CHEMICAL STUDIES ON ANTIBIOTICS AND OTHER BIOACTIVE MICROBIAL PRODUCTS BY PROF. HAMAO UMEZAWA

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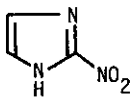
As written briefly by Dr. Umezawa himself in the preface of this book, the study of antibiotics in Japan was started in the beginning of 1944 in order to produce penicillin. In September of 1944, the first penicillin in Japan was extracted from culture filtrates of Penicillium Y-176 cultured in Erlenmeyer flasks placed on his table. This strain was selected from the strains supplied by the late Dr. Teijiro Yabuta to Dr. Umezawa on the basis of its activitv to inhibit the growth of Staphlococci but not E. coli and its formation of a golden pigment in its cultured broth. At that time, there was no laboratory equipped with a cold incubator for a 25°C culture in Tokyo. Dr. Umezawa was at that time in the Institute of Infectious Disease of the University of Tokyo (at present called Institute of Medical Sciences of the University of Tokyo). Fortunately this institute was not bombed during the last world war, but all Japanese researchers suffered from the shortage of glasswares, reagents, equipments and foreign journals. Moreover, no Japanese journals were published from about 1944 to 1948. Japanese Antibiotic Research Association (at that time called Japanese Penicillin Research Association) was established in August of 1946 and held monthly meetings for the presentation of papers since September of 1946. This promoted the research of antibiotics in Japan. This association started the publication of the Journal of Antibiotics (called Journal of Penicillin at that time) since the end of 1947. Both the monthly meetings and this journal promoted antibiotic research in Japan. Dr. Umezawa also started the screening of new antibiotics. Before October 1946, he obtained patulin from Penicillium and Aspergillus, a red crystalline antibiotic identified as actinomycin and streptothricin group antibiotics, from actinomyces strains cultured in bottles. At that time, the late Dr. Foster, Professor of Texas University

came to Japan, introduced penicillin studies in U.S.A. and brought penicillin-producing strains including the strain Q-176 for deep aerated fermentation. In May, 1947, half of the building and the people of the Institute of Infectious Diseases of the University of Tokyo was transferred to the newly established National Institute of Health of Japan and Dr. Umezawa together with his laboratory was transferred to the Department of Antibiotics of this new institute. As the director of this department, he devoted himself exclusively to the study of antibiotics and other microbial products. From Dr. Umezawa's study in the last 35 years, we can observe a great rapid progress of modern organic chemistry in these areas.

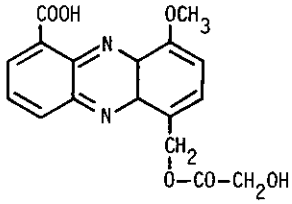
EARLY STUDIES ON ANTIBIOTICS (1950 - 1953)

In the beginning of 1947, he found that strain Q-176 which produced penicillin in high yields with deep aerated fermentation also produced high yields of penicillin in a synthetic medium of the bottle culture. This process helped in the production of penicillin at that time. At about the same time, the cylinder plate assay method was introduced to Japan. This method gave very accurate results as confirmed by Dr. Umezawa's statistics study. This method developed by Heatley and others at Oxford University contributed a lot to the progress of antimicrobial antibiotics. Dr. Umezawa applied the principle of this method to the tuberculin assay on guinea pig skin to establish a quantitative method of tuberculin assay. In 1947, a shaking culture machine was placed in a tin plate box of this laboratory in the basement and was used for a year until a pilot plant which had four 400 liter fermenters was built in the storehouse of the institute in 1948. After the penicillin study, he was asked by the Japanese Government to study the production process of streptomycin, for tuberculosis was the biggest cause of death of the Japanese people at that time. It caused many miserable deaths of the young or prevented them from their wish for education or work. During the screening of streptomycin-like antibiotics, he found neomycin from two strains classified as *S. fradiae* in 1948. He identified this antibiotic from streptothricin on the basis of its antibacterial spectrum and its low toxicity and named it streptothricin B. In his screening study in 1947, an interesting white crystal inhibiting both Gram negative and positive bacteria was obtained. Immediately thereafter, chloramphenicol was published and was confirmed to be identical with this crystalline antibiotic. From 1945 to 1953, it may be said that these were the years of his random screening of antimicrobial antibiotics and the following new compounds were reported from

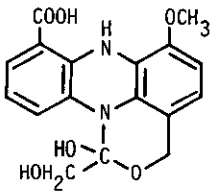
1948 to 1953 in the Journal of Antibiotics and the Japanese Medical Journal both of which were started in 1948:
Streptothricin B (neomycin, 1948), aureothricin (1948), griseolutein A and B (1950), nitrosporin (1951), abikoviromycin (1951), moldin (1952), pheofacin (1952), exfoliatin (1952), sarcidin (1953), achromoviromycin (1953), azomycin (1953), pyridomycin (1953), phthiomycin (1953), thiazolidone antibiotic (1953). Among these compounds, the structures of azomycin (1955), griseoluteins A (1959) and B (1964), and pyridomycin (1967) were determined later in his laboratories. Their action mechanisms were also later studied: griseolutein A interacted with the cell membrane causing inhibition of DNA and RNA synthesis (1978) and azomycin inhibited ribonucleotide reductase (1974). Pyridomycin was shown to inhibit some process before orotic acid synthesis during the biosynthesis of pyrimidine bases (1970).



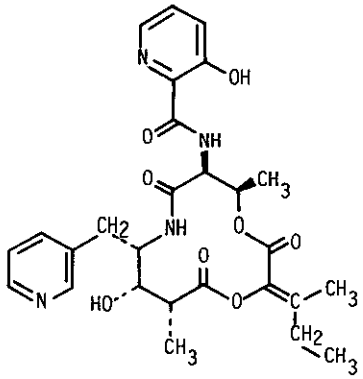
Azomycin (1955)



Griseolutein A (1959)



Griseolutein B (1964)



Pyridomycin (1970)

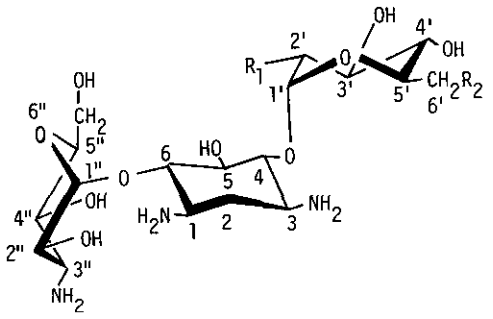
As shown by the findings of abikoviromycin and achromoviromycin which inactivated encephalitis viruses, Dr. Umezawa tried the screening of antiviral compounds for about two years from 1949 to 1950. However, it was difficult for him to find antiviral substances which inhibited the viral growth without interfering with the growth function of host cells. Moreover, he suspected the possibility of applying antiviral substances for the chemotherapy of virus diseases, because the amount of

virus was maximum in patients diagnosed by doctors as having virus diseases. He also thought that an antiviral substance might be useful to protect people from influenza virus infections but with extremely low toxicity in this case. On the other hand, during his study of antiviral substances, he noticed that microorganisms produced cytotoxic compounds in high frequency. Therefore, as it will be described later, he initiated the study of antitumor substances in 1951.

DISCOVERY OF KANAMYCIN AND KASUGAMYCIN, RESISTANCE MECHANISMS AND DEVELOPMENT OF ACTIVE DERIVATIVES AGAINST RESISTANT INFECTIONS

During the random screening of antimicrobial antibiotics, he concentrated his study into the screening of new antibiotics useful in tuberculosis chemotherapy. He endeavoured to find new antibiotics inhibiting the growth of nonpathogenic mycobacteria and found pyridomycin (1953) as mentioned above. Pyridomycin showed good activity against tubercle bacilli *in vitro*, but in the mouse test it did not show any therapeutic activity. Its interesting structure was determined in 1967. This antibiotic also inhibited Gram negative bacteria and had low toxicity.
In 1953, he found thiazolidone antibiotic and phthiomycin. Both inhibited tubercle bacilli. Thiazolidone antibiotic was found by several research groups at the same time. The action of this antibiotic was counteracted by biotin and was not active *in vivo*. On the other hand, phthiomycin which was water-soluble and basic was identified to be an antibiotic closely related to viomycin. It showed activity against experimental tuberculosis in mice. This proved one of Dr. Umezawa's findings that water-soluble basic antibiotics which showed inhibition of growth of tubercle bacillus *in vitro* can exhibit the effect also *in vivo*. He continued the screening of water-soluble basic antibiotics inhibiting the growth of mycobacteria which have no or low delayed-toxicity. Antibiotics in culture filtrates were extracted by a carboxyl resin process and the toxicity of the extracts to mice was tested. In this study, he found phleomycin (1956), kanamycin (1957) and alboverticillin (1958).
Kanamycin exhibited a similar effect as streptomycin on experimental tuberculosis in mice and guinea pigs. It was effective also against the infection of tubercle bacilli resistant to streptomycin. At that time in hospitals, most tubercle bacilli of patients became resistant to streptomycin after streptomycin treatment, therefore, kanamycin replaced streptomycin in the treatment of them. Moreover, it

inhibited the growth of Gram positive and negative bacteria both in vitro and in vivo. Staphylococci and Gram negative bacteria resistant to all drugs appeared in 1957. Thus, this antibiotic was evaluated on the merits of its effect on these resistant infections. Generally, at that time it was difficult to crystallize aminoglycoside antibiotics. Incidentally, during evaporation of a fraction obtained by carbon chromatography, kanamycin crystallized. This was shown to be its monosulfate, and the pH of its water solution was 8.0. Thereafter, the adjustment of the pH of the kanamycin aqueous solution to 8.0 followed by evaporation and addition of ethanol or acetone easily gave crystals of kanamycin monosulfate. Starting from this crystalline material, the structure was determined quickly in 1958. At that time, nmr was not useful and the structure was elucidated by fragmentation to known hexopyranoses and 2-deoxystreptamine, by the results of the peridodate reaction, by the isolation of 5-O-methyl-2-deoxystreptamine by the hydrolysis of totally methylated derivative and by the application of the Hadson rule. This structure was confirmed by X-ray crystal analysis to be kanamycin monosulfate in 1968.



