Scientific Contribution by Prof. Tsuda

Nobuo Ikekawa

Niigata College of Pharmacy, Kamishinei-chou 5–13–2, Niigata-shi 950–2081, Japan

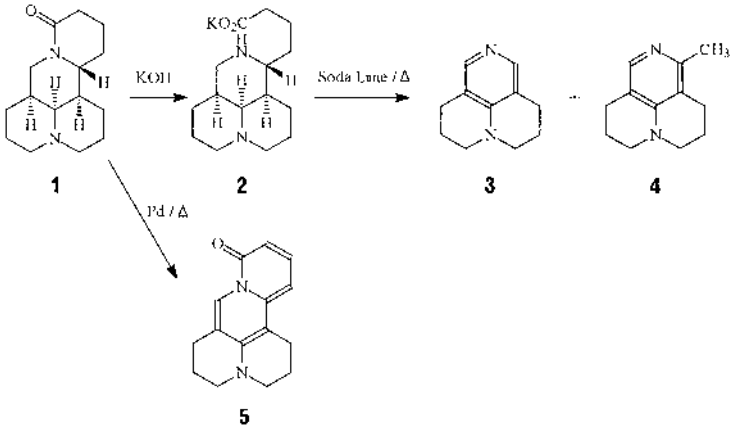
Research accomplishment of Professor Kyosuke Tsuda was summarized and published as "A list of publications by Professor Kyosuke Tsuda and his collaborators"[1] at his retirement from University of Tokyo at the age of 60. His memoir and treatise by his collaborators were published. In addition, the journal Heterocycles published as a commemorative issue for his 75th birthday, in which appeared at the head of the issue "Profile and Scientific Contribution of Professor K. Tsuda"[2] written by Okuda and Ikekawa. Further, as a commemorative event to celebrate his 85th birthday, a gorgeous volume with 500 pages entitled "Sixty fire years with Pharmaceutical Science",[3] was published seven years ago by a goodwill of Hirokawa Publishing Company, containing memorable photographs, his autobiography, his research and its development, his activities in education and in scientific societies as well as reminiscence by 80 individuals. Thus I feel somewhat hesitant to repeat his academic contributions and his personality in this memorial issue as they already seemed to have been described sufficiently. Prof. Tsuda, however, was one of the founders, and also the editor-in-chief of this journal, and his research accomplishments should be considered to represent the development of pharmaceutical sciences in the twentieth century. When one considers these facts, one realizes the significance of retracing his footprints in this journal, hence I have decided to uptake the task of writing this article even though many repetitions are unavoidable. As complete lists of his research articles have appeared in the above three publications, only main articles are described here. Also, courtesy titles of collaborators are omitted here.

Professor Tsuda was borne in 1907, in the city of Kiirun, Taiwan, as the fourth and youngest child to Mr. Sosuke Tsuda, who had been born to a warrior class family, and was an official of the Government-General of Taiwan and engaged, at that time, in the harbor construction of Kiirun. After finishing a primary school in Kiirun, Prof. Tsuda finished a five-year middle school in Taipei in four years, then passed the entrance examination and admitted to Urawa High School at his home area, in the Department of Science, in 1923. He lost his mother when he was in the second year at the high school. He belonged to the swimming team in the high school, and the coach of the club was a young chemist graduated from University of Tokyo. Upon his urging, Prof. Tsuda decided to study at the Department of Pharmaceutical Science, University of Tokyo. The Department at that time had only four laboratories, and upon graduation, he became, at the direction of Prof. Heizaburo Kondo, an assistant to the assistant professor, Dr. Eiji Ochiai in 1929. In the following year, Dr. Ochiai was promoted to an associate professor, and Dr. Tsuda to an assistant professor.

A study was initiated in 1885 by Prof. Nagayoshi Nagai on a Chinese herbal medicine, Kujin (SOPHORAE RADIX) which was prepared from dried roots of a leguminous plant, Kurara, and the bitter tasting component was isolated and named **matrine** (**1**) by Prof. Nagai. The first report on the study on its structure was published in the ninth volume of Yakugaku Zassi in 1889 by Nagai and Tahara. The struggle of Prof. Tsuda with matrine continued to 1935. During this struggle, Dr. Ochiai went to Germany to study for two years, and Prof. Tsuda was left to do the work by himself. He even worked on the day of his own wedding at his bench in the laboratory. Such dedication would not even be imaginable to the youth of the present time, I suppose. All these were done before the invention of chromatography, and it must have been of utmost difficulty to determine the structure of the oily substance that were produced by chemical reactions.

