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THE BETACYANINS AND THEIR DISTRIBUTION[1]

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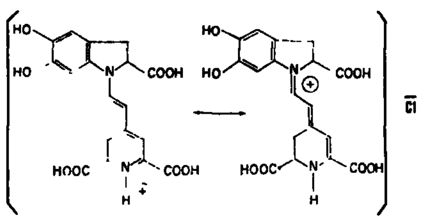
Abstract—Thirty-eight species, including thirteen previously uninvestigated genera, in ten families usually grouped in the Centrospermae were surveyed by electrophoresis and chromatography for the presence of betacyanin and anthocyanin pigments. Betacyanins were observed in all thirty-eight species but no anthocyanins were detected.

THE knowledge[2] that betacyanins, formerly considered to be "nitrogenous anthocyanins", represent a new class of plant pigments of restricted distribution, has stimulated interest in the phylogenetic significance of these compounds. The history of these pigments from the 1860's to 1960 has been reviewed comprehensively by Dreiding.[3] More recently Mabry[1] has discussed the structure of the betacyanins and reviewed their systematic distribution, particularly with respect to phyletic implications.

The formula was deduced[1,2] for betanidin hydrochloride, the aglycone salt of betanin, the betacyanin from *Beta vulgaris*. It is apparent that the betacyanins are unrelated



chemically to the well-known flavylium salt structures of the anthocyanins, although the two classes of compound are visually indistinguishable, both having λ_{max} around 540 mμ. The unusual chromophoric polymethylene cyanine group is shown in two resonance structures above.

Distribution of the betacyanins is limited, so far as is known, to ten families usually grouped in the Centrospermae: Chenopodiaceae, Didieraceae, Amaranthaceae, Nyctaginaceae, Stegnospermaceae, Phytolaccaceae, Ficoidaceae, Portulacaceae, Basellaceae and Cactaceae. All of these families but the Stegnospermaceae and Dideraceae had already been known to contain members producing betacyanins. In 1961 Rauh and Reznik[4] observed betacyanins in the Didieraceae and concluded that this family belonged in the

[1] Presented, in part, by T. J. MABRY, *Proceedings of the International Conference on Taxonomic Biochemistry, Physiology and Serology*, Sept. 4–6, 1962, University of Kansas, Lawrence, Kansas. Ronald Press, New York (in press).
[2] T. J. MABRY, H. WYLER, G. SASSU, M. MERCIER, J. PARIKH and A. S. DREIDING, *Helv. Chim. Acta* **45**, 640 (1962).
[3] A. S. DREIDING, *Recent Developments in the Chemistry of Natural Phenolic Compounds*, (Edited by W. D. OLLIS), p. 194, Pergamon Press, London (1961).
[4] W. RAUH and H. REZNIK, *Bot. Jb.* **81**, 95 (1961).

Centrospermae. Such an arrangement had been suggested earlier by Radlkofer[5] on morphological grounds (see also Dalla Torre and Harms[6]). We have now found betacyanins in

TABLE 1. NEW ADDITIONS TO THE LIST[1] OF BETACYANIN SPECIES

	Voucher numbers*
Chenopodiaceae	
Atriplex barclayana	190314
Atriplex lentiformis	183946
Chenopodium ambrosioides	171885
Chenopodium berlandieria	Mabry 11
Chenopodium glaucum	169317, 169318
Coriospermum nitidum	197422
Cycloloma atriplicifolium	195354
Salicornia perennis	Mabry 12
Suaeda linearis	53586
Amaranthaceae	
Amaranthus acanthocarpa	Mabry 15
Amaranthus palmeri	Mabry 4
Amaranthus pringlei	200689
Froelichia drummondii	Mabry 13
Gomphrena decumbens	193081, 189691
Gomphrena nealleyi	179298
Gomphrena sonorae	190367
Tidestromia lanuginosa	195909
Nyctaginaceae	
Abronia ameliae	178059
Abronia cycloptera	172412
Abronia fragrans	166083
Abronia villosa	54616
Allionia incarnata	174249, 200677
Boerhaavia erecta	193034
Boerhaavia intermedia	200657
Boerhaavia spicata	200655
Cyphomeris gypsophiloides	206819
Mirabilis lindheimeri	Mabry 1
Nyctaginia capitata	Melchert 246
Stegnospermaceae	
Stegnosperma halimifolium	190384
Portulacaceae	
Claytonia linearis	135561
Claytonia megarrhiza	175433
Claytonia virginica	Mabry 16
Montia perfoliata	200438, 200579
Portulaca pilosa	Mabry 7
Spraguea umbellata	175430
Aizoaceae	
Trianthema portulacastrum	Mabry 14
Cactaceae	
Opuntia leptocaulis	Mabry 5
Opuntia lindheimeri	Mabry 6

* Except where otherwise noted, the numbers refer to accession numbers of the University of Texas Herbarium where all specimens have been deposited.