kanamycin A: R_1=OH, R_2=NH_2

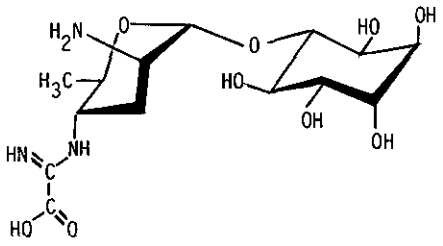
kanamycin B: R_1=NH_2, R_2=NH_2

kanamycin C: R_1=NH_2, R_2=OH

In 1962, Institute of Microbial Chemistry was built and its extention building was added in 1966. This institute was fully equipped for structure determination. Moreover, Professor Iitaka started to collaborate with Dr. Umezawa on structure determination by X-ray crystal analysis. Thus, the amount of study undertook by Dr. Umezawa was markedly enlarged.

In Japan, an effective agent against Pyricularia oryzae was requested, for this infection was causing great damage to rice plants and was creating enormous loss in rice harvests. Moreover, phenyl mercuric compounds which had been used had to be withdrawn. Dr. Umezawa started the screening of active agents against this rice plant disease in 1962: the actinomycetes strains isolated from soils were carefully selected and the activity to suppress the infection of this fungus on rice plants was tested by a pot test. This process was an unique screening process

at that time. Very quickly, a streptomyces strain was selected. Its culture filtrate did not show any activity to inhibit Pyricularia oryzae in the usual testing method at that time. But when the activity against Pyricularia oryzae was tested by using a medium prepared from rice plant juice, it showed great activity. This was an acidic medium, that is, a medium of pH lower than 6.0. In this step, pH 5.5 was confirmed to be the known pH of rice plant leaves. The active compound which inhibited this fungus in the acidic media was extracted and purified rapidly and was named kasugamycin (1965), because the soil sample was collected from the garden of Kasuga Shrine in Nara city. The structure was very rapidly determined by studying the hydrolysis products and performing X-ray analysis of its hydrobromide (1966). This was also totally chemically synthesized soon after (1968). More than 100 tons calculated as pure kasugamycin has been used every year in Japan, Korea, Formosa, Columbia, Brazil etc. since then. This antibiotic also inhibits Pseudomonas aeruginosa, and its therapeutic effect on pseudomonas urinary infection was confirmed by clinical studies. As shown by this clinical study, it has extremely low toxicity and is a safe agricultural drug. After the introduction of kasugamycin, phenyl mercuric compounds were withdrawn from use. After the introduction of kasugamycin, there was no crop decrease caused by Pyricularia oryzae.



Kasugamycin (1966)

Kanamycin and kasugamycin both are members of aminoglycosidic antibiotics. By looking at the course of the structure determination of kasugamycin in comparison with kanamycin, it is apparent that there was a great progress in organic chemistry in structure determination in the five years after 1957. It can be said that organic chemistry promoted the progress of antibiotic research. On the other hand, in 1967, Dr. Umezawa found and reported resistance mechanism and this promoted a rapid progress in sugar chemistry and dehydroxylation reactions.

In 1965, kanamycin-resistant Gram negative bacteria appeared in hospital patients and Dr. Umezawa undertook the study to elucidate its biochemical mechanism. He found and isolated 6'-N-acetylkanamycin and kanamycin 3'-phosphate as the reaction products of the homogenates of resistant cells with kanamycin in the presence of

CoA or ATP (1967). Most resistant strains produced the latter enzyme (3'-O-phosphotransferase). Therefore, Dr. Umezawa decided to synthesize 3'-deoxykanamycin and 3'-O-methylkanamycin. If these or one of these kanamycin derivatives inhibited the growth of resistant strains, it should be conclusive proof of the enzymic mechanism of resistance. Moreover, the active derivatives should be useful in the treatment of resistant infections. Parallel to syntheses of these derivatives, the chemical synthesis of 3',4'-dideoxykanamycin B was also started, because it seemed as if this could be more easily synthesized. Dr. Sumio Umezawa collaborated with Dr. H. Umezawa in the syntheses of these compounds, for Dr. S. Umezawa has been successful in the total syntheses of kanamycin A, B, and C.

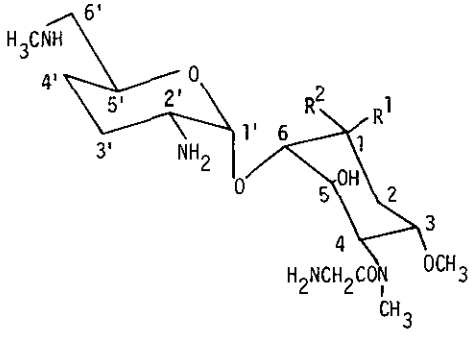
3'-Deoxykanamycin A and 3',4'-dideoxykanamycin A synthesized (1971, 1971) inhibited both sensitive and resistant strains. Most strains of Pseudomonas aeruginosa were resistant to kanamycin since the discovery of this antibiotic. One surprise was that these resistant pseudomonas strains were also inhibited by these derivatives. Thus, the enzymic resistance mechanism was conclusively determined. The other surprise was that 3'-O-methylation of neamine (1972) and kanamcyin inactivated these antibiotics.

Dr. H. Umezawa also analyzed the structures of the inhibitors and the substrates of 3'-O-phosphotransferase and reported the results in 1969 and 1970. It indicated that one or both of 1-NH_2 and 3-NH_2, 2'-OH (or 2'-NH_2), 3'-OH, 6'-NH_2 (or 6'-OH) were involved in the binding with 3'-O-phosphotransferase. The 3-amino-3-deoxyglucose moiety is not involved in the binding. Therefore, the modification of one of these groups involved in the binding with the enzyme can give derivatives active against both sensitive and resistant strains. In 1970, butirosin was reported to contain 2S-2-hydroxyl-4-aminobutyryl group on 1-NH_2. Amikacin synthesized by Kawaguchi is 1-N-(2S-2-hydroxy-4-butyryl)kanamycin A and exhibits a wide antibacterial spectrum against resistant strains. 3',4'-Dideoxykanamycin B and amikacin have been widely used. The characteristic point of the former is its low ototoxicity and that of the latter is a wide antibacterial spectrum against resistant strains. Thus, a new research area was opened up by Dr. H. Umezawa to obtain new effective drugs in derivatives and analogs of aminoglycoside antibiotics. As reviewed by Dr. H. Umezawa in 1974 (Advances in Carbohydrate Chemistry and Biochemistry, 30, 183-225), other enzymes such as 3-N-acetyltransferase, 2'-N-acetyltransferase, 4'-O-phosphotransferase, 2"-O-nucleotidyltransferase etc. are involved in resistance of other resistant strains. Most of these enzymes were found by Dr.

Umezawa. On the basis of these enzyme reactions, derivatives active against known resistant strains have been synthesized. Dr. Umezawa demonstrated the application of affinity chromatography containing an aminoglycoside antibiotic as the functional group for purification of these enzymes and the application of these enzymes in the solid phase for the rapid isolation of the products of the reaction of aminoglycosides with CoA or ATP (1974). Thus, it became very easy to isolate enzymes involved in resistance or enzyme reaction products rapidly. It has also been shown that the structures of the reaction products, that is, inactivated aminoglycoside antibiotics, are easily determined by the analysis of their proton or ^{13}C-nmr. The practical necessity of the synthesis of derivatives of aminoglycoside antibiotics promoted the progress of the methods for the protection of hydroxyl groups and the dehydroxylation.

Dr. H. Umezawa elucidated the mechanism of resistance to streptomycin in 1968: the 3-hydroxyl group of the N-methyl-L-glucosamine moiety is adenylated. There is another enzyme which transfers phosphate to this hydroxyl group. Dr. S. Umezawa was successful in the total synthesis of streptomycin and dihydrostreptomycin. In his collaboration, 3"-deoxydihydrostreptomycin was synthesized. This derivative inhibited resistant strains except for Pseudomonas aeruginosa. These pseudomonas strains were confirmed to produce a phosphotransferase which transferred phosphate from ATP to the 6-hydroxyl group of streptomycin. On the basis of this result, it became possible to synthesize streptomycin or dihydrostreptomycin derivatives active against resistant strains.

