CXXVIII. THE YELLOW PIGMENTS OF AUSTRALIAN ACACIAS.

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THE golden bloom of Australian Wattles, so favoured as the national flowers by Australian artists and poets, has afforded rich material for the study of the yellow pigments.

The Acacias constitute, with the Eucalypts, a large part of the unique endemic flora of Australia; more than 300 species are distributed throughout the continent, and in all seasons of the year one or more is to be seen in flower. The beautiful florescent masses consist largely of dense crowds of stamens, and their colour ranges from palest cream to deepest golden yellow.

Hitherto nothing was known of the chemical nature of these golden wattle-blossoms, or of the substances to which they owed their beauty. In this investigation the yellow pigments have been extracted, and their properties studied.

Plant pigments are considered biochemically in two distinct groups—the plastid pigments and the water-soluble pigments. In the former the molecule of the colour substance is combined in some obscure chemical way with the other components of the complex colloidal molecule of protoplasm; in this group chlorophyll, carotene, and xanthophyll are well known. Of the water-soluble pigments found in solution in the cell-sap, the yellow coloured substances are mostly flavone and xanthone derivatives, while the blue, red, and purple colours of flowers belong to the group of anthocyanins.

EXPERIMENTAL.

The Material. Four typical examples of Acacias were chosen for this investigation:

I. *Acacia discolor*. II. *Acacia linifolia*. III. *Acacia decurrens*, var. *mollis*. IV. *Acacia longifolia*.

Two of these possess the characteristic pinnate mimosa leaves, and two have their leaves replaced by phyllodes in response to their xerophytic environment. The flowers were collected in quantity at the period of maximum inflorescence, in the neighbourhood of Sydney and the Blue Mountains.

General Method of Extraction. The fresh flowers were immersed in small amounts at a time in boiling water, in order to destroy the enzymes, and the boiling continued till most of the soluble colouring matter was removed. The

large volume of fluid was concentrated and filtered from mucilage and coagulated proteins. The solution was then precipitated by lead acetate; the precipitate containing the colouring matter and tannin. The lead was removed, and the pigments were hydrolysed by boiling with sulphuric acid, during which operation the glucosides were decomposed into yellow pigment and sugars. The colour was extracted with ether, and the remaining aqueous fluid was reserved for the sugar. On distilling the ethereal extract the colouring matter was obtained in the crude state, and was then purified by a series of fractional crystallisations.

I. *ACACIA DISCOLOR.*

The flowers of *A. discolor*, the sunshine wattle, collected in April, were extracted and treated with ether. The absence of yellow pigment from this ethereal extract showed that no free flavone existed in the plant, and that the pigment was present wholly in the form of a glucoside. The latter was precipitated by lead acetate, and hydrolysed. This yielded (1) a black resinous substance insoluble in the hot fluid; (2) a deep brick-red deposit which formed on cooling; (3) a bright red solution.

The second and third of these gave with ether a yellow pigment. The lead filtrate was further treated with basic lead acetate, hydrolysed, and extracted with ether. The ethereal solutions were then agitated with alkali carbonates from which the yellow colouring matter was precipitated by acid; the alkaline solution was first green in colour, then became red, and changed to purple and violet.

Purification of the crude yellow pigment. The yellow crystalline deposits contained a red and an amorphous brown substance intermixed. By dissolving in hot alcohol a deep red solution resulted, and on carefully diluting this with hot water a point was reached when the yellow pigment could be crystallised out alone. When recrystallised in this way a number of times, the filtrate at last ceased to be red, and a mass of lemon-yellow, needle-shaped crystals was obtained. These possessed an almost constant melting point, and were submitted to a rigid investigation to determine their properties and constitution.

Products obtained from the above operations. 1200 g. of fresh flowers gave:
24 g. black resinous substance.

32 g. of brick-red deposit after hydrolysis, consisting of phlobaphenes.

2.4 g. of crude yellow pigment.

A solution containing the glucosidal sugars.

A brownish-yellow amorphous substance from the mother-liquors after crystallisation of the yellow pigment.

Deep crimson-coloured aqueous solutions exhibiting the properties of anthocyanins and tannins.

A viscid oil of strong acrid odour, obtained from the ether extract after the yellow pigment had been removed by alkali.

(a) *The soluble Yellow Pigment.*

In order to complete the purification of the yellow substance the whole was converted into its acetyl derivative. The compound separated from water as a brown crystalline mass, and after purifying with charcoal and recrystallising from hot alcohol, a pure white product was obtained. The acetyl derivative possessed a constant melting point, and was afterwards quantitatively hydrolysed. The yellow pigment obtained in this way was now assumed to be pure.

