Leuco-Anthocyanins

2. SYSTEMATIC DISTRIBUTION OF LEUCO-ANTHOCYANINS IN LEAVES

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In Part 1 (preceding paper) a method of demonstrating the presence of leuco-anthocyanins in plant tissues has been described. A modified version of this method, in which paper chromatographic separation of the anthocyanidins is not carried out, has been applied to the leaves of several hundred species of plants. As a confirmatory test, in the great majority of instances the vanillin reaction has been applied to methanolic extracts of the leaves. In extracts of plant tissues, this reaction is given only, so far as is known, by leuco-anthocyanins and catechins, and the latter do not, of course, give anthocyanidins under the conditions of the primary test. In every instance described below, where a specimen giving a positive anthocyanidin test has been examined chromatographically, anthocyanidin has, in fact, been present on the chromatogram.

METHODS

Anthocyanidin test. This is carried out by heating the sample of leaf tissue in 2N-HCl, and extracting the digest with *iso*amyl alcohol, as described in Part 1, except that the extraction can be carried out in the digestion tube in presence of the solid residue, and that the amount of *iso*amyl alcohol need not be so restricted as when the solution is to be used for chromatography. The shade and depth of colour of the *iso*amyl alcoholic layer are noted. Any colour from deep crimson to faintly pinkish is regarded as positive for anthocyanidin (subject to confirmation by the vanillin test or by chromatography) and is scored according to depth (see Table 1).

Vanillin test. A similar sample of fresh leaf tissue is macerated with methanol (about 2 ml.) in a test tube for approx. 1 min. The (usually) bright green extract is decanted into another tube and shaken with light petroleum to remove chlorophyll. (If the tissue is left in contact too long with methanol the phytyl group of chlorophyll will be transesterified by chlorophyllase, when present, with methyl and the chlorophyll is not then so readily extracted from the methanolic layer.) The extraction with light petroleum is repeated until the methanolic layer is no more than faintly green. One-half of the methanolic solution is then transferred to a third tube, and 3–5 drops of conc. HCl are added to each tube; 3–5 drops of a saturated solution of vanillin in ethanol are added to one of them. A positive reaction varies from one in which a deep cherry-red coloration is produced immediately to one which becomes only perceptibly warmer in tint than the control. When the vanillin reaction is negative, both the control and the test samples usually become bluer in tint, because of the development of a reaction between carotenoid compounds and HCl (cf. Bate-Smith, 1953). This reaction is slower in development than the vanillin reaction, and, moreover, it appears to be inhibited in presence of leuco-anthocyanins. When the latter are present, in high concentration, anthocyanidin is slowly developed in the HCl control sample even in the cold; the blue carotenoid reaction is not only suppressed, but is replaced by a scarlet anthocyanidin reaction.

RESULTS

In Tables 1 and 2 are recorded the results of applying the anthocyanidin test to the leaves of over 500 species of plants. The vanillin test was carried out on 448 (86 %) of these plants and only in four instances was there any clear qualitative disagreement between the results of the two tests.

Discussion of Table 1

The results in this table support the earlier conclusion (Bate-Smith, 1953) that leuco-anthocyanins occur much more commonly in the tissues of woody plants than in those of herbaceous plants. This generalization is strikingly brought out by relating the results to the phylogenetic scheme of Hutchinson (1946, 1948), which is based essentially on a division of dicotyledonous plants into two groups, one, Lignosae, predominantly woody, the other, Herbaceae, predominantly herbaceous. In the Lignosae, the only families so far examined of which the members contain no leuco-anthocyanins are Oleaceae, Loganiaceae, Cornaceae, Araliaceae, Greyiaceae,* Myrsinaceae, Linaceae, Erythroxylaceae, Buxaceae and those in the group Thymeleales to Cucurbitales. Most of these are regarded as 'apical' families in their respective phyletic series. Several of the other 'apical' families such as Asclepiadaceae, Caprifoliaceae, Rubiaceae, Rutaceae, Verbenaceae, and Papilionaceae (Hutchinson) contain many members without leuco-anthocyanins. On the other hand, families recognized as relatively primitive—for example, Magnoliaceae, Theaceae,

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* Willis (1948), following Engler and Prantl, places *Greyia* in Melianthaceae.

Dilleniaceae, Anonaceae, Tiliaceae and Rosaceae—regularly contain leuco-anthocyanins, often in great quantity. In the Herbaceae, the only families in which leuco-anthocyanins have been detected are Saxifragaceae (Hutchinson), Polygonaceae, Ficoidaceae (=Aizoaceae E.P.), Oxalidaceae, Limnanthaceae, Balsaminaceae, and Plumbaginaceae.* The first are 'extracted' by Hutchinson as a herbaceous group from the otherwise woody Saxifragaceae (E.P.). Of the remainder only Limnanthaceae and Balsaminaceae are 'wholly herbaceous' (Metcalfe & Chalk, 1950).

The incidence of leuco-anthocyanins within some of those families which give partly positive and partly negative reactions appears at first sight to be quite irrational. On examination, however, factors can be discerned which seem to influence the presence or absence of leuco-anthocyanin in these cases. The best example for detailed consideration is that of Leguminosae. Although more than sixty species of Leguminosae have been examined, this number is still too small to cover this enormous family adequately. Of the three subfamilies, the Papilionatae have been studied most thoroughly, members of all ten of its tribes being represented in the forty-seven species examined.

