ANTIMICROBIAL AGENTS

AND CHEMOTHERAPY - 1970

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Proceedings of the Tenth Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 18–21 October 1970

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PREFACE

The Tenth Interscience Conference on Antimicrobial Agents and Chemotherapy and the publication of this volume of *Antimicrobial Agents and Chemotherapy* represent an occasion to review the accomplishments of the series and to assess its future.

The original concept of ICAAC, to bring together scientists from various disciplines and to foster exchange of information and ideas, has been fully realized in the cooperative participation and support by microbiologists, biochemists, clinicians, and industrialists. The juxtaposition of meetings of the Infectious Diseases Society of America and ICAAC has encouraged and permitted extensive participation of clinicians in this group effort, an indispensable element in the evaluation of methods to alleviate and control microbial diseases.

Antimicrobial Agents and Chemotherapy makes available to all interested individuals the important results of the Interscience Conference.

The future of the Interscience Conference depends to a great extent on the interest and effort devoted to it by the various groups represented. Chemical substances, whether of biological or industrial origin, are the major weapon in the arsenal of tools available in the fight against disease. It seems certain that they will have increasing importance as genetics, microbiology, biochemistry, immunology, and medicine provide new insight into opportunities to influence the course of disease by judicious chemical modification of the internal environment. As long as the Interscience Conference sparks such insight, it has an assured place in the schedules for medicine, science, and industry.

The results presented at the 1970 Chicago meeting, under the leadership of Dr. George Whitfield and his committee, and with the cooperation of the many participants, are the very evident justification for the efforts culminating in the Tenth Interscience Conference. The commitments to the present publication by Editor Gladys Hobby, the Editorial Board, and the many authors make this volume a notable addition to its predecessors in the series.

Robert E. Hungate, *President*
American Society for Microbiology

Publisher's Statement

Antimicrobial Agents and Chemotherapy is a serial publication, published annually by the American Society for Microbiology. Volumes in the series contain reports presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy, held in the fall of each year; the volumes are published during the spring of the following year. The present publication is the tenth in the series.

All volumes in the series are available from the American Society for Microbiology, 4715 Cordell Ave., Bethesda, Md. 20014. The price is $15 per copy (with 50% discount to members of ASM).

Reprints of the papers in each volume were made available to authors, and readers should request reprints by writing directly to authors.

Papers in *Antimicrobial Agents and Chemotherapy* are indexed in *Index Medicus*, and are abstracted in *Biological Abstracts* and in *Chemical Abstracts*.

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Antimicrobial Agents and Chemotherapy—1970

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Infectious Diseases and Social Change

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It is a happy privilege, extended annually to the President of the Infectious Diseases Society of America, to address this meeting at its opening session. My distinguished predecessors have each dealt with pertinent concerns, predictions, perceptions, and historical developments in the field of infectious diseases. The standards of the past throw a heavy burden on the present speaker, and not the least is the certain knowledge that the only time the whale gets harpooned is when he comes up to spout.

However, I am led to spout, despite the hazards, by the same concerns that beset us all. We are experiencing with much pain an almost revolutionary restructuring of the programs of support for medical care, medical investigation, and medical education. We find ourselves puzzled, frustrated, and often so angry that we are occasionally led to the brink over which some of our young people have fallen, in which we are tempted to use obscenity because we fear that rationality has failed. We resent the abrupt manipulations of our good intentions when all that we have asked for is to be able to continue our good works in an atmosphere that will put to effective social use the fruits of our earnest efforts.

Now there is basically nothing wrong with this charming scenario of the white-coated medical scientist distributing good works like free beer at a political picnic, although it does seem to have been written by the least sophisticated of writers for the Sunday supplements; nor is it necessary that we abjure personal benefit in order to perform socially constructive work, for it is not at all clear how much would happen if there were not a combination of material reward and social motivation in our daily activities.

