CCLXXIV. STUDIES ON CAROTENOIDS.[1]

III. AN ISOMERIDE OF LUTEIN ISOLATED FROM THE FURZE (*ULEX EUROPAEUS*).

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(*Received 7 August 1936.*)

We have isolated from the yellow flowers of the furze a new carotenoid which in its properties is very similar to lutein. It crystallizes from absolute methyl alcohol in stellate groups of pale yellow twinned prisms. The crystals are dichroic and have a strong birefringence. Like lutein this yellow form, which does not contain methyl alcohol of crystallization, changes in contact with the solvent in the course of 2 or 3 days into a red form of metallic brightness containing methyl alcohol, which cannot be distinguished from lutein by the shape of the crystals. The absorption bands in different solvents are the same as those of lutein, in carbon disulphide 508, 475 mμ, in ethyl alcohol 475, 447 mμ, in methyl alcohol 473, 445 mμ. During chromatographic analysis in mixture with lutein on calcium carbonate, the new carotenoid forms a uniform orange-coloured zone, and in this way it cannot be separated from lutein. It shows, however, a marked difference from lutein in its M.P., which for the yellow methyl alcohol-free form was found to be 201–202° (uncorr., in evacuated tube; corr. 205–206°). The red crystals lose methyl alcohol at 120–130° and melt at 199–200° (uncorr.). Pure lutein from *Genista tridentata* melted 10° lower, at 190° (uncorr.).

For the reasons above mentioned we suppose that we are dealing with a stereoisomeric form of lutein. An analysis could not be made because of the small quantity available. The carotenoid is sparingly soluble in cold carbon disulphide, very sparingly in cold ethyl alcohol and still more sparingly in cold methyl alcohol.

The carotenoids of the furze (Ulex europaeus).

We have begun a systematic investigation of the carotenoids of diverse furze and broom species of the family of Leguminosae the preliminary results of which we report in this and the following papers. The yellow flowers of the furze were investigated long ago by Schunck [1903], who demonstrated the presence of a xanthophyll—named by him xanthophyll Y—which is distinguished by a strong blue colour reaction with concentrated hydrochloric acid. Schunck gives a photograph of the absorption spectrum of the xanthophyll as well as the spectrum of the product formed by hydrochloric acid, and from his data one may conclude that he dealt with violaxanthin.

Methods and results.

A preliminary investigation showed that the carotenoids of the furze are a rather complicated mixture. The flowers do not keep well in a fresh state and oxidize with ease. Therefore we have not attempted to separate the yellow

[1] These investigations are assisted by a grant from the van't Hoff Foundation of the Royal Academy of Sciences, Amsterdam, for which the author expresses his indebtedness.

petals of the flowers from the green ones of the calyx which represent the greater part of the weight of the flowers. The flowers were dried in a current of warm air at 50°. This procedure for a quantity of 25 kg. of fresh flowers required 8 days, and in consequence of this a large part of the carotenoids was already oxidized. The dry flowers weighed 5·5 kg., which corresponds to a weight of the yellow petals of 1·5 kg. They were ground in a mill to a very fine powder. As the yellow petals do not contain free xanthophyll, the powder was extracted three times with light petroleum in a total volume of 60 litres. The yellow solution was concentrated *in vacuo* in a current of carbon dioxide at 35–40° to 1 litre and freed from a very fine precipitate by centrifuging, and 2 litres of absolute ethyl alcohol were then added. After remaining 2 days in the ice-box a colourless wax-like mass had separated which was removed by suction, the residue being washed on the filter with a little ethyl alcohol. The filtrate was evaporated *in vacuo* and the resulting viscous oil kept in the ice-box for several days, where a further quantity of colourless material settled. After centrifuging, the oil was poured into 2·5 litres of pure acetone by which procedure a considerable quantity of colourless material was precipitated which increased during 1 day in the ice-box. After being again centrifuged, the solution was evaporated to dryness *in vacuo*. The residue was a dark coloured viscous oil of characteristic odour and weighed 330 g.

