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A NOVEL COLOUR REACTION OF SOME *EUPHORBIA* AND *OXYANTHUS* SPECIES

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Abstract—The woody stems of a few species of *Euphorbia* and of *Oxyanthus* have been found to give a brilliant orange colour when treated with hydrochloric acid in methanol. Attempts to isolate the orange pigment, or its colourless precursor, from *Euphorbia pulcherrima* have been unsuccessful. In the course of these attempts, germanicyl acetate and β-sitosterol have been isolated, and glucose, arabinose and ten of the common amino acids identified by paper chromatography.

INTRODUCTION

IN 1945 Isenberg and Buchanan[1] observed that shavings of some woods when soaked in 2·5% concentrated hydrochloric acid in methanol give a purple or magenta colour (a positive reaction); other woods treated similarly give no trace of purple (a negative reaction). They found that this test, which Adler[2] later concluded is due to the presence of catechol tannins, is of some taxonomic significance.

We have adopted this Isenberg–Buchanan[1] (I./B.) test and applied it to the sapwoods of all woody plants available to us. The results have been reported in part.[3] Some families of flowering plants seem constantly to be positive to this test; others as constantly negative. Some families have some genera which are positive and others which are negative. In a few genera some species are positive; others negative. Only very rarely does a single species seem variable from individual to individual or from time to time. Only very rarely, too, does one obtain an "off-beat" reaction. One such is discussed here.

RESULTS AND DISCUSSION

Colour Reactions of Euphorbia *and* Oxyanthus *Species*

In early March, 1961, we tested the sapwood of a *Euphorbia* (*E. nudiflora* Jacq.) freshly collected in Jamaica, and obtained a striking *orange* rather than purple reaction—recorded as "deep chrome"—using Ridgway's *Color Standards and Color Nomenclature*.[4] Later in the

[1] I. H. ISENBERG and M. A. BUCHANAN, *J. Forestry* **43**, 888 (1945).
[2] E. ADLER, *Svensk Papper stid.* **54**, 445 (1951).
[3] R. D. GIBBS, *Trans. Roy. Soc. Can. Sect. III* **48**, 1 (1954).
[4] R. RIDGWAY, *Color Standards and Color Nomenclature*. Washington (1912).

month *E. heterophylla* L. was tested and recorded as "capucine orange". A variety *E. heterophylla* var. *graminifolia*, gave a similar colour. It was later found that the common Poinsettia (*E. pulcherrima* Willd.), which is closely related to these plants, also gave an orange colour, recorded as "bittersweet orange". The colour was stable and did not leach into the reagent, and the reagent could be changed daily for a week or more without any noticeable fading of the colour. The colour is due to a pigment which acts as an acid-base indicator, changing to a pale yellow at pH 8.

We found that we had recorded a similar colour with wood of *Oxyanthus tubiflorus* DC. (Rubiaceae) nearly 10 years ago. The colour in this case was recorded as "mikado orange". When tested recently a specimen of the same species gave a fainter colour—"light orange yellow". A second species—*O. natalenis* Sond.—tested recently, seemed also to vary. One specimen gave "cadmium orange", another a much fainter orange.

Other woods, such as those of the Berberidaceae, may give a yellow colour with the reagent, but the colour leaches into the liquid and the wood itself becomes almost colourless after one or two changes of reagent.

Limitations of Colour Reaction

We have looked for the orange reaction in other woody members of the Euphorbiaceae and Rubiaceae, but without success. In our experience this "off-beat" colour is given only by a few closely related species of *Euphorbia* and by the two species of *Oxyanthus* available to us.