The Leguminosae are included by Hutchinson in Lignosae, but the Papilionatae are mainly herbaceous. Of the trees (or near-trees) in this subfamily, *Cladrastis*, *Robinia*, *Wistaria*, *Derris*, *Machaerium* and *Pterocarpus* are positive for leuco-anthocyanin, while *Sophora* and *Laburnum* are negative. Even among the herbaceous members, although the great majority are negative, there is one tribe, viz. the Hedysareae, persistently positive for leuco-anthocyanin. Clearly, woodiness is not the only factor determining presence or absence of leuco-anthocyanin. Some assistance in accounting for this unevenness in distribution is given by Dormer's (1946) division of the Papilionatae into 'pulvinate' and 'epulvinate' groups, depending on the presence or absence of a pulvinus at the base of the leaf. The former includes Sophoreae, Dalbergieae, Phaseoleae and parts of Galegeae and Hedysareae, the latter Podalyrieae, Trifolieae, Loteae, and Vicieae, with the remaining subtribes of Galegeae and Hedysareae. The distribution of species positive and negative for leuco-anthocyanin follows this division remarkably closely. *Sophora* in Sophoreae, and four of the Hedysareae are the only examples which fall outside this generalization. Much significance must be attached to Dormer's conclusion that the pulvinate condition is the primitive one, and his observation that 'there is a distinct correlation between the presence or absence of a foliar pulvinus and the general habit of the plant. Every tribe, subtribe, or genus mentioned in the epulvinate column of the table is predominantly herbaceous, whereas in the pulvinate series there are several groups which are predominantly or entirely woody. Furthermore, while the pulvinate series contains some dozens of genera of large forest trees, it is exceedngly rare for an epulvinate species to exceed the stature of a shrub....It is difficult to escape the conclusion that the whole metabolism is different in the two groups—in particular the pulvinate forms are characterized by much more pronounced secretory activity.' The important concept here is that a metabolic pattern associated with woody habit may be found in related species which are themselves of herbaceous habit.

We appear to have, therefore, as factors determining the presence or absence of leuco-anthocyanin in a species: (1) the general character and systematic position of the family to which it belongs, and (2) the stage of evolution of the species within the family, especially in relation to its habit, whether arboreal, climbing, shrubby, or herbaceous. A further question which has to be faced is the reality of the grounds on which affinities, sufficient to warrant the circumscription of species within the bounds of a family, are claimed. Many families—the Euphorbiaceae for instance—are notoriously of doubtful homogeneity, and it is not surprising, therefore, to find their members differing markedly in chemical characters.

Bearing these considerations in mind, the situation in such families as Rosaceae, Saxifragaceae (E.P.), Caprifoliaceae, and Rutaceae appears more rational. In these families the incidence of leuco-anthocyanins decreases as the habit approaches the herbaceous. Leuco-anthocyanins may be absent from shrubs and woody climbers of some tribes, but may nevertheless be present in herbaceous forms of other tribes of the family. As in Moraceae, they may be absent from trees in one division, yet present in herbs of another division of a family; especially if the divisions are so divergent in character as to be treated by some authors as separate families. Essentially, the association of leuco-anthocyanins with woody character underlies the pattern of their distribution in these families. At the same time, and perhaps not altogether independently, there is a tendency for leuco-anthocyanins to be present in the more primitive, and absent from the more advanced, members of a phyletic series. If this criterion were the only one which applied, the Herbaceae as a whole would qualify to be regarded as phylogenetically advanced beyond the Lignosae.

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* *Note added* 23 *April* 1954. Since this paper was submitted for publication, several species of *Primula* have been examined, with results as follows (cf. Table 1): *Primula denticulata*, + +; *P. obconica*, −; *P. verticillata*, + + +; *P. vulgaris*, + +. Primulaceae have therefore to be added to the families of Herbaceae which are positive for leuco-anthocyanin.

Table 1. *Anthocyanidin reaction in leaves of dicotyledonous plants*

The conventions used in recording the strength of the reaction are: + + + +, exceedingly strong; + + +, very strong; + +, strong; +, moderate; (+), weak; ±, uncertain; −, negative; ×, reaction obscured by development of interfering coloration. In each major subdivision the families are arranged in alphabetical order. Willis (1948) has been used as a guide to the further subdivision of families marked with an asterisk.

