CHAPTER II

LITERATURE REVIEW

In 1929, Alexander Fleming discovered that the fungus *Penicillium notatum* produced and excreted a compound which inhibited the growth of *Staphylococcus* species. There was no further research on the finding until the lack of antibacterial drugs for wound treatment was pronounced during the World War II. Then, extensive studies of *Penicillium notatum* were conducted and led to the excellent drug penicillin for the infectious treatment. Subsequently, there were a number of research programs searching for other antibiotics from microorganisms (Samuelsson, 1992), the greatest source of antibiotics is bacteria in the order Actinomycetales, especially the genus *Streptomyces* (Okami and Hotta, 1988).

1. Characteristics of the genus *Streptomyces*

The genus *Streptomyces* belongs to the family Streptomycetaceae. Other genera in this family are *Intrasporangium*, *Sporichthya*, and *Streptoverticillium*. The genus *Streptomyces* is gram-positive bacteria commonly distributed and abundant in soil. The *Streptomyces* grow from spore and formed discrete and lichenoid, leathery or butyrous colonies. At first, colonies are relatively smooth surface but later become a weft of aerial mycelium that might appear floccose, granular, powdery or velvety. They produce a variety of pigments that cause the color of the vegetative and aerial mycelia. Colored diffusible pigments might also be produced. The cell wall peptidoglycan contain L-diaminopimelic acid (L-DAP) as a major component. They use a wide range of organic compounds as source of carbon for energy and growth. Many strains of *Streptomyces* produce one or more antibiotics (Cross, 1994).

Cultural characteristics on various culture media such as the color of the diffusible pigment, the vegetative, aerial mycelium and spores and the micromorphology of the sporulation structures are used as criteria to define the species of *Streptomyces*. Physiological and biochemical characteristics are also used to describe the strain and species of *Streptomyces*.

The *Streptomyces* strains are found in soil and aquatic habitats such as lake mud, river sediment and marine sediment. They also occur in estuarine environment such as sandy sediment, intertidal sediment and sediment samples collected from the estuarine environment at the mouth of stream (Goodfellow and William, 1983; Jensen, Dwight and Fenical, 1991).

One member of this genus, *Streptomyces hygroscopicus* is placed in Gray series (Shirling and Gottlieb, 1972) is an interesting species due to its ability to produce large numbers and various groups of antibiotics (Glasby, 1993). The strain TRA 9875-2 has morphological and cultural characteristics similar to those of the *Streptomyces hygroscopicus*.

In 1931, "*Actinomyces hygroscopicus*" formerly described as aerial mycelium became moist with dark shiny patches which, when touched with a needle, was found to be a moist and smeary spore mass (Jensen, 1931). The type culture was no longer extant according to Shirling and Gottlieb. In 1948, "*Actinomyces hygroscopicus*" was redefined as *Streptomyces hygroscopicus* (Jensen) Waksman and Henrici (Shirling and Gottlieb, 1972).

Streptomyces hygroscopicus and *Streptomyces platensis* (a culture which has the characteristic "moist black patches") were lumped by Tresner and Backus in 1956 on the basis of cultural and light microscope studies (Tresner and Backus, 1956). Newer techniques (especially electron microscopy) were used to inform that the species were indeed distinguishable and therefore validly special (Tresner, Backus and Hayes, 1967).

2. Review of ansamycins

2.1 Chemistry of ansamycins

The ansamycins or ansa-macrolides are a class of macrocyclic compounds. Their structure consist of an aromatic nucleus in which two non-adjacent positions are linked by a long aliphatic chain of up to twenty-four atoms (Latin: ansa = handle). The aromatic portion may be benzenoid, naphthalenic, or quinonoid. The

macrocycle in the ansamycin is closed by an amide linkage rather than an ester linkage (ansamycins were lactams). The ansa chain or bridge usually lie above the aromatic system (Thomson, 1987; Dewick, 1997).

The name, ansamycin, originally suggested by Prelog, is derived from the term "ansa compound" coined by Lutteringhaus in 1942. It is the structures of the ansamycins which have provided the name of these antibiotics (Rinehart, Jr. and Shield, 1976).

The chemical structures of ansamycins are classified into four groups based on their aromatic moiety as follows: - benzenoid group, naphthalenic group, quinonoid group and hydroquinoid group (Table 2.1).