Thus, of the Euphorbiaceae, a family of mixed response to the test, the following have all been recorded as 0 (no colour, or very pale yellow with the I./B, reagent).—*Euphorbia antisyphilitica* Zucc., *E. balsamifera* Ait., *E. canariensis* L., *E. degeneri* Scherf., *E. fulgens* Karw., *E. grandidens* Haw., *E. intisy* Drake, *E. leucocephala* Lotsy, *E. longifolia* D. Don, *E. milii* Desmoul. (*E. splendens?*), *E. schimperi* Presl, *E. splendens* Boj., *E. stygiana* H. C. Wats., *E. tirucalli* L., *E. virosa* Willd., *Acalypha godseffiana* Masters, *A. hamiltoniana* Hort., *Alchornea latifolia* Sw., *Andrachne colchica* Fisch. & Mey., *Claoxylon australe* Baill., *Croton linearis* Jacq., *Hemicyclia australasica* Muell.-Arg., *Hura crepitans* L., *Mallotus discolor* F. Muell., *Pedilanthus tithymaloides* Poit., *Putranjiva roxburghii* Wall., and *Ricinus communis* L.

Several members of the family have given very faintly positive reactions (0?) to strongly positive reactions (4) as follows:

Antidesma bunius Spreng. (3–4), *Baloghia lucida* Endl. (1), *Beyeria leschenaultii* (*B. opaca* F. Muell.?) (1), *Breynia oblongifolia* Muell.-Arg. (4), *Codiaeum variegatum* Bl. vars. (1), *Colliguaja integerrima* Gill. & Hook. (0–2), *C. odorifera* Molina (3), *Croton humilis* L. (0?), *Excoecaria agallocha* L. (2), *Gymnanthes lucida* Sw. (3), *Hevea brasiliensis* Muell.-Arg. (2), *Mallotus japonicus* Muell.-Arg. (0?), *Phyllanthus calycinus* Labill. (4), *P. grandifolius* L. (3), *P. speciosus* Jacq. (4), *Ricinocarpus pinifolius* Desf. (2–3), *Sapium jamaicense* Sw. (*S. aucuparium* Jacq.?) (1?).

Of the Rubiaceae we have tested the following:

Alberta magna E. Mey. (2–3), *Brachytome wallichii* Hook. f. (0), *Canthium coprosmoides* F. Muell. (1), *Catesbaea spinosa* L. (0), *Cephaelis elata* Sw, (0), *Cephalanthus occidentalis* L. (2), *Cimarrhis cymosa* Jacq. subsp. *jamaicensis* Urb. (2–3), *Chiococca alba* (L.) Hitchc. (0), *Cinchona succirubra* Pav. (1–3), *Coffea arabica* L. (0), *Coprosma arborea* T. Kirk (0), *C. baueri* Endl. (0), *C. repens* Hook. f. (Rich.?) (0), *C. robusta* Raoul (0), *Gardenia cornuta* Hemsl. (0), *G. globosa* Hochst. (2), *G. jasminoides* Ellis (0), *G. thunbergia* L. (0), *Guettarda*

uruquensis Cham. & Schlecht. (1), *Hamelia patens* Jacq. (0–1), *Hamiltonia suaveolens* Roxb. (0), *Hoffmannia* sp. (0), *Ixora barbata* Roxb. (1–2), *I. coccinea* L. (1–2), *I. lutea* Hutch. (2), *I. macrothyrsa* Teijsm. & Binn. (1 ?), *Luculia gratissima* Sweet (1–4), *L. pinceana* Hook. (0–1), *Macrocnemum jamaicense* L. (2), *Mitriostigma axillare* Hochst. (0), *Morinda jasminoides* A. Cunn. (0), *M. paniculata* (0), *Mussaenda erythrophylla* Schum. & Thonn. (1), *Nauclea esculenta* (Afzel.) Merr. (2), *Paederia scandens* (Lour.) Merr. (0), *Palicourea alpina* DC. (0 ?), *P. crocea* (Sw). Roem. & Schult. (0), *Pavetta gracilis* Klotz. (0), *P. montana* Reinw. (1), *Phialanthus* sp. (1–2), *Portlandia* sp. (0), *Posoqueria latifolia* Roem. & Schult. (0), *Psychotria brasiliensis* Vell. (1 ?), *P. carthagensis* Jacq. (2–3); *P. corymbosa* Sw. (0), *P. uliginosa* Sw. (0), *Putoria calabrica* Pers. (bright yell., but leaching out to almost colourless), *Randia benthamiana* F. Muell. (1), *R. maculata* (1), *Rondeletia cordata* Benth. (1), *R. odorata* Jacq. (1 ?), *Sabicea hirta* Sw. (1 ?), *Serissa foetida* Lam. var. (0), *Vangueria infausta* Burch. (2), *V. madagascariensis* J. F. Gmel (0).

