LXXXVI. NATURAL ANTHOCYANIN PIGMENTS.

II. THE MAGENTA FLOWER PIGMENT OF *PRIMULA POLYANTHUS*.

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(Received May 1st, 1930.)

INTRODUCTION.

IN 1927 Karrer and Widmer [1927, 3] isolated and identified the anthocyanin pigments from three species of alpine *Primula*. They found that the flowers of *P. viscosa* and *P. integrifolia* both contained a pigment identical with malvin, a diglucoside of delphinidin-3':5'-dimethyl ether, while from the flowers of *P. hirsuta* they isolated a diglucoside of a new *O*-trimethyldelphinidin, which they named hirsutidin. The pigment extracted from *P. integrifolia* was also shown to contain traces of some anthocyanin less highly methylated than malvin—a contamination to which these authors [1927, 1] had already drawn attention in the cases of other malvidin glucosides, such as oenin from grapes and myrtillin from bilberries.

Primulin, the anthocyanin pigment found in magenta and dark red flowers of *P. polyanthus*, is an interesting addition to this series. Like oenin, it consists chiefly of a 3-monoglucoside of malvidin, contaminated by traces of a lower methylated compound. Thus, although primulin furnishes the same aglucone as that found in two of Karrer's pigments, it differs from them in the nature and position of its glucosidal residue.

P. polyanthus is a horticultural hybrid probably derived from the cowslip and the common primrose. It is to be found in a large range of colours including various shades of yellow, magenta, crimson, orange and brick-red. Only magenta and crimson flowers were used for this investigation, as it is possible that a second anthocyanin is present in the orange and flame-coloured flowers.

The method of extraction and isolation was adapted from Anderson's [1923] modification of Willstätter and Zollinger's [1914] picrate method for the preparation of oenin chloride from purple grapes, in which 1 % aqueous hydrochloric acid instead of glacial acetic acid is used for the extraction of the pigment from the fresh flowers. Owing to the difficulty experienced in obtaining large quantities of material, only about 1 g. each of the pure crystalline hydrochlorides of the glucosidal and sugar-free pigments could be prepared.

The determination of the constitution of these pigments is based upon their appearance, reactions, solubilities, combustion analyses and absorption spectra, and upon the products obtained from the alkali fission and perhydrol oxidation of primulidin chloride.

The conclusion that primulin is not composed entirely of a monoglucoside

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of malvidin is based firstly upon the low values obtained on estimating the methoxy-content of primulidin chloride, and secondly upon the transitory purple colour obtained with its alcoholic solutions on addition of ferric chloride. This colour, which is also given by oenidin chloride, must be due to the presence of small amounts of delphinidin, or some methylated delphinidin which does not contain the syringic acid nucleus, or of cyanidin, since it is only obtained with pyrylium salts possessing free hydroxyl groups at positions 3' and 4'.

Since oenin, the pigment of purple grapes, also consists of a monoglucoside of malvidin, together with traces of a less methylated compound, a comparison has been made between the reactions of primulin and oenin chlorides, and of the chlorides of primulidin, oenidin and pure synthetic malvidin. Oenin chloride was prepared from purple grapes by a method similar to that used for the isolation of primulin chloride. The specimen of malvidin chloride, together with samples of three synthetic methylated malvidin compounds, was very kindly sent me by Prof. R. Robinson, to whom I am greatly indebted.

A comparison between the appearance, solubility and reactions of primulin and oenin chlorides shows that they resemble each other in most respects, but differ in their "distribution number."

Primulidin chloride resembles pure malvidin chloride in its sodium carbonate reaction and in its sparing solubility in hydrochloric and sulphuric acids. In crystalline form, difficulty of recrystallisation and ferric chloride reaction it resembles oenidin chloride. The absorption spectra of these three pigments are almost identical.

These anthocyanins and anthocyanidins were further compared by means of their colour reactions and stability in a series of buffered solutions [Robertson and Robinson, 1929]. Although primulin and oenin chlorides gave an almost similar range of colours, above p_H 6.8, those of primulin were slightly more blue. The chlorides of primulidin and oenidin differed in their behaviour slightly but definitely both from each other and from pure malvidin chloride. These comparisons further demonstrated the probability that, although both consist chiefly of malvidin monoglucoside, primulin and oenin are not identical, since they differ in the amount of contaminating pigment present.