Acanthaceae, *Asystasia bella*, −; Aceraceae, *Acer laxiflorum*, + +; *A. negundo* v. *auratum*, (+); *Dipteronia sinensis*, + + +; Actinidiaceae, *Actinidia chinensis*, + +; Aizoaceae, *Carpobrotus acinaciformis*, +; *Tetragonia expansa*, −; *Vanzijlia angustipetala*, −; Amaranthaceae, *Amaranthus cordatus*, −; Anacardiaceae, *Cotinus americanus*, + +; *Mangifera indica*, +; *Cotinus coggygria*, + +; *Rhus illinoensis*, + +; *R. typhina*, ±; Anonaceae, *Asimina triloba*, + +; Apocynaceae, *Acokanthera venenata*, −; *Allamanda neriifolia*, −; *Apocynum androsaemifolium*, + +; *Landolphia florida*†, ×; *Landolphia klainei*†, ×; *Nerium oleander*, −; *Plumeria bicolor*, (+); *Vinca major*, + +; Aquifoliaceae, *Ilex aquifolium*, −; *I. integra*, −; *I. latifolia*, (+); *I. paraguayensis*, (+); Araliaceae, *Hedera helix*, −; Aristolochiaceae, *Aristolochia clematitis*, −; Asclepiadaceae, *Asclepias incarnata*, −; *Periploca graeca*, +; *Vincetoxicum fuscatum*, −; Balsaminaceae, *Impatiens noli-tangere*, + +; *I. parviflora*, + +; Berberidaceae, *Mahonia aquifolium*, −; Betulaceae, *Alnus glutinosa*, +; *Corylus avellana*, + + +; Bignoniaceae, *Bignonia capreolata*, −; *Campsis radicans*, −; *Catalpa bignonioides*‡, ×; *Glaziova bauhinioides*, −; *Incarvillea olgae*§, ×; Bixaceae, *Bixa orellana*, +; Bombacaceae, *Bombax malabarica*, −; *Ceiba pentandra*, +; Boraginaceae, *Nonnea lutea*, ±; Burseraceae, *Bursera* sp., + +; Buxaceae, *Buxus sempervirens*, −; *B. sinensis*, −; *Pachysandra terminalis*, −; Cactaceae, *Zygocactus truncatus*, −; Calycanthaceae, *Chimonanthus praecox*, −; Campanulaceae, *Campanula muralis*, −; *Platycodon grandiflorum*, −; *Specularia hybrida*, −; Caprifoliaceae, *Abelia grandiflora*, +; *Diervilla floribunda*, + +; *D. venosa*, + +; *Dipelta floribunda*, + +; *Lonicera alpigena*, −; *L. glaucescens*, −; *L. nitida*, −; *L. periclymenum*, −; *L. pileata*, −; *L. tatarica*, −; *Sambucus ebulus*, −; *S. nigra*, −; *Viburnum davidii*, + +; *V. lantana*, +; *V. tomentosum* var. *plicatum*, +; Caryophyllaceae||, *Acanthophyllum spinosum*, −; *Arenaria grandiflora*, (+); *Cerastium biebersteinii*, −; *Dianthus barbatus*, −; *Dianthus* 'Border carnation', −; *Dianthus* 'Cantab', −; *Herniaria ciliata*, −; *Paronychia capella*, −; *Saponaria officinalis*, ×; *Silene fimbriata*, −; *Spergula arvensis*, −; *Stellaria media*, −; Casuarinaceae, *Casuarina glauca*, +; Celastraceae, *Euonymus alatus*, + +; *E. fortunei* 'Silver Queen', + +; *E. japonicus*, +; *E. radicans minimus*, +; Chenopodiaceae, *Atriplex hastata*, −; *Beta maritima*, −; Cistaceae, *Cistus incanus*, + +; *Helianthemum verum* 'Old Gold', (+); Combretaceae, *Terminalia chebula*, +; Compositae, *Baccharis halimifolia*, −; *Cassinia fulvida*, −; *Centaurea cyanus*, −; *Dahlia merckii*, −; *D. variabilis*, −; *Helichrysum bracteatum*, −; *Kleinia cylindrica*, −; *Microglossa albescens*, −; *Olearia gunniana*, −; *O. haastii*, −; *O. macrodonta*, −; *O. virgata*, −; *Senecio fulgens*, −; *Tagetes patula*, −; Convolvulaceae, *Ipomoea learii*, −; Coriariaceae, *Coriaria nepalensis*, −; Cornaceae, *Aucuba japonica*¶, ×; *Cornus amomum*, −; *C. florida*, −; *C. foemina*, −; *C. mas* 'variegata', −; Crassulaceae**, *Bryophyllum tubiflorum*, −; *Sedum aizoon*, ×; *S. middendorffianum*, +; Cruciferae, *Cheiranthus cheiri* (orange)††, ×; *C. cheiri* (yellow), −; *Iberis sempervirens*††, ×; *Isatis glauca*††, × ×; *Lepidium latifolium*, −; *Peltaria alliacea*, −; Cunoniaceae, *Callicoma serratifolia*, + + + +; *Cunonia capensis*, + +; Dilleniaceae, *Candollea tetrandra*, + +; *Hibbertia volubilis*, + +; Dipsacaceae, *Cephalaria ambrosioides*, −; *Scabiosa graminifolia*, −; Ebenaceae, *Diospyros lotus*, +; Elaeagnaceae, *Elaeagnus orientalis*, +; Ericaceae, *Arbutus menziesii*, + +; *A. unedo*, + + +; *Gaultheria cuneata*, + +; *Macleania punctata*, +; *Rhododendron smirnowii*, + + +; *Vaccinium intermedium*, + +; Erythroxylaceae, *Erythroxylum coca*, (+); Eucommiaceae, *Eucommia ulmoides*, + +;

Euphorbiaceae*:

- Phyllantheae, *Andrachne colchia*, + +; *Phyllanthus angustifolius*, + +; *Phyllanthus mimosoides*, + +;
- Daphniphylleae, *Daphniphyllum macropodium*‡‡, ×;
- Acalypheae, *Mercurialis perennis*, −; *Ricinus communis*, −;
- Adrianeae, *Manihot utilissima*, ±;
- Cluytieae, *Codiaeum angustifolium*, +; *Codiaeum variegatum* var. *aucubifolium*, + +;
- Euphorbieae, *Euphorbia aphylla*, −;

Fagaceae, *Fagus sylvatica*, + +; *Quercus macrocarpa*, + +; *Quercus suber*, +; *Quercus warburgii*, +;

Flacourtiaceae*:

- Pangieae, *Hydnocarpus wightianus*, + +; *Kiggelaria africana*, −;
- Flacourtieae, *Azara gilliesii*, −; *Azara microphylla*, +; *Idesia polycarpa*, −; *Poliothyrsis sinensis*, (+);
- Casearieae, *Arechavaletaia uruguayensis*, + +;

