The Colouring Matters of Galls

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Dryophantin, $C_{23}H_{26}O_{15}$, the pigment of the 'Red Pea' gall, produced by *Dryophanta divisa* Adl. on the leaves of *Quercus pedunculata* Ehrh., yields on hydrolysis with dilute sulphuric acid 1 mol. of purpurogallin, $C_{11}H_8O_6$, and 2 mol. of glucose (Nierenstein, 1919). Extension of this work to other galls, produced either by insects or fungi, gave identical results, only whereas dryophantin is red, some of the pigments were differently coloured. Since some of them were green we examined each pigment spectroscopically for chlorophyll and also tested qualitatively for nitrogen. The results were negative, which thus excludes contamination with chlorophyll.

Although purpurogallin was first obtained a hundred years ago by Buchner (1847) it is still somewhat of a chemical enigma. Two structural formulae have been suggested for purpurogallin, namely, Formula (I) by Dean & Nierenstein (1913) and Formula (II) by Willstätter & Heiss (1923), neither of which, however, fully accounts for the chemical behaviour of purpurogallin, as already pointed out by Nierenstein (1934). Speculations as to the causes of these differences in colour of these glucosides of purpurogallin appear to us at the present premature and must be left open until purpurogallin has been synthesized. As work in this direction is in progress we would like to mention an observation made by Dr N. Green in 1928, which to some extent seems to undermine the validity of the Willstätter formula. Purpurogallin is known to yield on distillation with zinc dust naphthalene (Nietzki & Steinmann, 1887; Hooker, 1887; Perkin & Steven, 1903; Nierenstein & Spiers, 1913), and this is also the case when substance (III) is submitted to distillation with zinc dust (Dean & Nierenstein, 1916). According to Green, whose work will be published in due course, no trace of naphthalene is produced when substance (IV) is distilled with zinc dust:

Whatever ultimately the constitution of purpurogallin proves to be, there is no doubt whatever that it contains four hydroxyl groups, so that four isomeric biosides and six isomeric di-monosides are possible, thus giving a total of ten possible glucosides. The present communication describes eight such glucosides, named according to the gall producer, the method already adopted in the case of dryophantin.

EXPERIMENTAL

This investigation has been in progress from 1921 to 1942, with the result that after many experiments we have elaborated the following general method: (1) The materials, dried at 100° , were milled and percolated with ethanol followed by chloroform and carbon tetrachloride so as to remove chlorophyll, waxes, cyclogallipharic acids, etc. We found that percolation with the individual solvents does not give easily workable materials. After these solvents had evaporated, the dry powder, mixed with ten times its weight of dry silver sand, was exhaustively extracted in a Soxhlet with boiling water until the overflow became colourless. On evaporation to about one-third of its volume, the solution crystallized on standing in the laboratory or on ice. The products thus obtained were several times crystallized from water until the melting-points became constant. All pigments gave the colour reactions previously described by Nierenstein (1919). (2) Since, as previously observed, hydrolysis with sulphuric acid gives lower values for purpurogallin than theoretically required (Nierenstein, 1919), even if corrections for purpurogallin are made according to Willstätter & Stoll (1918), we used enzymic

hydrolysis with the purpurogallase already described by Nierenstein & Thomas (1941). (3) The difficulties in obtaining reliable melting-points for purpurogallin have already been dealt with by Nierenstein & Spiers (1913), and we have therefore identified it as tetraacetyl purpurogallin, which has a sharp melting-point. (4) The glucose residue was identified as the osazone and quantitatively estimated according to Geake & Nierenstein (1914). (5) All molecular weight estimations were made in ethanol. (6) For the identification of galls and gall producers, we used Appel (1899), Connald (1909), Ross (1911, 1916) and Böhner (1935). (7) The yields are poor and the maximum yield obtained by us was 0.83%.

ANALYTICAL AND DESCRIPTIVE DATA

(1) *Adelgesin*. Light brown needles, m.p. 204–206°, isolated from the 'Pineapple' gall produced by *Adelges abietis* L. on the bark of *Abies excelsa* de Cond. (Found: C, 50.61; H, 5.27; purpurogallin, 40.23; glucose, 63.32%; molecular weight (m.w.), 538, 541. $C_{23}H_{28}O_{15}$ requires: C, 50.73; H, 5.15; purpurogallin, 40.37; glucose, 63.21%; m.w., 544.) From the 'April bud' gall produced on the buds of *Quercus* species by *Spathogaster aprilinus* Giz., yellowish green needles were obtained which had the same melting-point and composition as adelgesin; a mixed melting-point showed no depression.