On hydrolysis, primulin chloride yielded one molecule of primulidin chloride and one of glucose.

On addition of sodium carbonate, solutions of the glucosidal pigment gave a violet colour, while primulidin chloride gave a pure blue. This indicates that the glucose molecule is attached at position 3, thus preventing the production of the clear blue which is obtained with the sugar-free pigment which has a free hydroxyl group at position 3 as well as at 5, 7 and 4' [Pratt and Robinson, 1925; Robertson and Robinson, 1927, 1928]. This position of the carbohydrate residue is further stressed by the fact that the colour given by primulin chloride on solution in a buffer of p_H 8.6 is identical with that given by the methylated malvidin compound synthesised by Bradley and Robinson [1928], in which the methyl group is at position 3.

Pure malvidin was first obtained as a crystalline chloride by Willstätter and Mieg [1914] from the wild mallow, in which it existed as the diglucoside malvin. Evidence that the methoxyl groups were in positions 3' and 5' was given later by Karrer and Widmer [1927, 1], who isolated syringic acid from the products of mild alkali fission, and decided to re-name the pigment *syringidin*. This structure was later confirmed by Bradley and Robinson's synthesis [1928] of a 3 : 5 : 7 : 4'-tetrahydroxy-3' : 5'-dimethoxyflavylium chloride identical with malvidin chloride.

The constitution of 3-monoglucosidylmalvidin chloride, the chief constituent of primulin chloride, may therefore be represented by the formula below.

EXPERIMENTAL.

The extraction and purification of primulin chloride.

After the removal of their yellow centres and colourless corolla tubes, 600 g. of fresh red flowers of *Primula polyanthus* were placed in a large Kilner jar, 600 cc. aqueous 1 % hydrochloric acid added, the flowers pressed under the liquid and the air bubbles dispelled. The jar was loosely covered and left to stand for two days. The dark purple extract was then filtered. After grinding in a mortar, the residue was left to extract for two more days with a further quantity of acid (400 cc.).

Fig. 1. Primulin picrate.

The first extract was warmed to 40°, and an equal volume of warm saturated picric acid added. The solution was kept at this temperature for a minute, and

then allowed to cool to room temperature. A flocculent precipitate of microscopic scarlet-red needles soon began to separate out. (See Fig. 1.)

After standing in the ice-chest overnight, the picrate was filtered off on a small Büchner funnel, washed free from picric acid with ether, and dried in a vacuum desiccator. As the red-orange filtrate still contained a considerable amount of pigment, it was saturated with picric acid and used to precipitate the anthocyanin from the second crude extract by the same method as before. After the separation of this second yield of picrate, an attempt was made to recover further quantities of pigment by making the extract 10 % acid with hydrochloric acid. More pigment was thereby precipitated, together with picric acid, but the bulk still remained in the mother-liquor and could not be utilised.

The two yields of picrate, together weighing 4.5 g., were very finely ground in a mortar, dissolved in 150 cc. methyl alcohol containing 7.5 cc. hydrochloric acid and filtered. On addition of about a litre of ether, most of the pigment was thrown down in dark red-brown granules with a bronze lustre. The mother-liquor not only retained the picric acid, but also some of the pigment, but the latter could not be fully precipitated even by the addition of light petroleum.

The chloride was ground finely, and washed with ether, until the washings no longer gave a yellow colour on addition of alkali. The yield of crude amorphous glucoside was 3.3 g.

To obtain the pigment in crystalline form, it was dissolved in a minimum quantity of an equal mixture of methyl and ethyl alcohol (about 10 cc.) and one-third the volume of 10 % aqueous hydrochloric acid added. On standing in a loosely covered vessel, purple rod-shaped crystals of primulin chloride separated out either singly or in aggregates. These were filtered off, washed with a little cold 10 % hydrochloric acid, sucked as dry as possible and air-dried at room temperature. They formed a purplish red powder which marked paper with a purple streak.

Part of this yield was recrystallised for analysis, the strongly coloured filtrate being used for the preparation of the sugar-free pigment.

