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THESIS  
presented at the  
UNIVERSITY of LYON FACULTY OF SCIENCES  
to obtain  
A PHYSICAL SCIENCES DOCTORATE  
by  
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1<sup>st</sup> THESIS – Research on Astaxanthin, a New Vitamin A Factor

2<sup>nd</sup> THESIS – proposals given by the school

Presented orally on October 23, 1950 before the Examination Jury

Mr. CORDIER.....President  
Mr. BERNARD.....Examiner  
Mr. MEUNIER.....Examiner

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21, RUE SAINTE-VERONIQUE  
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1	
2	TABLE OF CONTENTS
3	
4	Introduction: Importance of astaxanthin among natural carotenoids.
5	
6	Chapter I: Constitution and physical and chemical properties of astacine, astaxanthin and their derivatives.
7	
8	Chapter II: Astaxanthin in living creatures – Study of the <i>Aristaeomorpha foliacea</i> (Penaeidae).
9	
10	Chapter III: Biological Importance of Astaxanthin
11	
12	A. – Origin and destination of astaxanthin in living creatures.
13	B. Astaxanthin, the new Group A Vitamin:
14	I. – Vitamin A Activity in the oils of <i>Aristaeomorpha foliacea</i> .
15	II. – Antixerophthalmic activity of the <i>Aristaeomorpha foliacea</i> carotenoid pigment
16	1. Activity of the pigment extracted from the hepato-pancreas oil
17	2. Activity of the pigment extracted from the hypoderm.
18	III. – Location of astaxanthin in the treated rats.
19	Observation in the retina.
20	
21	Chapter IV: Discussion of Results
22	
23	1. Astaxanthin’s chemical constitution and vitamin activity.
24	2. Dissociation of astaxanthin’s antixerophthalmic activity and its effect on growth rate in the
25	vitamin A deficient white rat.
26	3. Astaxanthin and vision problems.
27	
28	General Conclusions
29	
30	

1  
2 Chapter I

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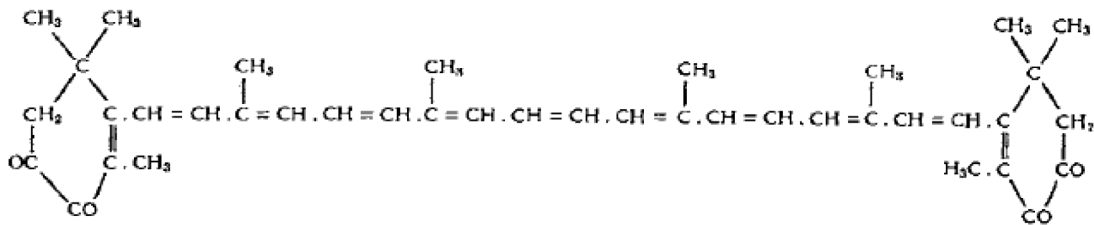
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5 THE CONSTITUTION, CHEMICAL AND PHYSICAL PROPERTIES OF ASTACINE, ASTAXANTHIN AND THEIR  
6 DERIVATIVES

7  
8 A. – ASTACINE AND ITS DERIVATIVES

9  
10 The carotenoid nature of astacine and the presence of oxygen in each of the  $\beta$ -ionone cores have been  
11 recognized by Kuhn and Lederer (45).

12  
13 In 1934, P. Karrer and colleagues (38) established the general formula,  $C_{40}H_{48}O_4$  and the constitution of the  
14 pigment which is that of a  $\beta$ -carotene-tetrone. This constitution was deduced from the general formula and  
15 oxygen group properties. Astacine, in fact, provides a dioxin with 4 active hydrogen atoms, two of which  
16 belong to oxime radicals, while the other two are fixed to two enolized carbonyls. The carotenoid has,  
17 therefore, 4 ketonic groups which are not equivalent. Ortho-phenylenediamine reacts and creates bis-  
18 phenazine astacine which indicates that each  $\beta$ -ionone core encloses two carbonyl groups. Oxidation with  
19 permanganate yields malonic dimethyl acid and oxidation of the diphenazine derivative leads to  $\alpha$ - $\alpha$ -dimethyl-  
20 succinate which establishes that the carbonyl groups occupy the 3, 4, 3', 4' positions. The formula for the  
21 constitution of astacine is, therefore, as follows:

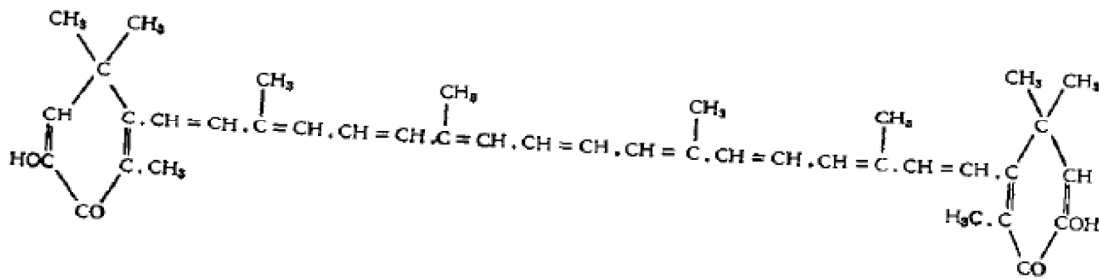
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Astacine (Ketonic Form)