[5] L. RADLKOFER, *Die naturlichen Pflanzenfamilien*, Vol. III (5), pp. 460–462, A. ENGLER and K. PRANTL, editors. Wilhelm Engelmann, Leipzig (1896).
[6] D. G. DE DALLA TORRE and H. HARMS, *Genera Siphonogamarum*, p. 606, Sumtibus Guilelmi Engelmann, Lipsiae (1900).

Stegnosperma halimifolium. The treatment of Stegnospermaceae as a family stems from Hutchinson's[7] elevation of the subfamily Stegnospermatoidiae of the Phytolaccaceae to the rank of family. Hutchinson, however, placed this newly erected family in the order Pittosporales, a woody group, phyletically far removed from the herbaceous Centrospermae. In addition to *Stegnosperma halimifolium* we have examined thirty-seven previously uninvestigated taxa, including thirteen genera, among the Centrospermae.

The results of electrophoretic and chromatographic examination of the plants, presented in Table 1, indicate the presence of betacyanins and the absence of anthocyanins.[8] Betacyanins and anthocyanins apparently do not co-exist in the same plant or even within the same family, although other classes of flavonoid pigments may occur together with betacyanins in the same plant. Our experimental procedure utilized a crude aqueous extract of dried plant material. The pigment extracts were compared by paper electrophoresis and paper chromatography against pure betanin, and a mixture of the pigment extract with pure betanin. In most analyses the pigment migrated slower than the pure betanin standard, but the extract-betanin mixture generally gave only one broad spot which corresponded to the spot observed for the extract alone. Further studies with purified extracts are required but these preliminary results suggest that the number of betacyanins may be fewer than previously suspected. Even purified extracts should be mixed with pure betanin for chromatographic and electrophoretic studies before this type of evidence can be reliably used for identification purposes.

Betanin, the only betacyanin which has been crystallized[9,10], has glucose attached at one of the two phenolic positions[2] in the betanidin structure. Significantly, all betacyanins which have been hydrolyzed produced betanidin as the aglycone.[11] Therefore secondary modifications of the structure shown above, involving the presence of different sugars or esterification of the carboxyl groups, probably account for the different betacyanins reported.[11]

For many years the correlation between the betacyanins and the Centrospermae was recognized. Reznik,[12] for example, has discussed the systematic value of these pigments, but at a time when these substances were considered to be flavonoid in nature. Ordinarily, major taxonomic importance would not be accorded a single chemical character, but we believe that the totally different structures of the two types of pigments, betacyanins and anthocyanins, which indicate different synthetic pathways, their mutual exclusion, and the limited distribution of the betacyanins make the presence of these latter substances of particular taxonomic significance. It is suggested that the order Centrospermae (Chenopodiales), as classically constituted, and including the Cactales, be reserved for the betacyanin-containing families, and that those anthocyanin-containing families such as the Caryophyllaceae and Illecebraceae be treated as a separate phyletic group whose relationship is close but not within the betacyanin producing order. A more comprehensive analysis of the Centrospermae and the significance of the betacyanins is in progress.

EXPERIMENTAL

The dried plant material was extracted for several hours at room temperature with water. The pigment extracts were concentrated under vacuum and, in most instances,

[7] J. Hutchinson, *Families of Flowering Plants*, Vol. I, II. Clarendon Press, Oxford (1959).
[8] Anthocyanins only were observed in *Rumex*, *Eriogonum*, *Antigonon* and *Chorizanthe* of the Polygonaceae, *Paronychia* of the Illecebraceae, *Krameria* of the Krameriaceae and *Hydrocotyle* of the Umbelliferae.
[9] H. Wyler and A. S. Dreiding, *Helv. Chim. Acta* **40**, 191 (1957).
[10] O. Th. Schmidt and W. Schonleben, *Z. Naturforsch.* **12b**, 262 (1957).
[11] H. Wyler and A. S. Dreiding, *Experientia* **17**, 23 (1961).
[12] H. Reznik, *Z. Botan.* **43**, 499 (1955); *Planta* **49**, 406 (1957).

spotted directly on Whatman No. 3 paper strips. With some extracts the pigment was precipitated with aqueous lead acetate, redissolved in methanolic hydrochloric acid and filtered to remove the lead chloride. The filtrate was concentrated before use. The pigments were compared by paper electrophoresis (0·1 M formic acid)[13] and paper chromatography (0·1 M formic acid and 0·1 M pyridine formate) with pure betanin and a mixture of the pigment extract and pure betanin. Frequently, the pigment was eluted from the paper strip with water and rechromatographed. A Lab-line electrophoresis apparatus was used at 450 volts. The descending paper chromatograms were run for about 4 hr.

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[13] Betacyanins are present as anions in solutions of about pH 2 or higher and thus migrate towards the anode.