UNIVERSITI PUTRA MALAYSIA

REACTION OF BETA·CAROTENE WITH METAL IONS - PRODUCTS ISOLATION AND CHARACTERISATION

YII MEI WO

FSAS 1998 21

REACTION OF BETA-CAROTENE WITH METAL IONS – PRODUCTS ISOLATION AND CHARACTERISATION

By

YII MEI WO

Thesis Submitted in Fulfilment of the Requirements for the
Degree of Master of Science in the
Faculty of Science and Environmental Studies
Universiti Putra Malaysia

June 1998

ACKNOWLEDGEMENTS

The author would like to take this opportunity to thank those who helped him to finish his project especially his supervisor, Assoc. Prof. Dr. Karen Badri and co-supervisors Prof. Dr. Badri Muhammad and Assoc. Prof. Dr. M.T.H Tarafder for their guidance, advice and their unlimited help.

He also will like to thank the laboratory officers Mr. Kamal Margona, Mr. Abdul Wahab, Mr. Sri Jegan , Mr. Nazri Ahmad, Mr. Zainal Abidin for their willingness to help in the analyses of the samples.

Besides, he would also like to thank his laboratory partners, Mdm. Hazimah Abu Hassan, Mdm. Kamaliah Haji Sirat and Dr. Abdul Rahim Khan for their help.

Thanks!

TABLE OF CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure **Page**

LIST OF EQUATIONS

Equation **Page**

LIST OF ABBREVIATIONS

CHN -- Carbon, Hydrogen, Nitrogen Analyses

HPLC -- High Performance Liquid Chromatography

IR -- Infra-Red

PORIM -- Palm Oil Research Institute of Malaysia

ppm -- parts per million

TGA -- Thermogravimetric Analysis

TLC -- Thin Layer Chromatography

UV-Vis -- Ultra violet - Visible

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of requirements for the degree of Master of Science.

REACTION OF BETA-CAROTENE WITH METAL IONS – PRODUCTS ISOLATION AND CHARACTERISATION

By

YII MEI WO

June 1998

Chairman : Associate Professor Karen Badri, Ph.D.

Faculty: Science and Environmental Studies

Reaction of β-carotene with ferric nitrate nonahydrate and anhydrous stannous chloride has been carried out. A π-complex of iron-β-carotene, $Fe(NO_3)_3.C_{40}H_{56}.2CH_2Cl_2$, been synthesised and characterised. No product was isolated from the reaction between stannous chloride and β-carotene. The structure of the iron-β-carotene complex was proposed on the basis of IR and UV-Vis spectra and elemental analyses.

Kinetic studies of the reaction of β-carotene with stannous chloride and stannic chloride were carried out in acetone and ethanol and that of ferric nitrate in 2:1 ethanol-CCl_4 mixture. Kinetic studies for the reaction of β-carotene with $SnCl_2$ failed. The decomposition reaction of ferric nitrate and β-carotene and the formation reaction of stannic chloride and β-carotene were found to be pseudo-first order with respect to β-carotene.

Thermodynamic parameters (viz. ΔH* and ΔS*) for the both reactions were determined. The negative value of ΔS* indicates that the transition state was more ordered and has less degree of freedom than the initial state.

The reaction between β-carotene and metal ions do not follow the Pearson's Universal Soft Acid-Soft Base Theories.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains.

TINDAK BALAS BETA-KAROTENA DENGAN ION LOGAM – PENGASINGAN HASIL DAN PENCIRIAN

Oleh

YII MEI WO

Jun 1998

Pengerusi : **Profesor Madya Karen Badri, Ph.D.**

Fakulti : **Sains dan Pengajian Alam Sekitar**

Tindak balas bagi β-karotena dengan besi(III) nitrat nonahidrat dan timah(II) klorida kontang telah dilakukan. Satu kompleks π bagi besi-β-karotena, $Fe(NO_3)_3.C_{40}H_{56}.2CH_2Cl_2$, telah disintesis dan pencirian dibuat. Tetapi tiada tindak balas berlaku di antara timah(II) klorida dengan β-karotena. Struktur bagi kompleks besi-β-karotena dicadangkan dengan berpandu kepada data-data dari IR, UV dan analisis unsur.