1 However, catalytic microhydrogenation reveals that there are not 11 but 13 double bonds. The extra two  
2 double bonds establish the possibility of the enolic form represented by the following diagram:  
3



4  
5  
6  
7

Astacene (enolic form)

8 In reality, free astacene is only very slightly enolized as demonstrated by the slow etherification speed with  
9 diazo-methane and the determination of mobile hydrogens (Zerewitinoff). Astacene's acidic nature disappears  
10 after hydrogenation.

11  
12 Astacene crystallizes from a mix of pyridine and water into purple needles with metallic reflection which melt  
13 at 228°C (Kuhn, Stene and Sorensen (48) <sup>(1)</sup>).

14  
15 Astacene is insoluble in water, is very difficult to dissolve in ether, petroleum ether or methane, is difficult to  
16 dissolve in benzene, ethyl acetate and acetic acid, is relatively easy to dissolve in carbon sulfur and is very easy  
17 to dissolve chloroform, pyridine (blood-red concentrated solution, diluted orangey solution) and dioxane.

18  
19 When the solution is agitated in petroleum ether with 90% methanol, astacene transforms into the hydro-  
20 alcoholic phase but after dilution with water, the pigment is easily dissolved in petroleum ether. In contrast, it  
21 remains in the aqueous phase if the latter is diluted with sodium or potassium after which it manifests its  
22 acidic nature. After cooling, the pigment separates from the alkaline solution in the form of red flakes which  
23 gather at the interphase. Acidification by acetic acid transforms the amorphous salt into shiny purple crystals  
24 of free astacene.

25  
26 Astacene dissolved in a mix of benzene and ligroin is absorbed rapidly in calcium carbonate. It is also absorbed  
27 in aluminum oxide from a benzene-ligroin or petroleum ether solution: it forms a purple area on the upper  
28 part of the column. The eluents are benzene and pyridine diluted with methanol as well as petroleum ether  
29 diluted with 5% potassium.

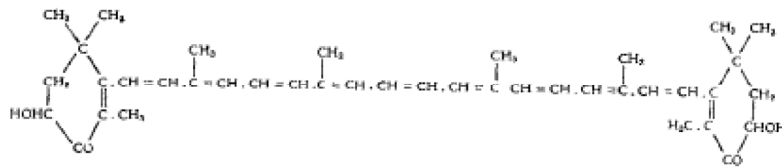
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31 (1) Other possible temperatures are 240 -243°C (Kuhn and Lederer [45]) and 241°C (Karrer and Benz [36]).  
32

1 The absorption spectrum of astacine is characteristic and present in a large band with a single peak (500 nm in  
2 pyridine and 510 nm in carbon-sulfur). The absorption spectrum and the pigment's behavior vis-à-vis sodium  
3 are the two main characteristics which identify it. Other, less specific, characteristics: in concentrated sulfuric  
4 acid, astacine dissolves into a deep blue color. The chloroform solution turns a blue-green color when diluted  
5 with antimony trichloride (R. de Carr and Price).

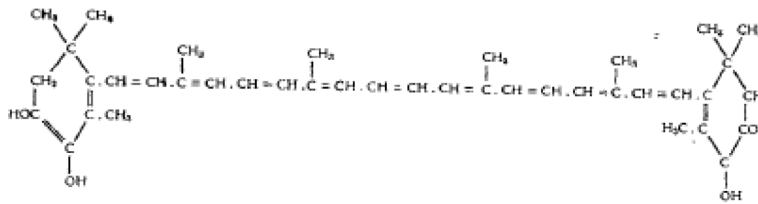
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7 Astacine is very resistant to oxidation in air. Kuhn, Lederer and Deutsch (46) have obtained astacine diacetate.  
8 Dipalmitate (astacene) has also been produced (Kuhn and colleagues [48]).

9  
10 B. – Astaxanthin and its Derivatives

11  
12 Kuhn and Sorensen (47) established that astaxanthin has four more hydrogen atoms than does astacine and  
13 has a general formula of  $C_{40}H_{52}O_4$ . In an alkaline solution in the presence of air, they have demonstrated that  
14 astaxanthin transforms into astacine by means of an enediol auto-oxidation in which the enediol absorbs two  
15 oxygen molecules and forms two molecules of oxygenated water. Deriving the structure of astaxanthin from  
16 that of astacine, they assigned it the following formula which is that of 3,3'-dihydroxy- $\beta$ -carotene-4,4'-dione:  
17  
18



19  
20  
21 Astaxanthin (Ketonic Form)  
22



23  
24  
25 Astaxanthin (Enolic Form)  
26  
27

28 The structural diagram for 3,3'-dihydroxy- $\beta$ -carotene-4,4'-dione can also be created. Based on energy order  
29 criteria, however, Kuhn  
30

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