2.2 Sources of origins

Most of the ansamycins are obtained from bacteria but one of benzenoid group, the maytansinoids, occurs in higher plants. They are plant-derived ansamycins from *Maytenus* spp. The genus *Maytenus* belongs to the family Celastraceae (Kupchan *et al.*, 1972, 1974; Thomson, 1987; Dewick, 1997).

The maytansenoids group also occurs in bacteria, *Nocardia* sp., ansamitocins. The chemical structures of them are similar to maytansine and related maytansinoids obtained from higher plants (Higashide *et al.*, 1977).

The only ansamycin currently used therapeutically is rifampicin, a semi-synthetic naphthalene-based macrocycle produced from rifamycin B (Dewick, 1997).

The sources of origin of the ansamycins are summarized in Table 2.1.

2.3 Biological activities of ansamycins

Ansamycins show many biological activities such as antibacterial activity, antifungal activity, antiprotozoa, antitumor activity, and herbicidal activity.

The ansamycins are notably active against gram-positive bacteria. Some broad-spectrum activity has been noted for certain rifamycin derivatives and some activity against gram-negative bacteria for streptovaricins (Rinehart, Jr., 1972).

The streptovaricins repress *Mycobacterium tuberculosis* and murine leprosy, and the rifamycins are reported to be clinically useful, especially in the treatment of tuberculosis (Rinehart, Jr., 1972).

One of the rifamycins, rifampicin, is marketed widely for treatment of tuberculosis and other infections caused by gram-positive organisms, while the other derivatives of rifamycins and streptovaricins are biological probes because of their binding to DNA-dependent RNA polymerase and their inhibition of reverse transcriptases (Rinehart, Jr. and Shield, 1976).

Geldanamycin differs from the other ansamycins in that its principal activity is against protozoa rather than against bacteria (Rinehart, Jr., 1972).

Maytansine and related compounds are powerful antitumor agents (Rinehart, Jr. and Shield, 1976).

The biological activities of ansamycins are summarized in Table 2.1.

Table 2.1 Sources and biological activities of ansamycins.

No.	Compounds	Types	Sources	Activities	References
1	Maytansine	Benzenoid	Higher plant *Maytenus*	Antileukemia	Kupchan *et al.*,
2	Maytanprine		*buchananii*		1972, 1974
3	Maytanbutine				
4	Maytanvaline				
5	Maysine				
6	Normaysine				
7	Maysenine				
8	Ansamicotin P1	Benzenoid	*Nocardia* sp. No. C-15003	Antitumor	Higashide *et al.*,
9	Ansamicotin P2				1977
10	Ansamicotin P3				
11	Ansamicotin P3′				
12	Ansamicotin P4				
13	Tolypomycin Y	Benzenoid	*Streptomyces tolypophorus*	Antibacterial	Kishi *et al.*, 1969
14	Ansatrienin A	[14]-[16]	*Streptomyces collinus*	Antifungal	Damberg *et al.*,
15	Ansatrienin A_2	Quinonoid, [17]			1982
16	Ansatrienin A_3	Hydroquinoid			Lazar *et al.*, 1983
17	Ansatrienin B				

Table 2.1 (continued)

No.	Compounds	Types	Sources	Activities	References
18 19	Mycotrienol I Mycotrienol II	[18] Quinoid, [19] Hydroquinoid	*Streptomyces rishiriensis*	Antileukemia, Antifungal	Glasby, 1993
20 21	Geldanamycin 17-*O*-demethylgelda-namycin	Quinoid	*Streptomyces hygroscopicus* var. *geldanus*	[20] Antifungal, Antibacterial, Antitumor and Herbicide	DeBoer *et al.*, 1970 Heisey and Putnam, 1986
22 23 24 25 26 27	Herbimycin A Herbimycin B Herbimycin C Dihydroherbimycin A Dihydroherbimycin B Dihydroherbimycin C	[22]-[24] Quinoid, [25]-[27] Hydroquinoid	*S. hygroscopicus* AM-3672	[22], [24], [25], [27] Cytotoxic, Herbicide	Omura *et al.*, 1979 Iwai *et al.*, 1980 Lin *et al.*, 1988
28 29	Macbecin I Mecbecin II	[28] Quinoid, [29] Hydroquinoid	*Nocardia* sp.	Antitumor, Antibacterial, Antifungal, Antiprotozoal	Muroi *et al.*, 1980, 1981 Thomson, 1987

