VIII.—*Researches on Colein.*

By A. H. CHURCH.

FOR several years past I have made experiments on a small scale with the hope of isolating the splendid colouring matter which renders the numerous varieties of *Coleus verschaffeltii* so attractive as ornamental-foliaged plants. This interesting Labiate contains, both in stem and leaf, a colouring matter having obviously many characteristics in

common with the singularly sensitive substance or substances to which violets, purple dahlias, iris, copper-beech, black grapes, and many other flowers, leaves, and fruits, ranging in colour from crimson to violet-blue, owe their varied hues. Although the investigation of colouring matters of this class is generally beset with difficulties, it appeared to me that a successful issue would be less unlikely to be reached in the case of the *Coleus* than of an ordinary plant. Commonly, an initial difficulty has arisen from paucity of material; the alterability of the colouring matter and its admixture with other colouring matters and with organic and mineral impurities have proved still more serious sources of vexation and delay. The first difficulty was removed effectually, thanks to Mr. Jackson, of Kingston, and to Dr. Hooker, Director of the Royal Gardens at Kew, from both of whom I received in the autumns of 1874 and 1875 an abundant supply of the stems of the desired plant. Indeed, I was obliged to decline several kind offers of further contributions of the raw material for my research, finding the rapid working-up of half-a-hundred weight of *Coleus* stems a task which taxed the resources of my laboratory to the utmost.

The method finally adopted in order to isolate the colouring matter to which I now wish to direct the attention of the Society, may be briefly outlined as follows:—The leaves of the selected plants are rejected, along with such paler portions of the stems as are greenish or deficient in pigment. The chosen parts are introduced without delay into an apparatus in which they can be reduced to a pulp. I found a porcelain sausage-machine far better than the large wedgwood mortar which I first employed for this purpose. During the crushing process, a slender stream of strong alcohol, faintly acidulated with sulphuric acid, falls upon the bruised mass. The crimson pulp thereby prepared is submitted to strong pressure (avoiding contact with metal), the deep crimson liquor from it being strained first through cambric and then through Swedish paper; the squeezed pulp is then again exhausted with strong alcohol. The united liquors may now be shaken with a little moist precipitated barium carbonate, and afterwards again filtered. They are then introduced into a capacious retort immersed in a water-bath, most of the alcohol being thus removed by distillation. If this process be carried far enough, a mass of deep-coloured, resinous-looking substance sinks to the bottom of the retort, while a rich-red liquid remains above it. The resin is the colouring matter, but not pure; the liquid also contains the same substance, part of which may be separated from it by further evaporation at a gentle heat. But the remaining liquor retains much colouring matter in solution, owing to the solvent action of the organic acids, sugar, and other substances which are likewise present. By dilution with much

water and subsequent repose, a further separation of more colouring matter may be caused.

So far we have described the extraction of the colouring matter in the form of a resinous mass, plastic when warm, and almost liquid when originally deposited. I did not at once ascertain the best and most thorough way of purifying this product, but was content to agitate its alcoholic solution with barium carbonate, and then to precipitate the colouring matter from the filtered liquid by pouring it into absolute ether. It will be convenient here to assign the provisional name of *colein* to the product now being described, not because I wish it to be regarded as new, but in order to avoid an awkward periphrasis, and the assumption that it is identical with similar colouring matters which have been previously named.

An early analysis of colein may be cited here, not because it afforded numbers which were subsequently confirmed, but in order to afford some notion of the tedious steps by which the inquiry progressed; for it was necessary to test every modification of the processes of purification and preparation by means of quantitative analysis.

Analysis I. Colein, prepared from red resin by repeated solution in alcohol, filtration, evaporation, and washing of residue with ether. This specimen was dried *in vacuo* over sulphuric acid.

·4224 gram gave ·7893 gram CO_2 and ·196 gram H_2O .

·3914 " ·0034 " ash = ·86 per cent.

·405 " ·0345 " PtAm_2Cl_6 by combustion with soda-lime = ·54 per cent. N.

1·2395 gram lost ·0247 in water-oven = 2·0 per cent.

Thus the obvious impurities amounted to 3·4 per cent., but in reality the nitrogen and the ash represented much larger quantities of intruding substances; for the ash was derived from the lime and potash salts of both organic and inorganic acids; while the nitrogen occurred mainly as a compound of an alkaloid closely resembling nicotine. It is, therefore, impossible to effect an adequate correction in the analytical numbers by means of a mere deduction of ash, moisture, and nitrogen from the substance taken, and of water from the total water weighed in the calcium chloride tube. But the resulting percentages are interesting, so far as they go, in connection with the later results. They are—

Carbon, 52·73 per cent. Hydrogen, 5·11 per cent.