Several hundred grams of potassium salt of matrinic acid (**2**) was crystallized from the reaction product with potassium hydroxide, and purification of the fraction upon the dry distillation with soda lime yielded at least five products. It took three years for him to identify these products. The work was painstakingly repeated to purify these products by distillation under reduced pressure, formation of their crystalline salts, isolation of the base, and again distillation under reduced pressure. Significant compounds among them were nordehydro-α-matrinidine (**3**) and dehydro-α-matrinidine (**4**) which were later synthesized.

He also succeeded in obtaining octadehydro derivative (**5**) of matrine by heating it with a palladium catalyst. Combining these reactions, he finally succeeded in elucidation two-dimensional structure of matrine.[4,5] This study constituted his doctoral dissertation, which took 6 years in the making. Prof. Kondo was very pleased with his work, and the six years of work formed the basic philosophy for Prof. Tsuda's research work, *i.e.*, the conviction that a man could find the way to accomplish his goal if he devoted himself thoroughly to his work.



About the time this work on matrine was finished, a new struggle was begun to make operable the micro elemental analyzer that Dr. Ochiai had brought back from Germany and to make it utilizable for research purposes. This type of analyzers had been brought back by some of those who had returned from their studies abroad around that time, but none of them were satisfactorily operable. With Tokutaro Ishii and Shusaku Sakamoto as the collaborators, Prof. Tsuda finally succeeded in establishing the elemental analytical method in micro scale for the first time at the Department of Pharmaceutical Science in University of Tokyo. Here also could we observe Prof. Tsuda's tenacity, and perhaps his keen awareness of the high humidity in our country. Results of this work were published in 1937, co-authored by Ochiai and Tsuda, as "Methods of micro and small scale organic analyses" (Nanzando), which became a bible-like classical reference for the microanalyses. Until this work, 100 mg samples would have been required for elemental analyses, but this work made only 3 mg samples to be sufficient, so it must have been really a great news to organic chemists.

Prof. Tsuda's research interests shifted toward soya sapogenins after 1935. He separated four kinds of sapogenins into pure forms, soyasapogenols A, B, C and D, by means of chromatography using large alumina column, a unique method used at that time. Using selen dry distillation, their structures were determined to have triterpene moieties.

The structures of these compounds were determined during the World War II in the laboratory of Prof. Ruzicka at the Zurich, Eidgenössishe Technische Hochschule (ETH). This fact later presented an opportunity for Prof. Tsuda to study at the ETH.

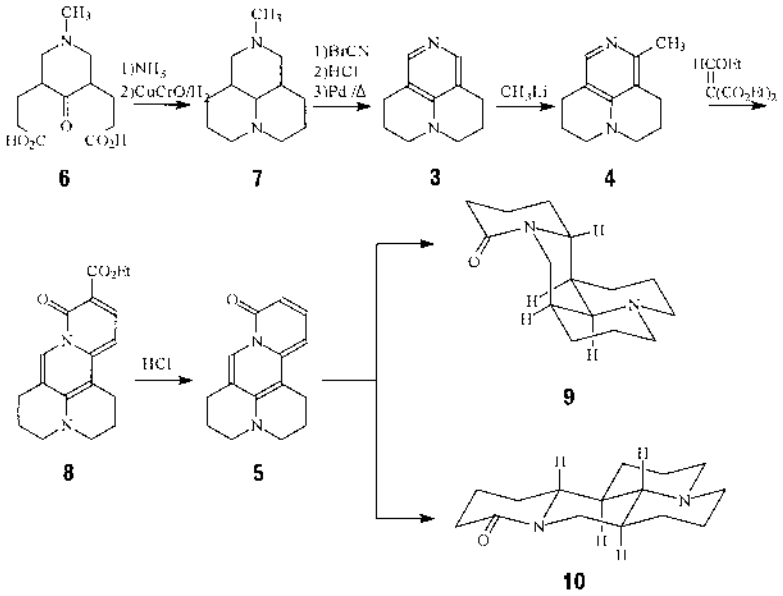
In 1939, Prof. Tsuda, upon the suggestion by Prof. Ochiai, spent his time in the laboratory of Prof. Shuji Hasegawa to study experimental chemotherapy, where Prof. Tsuda learned bacteriology. He held dual positions at the Department of Pharmacy and the Infectious Disease Research Institute, working both on chemical synthesis and animal experiments, thus he had an extremely busy period. He joined a research team on chemotherapy for malaria, and the study on sulfonamide synthesis he had begun then was continued until after the World War II. The color developing reagent he synthesized, Tsuda reagent, is listed in Pharmacopoeia as a quantitative reagent for sulfa drugs.

