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STRUCTURE OF AN ANTITUMOR ANTIBIOTIC, REDUCTIOMYCIN

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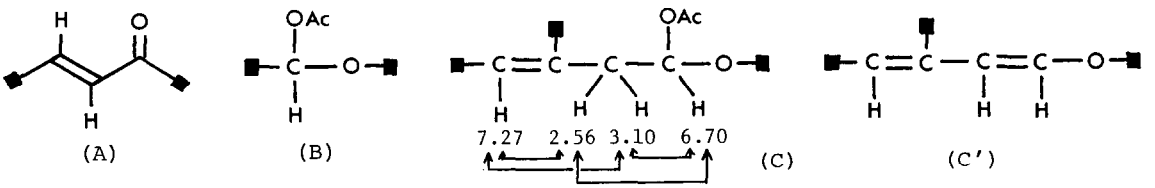
Abstract: The structure of an antitumor antibiotic isolated from a variant of Streptomyces orientalis was established as (1) by chemical and spectral evidence. The antibiotic was found to be identical with reductiomycin, and therefore the structure (X) of reductiomycin previously reported must be revised.

From a fermentation broth of a variant of Streptomyces orientalis, a crystalline antitumor compound (1) was isolated[1], which showed the following physical and spectral properties: 1[2], mp 215° (dec.) (MeOH), $C_{14}H_{15}NO_6$, $[\alpha]_D^{23}$ +273° (c 0.20, acetone); UV (MeOH) 286 nm (ε 25,200); IR (KBr) 3230, 2500 (broad), 1727, 1680 (weak), 1600 (broad), 1545, 1108, 1044, 988 cm^{-1}; ^{1}H-NMR (Table 1); ^{13}C-NMR (Table 2); MS 293 (M^+), 233, 204, 139, 121, 113. In the ^{1}H-NMR spectrum of 1 two signals at δ 6.37 (1H, d, J=15 Hz) and 7.42 (1H, d, J=15 Hz), shown to couple each other, were observed, indicating the presence of a trans-disubstituted α,β-unsaturated carbonyl group as in (A).

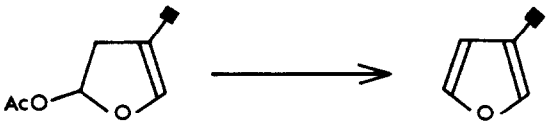
A hemiacetal acetate group (B) was deduced to be present in 1 by a doublet at δ 98.3 in the ^{13}C-NMR spectrum and a signal at δ 6.70 (1H, dd, J=7, 2 Hz) in the ^{1}H-NMR spectrum. The structural information around the hemiacetal acetate group (B) moiety in 1 was obtained from the ^{1}H-NMDR experiments, the result of which was shown by the partial structure (C): on irradiation of the signal at δ 6.70 a broad doublet at δ 2.56 became a sharp doublet and a broad doublet of doublets at δ 3.10 was changed into a doublet, revealing that the hemiacetal carbon is adjacent to a methylene carbon; irradiation of the signal (δ 2.56) due to one of the methylene protons caused a broad singlet at δ 7.27 and a doublet of doublets at δ 6.70 to collapse into a sharp singlet and a doublet, respectively, while irradiation of the signal (δ 3.10) arising from the other of the methylene protons resulted in the same change of the signal pattern at δ 7.27 as in the case of the irradiation of the signal at δ 2.56.



(A) (B) (C) (C')

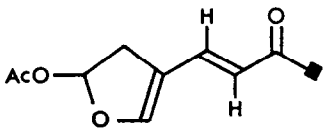
On heating the antibiotic (1) (AcOH, reflux, 3 h) elimination of one mole of acetic acid took place to afford an optically inactive compound (2)[2], $C_{12}H_{11}NO_4$, mp 255° (dec.). Comparison of the ^{1}H-NMR spectra of 1 and 2 revealed that the four signals at δ 2.56, 3.10, 6.70, and 7.27 together with the acetate Me signal at δ 2.07 present in 1 disappeared in 2 and instead three new signals at δ 6.65 (1H, d, J=2 Hz), 7.74 (1H, dd, J=2, 2 Hz), and 8.06 (1H, br.s) were observed in 2, other signals being essentially unchanged in both compounds. These three new signals in the ^{1}H-NMR spectrum of 2 corresponded well to those of mono-

substituted furan derivatives with respect to both chemical shifts and coupling constants. The ^{13}C-NMR spectrum of 2 also strongly supported the view that 2 was a monosubstituted furan derivatives (see Table 1 and 2). Based on the fact that the moiety undergoing the elimination of AcOH in the reaction, 1→2 was contained in the partial structure (C), and on the inference that the monosubstituted furan part was present in 2, the partial structure (D) was deduced to be present in 1. Therefore the structural change in the reaction, 1→2 was represented by the formulas, (D)→(D').