Analysis II.—The next specimen analysed had been prepared by repeated solution in alcohol and a final precipitation by means of absolute ether. It was nearly free from nitrogen and ash, but was not quite dry, having been merely kept over sulphuric acid *in vacuo* till

constant in weight. Burnt with copper oxide, in a platinum boat, in a current of oxygen, it gave these results*—

·288 gave ·594 CO₂, ·1396 H₂O, ·0015 ash.

Deducting ash, these numbers corresponding with the following percentages:—

Carbon, 56·54. Hydrogen, 5·41. Oxygen, 38·05.

A trace of alcohol was found to have been retained by this preparation, and thus the percentage of hydrogen was raised and that of carbon lowered.

Analysis III.—The sample employed for this analysis had been prepared from the lead-compound of colein, to which I have to refer again shortly. By decomposing this lead salt with sulphuric acid in strong alcohol, and then evaporating the alcoholic filtrate, and further purifying the colein present by the means already described, a nearly pure product was obtained. It was dried *in vacuo* first, and then at about 90° C. The results were: ·4968 gave 1·0329 CO₂. ·2144 H₂O. ·0013 ash. Deducting ash, these results correspond with the following percentages:—

Carbon, 56·85. Hydrogen, 4·80. Oxygen, 38·35.

Analyses IV, V, and VI. Colein purified by treatment with alcohol and ether as before, then dissolved once more in alcohol and precipitated by *water*. This process proved to be an excellent one, and was adopted in all subsequent operations. The three analyses here given were made with the same sample dried at about 90°. It was, however, found that traces of alcohol obstinately adhered to it.

	Colein taken.	CO ₂ .	H ₂ O.	Ash.
IV	·4602	·949	lost	·0035
V	·4125	·846	·1943	·0037
VI	·446	·9135	·2087	·0035

The corresponding percentages are:—

	IV.	V.	VI.
Carbon	56·67	56·41	56·30
Hydrogen	—	5·26	5·24
Oxygen	—	38·33	38·46

Analyses VII to XI. The colein for these analyses had been prepared and purified in the same manner as that last described; it was dried in the water-oven. The hydrogen of all the analyses showed an

* The weights referred to here and throughout the paper are grams and decimals of a gram; the same method of combustion was employed in all the analyses.

extreme range of no more than ·16 per cent., but the fluctuations in the carbon were too large to be satisfactory. It was found that a slight error, arising from imperfect removal of CO₂ from the oxygen used, had vitiated the results of Analyses VII to IX, so far as the carbon is concerned. Analyses X and XI were made after the chance of such experimental error recurring had been prevented.

	Colein taken.	CO ₂ .	H ₂ O.	Ash.
VII....	·4427	·9542	·2005	·001
VIII ..	·4285	·9257	·1935	·002
IX	·4415	·9499	·194	·0015
X	·4375	·9275	·1988	·001
XI	·4687	·9915	·2095	·0015

The corresponding percentages, after deduction of the ash, are—

	VII.	VIII.	IX.	X.	XI.
Carbon	58·91	59·19	58·88	57·95	57·69
Hydrogen ..	5·04	5·04	4·90	5·06	4·96
Oxygen	36·05	35·77	36·22	36·99	37·45

Analyses XII and XIII. Colein purified by alcohol, ether, and precipitation and much washing with water at 50°—60° C.; dried in air-bath at 100°. This sample was practically pure.

	Colein taken.	CO ₂ .	H ₂ O.	Ash.
XII	·447	·9429	·202	·001
XIII	·524	1·098	·24	002

The corresponding percentages are—

	XI.	XII.
Carbon	57·66	57·37
Hydrogen ..	5·02	5·10
Oxygen	37·32	37·53

Amongst the preceding thirteen analyses, there are five which I believe represent accurately, from one cause or another, the composition of pure colein. In other cases some defect in the mode of preparation or desiccation of the sample, or else in the combustion-process itself, has interfered with the accuracy of the results. I am, however, of opinion that the singular difficulty of dealing with substances of the class to which colein belongs, justifies and explains the range in the percentages deduced from the majority of the analyses cited: this range in fact is not a very wide one. The percentages from the five analyses, which, critically examined appear to me to be most deserving of acceptance, are here arranged—

No. of analysis.	Preparation.	Per cent. carbon.	Per cent. hydrogen.
III ..	From lead-salt	56·85	4·80
X. . .	{ Purified by alcohol, ether, and water. }	57·95	5·06
XI ..		57·69	4·96
XII. .	{ Treated similarly to last, but with water at 50°—60°. }	57·66	5·02
XIII. .		57·37	5·10

The mean percentages deduced from the above analyses are given below; and, for comparison, the percentages required by the formula $C_{10}H_{10}O_5$ —

	Experiment.	Theory.
Carbon	57·50	57·67
Hydrogen ..	4·99	4·76
Oxygen	37·51	37·57

We will now see how far the suggested formula is confirmed by the analysis of the lead-salt.