Members of the Verbenaceae tend on the whole to give yellowish to buff colours with the I./B. reagent, but in no case has an orange reaction been obtained.

Attempts to Isolate the Pigment or its Precursor from Euphorbia pulcherimma

Attempts to extract the orange pigment from chips of the woody stem of *Euphorbia pulcherimma* with benzene, acetone, methanol, dimethyl sulphoxide and acetic acid failed. Consequently, the untreated wood was extracted with the usual organic solvents in an attempt to remove the colourless precursor of the pigment; however, after each extraction the wood developed the usual orange colour on treatment with the I./B. reagent. These results indicated that possibly the colourless precursor and pigment were chemically bonded to cellulose or to lignin. Accordingly, the action of a number of species of fungi which attack cellulose or lignin was investigated. However, this treatment destroyed the precuror.

In the course of our extraction experiments, germanicyl acetate and β-sitosterol were isolated, and glucose, arabinose, and ten of the common amino acids were identified by paper chromatography. Germanicol,[5, 6] glucose,[7] arabinose,[7] and the same amino acids[8] have already been found in other species of *Euphorbia*.

EXPERIMENTAL*

Extraction of Wood of Euphorbia pulcherimma

Chips of the woody stem (4 kg) were extracted in a soxhlet apparatus with the following solvents, and on evaporation of solvent from the extract residues were obtained: Light petroleum (b.p. 60–80°): a yellow solid (22·1 g); Benzene: a brown oil (79 g); Ether: a brown oil (31 g); Ethanol: a brown oil (57 g); Acetic acid: a gum (28 g); Water: a gum (17 g).

The extracted chips still developed a bright orange colour when treated with 2·5% HCl in methanol. Other solvents (dioxane, nitrobenzene, nitromethane, dimethyl sulphoxide) also failed to extract the chromogen.

* Melting points are corrected. Chromatography was carried out on Grace and Davidson silica gel or Woelm neutral alumina. Microanalyses were by C. Daesslé, Montreal.

[5] J. C. E. Simpson, *J. Chem. Soc.* 283 (1944).
[6] S. David, *Bull. Soc. Chim. France* 155 (1949); S. Chapon and S. David, *Bull. Soc. Chim. France* 456 (1952).
[7] F. Moewus, *Biol. Zentr.* **69**, 181 (1950); *Chem. Abstr.* **44**, 9007 (1950).
[8] C. Montant, *Compt. rend.* **245**, 1454 (1957).

Isolation of Germanicyl Acetate

The light petroleum extract was chromatographed on 3% deactivated silica gel. Elution with benzene afforded a crystalline material (A), and with benzene–ether (95:5 v/v) a gelatinous material (B). The solid A was recrystallized from ether–methanol to give transparent plates of germanicyl acetate (1·21 g; 0·03% yield), m.p. 275–277°, $[\alpha]_D^{27}+27{\cdot}3°$ (*c*, 0·463 in chloroform) (Lit.[5] m.p. 274–276°, $[\alpha]_D^{20}+18{\cdot}1°$). Calc. for $C_{32}H_{52}O_2$: C, 81·99; H, 11·18%; mol. wt. 468. Found: C, 81·76; H, 10·90%; mol. wt. (mass spectroscopy), 468.

A solution of the acetate (0·080 g) in ether (15 ml) was added to a suspension of $LiAlH_4$ (0·15 g) in ether (20 ml) for 2 min. The mixture was refluxed for 90 min and then 10% NaOH (1 ml) was added dropwise with stirring. The precipitated inorganic salts were removed by filtration and washed with ether. Evaporation of the combined ether solutions gave a white crystalline material (0·073 g; 89% yield), m.p. 164–169°. Several recrystallizations from aqueous methanol yielded colourless needles of germanicol melting at 170–174° alone or in admixture with authentic germanicol (Lit.[5] m.p. 176–177°); $[\alpha]_D^{27}+19{\cdot}3°$ (*c*, 1·094 in chloroform) (Lit.[5] $[\alpha]_D^{17}+5{\cdot}8°$). Calc. for $C_{30}H_{50}O$: C, 84·44; H, 11·81%; mol. wt. 426. Found: C, 84·81; H, 11·60%; mol. wt. 426.

