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Chemistry of Astaxanthin

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Astaxanthin ($C_{40}H_{52}O_4$: 3, 3'−dihydroxy−β, β−carotene−4, 4'−dione) is the most commonly occurring carotenoid in aquatic organs. It has two carbonyl groups, eleven conjugated ethylenic double bonds and two hydroxy groups which make esterification possible. The pink to red coloring of many echinoderms and crustaceans, the skin of several fishes, and the meat of many salmonid fish consist partly or wholly of astaxanthin and its esters.[1-4]

Astaxanthin was first isolated from the shell of lobster as the form of astacene (β, β−carotene−3, 3', 4, 4'−tetraone) by Kuhn and Lederer[5] and it was proposed that astacene is partly combined with protein and partly esterified in animal tissues. Later astaxanthin was isolated from the lobster eggs[6,7] and the structure of 3, 3'−dihydroxy−β, β−carotene−4, 4'−dione was determined based on the structure of astacene by Karrer et al.[8,9]

Since astaxanthin was isolated from the lobster, it has been found in a number of organisms. It was originally believed that astaxanthin was a typical animal carotenoid. However, the isolation of astaxanthin from the green algae, *Haematococcus pluvialis,*[10] the flagellate, *Euglena heliorubescens,*[11] the yeast, *Phaffia rhodozyma*[12] and the higher plant, *Adonis annua* L.[13,14,15] and *A. aestivalis*[16] disproved the view that astaxathin is purely an animal pigment.

In the present paper, chemical constitution, chemical and physical properties and synthesis of astaxanthin are discussed.

Structure of Astaxanthin

The structure of astaxanthin was determined based on the structure of astacene. Karrer *et al.*[8,9] determined the structure of astacene based on characteristic reaction of astacene. Elementary analysis of the compound gave the molecular formula $C_{40}H_{48}O_4$, which differs from that of β−carotene only by the 8 fewer hydrogen and 4 additional oxygene atoms. Astacene (enol form) react with hydroxylamine (NH_2OH) and forms astacene dioxime. Astacene dioxime has four active hydrogen atoms. Two of those are derived from the oxime residues, while the other two must be due to the

enolization of the other two carbonyl groups. The four keto groups, therefore, differ in nature, two being capable of forming an oxime, and the other two undergoing enolization. Astacene (keto form) react with *o*－phenylene－diamine and forms the bis－phenazine derivative which shows that each pair of carbonyl groups must be adjacent. By the oxidation of astacene with permanganate, dimethylmalonic acid is produced, and α, α－dimethyl succinic acid is isolated by the oxidation of bis－phenazine derivatives. These reactions indicated that 4 carbonyl groups are at the 3, 3', 4, 4'－positions. Therefore, the structure of astacene is β, β－carotene－3, 3', 4, 4'－tetraone.

The structure of astaxanthin (3, 3'－dihydroxy－β, β－carotene－4, 4'－dione) was determined by Kuhn and Sorensen[6,7] based on the structure of astacene. This formulation is based on the fact that, in the absence of air, astaxanthin forms a deep blue salt in potassium hydroxide solution, while under aerobic conditions the pigment absorbed exactly 2 moles of oxygen in alkaline solution and is converted into astacene. The convertion of astaxanthin into astacene represents the autoxidation of a di－α－ketol. Fig. 1 shows the structural determination of astacene and astaxanthin.

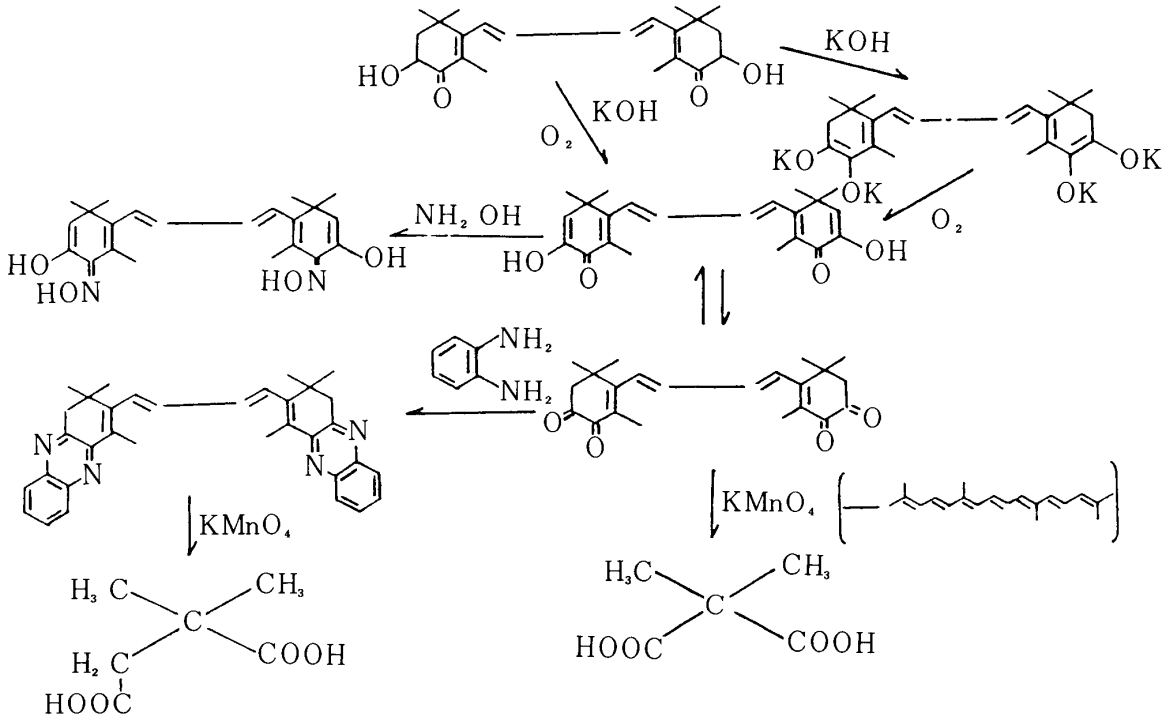

Fig- 1. Structural determination of astaxanthin and astacene

Chemical Reaction of Astaxanthin

Chracteristic chemical reaction of astaxanthin are discussed in the structural determination of astaxanthin (Fig. 1). Another reaction of astaxanthin and astacene, reduction, is also presented.