Garryaceae, *Garrya elliptica*¶, ×; Gentianaceae, *Menyanthes trifoliata*, −; Geraniaceae, *Erodium cicutarium*, −; *Geranium grandiflorum*††, ×; Guttiferae, *Hypericum androsaemum*, + +; Hamamelidaceae, *Distylium racemosum*, +; *Liquidambar styraciflua*, + +; *Parrotia persica*, +; Hydrophyllaceae, *Hydrophyllum canadense*, −; *Phacelia grandiflora*, −; *P. tanacetifolia*, −; Icacinaceae, *Pennantia cunninghamii*, + +; Juglandaceae, *Juglans regia*, + + +; Labiatae, *Origanum vulgare*, −; *Prostanthera ovalifolia*, −; *Salvia verbenaca*, −; *Teucrium scorodonium*, −; Lardizabalaceae, *Akebia quinata*, −; Lauraceae, *Lindera benzoin*, +; *Umbellularia californica*, +;

Leguminosae*:

- Mimosoideae: Acacieae, *Acacia alata*, + +; *A. armata*, +; *A. catechu*, (+); *A. decurrens*, −; *A. retinodes*§§, ×; *A. robusta*, + +; Eumimoseae, *Mimosa pudica*, + +;
- Caesalpinioideae: Bauhinieae, *Bauhinia yunnanensis*, +; *Cercis siliquastrum*, +; Cassieae, *Cassia tomentosa*, −; *Ceratonia siliqua*, + +; Eucaesalpinieae, *Caesalpinia echinata*, + +; *C. gilliesii*, −; *C. japonica*, + +; *Gleditschia sinensis*, −; *G. triacanthos*, +;
- Papilionatae: Sophoreae, *Cladrastis tinctoria*, +; *Sophora japonica*, −; *S. macrocarpa*, −; Podalyrieae, *Baptisia australis*, −; *Chorizema cordatum*, −; *C. ilicifolium*, −; *Piptanthus nepalensis*, −; *Thermopsis montana*, −; Genisteae, *Laburnocytisus adamii*, −; *L. vulgare*, −; *Petteria ramentaceae*, −; *Spartium junceum*, −; Trifolieae, *Medicago lupulina*, −; *M. sativa*, −; *Melilotus lutea*, −; *Ononis fruticosa*, −; *O. repens*, ±; *Trifolium dubium*, (+); *T. incarnatum*, −; *T. pratense*, −; Loteae, *Anthyllis vulneraria*, −; Galegeae, *Amorpha fruticosa*, + +; *Caragana frutescens*, ±; *Clianthus puniceus*, −; *Colutea arborescens*, ±; *C. orientalis*, ±; *Galega officinalis*, −; *Psoralea macrostachys*, (+); *Robinia pseudacacia*, + +; *Wistaria sinensis*, + +; Hedysareae, *Arachis hypogea*, (+); *Coronilla varia*, +; *Desmodium canadense*, + +; *Hedysarum multiflorum*, + +; *Hippocrepis comosa*, −; *Lespedeza thunbergii*, +; *Onobrychis sativa*, + + +; *Scorpiurus muricatus*, +; Dalbergieae, *Derris elliptica*, + +; *Machaerium firmum*, + +; *Pterocarpus fraxinifolius*, +; Vicieae, *Lathyrus luteus*, −; *L. venatus*, −; *Vicia dumetorum*, −; *V. faba*, −; Phaseoleae, *Glycine soja*, ±; *Phaseolus vulgaris*, −; *Rhyncosia phaseoloides*, + +;

Limnanthaceae, *Limnanthes douglasii*, + + +; Linaceae, *Linum grandiflorum*, −; *L. lewisii*, −; *L. perenne*, −; *Reinwardtia tetragyna*, −; Loganiaceae *Buddleia* sp., −; *Desfontainia spinosa*, −; Lythraceae, *Lagerstroemia indica*, −; *Lythrum salicaria*, −; *Nesaea salicifolia*, −; Magnoliaceae, *Liriodendron tulipifera*, + +; *Magnolia denudata* (= *M. conspicua*), +: *M. stellata*, −; *M. virginiana*, (+); Malvaceae, *Abutilon* 'Boule de Neige', −; *Abutilon* 'Thompsonii',

Table 1 (_cont._)

– ; _A. vitifolium_ var. _album_, + ; _Althaea rosea_, – ; _Gaya lyallii_, – ; _Gossypium herbaceum_, + + ; _Hibiscus rosa-sinensis_, – ; _H. syriacus_, – ; _Pavonia spinifex_, + ; Melastomaceae, _Schizocentron elegans_, – ; _Tibouchina semidecandra_, + + + ; _Tococa_ sp., + + ; Meliaceae, _Cedrela odorata_, – ; _Swietenia mahogoni_, + + + ; Melianthaceae, _Greyia sutherlandii_, – ;

Moraceae*:

Moroideae, _Dorstenia lujae_, – ; _Maclura pomifera_, – ; _Morus alba_, – ; _M. nigra_, – ;

Artocarpoideae, _Artocarpus incisa_, + ; _Cudrania javanensis_, – ; _C. tricuspidata_, – ; _Ficus carica_, – ; _F. elastica_, + + ; _F. lancifolia_, + + ; _F. religiosa_, – ; _F. sycomorus_, + + ;

Cannaboideae, _Cannabis sativa_, ± ; _Humulus lupulus_, + + + ;

