THE CHEMICAL DIFFERENTIATION OF SPECIES

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The differentiation of species by the biologist is based upon structural characters. If resolution be carried sufficiently far, the units of such a system constitute the elementary species of de Vries.¹ Each unit differs from another in several or in a number of morphological characters and it is part of the qualification of a systematic unit that its characters, within the limits of individual fluctuation, should be fixed, and should be transmitted unchanged from parent to offspring.

From biological investigation, we also learn that the structure of a species is of necessity adapted to its environment. Though there is no satisfactory evidence in support of the view that environment takes active part in the modelling of the plant form, the latter, nevertheless, can be modified to some extent during the life-time of the individual by changes in the environment. Such modifications, however, are regarded as somatic, i.e., modifications in degree only of existing morphological characters, and are not transmitted through the germ cells in heredity. Hence, in this sense, environmental changes have no permanent effect upon a species.

On the other hand, new varieties, and we must suppose, new species too, which are in themselves true entities, may at any time arise from an existing species through the formation of a new kind of germ-cell. These new forms differ from the type in certain morphological characters and also transmit these characters to their offspring. Among causes instigating this kind of variation, change in environment may be included, though there appears to be no direct connection between the nature of the change and the kind of variation produced. The new variety or species may or may not be able to adapt itself to the environment into which it is cast.

In all probability, classification in the vegetable kingdom will always be based on structure only. At the same time we cannot fail to realise that the various structural characters of species are in reality the expression

1. de Vries, *Species and Varieties : Their origin by Mutation*, 1905.

of certain phenomena of which the interpretation lies in another sphere of scientific research. Hence increased knowledge in the field of morphology, however exact and comprehensive it may become, must, from the nature of its methods, fail to give an insight into the underlying causes of specific form. To gain this insight the plant should be viewed from the bio-chemical standpoint.

Advance in bio-chemistry brings increasingly to the fore the conception of a chemical basis for vegetable structure. Many, for instance, of the so-called 'vital processes' which were at one time considered to be special functions of the living protoplasm, are now known to be controlled by complex chemical substances termed enzymes; at the same time it cannot be denied that there still remains a residuum of activities produced by living cells which no enzymes, so far known, are able to effect. A considerable number of these enzymes have been isolated from the plant and their activities have been demonstrated in vitro. Although much remains to be learnt of the actual nature and structure of enzymes, yet such knowledge as we possess with regard to these substances, tends to bring the reactions they control into line with other chemical reactions with which we have long been familiar.

Not only has our knowledge of the mechanism of metabolism increased, but progress has also been made along the lines of artificial synthesis of 'vital' products and likewise in the isolation and exact analysis of hitherto unknown compounds present in plants.

All bio-chemical results of the above kind afford evidence in favour of the supposition that the plant form is an expression of its chemical constitution.

From a scientific standpoint it is easy to realise that the plant body may have various appearances according to the conditions under which it is viewed. Magnification, for instance, considerably alters our idea of its construction and leads the biologist to look upon the plant as a complex of tissues built up of morphological units or cells, some living and of a highly organised structure, others dead.

To the bio-chemist, the underlying reality of the plant is a complex of chemical substances, some in solution, others in a solid or crystalline state. In addition, one must picture many of these substances taking part continually in a number of very complex chemical reactions which are highly susceptible to environment, i.e., presence of moisture, light, degree of temperature, etc.

We know that one organic compound differs from another in the number, kind and configuration of its component atoms. On similar lines we may suppose the number, kind and configuration of the chemical compounds which go to build up a plant to determine the plant form, and differences in the chemical components to be the underlying cause of difference in plant forms. Such a conception helps us to realise how each plant maintains its specific entity. Each plant may be supposed to inherit from its parent a certain definite chemical constitution and to be able to build up, by virtue of this constitution, compounds of a certain chemical type only and these products in their entirety give the outward morphological manifestation peculiar to the species.

