CCLXXV. STUDIES ON CAROTENOIDS.[1]
IV. THE CAROTENOIDS OF *GENISTA TRIDENTATA*.

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THE systematic investigation of the metabolic products of related plant species is of considerable biochemical interest, as it can provide us with a view of similar vital processes which occur during the metabolism and assimilation of the plants. Therefore we believe it useful to include in our studies of the carotenoids of the Portuguese flora a systematic investigation of the family of Leguminosae for later comparison of the results obtained.

In the preceding paper we have communicated the results of the investigation of two species of furze, and we propose to describe here our results obtained with the flowers of *Genista tridentata*.

The flowers of two other species, i.e. *G. racemosa* and *G. tinctoria*, have already been examined by Tammes [1900] and Courchet [1888] who determined the presence of carotenoids in these plants. Recent investigations of Baker & Robinson [1925; 1926; 1928] showed the presence in *G. tinctoria* of two water-soluble colouring matters, genistein, $C_{15}H_{10}O_5$ (3:7:4′-trihydroxy*iso*flavone), and luteolin, $C_{15}H_{10}O_6$ (5:7:3′:4′-tetrahydroxyflavone).

METHODS.

3·7 kg. of dried yellow flowers were ground in a mill to a very fine powder and this was then extracted several times with a total quantity of 30 litres of light petroleum. The extract was concentrated *in vacuo* in a current of carbon dioxide to 150 ml., and to the concentrate 450 ml. of absolute ethyl alcohol were added. On standing in the ice-box for several days a large amount of colourless material separated. This was removed by filtration, and water was added to the filtrate. After prolonged washing with water, the solution was dried over anhydrous sodium sulphate, filtered through a dry filter-paper and diluted to 600 ml. This solution was used for chromatographic analysis.

The colouring matter was adsorbed on to a column of activated aluminium oxide, 18 cm. long and 6 cm. in diameter. On washing the column with light petroleum nine different zones developed, of which, however, only seven were used for further treatment, the two upper zones being oxidation products.

Zone

1. Dark green, narrow and sharp
2. Yellow, narrow and sharp
3. Green, broad and diffuse
4. Light yellow, broad and diffuse
5. Orange, very broad and sharp
6. Orange-red, broad and sharp
7. Orange-red, narrow and diffuse

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The column was divided into three Parts, zones 1–4 forming Part I, zone 5 which occupies most of the column forming Part II and zones 6 and 7 forming Part III.

Part I. The colouring matter was eluted with light petroleum containing 1 % of methyl alcohol and the solution, which contains the chlorophyll pigments and a part of the esterified xanthophyll, was concentrated to 100 ml. *in vacuo*. To the concentrate were added 50 ml. of concentrated methyl alcoholic KOH and a quantity of absolute ethyl alcohol sufficient to make the mixture homogeneous. After complete saponification 500 ml. of light petroleum were added and water to reduce the alcohol concentration to about 40 %. The alcoholic layer was twice extracted with a total of 500 ml. of light petroleum, the united solutions of light petroleum were washed several times with water and then dried over anhydrous sodium sulphate. This solution served for a further chromatographic analysis.

The solution was adsorbed on to activated calcium carbonate and the chromatogram developed by washing with a mixture of light petroleum-benzene 4:1. Four distinct zones developed:

	Absorption bands in light petroleum (B.P. 80°) mμ
1. Narrow yellow zone	467, 441
2. Broad pale yellow zone	474, 441
3. Broad yellow-red zone	475 (very faint)
4. Broad orange-yellow zone	Very faint bands

From the first zone we have isolated a sterol which we shall describe later. It was, however, not possible to crystallize the colouring matter contained in this zone, which gives a strong blue colour with concentrated hydrochloric acid in ethereal solution.

The second and third zones, which have the same absorption spectra, were united and eluted with methyl alcohol. The solution was concentrated *in vacuo* to 10 ml. and kept in the ice-box for 1 day. A colourless substance crystallized, which was removed by suction. The filtrate was then evaporated to dryness *in vacuo* and the residue dissolved in light petroleum and kept in the ice-box. A crystalline precipitate appeared which was removed by filtration in the cold, as it was soluble at ordinary temperature. The xanthophyll was then extracted from the light petroleum solution with methyl alcohol (90 %), the solution concentrated *in vacuo* to 5 ml. and kept in the ice-box for 2 days. A mixture of xanthophyll with colourless substances crystallized. The crystals were filtered off, washed with light petroleum and twice recrystallized from absolute methyl alcohol. Pure lutein was obtained; M.P. 191–192° (uncorr., in evacuated tube). Absorption bands in methyl alcohol 471, 445mμ; in CS_2 504, 472mμ.

The fourth zone of the chromatogram which contained only a very small portion of colouring matter was not further investigated.

Part II. The colouring matter of this part of the chromatogram was eluted with light petroleum containing 1 % of methyl alcohol and the solution concentrated to a small volume *in vacuo*. 70 ml. of concentrated methyl alcoholic KOH and enough absolute ethyl alcohol to make the solution homogeneous were added. After complete saponification the solution was filtered from a colourless precipitate and a large quantity of water added. The formation of an emulsion took place, and by saturating this with sodium chloride the colouring matter was precipitated. It was dissolved in methyl alcohol and the solution reduced to a small volume *in vacuo*. A xanthophyll crystallized together with

colourless substances. The precipitate was dissolved in a small volume of methyl alcohol and three volumes of ether were added which caused the precipitation of a colourless water-soluble substance. After removing this by centrifuging the solution was evaporated to dryness *in vacuo* and the residue dissolved in methyl alcohol. This solution was again evaporated to dryness and the residue dissolved in hot light petroleum. On keeping the solution in the ice-box for 24 hours, a xanthophyll crystallized which after two further crystallizations from absolute methyl alcohol proved to be pure lutein; M.P. 192° (uncorr., in evacuated tube); absorption bands in methyl alcohol 473, 446 mμ; in CS_2 509, 474 mμ.