Where the scenario becomes distorted is in some of our assumptions and in some of our failures. We saw a grants system developing that virtually excluded education and direct application, and although we recognized the unbalanced system that was developing, we were not particularly effective (Did we really try harder?) in reordering these directions. In part, at least, we were afraid of rocking the boat. We were further sustained by the thought that in the long run research would provide easier and more effective ways to deliver medical care and teaching.

Once again, these were not objectionable points of view at all. The views simply failed to anticipate the political tides and the changing political pressures. Most particularly, we failed to realize that not much was happening to the statistics of mortality, survival, chronic illness, or causes of death even while the costs of illness and medical care were rising steadily; and the public now is demanding what we said we were providing, and is deeply concerned over what it is getting for its money.

The cost of the fighting in Vietnam and the inflationary pressures that accompanied it were the precipitants that forced a national policy of re-examination of objectives and allocations in the medical area. It is my conviction, however, that without this precipitant the day of reckoning would have come soon anyway. And while I have no sympathy with the unplanned, unstructured, and almost chaotic way in which this reckoning is being conducted, I cannot find it in my conscience to blame all of our troubles on our unhappy involvement in the problems of Southeast Asia. Nor can I state with any conviction the belief that without the Indochina war we would have reordered our priorities or have undergone searching re-examination of our allocations. We were like Mike falling from the top of a skyscraper and receiving solace from all the Pats distributed at each floor, each shouting out that everything was all right so far.

Why were we falling? First we had accepted

[1] President, Infectious Diseases Society of America. Address given at joint meeting of Infectious Diseases Society of America and Tenth Interscience Conference on Antimicrobial Agents and Chemotherapy sponsored by American Society for Microbiology.

some half truths and had stopped searching for the whole truths. The principal half truths were that medical research had stamped out the great killers of the past—tuberculosis, diphtheria, pneumonia, puerperal sepsis, etc.—and that medical research and our superior system of medical care were major factors in extending life expectancy, thus providing the American people with the highest level of health available in the world. That these are half truths is known but is perhaps not as well known as it should be.

Figure 1, for example, gives the data on deaths from tuberculosis in England and Wales. Similar data have been obtained in every industrialized country and throughout the United States, but these data are cited because they are reliable and begin in 1850. The data on deaths from tuberculosis show that the mortality rate from this disease has been declining steadily since the middle of the 19th century and has continued to decline in almost linear fashion during the past 100 years. There were increases in rates of tuberculosis during wars and under specified local adverse conditions. The poor and the crowded always came off worst of all in war and in peace, but the overall decline in deaths from tuberculosis was not altered measurably by the discovery of the tubercle bacillus, the advent of the tuberculin test, the appearance of BCG vaccination, the widespread use of mass screening, the intensive antituberculosis campaigns, or the discovery of streptomycin. Only the advent of isoniazid changed the mortality patterns, and by then the rate of tuberculosis had fallen to but a small fraction of its levels 100 years ealier.

It is important that this point be understood in its completeness. The point was made years ago by Wade Hamptom Frost, and more recently by René Dubos, and has been repeatedly stressed through the years by many observers of the public health. Our research efforts in dealing with tuberculosis have been of great value in the management of individual patients and in present-day public health practice, but they do not account for the linear decline in deaths during the past 100 years.

Similar trends in mortality have been reported with respect to diphtheria (Fig. 2), scarlet fever (Fig. 3), rheumatic fever, pertussis (Fig.

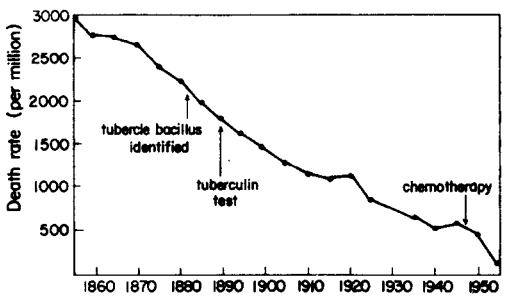
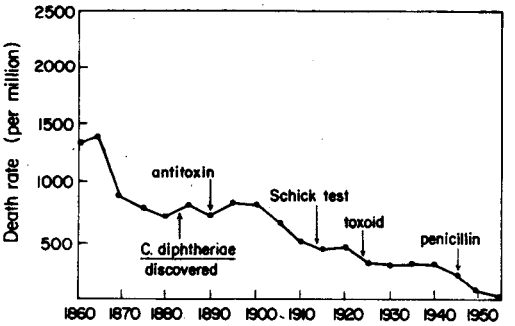