The IR spectrum of astacene indicated that it is present in the enol form in nature. Borohydride reduction of astacene gave a mixture of stereoisomers, one of which was identified as crustaxanthin, which was isolated from copepoda, *Arctodiaptomus salinus,* and the structure was determined as β, β-carotene-3, 3', 4, 4'-tetrol with bis-trans-α, α'-conformation.[17] Reduction of a natural astaxanthin diester with sodium borohydride gave a product different from crustaxanthin, particulaly with respect its behavior toward allylic elimination (Fig. 2). The reduced compound of astaxanthin

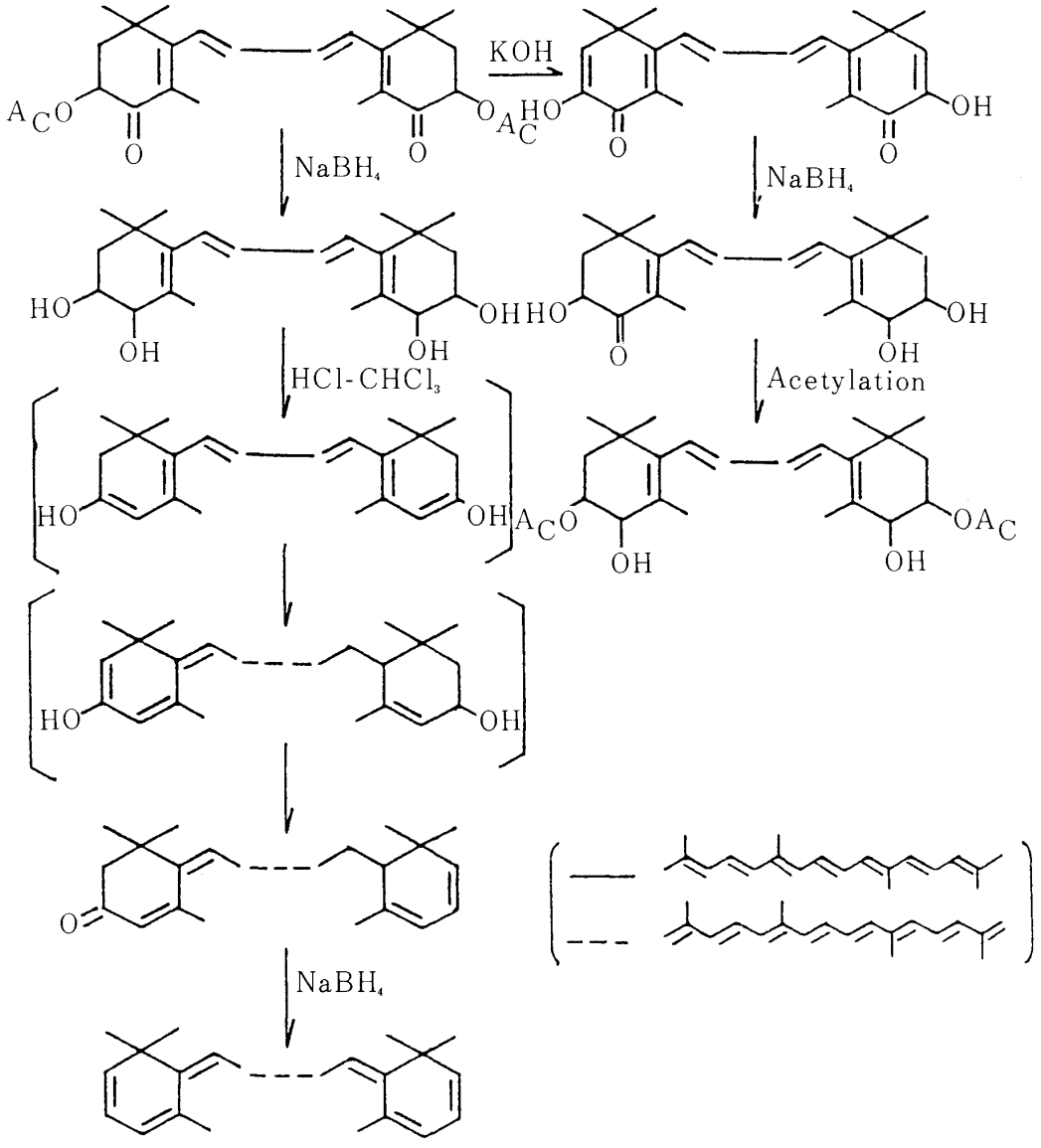

Fig- 2. Reduction of astaxanthin and astacene

diester produced 3−keto−retro−bishydrocarotene with acidic chloroform which indicated that the compound has di−cis−glycol conformation.[18] On borohydride reduction, 3−keto−retro−bisdehydro−carotene underwent spontenous dehydration to anhydro−echscholtzxanthin. Reduction of astaxanthin and astacene is shown in Fig 2.

Physical Properties of Astaxanthin

1. Melting Point (M. P.) : The M. P. of astaxanthin and astacene was reported by several investigators.[6, 9, 19] Kuhn and Sorensen[6] and Karrer *et al.*[9] measured the M. P. of natural astaxanthin and reported 216℃[6] and 212−215℃.[9] While Cooper *et al.*[19] measured the M. P. of synthetic astaxanthin and obtained 182−183℃. These reported M. P. differrences may due to the fact that one astaxanthin was from natural source and the other was synthesized chemically.