It has proved advantageous for the isolation of the different carotenoids to include in the process of purification at this point a chromatographic analysis with activated aluminium oxide. In this way a separation of the bulk of xanthophyll esters from carotene as well as from the chlorophyll pigments is easily obtained, and the main part of the xanthophylls is obtained in a state of great purity so that after saponification they readily crystallize.

The residue of the distillation was dissolved in 3 litres of light petroleum and shaken with methyl alcohol (80 %) and then with methyl alcohol (90 %) which extracted the small amount of free xanthophyll which had passed into solution from the green leaves of the calyx, as well as some chlorophyll. The solution was then washed with water to free it from the methyl alcohol and dried over sodium sulphate. It was then adsorbed on to activated aluminium oxide in two columns. By washing the adsorbed colouring matter with light petroleum nine different zones were formed. The rather large upper zone contained only colourless material, then followed four narrow green and reddish brown zones of chlorophyll and xanthophyll. Most of the column was filled with a yellow zone of xanthophyll esters below which followed three smaller orange-coloured zones which contained the carotenes. The column was divided into three parts (*a*) the three lowest zones, (*b*) the large uniform one, and (*c*) the four narrow upper zones.

(*a*) *Hydrocarbons.* It is possible to obtain a part of the hydrocarbons of the lower zones directly in a crystalline state by repetition of the chromatographic analysis and elution. It is, however, advantageous to proceed with an alkaline saponification of the solution before further treatment. The solution of the colouring matter obtained by eluting the lower zones of the chromatogram with light petroleum containing 1 % of methyl alcohol was concentrated *in vacuo* at low temperature to 150 ml. To the concentrate were added 25 ml. of a concentrated methyl alcoholic solution of potassium hydroxide, 50 ml. of ethyl alcohol (96 %) and the same volume of absolute ethyl alcohol, and the mixture was shaken in an atmosphere of nitrogen for 8 hours. After standing overnight, 20 ml. of water and 250 ml. of light petroleum were added, the alcoholic layer separated, the light petroleum solution several times shaken with methyl alcohol (90 %), then washed with water and dried over anhydrous sodium sulphate. The solution

was then adsorbed on to a column of activated aluminium oxide and washed with light petroleum. Three distinct zones developed:

	Absorption bands in light petroleum (B.P. 80°)* mμ
1. Narrow very sharp red-violet zone	451, 426
2. Broad orange zone	479, 450
3. Diffuse yellow zone	475, 445

* All spectroscopic measurements were made with a Hilger prism-spectroscope and a copper sulphate-ammonia filter.

Zones 2 and 3 were eluted together with light petroleum containing 1% of methyl alcohol, and the solution was evaporated to dryness *in vacuo*. The residue was dissolved in 20 ml. of light petroleum and to the solution 80 ml. of absolute ethyl alcohol were added. After several hours carotene began to separate in glistening crystals accompanied by colourless material. The quantity of crystals increased on keeping the solution in the ice-box for 1 day. The precipitate was filtered and freed from the colourless substance by boiling with 100 ml. of absolute ethyl alcohol. From this solution a sterol was obtained in a pure state. The remaining carotene was then twice crystallized from a mixture of benzene-methyl alcohol (1:3). Characteristic crystals of carotene were obtained; M.P. 181–182° (uncorr. in evacuated tube); absorption bands in light petroleum (B.P. 80°) 481, 450mμ; in CS_2 517, 482mμ. A separation into the isomerides was not attempted. A yield of 22 mg. was obtained, i.e. 20% of the total quantity. The rest of the carotene remaining in the mother-liquors could not be obtained in a crystalline state, as the separation of a great quantity of a colourless oil was not possible.