Very recently, a new group of aminoglycoside antibiotics, fortimicins, sporaricins, istamycins which have structural relationships with deoxystreptamine-containing



Istamycin A: $R^1=NH_2$, $R^2=H$

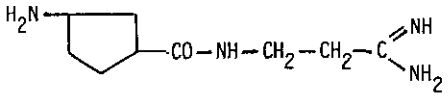
Istamycin B: $R^1=H$, $R^2=NH_2$

antibiotics were found in actinomycetes. Dr. Umezawa collaborated with the structure determinations of sporaricins, and his group found istamycins. Istamycin A was synthesized from 3',4'-dideoxyneamines. The structures of this group will also contribute to the analysis of structure-activity relationships.

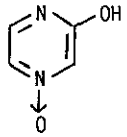
The random screening of new antibacterial antibiotics at present is not successful. There seems to be only a very small possibility of finding useful new types of antibiotics. This situation indicates the importance of the research of β-lactam and aminoglysocide antibiotics to develop useful chemotherapeutic agents. The latter research area was opened by Dr. Umezawa. It is the first time that a study of action mechanism was carried out in order to predict active structures.

ANTIBACTERIAL ANTIBIOTICS AND THEIR STRUCTURES OBTAINED BY SCREENING IN 1953-1979

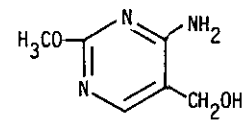
From 1953 to 1962, the following new antimicrobial antibiotics were found in his laboratory: seligocidin (antifungal, 1954), mediocidin (antifungal, 1954), fungicidin-rimocidin-chromin group (antifungal, 1954), eurocidin group (antifungal, 1954), trichomycin-ascosin-candicidin group (antifungal, 1954), tertiomycins A and B (antibacterial, 1955), antitoxoplasmic substance No. 534 (1955), mesenterin (antibacterial, 1955), althiomycin (antibacterial, 1957), unamycin (antifungal, 1958), niromycin (antiviral, 1960), amidinomycin (antiviral, 1960), phenazine compounds of S. griseoluteus (1961), ilamycins (antimycobacterial, 1962), pyrimidine compounds (reducing streptothricin toxicity, 1962), griseococcin (antibacterial, 1962), No. A-418-Z4 antibiotic (antibacterial, 1962). These antibiotics were found in the Department of Antibiotics of Japanese NIH. Dr. Umezawa also guided the discovery of the following compounds in the 6th Department of the Institute of Applied Microbiology of the University of Tokyo: blastomycin (antifungal, 1957), mikamycins A and B (antibacterial, 1958), variotin (antifungal, 1959), emimycin (weakly antimicrobial, 1960), bacimethrine (antibacterial, 1961). The following structures were reported in the years shown in parenthesis.



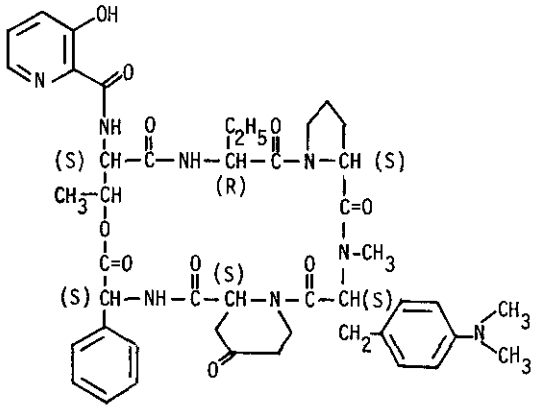
Amidinomycin (1961)



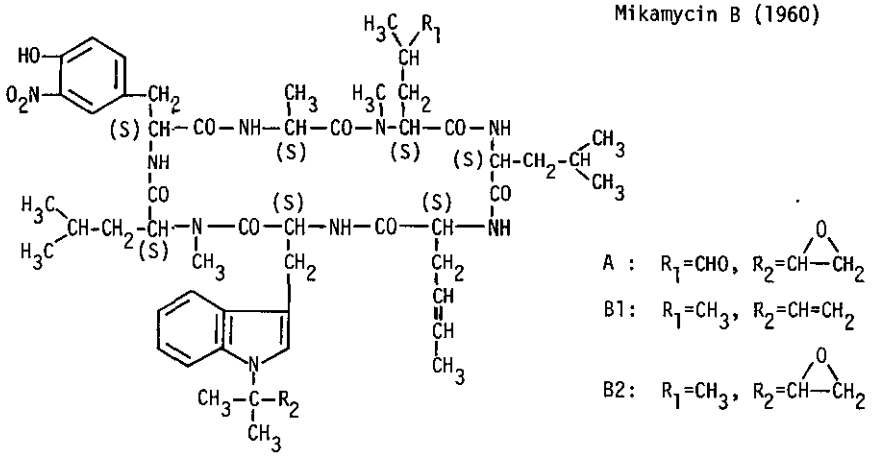
Emimycin (1960)



Bacimethrin (1961)



Mikamycin B (1960)



A : R_1=CHO, R_2=CH(O)CH$_2$

B1: R_1=CH_3, R_2=CH=CH_2

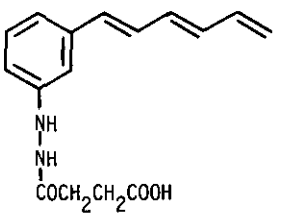
B2: R_1=CH_3, R_2=CH(O)CH$_2$

Ilamycins (1964)

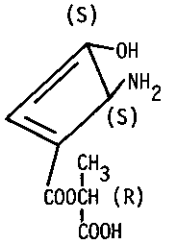
In these studies, Dr. Umezawa found that polyene antifungal antibiotics are produced by many strains of actinomycetes and he first discovered pentaene compounds named mediocidin. Dr. Umezawa studied the synergism between mikamycin A and B not only in the action to inhibit bacterial growth but also in the inhibition of protein synthesis on bacterial ribosomes and he extended this study to other antibiotics and found a strong synergism between O-carbamoyl-D-serine and D-4-amino-3-isoxazolidone (1964). These compounds inhibit the enzymatic reactions which work successively in the synthesis of peptidoglycan, and the reason of the synergism was understood.

From 1963 to now, the following antimicrobial antibiotics were found by Dr. H. Umezawa: monazomycin (antibacterial, 1963), spinamycin (antifungal, 1966), josamycin (antibacterial, 1967), leucinamycin (antibacterial peptide, 1967), laspartomycin

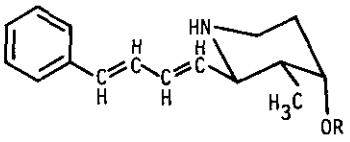
(antibacterial peptide, 1968), pepthiomycin (antibacterial and antifungal peptide, 1968), oryzoxymycin (antifungal, 1968), gougeroxymycin (antifungal, 1969), dienomycin (antimycobacterial, 1970), macarbomycin (antibacterial, 1970), negamycin (antibacterial, 1970), deoxynybomycin (antibacterial, 1970), leucylnegamycin (antibacterial, 1971), cyclamidomycin (desdanine, inhibitor of nucleoside diphosphate kinase, 1971), requinomycin (antibacterial, inhibitor of R-factor transfer, 1972), β-lactamase inhibitors (antibacterial, 1973), four minor antibiotics of a macarbomycin-producing strain (antibacterial, 1973), minosaminomycin (antibacterial, 1974), amicleonomycin (antibacterial, antagonized by biotin, 1974), calvatic acid (antibacterial, produced by a mushroom, 1975), SS-228Y (antibacterial, 1975), aplasmomycin (antiprotozoa and antibacterial, 1977), 3-epi-deoxynegamycin (antibacterial, 1977), leucyl-3-epi-negamycin (1977), pheganomycin (antibacterial, 1977), aplasmomycins B and C (1978). The following structures were reported in the years in parenthesis.



Spinamycin (1968)



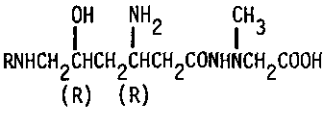
Oryzoxymycin (1974)



Dienomycins (1970)

A: R=$COCH(CH_3)_2$

B: R=$COCH_3$ C: R=H

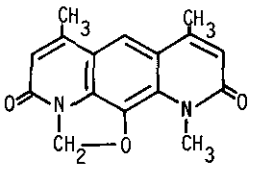


Negamycin (1971)

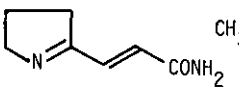
R=H

Leucylnegamycin (1971)

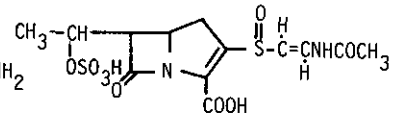
R=L-Leu



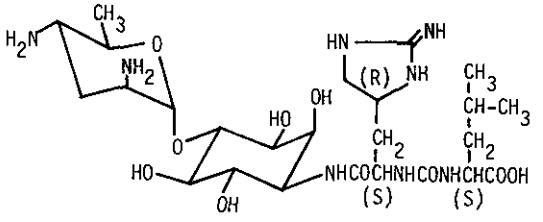
Deoxynybomycin (1970)



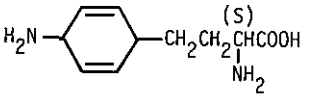
Cyclamidomycin
(1971)



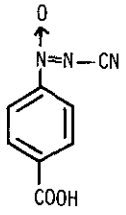
β-Lactamase inhibitor
(1977)



