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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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1st THESIS – Research on Astaxanthin, a New Vitamin A Factor

2nd 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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25	vitamin A deficient white rat.
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2 Chapter I

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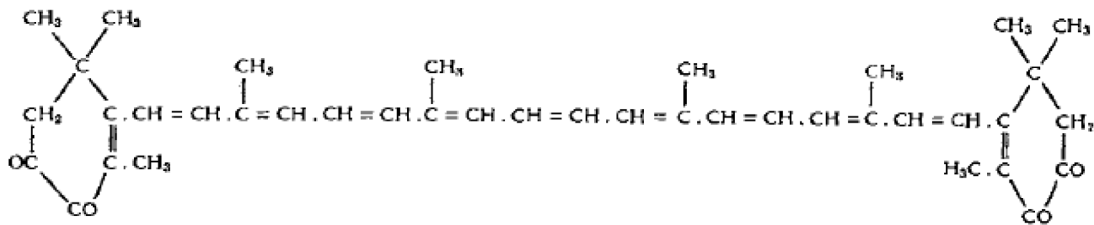
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5 THE CONSTITUTION, CHEMICAL AND PHYSICAL PROPERTIES OF ASTACINE, ASTAXANTHIN AND THEIR
6 DERIVATIVES

7
8 A. – ASTACINE AND ITS DERIVATIVES

9
10 The carotenoid nature of astacine and the presence of oxygen in each of the β -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 β -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 β -ionone core encloses two carbonyl groups. Oxidation with
19 permanganate yields malonic dimethyl acid and oxidation of the diphenazine derivative leads to α - α -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:

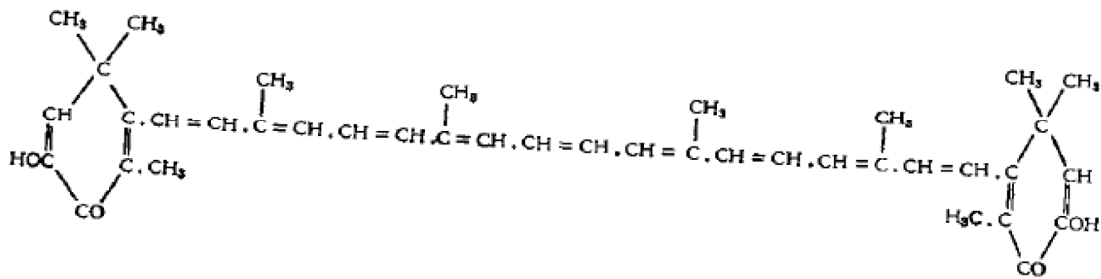
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Astacine (Ketonic Form)

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



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5
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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) ⁽¹⁾).

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.

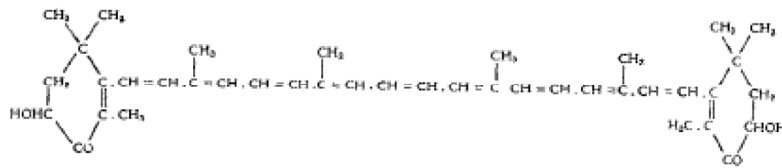
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31 (1) Other possible temperatures are 240 -243°C (Kuhn and Lederer [45]) and 241°C (Karrer and Benz [36]).
32

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).

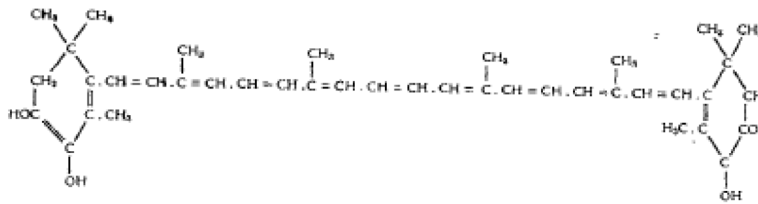
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7 Astacine is very resistant to oxidation in air. Kuhn, Lederer and Deutsch (46) have obtained astacine diacetate.
8 Dipalmitate (astacene) has also been produced (Kuhn and colleagues [48]).

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- β -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- β -carotene-4,4'-dione can also be created. Based on energy order
29 criteria, however, Kuhn
30

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