(1) *Properties.* The substance appeared under the microscope as beautiful masses of crystals, fine hair-like needles of a pale yellow colour, possessing a melting point of 274° . By adding a drop of ammonia to 10 cc. of a solution containing the smallest speck of the substance a deep yellow colour was produced; this is a characteristic reaction of flavones, which have little colour of their own in the glucosidal condition.

Lead acetate produced a bright orange-red precipitate.

Ferric alum in alcoholic solutions gave a purple colour.

Sulphuric acid dissolved the pigment to a bright yellow solution which, on standing some hours, exhibited a bluish-green fluorescence.

These indications that the substance belonged to the flavone group were confirmed by the following quantitative results.

(2) *Sulphuric acid derivative.* The substance, in acetic acid solution and under anhydrous conditions, united with sulphuric acid and yielded a mass of bright orange-red crystals in the form of minute thin needles. These when purified were found to decompose quantitatively, yielding the original substance.

0.1695 g. compound gave 0.1262 g. pigment = 74.45 %

0.0704 g. „ „ 0.0524 g. „ = 74.43 %

74.45 % of pigment represents a Mol. Wt. of 285.8

Calc. for $C_{15}H_{10}O_6$ 286

Five different flavones are known possessing this formula.

The property of combining with mineral acids in this way is probably a general reaction for the yellow colouring matters of the pheno- γ -pyrone class, as has been pointed out by A. G. Perkin. This property has been shown to be due to the oxygen atom in the pyrone ring becoming quadrivalent, and is also said to be characteristic of the γ -pyrone ring.

(3) *Disruption of the Pyrone Nucleus.* In order to determine the nature of the benzene rings attached to the pyrone nucleus, the yellow compound was submitted to fusion with potassium hydroxide at 200° . From the product, phenols and acids were separated. In the former, phloroglucinol was identified by its reactions and by the crystals of the tribromo derivative. The solution of organic acids gave with ferric alum a bluish-green colour, which changed to violet with sodium acetate, and to red with sodium carbonate; but no coloration was produced by a ferrous salt. Protocatechuic acid gives these colour reactions; but it also gives a violet colour with ferrous sulphate.

The small amount of substance left, when recrystallised, separated in radiating clusters of white needle-shaped crystals, melting at 192° . The discrepancy could not be removed at this stage, as the weight of material was quite inadequate for the preparation of salts or the molecular weight determination.

Later, a probable explanation was found in a paper by Barth and Hlasiwetz [1865]. Here protocatechuic acid was shown to form with *p*-hydroxybenzoic acid a compound of constant composition and a constant melting point of 192° . Ferric chloride, acting on the latter acid alone, produced a yellow colour, while the mixture gave the green of the former acid.

The results obtained by fusion of the yellow pigment with potash indicate, therefore, the presence of phloroglucinol, *p*-hydroxybenzoic acid, and protocatechuic acid, among the products of decomposition.

The highly purified pigment was not used for this experiment: it was reserved for the quantitative determination of acetyl and sulphuric derivatives. The sample of pigment used for the alkali fusion, from which it is not recoverable, consisted of the second and third crops of crystals, and although much time was spent in its purification it was subsequently found still to give faint colour reactions of tannins. In the later examination of the tannins protocatechuic acid was identified; and this is a possible source of the acid in the decomposition of the yellow pigment, since one only of these two acids can belong to the flavone molecule.

(4) *Acetylation*. The acetyl compound was prepared and purified. It consisted of fine, white, needle-shaped crystals of silky lustre, matted together into a network; these were anhydrous after drying at 130° , and melted at 179° .

The number of acetyl groups in this compound was then determined by hydrolysis with sulphuric acid, and the yellow pigment thus set free was deposited in its original form.

0.0327 g. acetyl derivative gave 0.0201 g. pigment = 61.5 %

1.3100 g. " " " " 0.8075 g. " = 61.6 %

In a substance of molecular weight 286, this result represents four acetyl groups. The original substance therefore contains four hydroxyl groups in its molecule.

Kaempferol contains four (OH) groups, and tetra-acetyl kaempferol melts at 181° . (See Fig. 2, p. 964.)

(5) *Methoxy-groups*. The testing for CH_3O -groups was carried out by Perkin's modification of Zeisel's method, but no methyl iodide was evolved, and the pigment was recovered unchanged from the mixture. Methoxy-groups are therefore absent from the molecule of the substance.