The glucosidal pigment, primulin chloride, $C_{23}H_{25}O_{12}Cl$.

Primulin chloride is very soluble in cold water, giving a deep red solution. On dilution it turns a plum colour, and then isomerises, with no precipitation of the colour-base.

The pigment has a maximum solubility in aqueous hydrochloric acid between concentrations of 1.0 % and 2.0 %, but is less soluble as the concentration increases. In all concentrations, the pigment is dissolved to a greater extent on heating.

Primulin is very soluble in methyl alcohol, and nearly as much so in ethyl alcohol. Both give intense crimson solutions, which, on dilution with more alcohol, at once turn mauve, and are then decolorised through isomerisation.

Primulin is insoluble in ethyl acetate; only slightly soluble in acetone when neutral, but more so on acidification.

The chief reactions of primulin chloride are given below.

<i>Ferric chloride.</i>	Plum colour in alcoholic solution, changing at once to a stable orange-red.
<i>Sodium carbonate.</i>	Violet colour in alcohol. Blue in thin layers, red-violet in thick layers.
<i>Sodium hydroxide.</i>	Clear blue.
<i>Sodium acetate.</i>	Plum-coloured solution in water, and in alcohol. A purple colour-base (insoluble in benzene) is sometimes precipitated from strong solutions.
<i>Lead acetate.</i>	Blue-violet precipitate.
<i>Fehling's solution.</i>	Reduced.
<i>Potassium acetate.</i>	Blue precipitate.
<i>Perchloric acid.</i>	Very soluble, no crystals of perchlorate formed.
<i>Picric acid.</i>	Soluble on warming, scarlet-red needles of picrate separate on standing. (See Fig. 1.)
7 % sulphuric acid.	Soluble even in the cold, no crystals of sulphate separate.

On drying *in vacuo* over phosphorus pentoxide at 105°, primulin chloride loses all of its water of crystallisation, but quickly regains half of it on standing in air.

On analysis, the following figures were obtained:

I.	0.2441 g. primulin chloride lost 0.0375 g. on drying	15.37 %
II.	0.4850 g. primulin chloride lost 0.0754 g. on drying	15.60 %
	Calculated for $C_{23}H_{25}O_{12}Cl, 5H_2O$	14.55 %
	Calculated for $C_{23}H_{25}O_{12}Cl, 5\frac{1}{2}H_2O$	15.85 %

Primulin chloride therefore appears to possess $5\frac{1}{2}$ molecules of water of crystallisation.

I.	4.386 mg. primulin chloride (dried) gave	8.390 mg. CO_2 and 1.91 mg. H_2O
	6.189 mg. " " "	1.682 mg. AgCl
II.	4.784 mg. " " "	9.100 mg. CO_2 and 2.08 mg. H_2O
	5.422 mg. " " "	1.480 mg. AgCl

The percentage compositions are therefore:

	C	H	Cl
By experiment. Sample I	52.1	4.82	6.75
Sample II	51.9	4.83	6.75
Calculated for $C_{23}H_{25}O_{12}Cl$ (oenin chloride)	52.2	4.74	6.74

The presence of methoxyl groups was demonstrated qualitatively by Zeisel's method.

The micro-analyses for carbon and hydrogen content of primulin chloride, and carbon, hydrogen and methoxyl content of the sugar-free pigment, were carried out by Dr Ing. Schoeller, of Berlin-Schmargendorf, and those for chlorine by A. Colwell, of this laboratory.

The colour reactions of primulin and oenin chlorides were compared in a range of buffered solutions, and found to be similar. Above p_H 6.8 the primulin chloride solutions were slightly more blue. The colours ranged from cherry-red at p_H 3.2, through rapidly fading violet-reds between p_H 3.6 and 5.6, and a fairly stable violet to violet-blue from p_H 6.8 to 8.0, to stable violet-blues (blue in thin layers) at higher p_H . In 10 % sodium hydroxide a very unstable blue was obtained. A violet colour-base was precipitated at all p_H values between 3.2 and 6.2.

Primulidin chloride, the sugar-free pigment, $C_{17}H_{15}O_7Cl$.