Fig. 1. *Respiratory tuberculosis. Mean annual death rate, England and Wales.*



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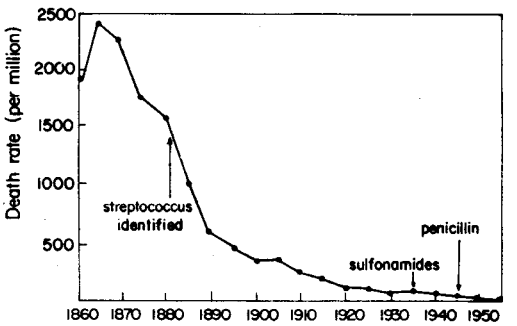

Fig. 3. *Scarlet fever. Mean annual death rate in children under 15, England and Wales.*

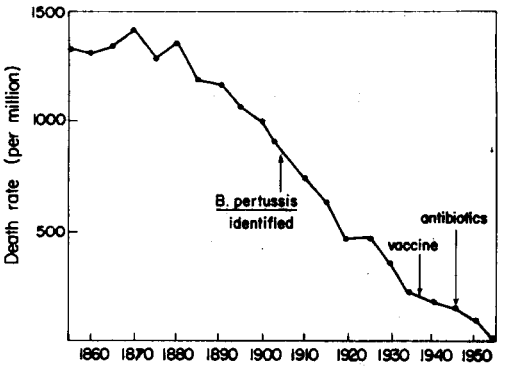

Fig. 4. *Whooping cough. Mean annual death rate in children under 15, England and Wales.*

5), measles (Fig. 5 and 6), and many other diseases. There are less reliably documented but suggestive similar trends with respect to carcinoma of the cervix, toxemia of pregnancy, stroke, and certain other disorders. This decline in rates of certain disorders, correlated roughly with improving socioeconomic circumstances, is merely the most important happening in the history of the health of man; yet we have only the vaguest and most general notions about how it happened and by what mechanisms socioeconomic improvement and decreased rates of certain diseases run in parallel. We know that for many infectious diseases, such as poliomyelitis and perhaps infectious hepatitis, the trend is opposite, and for some there is little or no socioeconomic effect. This does not detract from the overriding relationship that has been seen in most common communicable dieseases in which there is a strong relationship between socioeconomic status and rates of mortality and morbidity.

Currently fashionable is the view that nutritional improvements account for the decline in mortality from common infections and that nutritional inadequacy is a major factor in explaining the present predilection of the poor for certain communicable disorders. In fact, there is little useful evidence to support this view. Experimentally, the nutritional deficiencies that are needed to affect substantially resistance to infection are generally extreme and, in the case of certain viral disorders, such deficiencies may often *increase* resistance.

Clinically, there is not much evidence of manifest malnutrition in economically underprivileged populations in this or in other industrialized countries, using the available indices of malnutrition, even though it is evident that certain infectious disease problems such as tuberculosis and rheumatic fever are pretty much limited to the poor.

What other explanations are there for the effects of being poor? One explanation was developed in England more than 40 years ago (Fig. 6). It was shown that rates of rheumatic heart disease were almost linearly related to crowding in the home. This is understandable since spread by droplet infection is greatest in a narrow radius around an infected source and the home is, particularly for children, the place in which most prolonged contact will occur. Of course, this effect will be demonstrable in relation to any other locus for crowding.