Myoporaceae, _Myoporum acuminatum_, – ; _Oftia africana_††, × ; Myricaceae, _Myrica gale_, + + ; Myrsinaceae, _Myrsine africana_, – ; Nyctaginaceae, _Bougainvillaea glabra_ var. _sullivanii_, – ; _Oxybaphus nyctagineus_, – ; _Pisonia brunnoniana_††, × ; Nymphaeaceae, _Nymphaea_ sp., – ; _Victoria regia_***, ± ; Nyssaceae, _Davidia vilmoriniana_, – ; Oleaceae, _Fraxinus excelsior_, – ; _Fraxinus mariesii_, – ; _Ligustrum vulgare_, – ; _Phillyrea latifolia_, – ; Oxalidaceae, _Oxalis bupleurifolia_, + + ; _O. dispara_, + + ; _O. namaquana_, + ; _O. pes-caprae_, – ; _O. tuberosa_, ± ; _O. valdiviensis_, (+); Papaveraceae, _Dendromecon rigidum_, – ; Passifloraceae, _Passiflora coerulea_, – ; Phytolaccaceae, _Phytolacca americana_, – ; Piperaceae, _Piper futokadruna_, – ; Pittosporaceae, _Pittosporum crassifolium_, – ; Plantaginaceae, _Plantago major_, – ; Platanaceae, _Platanus acerifolius_, + + ; _P. orientalis_, + + ; _P. orientalis_ var. _cuneatus_, + + ; Plumbaginaceae, _Armeria maritima_, + ; _Ceratostigma willmottianum_, + ; _Limonium latifolium_, + + ; _L. sinuatum_ (cultivar), + + ; _Plumbago capensis_, + + ; Polemoniaceae, _Cobaea scandens_, ± ; Polygalaceae, _Polygala chamaebuxus_ var. _grandiflora_, – ; Polygonaceae, _Atraphaxis hispida_, + ; _Fagopyrum esculentum_, + ; _F. tataricum_, + + ; _Polygonum alpinum_, + ; _P. aubertii_, + ; _P. aviculare_, + ; _P. hydropiper_, + + ; _P. persicaria_, + ; _Rumex acetosa_, + + ; _R. maximus_, + + ; _R. patientia_, + ; Portulacaceae, _Portulaca grandiflora_, – ; _P. oleracea_, – ; Proteaceae, _Banksia serrata_, + + ; _Grevillea ornithopoda_, + + ; _G. robusta_, + + ; _G. sulphurea_, + ; _Hakea multilineata_, + + ; _H. teretifolia_, + + ; _H. ulicina_, + + ; _Macadamia ternifolia_, + + ; Ranunculaceae, _Paeonia humilis_, – ; _Zanthorhiza apiifolia_, – ; Rhamnaceae, _Ceanothus arnoldii_, + + ; _Colletia cruciata_, + ; _Paliurus australis_, + ; _Rhamnus cathartica_, + ; _R. purshiana_, + ; Rhizophoraceae, _Rhizophora_ sp., + + + ;

Rosaceae*:

Spiraeoideae: Spiraceae, _Neillia longiracemosa_, + + ; _Spiraea japonica_, + ; _S. salicifolia_, + + ; Quillajeae, _Exochorda racemosa_, + + ;

Pomoideae: _Chaenomeles speciosa_ (= _Cydonia japonica_), + ; _Cotoneaster horizontalis_, + ; _Crataegus crus-galli_, + ; _Mespilus germanica_, + + + ; _Osteomeles schwerinae_, + + + ; _Sorbus aucuparia_, + ;

Rosoideae: Kerrieae, _Kerria japonica_, – ; Potentilleae, _Fallugia paradoxa_, – ; _Fragaria_ (cultivated strawberry), + + ; _Potentilla alchemilloides_, + ; _P. fruticosa_, + + ; _P. montana_, + ; _Rubus deliciosus_, – ; Ulmarieae, _Filipendula ulmaria_, + + ; Sanguisorbeae, _Agrimonia eupatoria_, + + + ; Roseae, _Rosa bracteata_, (+); _R. damascena_, + ; _R. foetida_ var. _persiana_, + ; _R. multiflora_, + + ; _R. rubiginosa_, + ; _R. rugosa_, + ; _R. spinosissima_ 'lutea', + ; _R. soulieana_, (+); _R. xanthina_, + ;

Prunoideae: _Prunus laurocerasus_, + + ; _P. mahaleb_, + ;

Rubiaceae*:

Cinchonoideae: Cinchoneae, _Cinchona succirubra_, – ; Mussaendeae, _Mussaenda luteola_, + + ;

Caffeoideae: Ixoreae, _Coffea arabica_, + ; _C. robusta_, + + ; _Pavetta natalensis_, + + ; Galieae, _Galium aparine_‡‡, × ; _G. verum_††, × ;

Rutaceae*:

Rutoideae: Zanthoxyleae, _Barosma lanceolata_, + + ; _Choisya ternata_, – ; _Zanthoxylum bungei_, + + ; Ruteae, _Dictamnus fraxinella_, – ;

Toddalioideae: _Ptelea trifoliata_, – ; _Skimmia japonica_, – ;

Aurantioideae: _Aegle spinosa_ (= _Ponciras trifoliata_), – ;

Salicaceae, _Populus canescens_, + + ; _P. deltoides_, + ; _Salix acutifolia_, + ; _S. babylonica_, + + ; _S. caprea_, + ; _S. elaeagnos_ (= _S. incana_), + + ; Santalaceae, _Santalum album_, – ; Sapindaceae, _Koelreuteria paniculata_, + + ; _Nephelium longana_, + + ; _Sapindus saponaria_, – ; _Xanthoceras sorbifolia_, + + ;