Table 2.1 (continued)

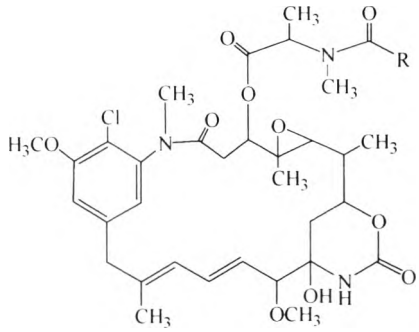
No.	Compounds	Types	Sources	Activities	References
30	Awamycin	Naphthalenic	*Streptomyces* sp. No. 80-217	Antitumor	Funayama *et al.*, 1985
31 32 33	Naphthoquinomycin A Naphthoquinomycin B Naphthoquinomycin C (Naphthomycin H)	Naphthalenic	[32]-[34] *Streptomyces* sp. No. S-1998 [34] *Streptomyces* sp. Y-83,40369	Fatty acid synthesis inhibitor in *Escherichia coli*	Mukhopadhyay *et al.*, 1985 Mochizuki *et al.*, 1986
34	Naphthomycin A	Naphthalenic	*S. diastatochromogenes* and *Streptomyces* sp. Y-83,40369	Antifungal, Antibacterial	Keller-Schierlein *et al.*, 1983,1984
35	Naphthomycin B	Naphthalenic	*S. galbus* subsp. *griscoporeus*	Antifungal, Antibacterial	Keller-Schierlein *et al.*, 1983
36	Naphthomycin C	Naphthalenic	*S. diastatochromogenes* var. *diastatochromogenes*	Antifungal, Antibacterial	Keller-Schierlein *et al.*, 1983
37 38	Diastovaricin I Diastovaricin II	Naphthalenic	*S. diastatochromogenes* subsp. *variabilicolor*	Effect on the induction of differentiation of Friend cell	Nakamura *et al.*, 1986
39	Rifamycin B	Naphthalenic	*S. mediterranei*	Antibacterial against gram negative bacteria	Glasby, 1993

Table 2.1 (continued)

No.	Compounds	Types	Sources	Activities	References
40	Rifampicin	Naphthalenic	Semi-synthetic produced from rifamycin B	Anti-TB, Antibacterial	Glasby, 1993
41	Rifamycin O	Naphthalenic	*S. mediterranei* and *S. tolypophorus*	Antibacterial against gram negative bacteria	Glasby, 1993
42	Rifocin	Naphthalenic	*S. mediterranei* and *Micromonospora chalcea*	Therapeutically effective in the treatment of pneumococcal, staphylococcal and streptococcal infections	Glasby, 1993
43	Rifamycin W	Naphthalenic	*Nocardia mediterrenei*	Antibacterial against gram positive bacteria	Glasby, 1993
44	Rifamycin X	Naphthalenic	*S. mediterranei*	Antibacterial against gram positive bacteria	Glasby, 1993
45 46 47	Halomicin A Halomicin B Halomicin C	Naphthalenic	*Micromonospora halophytica*	Antibacterial against gram positive bacteria	Glasby, 1993

Table 2.1 (continued)

No.	Compounds	Types	Sources	Activities	References
48	Protostreptovaricin I	Naphthalenic	*S. spectabils*	Inhibit Rauscher	Deshmukh *et al.*,
49	Protostreptovaricin II			leukemia virus	1976
50	Protostreptovaricin III			RNA-dependent	Glasby, 1993
51	Protostreptovaricin IV			DNA polymerase	
52	Protostreptovaricin V				
53	Streptovaricin A	Naphthalenic	*S. spectabilis*	Inhibit the	Rinehart Jr. *et al.*,
54	Streptovaricin B			incorperation of	1971
55	Streptovaricin C			nucleosides into	
56	Streptovaricin D			HeLa cells	
57	Streptovaricin E				
58	Streptovaricin F				
59	Streptovaricin G				

