

PROFILE AND SCIENTIFIC CONTRIBUTIONS OF PROFESSOR KYOSUKE TSUDA

Shigenobu Okuda

Institute of Applied Microbiology, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

Nobuo Ikekawa

Department of Chemistry, Tokyo Institute of Technology, 2-12-1, Ookayama, Meguro-ku, Tokyo 152, Japan

Professor Kyosuke Tsuda was born on February 10, 1907, in Kiirun, Taiwan, as the fourth and youngest child of Mr. Sosuke Tsuda, who was an official of the Government-General of Taiwan and engaged at that time in the harbor construction of Kiirun. After four years at a five-year middle school in Taihoku, he entered the Department of Science, Urawa High School in 1923. It was in April, 1926, that he became a student at the Pharmaceutical Institute, Medical Faculty, Tokyo Imperial University. There he conducted his graduation experiments (Synthesis of Quinaldic Acid from Quinaldine and Reductive Debenzylation of Vanillin Benzyl Ether) under the guidance of Professor Heizaburo Kondo, marking the first step in his research career which lasted for well over 40 years.

Professor Kondo was an authority on the studies on alkaloids. Before and even after he became the second Head of the Laboratory of Pharmaceutical Chemistry in 1917 to succeed Professor Nagayoshi Nagai, founder of modern pharmacy in Japan, he concurrently held a military post in pharmacy and remained in active service until he rose to the highest rank in the field, Army Medicine Major-General in 1926.

Professor Kondo was most considerate in training his students at experiments and eager in helping them to develop their own individualities. Blessed with such an excellent mentor, Professor Tsuda studied hard and at the same time gave himself to the sports which he began to love during his high school days — swimming and skiing. The laboratory at all times presented an "impressive" scene as his swimming wear was kept spread and dried over an experimental stand and his skiing kits he used for wax testing were left leaned against the wall. But his boss was definitely tolerant and spoke no words of complaint against his

conduct. With freedom of action thus granted he truly enjoyed his youthful life in the laboratory. In March, 1929, he graduated from the Pharmaceutical Institute, Medical Faculty, Tokyo Imperial University. Then he was assigned to the university's Laboratory of Pharmaceutical Chemistry, first as research fellow and subsequently as assistant, and assistant professor. He stayed there just until he was transferred in April, 1951, to University of Kyushu as Professor of Pharmaceutical Chemistry at the Pharmaceutical Institute, Medical Faculty.

As research fellow he took over "Studies on the Structure of 'Kujin (SOPHORAE RADIX)' Alkaloid Matrine," with which both Professor Nagai and Professor Kondo had labored for years, as the task subject for the Laboratory of Pharmaceutical Chemistry. In launching his research, he was fortunate enough to receive a "man-to-man" training from Professor Eiji Ochiai, who was assistant at that time and later became the third Head of the Laboratory of Pharmaceutical Chemistry, on extraction of matrine and up to preparations of derivatives. What was then described as the really intensive training continued for more than half a year until the trainer left to study under Professor Hermann Staudinger at University of Freiburg. This personal training meant the birth of the famous Ochiai-Tsuda partnership which, during a period of nearly half a century, produced outstanding achievements, thereby contributing tremendously to the progress in pharmaceutical sciences in Japan. In the first such feat, the two worked together to popularize in Japan techniques of elemental microanalysis, which Professor Ochiai introduced from Europe — an immeasurable contribution to the advancement of organic and pharmaceutical chemistry in Japan.

In the meantime, the pending study on the structure of matrine continued to exact effort and patience from him until 1936, when he succeeded in the elucidation of its plane structure, a key achievement which led to his doctorate thesis. After a 15-year interruption, he extended this work to consummation, for which he was awarded the 1957 Prize of Pharmaceutical Society of Japan and the 1966 Japan Academy Prize. The work will be outlined later.

In 1937, Professor Tsuda began to undertake a study of soyasapogenins. He separated the four species, soyasapogenol A, B, C, and D by means of alumina column chromatography, which was unique for those days. Then through the

dehydrogenation of soyasapogenol B by selenium, he confirmed that this substance is pentacyclic triterpene. This result was later supported by Professor Leopold Ruzicka's group in ETH with additional experiments to prove the structure. The scholarly relationship thus developed led to Professor Tsuda's postwar visit to ETH for study in 1954.

Professor Kondo left the Laboratory of Pharmaceutical Chemistry, Tokyo Imperial University, in March, 1938 for retirement, succeeded by Professor Ochiai. At the same time Professor Tsuda was promoted to assistant professor. Then he began work on experimental chemotherapeutica at the laboratory of Professor Shuji Hasegawa in the Institute for Infectious Diseases, Tokyo Imperial University. This meant exceptionally heavy pressure of work for him — conducting experiments on organic syntheses and on animals at both places, the Pharmaceutical Institute and the Institute for Infectious Diseases. Professor Tsuda reminiscently says that it was the period when he worked the hardest in his life. The work on the chemotherapeutica remained the principal theme for him until the end of World War II. Despite many adverse factors hindering his research, he published a number of reports on chemotherapeutica, mainly sulfonamide group.

As shock and chaos gripped Japan as a result of its defeat in the Pacific War, Professor Tsuda remained undaunted as a scientist. At that time he was conducting a basic study on aliphatic amine for the purpose of developing medicinal resources. In 1949, he embarked on a key pending task of the Laboratory of Pharmaceutical Chemistry, "Studies on the Toxin in Pufferfish," which is the most familiar natural toxin for the Japanese people, with the full backing of Sankyo Co., Ltd.

