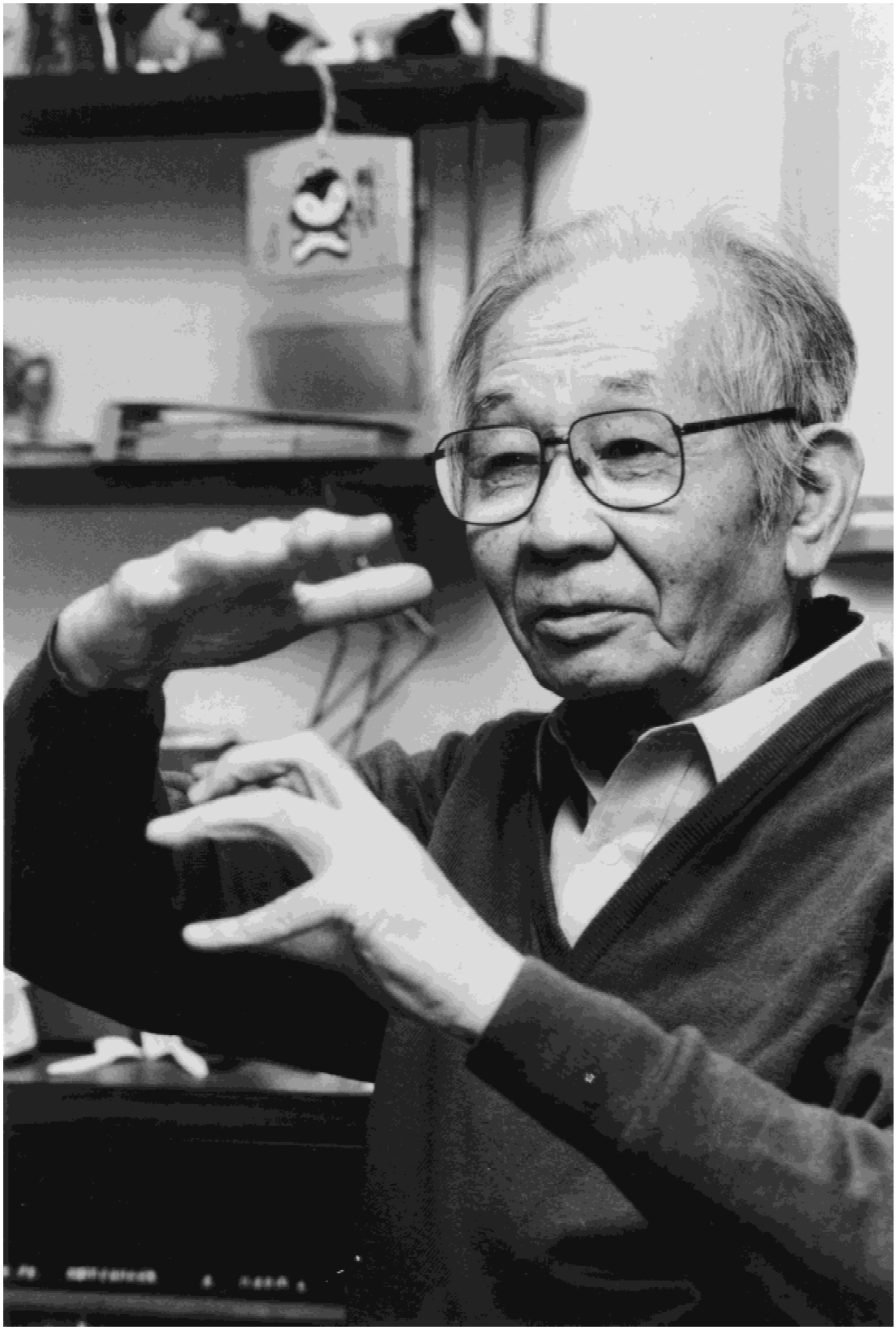

Professor Koji Nakanishi

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Koji Nakanishi's Enchanting Journey in the World of Chirality

NINA BEROVA*
Department of Chemistry, Columbia University, New York, New York

I am delighted to make some introductory remarks to this special issue, containing contributions from Koji Nakanishi's colleagues, friends, and former students.

For his outstanding contributions in various fields involving chirality, Professor Koji Nakanishi became the 1995 Chirality Medal Awardee at the Sixth International Conference on Chiral Discrimination in St. Louis, Missouri. This award was established some years ago and has been given to Jean Jacques, Vladimir Prelog, William Pirkle, Kurt Mislow, Emanuel Gil-Av, and Ernst Eliel. The special issue of *Chirality* dedicated to Koji Nakanishi is one of the first volumes that will be periodically published to honor the awardees of the international "Chirality Medal Award." I feel very grateful to all who supported the project and contributed to this volume. I believe the issue is a fine tribute to Nakanishi's seminal and pioneering achievements in different areas of organic and bioorganic chemistry and spectroscopy involving chirality. The articles cover a broad spectrum of research dealing with chirality phenomena and are in tune with Nakanishi's own interdisciplinary approach.

Having been closely involved with Koji Nakanishi in research at Columbia University for the past 10 years, I have the privilege to know his work, his multidisciplinary interests, his unending eagerness for trying new challenging steps, and perhaps some of his frustrations, which fortunately soon yield to his optimism. Koji Nakanishi has made significant scientific contributions in two different areas involving chirality, and for many years he has been regarded as one of the foremost scientists in these fields.

First, Koji Nakanishi has made many fundamental accomplishments in the fields of natural products. The compounds studied by him belong to various classes, but all have important biological activity. Many of them are endogenous to animal life, and some are the first members of a new type class of compounds. The establishment of their structure has made a great impact in advancing our understanding of nature. One of the main focuses of these studies includes the determination of the chirality, or the handedness of a molecule. Often this is a crucial structural factor for a molecule to be biologically active. When trying to determine the structure, especially when only a very limited amount of sample is available, Nakanishi has designed new, advanced methodologies for isolation and microscale structure determinations. Thus, he demonstrated that by combining spectroscopic and chemical derivatization methods of high sensitivity, we can study natural products and bioactivity beyond the conventional limit imposed by the minuscule quantity of material or by the complexity of the problem.

Of enormous importance, he has made outstanding spectroscopic contributions with broad impact in various fields where structure determination is involved. These include the first application of NMR nuclear Overhauser effect in structure determination (1966–1967); second derivative FTIR and UV difference spectroscopy (1985); and tandem MS for sticky peptide sequencing (1993). Of particular significance is the development of the exciton chirality method. In 1969, together with his graduate student Nobuyuki Harada, now professor at the Tohoku University, Japan, he pioneered and developed the nonempirical exciton chirality circular dichroic method (ECCD), which has proven over the years to have immense potential in determining the absolute configuration and conformation of chiral compounds in solution.