The crystalline sugar-free pigment, primulidin chloride, was obtained by hydrolysis of the glucoside with 20 % hydrochloric acid for 3 or 4 minutes. If heated too long, or if alcohol was present (as when the mother liquors from the crystallisation of primulin were hydrolysed), the pigment was partly amorphous.

Homogeneous crystals were prepared by dissolving the pure crystalline glucoside in hot 20 % hydrochloric acid and heating over a free flame for 4 minutes. The crystals began to appear after 2 minutes' boiling, and separated out almost quantitatively on cooling. Microscopically, they were pale brown or straw coloured rods, with blunt ends (see Fig. 2), their size depending upon the strength of acid used in the hydrolysis. The crystals were filtered, washed with a little cold 20 % hydrochloric acid and dried in air. They formed an almost black powder (pure malvidin chloride has a green reflex). The filtrate was later examined for the sugar residue.

Fig. 2. Primulidin chloride.

Unlike pure malvidin chloride, primulidin chloride resembles oenidin chloride in being very difficult to recrystallise. Most attempts only gave amorphous forms, but small crystals were finally obtained by dissolving the pigment in water, and acidifying carefully until the solution contained 3 % of hydrochloric acid.

Primulidin chloride is slightly soluble in cold water, giving a red-brown solution after standing for half an hour. On heating, it is more soluble, but soon loses all its colour by isomerisation. The colour-base is precipitated from strong solutions.

The pigment is very soluble in methyl, ethyl and propyl alcohols, giving intense crimson solutions which, on dilution, turn purple and then isomerise. (Pure malvidin chloride is reprecipitated in small crystals from methyl alcoholic solutions.)

In amyl alcohol the pigment dissolves easily, giving a violet colour if neutral and cerise if acid. Like all anthocyanidins, on adding an excess of

aqueous alkali, the pigment leaves the alcohol, turns blue, and dissolves in the water layer.

Ethyl acetate dissolves the pigment to a small extent; while acetone does so fairly easily, forming a red solution when acid, violet when neutral, and a clear blue if made alkaline with sodium bicarbonate.

Though practically insoluble in either water or concentrated hydrochloric acid, the pigment is slightly more soluble in concentrations of acid between 0.005 % and 1.5 %. Maximum solubility in the cold is found at a concentration of 0.03 %, while, when hot, 0.3 % acid has the greatest solvent power. This pigment therefore resembles malvidin chloride rather than oenidin chloride in respect of its solubility in water and aqueous acid.

The following reactions are given by primulidin chloride.

<i>Ferric chloride.</i>	Purple in alcoholic solution. Very unstable. (Malvidin chloride gives no colour reaction.)
<i>Sodium carbonate.</i>	Violet colour and then pure blue.
<i>Sodium hydroxide.</i>	Blue colour, more green than with carbonate.
<i>Sodium acetate.</i>	Red-blue dichroic solution in alcohol, purple in water.
<i>Potassium acetate.</i>	Blue colour, followed by precipitation of a colour base insoluble in benzene.
<i>Lead acetate.</i>	Dark blue precipitate of lead salt.
<i>Fehling's solution.</i>	Vigorously reduced.
<i>Perchloric acid.</i>	Soluble on standing, aggregates of badly formed hair-like crystals of perchlorate are formed.
<i>7 % sulphuric acid.</i>	Fairly soluble, especially on heating; almost complete precipitation of fine needles on cooling (as for malvidin chloride).
<i>Picric acid.</i>	Soluble on warming, fine red hair-like crystals in clusters separate on cooling.

The substance did not melt on heating to 300°.

On drying in a vacuum at 105° over phosphorus pentoxide, the air-dried crystals lose all their water of crystallisation.

