(1) were detected in the hydrolyzate of 6. Though N-terminal amino acid was determined to be Pro by dansyl method and also by Edman degradation, subsequent step of the Edman degradation did not proceed any more. Oxidative cleavage of 6 by bromine followed by alkaline treatment[7] gave H-Pro-Gly-N-MeVal-OH whose structure was determined by FAB-MS, amino acid analysis, and Edman degradation. In addition, 6 was positive to 2,4-dinitrophenylhydrazine test suggesting the presence of an aldehyde group. Consequently, the sequence of 6 was determined as shown in Fig. 2.

Another larger fragment peptide 7 of a molecular weight of 1165 (FAB-MS, M+H: 1166) was obtained by mild hydrolysis of dityromycin (cHCl-AcOH (1:1), r.t., 3h). Amino acid composition of 7 (hydrolysis condition: 6M HCl, 140°C, 36h) was the same as that of dityromycin. N-terminus of 7 was determined to be Pro by Edman degradation. Alkaline treatment of 7 produced 5 and 6, both of which were suggested to be connected by ester linkage in 7. Since aldehyde of 6 was certainly derived by retroaldol condensation of β-hydroxytyrosine moiety, there may be two modes of ester linkage for coupling of 5 and 6. If the carboxyl group in the moiety 6 is free in the molecule of 7, methylamine should be liberated from dehydroamino acid part in the treatment of 7 with $NaBH_4$ followed by hydrolysis[8]. However, it was not the case as shown in Table 3. This fact can be elucidated by an explanation that C=C double bond conjugated to the ester group in the structure of 7 was reduced with $NaBH_4$ by 1,4-addition, resulting in no liberation of CH_3NH_2. From these results, a coupling mode of 5 and 6, consequently, the structure of 7 was determined as shown in Fig. 3.

Finally, the missing structure was deduced to be unknown amino acid moiety of a formula $C_6H_7NO_3$ from comparison of the structure 7 and dityromycin in molecular weight and detection of Ile after reductive hydrolysis of dityromycin. Furthermore, all other experimental data such as liberation of one more additional ammonia[9], easy cleavage of the peptide bond before proline residue[10] as well as positive coloration reaction for epoxide[11] in the whole molecule strongly suggested a presence of epoxy hydroxy dehydroisoleucine moiety in dityromycin. Thus, a whole structure of dityromycin can be proposed as shown in Fig. 4, a

Table 3. Amino Acid Composition of 7

Pretreatment	–	$NaBH_4$*
N-MeVal	1.77(2)	2.30(2)
Pro	2.60(2)	1.92(2)
Gly	–	0.14(0)
Val	1.11(1)	0.92(1)
Pgl	1.00(1)	1.00(1)
NH_3	1.87	1.66
CH_3NH_2	1.27(1)	– (0)

Hydrolysis conditions: 6M HCl, 140°C, 36h or 24h($NaBH_4$)
* In Tris-buffer pH 8.5

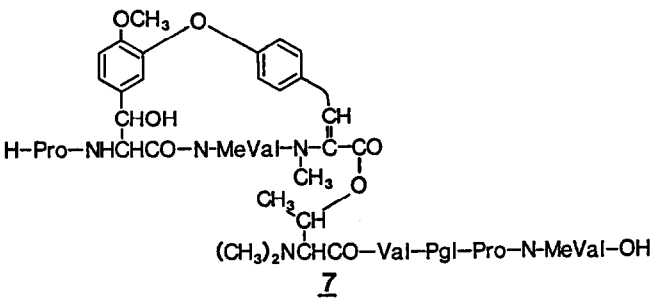

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Fig. 3. The Structure of 7

Table 4. Comparison of Dityromycin with 7

	MW	NH_3[a]	Ile[b]	NBP-test[c]	KSCN-test[d]
Dityromycin	1288	2	+	+	+
7	1165	1	–	–	–

a) 6M HCl, 140°C; b) 4 by HI-P (110°C), Ile and alloisoleucine by HI-P (155°C); c) 4-(4-Nitrobenzyl)-pyridine and NaOH; d) KSCN solution and phenolphthalein

very unique structure including bicyclic depsipeptide, diphenyl ether, and epoxide parts, although configurations of two double bonds are not yet clarified.