The M. P. of astacene was measured and 228−214℃ as assigned.[6, 9, 19]

2. Absorption Spectroscopy : Absorption spectroscopy has been used for the characterization of carotenoids for some fifty years. Light absorption spectra of carotenoids in various solvents are one of the most effective methods for the identification of carotenoids. The carotenoids which have the different types of end groups give different maximum absorption and the different shape of the absorption curves. The characteristic absorption spectrum of a carotenoid is a consequence of the conjugated polyene system present in the molecule and of various additional structural features. The effect of various substitutes on the position of the absorption maxima are summarized by Moss and Weedon.[20] The absorption maxima of the carotenoid in various solvents are reviewed by Davies[21] and Deritter and purcell.[22] Absorption spectra of astaxanthin and astacene in various solvent system have been studied.[23,24] Table 1. shows the absorption spectra of astaxanthin and astacene in 19 different solvents which were reviewed from the works of Buchwald and Jencks,[25] Davies[21] and Deritter and Purcell.[22]

3. Inflared Spectroscopy(IR) : In the past, IR has not played a mojor role in carotenoid chemistry, mainly because the conjugated polyene system gives rise to only very weak bands. It has, however, proven to be of value for detecting certain special structural features such as acetylenic, allenic, hydroxy and keto groups.

Hydroxy O−H stretching frequencies are typically in the region 3400−3700^{-1}cm. Carbonyl C=O streching frequencies occure in a region 1600−1740^{-1}cm. Carotenoids

Table- 1 The absorption spectrum of astaxanthin and astacene in various solvents (Buchwald and Jencks, 1968, Davies, 1976)

Solvents	max. (nm)		
	Astaxanthin		Astacene
	1)	2)	2)
Acetone	475	480	482
Acetonitril	474		
Benzen	488	478	
Carbondisulfide	506	503	500
Carbontetrachloride	485		
Chlorobenzene	493		
Chloroform	489	485	494
Cyclohexane	478		
Dichloromethane	486		
Dmethylsulfoxide	493		
Dioxane	482		
Ethanol	476	478	478
Ethylcinnamate	496		
Hexane	472	468	470
Iodomethane	496		
Methanol	473	472	
Petroleum ether	473	468	473
i-Propanol	478		
Pyridine	492		498

1) Buchwald and Jencks, 1968

2) Davies, 1976

with trans-disubstituted ethylene show a strong band near 965^{-1}cm. due to C-H out-of-plane deformation vibration.[25]

Many investigators have reported on the IR spectra of astaxanthin and astacene in $CHCl_3$ and KBr.[26, 27, 28] Khare *et al.*[24] showed that astaxanthin from Scottish salmon had the peaks at 3500 (OH), 3010, 2970, 2930, 2870 (C-H), 1660 (conjugated C=O),

1440 (CH_2), 1385, 1370 (gem. dimetnyl), 1310, 1280, 990 and 975^{-1}cm. (trans CH=CH). The similar peaks (3620, 3510, 1660, 1610 and 975^{-1}cm (in $CHCl_3$)) were also reported in synthesized astaxanthin from canthaxanthin. The IR spectra of astaxanthin and astacene are shown in Fig. 3.