Similar data were gathered during World War I by Glover, who showed that when beds in barracks were placed too close together rates of miningococcal infectior among troops rose abruptly. In Peru, the Communicable Diseases Center gathered data relating attack rates of meningococcal disease to crowding in the home. Recently, Lilienfeld and his associates in Balti-

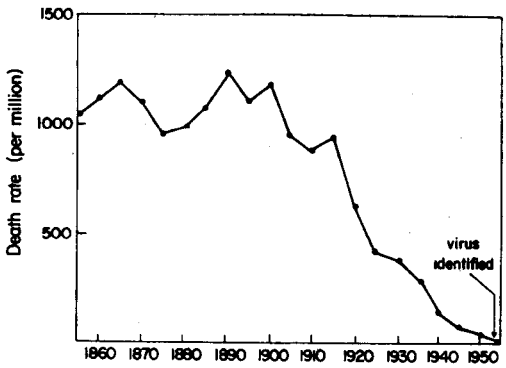

Fig. 5. *Measles. Mean annual death rate in children under 15, England and Wales.*

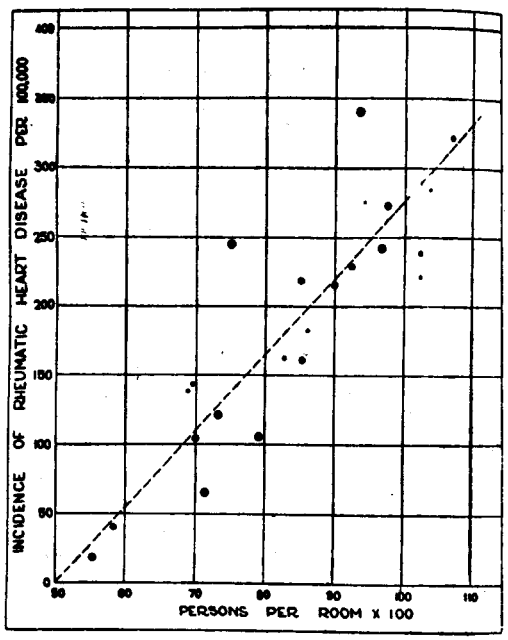

Fig. 6. *Correlation between the incidence of rheumatic heart disease per 100,000 and the number of persons per room (× 100) as found by Perry and Roberts in various districts of the city of Birstol, England, in 1927–1930. (The size of the dots roughly indicates the comparative population size of the districts.)*

more gained invaluable insight into this problem. They were distressed by the persistence of rheumatic fever in the black population of Baltimore and were puzzled because many of the black children with rheumatic fever came from homes that were middle class rather than ghetto in origin. Epidemiological analysis showed that the attack rates for rheumatic fever in these families were no longer related to low income or to lower educational levels, but were directly related to the number of people per bedroom. Evidently, in this group of families, when funds became more plentiful and the families began to move out of the ghetto they tended to choose new dwellings which stretched their capacity to pay. Given a choice, they tended to select more space in living and dining rooms, putting added space for bedrooms at a lower priority.

I have referred to the increased longevity of our population as one of the indices of improved medical care. However, this is due almost completely to decreased infant mortality. The age of death of those who have lived to adulthood has been extended very little over the past 50 years, and in some populations it has actually declined. Infant mortality, due largely to gastrointestinal and respiratory infections, was at a rate of several hundred per 1,000 births during the 19th century, at a time when in royalty the rate was 12 per 1,000 births. That is, before antibiotics and before contemporary methods of control, the infant mortality rate in royal families was lower than that which is found in the best national rates now being recorded in any country of the world. Clearly, rich is better.

The lessons from these and from many similar observations are numerous. Among these are the responsibilities that we as experts in the field must face. We must face the need to assist or to bring about maximal control of disease even while we devote ourselves to new possibilities. Thus, while we develop vaccines or new antituberculous drugs, we must be looking into more space per family unit or other ways of dealing with the spread of respiratory pathogens. While we try to determine why 50% of rheumatics drop out of penicillin prophylaxis programs, we may need to use concepts of human engineering to calculate the cost and benefits of having better air-flow systems in industry, or better housing, and we shall then need to compare these costs with the cost of multiple specific approaches to control of many different diseases that may have common methods of spread.