In 1951, Prof. Tsuda took a position in the newly opened Department of Pharmacy at the Medical School in Kyushu University as the professor of the laboratory of Pharmaceutical Chemistry. Dr. Issei Iwai was the associate professor, and Umezawa, Saeki and Ohki were research associates. As graduate students, Ikekawa and Tamura were transferred from University of Tokyo, thus the research group became quite large.

One of the research projects was the study on coal tar base, which I was the chief player. I did distillation of tar bases day and night, making distillation fractions into picrates, and recrystallizing them over and over every day. Prof. Tsuda was very thorough in pursuing this research, and made all of the third year students participate in the study as the themes of their graduation theses to identify these pyridine bases by synthesizing all seventeen isomers of polymethylpyridine.

Another project, mainly worked on by Iwai and Ohki, was the study on the structures of alantolactone, a component of root of *Inula Helemium*. This study provided evidence that the structural formula previously proposed by Ruzicka was wrong, and the correct structure was hence presented.[7)]

Studies on matrine was restarted, which had been suspended for more than ten years since the time of the structure study. The successful synthesis of nordehydro-α-matrinidine (**3**) was performed by Saeki and Imura in 1954,[8)] thus a passage was opened for the entire synthesis of matrine. Nordehydro-α-matrinidine (**3**) was derivatized into dehydro-α-matrinidine (**4**),[9)] then octadehydromatrine (**5**) was synthesized, and the structure of matrine



was determined by the total chemical synthesis.[10)]

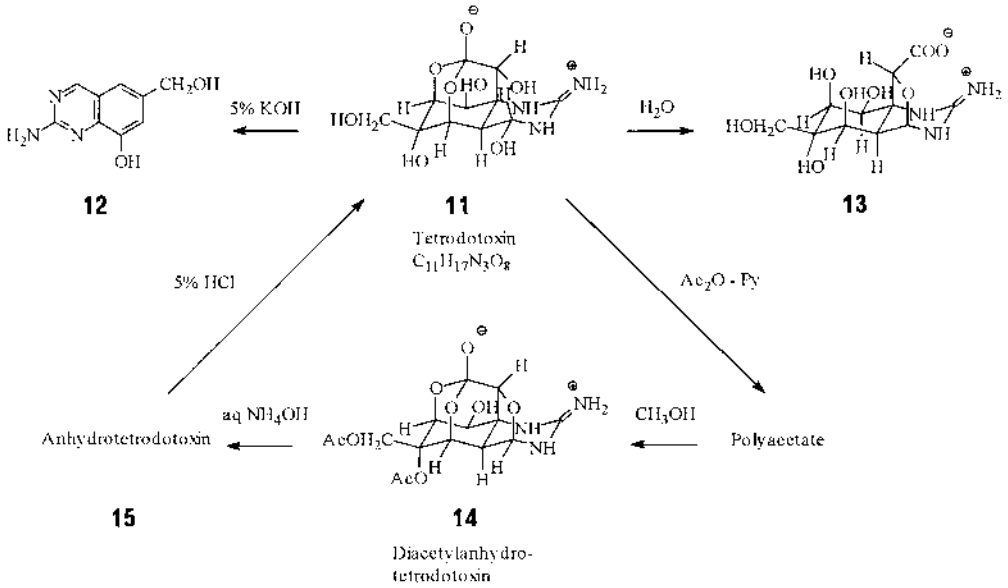
In 1954, Prof. Tsuda stayed at ETH for 8 months for study at the laboratory of Prof. Ruzick. In 1955, he was appointed as professor of chemistry at the Institute of Applied Microbiology, University of Tokyo, which was newly founded by Prof. Kin-ichiro Sakaguchi. At that time, Dr. Okuda was transferred to the new laboratory from Department of Pharmaceutical Science, University of Tokyo and Ohki and Ikekawa were also transferred from Kyushu University. They worked together to design a modern laboratory in the new institute.