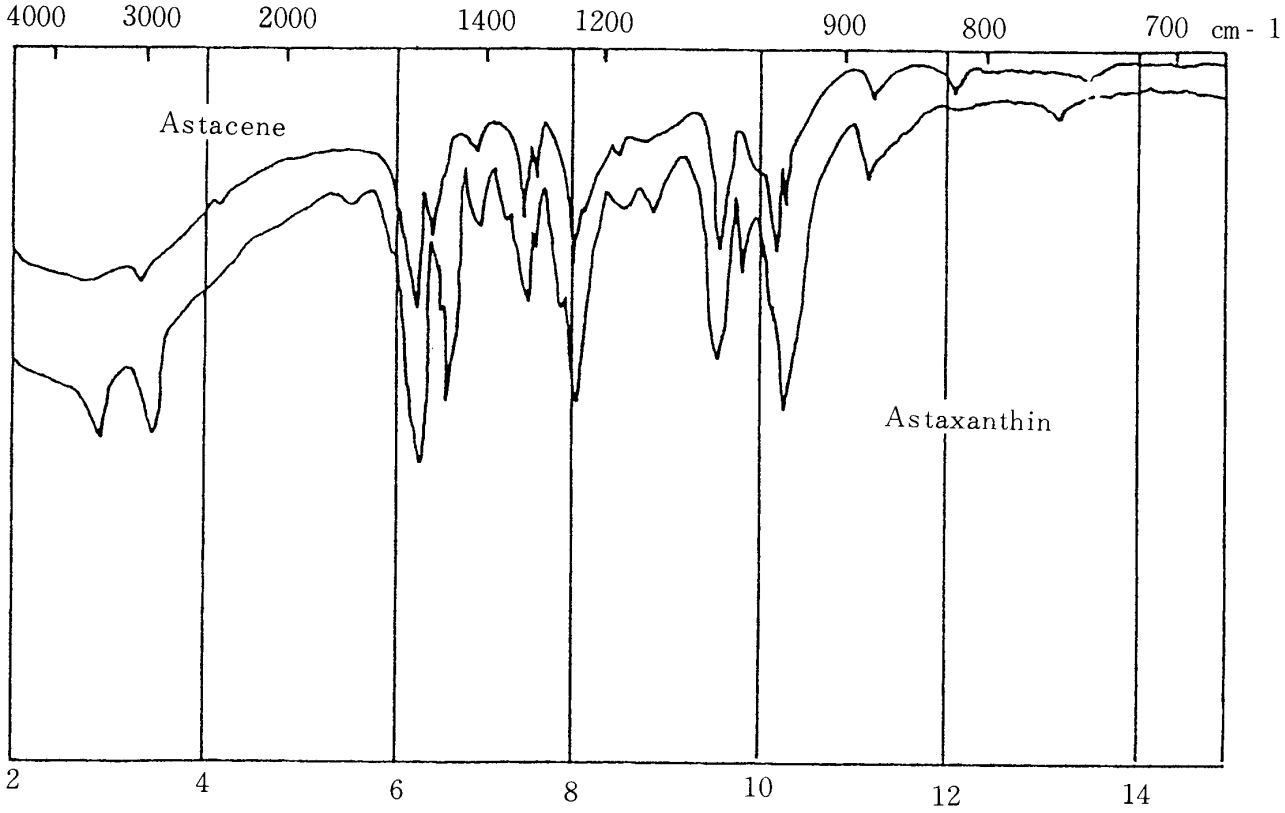

Fig-3. IR spectra of astaxanthin and astacene

4. MS Spectrometry (MS) : Mass spectrometry has tremendous impact on the elucidation of carotenoid structures, particularly when frequently not more than 100ug of a pigment are required for analysis. Not only will a high resolution instrument gave the molecular weight to within 2ppm but a study of the fragmentation pattern may lead quickly to a clear indication of the structure.[20]

One of the most characteristic fragmentation pattern observed with conjugated polyne carotenoid is the loss of C_7H_8 (toluene, M−92), C_8H_{10} (xylene, M−106) and $C_{12}H_{14}$ (M−154). The M−154 ion is often weak or absent. With about eight or more double bonds, M−92 and M−106 ions are usually observed. Their relative intensity is influenced by the length of the polyene chain and other substituents.[29] Typical fragmentation of carotenoid end groups have been extensively reviewed.[20, 30]

The mass spectra of astaxanthin are shown in Fig. 4. The following fragmentations are observed as the characteristic for astaxanthin : M−16 (O), M−154 ($C_9H_{14}O_2$), M−167 ($C_{10}H_{14}O_2$), M−207 ($C_{13}H_{18}O_2$), M−219 ($C_{14}H_{18}O_2$) and M−233 ($C_{15}H_{20}O_2$). M−92 (C_7H_8) and M−106 (C_8H_{10}) were also found.[31]

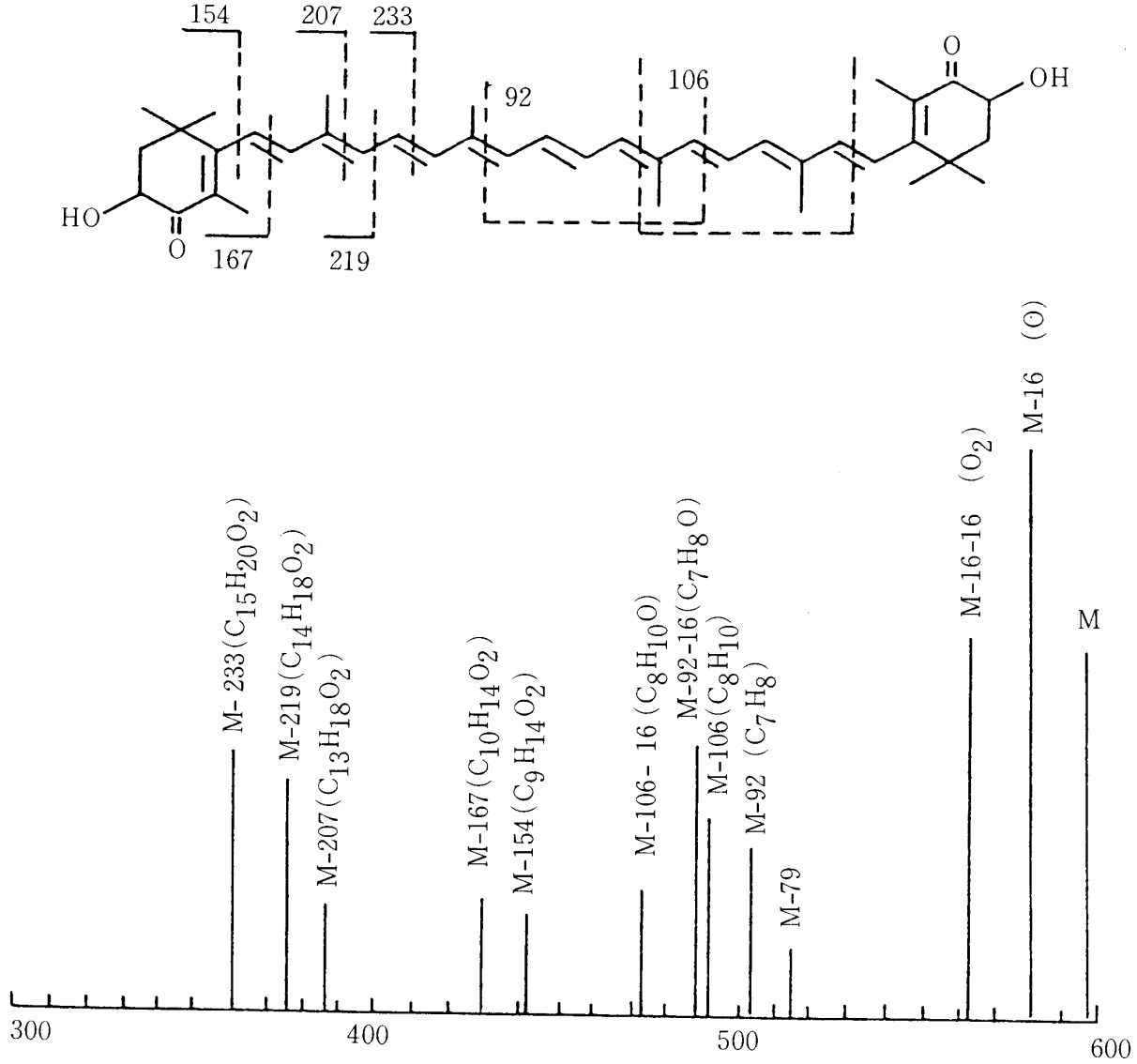

Fig- 4. Mass spectra of astaxanthin

5. Nuclear Magnetic Resonance Spectroscopy(NMR) : ^1H−NMR spectroscopy has played a major role in identifing and elucidating molecular structures in carotenoid chemistry. This spectroscopic technique was first used in carotenoid research by Barber *et al.* Since then, its application has been reported in a rapidly increasing number of papers, clealy demonstrating NMR is a very important and effecive tool for the elucidation of carotenoid structure. The technical shift ( ) of the signals, and the magnitude of the splitings ( ) caused by mutual spin−spin coupling of the proton,

directly reflect the structural properties of the different parts of the molecules. This means that chemical shifts and coupling constants are generally dependent only on the immediate surrounding of the different protons, and the interpretation of the $^{1}H-$NMR spectrum therefore yield detailed information on structural subunits present in an unknown molecule.