In 1951, he was appointed Professor of University of Kyushu, where he did much to establish the Pharmaceutical Institute at the Medical Faculty. This Institute was staffed largely by those who were transferred from University of Tokyo. They included Assistant Professor I. Iwai, Assistants B. Umezawa, E. Ohki and S. Saeki, and post-graduate students, S. Tamura and N. Ikekawa, who joined Professor Tsuda's team. Thus the Laboratory of Pharmaceutical Chemistry became operative with a total staff of about 20 including 10 undergraduate

students from University of Kyushu. They began studies in such fields as synthesis of matrine and chemistry of steroids, coal tar bases and alantolactone. The team immediately became one of the most active laboratories in University of Kyushu, which was now refreshed with a new academic feature in the chemistry of natural products. In epoch-making experiments, for instance, tetrodotoxin was purified as crystal in 1952, and in 1954 nordehydro- α -matrinidine was synthesized, which opened the way for subsequent total synthesis of matrine.

In March, 1954, he visited Switzerland for study at the Laboratory of Professor Leopold Ruzicka of ETH. There he worked on steroidal alkaloid with Professor Oskar Jeger until October of the same year, when he returned with a major impetus - the introduction of new microtechniques in organic chemistry.

In August, 1955, Professor Tsuda was appointed Professor of the Institute of Applied Microbiology, University of Tokyo, at the strong desire of the head of the Institute, Professor Kin-ichiro Sakaguchi, a great authority on fermentation science. He, helped effectively by Drs S. Okuda, E. Ohki, N. Ikekawa, and others, established the Division of Chemistry within the Institute. The laboratory, patterned in design after that of organic chemistry in ETH, was the most sophisticated of the kind in Japan at that time.

The Institute of Applied Microbiology, full of many prominent figures, was a mecca of studies on microbiology and chemistry of natural products. Professor Tsuda's laboratory with a store of young talented chemists especially attracted streams of visitors, both Japanese and non-Japanese, making itself a most lively research center.

Professor Tsuda continued on the studies which he began during the days in University of Kyushu, and at the same time, launched works on natural sterols and microbial transformation of steroids and alkaloids. Furthermore in 1963, he started on the studies of structures of mould metabolites produced by plant pathogenic fungi. The number of the research reports he published during his 12 years in the Institute totaled about 120.

His achievements while in office at the Institute won prompt academic

recognition: the Prize of Pharmaceutical Society of Japan in 1957 on "Total Synthesis of Octadehydromatrine," the Asahi Prize, Cultural Award, in 1965 on "Isolation and Structural Determination of Tetrodotoxin," and the Japan Academy Prize in 1966 on "Chemical Studies on 'Kujin' Alkaloids and Related Leguminosae Alkaloids."

In April, 1965, Professor Tsuda assumed the post of Director of the Institute of Applied Microbiology. In this position, he participated in the overall school administration, making untiring efforts to further improve the prestige of the university as a whole and the Institute in particular. In 1967, he retired from University of Tokyo with distinguished academic and administrative accomplishments and with appointment as Emeritus Professor. He was immediately invited to be installed as President of Kyoritsu College of Pharmacy in Tokyo, the position he has since held.

It is to be mentioned that Professor Tsuda has rendered meritorious services to the cause of natural product chemistry in Japan. He was one of the founding members of the Symposium on the Chemistry of Natural Products (Japan). He also served as General Secretary of the 3rd IUPAC International Symposium on the Chemistry of Natural Products held in Kyoto in 1964. Many participants including those from other countries still say they cannot forget the Kyoto conference which was organized and steered so excellently with lively and useful discussions.

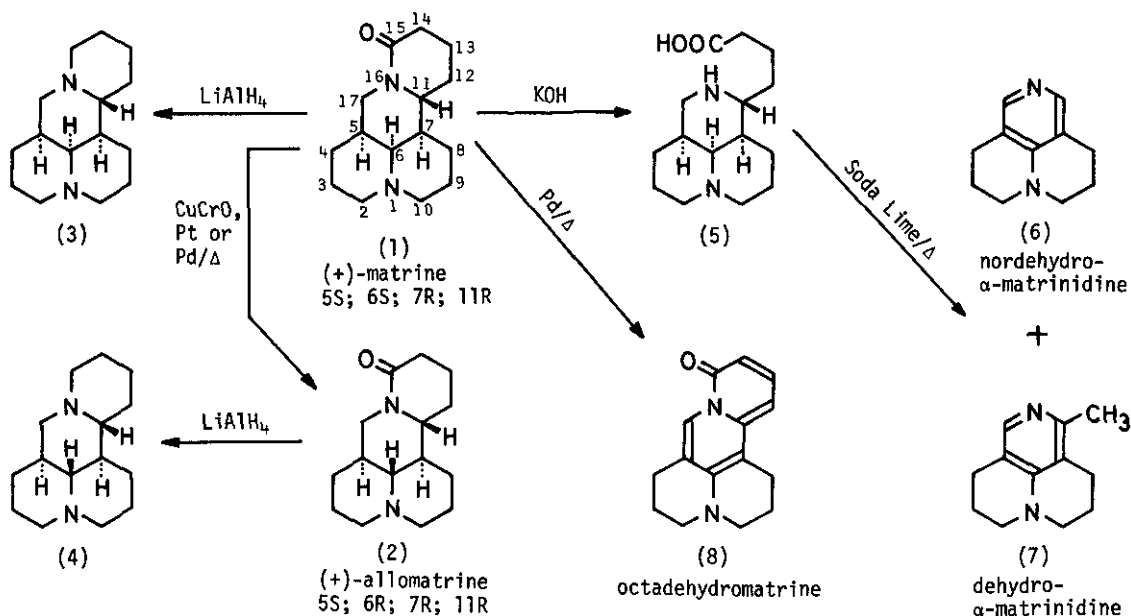
Professor Tsuda published over 200 reports together with a number of reviews and books during the 38-year period between 1929 when he graduated from Tokyo Imperial University and 1967 when he retired from University of Tokyo. All such works cover wide ranging areas. The major studies among them are on four themes, which are outlined below.