Those of us who are interested in infectious disease are in the fortunate position of working with systems that are immediately relevant. Furthermore, even more than in most fields, we have seen the advantages of the continuous interdependence of applied and undirected investigation.

At an earlier stage in scientific history, scientists, and particularly those of the physical sciences, were enjoined to stay dissociated from the social consequences of their work. The general acceptance of this attitude of noninvolvement by the scientist led to much powerful discovery, much of it used for the highest social purposes and much used to bring about more efficient ways of conquest, colonization, and the accumulation of wealth and power.

The present generation has questioned the wisdom of continuing a policy of advocacy of noninvolvement and of dissociation from the social consequences and social objectives of scientific work. It no longer follows that all discovery is progress and that all technical achievements improve the lot of humanity. As this formidable questioning of the most fundamental drives of science goes on, we may wish to examine our posture in relation to our field of interest.

Is it conceivable that, in conferring health and in taking care of our infirm and elderly, we can supply a source of drive for progress that can rival successfully the immense and productive stimuli that have come from wars and from the exploration of geographical frontiers? Can we find drives in social welfare that will direct and harness our productive and creative energies? If not, we are surely doomed. If we fail to develop viable alternatives to violence and adventurism as a source of stimulus to maximal creative activity, perhaps we deserve to be doomed. Lorenz has told us that man is probably the missing link between the anthropoid ape and the civilized human being. Can we continue to evolve?

My belief is simple and hopeful. Our field shares with only a few the stature of being socially acceptable, patently useful, intellectually stimulating, and economically productive. It is our responsibility to examine our functions and to allocate a sufficient share of our resources and abilities to permit the bringing to

society of the immediate benefits of what we have learned. We accept gladly the obligation to produce still further benefits within the limits of our capacities. We do these things in a framework that recognizes that the scientific method still offers the most valuable approach to the solution of problems, and that undirected investigation is a precious resource that must be preserved, but must be paid for by the prompt application of useful knowledge for the benefit of those who provide the basis for continuance.

It is depressing to contemplate, to cite but a small example, that for over 10 years it has been known how to prevent infection and death associated with indwelling catheterization of the urinary bladder, and yet we are still trying to convince physicians, nurses, hospital administrators, and government officials that the simple and inexpensive methods involved should be applied uniformly.

It is exhilarating, on the other hand, to begin to see a possible infectious basis for some of the excess prematurity among the poor with the realization that T-strain mycoplasmas may account for excess prematurity in certain population groups, and that these may be susceptible to simple and inexpensive treatment.

Can it be that most diseases that preferentially attack the poor are infectious in origin? Can we be sure that common chronic diseases are not due to infectious agents? Can we convince our increasingly skeptical public of the desirability of our continuing to ask and to explore such questions? I believe we can, but believe we must convince the public not by slick advertising tricks of which they are inherently suspicious, however gullible they may be, but by acting promptly and critically, by showing that we will set social objectives and that we will not allow gaps to appear between discovery and application—that we will deal with the full social problem and not with the more convenient but often less useful small portions that happen to command our individual attentions. Here we must distinguish between incompetent or self-seeking passion in those who use the right words but produce very little, and the thoughts of those who come to the problem with discipline and tough-minded capacity for analysis and action. As we recognize these distinctions and strike an effective balance between investigation and social action, we can look forward to continued satisfaction, and the realization that we have played a vital role in setting for our society new social goals.