At that time period, effects of stereochemistry on reactivity, mainly on steroids, were actively discussed, and attempts to systematize the effects were being made. Because of this, Prof. Tsuda's interest was mainly focused on the stereochemistry of (+)-matrine and more stable (+)-allomatine which was obtained from the same plant. From the dipole moments and IR spectra of the both compounds,[11)] and the difference in chemical reactivities (formation of quarternary bases, isomerization, rate of dehydrogenation, *etc*.), a conclusion was drawn that matrine had A/B, C/D *trans*, and A/C, B/C *cis* configurations and allomatorine had all trans configurations (**10**). This conclusion was also proven by the total synthesis of these compounds.

Later, Kujin alkaloids were studied thoroughly by Okuda, and Murakoshi (Chiba University, Dept. of Pharmacy), and more than twenty different alkaloids were isolated and their structures were determined, and further, pharmacological effects of matrine was elucidated by Yamazaki (Chiba University, Dept. of Pharmacy). The contribution by Prof. Okuda was enormous for Prof. Tsuda's studies on leguminous alkaloids, especially on matrine. When Prof. Okuda passed away suddenly in 1991, Prof. Tsuda told us that he greatly missed Prof. Okuda and he felt very lonely.

It was Dr. Yoshizumi Tahara, at the National Institute of Hygienic Science, who studied the purification of the toxin of puffer fish (fugu) first. A report of his study was published in 1909, in which he gave a name of **tetrodotoxin** to the concentrated crude fraction of the toxic substance. Though it was found later that the purity of the crude toxin in the glutinous material was less than 1%, a dilute aqueous solution of the concentrate was said to be used as a medicine for Rheumatoid arthritis during a period in the early 1900. It was in the 1949, just after the end of the World War II, when Prof. Tsuda, who became aware of the study of the glutinous preparation of tetrodotoxin, decided to purify the toxic component from it. Kawamura, who had been working at Sankyo Pharmaceutical Co., Ltd., the manufacturer of the preparation, was invited to University of Tokyo to pursue the purification work.

No structure had been known of the toxic component except that it was water soluble, hence, a paper chromatography that had just been started to be utilized for analyses of amino acids and sugars must have likely been used first. The position of the toxin migrated on a paper chromatogram was still unknown at all, thus, no other methods were devised but that the paper was cut into small pieces and the extract of each piece was prepared and injected to a mouse to find the location of the toxin. Hence, it must have been a really difficult task to crystallize the toxin, finally a crystal preparation was successfully obtained using multi-layer paper chromatography in 1952. Later, crystallization of the toxin was achieved in much larger scale using activated charcoal. The success in obtaining crystal preparation of the toxin was reported in newspapers, and the news caused quite an excitement in the



society. Sakai described an episode behind the news as follows.

Since the crystals were first obtained from the most toxic fraction, there was no doubt that it contained the toxin itself. Kawamura, however, injected the solution made from the crystal into mice to confirm its toxicity, he was quite shocked by the fact that no toxicity was detected. Then, a miracle occurred overnight. When he tried to reproduce the results by testing for the toxicity again, it showed strong toxicity. This was not due to technical mix-up, but it actually was a very important discovery for the elucidation of the structure of the toxin. The first non-toxic compound was an anhydrous form of the toxin (which has only 1/500th of toxicity of the fugu toxin itself) that resulted in changing its structure while the toxin was isolated chromatographically. The anhydrous form was altered back to tetrodotoxin while the non-toxic form was left standing in an acidic solution. This was later experimentally demonstrated.

It was a daunting task to remove anhydrotetrodotoxin (**15**) which was always present as a contaminant in preparations of tetrodotoxin (**11**), thus it was impossible to even definitively determine its molecular weight. The structure determination was initiated by studying the development of yellow color when the toxin preparation was heated in an alkali solution. As a product of such yellow color development reaction, $C_9H_9O_2N_2$ was obtained in a crystal form. In addition, oxalic acid was also obtained from the reaction products. The former was named as C_9-base (**12**), thus, a part of the structure started appearing. By the spectrum of the C_9-base and its oxidized product, the structure was speculated to be 2-amino-6-hydroxymethyl-8-hydroxyquinazoline (**12**),[12] and was confirmed by a total synthesis.