Saxifragaceae*:

Saxifragoideae, _Astilbe rosea_, + + ; _Bergenia crassifolia_, + + ; _Boykinia aconitifolia_, + + + ; _Tellima grandiflora_, + + ;

Francoideae, _Francoa ramosa_, – ;

Hydrangeoideae, _Hydrangea paniculata_, + + ; _H. petiolaris_, + + ; _Kirengeshoma palmata_, – ; _Philadelphus laxus_, + + ;

Escallonioideae||||, _Escallonia iveyana_, × ; _E. montevidensis_, + + ; _E. punctata_, × ; _E. virgata_, × ;

Ribesioideae, _Ribes grossularia_, + + ; _R. nigrum_, + + ; _R. sanguineum_, + + ; _R. speciosum_, (+);

Baueroideae, _Bauera rubioides_, + ;

Scrophulariaceae, _Paulownia tomentosa_††, × ; _Veronica acuminata_, – ; Simarubaceae, _Ailanthus altissima_, + ; _Cneorum tricoccum_, – ; _Picrasma quassioides_, – ; Staphyleaceae, _Staphylea trifolia_, (+); Sterculiaceae, _Fremontia californica_, – ; _Hermannia candicans_, + + ; Tamaricaceae, _Tamarix anglica_, (+); _T. tetrandra_, – ; Theaceae, _Camellia japonica_, + + ; _Stewartia pseudocamellia_, + + ; _Thea sinensis_, + ; Thymelaeaceae, _Daphne gnidium_, (+); _D. laureola_, – ; _D. odora_ 'marginata', – ; _Pimelia ferruginea_, (+); Tiliaceae, _Tilia europaea_, + + ; Tropaeolaceae, _Tropaeolum majus_, – ; Ulmaceae, _Ulmus campestris_, + + ; Umbelliferae, _Foeniculum capillaceum_, – ; _Sium sisarum_, – ; Urticaceae, _Boehmeria nivea_, ± ; _Parietaria diffusa_ (= _officinalis_), + + ; _Urtica dioica_, – ; Valerianaceae, _Centranthus rubra_, – ; _Valeriana sambucifolia_, – ; Verbenaceae, _Callicarpa japonica_, – ; _Caryopteris tangutica_††, × ; _Clerodendron fargesii_, – ; _Diostea juncea_, – ; _Petraea volubilis_, – ; _Tectona grandis_¶¶, × ; Violaceae, _Hymenanthera crassifolia_, – ; _Viola rugulosa_, – ; Vitaceae, _Parthenocissus quinquefolia_, + + ; Zygophyllaceae, _Peganum harmala_, – ; _Zygophyllum fabago_, –.

† Interference due to formation of blue-purple coloration.
‡ Interference due to formation of dark brown coloration, with heavy ppt.
§ Interference due to formation of deep blue-purple coloration.
|| Young leaves of several species gave positive or indeterminate anthocyanidin reactions, not given by mature specimens, and not confirmed by vanillin reaction.
¶ Interference due to formation of deep brown coloration (cf. Trim & Hill, 1952).
** Contain considerable quantities of catechin-like compounds.
†† Interference due to formation of purplish brown or brown coloration.
‡‡ Interference due to formation of deep steely blue coloration.
§§ Tissue becoming deep reddish brown; interference in vanillin reaction due to intense blue coloration.
|||| Interference due to formation of deep blue-black coloration.
¶¶ Interference due to formation of brown-scarlet coloration.
*** Leaves permanently pigmented. Peduncles gave positive reaction with both tests.

Table 2. *Anthocyanidin reaction in leaves of other groups of plants*

See Table 1 for explanation of symbols.

Monocotyledons

Araceae, *Philodendron racemosum*, +; *Zantedeschia elliottiana*, (+); Commelinaceae, *Tradescantia reflexa*, −; Cyperaceae, *Carex pendula*, +; Dioscoreaceae, *Dioscorea opposita*, ++; *Tamus communis*, (+); Gramineae, *Arundinaria japonica*, −; *Saccharum officinarum*†, −; *Sorghum halapense*, −; *S. sachanense*, −; Hydrocharitaceae, *Limnobium stoloniferum*, (+); Iridaceae, *Iris foetidissima*, −; *I. germanica* (cultivar), −; *I. pseudacorus*, ++; Lemnaceae, *Lemna minor*, (+); Liliaceae, *Smilax rotundifolia*, ++; Palmae, *Washingtonia gracilis*, ++; Typhaceae, *Typha latifolia*‡, −; Zingiberaceae, *Elettaria cardamomum*, ++; *Globba winitii*, ±; *Hedychium coccineum*, +; *H. spicatum*, +.

Gymnosperms

Cycadaceae, *Dioon edule*, (+); *Macrozamia riedleri*, ++; *Stangeria eriopus*, −; Ginkgoaceae, *Ginkgo biloba*, ++; Gnetaceae, *Ephedra americana*, +; *Gnetum gnemon*, −; Pinaceae, *Araucaria bidwillii*, +++; *Cedrus deodara*, ++; *Cunninghamia sinensis*, ++; *Metasequoia glyptostroboides*, ++; *Sequoiadendron giganteum*, ++; Taxaceae, *Podocarpus falcatus*, +++; *Taxus baccata*, +++.