I. Chemical Studies on Lupin Alkaloids, Especially the Alkaloid of Sophora flavescens.

Professor Nagai and Dr. Yoshizumi Tahara first took particular notice of

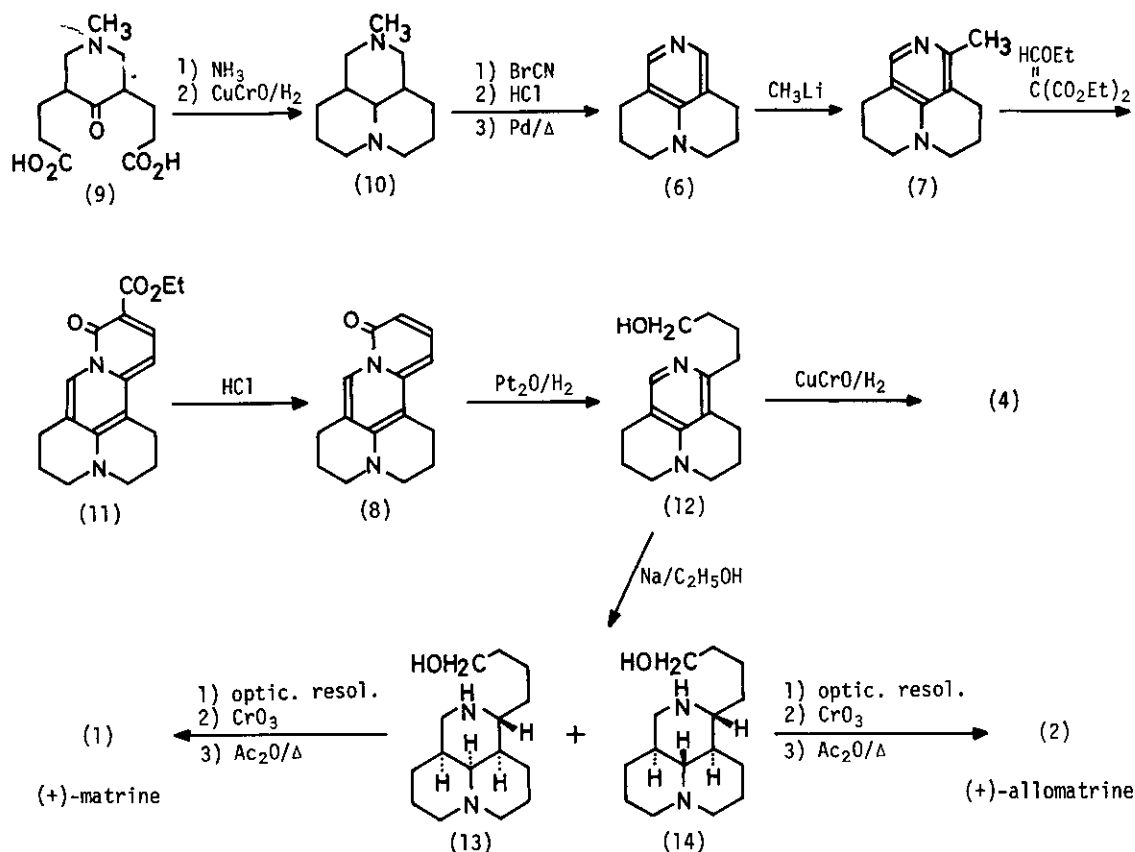
'Kujin,' a bitter agent in Chinese medicine and also a main component of a traditional proprietary drug called 'Shinkyo-gan' and sold popularly in Shiga Prefecture and its neighboring areas. They extracted and isolated the main alkaloid from 'Kujin' and named it "matrine" after the original plant's common name 'Matorigusa' (academically called 'Kurara' in Japanese and classified as Sophora flavescens). That was in the summer of 1885. From that time, studies on the chemical structure of this alkaloid became a pending project of the Laboratory of Pharmaceutical Chemistry. Professor Tsuda joined in the project in 1930. These investigations into 'Kujin' base, though interrupted twice, continued for as long as 80 years, the oldest in the history of pharmaceutical sciences in Japan. During this period increasingly new approaches were adopted in the investigations with the change of the times. Therefore, a review of these investigations gives us a glance at the history of Japan's natural product chemistry.

In 1936, the correct plane structure of (+)-matrine (1) was presented on the basis of the structural determination of various degradation products, such as nordehydro- α -matrinidine (6), dehydro- α -matrinidine (7), octadehydromatrine (8) and others.



Then, in 1958, the stereochemical structures of (+)-matrine (1) and (+)-allo-matrine (2), a stable stereoisomer of 1, were determined respectively. This followed the examination of the difference in chemical reactivity and studies on dipole moments measured for 1, 2, and their lithium aluminum hydride reduction products, (+)-matridine (3), and (+)-allomatridine (4). In 1966, the absolute configuration of (+)-matrine (1) was proved to be (5S;6S;7R;11R) by the chemical and physicochemical experiments.

Professor Tsuda established the general synthetic method concerning pyrrolidine, indolizidine, quinolizidine, and applied this method to the synthesis of 2-methylperhydropyrido[3,4,5-*i,j*]quinolizine (10) as starting material. Then he synthesized the degradation products of matrine, 6, 7, 8, the key compounds in the determination of plane structure, followed by the successful total synthesis of (±)-allomatridine (4).