Kajian kinetik bagi tindak balas β-karotena dengan timah(II) klorida dan timah(IV) klorida telah dilakukan dalam aseton dan etanol dan bagi besi(III) nitrat dalam campuran 2:1 etanol-CCl_4. Kajian kinetik timah(II) klorida dengan β-karotena telah gagal. Tindak balas penguraian bagi besi(III) nitrat dengan β-

karotena dan tindak balas pembentukan bagi timah(IV) klorida denganβ-karotena didapati ialah tindak balas tertib satu pseudo (palsu) terhadap β-karotena.

Parameter termodinamik seperti ΔH* dan ΔS* bagi kedua-dua tindakbalas telah ditentukan. Nilai negatif ΔS* menunjukkan bahawa keadaan peralihan adalah lebih teratur dan kurang darjah kebebasan berbanding keadaan awal.

Tindak balas di antara β-karotena dengan ion logam tidak mematuhi Teori Universal Asid Lembut - Bes Lembut Pearson.

CHAPTER I

INTRODUCTION

Carotene and Carotenoids

Carotene, a red crystalline substance, was first isolated in 1831 by Wackenroder[55] from carrots. Willstater and Meig[57] (1907) established that the carotenoids were the derivatives of isoprenoids. Zechmeister[60] (1928) realized that carotenoids were polyenes and these polyenes were shown to conjugate among themselves by Kuhn[31] in 1931. Kuhn and Brockmann[30] were able to isolate three isomers which are, 1) α-carotene (Figure 1.1), m.p 188°C, 2) β-carotene (Figure 1.2), m.p 184°C and 3) γ-carotene (Figure 1.3), m.p 178°C using a technique of applied column chromatography discovered by Tswett in 1906.[53,54]

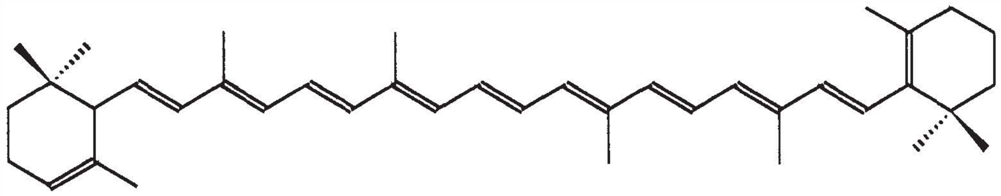


Figure 1.1 : α-Carotene

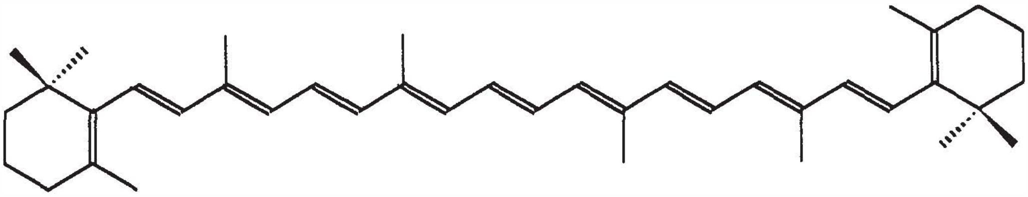


Figure 1.2 : β-Carotene

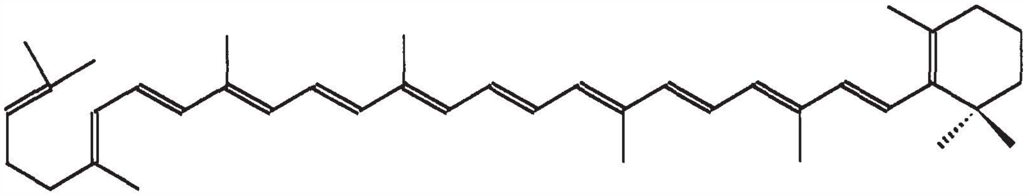


Figure 1.3 : γ-Carotene

Carotenoids are of importance in mammalian nutrition as pro-vitamin A.[18] Although they are highly valued nutritional products, carotenoids, in particular the carotenes, are easily destroyed and bleached during their refining and processing. Carotenoids are the only naturally occurring tetraterpenes consisting of eight isoprenoid residues.

