

CCIV. STUDIES ON CAROTENOIDS

V. GAZANIXANTHIN

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THREE xanthophylls of the formula $C_{40}H_{56}O$ are known, of which cryptoxanthin [Kuhn & Grundmann, 1933] is related to β -carotene, rubixanthin [Kuhn & Grundmann, 1934] to γ -carotene, and lycoxanthin [Zechmeister & Chlcnoky, 1936] to lycopene. In this paper we describe a fourth xanthophyll with one oxygen atom, which has been isolated from the petals of *Gazania rigens*, and which, judging from its behaviour, is related to γ -carotene and rubixanthin.

The flowers of *G. rigens* are very interesting from the biochemical standpoint since the great majority of its carotenoids belong to the γ -carotene series, which rarely occurs in other plants. The greater part of the carotenoids of this plant appears in the "carbohydrate fraction", which includes also the xanthophylls with one oxygen atom. This fraction contains four different carotenoids, three of which are present in about equal amounts, and the fourth—probably γ -carotene—only in much smaller quantities. All these four substances have practically the same absorption spectrum as γ -carotene; they can be separated chromatographically.

Chromatographic analysis of the saponified lipid extracts of *Gazania* flowers gives an upper zone of rubixanthin, which can easily be obtained in a pure crystalline state. The second coloured zone, separated from the first by a large colourless one, contains the new xanthophyll gazaniaxanthin. It is accompanied by another unknown carotenoid with the same absorption spectrum, adsorbed closely under the zone of gazaniaxanthin, and difficult to separate from it chromatographically. This substance has not yet been obtained crystalline. Below this zone, follows a small zone of γ -carotene.

Gazaniaxanthin crystallizes from a mixture of benzene-methyl alcohol (1 : 4) in brilliant rectangular leaflets of a deep red colour, whose macroscopic and microscopic appearance is very similar to that of cryptoxanthin. The crystals contain methyl alcohol from which they can be freed only with difficulty. In this respect gazaniaxanthin is rather different from rubixanthin, which crystallizes without methyl alcohol, in red-brown needles of a much lighter colour.

Gazaniaxanthin is readily soluble in benzene and light petroleum, very sparingly soluble in methyl alcohol. During partition between light petroleum and methyl alcohol, gazaniaxanthin behaves like rubixanthin. It melts at $136-137^{\circ}$ (uncorr., in evacuated tube).

The spectral behaviour of gazaniaxanthin is very similar to that of γ -carotene and rubixanthin, the absorption maxima being slightly displaced to shorter wave-lengths.

*Absorption maxima of gazaniaxanthin*¹

| | m μ |
|-------------------------------|-----------------|
| In carbon disulphide | 530, 495.5, 463 |
| In light petroleum (B.P. 80°) | 494, 461, 433.5 |
| In hexane | 492.5, 460, 432 |
| In benzene | 508, 476.5 |
| In absolute ethyl alcohol | 494.5, 462.5 |

Elementary analyses of gazaniaxanthin agree with the composition C₄₀H₅₄O or C₄₀H₅₆O. The presence of an alcoholic hydroxyl group was demonstrated by the formation of an acetyl derivative with acetic anhydride in pyridine solution.

Gazaniaxanthin in the crystalline state binds solvents and ash tenaciously and in spite of repeated crystallizations we did not succeed in obtaining absolutely ash-free preparations. We believe that this fact is the cause of the ease with which solutions and crystals of this carotenoid undergo autoxidation, whereby it is transformed in the course of a few days even in the cold into a colourless crystalline substance.

In its chromatographic behaviour gazaniaxanthin is intermediate between rubixanthin and lycopene. A mixture of these three substances can be easily separated in the alumina column.

The elucidation of the chemical structure of gazaniaxanthin will be possible only after further investigation. Kuhn & Grundmann [1934] have demonstrated that rubixanthin is a derivative of γ -carotene containing a hydroxyl group in the β -ionone ring; it is suggested that gazaniaxanthin may be an isomeride of this having the hydroxyl group in the aliphatic side chain, like lycoxanthin and lycophyll [Zechmeister & Cholnoky, 1936]. The correctness of this supposition can be tested by a biological assay, as a substance of the constitution proposed should be active as provitamin_A in contrast to rubixanthin.

EXPERIMENTAL

We are obliged to Dr Mendonça of the Botanical Institute of this University for the following notes about *Gazania* plants.