The modifications brought about in any species during its life-time by change in environment must be regarded as the expression of change, in degree only, in the chemical reactions possible to the metabolism of that species. The morphological character, pigmentation, affords examples of varying degrees of reaction due to change in environment. The leaves of a species, for instance, growing in a warm temperate climate, may normally show only a trace of red colour, because at that temperature the concentration of the products of metabolism does not permit the chemical reaction leading to pigment formation to take place to any extent. When transferred to a cold temperate climate, the leaves may become completely red, since the concentration of the metabolic products is now different and pigment formation is accelerated.

The origin of a variety must, on the other hand, be regarded as the expression of a fundamental change in the chemical constitution of the plant, whereby certain chemical reactions cease to take place and their resultant products to be formed, or new reactions are instigated and new products are produced. Thus a permanent change may be supposed to have taken place in the chemical constitution of a green-leaved type when it gives rise to a red-leaved variety, for example, Copper Beech. In the metabolism of the variety it is no longer possible for the chemical reaction inhibiting the formation of pigment to take place. Similarly in certain glabrous white-flowered varieties of *Matthiola*,¹ some portion of the chemical structure of the hairy purple-flowered type is lost and this loss is accompanied not only by total inability to form pigment but also by complete absence of a structural character, i.e., the outgrowth of hairs from the epidermal cells.

1. Bateson, Saunders, and Punnett, *Reports to Evolution Committee of the Royal Society*, I, II, and III.

In view of the fact that the structure of all the higher plants has, on the whole, a common basis, we should expect and do as a matter of fact find, that metabolism is conducted on the same general plan, and hence the main classes of plant products, i.e., proteins, carbohydrates, fats, aromatic compounds, etc., are represented in the majority of species.

It is also a well-recognised phenomenon that the same special morphological tendencies appear in different genera by no means closely allied. The herbaceous, woody, annual and perennial habits are characteristic of various members of a number of families among the higher plants. So also xerophytic, aquatic, saprophytic and parasitic forms appear throughout the vegetable kingdom. On analogy we may expect very similar or identical chemical compounds to appear in plants which are by no means closely related. Finally, if the differentiation of species is to be ultimately a matter of the configuration of the complex molecules which bring about what have been termed 'vital processes,' we should expect not only a different outward configuration but also the presence in different species, and groups of species, of characteristic types of chemical compounds.

That many chemical compounds are peculiar to certain species becomes obvious when an examination is made of any bio-chemical literature in connection with a group of organic compounds. There exists, in fact, a vast amount of knowledge of the chemical components of many species. The object of this kind of investigation has certainly not been the establishment of chemical differentiation, but in the majority of cases research has been stimulated owing to the fact that the substances investigated were used for medicinal or various commercial purposes, as, for example, the alkaloids, tannins, resins, oils, various vegetable dyes, etc. There has been very little application of these results in the direction of ascertaining whether any isolated plant product is peculiar to certain species only, to all species of a genus or to allied genera of a natural order. The differentiation of species upon a purely chemical basis would require a store of information which we are at present very far from possessing. I should like to take advantage of this opportunity to advance a plea for the application of such knowledge as we already have, or are likely to obtain in the future, to the furtherance of this scheme.

The investigations might be carried out along certain lines such as the following:—

(1) In case of isolation of a new substance, that its distribution among closely allied species and genera should be ascertained.

(2) That the distribution of known compounds which can be readily detected should be further investigated with reference to allied species and genera.

(3) That experiments should be made to detect as far as possible the various steps in the syntheses of plant products taking place in metabolism.

(4) That the knowledge we already possess of organic compounds occurring in plants should be co-ordinated with a view to the chemical differentiation of species.

As an example of systematic work of this kind, I may quote the case of the glucoside, *aucubine*. This substance was first isolated by Bourquelot and Hérissé¹ from the vegetative organs of *Aucuba japonica* (Cornaceae). Further researches by Hérissé and Lebas² demonstrated the presence of aucubine in several species of *garrya*, another genus of the Cornaceae. Aucubine is also said by Bourdier³ to be present in various species of *Plantago* (Plantaginaceae). It would be interesting to discover whether further investigation will provide any connecting links between the two, so far isolated, appearances of this compound.