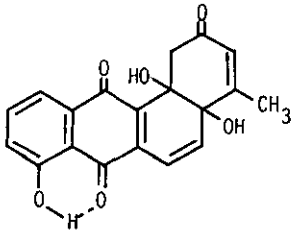
Minosaminomycin (1975)



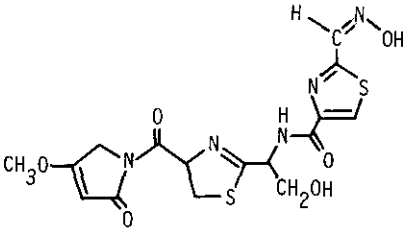
Amiclenomycin (1974)



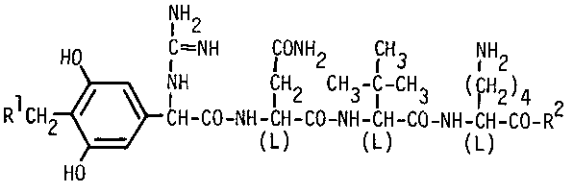
Calvatic acid
(1975)



SS-228Y (1975)

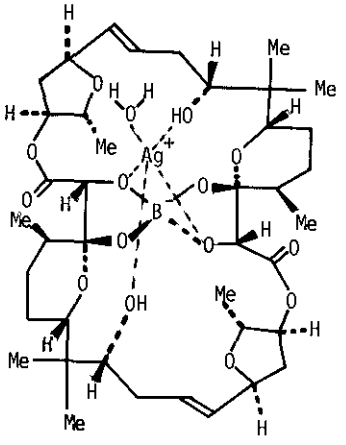


Althiomycin (1974)



Pheganomycin : R^1=OH, R^2=OH
Pheganomycin-D : R^1=OH, R^2=Asp
Pheganomycin-DR : R^1=OH, R^2=Asp-Arg
Pheganomycin-DPGT: R^1=OH, R^2=Asp-Pro-Gly-Thr
Deoxypheganomycin-D: R^1=H, R^2=Asp

Pheganomycins (1977)



Aplasmomycin-Ag (1977)

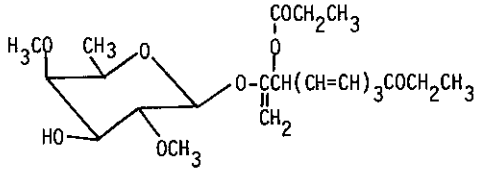
Among the antimicrobial antibiotics, josamycin has been developed for practical use. As already described, in 1953, Dr. Umezawa found tertiomycins A and B which were crystallized. They were macrolide antibiotics and had low toxicity but their oral administration did not produce a high enough blood level. Therefore, studies of tertiomycins were given up and Dr. Umezawa continued the screening of macrolides and found josamycin. Soon after the start of the clinical study, it was found that josamycin did not cause stomach irritation and produced a high enough blood level. Since 1970, this antibiotic has been widely used. It inhibited about a half of the erythromycin-resistant strains. The resistance of these strains was induced by erythromycin. After josamycin had been found, leucomycin A3 was found as a very minor component of leucomycin. Leucomycin A3 cannot be found in the present sample of leucomycin, therefore, josamycin has been used for the structure study of leucomycin A3. It can be said that josamycin produces the highest blood level after its oral administration as compared with other 16-member-ring macrolides.
Negamycin has low toxicity and inhibits the growth of Gram negative organisms including Pseudomonas aeruginosa both in vitro and in vivo. Negamycin inhibits the terminal step of protein synthesis on bacterial ribosomes. However, a hydrolysis product (N-methylhydrazinoacetic acid) inhibits glutamate pyruvate transaminase and the daily administration of negamycin to dogs caused reversible hepatic coma and the clinical study was stopped. Althiomycin which was discovered at the time of the discovery of kanamycin showed a strong action to protect mice from bacterial infections. Althiomycin inhibits protein synthesis on bacterial ribosomes but not on mammalian ribosomes. Its structure was finally proposed.
The bioactive chemical structures found by the screening of antibacterial activities may contribute to the study of structure-activity relationships. Especially, the structures of low toxic antibiotics such as negamycin and althiomycin are interesting. Their chemical studies may lead us to their derivatives or analogs which are useful in the treatment of resistant infections in the future.

INITIATION OF ANTITUMOR ANTIBIOTIC RESEARCH, DISCOVERY THEREOF, AND CHEMICAL STUDIES OF BLEOMYCIN

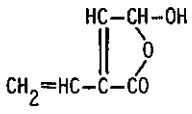
One of the most important chemical studies is to open up a new research area where new chemical structures with new bioactivities can be found.
As described in a previous paragraph, in about 1949-1951, Dr. Umezawa observed the production of cytotoxic compounds by many soil actinomycetes strains during his

study of antiviral substances. On the other hand, in about 1951, resistant pathogenic bacteria did not yet appear in hospitals and chemotherapy of bacterial diseases seemed to be completed by use of penicillin, streptomycin, chloramphenicol, aureomycin and terramycin. Therefore, Dr. Umezawa initiated the study of antitumor antibiotics. His publications on antitumor antibiotics obtained by the screening have been started in 1953. By screening for antitumor antibiotics which inhibited the growth of experimental tumors, he discovered finding No. 289 and sarkomycin. These papers stimulated the study of antitumor antibiotics in the world. The screening of antitumor antibiotics has contributed to the findings of useful cancer chemotherapeutic agents and has dug up various new chemical structures which had cytotoxic or antitumor activity. Up to now, Dr. Umezawa reported the findings of the following new antitumor antibiotics: No. 289 antitumor substance (1953), sarkomycin (1953), actinoleukin (1954), ractinomycin (1955), pluramycin (1956), phleomycin (1956), raromycin (1957), peptimycin (1961), cytomycin (1961), 3-carboxy-2,4-pentadienal lactol (1962), enomycin (1963), labilomycin (1963), formycin (1964), formycin B (1965), plurallin (1966), bleomycin (1966), phenomycin (1967), coformycin (1967), macromomycin (1968), coriolin (1969), neopluramycin (1970), diketocoriolin B (1971), macracidomycin (1975), revistin (1975), glyoxalase I inhibitor (1975), aclacinomycins A and B (1975), neothramycin (1976), rhodirubins (1977), baumycins A1, A2, B1, B2, C1, C2 (1977), anthracyclines M1, N1, S (1977), roseorubicins A and B (1979), auromomycin (1979), clazamycins A and B, 2-hydroxy-5-iminoazacyclopent-3-ene (1979).

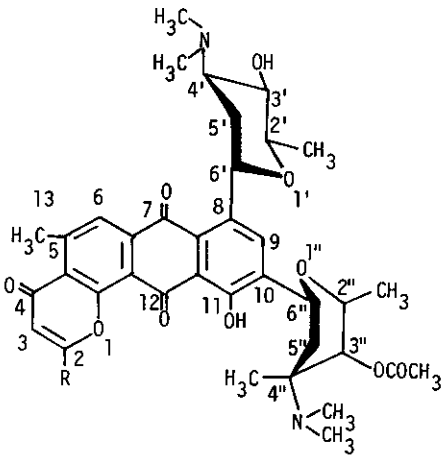
The following structures were determined (the year in parenthesis is that of the publication of the structure).



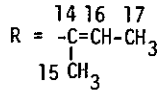
Labilomycin (1964)



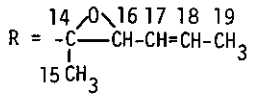
3-Carboxy-2,4-pentadienal lactol (1962)



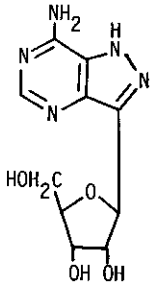
Neopluramycin:



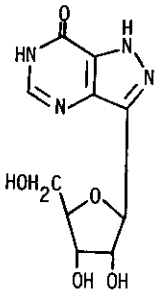
Pluramycin A:



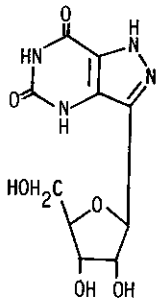
Pluramycin A and Neopluramycin (1977)



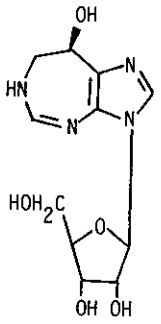
Formycin (1966)



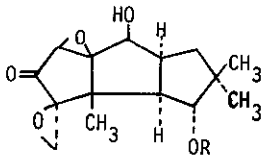
Formycin B (1966)



Oxoformycin B (1968)



Coformycin (1974)

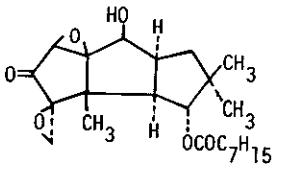


Coriolin (1971)

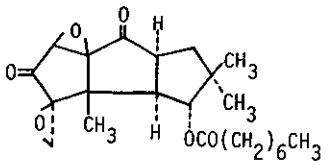
R = H

Coriolin C (1971)

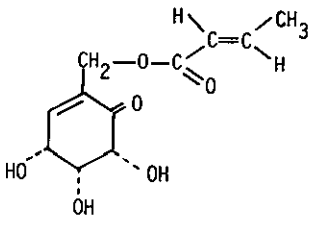
R = COCHC$_6$H$_{13}$ (OH)