Gazania rigens, of the family of Compositae, is native to Africa. It is cultivated in Portugal in the open air, whereas in the north of Europe it can only live in hot houses. The plant, though abundant in flowers, does not fructify in Portugal, and the flowers used for our investigation are from a specimen brought to the Botanical Garden of Coimbra about a century ago, and which has since then reproduced only asexually by means of suckers.

The plant flowers from March to the end of May, only a small part flowering at a time. We collected the flowers once a week. The plant contains a latex-like sap, which is exceptionally rich in lipoids.

The petals (about 200 g. collected in a week), are separated and dried in a stream of warm air at 50° for several hours. The dry material (40 g.) is finely pulverized in a mortar, and then exhaustively extracted with hot light petroleum (B.P. 30–50°) in an atmosphere of CO₂.

The solution, on standing in the ice box, deposits crystals of free xanthophyll and a part of the resinous substance. After filtering, the solution is evaporated *in vacuo* at a low temperature until it begins to foam. The residue is dissolved in 150 ml. of hot absolute ethyl alcohol. This solution, during slow refrigeration, precipitates a considerable quantity of colourless resinous substance, which is

¹ Determined with a Hilger prism-spectroscope and a copper sulphate-ammonia filter.

filtered off after about half an hour. It is important not to cool this alcoholic solution too much, or to let it stand for too long, as otherwise a part of the carotenoid pigments also precipitates.

The solution is filtered and 50 ml. of pure thiophen-free benzene and 5 ml. of saturated aqueous KOH are added and the homogeneous clear mixture is kept at room temperature (22°) in well filled stoppered bottles for 2 days. Then 300 ml. of light petroleum and 50 ml. of water are added and the mixture is agitated. Nearly all the colouring matter remains in the upper layer. The alkaline alcoholic layer is discarded as it contains much resinous matter and only a little xanthophyll.

The light petroleum solution is thrice extracted, each time with 50 ml. of methyl alcohol (90%), which dissolves a quantity of xanthophyll. The solution is then washed several times with water, to remove the methyl alcohol, dried with sodium sulphate and filtered. When kept in the ice box a quantity of colourless substances (various sterols) separate, which have been obtained in a pure state.

It proved of special importance for the chromatographic separation of the carotenoids present in the light petroleum solution to choose an adsorbing substance which is not too active, as otherwise in consequence of the many colourless substances present, and the relatively small differences in adsorbing affinity of the pigment, no sufficient separation can be obtained. We have found that, though separation may be effected with calcium hydroxide, this substance is not very appropriate because of its bad filtering qualities and the indistinct formation of the zones. The best material for the separation of the carotenoids of *Gazania* flowers is alumina. We used Merck's ordinary aluminium oxyd. puriss. anhydr., which, as it was still too active, was moistened with light petroleum and kept in the air for 1 day.

The solution is filtered through a column 15 cm. high and 5 cm. in diameter, without suction, and the chromatogram is developed with a mixture of benzene-light petroleum (1 : 3). Six zones develop. The first small, deep red, zone still contains a little xanthophyll, which was recognized to be pure lutein. Then follows a deep brown-red zone 5 mm. thick, which contains rubixanthin. The third zone, 3-4 cm. thick contains only colourless substances. Then comes a violet-red zone 4 mm. thick, immediately followed by another brown-red zone of double this thickness. From the former we have isolated gazanixanthin, while the latter has not yet furnished a crystalline coloured substance. The isolation and crystallization of gazanixanthin depends essentially on the sharp separation of the upper uncoloured and the lower coloured zones.

At some distance from the fifth zone, follows a small yellow ring which passes more rapidly to the filtrate and which shows the bands of γ -carotene. A very small first part of the yellow filtrate shows absorption bands of β -carotene.

Rubixanthin. The zone of the chromatogram containing rubixanthin is eluted with light petroleum containing 1% of methyl alcohol and evaporated *in vacuo*. The residue is dissolved in 10 ml. of hot light petroleum and kept for 2 days in the ice box. Deep red crystals and a red oil separate. The precipitate is filtered and the crystals remaining on the filter are freed from the oil by washing with cold light petroleum which contains a few ml. of absolute ethyl alcohol. The crude crystals have m.p. 145°. They are dissolved in a mixture of 15 ml. methyl alcohol and 10 ml. ether, and the solution evaporated on the water bath until crystallization begins. The solution is kept at room temperature for half an hour, then in the ice box overnight. The crystals obtained are once

more crystallized from a mixture of benzene-methyl alcohol (1 : 3). 5 mg. of fine red-brown needles are obtained with m.p. 160° (uncorr., in evacuated tube). Absorption bands in CS₂: 533·5, 498, 465 mμ; in light petroleum (B.P. 80°) 496, 461 mμ. From the mother liquors more rubixanthin of m.p. 150–153° was obtained.