Koji Nakanishi was born in May 1925 in Hong Kong and brought up in Egypt, France, and England, where his father worked as a Japanese bank executive. He received his B.Sc. degree from Nagoya University in 1947, and was one of the first postgraduate fellows from postwar Japan to work in the United States, at Harvard University with Louis Fieser from 1950 to 1952. He returned to Japan to complete his Ph.D. degree at Nagoya University under the direction of Professors Fujio Egami and Yoshimasa Hirata in 1954. He began his academic career as an assistant professor at Nagoya University (1955–1958), and then as professor at Tokyo Kyoiku University (1958–1963). From 1963 he spent 6 years in Sendai at Tohoku University before he moved in 1969 to Columbia University, New York. From 1980 he holds the title "Centennial Professor of Chemistry." During his first 10 years at Columbia he also served as a founding member and research director of the International Center of Insect Physiology and Ecology in Nairobi, Kenya, and later on, from 1979 till 1991, as Director of the Suntory Institute for Bioorganic Research (SUNBOR) Osaka, Japan.

STUDIES ON NATURAL PRODUCTS: ABSOLUTE CONFIGURATIONAL ASSIGNMENTS

Nakanishi's structure determinations of over 180 bioactive chiral compounds also include in most cases the establishment of their absolute configurations by chemical correlation methods, or more frequently, circular dichro-

*Correspondence to: Nina Berova, Department of Chemistry, Columbia University, New York, NY 10027.

ism (CD). Some selected examples of such natural products are given in the following: **Pristimerin,** a widely used ethnobotanical antibiotic;[1] **Ginkgolides,** a potent inhibitor of platelet activating factor;[2] **Illudin S (Lampterol),** a cytotoxic principle in the bioluminescent mushrooms;[3] **Antheridiogen,** an antheridia-inducing factor of a fern;[4] **Ponasterones** and **phytoecdysteroids,** insect/crustacean molting hormones; now over 80 ecdysteroids have been isolated from plants;[5] **Taxinine,** which possesses the skeleton of taxol, the clinically important anticancer drug;[6] **Chromomycins,** a once clinically used anticancer agent; currently used for mechanistic studies of intercalation;[7] **Cervicarcin,** an antitumor antibiotic;[8] **Fluorescent tricyclic tRNA base,** next to the anticodon (micro scale structure determination with about 300 mg);[9] **Isopavine alkaloids;**[10] **Spirobenzylisoquinoline alkaloids;**[11] **natural (+) abscisic acid,** an important plant growth regulator;[12] the potent carcinogens **Benzo[a]pyrene** and **7,12-Dimethylbenz[a]anthracene 5,6-Oxide** adducts with nucleic acids;[13] **Brevetoxin B,** a red-tide toxin;[14] **Periplanone-B,** a sex pheromone of the American cockroach;[15] **Ipomeamarone,** one of the first phytoalexins;[16] the elusive **mitomycin C/DNA** adduct (full structure);[17] **Lienomycin,** one of the earliest absolute stereochemical analysis of polyene macrolide;[18] **Andrimid,** the first intracellular bacterial symbion to be studied; it exhibits specific antipathogenic activity;[19] **Philanthotoxin,** potent noncompetitive inhibitor of glutamate (Glu-R) and nicotinic acetylcholine receptors (nACh-R);[20] **Hydroazulenoid Diterpenes** and **Sesquiterpenes** from a marine alga;[21] **Retro-Retinol,** new endogenous mammalian B-cell, T-cell growth factor;[22] Precocious sexual inducer (**psi Factor**) of fungi;[23] **Amphikuemin,** the first symbiosis-inducing substance to be identified from a marine source;[24] **Nemadectins** α and α_2, macrocyclic lactones with potent antiparasitic activity;[25] **Malonofungin,** an antifungal aminomalonic acid metabolite;[26] **4-Hydroxyretinals,** visual pigment chromophores in bioluminescent squid;[27] and **Diepoxins,** novel metabolites with antifungal and antibacterial activity.[28] Recent work is directed toward determining the helicity of the twisted conformation of natural **Retinal** in rhodopsin, the photoreceptor responsible for the dim-light vision in vertebrate species.[29]

STUDIES BASED ON CHIROPTICAL SPECTROSCOPY

Dibenzoate Chirality Rule, Early Stage of Development of the Exciton Chirality Method

The first two papers of Nakanishi's pioneering work on the exciton chirality method (see references 33,34), were published in 1968 and resulted from part of the doctoral thesis work of Nobuyuki Harada, who has continued his studies in this field and has become a leading international figure at Tohoku U., Sendai. Chiroptical spectroscopy was hardly used until optical rotatory dispersion (ORD) and circular dichroism (CD) were revived after 50 years of near dormancy, by C. Djerassi[30] and L. Velluz et al.,[31] respectively. The octant rule for substituted cyclohexanones described by Moffit et al.,[32] in 1961 greatly influenced the work on the determination of absolute configurations, but in some cases resulted in oversimplified applications. Nevertheless, the octant rule led to a series of chirality sector rules. One of them was "the benzoate sector rule,"[33] which was the first of Nakanishi's papers dealing with chiroptical spectroscopy (which now exceed 120 in number). Soon after, an attempt based on MO treatment of optical activity was made for theoretical justification of dividing the space surrounding the benzoate group into eight sectors.[34] However, as in other sector rules, the division of space into discrete sectors is often ambiguous. Moreover, the most stable benzoate conformation adopted in those papers turns out not to be correct based on modern evidence.