(6) *Oxidation*. The substance dissolved in dilute soda to a yellow solution. From this liquid the pigment could at first be reprecipitated by acid, but when a current of air was aspirated through the solution for 24 hours, a profound change was noted: the colour became dark brown, and the pigment, which was no longer precipitated by acid, could not be detected in, or recovered from,

the solution. Evidently it had been decomposed by oxidation and, when agitated with ether, a crystalline substance was obtained, which gave the reactions of phloroglucinol.

The nature of this result was shown by Perkin to be due to a particular hydroxyl group in the α position of the pyrone nucleus, the presence of which in a flavone molecule characterises the subgroup of the flavonols.

(7) *Metallic Derivative*. The attempt to prepare a flavonol potassium salt was unsuccessful. A very dark precipitate formed which settled as an Indian-red coloured granular powder, the superfluid being deep red. This granular deposit showed the same melting point as the yellow pigment. The typical orange prisms said to be obtained from the flavonols could not be prepared in this case, and one may conclude that the acidic properties of the substance in question are so feeble that the compound with a metal is difficult to form.

(8) *Conversion of Flavonol to Anthocyanidin*. When we compare the molecular configuration of yellow flavonols with red anthocyanidin pigments, it is observed at once that the difference, though fundamental, is very slight. Theoretically, in passing from one to the other, the change takes place in the 1:4 positions of the pyrone ring, where the oxygen atom changes in valency from 2 to 4, and the CO group is reduced to CH.

Fig. 1. Pyrone nucleus showing change from flavonol to anthocyanidin.

Such a change was effected practically by treating the alcoholic solution of the yellow pigment with nascent hydrogen. The colour at first became green, then changed suddenly to a brilliant red.

This product may be described as an artificial anthocyanidin, a reduced oxonium salt from flavonol. It exhibited all the general properties of anthocyanidins. When the red alcoholic solution was exposed to air and light it soon became colourless, owing to some allotropic change. When very dilute ammonia was added to the fresh red solution, a blue colour appeared, and with the decolorised solution yellow was formed, the other stages giving mixed green reactions due to both forms. When the red solution was agitated with acid and amyl alcohol the red pigment passed into the latter solvent. The red amyl solution was separated and, on the addition of a drop of sodium carbonate, a change of colour was observed, the solution passing rapidly from blue to green. Finally the alkaline amyl solution was shaken up with water, when the red anthocyanidin suddenly went back into the aqueous solution.

(b) *The Sugar of the Glucoside.*

During the process of extraction of the flavonol it was shown that the latter was present as a glucoside. The sugar resulting from the hydrolysis was isolated as a mass of white crystals. In the examination of these galactose was proved absent. The colour tests indicated the presence of pentose and when the crystals were boiled with sulphuric acid, methylfurfural was evolved, which gave the yellow aniline acetate test, changing to red with acid.

The sugar, thus shown to be a methylpentose, was subsequently proved to be rhamnose from the properties of its osazone.

The osazone crystals were purified by six crystallisations from dilute alcohol, and then fractionated. The first and last fractions had melting points of 187° and 188° respectively. There was therefore only one compound present.

The melting point of rhamnose has been recorded by E. Fischer as 185°, and by Votoček as 186°–187°.

(c) *The Tannins.*

A large amount of a reddish-brown granular substance was deposited on cooling the hydrolysed aqueous extract of the flowers; gallic acid also crystallised out from this solution. The dry red powder was fused with potash at 200°, and from the acidulated aqueous solution phloroglucinol and protocatechuic and gallic acids were isolated.

The red substance is therefore the phlobaphene or red anhydride of a tannin of the catechol group. Gallic acid is generally regarded as indicating the presence of a gallo-tannin, but A. G. Perkin has recorded a rare exception in the tannin of *Pistacia*, a small European tree (mastic tree), which gave both a red phlobaphene of the catechol series, and also gallic acid and phloroglucinol after fusion. Gallic acid also exists free in certain plants. The results indicate either the presence of two different groups of tannins—a gallo-tannin and a catechol tannin—or one similar to the rare instance mentioned, which appears not to coincide with the present classification of the tannins.

The deep red-coloured fluids obtained all through this work owe their colour to a certain extent to the phloroglucinol in the tannin molecule. During the acid hydrolysis, pentose sugars are formed from various sources such as lignin, pentosans, and the rhamnose glucoside. These give the phloroglucinol red when heated with acid.