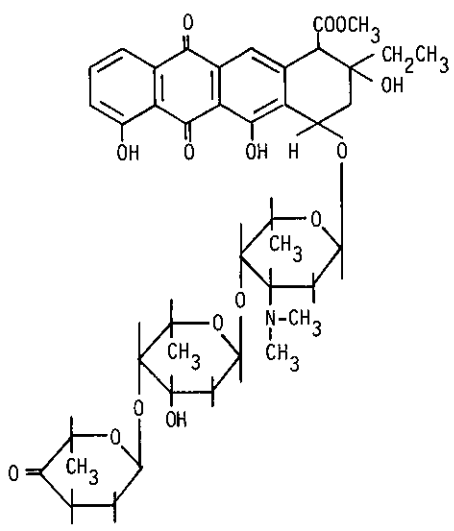
Coriolin B (1971)



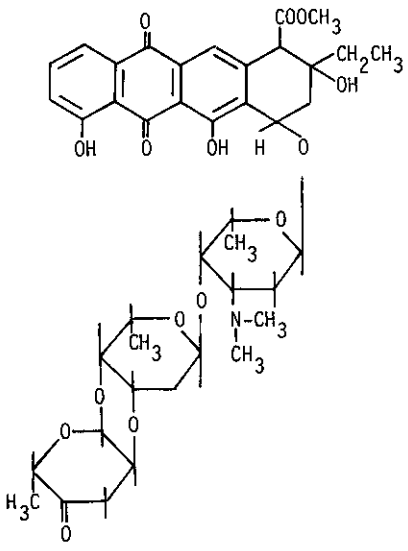
Diketocoriolin B (1971)



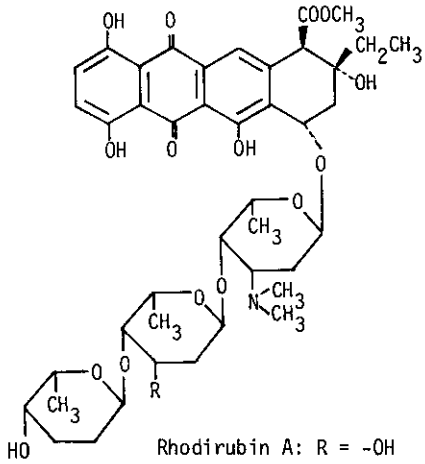
Glyoxalase inhibitor, glyo II (1975)



Aclacinomycin A (1975)



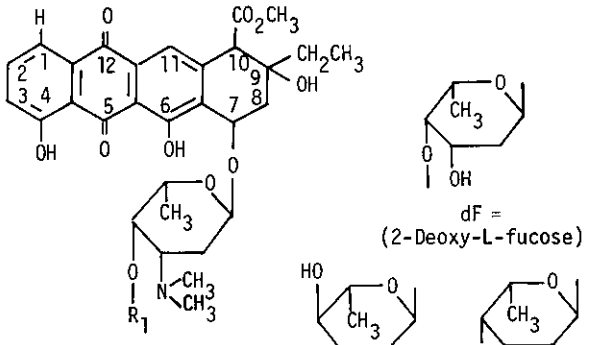
Aclacinomycin B (1975)



Rhodirubin A: R = -OH

Rhodirubin B: R = -H

Rhodirubins A and B (1977)



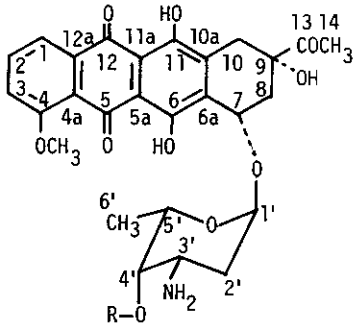
dF = (2-Deoxy-L-fucose)

Ami = (L-Amicetose)

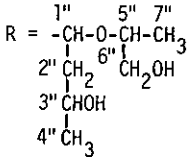
Rho = (L-Rhodinose)

	R_1
M1:	dF-Ami
N1:	dF-Rho
S1:	dF

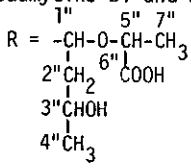
MA144M1, N1 and S1 (1977)

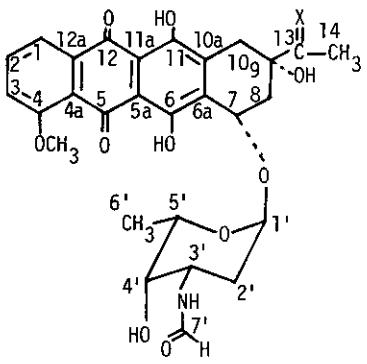


Baumycins A1 and A2



Baumycins B1 and B2

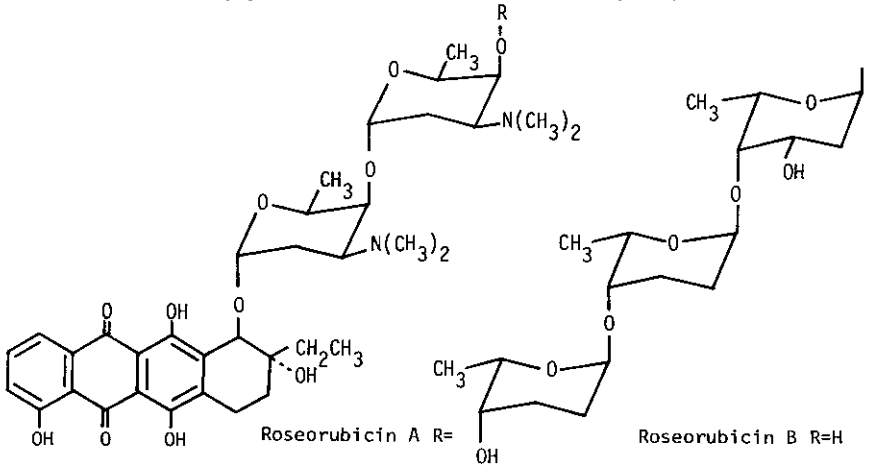




Baumycin C1 X=O

Baumycin C2 X = OH, H

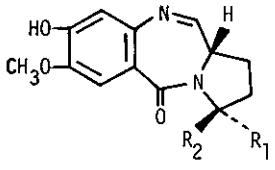
Baymycins A1, A2, B1, B2, C1 and C2 (1977)



Roseorubicin A R=

Roseorubicin B R=H

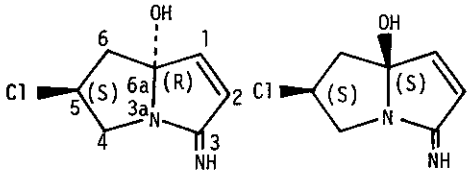
Roseorubicins A and B (1979)



Neothramycins (1976)

A: R_1=OH, R_2=H

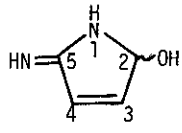
B: R_1=H, R_2=OH



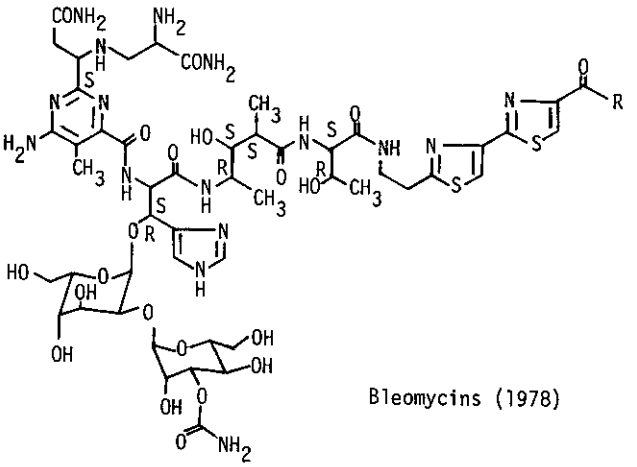
Clazamycin A

Clazamycin B

Clazamycins A and B (1979)



2-Hydroxy-5-iminoazacyclopent-3-ene (1979)



Bleomycins (1978)

A1: R=NH$(CH_2)_3SOCH_3$

A2: R=NH$(CH_2)_3S^+(CH_3)_2$

A2'-b: R=NH$(CH_2)_3NH_2$

A5: R=NH$(CH_2)_3$NH$(CH_2)_4NH_2$

B1': R=NH_2

B4: R=NH$(CH_2)_4$NHC(=NH)NH$(CH_2)_4$NHC(=NH)NH_2

Bleomycinic acid: R=OH

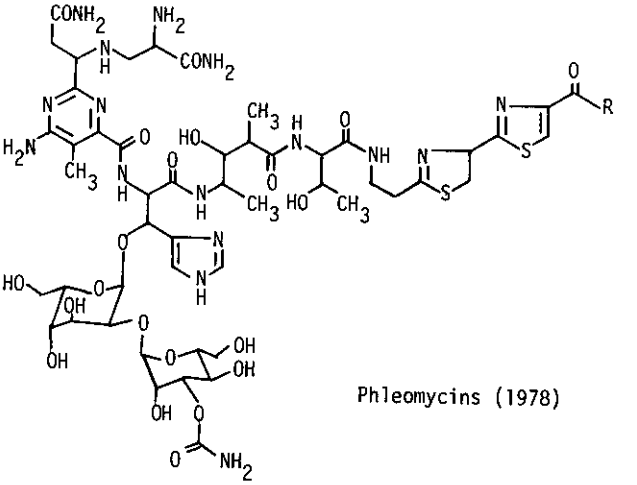
Demethyl-A2: R=NH$(CH_2)_3SCH_3$

A2'-a: R=NH$(CH_2)_4NH_2$

A2'-c: R=NH$(CH_2)_2$-(imidazole)