The physical and chemical properties of carotene determine its use in various industries such as in medicines, cosmetics, food colorants, vitamin A preparations and animal feeds.[22] β-Carotene was found to stimulate the body's immune defense mechanism by way of increased capacity of macrophages to kill tumor cells and to increase the production of tumor necrosis factor.[47]

In mammals, β-carotene readily undergoes oxidative cleavage at the central double bond, C9, to give two equivalents of an aldehyde which is known as retinal.[24] Von Euler[15] (1929) showed that crystalline carotene possesses high vitamin A activity, and a pioneering English biochemist, Moore[36] (1930) demonstrated that, in the rat, absorbed carotene is converted to vitamin A which is stored in the liver.

β-Carotene exists in several different configurations (isomers). Synthetic β-carotene is almost 100% trans-β-carotene. Meanwhile, β-carotene found in fruits and vegetables contains about 10% cis-isomers and β-carotene derived from algae contains about 50% of the 9-cis isomer.[4,16] Therefore, this shows that β-carotene appears mostly in the trans-form.[48,49]

Most carotenoids, including β-carotene, easily undergo decomposition under light and oxygen. This implies that studies should be carried out in an inert atmosphere, in subdued light and with highly purified solvents. Therefore, during an experiment, heating of the carotenoid compounds in solution should be done carefully, to prevent the carotenoids being destroyed before they react. The carotenoids should be stored in the dark, under an inert gas atmosphere and refrigerated.[13,20]

Natural Occurrence of Carotenoids

Carotenoids are widely distributed in higher plants and in some microorganisms. In higher plants, the carotenoids are found in the green leaves, together with chlorophyll. They are found in great abundance in dark yellow-orange vegetables (eg. carrots)[34] and dark green vegetables (eg. kale, broccoli and spinach), and also in potatoes, fruits (eg. apricots, cantaloupe, peaches, papayas)[46] and many others. It is found that carotenoids play an important role in coloration of flowers. Carotenoids are also found in the chloroplasts of many organisms such as red and green algae, yeast, fungi and photosynthetic bacteria.[19]

Carotenoids can be classified into two major groups,

a) Carotenes – hydrocarbons which are highly soluble in petroleum ether and carbon tetrachloride but only slightly soluble in ethanol and other polar solvents. e.g., α-carotene, β-carotene, γ-carotene and lycopene (Figure 1.4).[5]

b) Xanthophylls – oxygenated derivatives of the carotenes which are quite soluble in ethanol, methanol and petroleum ether. e.g., lutein (Figure 1.5) and cryptoxanthin (Figure 1.6)[5]

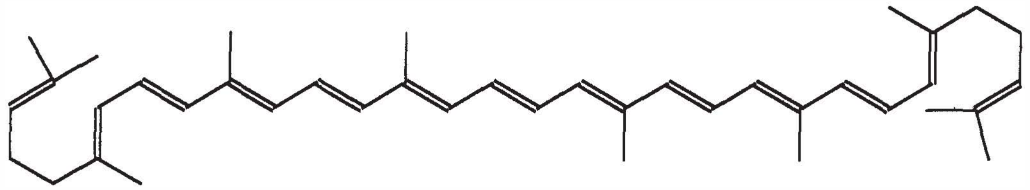


Figure 1.4 : Lycopene

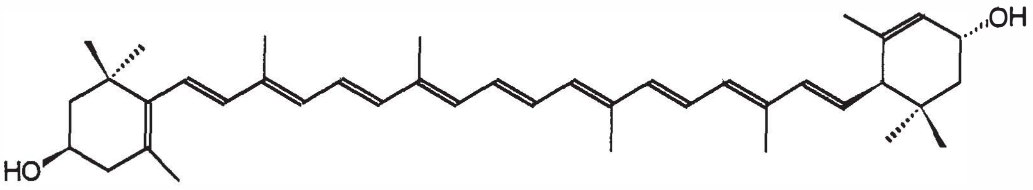


Figure 1.5 : Lutein

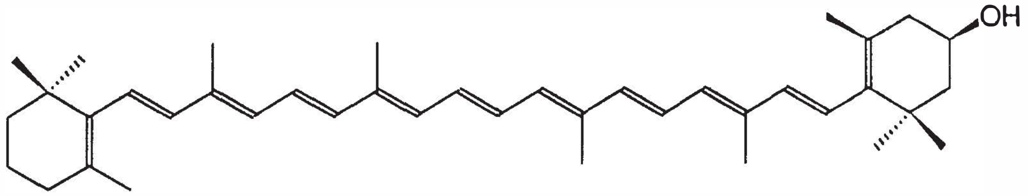


Figure 1.6 : Cryptoxanthin

Carotenoids, as noted above, are highly unsaturated polyenes and in some cases, like carotene, they contain eleven conjugated double bonds. The long chain of carbon atoms and the double-bonds which are located alternatively keep the molecule inflexible and elongated.