Around this time, Nakanishi was interested in the possibility of determining the absolute configurations of vicinal diols based on the CD of their acetonides. Harada therefore started investigating the CD of benzoates (λ_{max}227 nm). They soon found that "in glycol dibenzoates having interacting chromophores, the band gives two strong Cotton effects of the same amplitude ($\Delta\epsilon$ 9–18) but of opposite signs around 233 nm (first Cotton) and 219 nm (second Cotton). The splitting indicates that the two Cotton effects are mainly due to a dipole-dipole interaction between the electric transition moments of the two benzoate chromophores and that the Cotton effects are separated from each other by a Davydov splitting ($\Delta\lambda$)."[35] It was concluded that "if the chiralities of dibenzoates are defined as being positive or negative, respectively, according to whether they form a right- or left-handed screw, then the sign of the first Cotton effect around 233 nm is in accordance with the chirality ("dibenzoate chirality rule"). This was published in mid 1969[35] with references to the theoretical background provided by Davydov, Schellman, and Mason. In this first paper on the dibenzoate chirality, the method was also applied to the C-9/C-10 glycol system of taxinine. In a paper published together with Masato Koreeda (a classmate of Harada's, currently at Michigan U.), the dibenzoate chirality method was extended to tribenzoate systems such as steroidal 2,3,11-tribenzoates in which the third benzoate was non-adjacent. They reported that the effect of the third non-adjacent benzoate group was additive.[36] These results were later developed to the pairwise additivity rule.

The chirality method soon expanded by using chromophores other than benzoates. In 1969, Harada and Nakanishi applied the method to the interaction between the 4-methoxybenzoate (256 nm), chosen for its longer wavelength absorption and hence more efficient coupling with the other naphthalenoid chromophore (270 nm)in the clinically used chromomycin A_3 antitumor antibiotics to determine their absolute configurations.[7] The period around 1968–69 was quite productive for the Nakanishi group: they discovered the first insect molting hormones from plants, the phytoecdysteroids (1966),[5] which was followed by many other new ecdysteroids, determined the structures of the ginkgolides (1967), and found NOE, unknown at that time in natural products,[2] and determined the structure of the meiosis inducing substance from starfish (1969).[37] However, the biggest event was the move of Nakanishi from Tohoku University to Columbia University in August 1969, where he set up a new research group in natural products chemistry. Harada and Koreeda were in

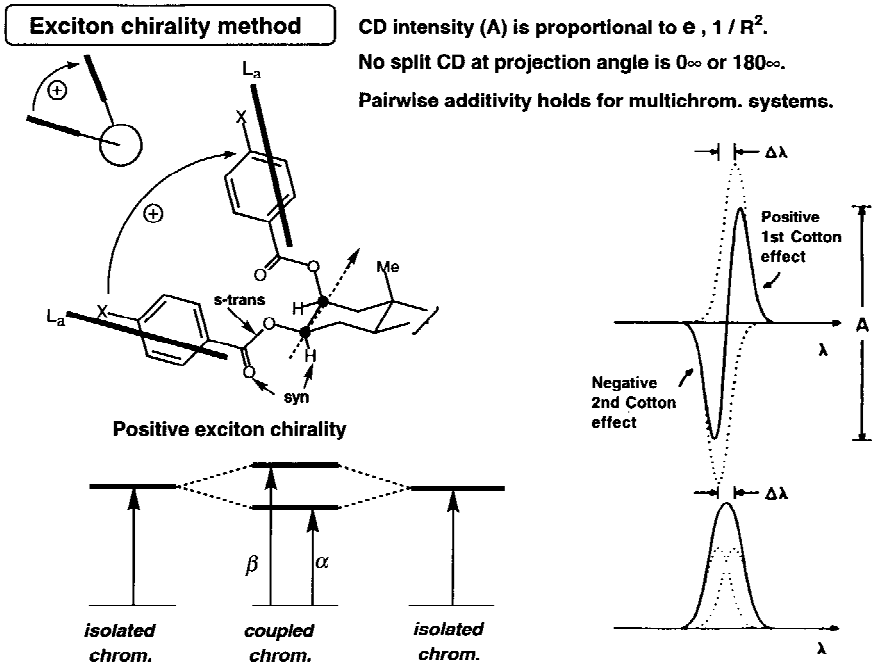

Fig. 1. Exciton coupling of two identical chromophores with positive chirality; in the preferred conformation the carbinyl hydrogen at the stereogenic centers are *syn* to the ester carbonyl and the (CO)-O-bond is *s*-trans. **Left:** The splitting of excited states. **Right:** Summation CD and UV curves (solid line) of two bisignate components of the exciton couplet (dotted line).

the team of the earliest postdocs to join the Nakanishi group at Columbia.

In New York, the group worked on the bis- and trisbenzoates of pyranoses, saponins, and genins,[38] the first entry into the sugars that later would provide solid experimental and theoretical evidence for the validity of the pair-wise additivity principle, a critical aspect of CD (see below). One of the first graduate students to join the group, Jim Dillon (now at Columbia U., Medical School) developed a method using the NMR shift reagents $Pr(DPM)_3$ and $Eu(DPM)_3$,[39] and then with Dave Schooley (U. Reno, Nevada) and others, applied it to determine the absolute configuration of the insect juvenile hormones.[40] However, this method, unlike ECCD, is empirical and hence not general and should be used with caution. ECCD was also applied to a variety of natural products containing various chromophores including alkaloids such as spirobezylisoquinolines[11] and the plant hormone abscisic acid.[12] At this time Nakanishi and Harada published their first review on the early stages of the exciton chirality method and its application in structural studies (Fig. 1).[41]

Trends in Exciton Chirality Method of Practical Significance and Some Early Applications

Although Nakanishi and Harada had already successfully applied the method to a wide variety of interacting chromophores, the need to clarify, both experimentally and theoretically, various factors which define the chromophoric interactions became a crucial point. Significant progress was achieved when they applied the 4-dimethylaminobenzoate chromophore, which is still one of the favorite chromophores for exciton coupling. By insertion of the first chromophore at C-3 of steroids and placing the other chromophore at various sites including the remote C-15, the study revealed two important trends in the CD exciton coupling: *the intensity of the split Cotton effect is (1) maximal at a dihedral angle of 70°, and is (2) inversely proportional to the square of the interchromophoric distance.*[42]

An important application of the ECCD method was determination of the structure and absolute configurations of the adducts formed between two potent carcinogenic polyaromatic hydrocarbons (PAH), benzo[a]pyrene[13a] and 7,12 dimethylbenz[a]anthracene 5,6-oxide.[13b] In the latter case, the point of attachment of the PAH moiety to the guanosine base was determined by estimation of the pK′ values of the base by titration and measurements the CD spectra; namely, raising the pH deprotonates the base and changes its UV absorption, and since the CD results from exciton coupling between the PAH and the purine base, the CD curve also changes with pH. This approach is very interesting and could be used as a sensitive micro method for pK′ measurements when two chromophores, at least one of which has a pH sensitive group, are exciton coupled.[43]

Principle of Pair-Wise Additivity: Exciton Coupling Between Identical Chromophores