A6: R=NH$(CH_2)_3$NH$(CH_2)_4$NH$(CH_2)_3NH_2$

B2: R=NH$(CH_2)_4$NHC(=NH)NH_2



Phleomycins (1978)

D1: R=NH$(CH_2)_4$NHC(=NH)NH_2

E: R=NH$(CH_2)_4$NHC(=NH)NH$(CH_2)_4$NHC(=NH)NH_2

Sarkomycin which Dr. Umezawa found in his earliest screening study had no chronic toxicity. Therefore, this was clinically studied, but clear results were not obtained. One of the reasons may be due to its unstable property. Pluramycin exhibited a strong effect on Ehrlich carcinoma, but it had chronic toxicity. The lethal dose of this compound as well as actinomycin, mitomycin and chromomycin caused bleeding in the intestine and the colon. Phleomycin which was first found as one of the water-soluble basic antibacterial antibiotics was found later to exhibit a strong inhibition against Ehrlich carcinoma and this antibiotic was isolated as a copper-containing blue powder. Dr. Umezawa who was interested in this chemical property selected this compound as the one to study in detail. The therapeutic index calculated from the ratio of the maximum tolerable dose of daily injection for seven days to the minimal daily dose given for seven days was 32-64. It was significantly high compared with the indices of other antitumor compounds: the indices of antitumor antibiotics isolated before 1960 were 4-8. But, phleomycin showed a strong renal toxicity to dogs. Dr. Umezawa gave up his plan to conduct clinical study of phleomycin and continued the screening to find antibiotics of a similar type. In this study, Dr. Umezawa found bleomycins which were shown by paper chromatography and found its stability to be different from phleomycins. A mixture of bleomycins caused hepatotoxicity, but did not show irreversible renal toxicity to dogs. As shown in his first and second papers on bleomycin in 1966, various bleomycins were produced in the same culture filtrate by fermentation and individual bleomycins which were purified showed a weaker activity against Ehrlich carcinoma than a mixture which consisted mainly of A2 (about 70%) and B2 (about 25%). Therefore, this mixture was presented for clinical study.

As shown by the structural formulae of bleomycins and phleomycins, their structure determination was not an easy one. However, by applying the reactions for the specific cleavage of aminoacylthreonine, or histidyl bond, transferring the carbonyl group of the histidyl moiety to the hydroxyl group of 4-amino-3-hydroxy-2-methylpentanoic acid moiety, the amino acid sequence was successfully determined. This structure determination process is a useful material in teaching peptide chemistry. It is also interesting that after testing hydrolysis conditions, the treatment of bleomycin for several hours in 0.3N sulfuric acid at 80°C gave the disaccharide moiety. The most difficult part for the structure elucidation was an amino acid moiety called pyrimidoblamyl moiety. As written by Dr. Umezawa in this book, the side chain of this amino acid was finally determined by X-ray crystal analysis of

a biosynthesis intermediate (demethylpyrimidoblamylhistidylalanine) and synthesis of model compounds of this amino acid.

As written by Umezawa in this book, the structure of the metal complexes of bleomycin gave the fundamental basis for the understanding of the action of bleomycin in causing DNA fragmentation, and the inactivation of bleomycin by bleomycin hydrolase which hydrolyzes the α-aminocarboxamide group of pyrimidoblamyl moiety leads to the interesting derivatives which were resistant to bleomycin hydrolase. The difference in the degree of renal and pulmonary toxicity was shown in various bleomycins which differed with each other in the terminal amine moiety, and less toxic bleomycins have been developed. Bleomycin is one of the group of compounds on which action mechanisms and structure-activity relationships have been studied in most detail. On the basis of this study, more useful bleomycins have been developed.

Formycin found by Dr. Umezawa is a structural analog of adenosine and replaces adenosine in almost all enzyme reactions in vivo where formycin is converted to formycin triphosphate. Coformycin was found in the same culture filtrate from which formycin was isolated. It inhibits adenosine deaminase strongly. This enzyme also deaminates formycin. It may be said that the structure of coformycin opened up a new nucleoside research area. Isocoformycin, one of the analogs, inhibits deaminase more weakly and has low toxicity.

In order to find effective cancer chemotherapeutic agents Dr. Umezawa extended his study to anthracycline antibiotics and demonstrated the presence of anthracyclines which had a low cardiac toxicity. As written in this book by Dr. Umezawa, anthracyclines which will be more effective than adriamycin is being developed.

Dr. Umezawa emphasized the necessity of the study of macromolecular antibiotics. He, himself, found enomycin, phenomycin, macromomycin, macracidomycin etc. It is interesting that enomycin and phenomycin inhibit protein synthesis on mammalian ribosomes but very rarely bacterial ribosomes.

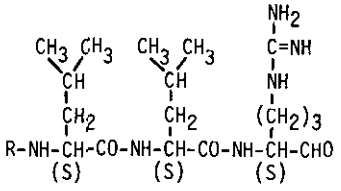
At present, it is one of the duties of chemists to contribute to the accomplishment of cancer chemotherapy. In this study, active structure should be provided to chemists and starting from this structure and its action mechanism, chemists can design the experiment to reach the goal. It can be said that in the last nearly 30 years Dr. Umezawa has dug up the useful chemical structures which exhibited antitumor action. From the compounds with these structures and on the basis of a high therapeutic index, he selected phleomycin as a worthwhile study and reached

bleomycin. Bleomycin thus found exhibited therapeutic effect on head, neck and skin cancers, Hodgkin's lymphoma and testis tumors. As written by Dr. Umezawa in this book, the relation between the bleomycin structure and action has been analyzed in detail, and on the basis of this relationships it became possible to design the chemical synthesis of active derivatives or analogs which might exhibit a stronger therapeutic effect than the present bleomycin did. It can be said that bleomycin and its structure were dug up by Dr. Umezawa for chemists as one of the most important basis in the development of cancer chemotherapeutic agents. It may be said that Dr. Umezawa is the pioneer of the chemistry of antitumor antibiotics useful in the treatment of cancer.

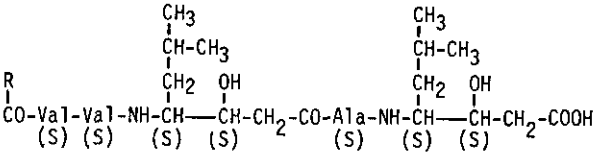
SMALL MOLECULAR ENZYME INHIBITORS

It is not easy or it is very rare to be successful in opening up a completely new research area. In about 1965, Dr. Umezawa planned the study of small molecular enzyme inhibitors produced by microorganisms, entered this research and found many important compounds. According to him, there were several reasons why he initiated this study: (1) In case of the study of small molecular inhibitors of enzymes, the rapid structure determination is the absolute requirement to understand the mechanism of the inhibition and in about 1965, from his experience in the structure determination of antibiotics, he thought that organic chemistry had been developed fully enough; (2) Still at present, it is often said that microorganisms in nature produce antibiotics to suppress the growth of others, but he suspected whether or or not antibiotics were produced in nature, because in general, the production of an antibiotic depends on medium compositions; (3) He thought that the many genes which caused the production of various microbial compounds had been generated and the compounds which were not necessary for the growth were produced; (4) He experienced that the ability of a strain to produce antibiotics was lost without causing the death of the strain. He thought it would be highly possible to find small molecular enzyme inhibitors in microbial culture filtrates. Moreover, on the basis of the biochemistry of diseases, he expected that compounds useful in the treatment of diseases would be found in enzyme inhibitors. The inhibitors found by him are described below. (1) Inhibitors of hydrolytic enzymes: leupeptin inhibiting plasmin, trypsin, papain, cathepsin B (1969), pepstatin (1970), pepstanone (1972), hydroxypepstatin (1973) inhibiting pepsin, cathepsin D and renin, chymostatin inhibiting chymotrypsins strongly and papain weakly (1970), antipain inhibiting trypsin,

papain, cathepsins A and B (1972), phosphoramidon inhibiting zinc endopeptidase (1973), elastatinal inhibiting pancreas elastase (1973), panosialin inhibiting clostridium sialidase (1971), siastatins A and B inhibiting sialidase (1974), pyridindolol inhibiting β-galactosidase (1975), isoflavone rhamnosides inhibiting β-galactosidase (1975), p-hydroxyphenylacetaldoxime inhibiting β-galactosidase (1979).

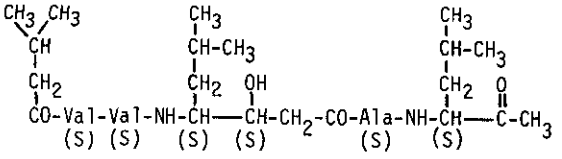


Leupeptins (1969)

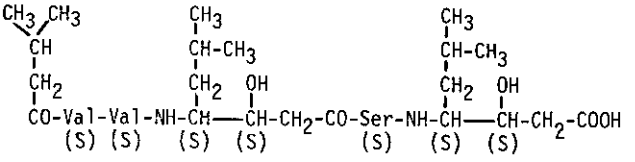


Pepstatins (1970) R=CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, ...etc.