In the latter half of this paper I have attempted to give an account of the distribution of a few of the various classes of organic compounds found in plants and to show as far as possible (1) that many compounds are peculiar to certain species, genera or natural orders; (2) that allied compounds of restricted distribution are often found in the same species or genus.

I have dealt at greater length with the aromatic series because this group is universally present in plants and yet shows a greater specificity in the distribution of many of its members than is shown in the case of other widely distributed classes, such as the sugars and acids of the aliphatic series.

1. Bourquelot et Hérissé, 'Sur l'aucubine, glucoside de l'*Aucuba japonica*,' *Annales de chimie et de physique*, 1905.

2. Hérissé et Lebas, 'Présence de l'aucubine dans plusieurs espèces du genre *Garrya*,' *Jour. Pharm. Chim.*, 1910.

3. Bourdier, 'Recherche biochimique des glucosides dans le plantain (aucubine) et dans la verveine (vérbénaline),' *Thèse doct. Univ. (Pharm.)*, Paris, 1908.

PHENOLS

The *pyrocatechin* $C_6H_4(OH)_2(1:2)$ complex exists in many substances widely distributed in plants, and it is highly probable that the free product occurs more frequently than is generally supposed. Both Grafe¹ and Weevers² are of the opinion that the oxidation of pyrocatechin is the cause of the darkening of the organs of many plants on injury or after death.

Hydroquinone, $C_6H_4(OH)_2(1:4)$, is more limited in its distribution than pyrocatechin. As a glucoside—*arbutin*—it is characteristic of many genera in the Ericaceae and Pyrolaceae. Its presence also in *Pyrus communis* and *Protea mellifera* shows that it is not strictly limited to the above orders. In some recent researches, Bourquelot and Fichtenholz³ have obtained most interesting results in connection with hydroquinone. These authors find 'that *Pyrus communis* alone contains arbutin, while none could be detected in *Cydonia vulgaris*, *Malus communis*, *Sorbus auenparia* or *S. torminalis*, all of which were formerly classified as *Pyrus*, but are now regarded by most botanists as distinct. There are therefore recognisable chemical characters correlated with the botanical characters, and the modern classification is justified on bio-chemical, as well as on morphological, grounds.'

Phloroglucin, $C_6H_3(OH)_3(1:3:5)$, is the most widely distributed of all the phenols. The phloroglucin complex is present in a large number of aromatic compounds, and in addition, phloroglucin forms esters with acids of the aromatic series.

AROMATIC ALCOHOLS, ALDEHYDES AND ACIDS

The relationships between the chief members of these derivatives, both in the benzene and the phenylolefine groups which are found in plants, are shown in the accompanying table.

Salicylic alcohol in the form of a glucoside—*salicin*—is a characteristic product of the Salicaceae (*Salix*, *Populus*), but is not

1. Grafe, 'Über die Dunkelfärbung von Rübensäften.' *Öst. Zeitsch. f. Zuckerindus. u. Landwirtschaft*, 1908.

2. Weevers, 'Die physiologische Bedeutung einiger Glykoside.' *Extrait du Recueil des Travaux botaniques Néerlandais*, Vol. VII, 1910.

3. Bourquelot et Fichtenholz, 'Nouvelles recherches sur le glucoside des feuilles de poirier ; son rôle dans la production des teintes automnales de ces organes,' *Jour. de Pharmacie et de Chimie*, 1911. See also *J.C.S. Abstracts*, February, 1911.

limited to this group, since it is said to occur also in Rosaceae (*Spiraea*), and in Compositae (*Crepis*).

Benzoic acid in the form of benzoyl salicin is found as a glucoside—*populin*—which is apparently limited to the Salicaceae.

Salicylic acid, either free, or in the form of a glucoside of the methyl ester, is very widely distributed:—Liliaceae (*Yucca*), Rutaceae (*Barosma*), Rosaceae (*Spiraea*, *Fragaria*, *Rubus*), Rubiceae (*Ipecacuanha*), Violaceae (*Viola*), Ericaceae (*Gaultheria*), Betulaceae (*Betula*), Pyrolaceae (*Monotropa*), Fagaceae (*Fagus*), Polygalaceae (*Polygala*), Lauraceae (*Lindera*) and Erythroxylaceae (*Erythroxylon*). *p. Oxy-benzoic acid* is found in Bignoniaceae (*Catalpa*).