The following values were obtained on analysis:

I.	0.0742 g. primulidin chloride lost 0.0048 g. on drying	6.47 %
II.	0.2963 g. primulidin chloride lost 0.0187 g. on drying	6.32 %
	Calculated for $C_{17}H_{15}O_7Cl, 1\frac{1}{2}H_2O$	6.87 %
I.	4.705 mg. primulidin chloride (dried) gave	9.16 mg. CO_2 and 1.9 mg. H_2O
	4.260 mg. " " "	1.577 mg. $AgCl$
	3.502 mg. " " "	3.20 mg. AgI
II.	4.721 mg. " " "	9.63 mg. CO_2 and 1.75 mg. H_2O
	5.135 mg. " " "	1.863 mg. $AgCl$
	3.195 mg. " " "	3.22 mg. AgI

The percentage compositions are therefore:

		C	H	Cl	OCH_3
By experiment.	Sample I	55.3	4.28	9.10	14.76
	Sample II	55.6	4.12	8.96	13.40
	Calculated for $C_{17}H_{15}O_7Cl$ (malvidin chloride)	55.9	4.11	9.68	16.90

These figures are in agreement with the dimethyl formula $C_{17}H_{15}O_7Cl, 1\frac{1}{2}H_2O$. The low values obtained for the (OCH_3) content indicate the presence of traces of a lower methylated delphinidin.

The colour reactions and stability of solutions of primulidin chloride were compared with those of oenidin and pure synthetic malvidin chloride in a series of buffered solutions. The different amount of contamination in oenidin and primulidin was demonstrated by the slight differences in the reactions of

Table I.

		Time of observation	Oenidin chloride (O)	Primulidin chloride (P)	Malvidin chloride (M)
10 % HCl					
I	3.2	Initial	Bluish red, stable	Same as O	Same as O
		30 min.	Bluish red, fading Very faint pink	" Colour gone	" Colour gone
II	3.6	Initial	A little bluer than I, fading	Same as O	Same as O
		30 min.	Very faint pink	Colour gone	Colour gone
III and IV	4.4 and 5.0	Initial	Same as II, but more intense	Same as O	Same as O, a little less intense
		30 min.	Very faint pink	All colour gone	All colour gone
V	5.6	Initial	Plum colour, more intense	Same as O	Same as O
		3½ hr.	Very pale pink	"	"
VI	6.2	Initial	A little more violet and more intense than V	Even more in- tense than O	Same as O
		3½ hr.	Very pale mauve	Same as O	"
		18 hr.	Very pale blue	"	"
VII	6.8	Initial	Much bluer, pure blue in thin layers	Slightly more mauve than O	Same as O
		3½ hr.	Very pale blue	Same as O	"
		18 hr.	"	"	"
VIII	7.4	Initial	Same as VII	Slightly more mauve than O	Same as O
		3½ hr.	Paler and greener	Same as O	"
		18 hr.	Very pale blue	"	"
IX, X and XI	8.0-9.2	Initial	Pure blue	Slightly more intense than O	Same as O
		3½ hr.	Paler and greener	Same as O	"
		18 hr.	Very pale blue	"	"
XII	9.8	Initial	Same as XI	Slightly more intense than O	Same as O
		3½ hr.	Paler and greener	Same as O	Pale green-blue
		18 hr.	Dirty blue-grey	Dirty grey- yellow	Dirty grey- yellow
XIII	10.4	Initial	Slightly greener than XII	Pure blue as for XII	Same as P
		20 min.			Green-blue
		3½ hr.	Paler and greener	Same as O	"
		18 hr.	Yellow (blue tinge)	Yellow	Yellow
XIV	11.0	Initial	Blue, greenish tinge	Slightly greener than O	Same as P
		20 min.	Pale blue	Pale blue	Pale green
		3½ hr.	Grey-blue	Grey-mauve	"
		18 hr.	Yellow	Yellow	Yellow
XV		Initial	Same as XIV	Same as XIV	Same as P
		20 min.	Pale blue	Dirty blue	Pale green
		3½ hr.	Grey-blue	Yellow	Clear yellowish green
		18 hr.	Yellow	"	Yellow
XVI		Initial	Same as XIV	Same as XIV	Same as P
		20 min.	Pale blue	Dirty blue	Pale green
		3½ hr.	Grey-blue	Yellow	Yellow
		18 hr.	Yellow	"	"
XVII		Initial	Same as XIV	Same as XIV	Same as P
		20 min.	Pale blue	Grey blue	Pale green
		½ hr.	Grey-blue	Brown	"
		3½ hr.	Yellow	Yellow	Yellow

the three chlorides, which are shown in Table I. It will be seen that below p_H 11 the primulidin chloride solution is slightly more mauve and more intense than the corresponding solutions of oenidin and malvidin chloride. At p_H 11 both primulidin and malvidin chloride are more mauve than oenidin, and at higher p_H these two pigments are destroyed in a shorter period of time. The resemblance between primulidin chloride and malvidin chloride appeared to be slightly greater than that between oenidin chloride and malvidin chloride. The buffered solutions were prepared by the addition of the increasing amounts of standard 0.2 *N* sodium hydroxide to the British Drug Houses "Universal Buffer Solution." Up to p_H 11.0, the p_H could be calculated accurately.