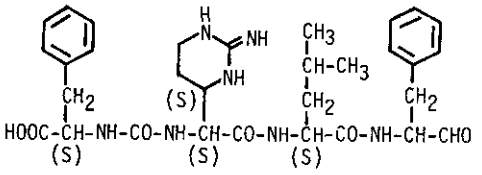
Ac-LL: R=CH_3CO, Pr-LL: R=CH_3CH_2CO



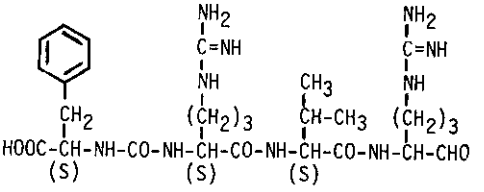
Pepstanone A (1972)



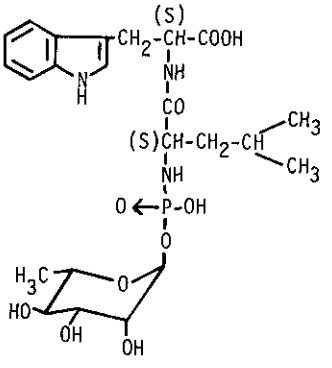
Hydroxypepstatin A (1973)



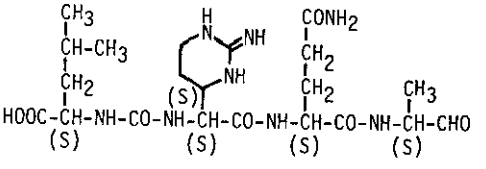
Chymostatin (1973)



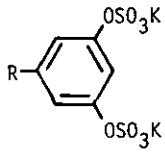
Antipain (1972)



Phosphoramidon (1973)

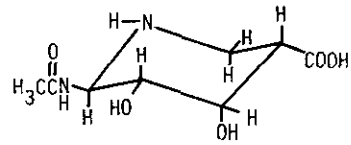


Elastatinal (1975)

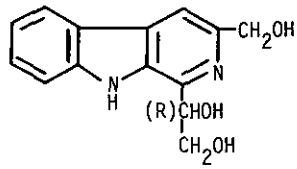


Panosialins (dipotassium salts) (1971)

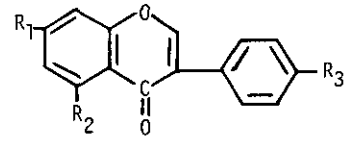
R=$(CH_3)_2CH(CH_2)_{12}$-, $CH_3(CH_2)_{14}$-, $(CH_3)_2CH(CH_2)_{13}$-



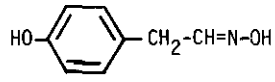
Siastatin B (1974)



Pyridindolol (1975)



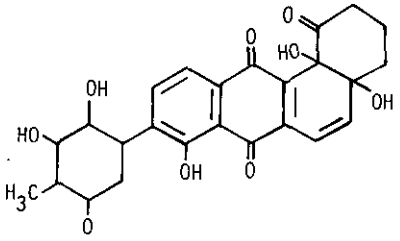
R_1	R_2	R_3	
O-Rha	H	O-Rha	daidzein 4',7-di-α-L-rhamnoside
O-Rha	H	OH	daidzein 7-α-L-rhamnoside
O-Rha	OH	O-Rha	genistein 4',7-di-α-L-rhamnoside
O-Rha	OH	OH	genistein 7-α-L-rhamnoside



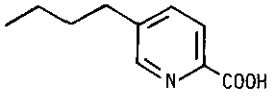
Hydroxypnenylacetaldoxime (1979)

New types of structures which inhibit serine thiole proteases were found in leupeptin, antipain, chymostatin and elastatinal. They contain argininal, phenylalaninal or alaninal to inhibit the enzymic hydrolysis of arginyl, phenylalanyl or alanyl bond in peptides. Pepstatin presented a very active inhibitory structure of carboxyl peptidases. The unusual amino acid, 3S,4S-4-amino-3-hydroxy-6-methylheptanoic acid, is the key structure and this amino acid is called statin. Phosphoramidon indicated that the leucine phosphate should be the structure of a strong inhibitor of zinc endopeptidases. Zinc endopeptidases hydrolyze the peptide bond at the amino side of hydrophobic amino acid. These inhibitors of proteases found by Dr. Umezawa have been used by biochemical, biological and medical researchers in the world. Leupeptin, antipain and pepstatin have also been reported to have a potential usefulness in the treatment of muscular dystrophy.

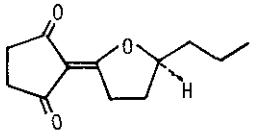
(2) Inhibitors of enzymes involved in biosynthesis and metabolism of catecholamines: aquayamycin inhibiting tyrosine hydroxylase and dopamine β-hydroxylase (1968), chrothiomycin inhibiting tyrosine hydroxylase and dopamine β-hydroxylase (1969), fusaric acid (5-butylpicolinic acid) inhibiting dopamine β-hydroxylase (1969), oudenone inhibiting tyrosine hydroxylase (1970), oosponol inhibiting dopamine β-hydroxylase (1972), dopastin inhibiting dopamine β-hydroxylase (1972), methylspinazarin and dihydromethylspinazarin inhibiting cathechol-O-methyltransferase (1973), monoamine oxidase inhibitors (1973), 7-O-methylspinochrome B and its 6-(3-hydroxy-n-butyl)-derivatives (1973), new isoflavones inhibiting cathechol-O-methyltransferase (1975), new isoflavones inhibiting dopa decarboxylase (1976), dehydrodicaffeic acid dilactone inhibiting cathecol-O-methyltransferase (1976), tetra-O-methyldehydrodicaffeic acid dilactones inhibiting cathechol-O-methyltransferase (1977).



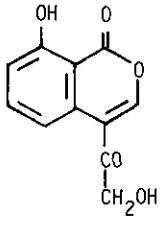
Aquayamycin (1970)



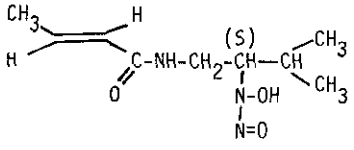
Fusaric acid (1969)



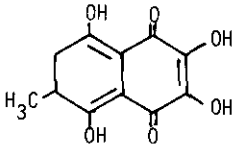
Oudenone (1971)



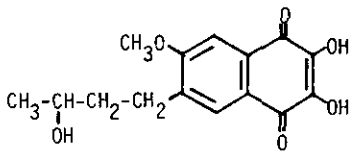
Oosponol (1972)



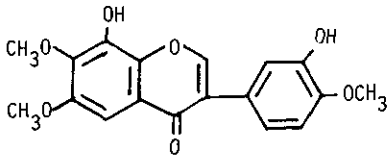
Dopastin (1973)



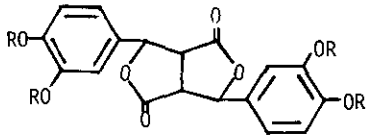
Dihydromethylspinazarin (1973)



6-(3-Hydroxy-n-butyl)-7-O-methyl-spinochrome B (1973)

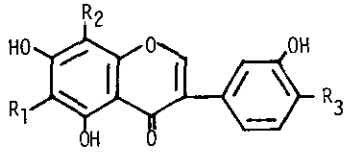


Specific inhibitor of catechol O-methyltransferase (1975)



(+) and (-)-Dehydrodicaffeic acid dilactone (R=H, 1976)

tetra-O-methyldehydrodicaffeic acid dilactone ($R=CH_3$, 1977)



New isoflavones inhibiting dopa decarboxylase (1976)

I: R_1=H, R_2=OCH_3, R_3=OH

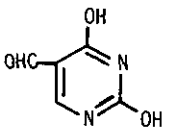
II: R_1=OCH_3, R_2=H, R_3=OCH_3

III: R_1=H, R_2=OCH_3, R_3=OCH_3

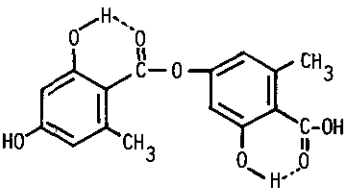
The inhibitors of enzymes involved in the biosynthesis of noradrenaline such as tyrosine hydroxylase, dopa decarboxylase and dopamine β-hydroxylase exhibited hypotensive effect on hypertension of spontaneously hypertensive rats. Among them, oudenone and fusaric acid have been studied clinically. Especially the latter showed a strong hypotensive effect and is becoming a useful hypotensive agent. The inhibitors of tyrosine hydroxylase and dopamine β-hydroxylase have no structural relationships with tyrosine and dopamine. This may be due to the complicated structures of these enzymes. The enzymes involved in the biosynthesis or the metabolism of norepinephrine have no function in microorganisms and their inhibitors were produced by microorganisms. This is one of the facts that microorganisms produce various compounds which are not utilized by microbial cells.