One of the most important features of ECCD, especially from a practical viewpoint is the "principle of pair-wise additivity." The additivity relation in optical activity was proposed a long time ago by Kauzmann et al.[44] and Brewster.[45] However, these earlier studies encountered severe difficulties since they were based on measurements of usu-

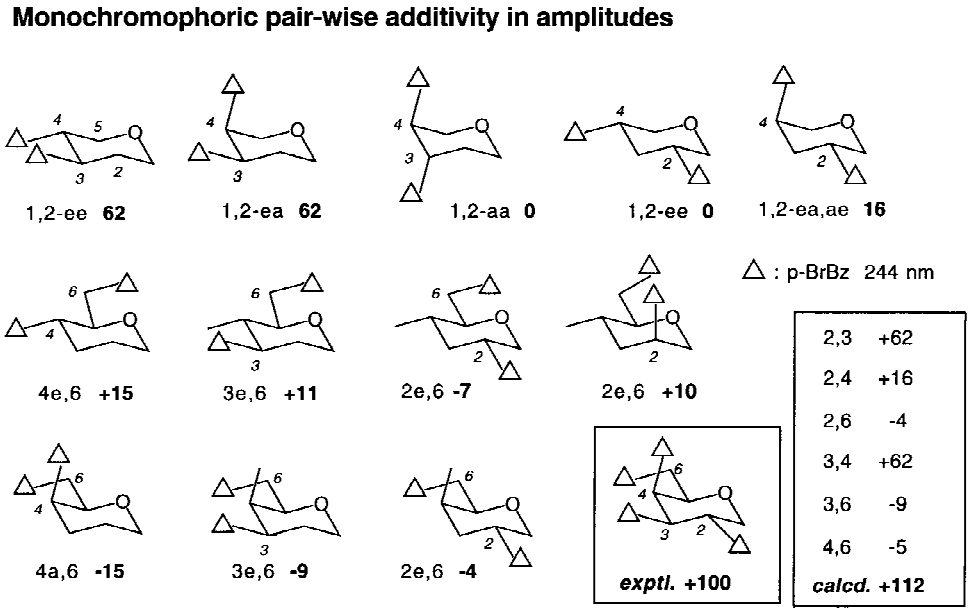

Fig. 2. CD additivity in the presence of more than two identical chromophores: the A value of a multichromophoric system can be approximated by summation of A values of all constituent pairs; the CD amplitude, A value is given in bold; for experimental details see reference 46.

ally weak values of the optical rotations at 589 nm (sodium D line).

The interest to prove this principle in exciton coupling between three or more interacting chromophores led Nakanishi and co-workers to accumulate massive experimental data over the years. Based on the experimental results and very recent theoretical calculations (*see below*), it is possible now to make the following statement: *the amplitudes (A values) of split CD curves can be approximated by the sum of constituent pair-wise interactions between the chromophores; when chromophores of different types are coupled, the entire CD curve can be represented by pair-wise summation of interacting chromophores, thus producing a fingerprint CD* (Fig. 2).

A look through Nakanishi's papers showed that this trend was already seen in his very early data.[38,40] Ben Liu (Minnesota U.) performed an exhaustive systematic study by making over 40 bis-, tris-, and tetrakis(p-bromobenzoates) of various methyl pyranosides covering the Glu, Man, Gal, and desoxysugars, and showed that excellent agreement existed between the experimental and pair-wise summation of A values; for example, the amplitude of a tetrakis(benzoate) can be approximated by adding the amplitudes of the six component bis(benzoate) units.[46] This additivity relation, in addition to the large amplitudes of the coupled CD, formed the basis for the development of a microscale method for determining oligosaccharide structures without reference compounds.[47] In parallel studies, it was found that the additivity relation is a more general property operating also between various chromophores such as benzoates, enones, and furans.[48] The additivity studies were also applied to the trichothecenes, tetracyclic sesquiterpenes that are important toxins causing diseases in humans and agricultural animals; despite the congested cage structures containing up to 4 hydroxyl groups, the additivity was still valid and allowed Nakanishi et al. to determine the structure of a new trichothecene at the sub-microgram level.[49] Importantly, the additivity principle led to a simple microscale method for determining the branching point in oligosaccharides; namely, the sugars are permethylated, hydrolyzed, and the liberated hydroxyls, which are tags of the linkage point, are bromobenzoylated; each sugar benzoate is then readily identified from the CD amplitude (microgram level).[50]

It was further found that the additivity relation is true also for benzyl chromophores such as p-phenylbenzylates of sugars, indicating that the benzyl groups adopt a fixed conformation similar to the benzoates.[51] This additivity was proven by preparing all permutations of bis, tris, and tetraquis-benzylates of Glu, Man, and Gal; importantly, the benzylates can be oxidized in 60% yield to the corresponding benzoates, in which the sensitivity is increased by 5-fold, because of their more intense absorptions.[51] The advantage of benzylates is that, unlike the benzoates, they are not cleaved under methanolysis conditions employed in saccharide cleavage; also benzylation can be performed on hindered hydroxyl groups.

Principle of Pair-Wise Additivity: Exciton Coupling Between Different Chromophores

In 1983 Harada and Nakanishi published a superb monograph describing the basic principle, the broad range of practical applications, and the theoretical background of circular dichroic exciton chirality method.[52] This monograph soon became one of the most valuable and frequently quoted books on chiroptical spectroscopy, which provided to organic chemists an overview of the immense potentiality of the CD based on exciton coupling. Both experimental and theoretical results showed that when vicinal diols are acylated with different para-substituted

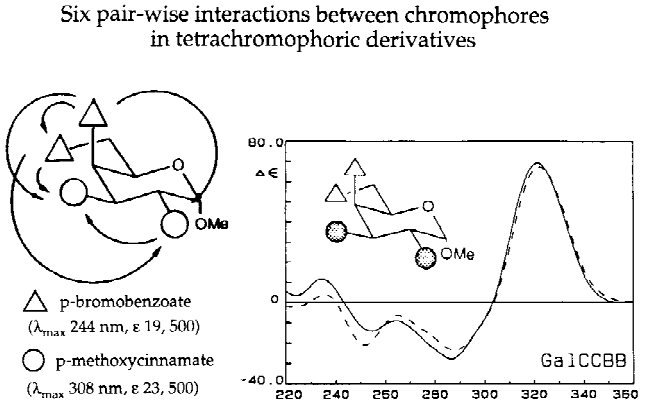

Fig. 3. Summation curve of the six interacting CD pairs (solid line) and observed (dashed line) CD of Gal-4,6- bis -p-bromobenzoate-2,3-p-methoxycinnamate in acetonitrile.

