# RESORTHIOMYCIN, A NOVEL ANTITUMOR ANTIBIOTIC

## II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE ELUCIDATION

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Resorthiomycin was revealed to be a new antibiotic with a molecular weight of 284 and a chemical formula of $C_{14}H_{20}O_4S$ as determined by MS and elemental analysis. The structure of resorthiomycin was determined to be 6-acetyl-4-(3-hydroxybutyl)-2-methyl-5-methylthioresorcinol by IR spectrum and $^1H$ and $^{13}C$ NMR.

In the preceding paper[1], taxonomy of the resorthiomycin-producing organism, production, purification and some biological activities of the antibiotic have been described. This paper reports the physico-chemical properties and structure elucidation of resorthiomycin.

### Physico-chemical Properties

Resorthiomycin was obtained as pale yellow oil, which was soluble in methanol, ethanol, chloroform and ethyl acetate, but hardly soluble in water and *n*-hexane.

The EI-MS of resorthiomycin revealed the molecular ion peak at *m/z* 284 ($M^+$) and 237 ($M^+-47$). The UV absorption spectra showed maxima at 206 (ε 14,800), 224 (6,000, sh) and 287 nm (1,800) in methanol, and 206 (11,900), 248 (2,870) and 351 nm (6,700) in 0.01 N NaOH-methanol (Fig. 1). The IR spectrum (chloroform) was consistent with the

Fig. 1. UV absorption spectra of resorthiomycin.
—— MeOH, – – – 0.01 N HCl-MeOH,
—·— 0.01 N NaOH-MeOH.

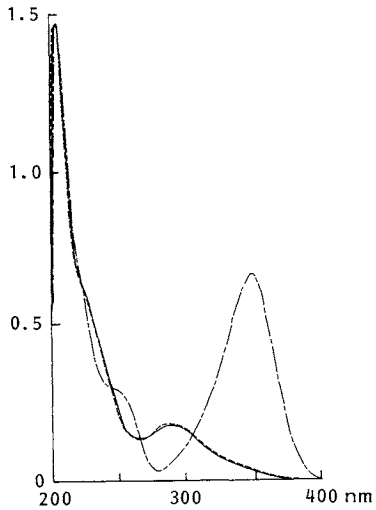


Table 1. $^1H$ NMR spectral data for resorthiomycin (400 MHz, $CDCl_3$).

| Proton | Chemical shift (ppm) | Multiplicity (*J*, Hz) | |
|---|---|---|---|
| 1-OH | 9.80[a] | s | |
| 3-OH | 8.0 | br s | |
| 7-$H_3$ | 2.14 | s | |
| 8-$H_A$ | 2.68 | ddd | (15.3, 5.5, 4.1) |
| 8-$H_B$ | 2.84 | ddd | (15.3, 11.4, 5.2) |
| 9-$H_A$ | 1.63 | dddd | (14.4, 10.0, 5.2, 4.1) |
| 9-$H_B$ | 1.72 | dddd | (14.4, 11.4, 5.5, 3.0) |
| 10-H | 3.75 | ddq | (10.4, 6.1, 3.0) |
| 10-OH | 1.7 | br s | |
| 11-$H_3$ | 1.25 | d | (6.1) |
| 12-$H_3$ | 2.55 | s | |
| 14-$H_3$ | 2.45 | s | |

[a] TMS (0 ppm) was used as an internal standard.

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Fig. 2. *IR spectrum of resorthiomycin in $CHCl_3$.*

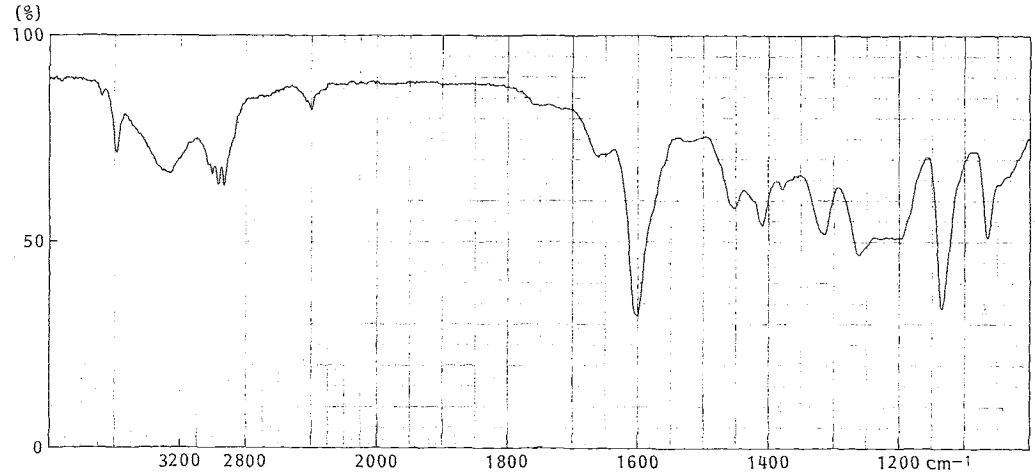



Table 2. $^{13}C$ NMR spectral data for resorthiomycin (100 MHz, $CDCl_3$).