Part III. After elution of the colouring matter with a mixture of light petroleum and methyl alcohol, the solution obtained was concentrated *in vacuo* to 300 ml. To the concentrate 50 ml. of a concentrated methyl alcoholic solution of KOH, 50 ml. of ethyl alcohol (96 %) and 100 ml. of absolute ethyl alcohol were added and the mixture was saponified for 2 days with occasional shaking. Part of the colouring matter passed into the alcoholic layer. After separation of the two layers, the light petroleum solution was twice washed with methyl alcohol (90 %) and the alcoholic layers were united. Water was added to lower the concentration of the alcohol to 80 % and the solution then extracted with light petroleum. In this way two solutions were obtained, an alcoholic solution containing xanthophyll and a light petroleum solution containing hydrocarbons.

(*a*) *Xanthophyll.* To the alcoholic solution a little light petroleum and then a considerable quantity of water were added with continuous shaking. As an emulsion formed, the solution was saturated with sodium chloride which caused the precipitation of 650 mg. of xanthophyll in a crystalline state. After two recrystallizations from absolute methyl alcohol we obtained 204 mg. of very pure lutein with M.P. 193° (uncorr., in evacuated tube) and absorption bands in CS_2 507, 473 mμ. From the mother-liquors more lutein of a less degree of purity was obtained by concentration.

(*b*) *Hydrocarbons.* The light petroleum solutions were thrice washed with methyl alcohol (90 %) and then several times with water, dried over anhydrous sodium sulphate and then passed through a dry filter. The solution was then adsorbed on to activated aluminium oxide and the column washed with light petroleum. Three different zones formed:

	Absorption bands in light petroleum (B.P. 80°) mμ
1. Very narrow red-violet zone	453, 424
2. Broad sharp orange zone	483, 448
3. Narrow diffuse yellow zone	476, 446

The first zone contained a very small amount of a carotenoid which shows similar absorption bands to those of flavoxanthin and which is identical with a carotenoid found in the furze, described in the preceding paper. It could not be obtained in a pure crystalline state.

The second zone contains the bulk of the colouring matter and consists of β-carotene. It was eluted with light petroleum containing 1 % of methyl alcohol. This solution after colorimetric determination [Kuhn & Brockmann, 1932] was found to contain 191 mg. of carotene. It was concentrated *in vacuo* to 100 ml. and 50 ml. of absolute ethyl alcohol were added. After standing in the ice-box for 12 hours, 76 mg. of pure β-carotene had separated in beautiful crystals of permanganate-like colour; M.P. 181–182° (uncorr., in evacuated tube); absorption bands in CS_2 521, 487 mμ.

The third zone was also eluted with the mixture of light petroleum-methyl alcohol and evaporated to dryness *in vacuo*. The residue was dissolved in 5 ml. of light petroleum and 30 ml. of absolute methyl alcohol were added. As the colouring matter did not crystallize, the solution was brought to dryness and the residue dissolved in 10 ml. of hot methyl alcohol. From this solution about 1 mg. of α-carotene crystallized, which, however, was not pure. Owing to the small quantity, we have not purified this substance further.

Sitosterol. This sterol was isolated as a by-product from zones 2 and 3 of the chromatogram of Part I. It crystallizes from methyl alcohol in characteristic leaflets with strong birefringency; M.P. 131° (uncorr.); $[\alpha]_D$ −34·1° (in ethyl acetate). With the reagents of Salkowski and of Liebermann-Burchardt the sterol gives the colour reactions characteristic of sitosterol. We obtained 177 mg. of the pure product.

In addition to sitosterol another sterol was isolated in very small amount from zone 1 of the same chromatogram, which gives the same colour reactions as the sterol $C_{30}H_{50}O$ described in the preceding paper. The low M.P. of 105–106° (uncorr.) indicated that it was probably not pure, but owing to the small amount of crystals we could not purify it further.

Discussion.

There is a striking difference between the carotenoids of the furze and those of *G. tridentata* in respect of their xanthophyll contents. Whilst in the first the xanthophylls are a mixture in which violaxanthin predominates, with taraxanthin and only a little lutein, other xanthophylls being probably present, the xanthophyll of the latter is almost pure lutein. The lutein occurs in the plant in an esterified form and, by means of chromatographic analysis, we were able to demonstrate that there exist at least three different esters of lutein.

On the other hand, the carotene of *G. tridentata* is almost pure β-carotene, only traces of the α-isomeride being present. From this it is clear that the hydrocarbons belong to the β-carotene series, whereas xanthophyll in its chemical structure is related to α-carotene. A similar relation exists in a less accentuated degree between the carotene and xanthophyll of the green leaves of almost all plants. It is possible to admit that *in vivo* lutein may be related to β-carotene, in contrast to the relation *in vitro*.

Summary.

1. The carotenoids of *Genista tridentata* have been investigated. α-Carotene, β-carotene and lutein have been isolated in a crystalline state. The occurrence of other carotenoids is probable.

2. Sitosterol and another sterol were isolated.

3. A possible biological relationship between β-carotene and lutein is discussed.

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