Pteridophyta

Equisetaceae, *Equisetum arvense*, −; *E. telmateia*, +; *E. variegatum*, −; Isoetaceae, *Isoetes lacustris*§, ×; Lycopodiaceae, *Lycopodium selago*, −; Marattiaceae, *Angiopteris evecta*, +; *Marattia fraxina*, +; Polypodiaceae, *Dryopteris elongata*, ++; *Pellaea rotundifolia*, ++; *Pteridium aquilinum*, ++; Psilotaceae, *Psilotum triquetrum*, −; Salviniaceae, *Azolla filiculoides*, ++; *Salvinia auriculata*, ++; Schizaeaceae, *Lygodium circinatum*, +; Selaginellaceae, *Selaginella apus*, −; *S. caulescens*, −; *S. kraussiana*, −.

Bryophyta

Dicranum scoparium, −; *Leucobryum glaucum*, −; *Mnium hornum*, −.

† Positive anthocyanidin and vanillin reactions given by roots.
‡ Strongly positive anthocyanidin and vanillin reactions given by roots.
§ Interference due to formation of deep blue coloration.

Discussion of Table 2

Groups other than the dicotyledons have so far been only scantily covered.

In monocotyledons a distinction between woody and herbaceous species is no longer observed; positive and negative reactions are more or less equally divided among the herbaceous forms. In several instances (*Typha*, *Saccharum*) in which the leaves are negative for leuco-anthocyanin, the rootstock reacts positively. All parts of *Iris pseudacorus* except the petals give an intense reaction; neither leaves nor rhizomes of *I. germanica* and *foetidissima*, however, give any reaction. The strong reactions of the fleshy *Dioscorea*, and of the Zingiberaceae are especially remarkable. *Smilax rotundifolia*, although climbing in habit, is essentially woody.

Among the gymnosperms the reaction is general. Its occurrence in *Ginkgo* is worthy of remark, as this is regarded as a form of great antiquity.

In Pteridophyta, the reaction is general among the ferns examined, is present in Equisetaceae, and although any anthocyanidin formed is obscured by a strong darkening of the tissues when heated with hydrochloric acid, judging from the positive vanillin reaction, is also positive in *Isoetes*.

DISCUSSION

That the presence of leuco-anthocyanins in vascular plants is a primitive character is strongly indicated by (1) the generality of their presence in the lower and more primitive forms of these plants—for example, the Equisetaceae, Polypodiaceae, Cycadaceae, *Ginkgo*, and *Metasequoia*—and (2) their absence from those families which, whether woody or herbaceous, are recognized as advanced in their various lines of development. It would seem that the production of leuco-anthocyanins is part of a primitive metabolic pattern associated with, but not essential to, a tree-like or woody habit of growth.

Relationship to lignin

Consideration of a possible role for leuco-anthocyanins in the process of lignification as such is hampered by our present limited knowledge of the chemistry of both leuco-anthocyanins and lignin. As regards the first, there is reason to suppose (cf. Part 1) that the molecule is of the flavonoid C_6–C_3–C_6 pattern substituted with OH in positions C-3 and C-4 or C-3, C-4 and C-5 of one benzene nucleus (see Formulae in Part 1); and as regards lignin, that it is largely composed of C_6–C_3 fragments substituted at C-4 with OH, either alone or with one or both of positions 3 and 5 substituted with OCH_3. It is, in fact, of some importance to have established, as has been done in Part 1, that the leuco-anthocyanins are narrowly restricted, in leaves at least, to unmethylated types. Methylation of the hydroxyl groups at C-3 and C-5 would seem to be an essential feature distinguishing the lignin nucleus from the leuco-anthocyanin (and also the catechin) molecule.

Creighton, Gibbs & Hibbert (1944) and Towers & Gibbs (1953) have made a start on a systematic survey of the distribution of guaiacyl (4-OH, 3-OCH_3) and syringyl (4-OH, 3:5-di-OCH_3) residues in lignins from a wide range of plants. For the most part, syringyl residues are absent from coniferous lignins (present only in *Podocarpus*) but present, together with *p*-hydroxyphenyl residues, in monocotyledonous lignins and present also in dicotyledonous lignins. Reference to Table 3 of Part 1 will show immediately what little resemblance there is between the systematic distribution of 3:4- and 3:4:5-substituted types in the leuco-anthocyanins and that in the lignins. There is, in fact, as little sense to be made of the distribution of leuco-anthocyanin types in leaves as there is of the different anthocyanin types in flowers (cf. Lawrence, Price, Robinson & Robinson, 1939). The only really outstanding feature of the distribution of leuco-anthocyanin types is, in fact, the absence of leuco-delphinidin from all species of Rosaceae. Otherwise cyanidin and delphinidin types appear to occur indiscriminately within a family, often occurring together in species. It may well be that all these plants possess the capacity, i.e. the enzymic equipment necessary to produce both types, but that in any particular instance one may be formed preponderantly, often to the complete exclusion of the other.

It is important, too, to note that neither type is conspicuously the more 'primitive'. Some Pteridophyta, including *Equisetum*, have leuco-cyanidin alone (or mainly) while others have both types; *Ginkgo* and *Sequoiadendron* have leuco-delphinidin, *Metasequoia*, leuco-cyanidin, other Coniferae both. There is in this context no support for the view (Lawrence *et al.* 1939) that cyanidin is, of the three main anthocyanidin types, the primary (and primitive) product of metabolism; but it must also be borne in mind that there is no evidence that any connexion exists between the metabolism of leuco-anthocyanins and anthocyanins.