Physical and Chemical Properties of β-Carotene

As was indicated above, carotenes are insoluble in water, slightly soluble in fats, moderately soluble in aliphatic hydrocarbons, and very soluble in aromatic and chlorinated hydrocarbons such as benzene and carbon tetrachloride.[28] Their melting points are fairly high (around 200°C) and increase with increasing molecular weight and number of functional groups. When they are oxidised, carotenes lose their red-orange colour and turn colourless.[19] The highly unsaturated structure of β-carotene enables this compound to undergo oxidation, isomerisation and thermal degradation very easily.[6]

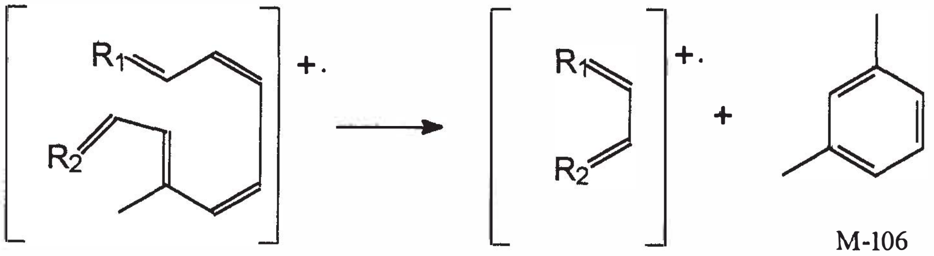
The structure of the carotenoids which are made up of eight isoprenoid structures, formed by head to tail condensation of four isoprenoid units that joined tail to tail was shown by Zechmeister (1928).[59]

For molecules containing conjugated double bonds, the ultra-violet or visible spectra give an indication of the nature of the polyene systems. The conjugated double bond system gives colour to the molecule. As example, β-carotene has three absorption maxima at 425 nm, 451 nm and 482 nm.[18] All these three bands are located in the visible region. The first band has intense absorption and gives the colour characteristic of this class. There is another weak band at about 220 nm in the ultra-violet which indicates the presence of the long conjugated chain. The wavelengths of the three absorption bands in the visible spectrum increase with each extension to the conjugated system.[37] This is important for the analysis of the carotenes[13] since the absorption of samples of the same concentration will give different values depending on the number of conjugated double bonds present. The solvent in which the absorption spectrum of a carotenoid is measured also has a marked effect on the position of the maxima and on the molecular absorbance of the compound.[20]

Advances in chromatographic techniques, leading to a marked improvement in sensitivity and resolution, have paved the way for great development in the identification of carotenoids. Through working with the counter current extraction techniques applied to solutions containing carotenoids and chlorophylls, Tswett (1906) first isolated carotenoids and then adopted the application of the column chromatographic technique to separate the components.[53,54]

However it was not until 1931, that the separation of isomeric β-carotene and α-carotene[30,31] and the enrichment of vitamin A[26] were achieved. Chromatography on various forms of paper for separation of mixed compounds was first introduced around 1950, and was followed by the more efficient thin-layer chromatography (TLC). Later, with the introduction of specialized uniform stationary phases and refined instrumentation, column chromatography evolved into High Performance Liquid Chromatography (HPLC), which permits the highly efficient separation of mixtures of carotenoids that are of very similar polarity, and their sensitive detection and accurate quantitative analysis.

A useful tool for structural identification of carotenoids is Mass Spectroscopy.[37] Schweiter[43] proposed a mechanism to explain that the elimination of M-92 and M-106 ions recorded in the mass spectrum is a result of the fragmentation of the carotenoids (like β-carotene) actually corresponded to toluene (C_7H_8) and m-xylene ($C_{12}H_{18}$) respectively.