(d) *The Plastid Pigments.*

Two different tints of yellow may be observed in the inflorescence of *Acacia discolor* when in full bloom: the anthers possess a shining golden yellow colour, while the long filaments are pale cream. If fresh flowers be submitted to the action of ammonia vapour the flavone reaction at once appears, and the previously pale parts of the stamens assume the characteristic deep yellow tint. If some cut flowers be laid aside for a few days and afterwards examined with a lens, the withering is seen in the pale filaments which have become brown,

while the bright yellow of the anthers persists. The brown portions now respond much less to the action of ammonia, for probably the flavonol pigment has been partly oxidised. The unchanged golden yellow parts are most probably those in which the colour is fixed in the chromoplasts as carotene and xanthophyll.

The latter were isolated from the flowers by first removing the water-soluble pigments, and saponifying the chlorophyll in cold alcoholic potash. Carotene and xanthophyll were then obtained by extracting with ether. The weight was equivalent to 0.9 % of the flowers (dried at 100°).

The solution of carotene and xanthophyll showed the complete absorption of the violet and blue rays from λ 495 μ .

II. *ACACIA LINIFOLIA*.

The pale cream-coloured flowers of this species were collected in June, and treated as in the previous case.

From 2 kilos. of flowers about half a kilo. of phlobaphenes was obtained. The yellow pigment was similar to that described above, and the yield was 0.17 % of the air-dried material. The plastid pigments formed 1 %, and an approximate separation gave 0.75 % of carotene, and 0.25 % of xanthophyll.

III. *ACACIA DECURRENS*.

The golden yellow flowers were collected in December, and yielded the same pigment in small amount.

Gallic acid was obtained here, as in *A. discolor*, in considerable quantity, along with the phlobaphene of the catechol tannin.

IV. *ACACIA LONGIFOLIA*.

The deep yellow flowers of the "Golden Wattle," collected in January, yielded the same pigments. The ethereal extracts of this species presented some peculiarities not observed in the previous extracts: for instance, in place of the bright yellow coloured flavone solutions of the three species, this one gave a solution which was quite colourless by transmitted light, while by reflected light it appeared brilliant purple, and on evaporating the solvent the yellow pigment separated.

CONCLUSIONS.

The yellow pigments isolated from the flowers of the Acacias gave the following data:

	<i>Acacia discolor</i>	<i>A. linifolia</i>	<i>A. decurrens</i>	<i>A. longifolia</i>	Kaempferol
Melting point of flavonol	274°	275°	274°	276°	274°
" " acetyl-flavonol	179°	180°	181°	181°	181°
Amount of flavonol	.08	.07	.006	.06 % of fresh flowers	
Carotene, xanthophyll	.27	.33	—	.14	" "

The water-soluble pigment is proved to be a flavonol glucoside of kaempferol and rhamnose.

It is noteworthy that *A. linifolia*, with the palest yellow flowers, contains the largest amount of carotene and xanthophyll, while less than half the amount was obtained from the deep golden yellow flowers of *A. longifolia*.

In conclusion, the author desires to express his indebtedness to Professor H. G. Chapman for affording laboratory accommodation and facilities for carrying out this research.

Fig 2. Kaempferol.

SUMMARY.

Four different species of Australian acacias have been examined to ascertain the nature of the colouring matter of their yellow inflorescence.

The water-soluble yellow pigments isolated from each species were identical, and were proved to be a glucoside of kaempferol. The kaempferol consisted of fine hair-like needles of a lemon-yellow colour. In exceedingly dilute solutions alkali produced a very deep yellow colour, and in sulphuric acid the solution exhibited a bluish-green fluorescence.

An oxonium salt was formed with sulphuric acid, of bright orange crystals, from which by quantitative decomposition the molecular weight of the pigment was obtained as 286.

By fusion of the pigment with potash, phloroglucinol, and *p*-hydroxybenzoic and protocatechuic acids were identified, the two acids being combined as a compound of constant composition.

The kaempferol exists in the flowers as a rhamnose glucoside: no free flavonol was found.

The yellow flavonol could be transformed by nascent hydrogen to a red solution, resembling the anthocyanins.

The acacia tannins were composed of phloroglucinol and protocatechuic and gallic acids, and deposited on hydrolysis large amounts of the red phlobaphene anhydrides.

The carotene and xanthophyll as plastid pigments were present in amounts of from 0.14 to 0.3 %, and the flavonol of about 0.06 % of the fresh flowers.

REFERENCE.

Barth and Hlasiwetz (1865). *Liebig's Annalen*, **134**, 276.