The first zone after elution with light petroleum and methyl alcohol was evaporated to dryness *in vacuo*. The residue was boiled with the purest methyl alcohol, a colourless substance passing into solution. During repeated boiling with the same solvent nothing more went into solution. We have tried to crystallize the colouring matter from different solvents but could not succeed in obtaining it in a pure state. This pigment is a hitherto unknown carotenoid which in its spectroscopic properties agrees with flavoxanthin [Kuhn & Brockmann, 1932]. Absorption bands in CS_2, 478, 450mμ; in light petroleum, 451, 426mμ; in methyl alcohol, 448, 422mμ.

When shaken with light petroleum and methyl alcohol (90%) the pigment remains completely in the upper layer. In ethereal solution it gives no colour reaction with concentrated hydrochloric acid. By means of chromatographic analysis with aluminium oxide, on which it is strongly adsorbed, the carotenoid can be separated from carotene with the same ease as it can be separated from lutein with calcium carbonate, by which it is scarcely adsorbed. In these properties the carotenoid is very similar to the xanthophylls of the formula $C_{40}H_{56}O$, cryptoxanthin and rubixanthin [Kuhn & Grundmann, 1933; 1934]. It occurs not only in the furze but also in Planchon's furze (*Ulex galli*) and in very small quantity in *Genista tridentata* (see the following paper). In somewhat greater quantity it occurs in the flowers of the wild clover (*Oxalis cernua*), of which we shall make a report later.

(*b*) *Xanthophylls*. The large middle zone of the first chromatographic separation contains the bulk of the xanthophylls in a moderately pure form. We have tried to isolate a uniform crystalline coloured wax but without success. The pigment after elution with light petroleum containing 1% of methyl

alcohol was concentrated *in vacuo* to 100 ml. Then for saponification were added 25 ml. of concentrated methyl alcoholic KOH, 20 ml. of ethyl alcohol (96 %) and a quantity of absolute ethyl alcohol sufficient to make a homogeneous mixture. The liquid was kept in a well-filled and stoppered bottle for 2 days at room temperature. After complete saponification a considerable quantity of water was added and the free xanthophylls were extracted with ether. The ethereal solution was washed several times with water, dried over anhydrous sodium sulphate and concentrated *in vacuo* to 10 ml. 150 mg. of xanthophyll crystallized, which were twice recrystallized from methyl alcohol. 80 mg. of xanthophyll of M.P. 193–194° were obtained; absorption bands in CS_2, 500·5, 468 mμ. With strong hydrochloric acid a deep blue colour develops. As the xanthophyll did not yet seem to be uniform, it was dissolved in benzene, diluted with four times its volume of light petroleum and adsorbed on to a column of calcium carbonate. After prolonged washing with a mixture of benzene-light petroleum, 1:2 and then 1:1, three well-defined zones developed. The upper brown-yellow zone contained violaxanthin, under which there was a narrow lighter yellow zone of taraxanthin [Kuhn & Lederer, 1931] and at a distance of 2 cm. a third orange-yellow zone, from which the isomeride of lutein was isolated.

Violaxanthin. The zone containing this xanthophyll was eluted with pure methyl alcohol and the solution evaporated to dryness *in vacuo*. The residue was crystallized from methyl alcohol, the crystals boiled with light petroleum and then recrystallized from absolute methyl alcohol. We obtained 35 mg. of crystals which formed brown-red needles of M.P. 197° (uncorr., evacuated tube); absorption bands in CS_2, 500·5, 469 mμ; in methyl alcohol 469, 411 mμ.

Taraxanthin. The second zone was eluted with methyl alcohol and evaporated to dryness *in vacuo*. The residue was twice crystallized from the purest methyl alcohol and 15 mg. of long needles grouped in clusters were obtained; M.P. 185–186° (uncorr., in evacuated tube); absorption bands in CS_2, 501, 469 mμ; in absolute ethyl alcohol, 470, 441 mμ; in methyl alcohol, 468, 440 mμ. With strong hydrochloric acid in ethereal solution no coloration occurs.

