CHEMICAL MODIFICATION OF RAVIDOMYCIN AND EVALUATION OF BIOLOGICAL ACTIVITIES OF ITS DERIVATIVES

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Several derivatives of the antitumor antibiotic ravidomycin were synthesized and their antitumor and antimicrobial activities and mode of action were evaluated. Deacylation produced a compound with higher biological activity than the parent. Structure-activity relationship of the derivatives is discussed.

Ravidomycin, a potent antibacterial, antitumor antibiotic isolated from the fermentation broth of *Streptomyces ravidus*[1], has been characterized as the *C*-glycoside I[2]. The tetracyclic aglycone is common to three recently described antibiotics: toromycin[3], gilvocarcin[4] and chrysomycin[5]. The present communication describes the antimicrobial and antitumor activities and the mode of action of ravidomycin and its derivatives illustrating the structure-activity relationship.

Chemistry

In order to ascertain the essential features for the antimicrobial and antitumor activities, simple modifications of the parent antibiotic was undertaken (Fig. 1). Deacetylation of ravidomycin was readily achieved by refluxing the parent compound in methanol. The structure of the product (**1**) was easily assignable from the spectral data. It showed the desired M^+ peak at 521 *m/z*. In the NMR spectrum, the acetyl peak at δ 2.12 was absent and the *N,N*-dimethyl group appeared at δ 2.62 indicating vicinal relationship of the acetyl group (δ 2.52) in ravidomycin. Acetylation of ravidomycin with acetic anhydride-pyridine at room temperature yielded the diacetyl derivative (**3**, M^+ 647 *m/z*). The NMR spectrum showed two additional acetyl groups indicating two acylable hydroxyl groups in ravidomycin. One of the acetyl group is at rather high field (δ 1.58) indicating some kind of shielding effect [aromatic *C*-glycoside]. The *N,N*-dimethyl group is also shifted from δ 2.52 to δ 2.42 confirming vicinal relationship with it. Hydrogenation of ravidomycin in the presence of 5% Pd/C at atmospheric pressure yielded the dihydro derivative (**2**) arising from the reduction of the vinylic bond. The NMR spectrum of dihydroravidomycin lacked the original olefinic protons, but in turn produced an ethyl group from its reduction. Deacetylation of this was readily achieved in a similar manner as of ravidomycin by refluxing in methanol. The dihydrodeacetylravidomycin (**5**) was also obtained by the reduction of deacetylravidomycin by hydrogen in the presence of 5% Pd/C.

Biological Activity

Antitumor Activities

Antitumor activities of ravidomycin and its analogs were evaluated against the P388 leukemia according to the protocol established by the National Cancer Institute, Division of Cancer Treatment, and results are recorded in Table 1. BDF_1, male mice were injected intraperitoneally with 1×10^6 viable P388 leukemic cells on day 0. A single injection of treatment was given on day 1, also intraperitoneally.

Fig. 1. Chemical transformation of ravidomycin.