As no other chemical methods existed at that time to determine the structure, X-ray crystallography of a stable reaction product seemed to be the only method for the elucidation of the structure. Among several derivatives, a compound that was the most stable and easiest to handle was tetrodonic acid (**13**), which was nontoxic, and was recrystallized from an aqueous solution. The HBr salt of this compound was subjected to an X-ray crystallographic analysis and a part of the molecular structure of tetrodotoxin became clear.[13] It was quite difficult to purify the polyacetate which was obtained by an acetylation of tetrodotoxin, but a stable diacetate (**14**) was obtained by leaving this compound in methanol, and it was determined to be an anhydrous form of tetrodotoxin according to the elemental analysis.[14] X-ray analysis of its HI salt was then performed.[16] Tetrodotoxin is not a carboxylic acid as is tetrodonic acid, and its pK_a value of 8.84 (in water), 9.54 (in 60% EtOH) suggested that it might be an ortho ester structure. The X-ray crystallography of anhydrotetrodotoxin diacetate HI (**14**) definitively proved its structure as an ortho ester form.[15] The structure of tetrodotoxin was elucidated from this ortho ester structure, *i.e.*, when the anhydrous form (**15**) was incubated at room temperature in a 5% HCl solution, it changed into tetrodotoxin. This reaction was explained as that the ether was cleaved and the hydroxyl group was attacked from the back side. This was also later proven using NMR, but the structure of tetrodotoxin (**11**) was determined first using an X-ray crystallography.[17]

The structure of tetrodotoxin was reported at the International Congress on the Chemistry of Natural Products held in Kyoto in April, 1964, simultaneously by three groups, Tsuda group consisted of Tachikawa, Sakai, *et al.*, Hirata-Goto group of Nagoya University and Woodward of Harvard University, thus these reports were high-

lights of the meeting. Individually unique X-ray crystallographic methods were used by these groups to elucidate the structure of tetrodotoxin, the Hirata-Goto group used bromo anyhdrotetrodoic lactone HBr, and the Woodward group used 4-methyoxy-6,11-acetonide.

The tremendous efforts by Prof. Tsuda in the preparation for his report at the congress were vividly described in a review by Sakai.[3] The presentation of the structure by Prof. Woodward was made as a special lecture held in an evening, and his logically sound, excellent lecture lasting as long as three hours is still unforgettable to this day. In a seminar Prof. Woodward had given at Harvard University before his travel to Japan, his high praise of the chemical study of tetrodotoxin by Prof. Tsuda made Nozoe who was studying at Harvard at that time felt extremely proud, according to his letter sent to Japan.

Thus, the structure determination of fugu toxin marked the start of the research of natural product chemistry, biochemistry and physiology of tetrodotoxin. In this research, the reaction in alkali to yield C_9-base quantitatively contributed greatly to the structure determination and quantification of tetrodotoxin. Studies on its biosynthesis by Profs. Hashimoto and Shimizu of University of Tokyo and Prof. Yasumoto of Tohoku University developed into unexpected directions, however.

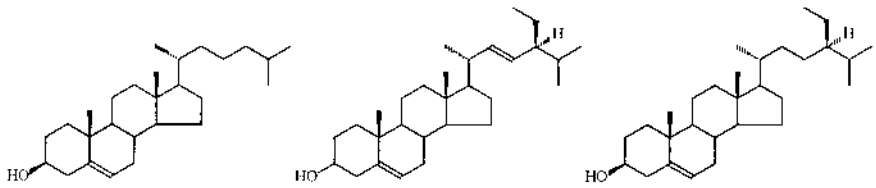
The toxin was produced by microorganisms which was ingested by small animals inhabited at the bottom of the sea. Thus, the toxin was accumulated in those animals, and in turn, accumulated further through the food chain. In such a concentration process, only those that had strong resistance to the toxin would survive and accumulate the toxin. Prof. Shimizu's hypothesis tells us a horror story that several million tons of tetrodotoxin must have been accumulated at the bottom of the ocean. It has also been discovered that tetrodotoxin causes paralysis by selectively blocking the intake of sodium ion. The toxin hence has become an indispensable reagent for physiological research. Thus, research on the fugu toxin is still being continued long after the discovery of its structure. It is a rare natural substance unparalleled by others, for which new research subjects appear continuously.