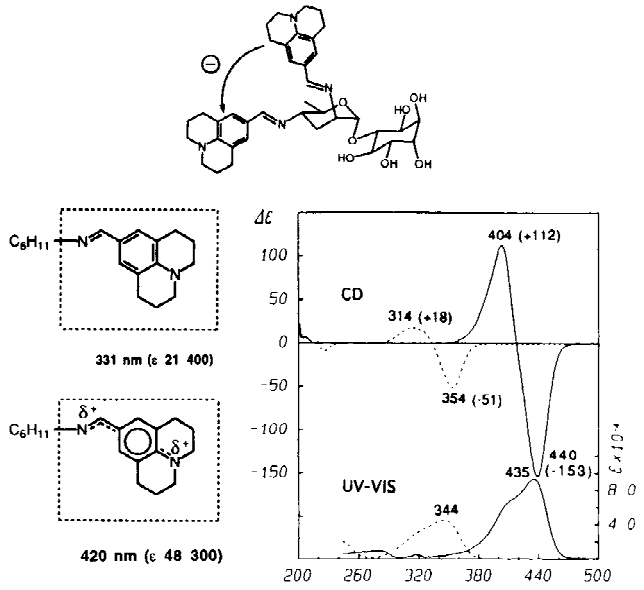

Fig. 4. UV-vis and CD spectra of the neutral (dashed line) and protonated (solid line) species of kasugamine bis julolidine derivative in acetonitrile.

benzoates, *the two chromophores can still couple even if the absorption maxima differ by 90 nm*.[52] This led to the conclusion that the absolute configurations of allylic benzoates, acyclic as well as cyclic, could be determined simply from the sign of the first Cotton effect with approximately λ_{max} 227 nm, ascribed to the ^1L_a benzoate transition, even though the measurement of the Cotton effect arising from the 195 nm double bond absorption may be difficult. In 1982 the "allylic benzoate method" for determining the absolute configurations of cyclic and acyclic allylic alcohols was published;[53] this provided rationalization for the empirical rules forwarded by Mills[54] and Brewster.[45] This simple method was used in establishing the absolute configuration of the important antiparasitic macrocyclic lactone nemadectin[25] and extended further to deal with hydroxyl groups flanked by allylic and homoallylic bonds (Fig. 3).[55]

Another important advancement in ECCD with great potentialities was the finding by Bill Wiesler, Jesus Vázquez (currently at U. de La Laguna), and Nakanishi that the principle of pair-wise additivity observed by identical chromophores could be extended to multichromophoric systems containing two different type chromophores, i.e., the additivity in A value in case of identical chromophores could be extended to encompass the entire CD curve.[56] This was demonstrated by using methyl α-D-glucopyranoside as a model system with p-bromobenzoate (λ_{max} 245 nm) and p-methoxycinnamate (λ_{max} 307 nm) as chromophores. All 24 dichromophoric diacetates (6 dicinnamates, 6 dibenzoates, and 12 monocinnamate monobenzoates) were prepared to represent the pair-wise interactions contributing to the complex CD curves. In 20 tri- and tetrachromophoric cases prepared to check the additivity, the spectral summation of the three or six corresponding constituent pair-wise interactions, respectively, yielded excellent empirical calculation of the observed CD curves throughout the 200–400 nm region.[56] This was then extended to include all other Gal and Man sugars, amino sugars, and desoxy sugars to give reference spectra of 150 different glycopyranosides; these characteristic curves provide valuable data for the development of an alternative means to conventional methylation analysis of oligosaccharides.[57]

Chromophores With Red-Shifted Absorption Maxima

It was already known that the amplitude of the CD exciton couplet is inversely proportional to the square of interchromophoric distance[42] and proportional to the square of extinction coefficients[52a,58] of the coupled chromophores. Therefore, the stronger the absorption, the more sensitive the method. Furthermore, in order to limit the complications arising from overlap with preexisting chromophores in the substrate, or as in the case of biochemical preparations, to avoid overlap with the approximately 280 nm band arising from proteins, nucleic acid bases, or impurities, much of Nakanishi's recent effort has been to develop highly sensitive and "red-shifted" chromophores for hydroxyl, amino, and other groups.

These studies started when Greg Verdine (now at Harvard University) used the p-dimethylaminocinnamate chromophore, λ_{max} 362 nm (ϵ 30,400) to determine the stereochemistry of the aziridine ring cleavage in chromomycin C,[59] which was performed during the crosslinking and other studies of this antitumor antibiotic to DNA.[17] It was in the mid-1980s when Derguini (Memorial Sloan-Kettering Cancer Center), Nakanishi, and others prepared with merocyanine a new bacteriorhodopsin analog exhibiting a strong red-shifted absorption from the regular 560 to 662 nm due to the cyanine dye structure of the corresponding protonated Schiff base.[60]

An important development in the early CD studies of red-shifted chromophores was the employment of this remarkable cyanine dye for exciton coupling. The biscyanine dye formed from trans-1,2-cyclohexyldiamine and merocyanine even showed a separation, a very rare phenomenon, in its electronic absorption bands at λ_{max} 550 (ϵ

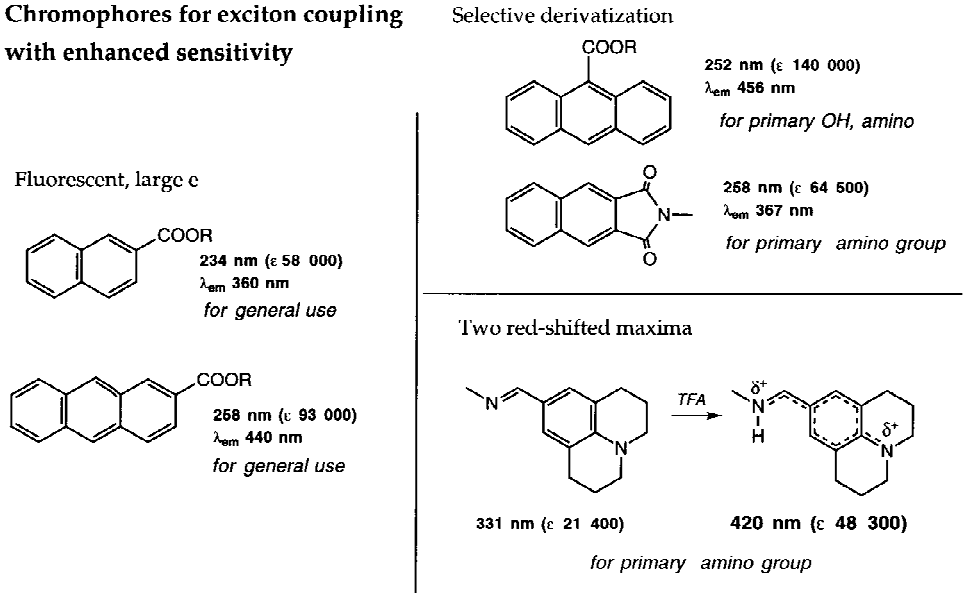

Fig. 5. Chromophores with favorable properties for CD studies.