Antimicrobial Agents and Chemotherapy—1970

Chemistry of Enduracidins, New Antibiotics

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Enduracidin was produced by *Streptomyces fungicidicus* B-5477. It has been separated into two main components, enduracidin A and enduracidin B, by chromatography on Amberlite XAD-2. They are chemically characterized by the following constituents: 10-methylundeca-2(*cis*)-4(*trans*)-dienoic acid (C_{12}-dienoic acid, from enduracidin A), 10-methyldodeca-2(*cis*)-4(*trans*)-dienoic acid (C_{13}-dienoic acid, from enduracidin B), α-amino-4-hydroxyphenylacetic acid, α-amino-3,5-dichloro-4-hydroxyphenylacetic acid, alloenduracididine, enduracididine, and eight kinds of other known amino acids (from both enduracidins A and B). Hydrolyses of enduracidins A and B with barium hydroxide solution gave C_{12}-dienoylaspartic acid and C_{13}-dienoylaspartic acid, respectively. Saponification of enduracidins A and B cleaved the lactone to give linear long peptides containing different dienoic acids, enduracidic acids A and B, respectively. The amino acid sequences of enduracidic acids A and B are identical as determined by the usual methods. The lactone structure of enduracidin A and B was established by reduction with $LiBH_4$, and by reaction with phenylisocyanate. Thus, the chemical structures of both antibiotics are tentatively proposed. Many derivatives of enduracidin have also been prepared for investigations of the contribution of the functional groups to the biological activities.

Enduracidin, a basic peptide antibiotic, was obtained from the mycelium of *Streptomyces fungicidicus* B-5477 (5). The isolation, characterization, and biological properties of enduracidin were reported in the previous paper (2). This antibiotic showed strong bactericidal activity both in vitro and in vivo against various gram-positive bacteria which are resistant to other antibiotics (11). The bactericidal activity was also long-acting in vivo. Therefore, this new antibiotic was named "Enduracidin," and recently "enramycin" was proposed as a suitable generic name ("Enradin injection" is given as the trade name of the preparation of enduracidin, and will be supplied by Takeda Chemical Industries, Ltd.). The present paper deals with the separation of two components, enduracidin A and enduracidin B, determination of their chemical structures, and preparation of some derivatives of them.

EXPERIMENTAL

Separation of enduracidins A and B. Good separation of enduracidins A and B was achieved by Amberlite XAD-2 column chromatography with the gradient elution of 0.05 N NaCl to 0.006 N HCl in 50% aqueous methanol. Enduracidin A was eluted first and enduracidin B later. Each of them was isolated in the form of a colorless crystalline monohydrochloride. The physicochemical and biological properties of the two compounds are similar (Table 1) and closely resemble those of the original enduracidin (2).

Structures of new fatty acids in the N-terminal moieties. Enduracidin A was refluxed in aqueous barium hydroxide for 7 hr to give a mixture of several acids, all of which have an ultraviolet absorption at 261 nm. The mixture

Table 1. *Properties of enduracidins A and B*[a]

Compound	Melting point (C)	Molecular formula
Enduracidin A hydrochloride	240–245	$C_{107}H_{138}N_{26}O_{31}Cl_2 \cdot HCl$
Enduracidin B hydrochloride	238–241	$C_{108}H_{140}N_{26}O_{31}Cl_2 \cdot HCl$

[a] The following properties were the same for the two compounds: molecular weight (X ray), ca. 2,500; $[\alpha]_D^{23}$ (0.5% dimethylformamide), +92°; ultraviolet absorption (λ_{max} 0.1 N HCl), 231 and 272 nm.

of these acids was converted into methyl esters, and a main product was purified by thin-layer chromatography to give compound I. The elemental analysis of compound I corresponded the molecular formula $C_{18}H_{29}NO_5$. Acid hydrolysis of compound I gave L-aspartic acid. The mass spectrum [*m/e*, 339 (M^+)] and the nuclear magnetic resonance (NMR) spectrum [δ, 0.84 (doublet, $\underline{CH_3}>C-\underline{CH_3}$), 5.54 (doublet, HC = CH – CO), and 7.44 (double doublets, HC = $C\underline{H}$ – CH = C) ppm], with extensive decoupling experiments, led to the assumption of the structure of compound I to be dimethyl *N*-[10-methylundeca-2(*cis*)-4(*trans*)-dienoyl]-aspartate (Fig. 1). Furthermore, 10-methylundeca-2(*cis*)-4(*trans*)-dienoic acid and 10-methylundecanoic acid were isolated by acid hydrolysis from enduracidin A and tetrahydroenduracidin A, respectively. 10-Methylundecanoic acid was identified by comparison with that described in the literature (9).