In the next stage, total syntheses of natural (+)-matrine (1) and (+)-allo-matrine (2) were accomplished by successive reduction of (1) to (+)-matrinol (13) and (+)-allomatrinol (14), followed by their optical resolution via salts of dibenzoyltartaric acid, oxidation and finally cyclization. This synthetic route is quite significant in the structure of compound which serves as a key to structural determination was confirmed step by step through synthesis and ultimately total synthesis of the object was perfected, thereby giving a firm proof that the structure he presented is correct.

It was already known in the 1930s that a large amount of (+)-oxymatrine, N-oxide of (+)-matrine, are contained in fresh 'Kurara' root. However, no investigation on the existence and the structure of the minor bases had been carried out until the end of the 1950s. Professor Tsuda investigated component alkaloids of the root of 'Kurara' (collected in June, 1960, just before the flowering period, at Makuhari, Chiba Prefecture) using column chromatography, and paper partition chromatography. He then isolated (-)-sophoranol, (-)-sophocarpine, (-)-anagyrine, (-)-baptifoline, (-)-N-methylcytisine, in addition to the two alkaloids mentioned above. He also succeeded in converting (+)-matrine (1) into (-)-sophocarpine and (-)-isosophocarpine, and perfected the total syntheses of these compounds.

Lupin alkaloids, almost all of whose structures had been determined by the 1960s, were roughly classified into four groups — lupinine-type, cytisine-type, sparteine-type and matrine-type. However, in regard to the absolute configuration, only Cookson's estimation on lupinine-type alkaloid had been published. Professor Tsuda precisely re-examined Cookson's estimation with modern technique and confirmed that it is accurate. Based on this confirmation, he clarified the absolute configuration of sparteine-type and cytisine-type alkaloids by the chemical interrelation of them with the lupinine-type. These studies on clarification of the absolute configuration provided the base for consideration of the mutual relations, regarding absolute configurations and biosynthetic pathways, among those lupin alkaloids which co-exist in the same plant and have different mother ring skeletons.

II. Studies on Tetrodotoxin

Pufferfish, or Fugu in Japanese, inhabit waters of mild temperatures throughout the world. The shape of the fish with its body inflated was used for a hieroglyphic character in ancient Egypt to mean "dissatisfaction." Ancient Chinese books on medicine also make mention of the fish as poisonous. But in western Europe, where people do not eat the fish, it did not appear in any literature until Engelbert Kämpfer (German), a Dutch ship doctor, referred to it in his book, "History of Japan (1727)," which summarized his visit to Nagasaki (1690-1692).

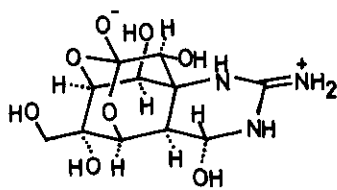
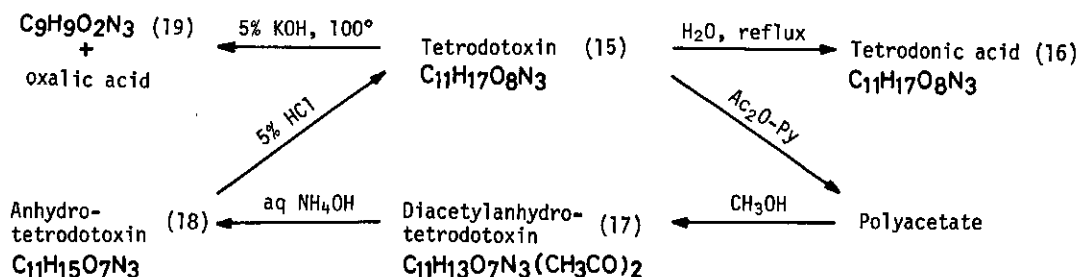
Many Japanese highly appreciate the taste of pufferfish and therefore they have shown great interest in its poison since olden times. Past surveys indicate that the fishes with toxin are found concentratedly among those in the family of Mafugu or Tetraodontidae. Their ovaries and livers contain toxin, which is also found in the intestines and skins of some classes of the fishes. All the pufferfish taken for food in Japan contain toxin except Sabafugu (Lagocephalus lunaris spadiceus). The amount of toxin increases gradually before the spawning season. The toxin content varies markedly according to individual fishes even of the same family. The toxic symptoms begin with mouth and lips numbed, vomiting, skin sensation and sense of taste slowed down, movements of hands and legs paralyzed, bone core muscle let loose, blood pressure lowered, and then end with respiration halted to death.

One of Professor Tsuda's predecessors in the study on the toxin in pufferfish is Dr. Yoshizumi Tahara, who headed what is now the National Institute of Hygienic Sciences. While in office, he extracted the ovaries of Torafugu (Fugu rubripes rubripes) and obtained toxin like glutinous rice-jelly (LD_{50} :4100 μ g/kg mouse) in 1909. He named it "tetrodotoxin" after the name of the Mafugu family, Tetraodontidae, and also the then genetic name of Torafugu, Tetraodon. That toxin, when compared with the present day pure sample, shows a purity rate of about 0.2 %.

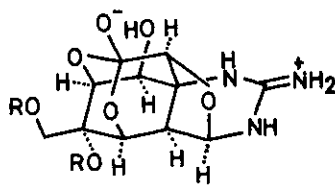
Later in 1950, Professor Akira Yokoo successfully crystallized tetrodotoxin (LD_{50} :8.7 μ g/Kg mouse), followed by Professor Tsuda in 1952. Professor Tsuda

went further with improved purification techniques to the point where he succeeded in the production of 8-to-10 grams of pure tetrodotoxin from one ton of ovaries. This success meant that he now placed the structural studies of tetrodotoxin on the right track and set the stage for the test materials for biological experiments to be made available to the like researchers in other countries.