(2) *Dryophantin*. Terra-cotta coloured needles, m.p. 220–221°, from the 'Red Pea' gall, produced by *Dryophanta divisa* Adl. on the leaves of *Quercus pedunculata* Ehrh. and from the 'Cherry' gall produced by *Dryophanta Taschenbergii* Schulz on the leaves of *Quercus sessiliflora* Sal. (Found: C, 50.61; H, 5.21; purpurogallin, 40.26; glucose, 63.21%; m.w. 544. $C_{23}H_{28}O_{15}$ requires: C, 50.73; H, 5.15; purpurogallin, 40.37; glucose, 63.21%; m.w. 544.) From the 'Two-cell' gall produced by *Dryophanta disticha* Htg. on the shoots of various species of *Quercus*, dull orange-coloured needles, and the gall produced by *Asphondylia genistae* Lew. on the leaves and stems of *Genista tinctoria* L., brown-coloured needles, which had the same melting-point and composition as dryophantin; mixed melting-points showed no depressions.

(3) *Cecidomin*. Flame-coloured orange needles, m.p. 227–231°, produced by *Cecidomyia tiliae* Schk. on the twigs and young shoots of *Tilia vulgaris* L. (Found: C, 50.69; H, 5.21; purpurogallin, 40.16; glucose, 63.18%; m.w. 556, 561. $C_{23}H_{28}O_{15}$ requires: C, 50.73; H, 5.15; purpurogallin, 40.37; glucose, 63.21%; m.w. 544.) From the 'Lighthouse' gall produced by *Cecidomyia bursaria* Bret. on the leaves of *Nepeta hederacea* Ter. and the 'Hedgebank' gall, produced by *Xestophanus potentillae* Cam. on the leaves of *Potentilla reptans* L., both of which crystallized in orange-coloured needles, which had the same melting-point and composition as cecidomin; mixed melting-points showed no depression.

(4) *Melampsin*. Pale yellow needles, m.p. 233–237°, produced by *Melampsora goepportiana* Htg. on the leaves and roots of *Vaccinium vitis idaea* L. (Found: C, 50.68; H, 5.22; purpurogallin, 40.41; glucose, 63.41%; m.w. 531, 549. $C_{23}H_{28}O_{15}$ requires: C, 50.73; H, 5.15; purpurogallin, 40.37; glucose, 63.21%; m.w. 544.) From the 'Pitmaking' gall produced by *Eriophyes ajugae* Nal. on the leaves of *Ajuga reptans* L., dark yellow needles which had the same melting-point and composition as melampsin; a mixed melting-point showed no depression.

(5) *Raestelin*. Bright green needles, m.p. 242–244°, isolated from the gall produced by *Raestelia lucerta* Sbg. on

the twigs of *Caraegus oxyacantha* L. (Found: C, 50.67; H, 5.24; purpurogallin, 40.37; glucose, 63.27%; m.w. 526, 541, 549. $C_{23}H_{28}O_{15}$ requires: C, 50.73; H, 5.15; purpurogallin, 40.37; glucose, 63.21%; m.w. 544.)

(6) *Eriophyesin*. Brownish red needles, m.p. 257–261°, produced by *Eriophyes macrotrichus* Nal. on the leaves of *Caprinus betulus* L. (Found: C, 50.71; H, 5.17; purpurogallin, 40.22; glucose, 63.27%; m.w. 531, 543. $C_{23}H_{28}O_{15}$ requires: C, 50.73; H, 5.15; purpurogallin, 40.37; glucose, 63.21%; m.w. 544.) From the 'Nail' gall produced by *Eriophyes gracilis* Nal. on the leaves of *Rubus idaeus* L., dark red needles; the 'Witches Broom' produced by *Ezoascus turgidus* Sad. on the twigs of *Betula alba* L., dark green needles; the 'Bean' gall produced by *Nematus gallicola* Staf. on the leaves of *Salix fragilis* L., light green needles; the 'Blister' gall produced by *Nematus Bridgemanii* Cam. on the leaves of *Salix caprae* L., dark green needles, and the 'Cherry' gall produced by *Neuroterus lenticularis* Oliv. on the leaves of *Quercus pedunculata* Ehrh., golden plates. All of these had the same composition as eriophyesin, the same melting and mixed melting-points.

Eriophyesin on methylation with diazomethane and hydrolysis yields 2:3:4-trimethoxypurpurogallin (Perkin & Steven, 1903; Willstätter & Heiss, 1923; Evans & Dehn, 1930) identified by its melting and mixed melting-points with authentic 2:3:4-trimethoxypurpurogallin. In both formulae I or II nucleus (A) is the same, which thus goes to prove that in eriophyesin the biglucose residue is attached to the hydroxyl group in nucleus (B).

(7) *Xestophanesin*. Bronze-coloured needles, m.p. 263–266°, isolated from the 'Hedgebank' gall produced by *Xestophanus potentillae* Cam. on the leaves of *Potentilla reptans* L. (Found: C, 50.49; H, 5.31; purpurogallin, 40.29; glucose, 63.18%; m.w. 537, 546, 557. $C_{23}H_{28}O_{15}$ requires: C, 50.73; H, 5.15; purpurogallin, 40.37; glucose, 63.21%; m.w. 544.)