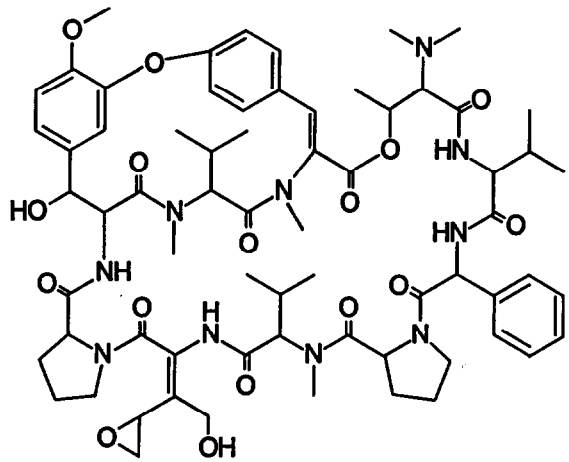
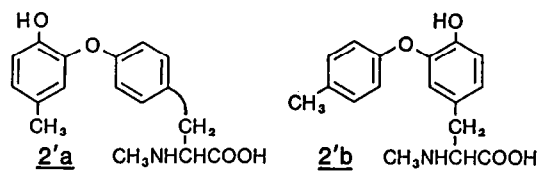


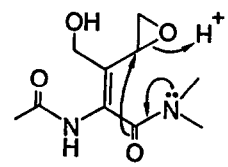
Fig. 4. The Total Structure of Dityromycin

References and Notes

1) S. Ōmura, Y. Iwai, A. Hirano, J. Awaya, Y. Suzuki, and K. Matsumoto, Agric. Biol. Chem., 41, 1827 (1977).
2) The absolute configurations of all amino acids obtained from the hydrolyzate by 6M HCl were determined to be L form by comparison of ORD curves with those of the authentic samples.
3) N,N-Me_2-L-Thr-L-Val-OH was obtained by reductive methylation of H-L-Thr-L-Val-OH with formalin and $NaBH_3CN$. N,N-Me_2-DL-Thr-L-Val-OH and N,N-Me_2-DL-aThr-L-Val-OH (aThr: allothreonine) were also synthesized as a mixture of diastereoisomers. Under condition of HPLC where four diastereoisomers thus prepared could be separated, the retention time of natural sample was identical with that of N,N-Me_2-L-Thr-L-Val-OH.
4) In order to determine the structure of 2', two possible isomers were synthesized: The compound 2'a was synthesized by the coupling of creatinine and 4-(2-methoxy-5-methylphenoxy)-benzaldehyde obtained from 3-(4-(methoxycarbonyl)phenoxy)-4-methoxybenzaldehyde[a] followed by reductive hydrolysis with HI-P and alkaline hydrolysis. The compound 2'b was synthesized from isovaniline and p-bromotoluene in a similar manner. On HPLC analysis 2' was identical with 2'a but not with 2'b, both of which can be distinguished easily by different retention time. a) M. Iyoda, M. Sakaitani, H. Otsuka, and M. Oda, Tetrahedron Lett., 26, 4777 (1985).



5) C. M. Harris and T. M. Harris, J. Am. Chem. Soc., 104, 363 (1982).
6) γ-Hydroxyisoleucine lactone was obtained as a mixture of four diastereoisomers. Its structure was determined by NMR, FAB-MS and chemical cleavage (Treatment of this compound with HI-P at 155°C for 24h gave Ile and alloisoleucine).
7) A. Patchornik and M. Sokolovsky, J. Am. Chem. Soc., 86, 1206 (1964).
8) Amino acid composition of 6 after treatment with $NaBH_4$ followed by hydrolysis was as follows; N-MeVal: 1.10(1), Pro: 0.92(1), Gly: 1.00(1), CH_3NH_2: 1.05(1).
9) The molar ratio of NH_3 derived from contamination was negligible since it was estimated less than 0.5 in the amino acid analysis using more than 100 nmol of the sample.
10) A peptide bond connected to an amino acid residue with epoxide ring on γ and δ carbons may be cleaved easily on acid hydrolysis by the mechanism as shown here.



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