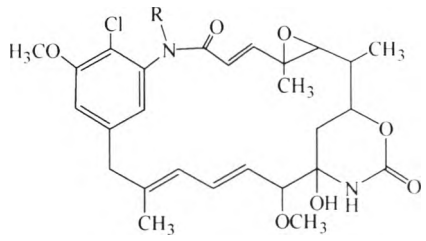


[1] R = CH_3

[2] R = CH_2CH_3

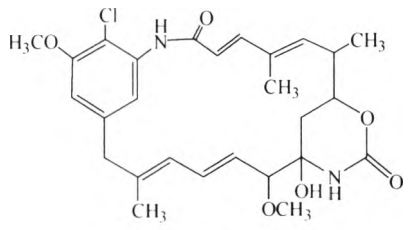
[3] R = $CH(CH_3)_2$

[4] R = $CH_2CH(CH_3)_2$



[5] R = CH_3

[6] R = H



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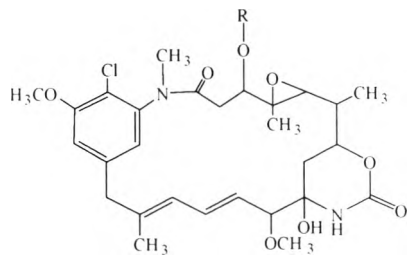


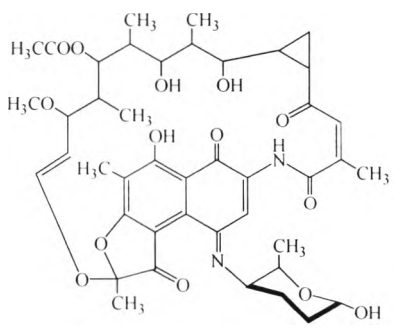
[8] R = COCH3

[9] R = COCH2CH3

[10] R = $COCH(CH_3)_2$

[11] R = $COCH_2CH_2CH_3$

[12] R = $COCH_2CH(CH_3)_2$



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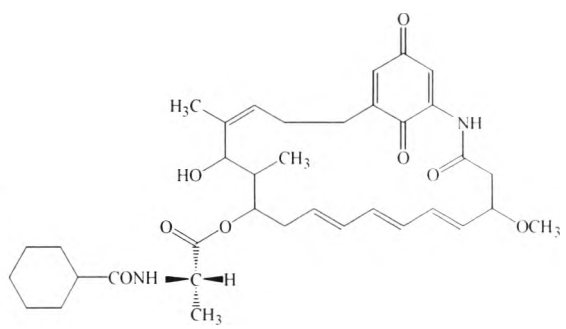

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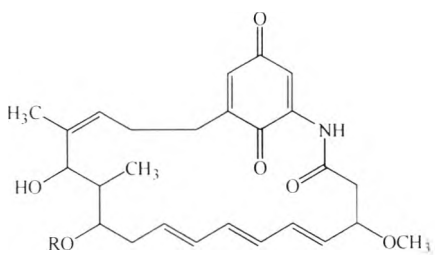
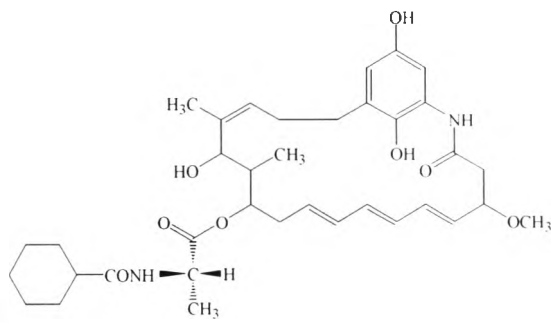


[15] R = $COCHCH_3NHCOCHCH_3CH_2CH_3$

[16] R = $COCHCH_3NHCOCH_2CH(CH_3)_2$



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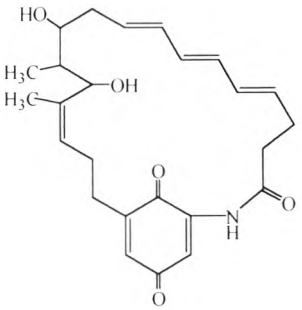

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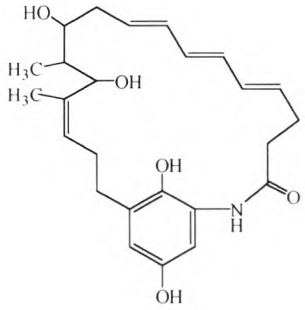