Prof. Tsuda's research on **steroids** began in 1953. The first project was the isolation of bis-cholestapolyene with cholesterol from non-saponifiable materials in the fatty substance in fugu ovary. He also recognized marine algae as a natural resource for steroids, and found many different sterol substances in it. The experiments to identify sterol components in algae were performed by Akagi, Kishida, Sakai and Hayatsu of Sankyo Research Institute. Large amounts of 30 different kinds of marine algae were collected with the cooperation of the staff of the Research Laboratory of marine algae, Hokkaido University, in Muroran, and they were dried, ground and extracted. The purification method of sterol at that time was to separate *p*-phenylbenzoates using silicic acid/cellite columns, and fractions were individually recrystallized, hence it must have been extremely difficult to obtain pure substances. As the component of sterols were identified using melting points and refraction rates of their acetates and benzoates, the procedures required extreme cautions with precision. Later, I analyzed algae sterols using gas chromatography, and learned that these components were mixtures of many different compounds.

The most significant discovery among what Prof. Tsuda accomplished in the study on algae sterols was the discovery of cholesterol (**16**) in the sterols of a species of red algae, and it caused a sensation when this discovery was reported in Science in 1957.[18] He also discovered cholestanol was present in some species of red algae. I learned, later, using gas chromatographic analyses, that some species of red algae contained cholesterol, and other contained cholestanol as the main component respectively. These components, I believe, should be re-examined for nutritional qualities of the seaweed.

At around 1957, it was generally believed that cholesterol was an animal sterol, and plant sterols were those with an alkyl moiety on the 24-position, hence this discovery of the presence of cholesterol in seaweed caused quite a sensation. Also found was fucosterol (**20**) as the main sterol component of brown algae, and research to utilize fucosterol as a source of steroid hormone production was begun. Further, Prof. Tsuda also discovered new sterols such as 24-methylenecholesterol, 22-hydroxycholesterol, 22-dehydrocholesterol, *etc*.

It was one of Prof. Tsuda's great accomplishments to have determined the configurations of 24-alkyl moieties in natural sterols. He determined chemically that side chain alkyl groups of stigmasterol (**17**) and sitosterol (**18**) were in the 24α-position, and that of brassicasterol and ergosterol were in the 24β-position.[20] Thus, his work helped organize stereochemistry of side chains of plant sterols. This study on the stereochemistry of side chains was utilized in a great deal in my work on vitamin metabolites at a later date. The first research project assigned to Ikekawa when he was back from the study at the NIH was to reexamine sterol components of a brown algae, Ohbamoku. This involved a search of sargasterol which was reported by Hayatsu. I was unable to prove the presence of sargasterol, but during this work, I was able to purify rather a large amount of fucosterol. This was the reason why I was able to start the synthesis of the active metabolites of vitamin D immediately upon the report by Prof. H. F. DeLuca of its discovery. The 1, 24(*R*)-dihydroxycholecalciferol (**21**) which I synthesized during the initial phase of my work on vitamin D metabolites was developed by a research group at Teijin Co., Ltd. as a thera-



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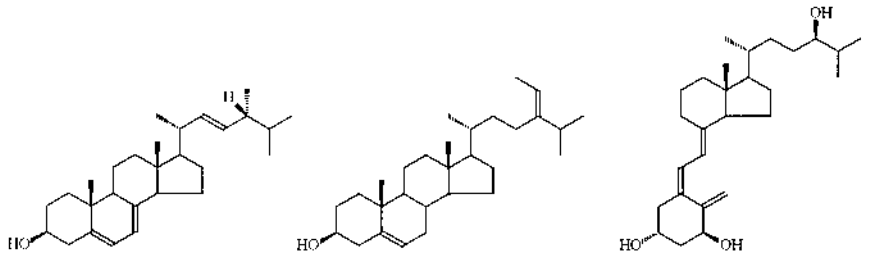

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peutic drug for psoriasis, and it is widely used in Japan, Europe, *etc*., in the name of Bonalpha.

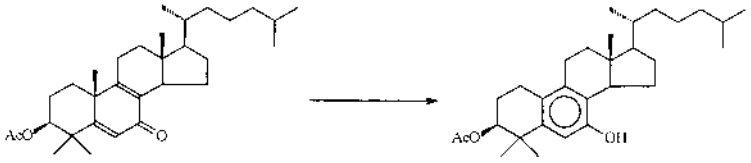
I must mention gas chromatography (GC) when I talk about the studies of steroids by Prof. Tsuda. In 1960, at the NIH where I was studying at that time, Dr. E. C. Horning succeeded in a separation of steroids using GC. Reading the news on Chemical Engineering News, Prof. Tsuda immediately wrote me a letter telling me to study thoroughly regarding the GC analysis of steroids. After I returned to Japan, a GC apparatus of Barbar Colman was imported for the first time in our country to Prof. Tsuda's laboratory. This apparatus was of course used in the steroid research, and the steroid analysis with GC gave enough impact on microanalytical methodologies in many fields that it helped them revolutionized. When one considers the process of the development of steroid research in an epoch-making fashion, and also development process of GC to GC-MS, HPLC, then to HPLC-MS, one would realize Prof. Tsuda's perseverance in pursuing separation technology.