The characteristic chemical shift and coupling constants of end groups of carotenoids have been reviewed by Vetter *et al*,[32] Moss and Weedon[20] and Englert.[33, 34] $^{13}C-$NMR has also become increasingly applied in the carotenoid field. Englert[33, 34] reported $^{13}C-$NMR chemial shifts of 62 end groups of carotenoids.

$^{1}H-$NMR chemical shifts of astaxanthin appeared at 1.21, 1.32 (1, $1-CH_3$), 1.94 ($5-CH_3$), 2.00 ($9-CH_3$, $13-CH_3$), 4.28 ($3-H$),[24] Kamata and Simpson[35] measured $^{1}H-$NMR spectra of astaxanthin diester extracted frpm the flower, *Adonis aestivalis*, and similar results were obtained (Fig. 5). $^{13}C-$NMR chemical shifts appeared at 26.2, 30.7 (1, $1-CH_3$), 14.0 ($5-CH_3$), 12.6 ($9-CH_3$)[34]

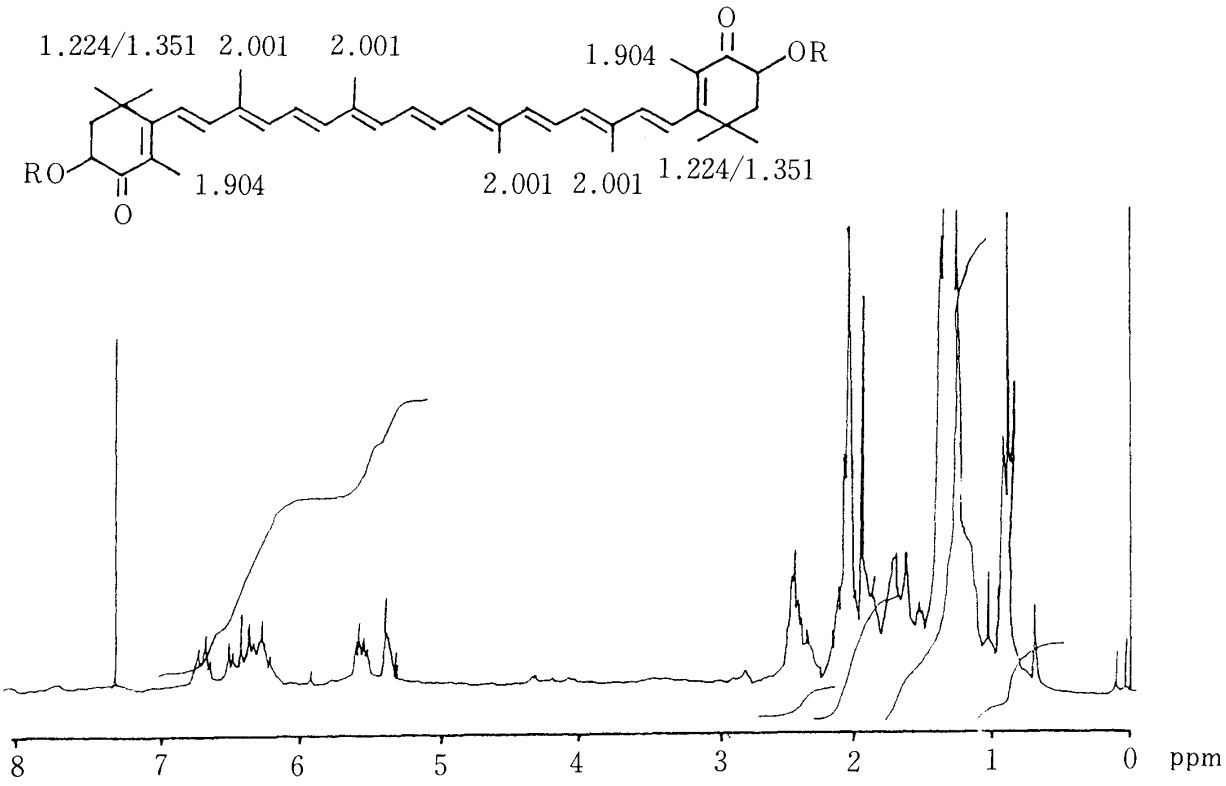

Fig-5. ^{1}H-NMR spectrum of astaxanthin diester extracted from *Adonis aestivalis*, at 270 MHz (in $CDCl_3$)

6. Circular Dichroism (CD) : Until recentlly, CD measurement of carotenoids was confined to the ultra violet region because of the intenes absorption of the compound in the visible region of the spectrum. Since its introduction, circular dichroism has permitted measurement of good reproducible spectra even in the region of the main absorption maxima. Although there is not an adequate theory to explain the observed spectra of carotenoids, they do present a usefull method of correlating absolute configurations. CD spectra of some carotenoids have been provided by Moss and Weedon.[20] CD spectra of (3S, 3'S) −astaxanthin and (3R, 3'R) −astaxanthin are shown in Fig. 6.