The initial period of several years in the structural studies of tetrodotoxin presented tremendous difficulties to Professor Tsuda and his team. However, in 1960, he found that oxalic acid and $C_9H_9O_2N_3$ (mp 200° , yellow crystal) can be obtained by heating tetrodotoxin in 5 % KOH solution at $90-100^\circ$. In 1962, he clarified this substance as 2-amino-6-hydroxymethyl-8-hydroxyquinazoline (19)

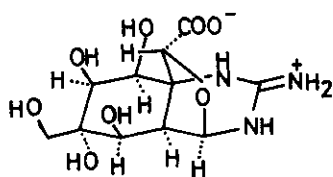


(15)

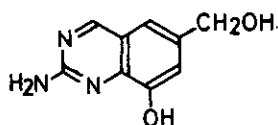


(17) R=Ac

(18) R=H



(16)



(19)

by identifying it with a synthesized authentic sample. Though the investigations that followed still called for painful efforts, Professor Tsuda stood firm and succeeded in elucidating the structure of tetrodotoxin, using not only IR- and UV-, but also NMR-spectra, and at the same time resorted to, as a decisive key to structural determination, the X-ray crystallographic analysis, requiring maximum use of large electronic computers, which became accessible only from that time.

First, tetrodonic acid, $C_{11}H_{17}O_8N_3 \cdot H_2O$, mp $> 300^\circ$, (16), obtained from tetrodotoxin on refluxing with water, was derived to its hydrobromide, the structure of which was elucidated using X-ray crystallographic analysis. What proved to be the finally contributing factor in the structural determination is 16,17-diacetylanhydrotetrodotoxin, $C_{11}H_{13}O_7N_3(CH_3CO)_2$ (17). This substance can be obtained as follows; first, acetic acid salt of tetrodotoxin was acetylated with acetic anhydride-pyridine. Then the afforded polyacetate was allowed to stand in methanol to give the compound (17) in question. Deacetylation of 17, followed by treatment with 5% HCl, recovered tetrodotoxin. He therefore believed that this compound was extremely similar in structure to tetrodotoxin. The diacetate (17) was derived to its hydroiodide, the structure of which was determined through X-ray crystallographic analysis. In 1964, he proved, on the basis of the structure of 17 and his accumulated knowledge in chemistry and physical chemistry, that the whole structure, including the absolute configuration and stereochemistry, of tetrodotoxin is 15.

Around that time, the two other study groups, one led by Professor Yoshimasa Hirata, and the other by Professor Robert B. Woodward, arrived at the same conclusion on the basis of the information obtained from the X-ray crystallographic analyses. It is noteworthy that each of the three teams had taken creative pains to prepare its own derivative before subjecting it to such an analysis. The results of the studies conducted by these groups happened to be simultaneously announced at the 3rd IUPAC International Symposium on the Chemistry of Natural Products, held in Kyoto, in April, 1964, which left all participants deeply impressed.

Tetrodotoxin, at the time of its structural determination, was the most

powerful toxic compound with low molecular weight. It shows a unique physiological action which specifically inhibits sodium ion activation mechanism. For this reason it has continued to attract worldwide attention as a reagent of neurophysiology and muscular physiology.

Tetrodotoxin does not exist in pufferfish alone. It has since been isolated from other sources — a newt (Taricha torosa) in California, Tsumugi-haze (Gubius cringer) on Ishigakijima Island, poisonous frog (Atelopus varius, A. chiriquiensis) in Costa Rica, and Hyomondako (Octopus maculosus) on Australian coasts. These facts have naturally led many scientists to take deep interest in the biosynthesis of tetrodotoxin. In a recent example, a team of Professor Shoji Konosu, Department of Fisheries, University of Tokyo, reared pufferfishes for biosynthetic investigation. The team checked those artificially bred pufferfishes to see if they produced any tetrodotoxin. But not a trace of it was detected. This and other related findings have opened up interesting problems of importance about biosynthesis in connection with the might of nature. It is hoped that these problems will be solved promptly.

All in all, light has now been shed on real state of natural poison, the focus of major international attention long since Tahara's extraction of the crude toxin. This achievement is indeed a big scientific feat Japan can be proud of as the first brilliant monument of the studies on marine physiologically active natural products.