That a metabolic relationship exists in the Salicaceae between salicylic alcohol and pyrocatechin has been shown by Weevers¹ in an interesting paper on glucosides. The author finds that a decrease of saligenin is accompanied by an increase of pyrocatechin, and vice versa. Weevers was also able to obtain three enzyme actions from 'Rohenzym' extracted from *Salix*, though the enzymes were not isolated. One enzyme—salicase—brings about the reversible action: salicin + water = saligenin + glucose; the second—saligeninase—oxidises saligenin to pyrocatechin; and the third—catecholase—oxidises pyrocatechin to a black amorphous substance.

There is also obviously a relationship between the salicylic alcohol and salicylic acid in *Spiraea*.

Protocatechuic acid. The protocatechuic acid nucleus forms a constituent of many aromatic plant compounds, and the acid itself is formed as a product in the potash fusion of these substances. Native, the acid, occurs in Magnoliaceae (*Illicium*) and in Vitaceae (*Vitis*).

Vanillin occurs as a glucoside in Orchidaceae (*Vanilla*), and vanillin-producing substances are probably fairly widely distributed:—Orchidaceae (*Vanilla*, *Nigritella*), Graminaceae (*Avena*), Leguminosaeae (*Lupinus*), Liliaceae (*Asparagus*), Chenopodiaceae (*Beta*), Aquifoliaceae (*Ilex*), and Coniferae (*Pinus*).

Piperonal accompanies vanillin in *Vanilla* and *Nigritella*, and it is also found in *Heliotropium* (Boraginaceae).

Veratric acid is only known to occur in *Veratrum* (Liliaceae).

The phenyl-olefine series may next be considered.

Cinnamic acid is widely distributed, though not very frequent:—

1. Weevers, *loc. cit.*

Erythroxylon (Erythroxylaceae), *Thea* (Theaceae), *Scrophularia* (Scrophulariaceae), *Globularia* (Globulariaceae), and *Alpinia* (Zingiberaceae).

The phloroglucin ester of p-coumaric acid—*naringenin*—is present as a glucoside—*naringin*—in *Citrus decumana* (Rutaceae), and appears to be confined to that genus.

From the coumaric acids it is possible to pass to the phenyl fatty acids.

o-Hydrocoumaric acid has been found only in *Melilotus* (Leguminosae). *p-Hydrocoumaric acid*, as pointed out by Czapek, is of interest because of its connection with tyrosine.

Coumarin is a very widely distributed substance:—*Phoenix* (Palmaceae), *Anthoxanthum*, *Hierochloë*, *Milium*, *Cinna* (Graminaceae), *Angraecum*, *Nigritella*, *Orchis*, *Aceras* (Orchidaceae), *Herniaria* (Caryophyllaceae), *Ruta* (Rutaceae), *Prunus* (Rosaceae), *Dipteryx*, *Melilotus*, *Toluifera* (Leguminosae), *Myroxylon* (Flacourtiaceae), *Vitis* (Vitaceae), *Peristrophe* (Acanthaceae), *Galium*, *Asperula* (Rubiaceae), *Ageratum*, *Liatris* (Compositae).

Several oxy-coumarins have been found in various plants:—

Umbelliferone is found in *Daphne* (Thymeleaceae), and probably also in *Skimmia* (Rutaceae). Its methyl ester is said to be present in *Herniaria* (Caryophyllaceae).

Daphnetin as a glucoside—*daphnin*—is confined to *Daphne* (Thymelaeaceae).

Æsculetin occurs as a glucoside—*aesculin*—in *Æsculus* (Hippocastanaceae), *Gelsemium* (Loganiaceae), and *Euphorbia* (Euphorbiaceae).

Scopolin, a methyl aesculetin glucoside, occurs in *Scopolia* and *Atropa* (Solanaceae).

Limettin is found in *Citrus* (Rutaceae).

