Betalamic Acid, a New Naturally Occurring Pigment

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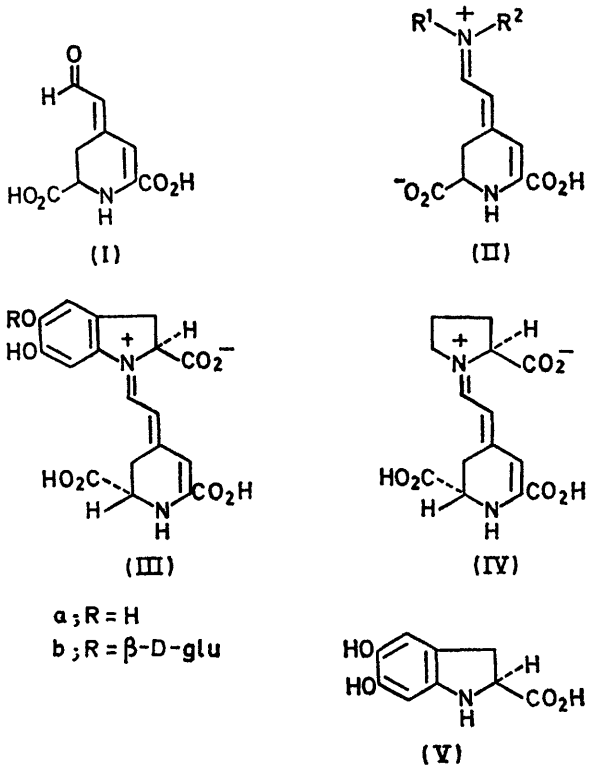
Summary Betalamic acid, the probable key intermediate in the biogenesis of all betalains, has been detected for the first time as a natural product in numerous species of plants belonging to families of the Order Centrospermae, has been obtained directly from the cleavage of the red beet pigment betanin, and has been characterized by the formation of betanidin when mixed with synthetic cyclodopa, and indicaxanthin on reaction with L-proline.

WE report the detection and characterization of betalamic acid (I) as a natural product in betalain-producing plants and as a cleavage product from betanin (IIIb), the red beet (*Beta vulgaris*) pigment. The elusive betalamic acid has long been suggested[1] to be the key biogenetic precursor to the dihydropyridine moiety present in all betalains [general formula (II)]: the red-violet [*e.g.* betanidin (IIIa) and betanin (IIIb)] and yellow [*e.g.* indicaxanthin (IV)] pigments which are restricted[2] to species belonging to the Order Centrospermae. Although it has been established[3] that the dihydropyridine moiety of betanin (IIIb) is derived by an extra-diol oxidative cleavage of dopa (5,6-dihydroxyphenylalanine), until the present investigation dopa-cleaved intermediates between dopa and betanin had not been detected.

We first obtained betalamic acid by separating the products from the alkaline treatment of betanin (IIIb) (deoxygenated NH_4OH, pH 10·5; 16 h); the separation, which was effected by passing the reaction mixture over an ECTEOLA cellulose anion-exchange column, gave an aqueous yellow solution (pH 9, λ_{max} 430 nm) of betalamic acid; positive 2,4-dinitrophenylhydrazine and Fuchsin aldehyde tests. The isolated yield, based on an estimated ϵ of 30,000, was 25%. Although stable in a pH 9 solution, betalamic acid is sensitive to air, acids, and strong alkali. The preparation contained a single substance by t.l.c. (ECTEOLA, 5 different pH's) and electrophoresis (E_B = 2·19).†

The betalamic acid preparation was further characterized by treating it directly or after purification by electrophoresis with synthetic cyclodopa (V)[4] to give in a few minutes (at pH 2) a red-violet pigment identical by electrophoresis and u.v. with authentic betanidin (IIIa). Similarly, betalamic acid readily reacted with L-proline giving in high yield a yellow pigment which was identical by u.v. and electrophoresis with authentic indicaxanthin.



We are aware that the availability of betalamic acid suggests the synthesis of new colouring matters; for example, when the betalamic acid preparation was mixed with aniline, a brilliant, salmon-pink pigment was produced (λ_{max} 506 nm) presumably having structure (II; R^1 = Ph, R^2 = H).

† E_B = the electrophoretic mobility of betalamic acid in a pyridine–acetic acid buffer (pH = 6·5) relative to betanin having E_B value of 1, other relevant compounds exhibited the following E_B values: betanidin, E_B = 1·02 and indicaxanthin, E_B = 1·68.

Finally, betalamic acid has been detected as a natural constituent of numerous species (for example, red and yellow *Beta vulgaris*, *Celosia cristata* var. Fireglow, and *Portulaca grandiflora*) by the electrophoretic analysis at pH 7·8 of fresh plant extracts. All the species belong to the betalain-producing families of the Order Centrospermae; betanin and related pigments are not converted into betalamic acid for several hours under these conditions.

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[1] T. J. Mabry, "The Betacyanins, A New Class of Red-violet Pigments, and Their Phylogenetic Significance", in "Taxonomic Biochemistry and Serology", ed. C. A. Leone, Ronald Press, New York, 1964, pp. 239–254; H. Wyler, M. E. Wilcox and A. S. Dreiding, *Helv. Chim. Acta*, 1965, **48**, 361.

[2] T. J. Mabry and J. A. Mears, "Alkaloids and Plant Systematics" in "Chemistry of the Alkaloids", ed. S. W. Pelletier, Van Nostrand Reinhold, New York, 1970, pp. 719—746.

[3] H. E. Miller, H. Rösler, A. Wolhpart, H. Wyler, M. E. Wilcox, H. Frohfer, T. J. Mabry, and A. S. Dreiding, *Helv. Chim. Acta*, 1968, **51**, 1470; N. H. Fischer and A. S. Dreiding, paper presented at the Southeast-Southwest Combined Regional Meeting, American Chemical Society, New Orleans, Dec. 2—4, 1970. Abstracts, p. 120.

[4] We thank Dr. Hugo Wyler, Univ. Lausanne, Switzerland for a synthetic sample of *NOO*-triacetylcyclodopa methyl ester; *cf.* H. Wyler and J. Chiovini, *Helv. Chim. Acta*, 1968, **51**, 1476.