The presence of leuco-anthocyanins is evidently not essential to the process of lignification, nor is it essential to the development of a woody habit of growth, since lignification in some degree occurs in all vascular plants at and above the level of Pteridophyta, and many perfectly good trees do not possess leuco-anthocyanins either in the foliage or in the wood itself. Whatever therefore might be the function of leuco-anthocyanins with respect to the development of woody tissue, that function can be assumed by other molecules. It is possible for instance that, in some cases, the coumarins might assume this function. It is probably not merely a coincidence that the recorded occurrence of the coumarins (cf. Bergmann & Gierth, 1932) is virtually confined to those families of plants in the leaves of which leuco-anthocyanins do not occur. If this were so, it would indicate one way in which a primitive metabolic pattern, of which the leuco-anthocyanins form a feature, might be modified in advanced forms without loss of the ability to form wood, but with, perhaps, metabolic economy in such circumstances as the herbaceous condition where suppression of wood formation is a concomitant of their habit of growth.

Relationship to tannin

The relationship of leuco-anthocyanin to 'tannin' in plants is more easily discerned. Amongst naturally occurring substances, the vanillin reaction, one of the reactions used for the histochemical detection of tannins, is given, so far as is known, only by catechins and leuco-anthocyanins. These substances also react in a manner characteristic of tannins with other reagents used for the histochemical detection of tannins, such as ferric salts. It is not surprising, in view of the widespread distribution of the leuco-anthocyanins revealed by the present survey, to find a considerable degree of congruence between their distribution and that recorded for 'tannins' in the botanical literature. The extent of this congruence can be judged from the fact that, out of twenty-five of the families of dicotyledons noted by Metcalfe & Chalk (1950) as regularly tanniniferous, only two (Tamaricaceae and Crassulaceae) have not been found to contain leuco-anthocyanins, and of these, the Crassulaceae contain strongly vanillin-positive, catechin-like substances. The leuco-anthocyanins have, in fact, many properties of tannins besides those mentioned above. They are, for instance, precipitated by hide powder and are astringent in taste (Bate-Smith & Swain, 1953). It is difficult to avoid the conclusion that they are, in fact, the commonest and most typical representatives of the class of substances rather indefinitely described in the botanical literature as 'tannins'. The tannins of commerce are much more definitely characterized under their specific commercial designations, and by their specific property of tanning leather. It is not known to what extent, if any, they owe their tanning properties to leuco-anthocyanins.

Taxonomic implications

Although there would seem to be useful implications for the taxonomist in the results of this survey, it is not intended to attempt to press these to any firm conclusions because, although a wide area has been covered, the number of specimens studied is still so very small. In dicotyledons, confinement of leuco-anthocyanins to Hutchinson's Lignosae (with the exception of a small group of families in Herbaceae) appears to us to provide some validation of the system put forward by Hutchinson (1946) mainly on morphological grounds; and to encourage, perhaps, reconsideration of the placing of some of the few families containing leuco-anthocyanins which are at present included by him in the Herbaceae.

SUMMARY

1. The presence or absence of leuco-anthocyanins in the leaves of vascular plants is related to their systematic position. They are generally present in ferns, but have not been found in Selaginellaceae, Psilotaceae, or Lycopodiaceae, nor in mosses. They are also generally present in gymnosperms, but are absent from *Gnetum*.* Their occurrence in monocotyledons is common but scattered.

2. In the leaves of dicotyledonous plants, the presence or absence of leuco-anthocyanins appears to be connected with a woody habit in the plant, or in the forms closely related to it. Hutchinson's classification of the families of dicotyledons into a predominantly woody group (Lignosae) and a predominantly herbaceous group (Herbaceae) provides a fairly accurate division into those containing members which have, and those whose members do not have, leuco-anthocyanins in their leaves. There are, however, many members of the Lignosae the leaves of which do not contain leuco-anthocyanins; these are usually either herbaceous in habit, or belong to families containing herbaceous members. A few families of Herbaceae, viz. Saxifragaceae (Hutchinson), Polygonaceae, Oxalidaceae, Limnanthaceae, Balsaminaceae, Aizoaceae and Plumbaginaceae, contain leuco-anthocyanins. In Crassulaceae substances reacting with vanillin occur, but these are not leuco-anthocyanins.

3. The distribution of leuco-anthocyanins in the leaves of Papilionatae has been especially studied. They occur in plants of woody habit in Sophoreae, Dalbergieae, Phaseoleae and Galegeae, and in the herbaceous Hedysareae, but are absent from Podalyrieae, Trifolieae, Loteae and Vicieae. This distribution follows closely the division of Papilionatae by Dormer (1946) on grounds of vegetative morphology.

4. The systematic distribution of leuco-anthocyanins closely follows the recorded incidence of tannins in the botanical literature. They have, in fact, the properties of tannins and are probably the substances most commonly responsible for the reactions in plant tissues attributed to tannins.

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* See also footnote p. 127.

On the Occurrence of Adrenaline and Noradrenaline in Blood

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Results obtained with a fluorimetric method of estimation (Weil-Malherbe & Bone, 1952, 1953) indicate a concentration of about 2–3 μg. adrenaline and 5–7 μg. noradrenaline/l. human venous plasma under basal conditions. Although these figures are lower than many proposed by previous investigators (see review by Pekkarinen, 1948), even lower levels are postulated by some physiologists. Evidence in support of the specificity of the method has been submitted in our earlier publications (Weil-Malherbe & Bone, 1952, 1953). This paper contains a more detailed report of experiments in which the occurrence and concentration of adrenaline and noradrenaline in blood were studied by paper-chromatographic methods.

A comparison of the R_F values of the fluorogenic substances present in blood with those of adrenaline and noradrenaline provided qualitative evidence