Fraxetin accompanies aesculin in *Æsculus* (Hippocastanaceae), and is also found in *Fraxinus* (Oleaceae).

Caffeic acid is probably widely distributed:—*Conium* (Umbelliferae), *Coffea*, *Chiococca* (Rubiaceae), *Ilex* (Aquifoliaceae), *Scrophularia* (Scrophulariaceae), *Strychnos* (Loganiaceae).

Coniferyl alcohol as a glucoside—*coniferin*—is a characteristic product of the Coniferae. It is found also in *Beta* (Chenopodiaceae), *Asparagus* (Liliaceae), *Scorzonera* (Compositae).

Syringenin, methoxy-coniferyl alcohol, occurs in *Ligustrum* and *Syringa* (Oleaceae), and also in *Robinia* (Leguminosae).

Ferulic acid has been found in the resin of *Asafœtida*.

Hesperitinic acid as a phloroglucin ester—*hesperidin*—is a product of many Rutaceae, i.e., *Citrus*, *Barosma*, *Toddalia* and *Skimmia*, and is also found in *Scrophularia* (Scrophulariaceae).

When we consider the question of allied products, the following facts are of interest.

Coniferyl alcohol is readily oxidised to vanillic alcohol, though it appears probable that hadromal is the mother substance of vanillin. Nevertheless, both nearly related products do appear in the same genera.

Vanilla and *Nigritella* (Orchidaceae) contain vanillin, piperonal and coumarin.

Pinus (Coniferae), *Asparagus* (Liliaceae) and *Beta* (Chenopodiaceae) contain both vanillin and coniferyl alcohol.

Scrophularia nodosa (Scrophulariaceae) contains cinnamic, caffeic and hesperitinic acids.

Daphne, umbelliferone and daphnetin.

Ilex, vanillin and caffeic acid.

Æsculus, aesculin and fraxetin.

Herniaria, umbelliferone and coumarin.

Melilotus, o-hydrocoumaric acid and coumarin.

In the Rutaceae, many substances allied to cinnamic acid are found, i.e., naringin, umbelliferone, coumarin, limettin and hesperitin.

PROTEINS

These substances form an essential constituent of every plant, yet of all classes of compounds they are probably the least adapted to purposes of chemical differentiation. To quote Osborne on 'The Vegetable Proteins':—'The possibility of making preparations of proteins which may fairly be considered as chemical individuals deserves special attention. These substances present unusual difficulties in this respect, for they have none of those physical and chemical characters which the chemist usually depends upon to show the individuality of an organic chemical compound; in other words, the proteins have no single property by means of which any judgment can be formed in regard to the strict chemical individuality of any one of them. The best that can be done at present is to establish a constancy of the ultimate composition of successive fractional precipitations of the protein under consideration, and to show the constancy of the physical properties and products of hydrolysis of these fractions as far as possible . . . Many, however, of the proteins with which we are at present familiar have shown such constancy of composition and properties that we feel justified in now considering them as substances of reasonably definite character.'

There is, nevertheless, evidence for regarding many of the known proteins as distinctive of certain species or groups of species. Definite names, for instance, have been given to the following globulins:—

Leguminosae: legumin in *Pisum sativum*, *Vicia faba*, *V. sativa*, *Ervum lens*; vignin in *Vigna sinensis*; glycinin in *Glycine hispida*; phaseolin, *Phaseolus vulgaris*, *P. radiatus*, *P. lunatus*; conglutin, *Lupinus* sp; vicilin, *Pisum sativum*, *Vicia faba*, and *Ervum lens*.

Corylaceae: corylin in *Corylus avellana*.

Rosaceae: amandin in *Prunus amygdalus*, *P. persica*.

Juglandaceae: juglansin in *Juglans regia*, *J. nigra*, *J. cinerea*.

Lecythidaceae: excelsin in *Bertholletia excelsa*.

Cannabaceae: edestin in *Cannabis sativa*.

Graminaceae: avenalin in *Avena sativa*; maysin in *Zea mays*.

Fagaceae: castanin in *Castanea vulgaris*.