182,000) and 480 nm (ϵ 19,100) due to intense exciton coupling and narrow half-bandwidth.[61] The "cyanine dye" idea of Nakanishi proved to be fruitful. Later on five more red-shifted chromophores, all cyanine dye type, were developed, the simplest being with p-dimethylaminobenzaldehyde, and used in microscale configurational studies (Fig. 4).[62] Since these aldehydes contain a p-amino function, the Schiff base, formed spontaneously with primary amino group in the substrate, yields upon protonation a cyanine type dye with greatly enhanced absorption intensity and very large red-shift. For example, dimethylaminobenzaldehyde gives with cyclohexylamine a Schiff base with an absorption maximum at 305 nm (24,300), which upon addition of one drop of TFA shifts to 395 nm (51,700); the protonated Schiff base is hydrolyzed overnight by addition of water, and the starting amino compound is recovered.[63]

Fluorescent CD Chromophores: Application to Acyclic Polyols, Aminopolyols, and Sphingosines

For the past 10–15 years, acyclic polyols and aminopolyols have always been in the focus of Nakanishi's interests. Although many acyclic polyols exist in nature and are biologically very important, as exemplified by polyene macrolides, bacteriohopanoids, and brassinosteroids (acyclic side chain), the determination of their relative and absolute configurations still remains a difficult task and depends mainly on synthesis unless X-ray crystallographic studies can be performed. Many polyols, including those derived from polyene macrolides, have terminal primary hydroxyl or amino groups. Moreover, many of the acyclic systems exist in nature in minuscule amounts. For this reason Nakanishi's efforts aimed to develop highly sensitive microscale chemical/chiroptical methods based on employment of new fluorescent CD chromophores (Fig. 5).

With this in mind, Wiesler designed a simple two-step microscale chemical/chiroptical protocol for assigning configurations to 1,2,3-triols, 1,2,3,4-tetrols, and 1,2,3,4,5-pentols.[64] Namely, a selective 9-anthroylation of the primary hydroxyls followed by per-p-methoxycinnamoylation of sec-hydroxyls affords bichromophoric derivatives, the CD spectra of which are characteristic and predictable for each stereochemical pattern. The fingerprint CD curves obtained are rationally interpreted by considering the additive effects of pair-wise interchromophoric exciton coupling, which occur in the conformations indicated by NMR.[65] The knowledge of the characteristic trends of CD spectra makes possible the determination of the full side chain amino-pentol configuration of aminobacteriohopane polyols without reference compound on a microgram scale.[66] Likewise, it was possible to deduce general trends for the entire set of Emil Fischer's sugar tree family up to pentols.[67]

A similar approach was developed by Peng Zhou for acyclic 1,3-skipped polyols where the application turned out to be much easier because the trends accompanying 1,3-chain extension were more uniform and easy to predict.[68] The most recent studies in the acyclic polyol series dealt with the more challenging 1,2/1,3-mixed pentols; again the bichromophoric method involving anthroylation and p-methoxycinnamoylation step led to the determination of two bacteriohopanoids on microgram scale (Fig.6).[69]

A seemingly simple but very challenging project was to develop an uncomplicated and efficient method for configurational assignments of sphingosines containing only two stereogenic centers. The breakdown products of sphingolipids such as ceramide, sphingosine, and sphingosine-1-phosphate are involved in cell regulation and exhibit a wide variety of activities related to signal transduction, one being the inhibition of protein kinase C. The sphingolipids occur in minuscule quantities; furthermore, some dihydro-

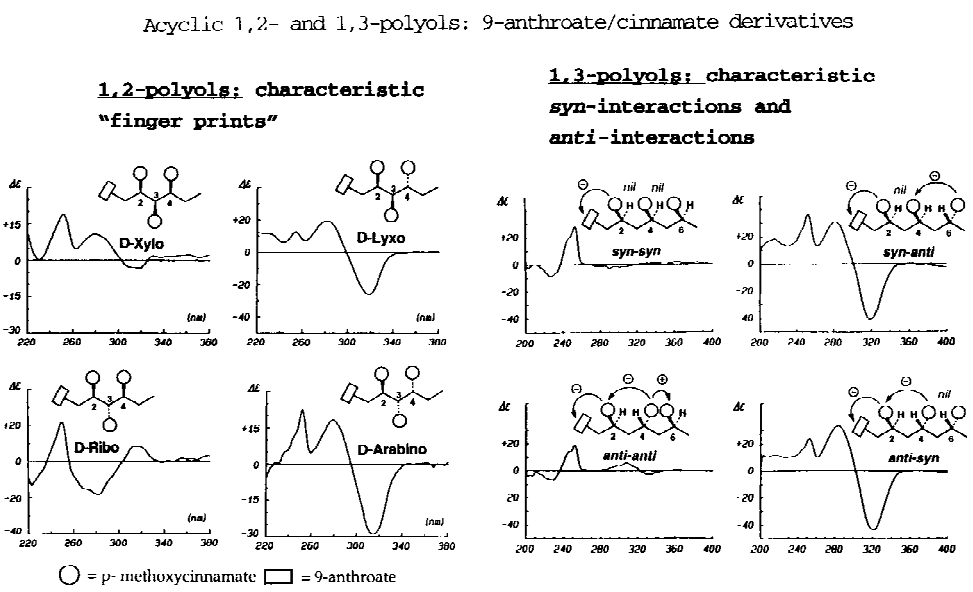

Fig. 6. Reference CD curves of 9-anthroate/p-methoxycinnamate derivatives of some 1,2-polyols (in methylcyclohexane) and 1,2/1,3-polyols (in acetonitrile).