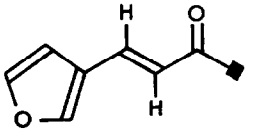


(D) (D')

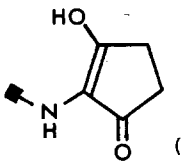
Treatment of 2 with methyl iodide under basic conditions (NaH, DMF, r.t., 30 min.), gave a dimethyl derivative (3)[2], $C_{14}H_{15}NO_4$ (amorphous), the ^{1}H-NMR spectrum of which showed two new singlets due to an OMe and an NMe group. Oxidation of the dimethyl derivative (3) with OsO_4 (THF-Py, 17°, 1 h) afforded a 1,2-diol (4), $C_{14}H_{17}NO_6$ (amorphous). The trans-disubstituted double bond in (A) was shown to undergo oxidation with OsO_4 on the basis of the ^{1}H-NMR spectral analysis (see Table 1). The diol 4 was subsequently oxidized with $NaIO_4$ [H_2O-EtOH (1:1), 17°, 1 h]. From the oxidation products, 3-formylfuran[2] was obtained as the 2,4-dinitrophenylhydrazone[2], mp 232-235°, which was identical with an authentic specimen obtained by oxidation of 3-hydroxymethylfuran with MnO_2 and subsequent treatment with a 2,4-dinitrophenylhydrazine solution by spectral comparison and mixed mp. The above findings made it possible to correlate two partial structures, (A) and (D), leading to the new partial structure (E). The partial structure (E') was therefore shown to be present in 2. With the partial structure (E) in hand, the remaining problem to be settled was the elucidation of the structure corresponding to the formula $C_5H_6NO_2$ in 1.



(E)

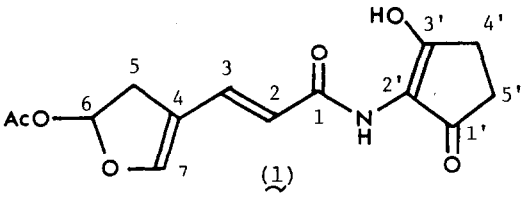


(E')



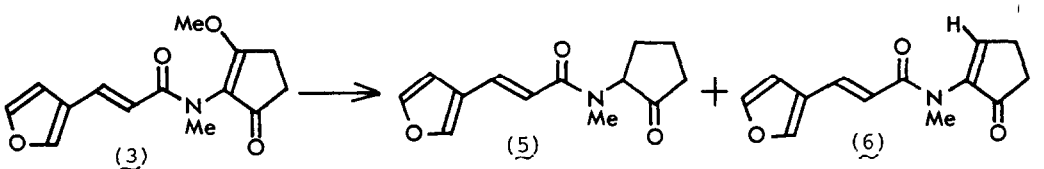
(F)

Reduction of 3 with DIBAL (CH_2Cl_2, -20°, 25 min.) afforded two products, a dihydro-demethoxy derivative (5)[2], $C_{13}H_{15}NO_3$ (amorphous), and a demethoxy derivative (6)[2], $C_{13}H_{13}NO_3$ (amorphous). From the ^{1}H-NMR spectral data of 5 and 6, the part (E') was shown to be intact and the moiety undergoing reduction was evidently the part corresponding to $C_5H_6NO_2$. In the IR spectrum of 5 an absorption band at 1747 cm^{-1} was observed, which was due to the five-membered ring ketone, and in the ^{1}H-NMR spectrum there were observed a signal at δ 4.35 (1H, br.t, J=10 Hz) and signals between δ 1.9 and 2.5 (6H, m). In the IR spectrum of 6 an absorption band at 1712 cm^{-1} appeared, which was ascribed to a conjugated five-membered ring ketone, while a signal at δ 7.64 (1H, t, J=3 Hz) and signals at δ 2.48 and 2.75 (4H, the A_2B_2 part of an A_2B_2X type) were newly observed in the ^{1}H-NMR spectrum of 6. On the basis of these IR and ^{1}H-NMR spectral analyses of 5 and 6 together with the mechanistic consideration of the DIBAL reduction, the remaining part corresponding to the formula $C_5H_6NO_2$ in 1 and 2 was deduced to be represented by (F)[3]. Combination of the two partial structures (E) and (F)



(1)

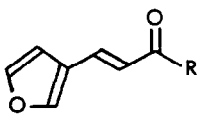
led to the whole structure of the antibiotic, which was represented by (1). Therefore, the reaction, 3 → 5 + 6 was expressed as shown below.



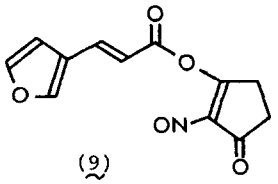
(3) (5) (6)

The structure (1) of the antibiotic was further confirmed by the synthesis of 2, a derivative obtained by elimination of AcOH from the antibiotic.