| Carbon | Chemical shift (ppm)[a] | m | Carbon | Chemical shift (ppm)[a] | m |
|---|---|---|---|---|---|
| C-1 | 155.3 | s | C-8 | 21.9 | t |
| C-2 | 110.0 | s | C-9 | 37.2 | t |
| C-3 | 157.5 | s | C-10 | 66.8 | d |
| C-4 | 118.9 | s | C-11 | 23.8 | q |
| C-5 | 134.0 | s | C-12 | 18.4 | q |
| C-6 | 117.7 | s | C-13 | 198.2 | s |
| C-7 | 8.5 | q | C-14 | 13.1 | q |

[a] TMS (0 ppm) was used as an internal standard.
m: Multiplicity.

presence of *o*-hydroxyarylketone (1605 $cm^{-1}$) (Fig. 2). $^1H$ and $^{13}C$ NMR spectral data for resorthiomycin, measured in $CDCl_3$ at 400 and 100 MHz, respectively, are summarized in Tables 1 and 2.

The elemental analysis was as follows:

*Anal* Calcd for $C_{14}H_{20}O_4S \cdot \frac{1}{4}H_2O$: C 58.21, H 7.15, O 23.54, S 11.10.
Found: C 57.68, H 7.10, O 23.23, S 10.76.

The HREI-MS was as follows: Calcd for $C_{14}H_{20}O_4S$: 284.1083. Found: 284.1071.

## Structure Elucidation

The results of elemental analysis and EI-MS ($M^+$ 284) of resorthiomycin gave the molecular formula of $C_{14}H_{20}O_4S$. The $^1H$ and $^{13}C$ NMR suggested that resorthiomycin has a structure of 6-substituted benzene ring.

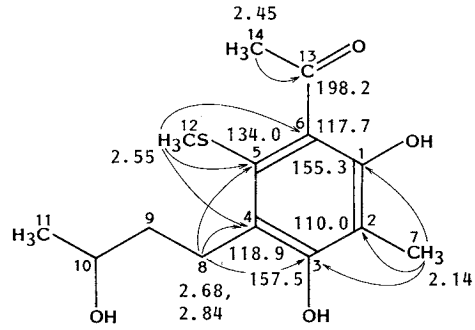
The UV absorption at 287 nm in methanol and its alkaline-shift to 351 nm suggested the existence of *p*-acetylphenol. The other phenolic proton signal ($\delta_H$ 9.80 ppm) was so sharp, that it is considered to be substituted at the *ortho* position of the acetyl group. Thus a 6-acetylresorcinol moiety was determined to be present.

When the methin proton signal ($\delta_H$ 3.75 ppm) was irradiated in a decoupling experiment, the doublet methyl signal ($\delta_H$ 1.25 ppm) was collapsed to singlet, and the multiplet methylene signal ($\delta_H$ 1.63 and 1.72 ppm) was also simplified. This result, coupled with the presence of multiplet methylene signal ($\delta_H$ 2.68

and 2.84 ppm), shows the existence of 2-hydroxybutyl group. Since one of the methyl groups is present as a methylthio form, as suggested by the existence of sulfur atom (confirmed by elemental analysis) and the fragmentation of *m/z* 47 on EI-MS, the remaining methyl group should be directly bound to the benzene ring. The positions of these three substituting groups were determined by long range selective proton decoupling (LSPD) experiments (Fig. 3).

Fig. 3. LSPD and chemical structure of resorthiomycin.

⟶ Long range coupling observed by LSPD experiment.




All the proton signals were assigned by $^{13}C$-$^{1}H$ chemical shift-correlated spectroscopy, and the structure of resorthiomycin was determined to be 6-acetyl-4-(3-hydroxybutyl)-2-methyl-5-methylthioresorcinol, as shown in Fig. 3, indicating that this is a new antibiotic.

## Discussion

Resorthiomycin is a hexa-substituted benzene analog and positions of the substituent groups were determined by LSPD experiment. The antibiotic was named resorthiomycin because it is a derivative of resorcinol and is unique in having a methylthio group. Some derivatives of resorcinol have been found as metabolic products of microorganisms. Lecanoric acid, which was first isolated from lichen and then fungi, was reported to inhibit histidine decarboxylase[2]. Curvulic acid, a metabolite from *Penicillium janthinellum* C-268, was found to have antimicrobial activity[3], and the antifungal activity of 2,4-dihydroxyacylophenones was more potent than that of amphotericin B[4]. Recently, resorcinomycin A from *Streptoverticillium roseorverticillatum* was reported to have an antibacterial spectrum directed towards mycobacterial species[5]. To this group of resorcinol derivative with various biological activities, resorthiomycin has now been added, which not only has antitumor activity but also the ability to potentiate certain other antitumor drugs[1,6].

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