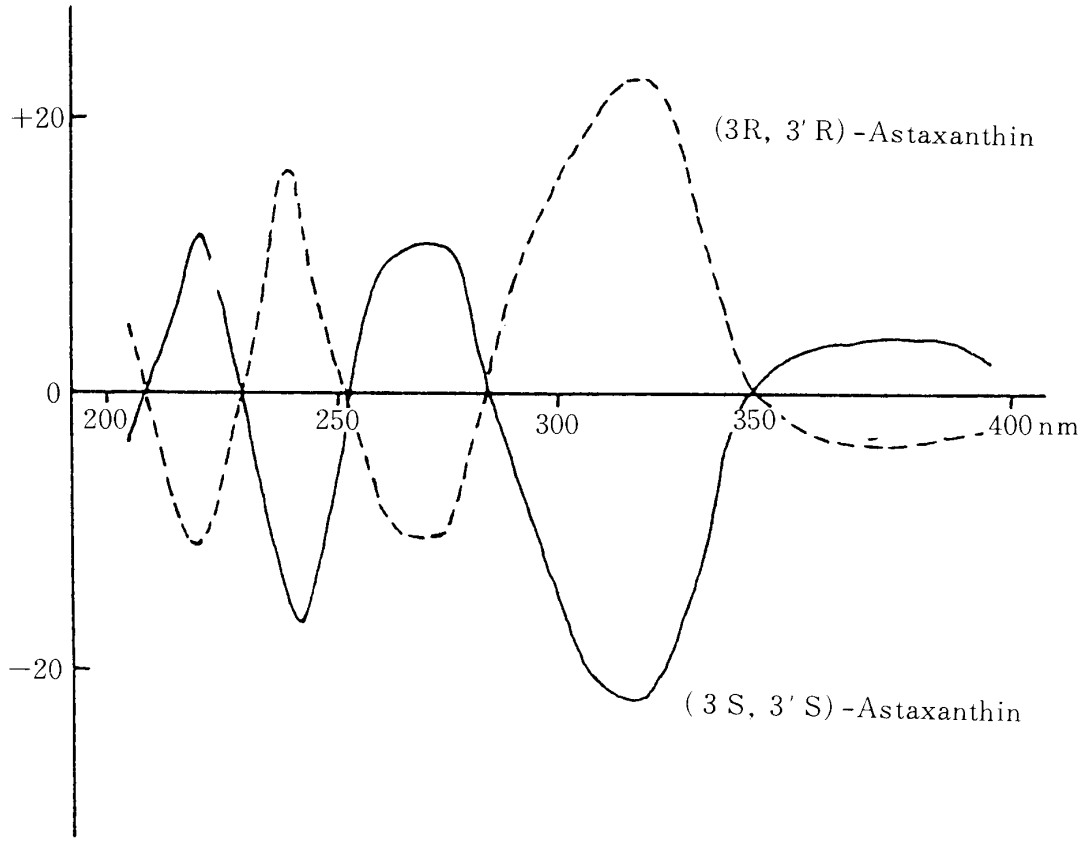

Fig- 6. CD spectra of (3 S, 3'S) - and (3R, 3' R) -astaxanthin

Chemical Synthesis of Astaxanthin

In the few years, major efforts have been made toward the synthesis of optically active carotenoids. The first total synthesis of carotenoids, β −carotene, was reported by Karrer and Eugster[36] and Miluas *et al.*[37] Since then, large numbers of natural and unnatural carotenoids as well as related compounds have been totally synthesized. A complete and excellent review on the total synthesis of carotenoids have been given by Mayer and Isler.[38] Mayer[39] reported the synthesis of (3S, 3'S) −, (3S, 3'R) −and

(3R, 3'R) −astaxanthin. The first synthesis of (3S, 3'S) −astaxanthin was studied by Kienzel and Mayer[40] and Mayer.[39] (3S, 3'S) −, (3S, 3'R) −and (3R, 3'R) −astaxanthin were synthesized by Widmer *et al.*,[41] and Zell *et al.*[42] Recently, Wildmer[43] reported that 6−oxo−isophrone is an ideal starting material for the synthesis of numerous carotenoids possessing cyclic end groups, including some arene− and cyclopentane− analogs.

The synthesis of astaxanthin has been studied by a number of investigators.[19,27,39,40,43] In this section, the partial synthesis of astaxanthin from β−carotene and canthaxanthin and total synthesis of astaxanthin are discussed.

1. Partial Synthesis of Astaxanthin: The partial synthesis of astaxanthin from canthaxanthin has been reported by Cooper *et al.*[19] When a solution of canthaxanthin in t−butyl alcohol, containing potassium t−butoxide, was shaken in oxygen, astacene was produced. Phoeniconone was isolated as intermediate diosphenol from incomplete autoxidation. Astacene (enol form) produced the corresponding mono−or bis−α−glycols by the reduction with potassium borohydride. When the tetrol compound was treated with dichlorodicyano−p−benzoquinone, astaxanthin was produced in ca. 2% yield. The acetylenic tetrols gave 15, 15'−didehydroastaxanthin which, on partial hydrogenation of the triple bond and stereomutation of the compound gave astaxanthin. The synthesis of astaxanthin is presented in Fig. 7.