Gazaniaxanthin. The zone of the chromatogram which contains the gazaniaxanthin is carefully separated from the other zones above and below and eluted with light petroleum containing 1 % of methyl alcohol. The solution is evaporated to dryness *in vacuo*. The residue solidifies within a few seconds. It is dissolved in the minimum necessary amount of hot light petroleum (5 ml. approximately) filtered and kept in the ice box. After half an hour gazaniaxanthin begins to crystallize in brilliant orange leaflets. After 20 hr. the crystals are filtered, and twice crystallized from a mixture of benzene-methyl alcohol (1 : 4). From this mixture the carotenoid crystallizes in brilliant rectangular crystals of a deep red colour with strong dichroism, and birefringence. From a mixture of light petroleum and methyl alcohol brown-red crystals of more irregular shape are obtained. Both these crystals contain methyl alcohol, of which the greater part is lost during heating *in vacuo* to 80–90°. The m.p. determined in a circular bath of sulphuric acid was found at 136–137° (uncorr., in evacuated tube).

During heating in high vacuum at 80° the carotenoid lost 0·92, 0·89 mol. of methyl alcohol.

Found (for dry and ash-free substance): C, 87·12, 87·39 %. H, 10·75, 10·73 %. Calculated for C₄₀H₅₆O: C, 86·89%; H, 10·22 %. C₄₀H₅₄O: C, 87·27%; H, 9·90 %.

Gazaniaxanthin acetate. 10 mg. of gazaniaxanthin were dissolved in 23 ml. of pyridine and 2 ml. of acetic anhydride added. The mixture was kept for 1 day in a well-filled, stoppered bottle. Then 75 ml. of benzene were added and the solution shaken many times with water to remove all pyridine. The solution was dried with sodium sulphate, filtered and concentrated *in vacuo* to 3 ml. 9 ml. of absolute methyl alcohol were added and the mixture kept in the ice box. In the course of several days, the acetate crystallized. It was twice recrystallized from a mixture of benzene-methyl alcohol. The acetate tends to crystallize slowly, in the course of several days only.

Gazaniaxanthin acetate is more soluble in benzene and light petroleum than the free xanthophyll. It crystallizes from a mixture of benzene-methyl alcohol in thick star-shaped orange needles. From light petroleum-methyl alcohol fine, long, needles appear, frequently curved; m.p. 83–85° (uncorr., in evacuated tube) with strong foaming, probably losing solvent.

The absorption spectrum is identical with that of gazaniaxanthin; in CS₂: 530, 494 mμ; in benzene 508, 476, 445 mμ.

Xanthophylls. The methyl alcohol extracts obtained after saponification are, after addition of 10 ml. of light petroleum, carefully diluted with water. Crystalline xanthophyll precipitates. The ethereal solution of this crude xanthophyll gives a faint blue colour with conc. HCl, which, however, disappears after further crystallizations. The xanthophyll is recrystallized from a mixture of chloroform-light petroleum, then twice from a mixture of methyl alcohol-ether. A small portion of "leaf xanthophyll" is obtained; m.p. 204° (uncorr.). Absorption bands in CS₂: 509, 478 mμ. During chromatographic adsorption of the xanthophyll on to calcium carbonate, two zones are observed, the upper orange yellow, the lower light yellow. We have not tried to isolate the components in a pure state.

From the first zone of the chromatogram of the hydrocarbon fraction we obtained by elution with methyl alcohol a solution, from which after evaporation

in vacuo and two crystallizations from a mixture of methyl alcohol-ether, pure lutein was isolated. It crystallized in yellow needles, which, in contact with the methyl alcohol, were transformed in the course of several days into deep red crystals, which contained methyl alcohol. M.P. 195° (uncorr., in evacuated tube). Absorption bands in CS₂ 508, 476, 445 m μ .

SUMMARY

1. A new xanthophyll with one oxygen atom, gazaniaxanthin, has been isolated from the flowers of *Gazania rigens*. Its physical and chemical properties are similar to those of rubixanthin.

2. In the flowers of *Gazania rigens* there occur, besides gazaniaxanthin, rubixanthin, lutein and "leaf xanthophyll", which were isolated in a pure crystalline state, and probably γ -carotene. Another carotenoid with the same absorption spectrum as γ -carotene could not be isolated in the crystalline state.

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