(8) *Pontanin*. Orange-yellow needles, m.p. 281–287°, isolated from the galls produced by *Pontania proxima* Lepel on the leaves of *Salix fragilis* L. (Found: C, 50.61; H, 5.23; purpurogallin, 40.31; glucose, 63.11%; m.w. 542, 553. $C_{23}H_{28}O_{15}$ requires: C, 50.73; H, 5.15; purpurogallin, 40.31; glucose, 63.21%; m.w. 544.) From the 'Cotton-wool' gall produced by *Eriophyes Thomasi* Nal. on the leaves of *Thymus serpyllum*, blood red needles; from the 'Cupped Spangle' gall produced by *Neuroterus fumipennis* Hartg. on the leaves of *Quercus sessiliflora* Sal., golden plates; from the 'Smooth Pea' gall produced by *Rhodites elegantariae* Hartg. on the leaves of *Rosa canina* L., yellow needles, and from the 'Fig' gall produced by *Pemphigus ulmi* de Geer on the bark of *Ulmus campestris* L., dark brown needles. All of these had the composition and melting-point of pontanin and showed no depression on admixture with it.

SUMMARY

1. The isolation and characterization of eight colouring matters from various galls is described.

2. All the pigments described were found to be diglucosides of purpurogallin. In the case of one of them (eriophyesin) it is shown that the biglucose residue is attached to the hydroxyl group of the 'B' nucleus in purpurogallin.

We wish to thank Miss K. W. Sullvin, B.Sc., for the description of these pigments and for the trouble she has taken in comparing our dryophantin with the original dryophantin, which she has found to be identical in every respect.

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Tissue Nucleic Acids

2. THE ISOLATION AND PROPERTIES OF LIVER RIBONUCLEIC ACID

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Both ribonucleic acid and desoxyribonucleic acid have recently been shown to be present in the tissues of the sheep (Davidson & Waymouth, 1944*a*), and the current view is that the ribonucleic acid is in general a cytoplasmic constituent (Mirsky, 1943; Pollister & Mirsky, 1944), while desoxyribonucleic acid is confined to the nucleus.

So far, although the presence of pentose nucleic acids has been demonstrated in many biological materials, only three such acids have been isolated and characterized. The first to be isolated was yeast ribonucleic acid, whose structure has been reviewed by Gulland (1938, 1944). In addition to *d*-ribofuranose it contains small amounts of *l*-lyxose (Gulland, Barker & Jordan, 1943; Gulland & Barker, 1943) and is usually considered to be composed of tetranucleotide units in which the bases are adenine, guanine, uracil and cytosine in equimolecular proportions. The pancreas pentose nucleic acid was considered by Jorpes (1928, 1934) to be probably a pentanucleotide containing three molecules of purine (guanine:adenine ratio = 2:1) and two of pyrimidine. Jorpes (1924) isolated the crystalline brucine salts of cytidine and uridine phosphoric acids from hydrolysates of this nucleic acid, and concluded that the pyrimidines were cytosine and uracil.

The third pentose nucleic acid to be characterized was isolated from tobacco mosaic virus (Loring, 1939; Cohen & Stanley, 1942). It appears to be

very similar to yeast ribonucleic acid and on hydrolysis has yielded guanine, adenine, cytosine and the brucine salt of an acid similar to, and probably isomeric with, yeast uridylic acid (Loring, 1939).

Liver tissue contains a desoxyribonucleic acid very similar to thymus desoxyribonucleic acid (Levene, 1922; Peters, 1911; Ishiyama, 1928; Greenstein & Jenrette, 1940). This nucleic acid has been prepared from isolated liver nuclei by Dounce (1943). The cytoplasm of liver cells contains particles of at least two sizes (Claude, 1941, 1943*a*, *b*; Bensley, 1942) composed of phospholipin nucleoprotein complexes containing a pentose nucleic acid which has not yet been isolated and characterized.

It has previously been shown that sheep liver is a tissue with a relatively high content of pentose nucleic acid (Davidson & Waymouth, 1944*a*). From a dry ethanol-ether powder of sheep liver the nucleic acids were extracted with 10% sodium chloride and precipitated as lanthanum salts. The precipitate was rich in pentose. Moreover, the sodium chloride extract contained a substrate for the enzyme ribonuclease, and the amount of this substrate, in terms of phosphorus, indicated a ribonucleic-acid content of the same order as that found by the pentose estimations. The evidence for the presence of a pentose nucleic acid, presumably a ribonucleic acid, in liver is thus fairly conclusive (Davidson & Waymouth, 1944*b*), but it has seemed desirable that this nucleic acid should be isolated and characterized.