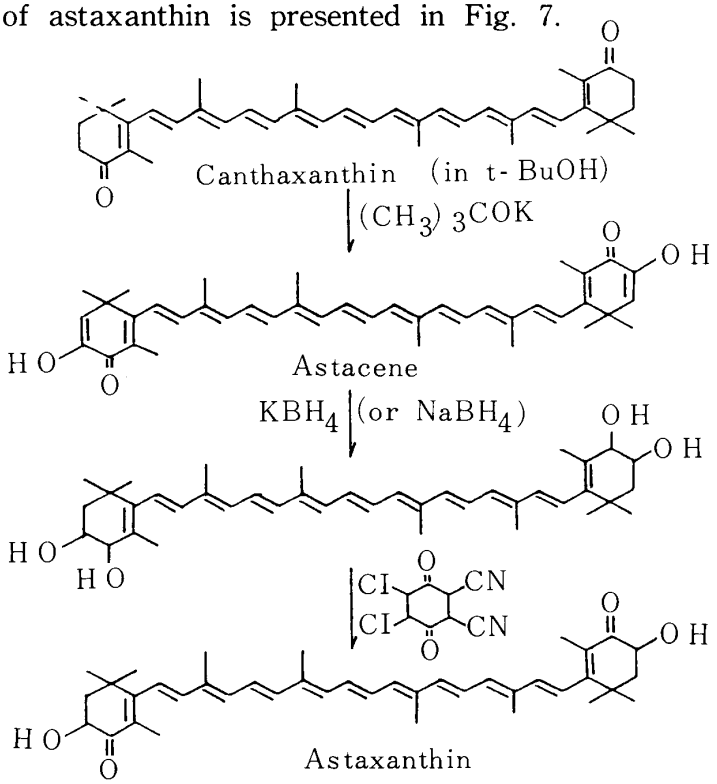

Fig-7. Partial synthesis of astaxanthin from canthaxanthin

2. Total Synthesis of Astaxanthin: Astaxanthin is known to undergo autoxidation, especially under basic condition, to give astacene. Moreover, racemization of the α-ketol system may render the preparation of optically pure materials impossible. This emphasized that the care must be given to the reaction conditions employed in a protected synthetic route.

The first total synthesis of (3S, 3'S)-astaxanthin was successfully completed by Kienzel and Mayer[40] and Mayer.[39] The C9 compound, (4R, 6R)-4-hydroxy-2, 2, 6-trimethyl-cyclohexanone, was used as a starting compound(I). (I) was converted to (4S)-4-hydroxy-2, 6, 6-trimethyl-2-cyclohexanone-1-on (III) via (4R, 6R)-4-acetoxy-2, 2, 6-trimethyl-cyclohexanone (II). A Grignard addition of the protected acetylenic alcohol, (E)-trimethylsilyl-(3-methyl-2-penetn-4-on-1-yl), to (III b) gave a 4:1-mixture of (E)-(1S, 4S)-1-(5-hydroxy-3-methyl-3-penten-4-on-1-yl)-2, 6, 6-trimethyl-2-cyclohexane-1, 4-diol (V) and (1R, 4S) isomers(VI). The reduction and oxidation of (V) and (VI) formed (XI) and (X). Compound (IX), (E)-5-((S)-4-hydroxy-2, 6, 6-trimethyl-3-oxo-1-cylohexane-1-yl)-3-methyl-2-pentene-4-enol, was separated from (X) by chromatography. The partial hydrogenation of (IX) with Lindlar-catalyzer produced C15-ketoaldehyde, (3E, 4Z)-5-((S)-4-hydroxy-2, 6, 6-trimethyl-3-oxo-1-cyclohexane-1-yl)-3-methyl-2, 4-pentadienal. The aldehyde group of compound (XI) is selectively reduced with $NaBH_4$ to alcohol (XII). (XII) then reacted with PBr_3 to form bromide (XII b). With triphenylphosphin, (XII b) produced phosphonium salt (XIII), ((2E, 4E)-3-methyl-5-((S)-2, 6, 6-trimethyl-3-oxo-4-(phenoxyacetoxy)-1-cyclohexane-1-yl)-2, 4-pentadienyl)-triphenyl-phosphonium bromide. In a final step, two pathways were employed. The first pathway was Witting condensation of 2 moles (XIII) and one of C10-dialdehyde. In the other pathway the C10-trienaldehide reacted with (XIII) and formed (3S, 3'S)-astaxanthin through (3S, 3'S)-15, 15'-didehydroastaxanthin and (3S, 3'S)-15, 15'-cis-astaxanthin. The total synthesis of (3S, 3'S)-astaxanthin is shown in Fig. 8A. and Fig. 8B.