The same treatment of enduracidin B gave compound II, $C_{19}H_{31}NO_5$. Hydrolysis, mass spectrum, and NMR spectrum revealed the structure of compound II as dimethyl *N*-[10-methyldodeca-2(*cis*)-4(*trans*)-dienoyl]-aspartate (Fig. 2). Acid hydrolysis of enduracidin B and tetrahydroenduracidin B yielded 10-methyldodeca-2(*cis*)-4(*trans*)-dienoic acid and (+)-10-methyldodecanoic acid, respectively. The latter was identified by comparison with that described in the literature (9). Consequently, enduracidins A and B were found to contain *N*-[10-methylundeca-2(*cis*)-4(*trans*)-dienoyl]-aspartic acid and *N*-[(+)-10-methyldodeca-2(*cis*)-4(*trans*)-dienoyl]-aspartic acid, respectively, as their N-terminal moieties.

Amino acid composition. Enduracidins A and B were hydrolyzed with 6 N HCl at 105 C for 24 hr, and then were subjected to amino acid analysis. They have the same amino acid composition, namely, 17 amino acid residues (Table 2). Three (K_2, Y_1, and Y_2) among 12 kinds of amino acids are unique, and one (K_1, α-amino-4-hydroxyphenylacetic acid) is rare, having been already reported to be a constituent of actinoidin (3). (Y_1 and Y_2 were named enduracidine and alloenduracididine, respectively.) The chemical structures of K_2, Y_1, and Y_2 have already been reported as α-amino-3,5-dichloro-4-hydroxyphenylacetic acid for K_2 (2), as α(S)-amino-β-4(R)-(2-iminoimidazolidinyl)-propionic acid for Y_1 (6), and as α(R)-amino-β-4(R)-(2-iminoimidazolidinyl)-propionic acid for Y_2 (6; Fig. 3). The configuration of the amino acids was established by specific rotation or by treatment with D- and L-amino acid oxidase.

Enduracidic acid. The infrared spectrum of enduracidin A possesses a characteristic absorption at 1,750 cm^{-1}, corresponding to an ester or lactone function. On treatment with 1 N sodium hydroxide at room temperature, the infrared absorption at 1,750 cm^{-1} disappeared, but no change was observed in the ultraviolet spectrum or the amino acid composition. This compound