It was also at Prof. Tsuda's laboratory that a discovery was made that the sitosterol contained in leaves of mulberry was converted in the body of silkworm into cholesterol upon ingestion. At the time, it was yet unknown that the carbon skeleton of ecdysone was cholesterol. This study developed into the elucidation of the biochemical mechanism of conversion of sitosterol into cholesterol by insects, and the study on biosynthesis of ecdysone, *etc*., which were achieved in my laboratory at the Tokyo Institute of Technology at a later date.

Steroid research of Prof. Tsuda were multi-faceted, and he discovered many new chemical reactions. Among them, especially noteworthy was the discovery of the A-ring aromatization reaction accomplished by Ohki and Nozoe. This discovery of the new reaction was initiated with that, when 5, 8-diene-7-one derivative of lanosterol (**22**) was treated with zinc, it split off methane and the B-ring was converted into an aromatic ring (**23**).[21] When this reaction was performed with Δ^9-1,4-diene-3-one system (**24**), methyl moiety at the 10 position would be split off and Δ^9-estrone (**25**) was obtained at a high yield.[22] This reaction can be applied to many similar situations and it can beutilized in the syntheses of estrogen like compounds and 19-norsteroids.

Shortly after Prof. Tsuda had assumed a position at the Institute of Applied Microbiology, he organized project teams for microbiological conversion of steroid, thus a lot of screening work for microorganisms was performed. Studies on structures of microbial products were led by Ohki and Sato. Out of 264 preserved strains of *Rhizopus*, 21 strain showed an activity to produce 11α-hydroxylated progesterone. Also, activities of many molds were discovered. Three species of mold converted progesterone to its 7β,15β-diol, and some species converted it to the 6β,15β-diol.

Many microorganisms found in petroleum and natural gas deposits were tested for their activities. Among them, *Bacillus pulvifaciens* strain SANK LAM N 19-2 was found to produce prednisolone (**27**) at high yield by introducing a double bond to the 1,2-position of cortisol (**26**).[23] This was developed into industrial scale production of prednisolone. At that time, many studies were being done for the microbiological conversion of steroid domestically and in abroad. Especially, it was an epoch making discovery at the laboratory of Prof. Arima of Faculty of Agriculture, University of Tokyo, to find a reaction to produce steroid hormones using microbial cleavage of the side chain of cholesterol. This technology was further developed and utilized in industrial production of steroid hormones.



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In Prof. Tsuda's laboratory, Okuda was the chief investigator in the microbiological conversion of alkaloids, which was rather rare type of study at that time, but later the technology was developed into active research for the use of micro quantities of enzymes in synthetic reactions, especially for asymmetric syntheses. There was one valuable study in basic science in the laboratory of Prof. Tsuda that was later developed into practical use. It was the development of antilipemia drug, Plavastatin, which was discovered at Sankyo Research Institute. Its industrial production became successful first by using the hydroxygenation reaction by an streptomyces sp. discovered by Naito.

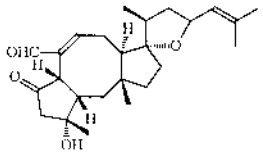
Prof. Tsuda was hoping to start his study on metabolites of plant pathogens in the beginning of 1960's. Around that time, a group led by Ishibashi and Nakamura of Sankyo Fermentation Research Institute had already obtained from metabolites of plant pathogens a few substances that had strong antimicrobial activities. Because no NMR technology was yet available at that time, and only oily reaction products was obtained for the structural determination, the study had to be suspended. When Nozoe came back from his study in the United States, Prof. Tsuda gave this project to him. With Nozoe leading the effort, graduate students at that time participated in the project. Owing to their work, a new research area of natural substances was developed. The NMR technology became available by this time, and it was used to partially elucidate structures of compounds of interests. As an automation of the structural analysis by X-ray crystallography had just become available around that time, Prof. Tsuda decided to use this technology in structural determination, thus a cooperative work with Prof. Iitaka of Department of Pharmaceutical Science was initiated.