sphingosines appear to exhibit their own bioactivity. After numerous attempts (an unusual large number of Nakanishi's co-workers were involved in this project!) Verena Dirsch finally succeeded in developing a rapid and general bichromophoric CD method to distinguish all four possible stereoisomers of sphingosines at the level of about 1 μg. The protocol consists of using two new CD chromophores, 2,3-naphthalene dicarboxylic acid anhydride to convert the primary 2-amino function into the fluorescent naphthimide derivative, followed by conversion of the 1,3-diol to the fluorescent 2-naphthoate.[70] Surprisingly, the dihydrospingosines bichromophoric derivatives in which the allylic double bond of sphingosines are simply reduced showed different CD spectra from the parent compounds. Hence, the previous bichromophoric method was extended to include the dihydro derivatives, thus bringing the total number of isomers to be distinguished to eight. Furthermore, Akira Kawamura, a graduate student, found that by the use of chiral HPLC columns, the eight isomeric fluorescent naphthimide derivatives could be differentiated; by performing the ultra microscale derivatization in a melting point tube and heating in a melting point apparatus, now characterization of the eight isomers could be performed with about 1 ng of sample.[71] At this level it should be possible to carry out metabolic studies to clarify the physiology of the intriguing and little understood sphingosines.

Tetraarylporphyrins-Potent CD Chromophores

For some time Koji Nakanishi had been interested in applying porphyrins to structural studies based on exciton coupled CD (Fig. 7). I remember also he was very impressed by Dr. E. Vogel's results (Köln University) in the

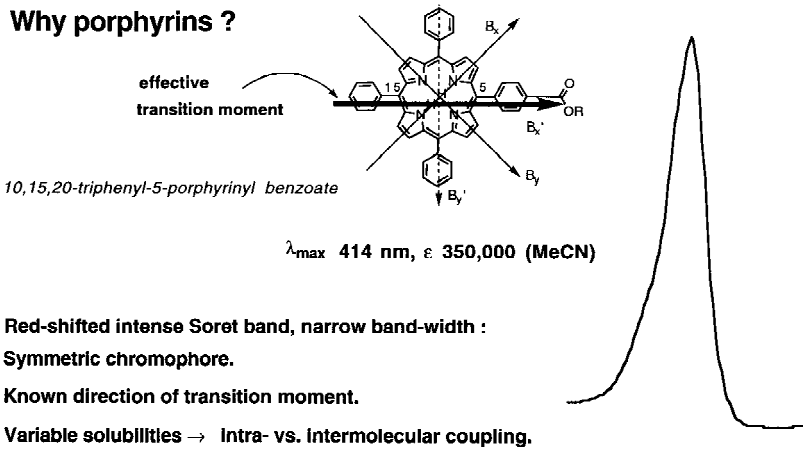

Fig. 7. Tetraarylporphyrins as powerful CD chromophores.

early 1990s on synthesis of new porphyrins with unusual high molecular extinction coefficients close to 1 million! When in 1994 Stefan Matile (Zürich University) joined Nakanishi's group, bringing his excellent experience in porphyrin chemistry, we soon found that tetraarylporphyrins are superb chromophores for ECCD, and that they have many advantages when applied to biopolymers.[72] Among these are the following properties:

1. They exhibit intense and sharp Soret bands at 414 nm, thus avoiding overlap with most other bands.
2. The intensity of ϵ 350,000 makes certain porphyrins highly sensitive chromophores, thereby further reducing the amount of sample needed for analysis. For example, the amplitude of a steroidal 3,6-bisdimethylaminobenzoate was +89, whereas the amplitude of the corresponding porphyrin derivative was enhanced by 7.6-fold, i.e., +675 (Fig. 8).[72b]
3. The intense absorption also led to a through space coupling over a long distance. When porphyrins were attached to both ends of the 30-Å-long red tide toxin brevetoxin B, exciton couple CD was observed despite the interchromophoric distance of about 50 Å.[72b].
4. From the viewpoint of exciton coupled CD, the polarization of the electric transition moment can be regarded as being in the same direction as the bond connecting the substrate to the porphyrin chromophore, i.e., as in other acylates.[72b]
5. By changing the pendant groups from phenyls to 3-pyridine, to pyridine methiodides, porphyrins covering a wide range of hydrophobicity/hydrophilicity can be prepared. A variety of porphyrin chromophores were attached to brevetoxin B and submitted to detailed CD studies, which showed for the first time that, contrary to common belief, the brevetoxins themselves form sodium specific transmembrane channels.[73]
6. Depending on the extent of the hydrophobic/hydrophilic nature of the porphyrin moiety, the molecules can be induced to undergo intermolecular or intramolecular stacking. All these attributes are being taken advantage of in ongoing studies. The intramolecular stacking properties of a chiral molecule to which two hydrophobic porphyrins have been linked have led to a novel ECCD method for determining the absolute configuration of a single stereogenic center.[74] Recently Koji Nakanishi's interests in porphyrins are taking new directions. Studies using the porphyrin chromophore for other purposes, including biopolymers, are in progress.

Theoretical Treatment of Exciton Coupling

In my opinion, Koji Nakanishi's interests in the theoretical treatment of CD always have been related to one or another of the ongoing structural studies and provided another alternative for solving difficult problems. The vinblastine alkaloids, important anticancer drugs, are a case in point. During the studies on these alkaloids, it became well known that the configuration at the C-16′ stereogenic center, which links the indoline and indole moieties, should be *S*. The physiologically active bisindole alkaloids with this configuration exhibit characteristic CDs in which the Cotton effects at about 210 and 220–230 nm are negative and positive, respectively, and vice versa in case of inactive analogs.[75] Another Nakanishi graduate student, Jian-Guo Dong, recently performed SCF-Cl-dipole velocity MO calculations (the computational program, CD/NH-Sendai) and has shown that the bisignate CD curves are due to exciton coupling between the indoline and indole moieties.[76] The theoretical clarification of the vinblastine CD should play an important role in the studies of the mode of binding of these alkaloids to tubulin and contribute to the understanding of their mode of action.