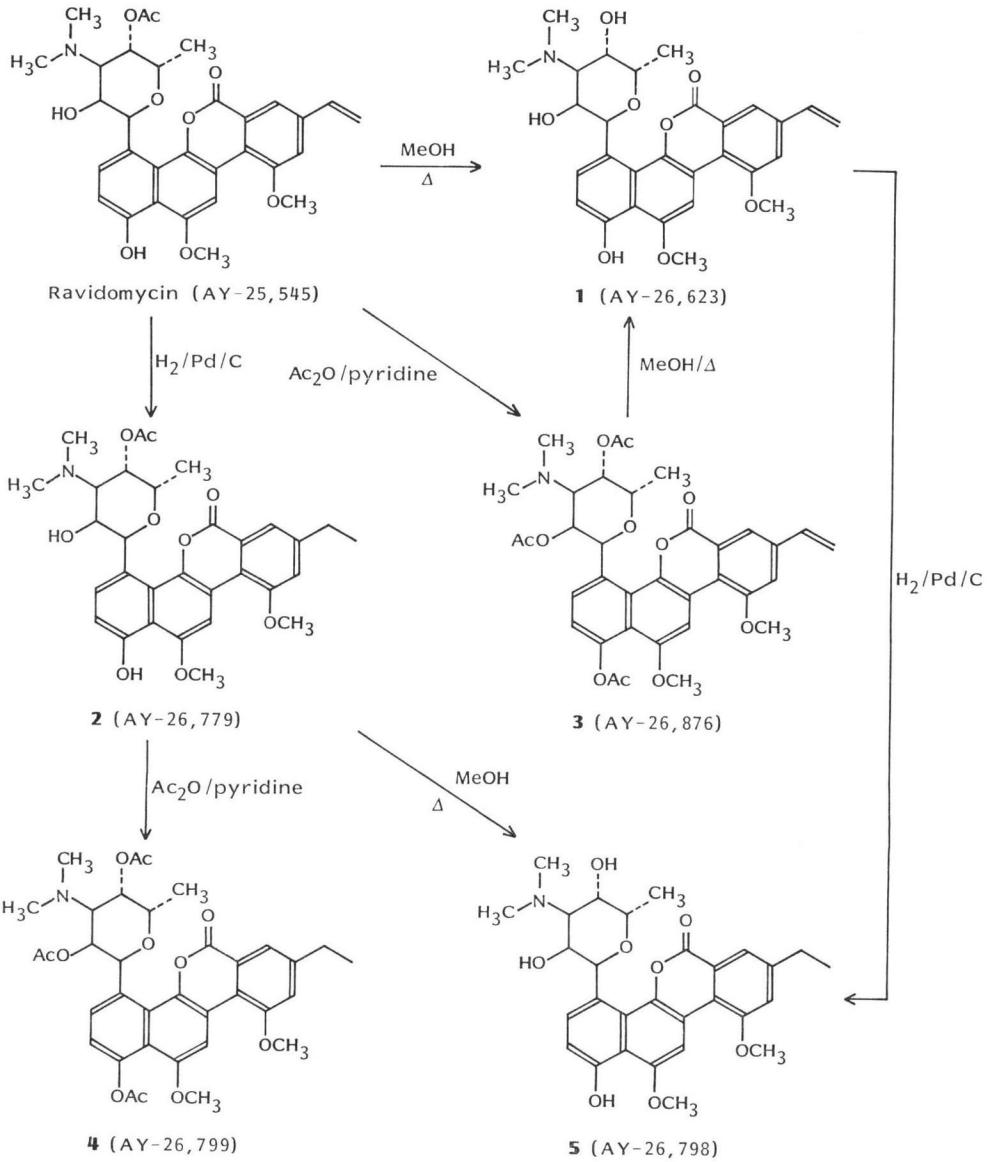


Table 1. Anti P388 activity expressed as T/C%.

Doses (mg/kg)	AY-25,545	AY-26,623	AY-26,779	AY-26,876	AY-26,799	AY-26,798
400	195	Toxic	Toxic	100	90	Toxic
200	205	Toxic	Toxic	100	100	Toxic
100	175	Toxic	Toxic	100	95	Toxic
50	140	205	135	95	100	140
25	150	185	145	90	105	133
12.5	110	180	130			128
6.2	100	170	130			124
3.1		165	115			

Table 2. Antimicrobial activities of ravidomycin and its derivatives.

Bacteria	MIC (μg/ml)					
	AY-25,545	AY-26,623	AY-26,779	AY-26,876	AY-26,799	AY-26,798
Staphylococcus pyogenes (penicillin^S)	6.4	3.2	12.5	100	100	100
S. pyogenes (penicillin^R)	6.4	3.2	12.5	100	100	100
Streptococcus faecalis	3.2	1.6	3.2	100	100	12.5
Escherichia coli	100	100	100	100	100	100
Bacillus subtilis	2.5	100	2.5	10	5	10
Salmonella pullorum	100	100	100	100	100	100
Pseudomonas aeruginosa	100	100	100	100	100	100
Proteus mirabilis	100	100	100	100	100	100
P. vulgaris	100	100	100	100	100	100
Klebsiella pneumoniae	100	100	100	100	100	100

Penicillin^S: Penicillin sensitive strain.
Penicillin^R: Penicillin resistant strain.