Colein lead-salt—The most satisfactory compound of colein which I have yet been able to obtain was this lead-salt; its preparation was conducted thus:—Ordinary neutral lead acetate was dissolved in alcohol of 70 per cent., the solution was filtered. Pure colein in powder was then dissolved in another portion of the same alcohol; the two solutions were then mixed, decided excess of lead acetate being used. The dull indigo precipitate was washed with weak alcohol and then with water free from carbonic acid, until all soluble lead-salts were removed; it was then dried *in vacuo* over sulphuric acid. Such part of the dried mass as had been in contact with the porcelain surface of the basin showed a faint coppery lustre.

Analysis XIV. Colein lead-salt, dried in water-oven. ·638 gave ·924CO₂, ·1803H₂O.

Analysis XV. Colein lead-salt, another preparation, dried at 100° 4518 gave ·6626 CO₂, ·131 H₂O.

Analysis XVI. Colein lead-salt, another and purer preparation, dried at 100°. ·453 gave ·6299 CO₂, ·121 H₂O.

Analysis XVII. Colein lead-salt, same as last, dried at 100°. ·491 gave ·2365 PbSO₄.

Translated into percentages, these results become—

	XIV.	XV.	XVI.	XVII.
Carbon	39·49	39·99	37·92	—
Hydrogen ..	3·14	3·23	2·96	—
Lead	—	—	—	32·9
Oxygen	—	—	—	—

The preparation represented by analyses XVI and XVII was subsequently ascertained to be purer than the earlier specimen analysed,

which contained some free colein. We must therefore employ the percentages derived from the two last analyses in comparing experiment and theory.

	Experiment.	Theory, $C_{20}H_{18}PbO_{10}$.
Carbon	37·92	38·40
Hydrogen	2·96	2·88
Lead	32·90	33·12
Oxygen	26·22	25·60

I think that the empirical formulæ which I have suggested for colein and its lead-salt are rendered extremely probable by the results of the analysis of those preparations in the purity of which I feel the greatest confidence. Further endeavours to estimate the molecular weight of colein by means of watching the changes of colour wrought in its solution by means of standard acids and alkalis have not done much in elucidating this question. I hope that some new metallic derivatives of colein and some compounds with organic bodies, which I expect to be able to prepare, may decide this point.

It remains now to give a description of the physical and chemical properties of colein, to compare it with kindred compounds, and to note its mode of occurrence in the particular plant from which my supplies have been derived.

Colein when pure is a brittle solid of resinous aspect and conchoidal fracture. The colour of its powder is reddish-purple, but it usually shows a colour verging upon crimson, owing to the presence of a trace of acid. It dissolves freely in moderately strong alcohol with a colour, which is perhaps best described as somewhere between crimson and violet; in absolute alcohol (distilled fresh from sodium), even when warm, it is, when itself dry, much less soluble. The alcoholic solution of colein, if neutral, soon loses its colour, owing, as I conclude, to no reduction of the colein, but merely to its union with a portion of the alcohol. The full intensity of the original colour may be restored by the removal of the alcohol in a current of hydrogen, while a drop of sulphuric acid gives an equally marked red reaction with an alcoholic solution which has been kept till nearly colourless, and with one that has but just been made. It is beautiful to watch a nearly colourless solution of colein recover its hue as it is being warmed, to drive off the alcohol, on the steam-bath. The disappearance or fading of the colour of colein solutions, both alcoholic and aqueous, is prevented by the presence of acids, and, in some degree, of certain salts and organic compounds. Colein is but slightly soluble in water, and quite insoluble in ether, both of these liquids precipitating it from its alcoholic solution—the latter completely, the former imperfectly and somewhat slowly. If the colein,

after precipitation by water, be gently warmed under water, it will partially fuse at 50° — 60° C., and in that condition scattered particles may be welded into a single mass or drawn out into threads. When dry it is not fusible at 100° . At higher temperatures it gives off traces of a thymol-like substance and leaves a bulky charcoal.