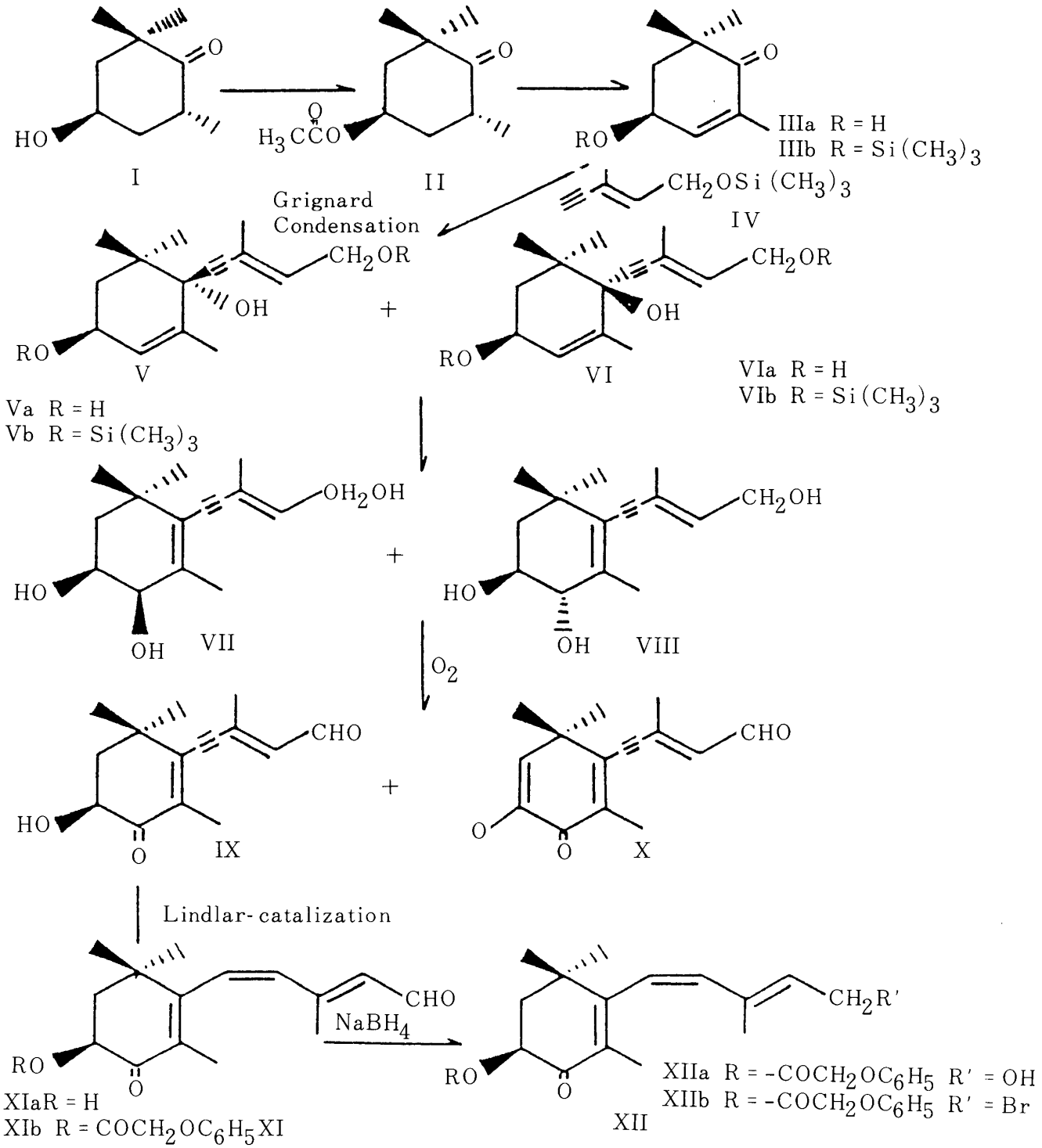

Fig-8A Total synthesis of (3S, 3′S)-astaxanthin

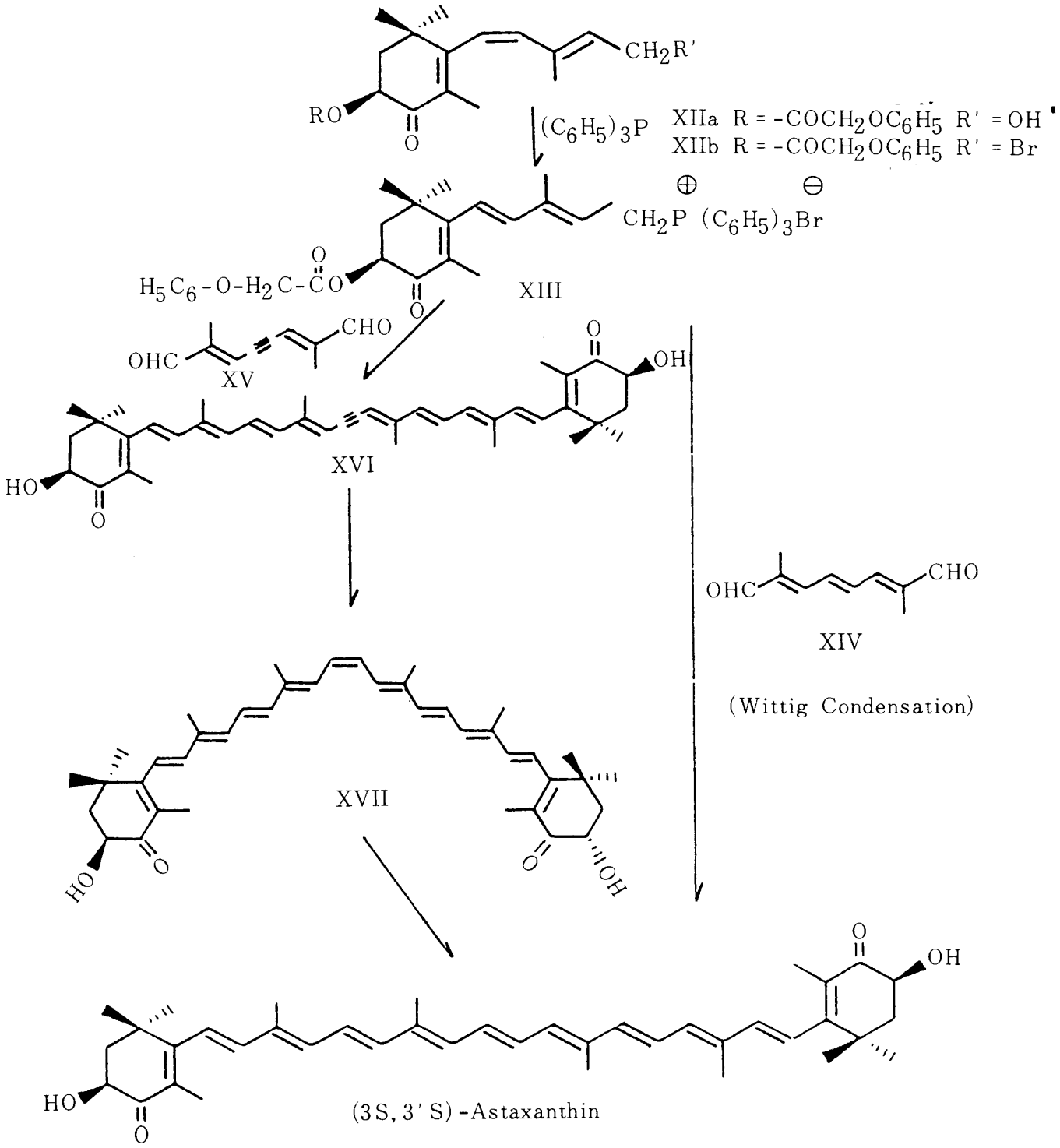

Fig- 8 B Total synthesis of (3 S, 3' S) -astaxanthin

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