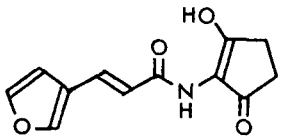
Condensation of 3-formylfuran and malonic acid (Py, 90°, 2.5 h) afforded β-(3-furyl)-acrylic acid (7)[2], mp 152.5-154°, which was converted to the corresponding acid chloride (8) by oxalyl chloride (60°, 15 min.). The acid chloride (8) was reacted with 2-nitrosocyclopentane-1,3-dione[4] to give an O-acylated product (9), which was immediately reduced (Zn/THF, -30°, r.t., 1.5 h, then Zn/AcOH-conc. HCl, r.t., 5 h), affording the crystalline product (2). During the reduction, the intramolecular acyl migration took place. The synthetic 2 was proved to be identical with the natural 2 by spectral (IR, ^{1}H-NMR, MS) and chromatographic comparison.



(7) R = OH
(8) R = Cl

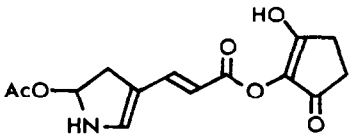


(9)



(2)

Recently, the structure of reductiomycin isolated from Streptomyces griseorubiginosus nov. sp. was reported to be (X) based on the X-ray crystallography[5], which is similar to but not identical with the structure of the antitumor antibiotic 1 in the present study. Since the physical and spectral properties of reductiomycin seemed to be quite similar to those of the antibiotic 1, direct comparison of the spectral (IR, ^{1}H-NMR, ^{13}C-NMR, MS) and chromatographic properties were made through the courtesy of Prof. G. Tamura and Dr. K. Shirahata, proving that our antibiotic 1 was identical with reductiomycin. The structure of reductiomycin must therefore be revised and represented by the formula (1).



(X)

Table 1 ^{1}H-NMR Spectral Data (100 MHz)[a)]

	H-2	H-3	H-5		H-6	H-7	H-2'	H-3'	H-4'	H-5'
1	6.37 d, 15	7.42 d, 15	2.56 br.d, 16	3.10 br.dd, 16, 7	6.70 dd, 7, 2	7.27 br.s	-	-	2.47 s	2.47 s
2	6.90 d, 15	7.51 d, 15	6.65 d, 2		7.74 dd, 2, 2	8.06 br.s	-	-	2.46 s	2.46 s
3	6.15 d, 15	7.55 d, 15	6.46 d, 2		7.38 dd, 2, 2	7.60 br.s	-	-	2.66 A_2B_2	
4	4.67[b)] br.m	4.06[b)] br.m	6.40 br.m		7.40 br.m	7.40 br.m	-	-	2.70 A_2B_2	
5	6.55 d, 15	7.56 d, 15	6.57 d, 2		7.41 dd, 2, 2	7.61 br.s	4.35 br.t 10	*	*	*
6	6.49 d, 15	7.48 d, 15	6.70 d, 2		7.54 br.s	7.86 br.s	-	7.64 t, 3	2.75 m	2.48 m

a) Chemical shifts are reported in ppm downfield from TMS. Coupling constants are given in Hz. Spectra were taken in DMSO-D_6 (1 and 2), in $CDCl_3$ (3 and 5), and in acetone-D_6 (4 and 6).
b) Assignments may be interchanged. The multiplicity could not be determined, because compound 4 is a mixture of two rotational isomers concerning the amide group.
* This signal appeared in the region of δ 1.9 - 2.5.

Table 2 ^{13}C-NMR Spectral Data (25 MHz)[a)]

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-1'	C-2'	C-3'	C-4'	C-5'
1	166.2 s	116.2 d	134.3 d	114.9 s	33.6 t	98.3 d	150.5 d	196.4 br.s	114.9 s	174.0 br.s	25.3[b)] br.t	31.8[b)] br.t
2	165.9 s	118.6 d	132.4 d	122.4 s	107.2 d	145.0[b)] d	145.4[b)] d	195[c)]	114.7 s	175[c)]	29[c)]	29[c)]
3	166.6 s	117.4 d	132.3 d	122.9 s	107.5 d	144.0 d	144.0 d	199.6 s	121.8 s	182.2 s	24.1[b)] t	31.7[b)] t

a) Chemical shifts are in ppm relative to TMS. Spectra were taken in DMSO-D_6 (1 and 2) and in $CDCl_3$ (3).
b) Assignments may be interchanged.
c) The multiplicity could not be determined because of the broad shape of this signal.

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REFERENCES AND NOTES

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2. Satisfactory microanalyses or high resolution mass spectral data were obtained.
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