Isomeric lutein. The solution obtained by eluting the lowest zone of the chromatogram with methyl alcohol was evaporated to dryness *in vacuo* and the residue crystallized from methyl alcohol. 10 mg. of xanthophyll were obtained, which were boiled with light petroleum and then four times recrystallized from purest methyl alcohol. 3 mg. of crystals were obtained which macroscopically have a reddish brown aspect similar to violaxanthin. Under the microscope yellow dichroic crystals with strong birefringence are observed. The substance gives no colour reaction with concentrated hydrochloric acid; M.P. 201–202° (uncorr., in an evacuated tube) (corr. 205–206°). These crystals change in the course of 2 or 3 days in contact with methyl alcohol into a red form, which contains methyl alcohol of crystallisation. Simultaneous comparison of the melting points of the isomeride with pure lutein from *G. tridentata*: lutein M.P. 190° (uncorr.), isomeric lutein (red form) M.P. 199–200° (uncorr.).

(*c*) *Other xanthophylls.* The xanthophylls contained in the narrow upper zones of the first chromatogram in mixture with the chlorophyll pigments were eluted with light petroleum containing 1 % of methyl alcohol and the solution concentrated *in vacuo* to 20 ml. The concentrate was then saponified with methyl alcoholic KOH and absolute ethyl alcohol sufficient to make a homogeneous mixture. After adding water, the free xanthophylls were extracted with ether, and a deep red ethereal solution was obtained, which gave a strong blue colour reaction with concentrated hydrochloric acid. The solution was washed with water and then evaporated to dryness *in vacuo*. The residue was dissolved in

benzene, diluted with the same volume of light petroleum and adsorbed on to a column of calcium carbonate. By washing the chromatogram with a mixture of benzene and light petroleum 1:1 several zones developed, which have a spectrum similar to violaxanthin.

	Absorption bands in (B.P. 80°)	
	Light petroleum mμ	Ether mμ
1. Large yellow zone	465, 436	—
2. Small green-yellow zone	466, 436	466, 440, 420
3. Very large lemon-coloured zone	468, 438	465, 440
4. Yellow-brown zone	470, 440	(Violaxanthin)

We have tried without success to crystallize the xanthophylls from these zones. From all solvents only deep red oils separated, which solidified in the cold and became liquid again at room temperature. We suppose that those xanthophylls which are rich in oxygen are unstable to alcoholic KOH and that they lose the power of crystallization by this treatment like fucoxanthin [Willstätter & Page, 1914], or that they are altered in the course of chromatographic analysis with aluminium oxide or calcium carbonate.

The carotenoids of Planchon's furze (Ulex galli).

Planchon's furze differs from *Ulex europaeus* by being smaller in height and especially by having much smaller flowers. The investigation of the pigment of the flowers showed that it consists of the same carotenoids as that of the flowers of the furze. In the case of Planchon's furze carotene is isolated much more easily, as only few colourless substances are present in that fraction. We have also separated the carotene obtained into the isomeric forms and have obtained α- and β-carotene in a pure state.

β-Carotene: M.P. 181–182° (uncorr., in evacuated tube); absorption bands in CS_2, 520, 486mμ.

α-Carotene: M.P. 185° (uncorr., in evacuated tube); absorption bands in CS_2, 512, 482mμ; in light petroleum (B.P. 80°), 480, 448mμ.

In one case of the preparation of the xanthophylls we have observed during chromatographic analysis a small light yellow zone between the zones of violaxanthin and of lutein, from which we extracted a solution of a carotenoid which shows all the properties of flavoxanthin [Kuhn & Brockmann, 1932]. The absorption bands in ethyl alcohol (96%) are 446, 423mμ; in CS_2, 475, 452mμ. With strong hydrochloric acid in ethereal solution a deep blue colour develops. The pigment gives all the colour reactions described by Kuhn & Brockmann for flavoxanthin. We have not, however, succeeded in isolating this carotenoid in a crystalline state.