A solution which contained 1% carboxymethylcellulose in water was used as the vehicle solution. The median survival time (MST) in days was used as the parameter for evaluation. The T/C % value was calculated according to the formulae T/C%=MST (treatment/MST(control))×100. The compounds which produced T/C% values over 130 are considered active. Thus the dihydro and deacetyl analog of ravidomycin were found to be active whereas the acetylation failed to produce compounds with any noteworthy activity.

Antimicrobial Activities

The antimicrobial activities of ravidomycin and its derivatives were determined by standard serial dilution method against various Gram-positive and Gram-negative organisms and are recorded in Table 2. Only the deacylated product showed enhanced antimicrobial activity, whereas other changes like reduction or acylation brought about reduction in activity.

Mode of Action

It has been shown by one of us (K. SINGH, manuscript under preparation) that the primary mode of action of ravidomycin is to inhibit the synthesis of DNA in *Bacillus subtilis*. This would explain its antitumor activity. In order to determine the structure activity relationship between ravidomycin and its analog and to establish the essential features of the molecule for activity at the molecular level, knowledge of their mode of action is important. Therefore, the effect of ravidomycin and its analogs on the DNA synthesis in *B. subtilis* was followed by measuring the incorporation of labelled thymidine into the acid-insoluble precipitates. The results are shown in Table 3. It is clear that deacylation produces increased activity, any other modification produces reduction in biological activity.

Structure-activity Relationship

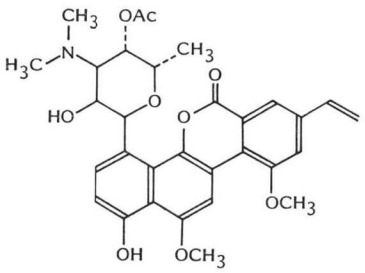
From the comparison of the antitumor and antimicrobial activities (Tables 1 and 2) of ravidomycin and its derivatives, it is apparent that the antitumor potency follow that of antimicrobial activities of the products. Acetylation of the antibiotic reduces activity in both the test systems. These derivatives are also incapable of inhibiting DNA synthesis (Table 3). Removal of the acetyl group on the other hand produces product that is several times more active than the parent. Reduction of the vinylic group of ravidomycin produce appreciable loss of *in vitro* and *in vivo* activity. Dihydro derivative does not affect the DNA synthesis indicating strongly the importance of the vinylic group in the molecule to retain in-

Table 3.

Antibiotic	% Inhibition of 0.5 μg/ml	Incorporation at 2.0 μg/ml
AY-26,779	None	9
AY-26,798	None	6
AY-26,876	10	25
AY-26,623	73	97
AY-25,545 (Ravidomycin)	58	96

The 1 ml reaction mixture contained 0.5 ml of log-phase cells (absorbancy$_{280}$=3), 0.46 ml of a solution containing 0.4% glucose and 0.2% yeast extract and 20 μl of antibiotic. After 10-minute incubation at room temperature 20 μl of [2-^{14}C]thymidine (1 mM, 10 mCi/mmol) was added and the tubes incubated at 37°C on a rotary shake. After 60-minute incubation 1 ml of 10% cold trichloroacetic acid was added and radioactivity in acid-insolubles determined.

Ravidomycin I



trinsic activity. The reported values for the antitumor activities of the closely related antibiotics—toromycin, gilvocarcin and chrysomycin are quite low in comparison to that of ravidomycin or deacetylravidomycin. This would strongly suggest the vital role of aminosugar moiety in biological activity. The amino sugar may play not only important role in the transport of the molecule, but it also has important role at the molecular level. For appropriate interaction at the molecular level to bring about inhibition of DNA synthesis (inhibition of incorporation of thymidine into DNA), the free hydroxyl groups in the aminosugar moiety seems to be essential because the acetylated product AY-26,799 was found to lack significant activity in the above test systems.

Experimental

Mass spectroscopic data were collected on a LKB 9000 S spectrometer. UV spectra were taken with a DMR 21, Zeiss spectrophotometer. NMR spectra were obtained in $CDCl_3$ with a Varian CFT 20 spectrometer using trimethylsilane as internal standard. IR spectra were taken on Perkin Elmer 225 spectrophotometer in $CHCl_3$ solution.