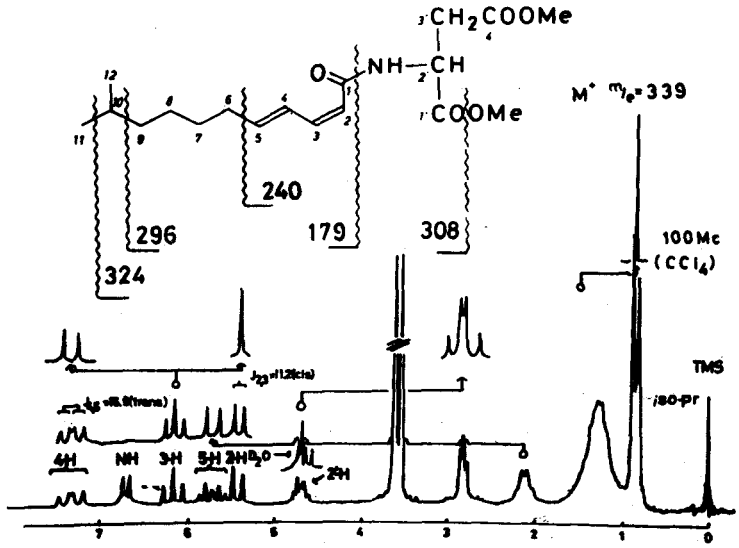

Fig. 1. *Structure of compound I (new fatty acid moiety of enduracidin A).*

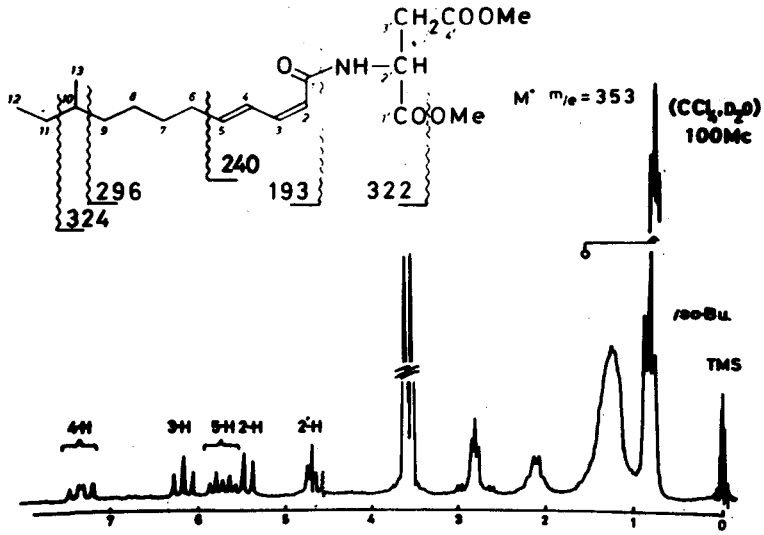

Fig. 2. *Structure of compound II (new fatty acid moiety of enduracin B).*

Table 2. *Amino acid composition of enduracidins A and B*

Amino acid	Moles/molecule
L-Asparagine	1
L-Threonine	1
L-alloThr	1
D-alloThr	1
D-Serine	1
L-Cit	1
Glycine	1
D-Alanine	1
K_1	5
L-K_2	1
D-Ornithine	1
L-Y_1	1
D-Y_2	1
Total	17

was designated as enduracidic acid A. No second fragment containing a hydroxyl group could be detected. This is consistent with a lactone structure for enduracidin A.

Amino acid sequence of enduracidic acid A. Enduracidic acid A was hydrolyzed with hydrochloric acid under various conditions, and many peptide fragments which served to estimate the amino acid sequence of enduracidic acid A were obtained. In the sequence assignment of these fragments, Edman degradation (7), dansylation (4), and dinitrophenylation (10) were used for the determination of the N-terminal sequence; tritium-labeling (8), reduction with lithium borohydride, and hydrazinolysis (1) were applied for the estimation of C-terminal amino acids.

Peptides obtained by partial hydrolysis with concentrated HCl at 37 C for 72 hr were separated by Dowex 50W X 2 column chromatography with pyridine acetate buffers. Among many peptides, seven (Table 3) were useful for the sequence determination of four sequences: Asp → Thr → K_1 → Orn; D-aThr → K_1 → K_1; L-aThr → Cit → Y_2 → K_1; and Ser → K_2 → Gly → Y_1 → Ala → K_1. All of the amino acids residues are contained in these sequences.

The N-terminal sequence of enduracidic acid A was determined by selective elimination of aspartic acid. As enduracidic acid A contains 1 mole of aspartic acid at the N-terminal amino acid as described above, selective cleavage of the peptide for the elimination of aspartic acid (12) appeared to be an appropriate method for obtaining a large peptide. Enduracidic acid A was boiled in 0.05 N HCl for 30 hr, and the resulting cleavage products were purified by Sephadex G-25 column chromatography. Thus, a hexadecapeptide containing all amino acid residues except aspartic acid was obtained. Edman degradation of the hexadecapeptide elucidated the sequence of five amino acids from the N-terminal end as Thr → K_1 → Orn → aThr → K_1.

Enduracidic acid A was hydrolyzed with 0.05 N HCl at 120 C in a sealed tube for 24 hr. The