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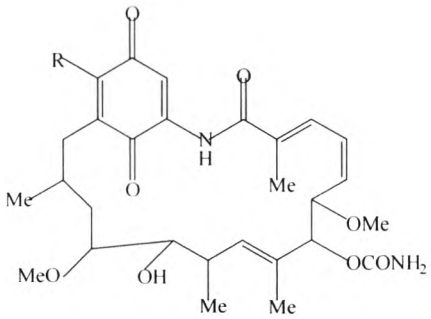
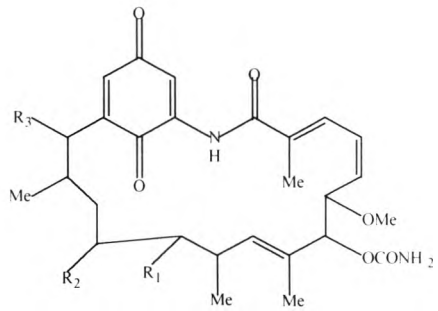
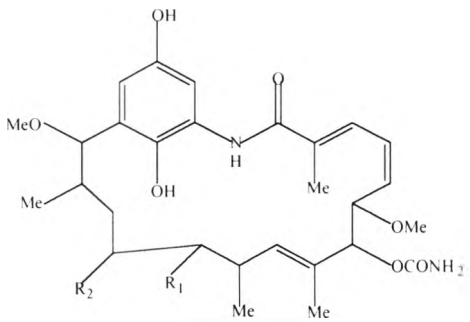


[20] R = OCH_3

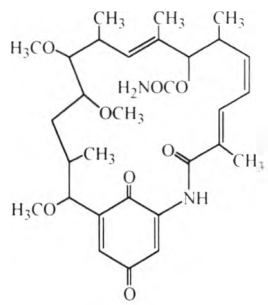
[21] R = H



	R_1	R_2	R_3
[22]	OCH_3	OCH_3	OCH_3
[23]	OH	OCH_3	H
[24]	OH	OCH_3	OCH_3



	R_1	R_2
[25]	OCH_3	OCH_3
[26]	OH	OH
[27]	OH	OCH_3



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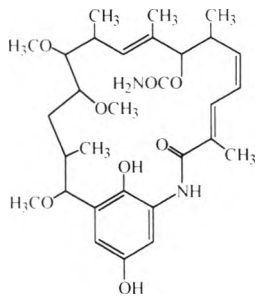

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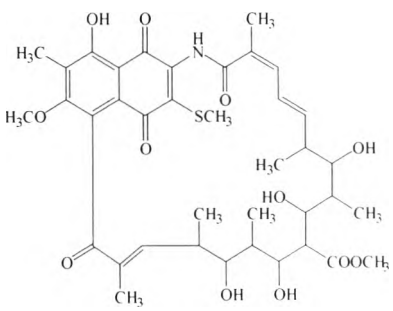

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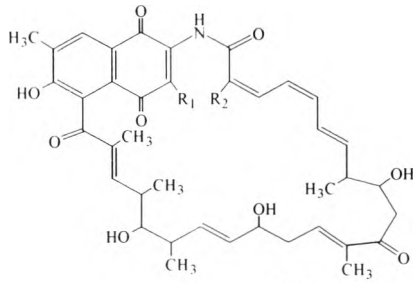
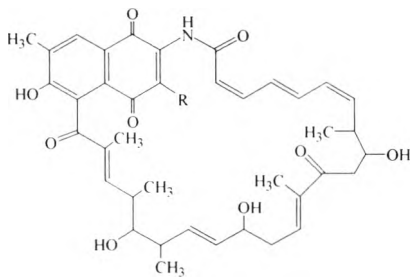


	R_1	R_2
[31]	OCH_3	H
[32]	SCH_3	H
[33]	Cl	H
[34]	Cl	CH_3

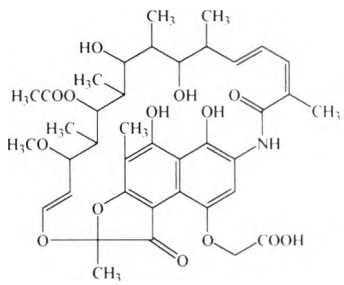


[35] R = Cl

[36] R = H

[37] R = OH

[38] R = $SCH_2CHCOOHNHCOCH_3$



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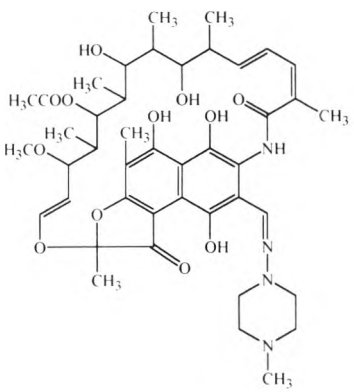