Solanaceae: tuberin in *Solanum tuberosum*.

THE MUSTARD-OILS

The mustard-oils, in combination with sugar as various glucosides, form as one group—sinigrin, sinalbin, sinapin, etc.—characteristic components of the Cruciferae, and as a second group—glukotropaeolin—of the Tropaeolaceae.

PURIN DERIVATIVES

Caffeine: *Coffea* (Rubiaceae).

Thea (Theaceae).

Paullinia (Sapindaceae).

Ilex (Aquifoliaceae).

Cola (Sterculiaceae).

Theobroma (Sterculiaceae).

Theobromine: *Theobroma* (Sterculiaceae).

Cola (Sterculiaceae).

Theophylline: *Thea*.

Xanthine: *Thea*.

Adenine: *Thea*.

As will be seen from the above list, several of these compounds (theophylline, xanthine and adenine) are known only in the genus *Thea* (Theaceae). Theobromine in *Theobroma* and *Cola* (Sterculiaceae). Caffeine is more widely distributed.

CYANOGENETIC COMPOUNDS

As glucosides, these are characteristic products of certain genera. *Amygdalin* and *laurocerasin* are found in genera of the Rosaceae, but there is evidence that amygdalin-like compounds are widely distributed among plants. Other cyanogenetic glucosides are *dhurrin*, peculiar to *Sorghum* (Graminaceae); *gynocardin*, to *Gynocardia*; *phaseolunatin*, to *Phaseolus lunatus* (Leguminosae), and *Linum* (Linaceae); *lotusin*, to *Lotus arabicus* (Leguminosae).

ALKALOIDS

The alkaloids form a group of organic substances specially characterised by the fact that every member of the group, with a few exceptions, is a product of metabolism in some one species only, in one genus only, or at most in several allied genera.

Considering the group as a whole, we find the alkaloids especially prominent in certain orders and cohorts, whereas they are entirely absent from others. The Monocotyledons are poor in alkaloids. Among the Dicotyledons, the chief alkaloid-containing groups are the Ranales (Ranunculaceae, Menispermaceae, Berberidaceae, and others); the Rhoeadales (Papaveraceae); the Contortae (Loganiaceae, Apocynaceae); the Solanaceae; the Rubiales (Rubiaceae).

From other large families of flowering plants, these substances are either entirely absent (Rosaceae, Labiateae), or are present in one or two genera only (Cruciferae, Umbelliferae, Compositae).

The alkaloids are generally classified into three groups, according to whether they are derivatives of pyridine, quinoline, or isoquinoline. Derivatives of each group are represented in various natural orders.

The pyridine group is mostly widely represented. The best known derivatives occur in the Solanaceae, Erythroxylaceae, and Leguminosae, but other members are present in a large number of orders, including Palmaceae, Liliaceae, Amaryllidaceae, Piperaceae, Zygophyllaceae, Rutaceae, Euphorbiaceae, Buxaceae, Celastraceae, Caricaceae, Cactaceae, Punicaceae, Myrtaceae, Umbelliferae, Caprifoliaceae, Cornaceae, Plumbaginaceae, Sapotaceae, Symplocaceae, Apocynaceae, Boraginaceae, Verbenaceae, Campanulaceae, Cucurbitaceae, and Compositae.

Members of the quinoline group are chiefly characteristic of the Loganiaceae (*strychnine* and *brucine*, and other derivatives in *Strychnos* spp.), and of the Rubiaceae (*quinine* and *cinchonine*, and other derivatives in *Cinchona* spp.).

Members of the isoquinoline group are found in Nymphaeaceae, Ranunculaceae, Berberidaceae, Anonaceae, Menispermaceae, Calycanthaceae, Lauraceae, and Papaveraceae.

It is rare to find an alkaloid distributed among the genera of more than one natural order. *Trigonellin*, which is present in the Leguminosae, Moraceae, Graminaceae, Apocynaceae, and *berberin* in the Ranunculaceae, Berberidaceae, Anonaceae, Menispermaceae, Leguminosae, and Rutaceae, both form examples of this kind of exception.