III. Studies on Steroids

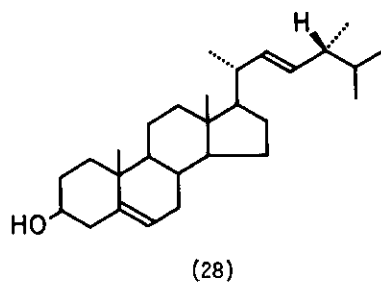
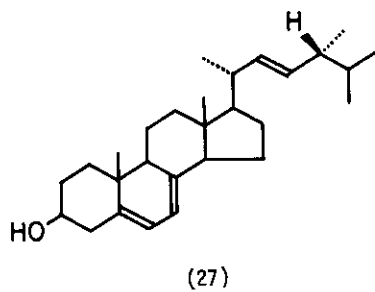
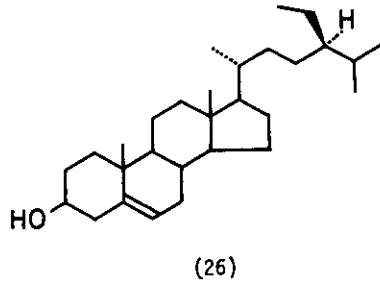
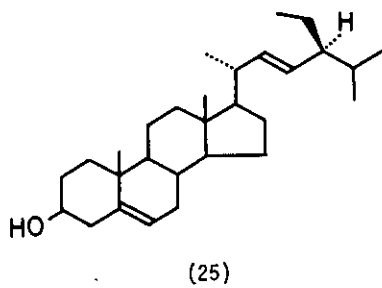
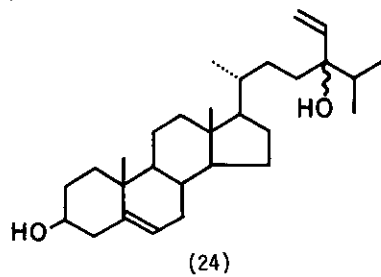
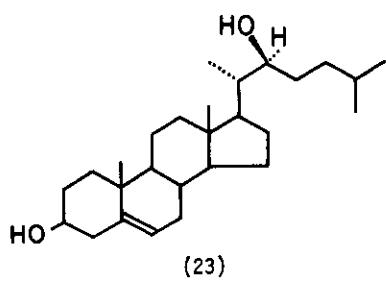
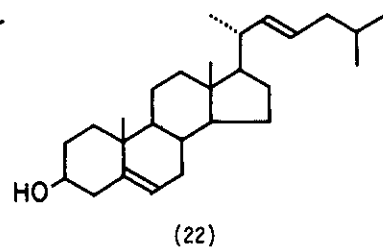
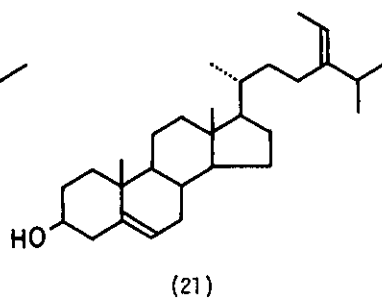
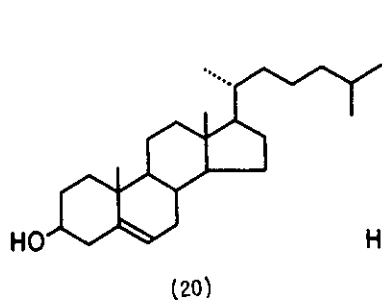
Professor Tsuda's studies on steroids, one of his life works, were conducted between 1950 and 1966, on which 75 reports were published. His investigation on the unsaponifiable fraction of the ovaries of pufferfish occasioned him to begin studies on steroids. First, he set his eyes on sterol resources in marine natural products and examined sterol constituents of various seaweeds.

In 1957, he discovered cholesterol (20) in a certain red algae and also found that cholesterol is widely distributed in red algae. Until that time it

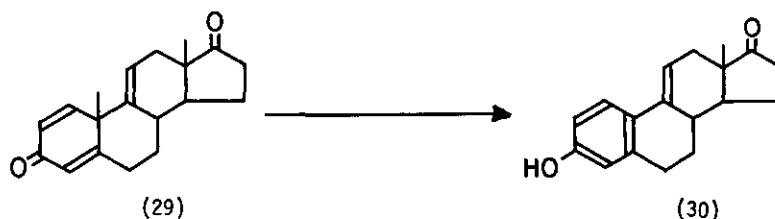
had been believed that cholesterol existed only in animals. Many researchers have since that time confirmed that cholesterol does exist in plants as well. Professor Tsuda made it clear that the main component sterol in Rhodophyta is cholesterol and that in Phaeophyta is fucosterol (21). This fucosterol in later studies was used as a starting material for syntheses of vitamin D metabolites. Furthermore, he found 22-dehydrocholesterol (22) and 22-hydroxycholesterol (23) in Rhodophyta, and sargasterol, the first 20-iso sterol, in Phaeophyta. Saringosterol (24) which has a hydroxyl group in C-24 position was also isolated.

At that time the absolute configuration at C-24 position of sterols had been assumed mainly by means of optical rotation. He determined the absolute configurations of two series of sterols at C-24 position by chemical means. Thus, correlation between (+)-2-ethyl-3-methylbutanal obtained from stigmasterol (25) and (-)-hydrocarbon from glucose assigned the configuration at C-24 of stigmasterol as 24 α in the extended Plattner convention. Furthermore, comparison of 2-oxo-5-ethyl-6-methylheptane (d-type), obtained by oxidation of sitosterol (26), with the same compound (l-type) from (+)-limonene determined the configuration of sitosterol as 24 α (24R). Ergosterol (27) has been determined to have the 24 β configuration by deriving this compound to 2,3-dimethylbutanal. This conclusion was confirmed by stereoselective synthesis of brassicasterol (28) from the 22-aldehyde.

Since natural source sterols are usually the mixture of homologues and the isolation at that time had to depend mainly on recrystallization, it was difficult to ascertain the constitutions accurately. In 1960, Dr. E. C. Horning succeeded in separation of steroids by gas chromatography (GC). This technique was immediately introduced by Professor Tsuda's group and the separation of natural sterol by GC was reported in 1961. Since then GC became indispensable technique for studies on natural sterols. He applied GC to analyze algae sterols, insect sterols and others, and clarified the profiles of these sterols. He also established the analytical method with GC of androstane- and pregnane-series steroids, bile acids, bile alcohols, and oxidation products of corticosteroids, and laid the foundation for use of GC in clinical analysis. These studies on GC of natural products have been extended to GC-MS analyses of steroids, the direct combination with the mass spectrometer.



Professor Tsuda found many new reactions of steroids. Treatment of 1,4-dien-3-one system (29) with zinc afforded estrogen derivative (30) through elimination of the angular methyl group as methane under mild conditions. This reaction opened a new route for syntheses of estrogen and 19-norsteroid. He also found a transformation reaction of 5,7,9(11)-triene to anthrasteroid.



