REDUCTIOMYCIN, A NEW ANTIBIOTIC

II. STRUCTURAL ELUCIDATION BY SPECTROSCOPIC STUDIES

KEN-ICHI SHIMIZU* and GAKUZO TAMURA

Laboratory of Microbiology, Department of Agricultural Chemistry
University of Tokyo, Bunkyo-ku, Tokyo, Japan

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The structure of reductiomycin was elucidated as 2-hydroxy-5-oxo-1-cyclopentenyl (2*E*)-3-(5-acetoxy-2,3-dehydropyrrolidin-3-yl) propenate by spectroscopic studies.

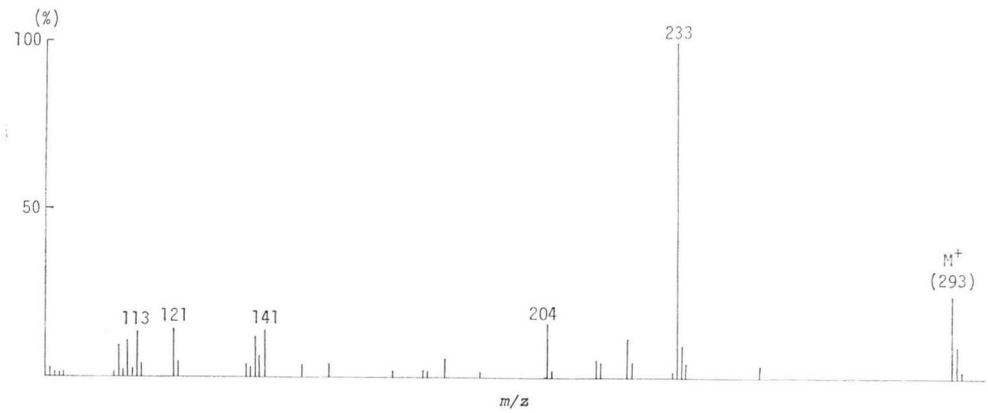
In the previous paper,[1] we reported fermentation, isolation, physico-chemical properties and biological activities of reductiomycin. Further investigation on reductiomycin by various spectroscopic methods elucidated its full structure. The structure was supported by X-ray diffraction studies which were reported in the separate paper.[2]

This paper describes the structural elucidation by spectroscopic studies and discusses the unique characteristics of reductiomycin.

As reported in the preceding paper,[1] a high resolution mass measurement determined the molecular formula of reductiomycin as $C_{14}H_{15}O_6N$ which was in good agreement with the elemental analysis. As shown in Fig. 1, the mass spectrum of reductiomycin showed a base peak at *m/z* 233 that was ascribed to $C_{12}H_{11}O_4N$. This suggested that reductiomycin readily lost CH_3COOH. The loss of CH_3COOH from reductiomycin was also observed when it was heated. The sublimate, obtained by heating at 215°C, possessed a molecular ion at *m/z* 233, the composition of which was determined as $C_{12}H_{11}O_4N$ by high resolution measurement. Its PMR spectrum did not exhibit a signal due to an acetoxy group which was observed at 2.12 ppm in that of the parent antibiotic (Tables 1 and 2).

The fragment peaks at *m/z* 113 and 141 were ascribed to $C_5H_5O_3$ and $C_6H_5O_4$, respectively. Their partial structures were proposed to be **I** and **II** as shown in Fig. 2. These partial structures were sup-

Fig. 1. EI mass spectrum of reductiomycin.



* Present address: National Research Institute of Brewing, 2–6, Takinogawa, Kita-ku, Tokyo 114, Japan.

Table 1. PMR spectrum of reductiomycin.

	Chemical shift (ppm)	Multiplicity	Proton integration	Coupling constant (Hz)
H_a*	13.75	s	H	
H_b*	7.86	s	H	
H_c	7.49	d	H	$J_{cf}=16$
H_d	6.86	s	H	
H_e	6.73	dd	H	$J_{eg}=7.7$
				$J_{eg'}=2.2$
H_f	5.88	d	H	
H_g	3.08	dd	H	$J_{gg'}=16$
$H_{g'}$	2.70	dd	H	
H_h	2.60	broad s	4H	
H_i	2.12	s	3H	

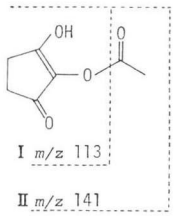
Run in $CDCl_3$ at 100 MHz. Frequencies in ppm downfield from Me_4Si as internal standard.
* Removed by the addition of D_2O.

Table 2. PMR spectrum of deacetoxy reductiomycin.

	Chemical shift (ppm)	Multiplicity	Proton integration	Coupling constant (Hz)
H_X	8.04	d	H	$J_{XY}=1.5$
H_d	7.72	s	H	
H_c	7.48	d	H	$J_{cf}=16$
H_f	6.83	d	H	
H_Y	6.65	d	H	
H_h	2.50	s	4H	

Run in DMSO-d_6 at 100 MHz.
Frequencies in ppm downfield from Me_4Si as internal standard.

Fig. 2. Partial structure I and II of reductiomycin.



ported by the presence of an absorption band at 1600 cm^{-1} (1,3-diketone) in the IR spectrum. Further evidence was the presence of an enolic hydroxy proton (H_a) at 13.75 ppm and the two equivalent methylene groups (H_h) at 2.60 ppm in the PMR spectrum (Table 1).