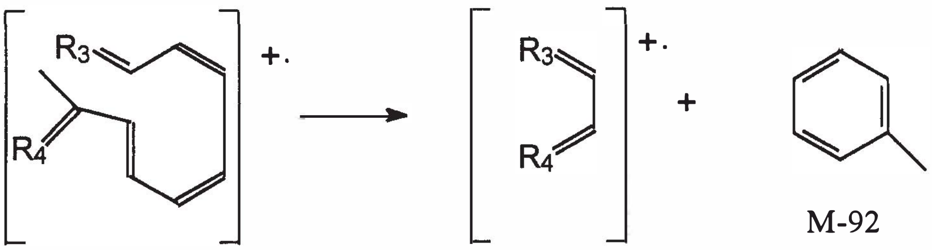


Figure 1.7 : Mechanism for the Fragmentation of Carotene

The above reaction occurs by the coiling of the polyene chain and the expulsion of the fragment.[33]

Infra-red Spectroscopy has been widely applied to carotenoids. The main peaks in the spectrum of pure β-carotene are

a) above 3000 cm^{-1}, due to the stretching of C—H bond in C=CH groups.

b) around 2900 cm^{-1}, resulting from the stretching of C—H single bonds in C—C—H groups.

c) near 970 cm^{-1}, due to the bending of the C—H bond of the C=CH grouping.[8]

Because of the presence of acyclic conjugated units, reactions involving hydrogenation, oxidation, methyl group migration and chain elongation or shortening may occur. Sometimes, antioxidants like tocopherol and butylated hydroxytoluene (BHT) are used to enhance the stability against oxidation.

The oxidation of β-carotene by strong reagents normally gives several derivatives.

For instance,

a) potassium permanganate oxidizes β-carotene to beta-apo-8’-carotenal and beta-apo-12’-carotenal.[25]

b) iodine reacts with it to form 5,5’,6,6’-tetraiodo beta-carotene.[20]

c) peracids such as peracetic acid combine with β-carotene form 5,6-diepoxide beta-carotene.[20]

Meanwhile, the normal trans β-carotene may undergo cis-trans isomerization under the following conditions,[28]

a) exposure to light,

b) heating in hydrocarbon solvents,

c) melting briefly in vacuo,

d) prolonged contact with an active surface, e.g. Al_2O_3,

e) treatment with acids.

Thermal degradation of β-carotene in solution gives several simple aromatic compounds such as 2,6-dimethylnapthalene, ionene, toluene and xylene.[17]

Biological Functions, Actions and Uses of Carotenoids

Most of the functions, applications and uses of carotenoids, are a consequence of the light-absorbing properties of the polyene chromophore.

Natural roles in coloration, photosynthesis and photoprotection are well established and are of major biological importance. The great importance of carotenoids in human and animal nutrition and health, however, is not based upon the light-absorbing properties of the molecules.

Colour and Coloration

Carotenoids, particularly carotenes are responsible for the colours of some parts of living organisms.[32] They are harmless colorants which can be used in foods and cosmetics.[9] Some of them have been used for centuries, especially bixin (Figure 1.8), crocin (Figure 1.9) and lycopene (Figure 1.4) which can be found easily in annatto, saffron and tomato, respectively. Even though nature-identical carotenoids are now produced synthetically and are used on a large scale as food colorants, the demand for the natural extract carotenoids is increasing greatly. Carotenes are used as additives to animal feed, for the purpose of imparting a desired colour either to animal tissues or to derived products (e.g. β-carotene, Figure 1.2, to cattle for cream or fat coloration; lutein, Figure 1.5, and zeaxanthin, Figure 1.10, to chickens for egg yolk and skin coloration) or to provide adequate supplies of vitamin A.[7]

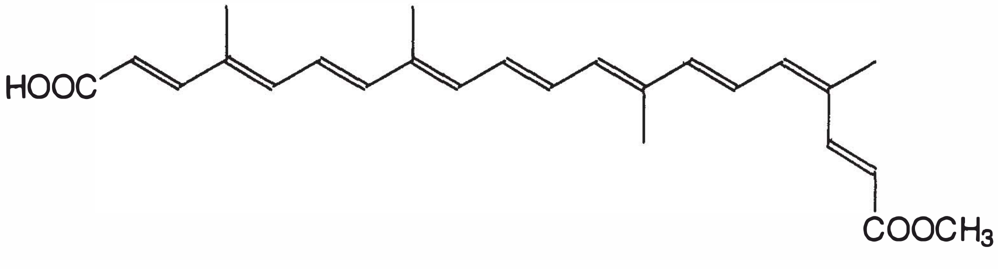


Figure 1.8 : Bixin