Fig. 3.

The absorption spectra of alcoholic hydrochloric acid solutions of oenidin, primulidin and malvidin chlorides were examined in the spectrophotometer, and the logarithmic curves were plotted to show the intensity of absorption of these three pigments within the visible spectrum. Each showed a band in the green portion, and a further one commencing in the violet and continuing into the ultra-violet region. The curves (see Fig. 3) were almost exactly alike, except at wave-lengths below $480\mu\mu$ where the figures were unreliable, owing to the difficulty in taking accurate readings in the violet region. The maximum absorption in each case was at $560\mu\mu$. The pigment solutions used were of such

a strength that the maximum log absorption values were at about 2.0. Owing to slight differences in these concentrations the curves obtained are parallel but not identical.

The breakdown of primulidin chloride by oxidation and alkaline hydrolysis.

After oxidation of primulidin chloride with hydrogen peroxide [Karrer and Widmer, 1927, 2], syringic acid was isolated from the decomposition products. About 0.2 g. of pigment was dissolved in 20 cc. hot water, and 15 cc. of 30 % hydrogen peroxide were added. After standing overnight, the brown residue was filtered off from the decolorised solution, which was then extracted several times with pure ether, and the ethereal solution washed free from peroxide by shaking twice with water. The ether was then evaporated off, and the pale brown residue was dissolved in a small amount of hot water, and decolorised by boiling with charcoal. On concentration of the solution in a vacuum desiccator, colourless needles separated. These were recrystallised twice, and identified as syringic acid by their melting point of 105° (uncorr.) and grey-green reaction with ferric chloride. The yield was very small.

A second sample of the pigment was treated for a few minutes with boiling 50 % potassium hydroxide, aerial oxidation being avoided. When hydrolysis was complete, the hot solution was poured cautiously into dilute sulphuric acid and extracted with ether. After treatment with sodium bicarbonate solution, through which carbon dioxide had been passed, it was necessary to extract repeatedly with ether to remove phloroglucinol. After evaporation of the ether, the pale yellow residue was taken up in water. The yield was very small, owing to the small amount of pigment available for the experiment, and the ease with which phloroglucinol is oxidised during the hydrolysis. It was possible, however, to identify it by the red colour obtained with a pine shaving and strong hydrochloric acid, by its orange-red diazo-benzene compound, and by the precipitation of a brick-red phloroglucide on boiling with rhamnose and 20 % hydrochloric acid.

On acidification of the aqueous layer, extraction with ether, and evaporation, a pale brown residue was obtained, which appeared from its acid reaction and colour with ferric chloride, to be a mixture of gallic and syringic acids.

The sugar residue.

The identification of the carbohydrate portion of the primulin molecule was carried out by means of qualitative tests and quantitative estimations.

The "distribution number" for primulin chloride was ascertained by dissolving 0.005 g. in 25 cc. 5 % hydrochloric acid, and shaking with two successive amounts of amyl alcohol (25 cc.). The amount of pigment extracted in each case was measured colorimetrically by comparison with a standard made from 0.005 g. of pigment, dissolved in 1 cc. of methyl alcohol and made up to

25 cc. with amyl alcohol. The amyl alcohol was pyridine-free and contained a trace of hydrochloric acid to prevent isomerisation.

The mean value obtained for the distribution number of primulin chloride was 8.6. This is somewhat below the values usually found for monoglucosides (9.8 to 11.0), but is considerably above those for diglucosides, which are all below 2.0. (Oenin chloride has a distribution number of 10.4.)

About 0.1 g. of glucoside was hydrolysed with 10 cc. of 20 % hydrochloric acid. After cooling, the sugar-free pigment was filtered off, and a small amount of colour which still remained in solution was removed by shaking with amyl alcohol. The absence of pentose from the filtrate was shown by a negative orcinol test.