These structures were also substantiated by the observation that methylation of the enolic group with diazomethane revealed two signals (2.50 and 2.73 ppm, in $CDCl_3$) corresponding to two methylene groups that were equivalent before the reaction.

The doublet (H_f) at 5.88 ppm was coupled with the doublet (H_c) at 7.49 ppm (AB system, $J_{cf}=$ 16 Hz) indicative of the presence of two *trans* hydrogens on a –C=C–CO– group. Thus an olefinic group is connected to the ester carbonyl group of the partial structure (**II**).

The partial structure (**II**) and the acetoxy group of reductiomycin leave C_4H_5N to be elucidated.

The PMR spectral data (Tables 1 and 2) showed the presence of a secondary amine proton (H_b) at 7.86 ppm. When the PMR spectral data of reductiomycin were compared with those of the sublimate, the signals corresponding to H_g and $H_{g'}$ of reductiomycin disappeared, and two methine signals in the aromatic region appeared in the sublimate. This indicated the introduction of a double bond with the loss of acetate and the center of asymmetry. In fact, the optical rotation of the sublimate was $[\alpha]_D^{28}$ 0° (*c* 0.15, acetone).

As shown in Table 1, H_e at 6.73 ppm attributable to the proton attached to the asymmetric carbon

Table 3. CMR chemical shifts of reductiomycin (in DMSO-d_6).

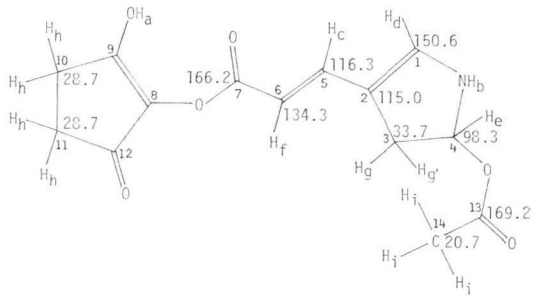
>C=O (s)	-NH-CH= (d)	-CH= (d)	>C= (s)	-O-CH- (d)	$-CH_2-$ (t)	$-CH_3$ (q)
169.2	150.6	134.3	115.0	98.3	33.7	20.7
166.2		116.3			28.7	

was coupled with the geminal protons (H_g and $H_{g'}$). The CMR spectral data (Table 3) supported this assignment, for the chemical shift of the methine carbon (C-4) was found at 98.3 ppm in agreement with the attachment of both oxygen and nitrogen atoms.

Thus the partial structure (**III**) is proposed for C_4H_5N (Fig. 3).

Fig. 3. Partial structure (**III**) of reductiomycin.

Fig. 4. Structure of reductiomycin.



The interconnections of these structural units, **II**, **III**, olefinic group (*trans*) and the acetoxy group were deduced through the following arguments. The linkage of the olefinic group to the ester carbonyl of the partial structure (**II**) was discussed in the foregoing section. The loss of the acetoxy group from reductiomycin caused the loss of asymmetry of C-4. Thus the acetoxy group must connect to C-4. These assignments concluded the total arrangement of the partial structures as shown in Fig. 4.

The CMR spectral data of reductiomycin in DMSO-d_6 is summarized in Table 3. Signals of 11 carbon atoms were observed, but 3 signals were not observed. They were also not detected in $CDCl_3$. Signals of 11 carbon atoms confirmed the presence of the functional groups indicated in Fig. 4.

Since signals of three carbon atoms were not observed in CMR spectrum, X-ray diffraction studies were conducted which supported the structure shown in Fig. 4.[2]

This structure is in complete agreement with IR, PMR, CMR and mass spectral data. The assignments of PMR and CMR are summarized in Fig. 4.

Discussion

The structure of reductiomycin elucidated by spectroscopic studies was supported by X-ray diffraction studies.[2] Reductiomycin consisted of unique moieties, a β-diketone and carbinolamine.

A β-diketone is present in uchinol (an antifungal compound),[3] asukamycin (an antibiotic),[4] oudenone (an inhibitor for tyrosine hydroxylase)[5] and reductic acid (a reducing agent).[6] This moiety seems to play an important role in their biological activities. The carbinolamine moiety has been shown to be present in various antitumor antibiotics including pyrrolo(1,4)benzodiazepine antibiotics,[7~10] mitomycin C,[11] ansamitocin[12] and naphtyridinomycin.[13] In pyrrolo(1,4)benzodiazepine antibiotics, the carbinolamine is essential for their biological activities. The formation of the antibiotic-DNA complex involving a SCHIFF base linkage has been proposed for the mechanism of action.[10]

Therefore, both moieties, β-diketone and carbinolamine present separately play important roles in biological activities of the above compounds.

In the case of reductiomycin, the presence of both the moieties provides additional properties to the molecule. The linkage of both moieties makes the molecule planar, and the size of the planar part measures about 11 Å.[2] The distance has been observed in polycyclic compounds that intercalate DNA.[14] Therefore, reductiomycin may intercalate DNA, affecting DNA and/or RNA synthesis, even though reductiomycin is not a polycyclic compound. In fact, reductiomycin caused elongation of *Bacillus subtilis* IAM 1026, suggesting that it affected DNA and/or RNA synthesis. Further related experiments are in progress.

Recently, we received a personal communication concerning an antibiotic similar to reductiomycin.[15] Although it is not clear whether the antibiotic is identical with reductiomycin, the same structure as shown in Fig. 4 is proposed for the antibiotic except for interchange between NH and O(connected C-8). Thus chemical studies may be necessary to confirm conclusively the structure of reductiomycin shown in Fig. 4.

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