Ample experimental evidence has shown that the principle of pair-wise additivity holds in exciton coupled CD systems consisting of three or more interacting chromophores. This additivity principle lies at the core of interpreting complex CD spectra and is one of the most important from a practical viewpoint. However, the theoretical proof of this empirical rule for a long time remained opened and intriguing to Koji Nakanishi. In order to check the additivity principle by calculation, ouabagenin 1,3-bis-, 1,19-bis-, 3,19-bis-, and 1,3,19-tris-naphthoates were chosen as models. Because the 2-naphthoate chromophore can adopt either the s-*cis* or s-*trans* conformation and the C-19 side chain of ouabagenin is flexible, they represent a more complex case than the symmetrically substituted p-substituted benzoates. The conformations of the naphthoate derivatives were calculated by Monte Carlo conformational search. The p-electron SCF-CI-DV MO calculation was then applied to all conformers within the 1 kcal/mol range from the lowest energy conformer. Good agreements between the experimental and theoretically calculated CD spectra were obtained for all ouabagenin bisnaphthoates and the trisnaphthoate. Summation of the three computed CDs of bisnaphthoates almost overlapped with the CD of the trisnaphthoate; the summation curve of the three experimental CDs was also in excellent agreement with the experimental trisnaphthoate CD. Thus the principle of pair-wise additivity has been shown to be valid both by experiments and by theoretical calculation.[77]

Ouabain is a rhamnose-containing saponin with one primary, five secondary, and two tertiary hydroxyls. The CD of all possible ouabain pentanaphthoate isomers that differ in the point of attachment of rhamnose, namely C-3 (i.e., ouabain), C-1, -19, -11, and -5 rhamnosides have been calculated. Eight of these isomeric pentanaphthoates have also been synthesized in addition to ouabain pentanaphthoate, and their CD spectra compared with the calculated spectra. The calculations were based on a combination of the Monte Carlo conformational search of molecular modeling and the p-electron SCF-CI-DV MO method. Except for one case, there was good agreement between the theoretical and the experimental data.[78] It is believed this is the first case where the CD calculations have been performed on a complex multichromophoric system such as ouabain pentanaphthoate. The experimental data combined with such theoretical calculations further enhance the potentiality of exciton coupled CD as a powerful and versatile physical tool.

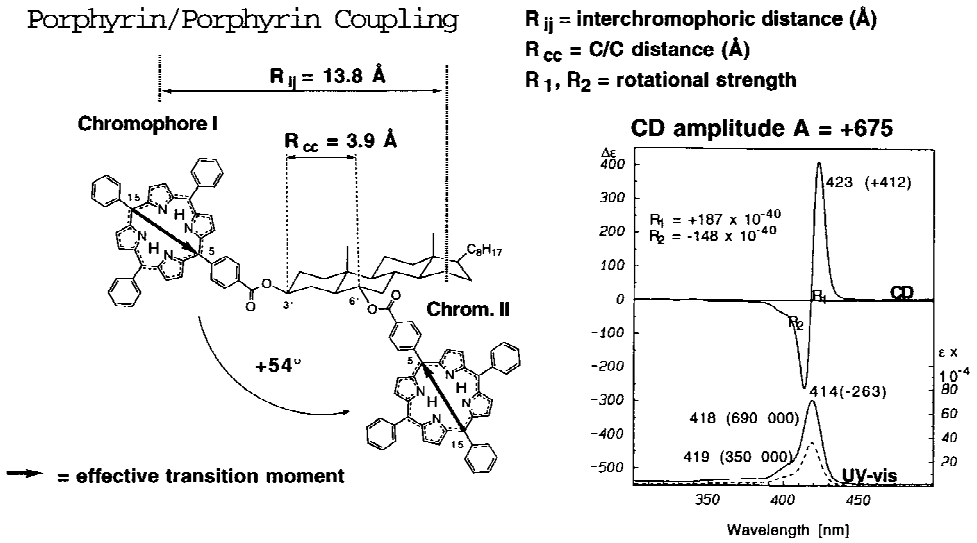

Fig. 8. CD and UV-vis data of the bisporphyrin derivative of 5α-cholestan 3β,6α-diol (solid line) and the UV-vis spectrum of the monoporphyrin derivative of (-) *trans*-(1R,2R)-1,2-cyclohexane diol in CH_2Cl_2.

CD Studies With Biopolymers

Over the past two or three decades, CD spectroscopy has made an enormous progress in many fields where biopolymers are involved. The CD data have in many cases provided invaluable information about the drug/receptor interactions and have demonstrated the close relevance of chirality to biological response. Many challenging bioorganic problems have also inspired Koji Nakanishi to try CD beyond conventional experience.

During our studies on rapamycin, a potent immunosuppressive agent, an approach based on CD and second derivative analysis was developed. CD measurements of the complex formed between FKBP (FK 506 binding protein) and three inactive rapamycin analogs (group I) and three bioactive rapamycins (group II) showed an interesting trend. The CD of group I rapamycin/FKBP complex were similar with the summation CD curve of FKBP and rapamycins. In contrast, the CD of the active complex differed from the FKBP/rapamycin summation CD. Namely, the second derivative CD of the active group II showed that in the complex, the CD with vibrational fine structure arising from the rapamycin triene moiety became more pronounced (with 1,600–1,650 cm^{-1} intervals) and also changed the sign of the Cotton effects.[79] The enhanced fine structure in the group II/FKBP complexes, relative to free compounds, demonstrates that the triene moiety adopts a more planar rigid conformation. CD suggests that the protruding triene undergoes subtle conformational changes upon binding to FKBP. It is possible that this conformational change dictates the binding of the rapamycin-FKBP adduct to its effector protein. Such information gained from the CD of a drug-protein complex should be valuable in elucidating the interaction on a molecular basis. CD, coupled with second derivative analysis method, offers a unique tool for studying subtle conformational changes arising from ligand-receptor interactions.

In the visual pigment rhodopsin, the 11-cis-retinal chromophore is present within the binding site in a nonplanar conformation with twists around two single bonds, the 6-s- and 12-s-bonds. The extent and direction of the twists play a central role in wavelength regulation of vision. The absolute sense of twist around the C-12/C-13 single bond has recently been established by incorporating two retinal analogs in which the six double bonds of the polyene moiety were separated into two halves by hydrogenating one of the central double bonds, namely C-11/C-12. The CD of the two rhodopsins incorporating these dihydro retinal analogs both showed bisignate CD curves arising from exciton coupling of negative chirality, thus establishing the sense of twist around C-12/C-13 as counter-clockwise.[29] These results further demonstrate the versatility of the exciton split CD method in probing subtle conformational aspects on a broad range of subjects involving ligand receptor interactions.

Finally, most fitting of all, perhaps, and certainly worthy of saying, is that Koji Nakanishi's outstanding accomplishments in various areas related to chirality are contributions not only of great scientific merit. Often they have suggested new challenging opportunities and directions for future research. As a person of great intellectual power, optimism, and generosity, he has also inspired and motivated many young scientists in different fields. The large response and support of his colleagues, friends, and former students to this issue is certainly a kind of reward. We all wish Koji Nakanishi many more years of professional and personal success.

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