Deacetylravidomycin (**1**)

A solution of 500 mg of ravidomycin in 250 ml methanol was refluxed under nitrogen atmosphere for 24 hours. The solution was evaporated to dryness under reduced pressure. The yellow residue was recrystallized from methylene chloride - acetone; mp 225~228°C (dec.); MS *m/z* 521 (M^+); UV λ_{max}^{MeOH} (ε) 246 (34,815), 279 (29,815), 286 (35,920), 307 (16,355), 319 (12,274), 334 (11,570) and 349 (9,150) nm; IR ν $CHCl_3$ 3570, 3370 (OH), 1715 cm^{-1} (C=O);

Anal. Calcd. for $C_{29}H_{31}NO_8$: C 66.78, H 5.99, N 2.69
Found: C 66.89, H 6.05, N 2.72

Dihydroravidomycin (**2**)

A solution 10 g of ravidomycin in 500 ml ethyl acetate was hydrogenated at room temperature and atmospheric pressure for a period of 18 hours, using 1 g of 5% Pd/C as catalyst. The catalyst was filtered off and the solvent was removed under reduced pressure to yield 9.5 g of the reduced product. It was crystallized from methylene chloride - methanol; mp 255~257°C (dec.); MS *m/z* 565 (M^+); UV λ_{max}^{MeOH} (ε) 245 (53,930), 266 (30,160), 274 (39,770), 305 (12,510) and 318 (12,430) nm; IR ν $CHCl_3$ 3370 (OH), 1733, 1715 cm^{-1} (C=O);

Anal. Calcd. for $C_{31}H_{35}NO_9$: C 65.83, H 6.24, N 2.48
Found: C 65.63, H 6.24, N 2.57

Diacetylravidomycin (**3**)

To a solution of 8 g of ravidomycin in 25 ml dry pyridine, 10 ml of acetic anhydride was added and

the solution stored at room temperature for 18 hours. Excess of acetic anhydride was carefully decomposed by addition of methanol in cold and the solution was poured into ice water. The resulting precipitate was collected and washed with water and air dried. The yellow product was crystallized from methanol - methylene chloride; mp 245~247°C (dec.); MS *m/z* 647 (M^+); UV λ_{max}^{MeOH} (ε) 249 (35,300), 267 (24,680), 276 (32,370), 322 (10,160) and 336 (10,610) nm; IR ν $CHCl_3$ 1730, 1715 cm^{-1} (C=O);

Anal. Calcd. for $C_{35}H_{37}NO_{11}$: C 64.91, H 5.71, N 2.16
Found: C 64.72, H 5.80, N 2.12

Dihydrodiacetylravidomycin (**4**)

A solution of 6.5 g of dihydroravidomycin in 15 ml dry pyridine and 6 ml of acetic anhydride was stored at room temperature for 18 hours. The excess of anhydride was carefully decomposed by methanol and poured into ice water. The resulting yellow precipitate was collected by filtration and washed with water and air dried. This was further purified by chromatography over silica gel using 60% ethyl acetate, methylene chloride as solvent to yield 7.0 g of the desired product. It was recrystallized from acetone - hexane; mp 244~245°C (dec.); MS *m/z* 649 (M^+); UV λ_{max}^{MeOH} (ε) 244 (43,840), 267 (28,450), 276 (40,880), 300 (10,630), 322 (11,760) and 336 (12,050) nm; IR ν $CHCl_3$ 1730 cm^{-1} (C=O);

Anal. Calcd. for $C_{35}H_{39}NO_{11}$: C 65.01, H 6.45, N 2.30
Found: C 64.87, H 6.37, N 2.12

Dihydrodeacetylravidomycin (**5**)

A solution of 1 g of dihydroravidomycin in 100 ml methanol was refluxed under nitrogen for 18 hours. The solution was concentrated under reduced pressure resulting in crystallization of the product. The crystals were collected and air dried to give the desired product (900 mg); mp 235~238°C (dec.); MS *m/z* 523 (M^+); UV λ_{max}^{MeOH} (ε) 244 (53,020), 267 (29,350), 275 (38,630), 305 (12,460), 318 (12,270) and 326 (11,760) nm; IR ν $CHCl_3$ 3550, 3350 (OH), 1710 cm^{-1} (C=O);

Anal. Calcd. for $C_{29}H_{33}NO_8$: C 66.53, H 6.35, N 2.68
Found: C 66.32, H 6.41, N 2.67

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