Acetylation of this compound (45 mg) with acetic anhydride (1 ml) and pyridine (1 ml) gave, after several recrystallizations from ether–methanol, germanicyl acetate, m.p. 268–271°, $[\alpha]_D^{27}+27{\cdot}5°$ (*c*, 0·512 in chloroform), shown by mixed m.p. to be identical with the compound originally isolated.

Isolation of β-Sitosterol

The gelatinous material B (see above) was dissolved in ether and shaken with 2% NaOH. The ether layer was removed, dried, and chromatographed on alumina. Elution with acetone removed a crystalline material (0·560 g; 0·01% yield), which was recrystallized from methanol–chloroform to give β-sitosterol, m.p. 132–134° (Lit.[9, 10] 136–137°, 138–138·5°), $[\alpha]_D^{25}-34{\cdot}5°$ (*c*, 0·970 in chloroform) (Lit.[9, 10] $[\alpha]_D^{25}-36{\cdot}6°$, $[\alpha]_D-35°$). Calc. for $C_{29}H_{48}O$: C, 84·40; H, 11·72%. Found: C, 84·66; H, 12·10%.

The acetate, prepared in the usual way with acetic anhydride and pyridine, melted at 124–125° after crystallization from methanol (Lit.[9, 10] 125–126°, 127–129°), $[\alpha]_D^{25}-44{\cdot}2°$ (*c*, 0·772 in chloroform) (Lit.[9, 10] $[\alpha]_D^{25}-41°$, $[\alpha]_D-45°$).

The 3,5-dinitrobenzoate, prepared with 3,5-dinitrobenzoyl chloride in pyridine, was recrystallized from ethyl acetate and obtained as needles, m.p. 210–713° (Lit. 202–203°[9], 211–213°[10]), $[\alpha]_D^{25}-10{\cdot}4°$ (*c*, 0·921 in chloroform) Lit.[9, 10] $[\alpha]_D^{25}-10{\cdot}4°$, $[\alpha]_D-12{\cdot}4°$).

Identification of Amino Acids

The ethanol fraction was submitted to two-dimensional ascending chromatography on Whatman No. 1 filter paper using the following solvent systems: butanol–acetic acid–water (5:1:4) (vols.); and phenol saturated with 3% aqueous sodium cyanide. Spraying with 1% ninhydrin in butanol–ethanol (1:1 v/v) gave ten well-defined blue zones having R_f values for leucine, phenylalanine, valine, tyrosine, alanine, threonine, glycine, serine, aspartic acid, and arginine.

[9] E. S. WALLIS and P. N. CHAKROVARTY, *J. Org. Chem.* **2**, 335 (1937).
[10] J. J. SCHWARTZ and M. E. WALL, *J. Am. Chem. Soc.* **77**, 5442 (1955).

Identification of Arabinose and Glucose

Samples of the acetic acid and aqueous extracts were submitted to ascending chromatography using pyridine–ethyl acetate–water as solvent, and the chromatogram was sprayed with aniline–hydrogen phthalate solution. Two zones corresponding in R_f and colour to glucose and arabinose were observed.

Action of Wood-rotting Fungi

Chips of *Euphorbia pulcherrima* were added to malt agar in culture tubes and sterilized. Inocula from actively growing cultures of *Polyporus albellus*, *P. hirsutus*, *P. versicolor*, *Corticium vellereum*, and *Stereum hirsutum* were added to the tubes and allowed to grow for 3 months. The chips were then removed and washed with water. On testing with methanolic hydrochloric acid they developed only a faint orange colour; no colour developed in the culture medium.

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