(3) Inhibitors of various enzymes: 5-formyluracyl inhibiting xanthine oxydase (1972), lecanoric acid inhibiting histidine decarboxylase (1974), 1-[2-(3,4,5,6-tetrahydropyridyl)]-1,3-pentadine inhibiting nonspecific N-methyltransferase (1974), coformycin inhibiting adenosine deaminase (1974), revistin inhibiting reverse transcriptase (1975), glyoxalase inhibitor (glyo I, MS-3) (1975), reticulol inhibiting cyclic AMP phosphodiesterase (1975), PDE-I and II inhibiting cyclic AMP phosphodiesterase (1978), 2,5-dihydro-L-phenylalanine inhibiting tryptophan hydrox-

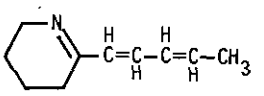
ylase (1977).



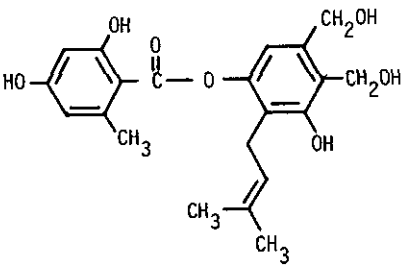
5-Formyluracil (1973)



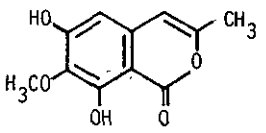
Lecanoric acid (1974)



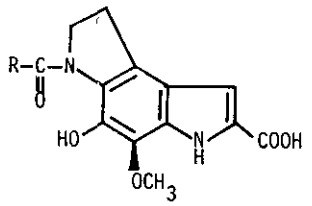
N-Methyltransferase inhibitor (1974)



Glyoxalase inhibitor, MS-3 (1975)

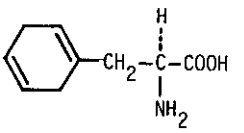


Reticulol (1975)



Phosphodiesterase inhibitors (1977)

PDE-I: R=NH_2
PDE-II: R=CH_3

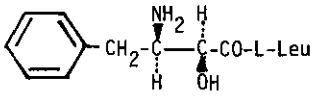


2,5-Dihydro-L-phenylalanine (1977)

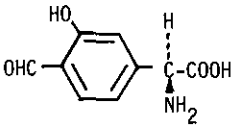
Lecanoric acid has been isolated from lichens and was found for the first time by the screening of an inhibitor or of histidine decarboxylase. Lecanoric acid is not effective in vivo because of a rapid enzymic hydrolysis. But, it suggested a specific inhibitory structure for the inhibition of histidine decarboxylase. The compounds which were structurally related to lecanoric acid but contained an amide group instead of the ester group have been confirmed to inhibit histidine decar-

boxylase. The compound MS-3 inhibits glyoxalase but it is not effective in vivo due to the hydrolysis of the ester group. Another, Glyo II, which was described in the previous paragraph, inhibited glyoxalase and showed antitumor activity also in vivo. Reticulol, PDE-I and PDE-II inhibited cyclic AMP phosphodiesterase and they were found by searching for inhibitors of cyclic AMP phosphodiesterase. Their structures are unique and they may be utilized for the design of chemical synthesis of phosphodiesterase inhibitors.

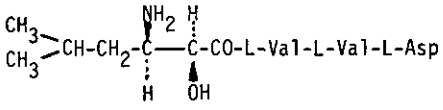
(4) Inhibitors of hydrolytic enzymes on the cellular surface (immunomodulators); bestatin inhibiting aminopeptidase B and leucine aminopeptidase (1976), forphenicine inhibiting chicken intestine alkaline phosphatase (1978), amastatin inhibiting aminopeptidase A (1978), esterastin inhibiting esterase (1978).



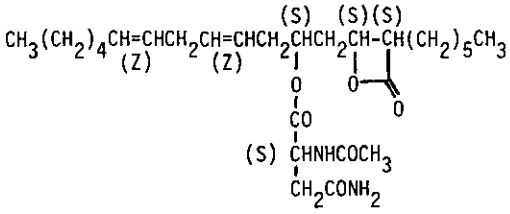
Bestatin (1976)



Forphenicine (1978)



Amastatin (1978)



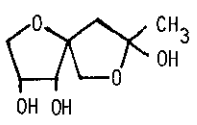
Esterastin (1978)

On the basis of a known fact that lectins such as concanavalin A, phytochemoagglutinin etc. bind to lymphocytes and cause mitogenesis, Dr. Umezawa thought that even small molecular compounds which bound to cells might cause mitogenesis and enhance immune responses. In order to find compounds which bind to the cell surface, he first examined the enzyme activities of the cellular surface and searched for inhibitors of enzymes on the cell surface. Thus, he found bestatin, amastatin, forphenicine and esterastin. As written by Dr. Umezawa in his paper in this book, bestatin, amastatin and forphenicine enhanced immune responses and esterastin suppressed them. This study is very unique. It can be said that again he opened a new research area to dig up new bioactive structures and new compounds with potential usefulness in treatment of cancer, resistant infections in immunodeficient

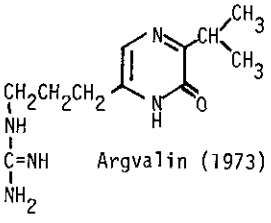
patients and autoimmune diseases.

NEW ASPECT OF MICROBIAL SECONDARY METABOLITE—GENETICS

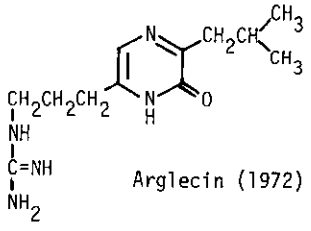
Although it is said that microorganisms produce antibiotics to suppress the growth of others, Dr. Umezawa searched for enzyme inhibitors in microbial culture filtrates and found compounds which had no antimicrobial activity. In another screening study, that is, in the screening of color reaction positive compounds, Dr. H. Umezawa found the following compounds: sphydrofuran, arglecin, argvalin and KD-16-U1.



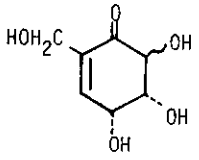
Sphydrofuran (1971)



Argvalin (1973)

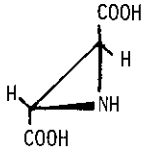


Arglecin (1972)

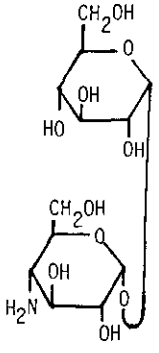


KD16-U1 (1974)

Furthermore, the following compounds were obtained as by-products.



S-2,3-Dicarboxyaziridine (1975)



4-Amino-4-deoxy-α, α-trehalose (1974)

Thus, it has been shown by Dr. Umezawa that compounds which have widely varied structures are produced by microorganisms. This led Dr. Umezawa to study the reason why the almost unlimited number of various compounds are produced by microorganisms. It can be said to be ingenious that he undertook this study from the viewpoint of genetics of characteristic structural parts of microbial secondary metabolites. Antibiotics can be divided into various groups on the basis of their structure relationships. Antibiotics of each group contain a characteristic structural part common to them. It indicates the presence of a special gene or a gene

set involved in the biosynthesis of this characteristic structural part and a wide distribution of such a gene or gene set among strains producing the antibiotics of the same group. As described in his paper in this book, he proposed a principle for genetics and biosynthesis of characteristic structural parts of microbial secondary metabolites. This can indicate the most possible reason why so many various microbial secondary metabolites can be found in microorganisms. He confirmed the involvement of multienzymes in the biosynthesis of leupeptin acid. The gene or gene set involved in the biosynthesis of this multienzyme was transferred by conjugation, indicating this gene or gene set is on a plasmid. Moreover his study on the biosynthesis of pepstatin and the peptide part of bleomycin etc. as described in his paper in this book suggested their synthesis by multienzymes specific to each of them. On the basis of these observations, he suggested that it might be interesting to study whether multienzymes are also involved in the biosynthesis of characteristic structural parts other than peptides. Biosynthesis of natural products is one of the research areas of organic chemists. Dr. Umezawa's study led these studies to their genetics.

CONCLUSION

The skillful application of principles and methods of organic chemistry, or the work of pioneering a new important application area is one of the great accomplishments in organic chemistry, and chemistry of bioactivities and the development of useful organic compounds on the basis of molecular mechanisms of bioactions are recent research areas of significant account in organic chemistry. It can be said that Dr. Umezawa pioneered an application area of organic chemistry for the development of useful aminoglycosides on the basis of resistant mechanisms and for the development of bleomycin group compounds on the basis of the relationships between structures and activity or toxicity, by extending the antibiotic research to small molecular enzyme inhibitors, he dug up new bioactive chemical structures, and he pioneered the research area in genetics of characteristic structural parts of microbial secondary metabolites on the basis of their chemical structures and was successful in proposing the reason why the almost unlimited number of various compounds are found in microorganisms.

As shown by Dr. Umezawa's study, it is now reasonable to search for useful bioactive compounds in microbial culture filtrates. If a quantitative exact screening method is used, compounds which have the purposed activity will be found in high

probability. As reported in Current Content (Life Sciences), No. 35, 1978, his works were cited in 5781 papers during the years 1961 to 1976. It is also interesting that most bioactive compounds found by him contain nitrogen, oxygen or both and many of them contain heterocycles.

In closing this report, we would like to add that we are honored and privileged to have studied under Professor Hamao Umezawa's pertinent guidance and we would like to express our sincerest congratulations to him on his 65th birthday and send him our best wishes for his personal and everlasting scientific future with our deepest gratitude.