The remaining sugar solution was shaken with ether to remove any amyl alcohol remaining and neutralised with lead carbonate. After filtering, the excess lead was removed with hydrogen sulphide, and the latter was expelled from the filtered solution by heating on a water-bath.

The osazone was prepared from this neutral solution in the usual way. It resembled glucosazone in crystal form and in its melting point of 210° (uncorr.), which did not change after three recrystallisations from methyl alcohol.

The amount of glucose in the primulin molecule was estimated by determining the proportions of primulidin chloride and reducing sugar produced on hydrolysis of the glucoside.

A weighed quantity of primulin chloride (dried at 105° as before) was hydrolysed with 10 cc. of acid and left in the ice-chest until the mother-liquor was barely pigmented. The crystalline sugar-free pigment was collected on a weighed Pregl glass filter, washed with a small amount of 30 % hydrochloric acid, dried *in vacuo* at 105° over P₂O₅ and weighed.

The filtrate was extracted with amyl alcohol, shaken with pure ether to remove any remaining alcohol, neutralised with sodium hydroxide and made up to a definite volume. The reducing sugar present was estimated by Bertrand's method and polarimetrically.

I.	128.9 mg. primulin chloride (dry)	gave 88.8 mg. primulidin chloride (dry)
	128.9 mg. " " "	reduced 80.0 mg. copper $\equiv$ 39.82 mg. glucose
II.	84.1 mg. " " "	gave 23.7 mg. glucose (25 cc., $l=2$ dm., $\alpha_D = +0.10^\circ$)
III.	53.9 mg. " " "	gave 36.5 mg. primulidin chloride (dry)

The percentage yields are therefore:

	% C ₁₇ H ₁₅ O ₇ Cl	% C ₆ H ₁₂ O ₆
By experiment I	68.8	30.9
II	—	28.2
III	67.7	—
Calculated for C ₂₃ H ₂₅ O ₁₂ Cl	69.4	34.1

The low sugar values obtained are due to the breakdown of an appreciable amount of glucose during the hydrolysis.

The analyses for the carbon, hydrogen and chlorine content of primulin chloride are also in agreement with the monoglucosidal formula. The polarimeter reading is correct for glucose, and not galactose.

The colour reactions of primulin, primulidin, oenin, oenidin and malvidin chlorides, and of three synthetic malvidin compounds methylated at 3, 5 and 7 respectively, were compared in buffered solutions of p_H 8. It was found that the chlorides of primulin, oenin and 3-*O*-methylmalvidin gave a similar violet colour (violet-blue in thin layers, and red-violet in thick layers). On the other hand, primulidin, oenidin and malvidin chlorides gave a similar pure blue, 5-*O*-methylmalvidin chloride gave a slightly less green-blue, and the 7-methyl compound gave a violet-blue (pure blue in thin layers, and blue-violet in thick layers), which was easily distinguishable from the violet colour given by the 3-substituted compounds. It is therefore reasonable to suppose that the glucose molecule in primulin is attached at position 3.

SUMMARY.

1. The magenta and dark red flowers of *Primula polyanthus* contain an anthocyanin pigment which has been named *primulin*.
2. By extraction of the pigment with dilute hydrochloric acid and purification through the picrate, pure crystalline primulin chloride has been prepared, and shown to consist of 3-monoglucosidylmalvidin chloride, together with traces of a lower methylated compound.
3. By a comparison of the properties and reactions of the chlorides of primulin and oenin and of the sugar-free pigments primulidin, oenidin and malvidin, it is shown that, although of nearly the same constitution, primulin and oenin are not identical.
4. Primulin furnishes the same aglucone as the pigments of *P. viscosa* and *P. integrifolia*, but differs from them in the nature and position of attachment of its glucosidal residue.

My thanks are due to the Hon. Mrs Wheldale Onslow for her help and advice, to Prof. R. Robinson for criticisms and suggestions and to Sir F. G. Hopkins for his interest in the work.

I am also indebted to the Department of Scientific and Industrial Research for the grant which has enabled me to complete this investigation.

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