For the syntheses of adrenocortical hormones, a number of studies were conducted in various countries since the beginning of the 1950s to introduce the hydroxyl group at C-11 position or the double bond at C₁-C₂ position of the steroid structure. As soon as Professor Tsuda took office at the Institute of Applied Microbiology, University of Tokyo in 1955, he started studies on the microbial transformation of steroids with the cooperation of the group of

Substrates		
	Progesterone	Reichstein's compound S
Microorganisms		
	15 β -OH	11 α -OH
<i>Syncephalastrum racemosum</i>	7 β ,15 β -, 6 β ,11 α -and 6 β ,15 β -(OH) ₂ 7 β ,14 α ,15 β -(OH) ₃	
<i>Helminthosporium sativum</i>	15 α -and 15 β -OH 7 β ,15 β -, 7 α ,15 β -and 14 α ,15 β -(OH) ₂	15 α -OH
<i>Diplodia tubericola</i>	7 β -OH 7 β ,15 β -(OH) ₂	7 ζ -OH

Professor Yoshinobu Asai and that of Professor Hiroshi Iizuka. Among the 284 strains of Rhizopus sp. preserved at that time in the Institute, 21 strains were found to have an ability to oxidize progesterone to 11 α -hydroxyprogesterone.

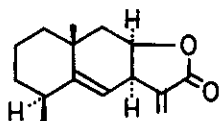
On the other hand, among 473 strains of Aspergillus sp., it was recognized that one of the strains shows an ability of hydroxylation at 11 α , and the other dihydroxylation at 6 β , 11 α along with 11 β -hydroxylation. After screening many kinds of strains of fungi, three strains were found to possess strong oxidative activities as shown in the table above. The structures of the compounds produced by these fungi were determined and the oxidation pathways were proved.

It was also found that bacteria Bacillus pulvifaciens IAM N-19-2 strain possesses activities such as dehydrogenation at C-1 position or oxidation of 17-hydroxyl group to 17-ketone and that in transformation of hydrocortisone with Pseudomonas chlororaphis IAM 1511 strain, prednisolone was obtained as a main product, along with 17-keto compound derived by the cleavage of the side chain.

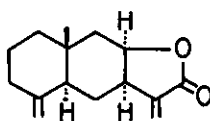
IV. Studies on Terpenoids

Professor Tsuda's studies on terpenoids were resumed in 1955 after an interruption of about 15 years since his investigations on soyasapogenols.

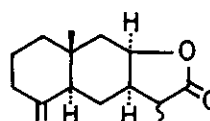
He presented new structures of alantolactone (31), isoalantolactone (32) and dihydroisoalantolactone (33), the constituents of the root of Inula Heleium, which were assumed incorrectly at that time. Absolute configurations of these compounds were also determined by their correlations with eudesmol.



(31)



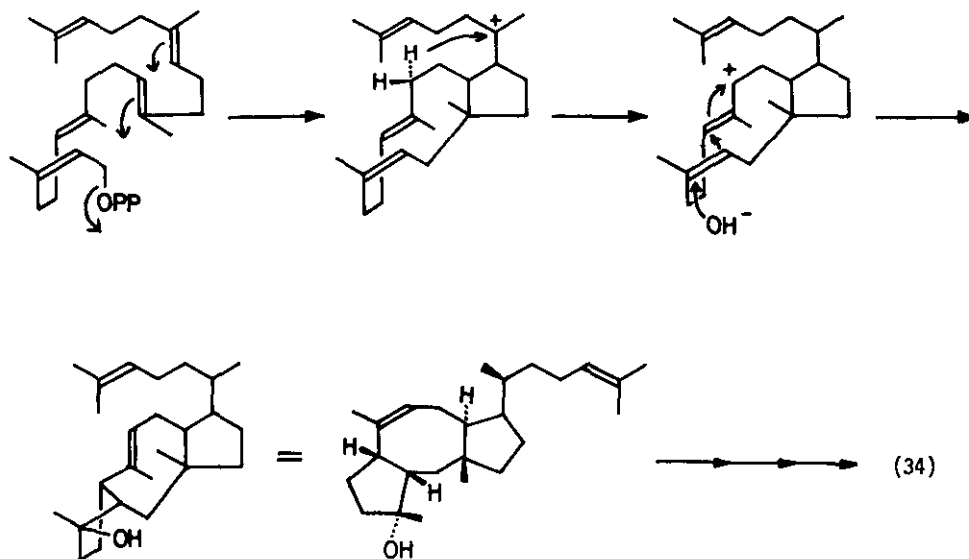
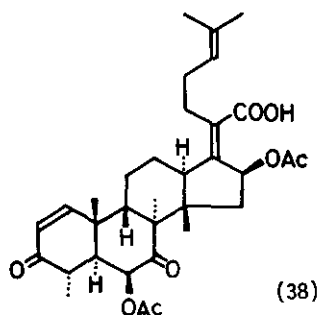
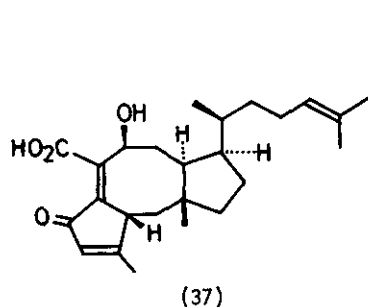
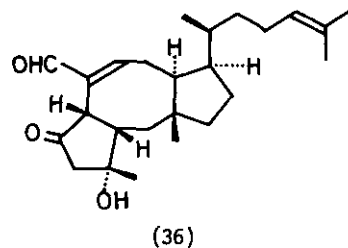
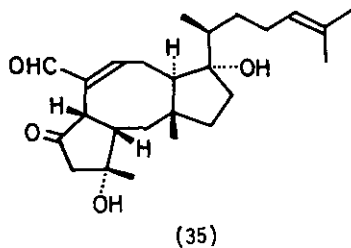
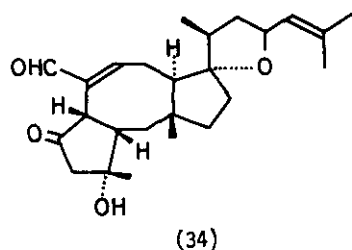
(32)