By acids a solution of colein is reddened, the quantity of acid necessary to produce this effect being very small, so small indeed, as to make it probable that colein is really a red acid itself, but that its colour is generally modified by a trace of ammonia in the acid solution; and the test-papers imbued with it keep their colour well. On the gradual addition of a weak solution of ammonia to a colein solution, a series of changes in its colour occur, the order being purple, violet, indigo, chrome-green, and greyish-yellow. When once the yellow stage has been reached, the colein has been profoundly altered and is no longer susceptible of being reddened by an acid. On examining the absorption spectra of these acid, neutral, and alkaline solutions, it will be found that their shaded portions do not present very definite features. However, it may be stated that it is possible to reproduce the exact spectrum of the coloured contents of a cell of the coleus stem by means of a neutral solution of colein, thus negating the idea that colein might be a product rather than an educt. It may be stated that the red acid solution of coleus shows a slight shade over the farther red of the spectrum up to about 31 on a scale where the E line is 71, the D line 50, and the C line 34. The space between 32 and 58 or 59 is bright, but a very decided absorption begins at 59, and cuts off the green and a little of the blue of the spectrum. The solution assumed to be neutral shows a narrower unobscured space lying between 32 and 51; the band at 51 is very decided up to 58 and shades off beyond. The green alkaline solution of colein has the red more obscured, especially about 32; the bright space between this and the second dark band is very narrow, the latter band beginning at about 41 and attaining its greatest blackness about 46: this band soon becomes very faint, and almost disappears at 59 in weak solutions.

I do not give these measurements as having any claim to exactness; but they may serve as helps in identifying colein with some of those colouring matters which have received different names, but which are certainly not far removed from it. Spectroscopic examination of such substances should, however, always be connected with the completest chemical investigation, including ultimate analysis, or differences will be found where none exist. The acid, neutral, or alkaline reaction of the solution, the presence of other compounds not themselves coloured, and the marked influence of different solvents will, in many cases, render spectroscopic results, unless supported by chemical analysis, most misleading.

With concentrated sulphuric acid colein forms an orange-coloured liquid which, on dilution with water, presents the red colour and other characters of an acid solution of unaltered colein. It is dissolved with a crimson colour by acid of sp. gr. 1.53. In either case, if only enough acid to dissolve it be used, much of the colein may be reprecipitated on the addition of water. Nitric acid converts colein into a brown resinous mass. Caustic alkalis—even ammonia—profoundly alter colein, but no definite products have yet been found. Of all the reactions which colein furnishes with metallic salts not yet named in this paper, that with stannic chloride is the most interesting. A splendid violet precipitate, apparently permanent, is produced when this chloride is added to a strong alcoholic solution of colein. This tin compound awaits investigation.

The distribution of the colouring matter in the plant demands a word or two of explanation. In the leaves it is confined mainly to the epidermal cells and to the hairs connected therewith. In the stem it occurs quite capriciously distributed, not only in the epidermis and hairs, but also in abundance in many parts of the parenchyma of the pith. Occasionally it may be seen in a few parenchymatous cells bordering the fibro-vascular bundles, but it is absent from the vessels themselves and from the whole cortical tissue. Its mode of occurrence in the hairs of the stem and leaf of the plant is most curious. A hair having, say six cells, may have the terminal cells richly tinted and all the others free from colein. It has clearly been formed throughout the plant *in situ*. It is also a substance of lower physiological position than the less oxidised chlorophyll.

A careful comparison of colein with such colouring matters as those named cyanin, erythrophyll, oenolin, &c., has yet to be made. I am quite aware of the importance of such comparison, but I cannot promise to undertake so laborious a study until I have further elucidated the real nature of colein itself. It must be confessed that all the chemical evidence yet obtained points to the identity of colein with the *anthocyan* of Marquart, the *cyanin* of Frémy and Cloëz, the *erythrophyll* of Morren, and with the *oenolin* extracted from red wines by Glénard. I confess to my ignorance of the results obtained by this last chemist until my own analyses had been completed. He obtained a colouring matter which agreed not only in all its recorded characteristics with colein, and indeed with the substances described under the three names just cited, but in centesimal composition also. Glénard analysed the substance itself and its lead-salt, and was thus led to adopt the same formulæ as those which my analyses support. Should all these colouring matters prove identical, as I strongly suspect, Glénard's name of oenolin may perhaps deserve

the preference, should his formula be confirmed. Anthocyan may claim priority of description, but not of analysis.

To my assistant, Mr. R. C. Woodcock, my best thanks are due for the care which he has bestowed upon the analytical work involved in this research.