The pathogenic organism from the cultured broth of Ophiobolus miyabeanus of rice was studied by Morisaki, and the structure of a metabolite, ophiobolin A (**28**), was elucidated in only a few months of work as a terpenoid with C_{25} structure.[24] Following this determination, cephalonic acid (ophiobolin D) (**29**) studied by Itai also was shown to have the same skeleton, a homolog with C_{25}-terpen.[25] Zizanin A (ophiobplin C), zizanin B (ophiobplin B) studied by Hirai were demonstrated with chemical conversion as homologs that shared the same carbon skeleton.

As these C_{25}-terpens had not been found in the plant kingdom, they were named **sesterterpens**. The discovery of these terpens was reported at the International Congress on the chemistry of Natural products, Stockholm (1966) by Nozoe, and created a sensation. Sesterterpens were later isolated from insects, lichens, ferns, and sponges, and constitute one of the groups of terpenoid.

Pyrenophorin (**30**) isolated from a pathogen attacking the leaf of oats was studied for its structure by Nozoe and Hirai. It was found to be the same as what Grove had isolated, but he had reported it having a 8-member ring. This was found wrong, and the correct structure was with a 16-member ring.[26]

Siccanin (**31**) is a metabolite produced by a parasite of rye-grass with a strong antifungal activity. The structure of siccanin was determined by Hirai, using X-ray crystallography upon introducing heavy atoms.[27] This compound was later marketed widely by Sankyo Co., Ltd. as a drug for athlete's foot. Research of microbial metabo-



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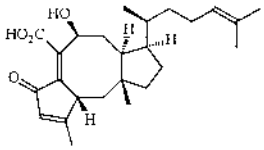

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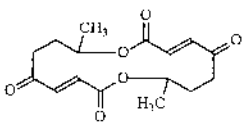

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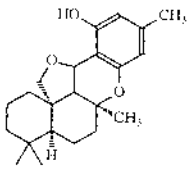

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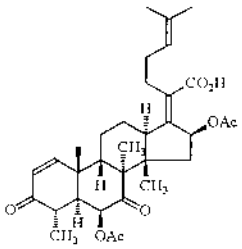

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lites was later developed splendidly with a leadership of Nozoe, involving studies on biosyntheses, chemical syntheses, and developments of new metabolites.

As another group of research on microbial metabolites, a structural study of helvolic acid (**32**), a steroid antibiotic, was begun in 1962. This study was done by Iwasaki, and the compound was identified as proto-lanostan type that seems to be an intermediate of lanosterol biosynthesis.[28] Protosterol was isolated, in addition, from a helvolic acid producing strain, and studies on their structures were organized by Hattori, Igarashi, *et al.* Iwasaki isolated many important compounds from metabolites produced by rice pathogens in a cooperative work with Agricultural Technology Research Institute, and he successfully elucidated the mechanisms for their physiological activities.

I have just summarized Prof. Tsuda's research activities. He determined the structures of matrine and pufferfish toxin, the two of the targets of long period of research among our pharmaceutical sciences since the time of Prof. Nagayoshi Nagai. When one considers the later development of these works, and also later development of his work in steroids and terpenoids, one realizes how epoch-making Prof. Tsuda's accomplishments were. He always was cognizant of new technologies, and taught us to enjoy research while himself was bravely challenging to solve big problems.

Profs. Kondo, Ochiai and Tsuda were awarded "the Order of Culture of Japan" in the Showa era (1926—1988) for their internationally recognized research of natural products. They were closely connected to each other in mentor-disciple relationships, as they were, respectively, Professor, Associate Professor and Assistant Professor at one time. Their monumental accomplishments in organic chemistry research in the 20th Century in Japan should be immortalized in history.

Prof. Tsuda's huge footprints are immeasurable when one considers his selfless contributions to the Pharmaceutical Society, his effort in the education in the pharmaceutical field as the president of Kyoritsu College of Pharmacy after retirement from University of Tokyo, and the succession of public work including those as the presidnet of the Central Pharmaceutical Affairs Council, the Chairman of the Research Foundation for Pharmaceutical Sciences and as the Chairman of Japan Health Sciences Foundation, etc.

Memorial addresses for Prof. Tsuda have been published in several journal.[29]

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