(33)

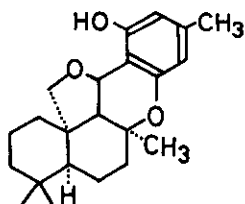
In the course of systematic screening of metabolites produced by plant pathogenic fungi, Drs Keiji Ishibashi and Michikazu Nakamura (Sankyo Co., Ltd.) found that the fungi belonging to Helminthosporium sp. are the most promising resource of new antibiotic metabolite. In 1963, Professor Tsuda started research on the elucidation of the structures of these metabolites. From

Ophiobolus miyabeanus, ophiobolin A (ophiobolin) (34), ophiobolin B (zizanin B) (35) and ophiobolin C (zizanin A) (36) were isolated and the structures of these compounds were elucidated by X-ray crystallographic analysis and by chemical correlations between them. Ophiobolin A (34) is the first compound whose

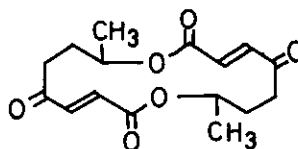


structure was elucidated as sesterterpene. Ophiobolin D (cephalonic acid') (37) and helvolic acid (38) were also isolated from Cephalosporium caerulens and the structures of them were determined. He also clarified the biosynthetic route of ophiobolin A (34) from geranylfarnesyl pyrophosphate by means of the experiments with intact cell and cell free system.

The structure of siccanin (39), isolated from Helminthosporium siccanis, was determined. Siccanin exhibits significant inhibitory activity against a variety of fungi, and is practically used as medicine for water-eczema. Pyrenophorin (40) was determined to have a 16-membered dilactonic structure rather than the 8-membered structure as had been incorrectly reported previously.



(39)



(40)

Professor Tsuda since his retirement from University of Tokyo has continued to devote his time and energy to the education of youth as President of Kyoritsu College of Pharmacy and also Vice President (1977-81), and President (since 1981) of the Japan Association of Private Colleges of Pharmacy. Also from 1975 to 1981, he, as President of the Central Pharmaceutical Affairs Council, played an indispensable role with his profound scholarly knowledge and impartial personality. In another distinguished service, Professor Tsuda worked as Editorial Member for the Asian Region of the Tetrahedron and Tetrahedron Letters, the journals most widely read by organic chemists in the world, for a little more than 10 years. He in such a capacity successfully made latest information on studies in Japan and other Asian countries available to the rest of the world.

He also has rendered significant services to the Pharmaceutical Society of

Japan first as Editor in Chief, Honorary Member, Vice President and then President. His efforts resulted in the construction of the Pharmaceutical Society House under the close cooperation with Professor Ochiai. Currently, he is the first president of a foundation for promotion of pharmaceutical researches which was recently established to mark the centenary of the founding of Pharmaceutical Society of Japan. In this post, he is passionately seeking to advance international cooperation in the academic area and support programs for promising young scientists. Since 1980, moreover, he has been Honorary Member of the Agricultural Chemical Society of Japan, the title given for his outstanding role in furthering cooperative ties with the Pharmaceutical Society of Japan.

In 1976, he was elected member of the Japan Academy in recognition of the meritorious contributions he made over so many years to the progress in the pharmaceutical sciences, organic chemistry and chemistry of natural products in Japan. On an even more glorious occasion, in January, 1979, he had the honor of attending the traditional New Year's Lectures at Imperial Court. In the presence of the Emperor, Professor Tsuda, as one of the selected lecturers, delivered a lecture on "Toxin in Some Fishes and Shellfishes," in a major introduction of his stocks of knowledge accumulated over years. In another honor, citation was conferred on him by the Japanese Government as Person of Cultural Merits in 1980.

Turning to one of the side-lights on Professor Tsuda, there are many people who associate him with skiing, the sport which he took to during his Urawa High School days. In fact, he laughingly says, "My friendship with skiing has been much longer than that with research!" In those prewar years when skiing was not yet a popular sport, Professor Tsuda used to visit still unexplored gelandes together with his youthful colleagues. In the immediate postwar period, he spent several skiing seasons at a completely desolate village on the slope of the Japan Alps. Later, he made it a practice to lead teams of his students on skiing trips. Conspicuous among such students have been those cheering girls from Kyoritsu College of Pharmacy! In a related episode, he suffered fracture of his leg bone while skiing in the Alps during his 1965 tour of Europe. This news left many of his friends both home and abroad worried at the time. Thus not a few people cherish the fond memory of Professor Tsuda as symbolized by his

skiing figure and growingly silver hair.

It is heartwarming to see that the professor and his wife Eiko, an accomplished lady, are a happy and contented couple, blessed with children and grandchildren.

We take everlasting pride and delight in the fact that we, as disciples of Professor Tsuda, have had the golden opportunity of participating in his research projects and receiving his personal guidance throughout our days at University of Kyushu and the Institute of Applied Microbiology.

In conclusion, it is our sincere hope that Professor Tsuda, celebrating his 75th birthday and attaining a life stage of ripeness, will even be more prosperous and healthy so that he, as our leader, may continue to light our way with a torch in the future.

Acknowledgements: We gratefully acknowledge the generous help we received from Mr. Mitsuo Onchi and his wife Dr. Kaoru Onchi, nee Abe, in preparing and reviewing the English manuscript of this article.

Shigenobu Okuda

Nobuo Ikehara