Some colourless substances accompanying the carotenoids of Ulex galli and U. europaeus.

Hentriacontane. This hydrocarbon was isolated from the mother-liquors of carotene from *Ulex galli*. It separated from the concentrated solution as a light yellow precipitate which was twice crystallized from ethyl alcohol with the addition of a little charcoal. It forms soft leaflets with a silvery lustre which have no birefringence; M.P. 64° (uncorr.).

Micro-analysis (Weiler): Found: C, 85·02%, H, 14·84%. $C_{31}H_{64}$ requires C, 85·22%; H, 14·78%.

Sterol $C_{30}H_{50}O$. This crystallizes together with carotene from *Ulex europaeus* from a mixture of light petroleum and absolute ethyl alcohol, and can be

separated from carotene by boiling with absolute ethyl alcohol. It crystallizes from ethyl alcohol (90 %) in nodular masses, from a mixture of acetic ether and methyl alcohol in fine stellate needles; M.P. 152–153° (uncorr.). The sterol is optically inactive, $[\alpha]_D = \pm 10°$. Colour reactions: Salkowski's test; acid light yellow with green fluorescence; chloroform uncoloured: Liebermann-Burchardt test; chloroform intense dark blue; the colour passes into the acid layer, where after some time it turns to violet.

The sterol is perhaps identical with a sterol $C_{30}H_{50}O.\frac{1}{2}H_2O$, isolated from *Gledschia triacanthus* [Dalmer, 1932].

Analysis: For analysis the substance was dried for 2 hours at 60° in a high vacuum, but it still contained water or alcohol of crystallization.

Found: C, 83·11, 83·07; H, 11·88, 11·57 %. Mol. wt. (Rast) found 385. $C_{30}H_{50}O$, $\frac{1}{2}H_2O$ requires C, 82·68; H, 11·72 %. Mol. wt. 435. $C_{30}H_{50}O$, $\frac{1}{2}C_2H_5OH$ requires C, 82·77; H, 11·89 %. Mol. wt. 449.

Acetate. The acetate was prepared by boiling the sterol with acetic anhydride. It crystallizes from a mixture of ether and methyl alcohol in rectangular leaflets or brilliant needles, which show a strong birefringence; M.P. 145–146° (uncorr.), the substance softening at 138°. The acetate is, like the sterol, optically inactive.

Analysis: Found: C, 81·96; H, 11·30 %. Mol. wt. (Rast) 434. $C_{32}H_{52}O_2$ requires C, 81·96; H, 11·21 %. Mol. wt. 468.

Sitosterol. This sterol was isolated from the xanthophyll fraction of *Ulex europaeus*. After two crystallizations from methyl alcohol it forms leaflets grouped in rosettes with strong birefringence. $[\alpha]_D - 43·2°$ (in ethyl alcohol (96 %)). Colour reactions: Salkowski's test; acid yellow-red with green fluorescence; chloroform uncoloured. Liebermann-Burchardt test: chloroform blue then green; acid violet.

SUMMARY.

1. The carotenoids of the furze (*Ulex europaeus*) and of Planchon's furze (*Ulex galli*) have been investigated. α-Carotene, β-carotene, violaxanthin, taraxanthin and an isomeride of lutein were isolated in pure crystalline state.

2. The occurrence is proved of an unknown carotenoid with an absorption spectrum similar to that of flavoxanthin, but with other chemical properties. The occurrence of flavoxanthin in *U. galli* is probable.

3. Hentriacontane, a sterol of the formula $C_{30}H_{50}O$ and sitosterol were isolated.

The author wishes to express his thanks to the Director of the Chemical Laboratory of the University, Prof. Dr E. Pinto-Basto for placing at his disposal the facilities of the Institute. Acknowledgement is also made to Dr Mendonça of the Botanical Institute for the determination of the flowers.

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