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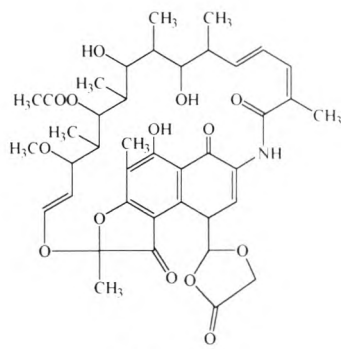

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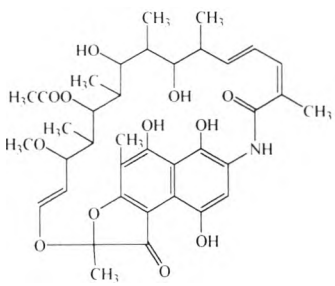

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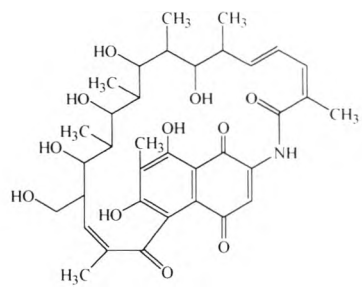

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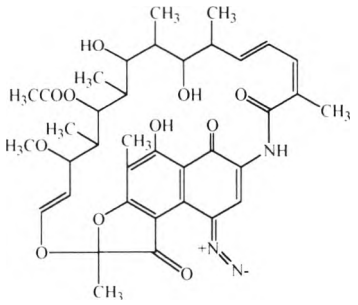

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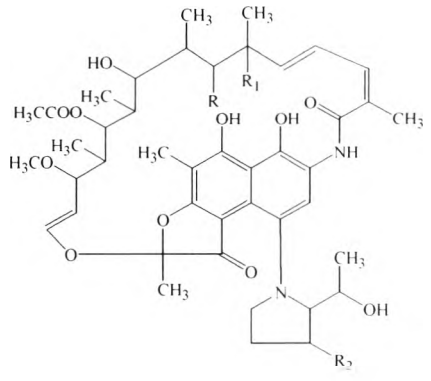
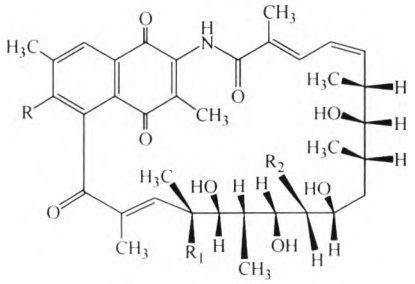
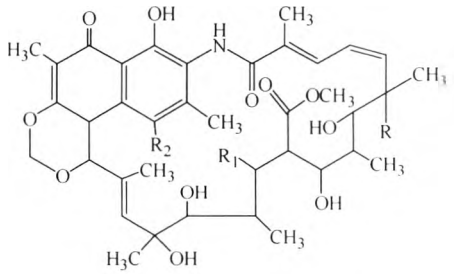


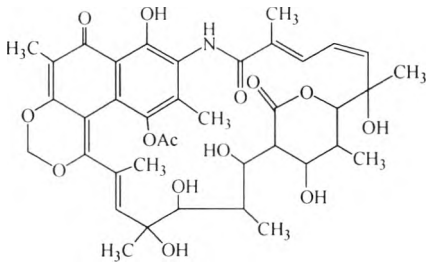
	R	R_1	R_2
[45]	H	H	OH
[46]	OH	H	H
[47]	H	OH	H



	R	R_1	R_2
[48]	OH	H	CH_3
[49]	OCH_3	H	CH_3
[50]	OH	OH	CH_3
[51]	OCH_3	OH	CH_3
[52]	OH	H	H



	R	R_1	R_2
[53]	OH	$OCOCH_3$	$OCOCH_3$
[54]	H	$OCOCH_3$	$OCOCH_3$
[55]	H	OH	$OCOCH_3$
[56]	H	H	$OCOCH_3$
[57]	OH	O	$OCOCH_3$
[59]	OH	OH	$OCOCH_3$



[58]