the growing concept that the reactivity of peroxidase with lignins, at least in cell-free systems, results at best in an internal rearrangement of the polymeric substrate (*cf.* refs [5, 8, 12, 13]), and not in depolymerization.

EXPERIMENTAL

Horseradish peroxidase (type VI) was purchased from Sigma Chemical Co., and acetovanillone and acetosyringone from Aldrich Chemical Co. Alphamethylguaiacyl alcohol and α-methylsyringyl alcohol were gifts from Dr. Carlton Dence, meadol MRM and MWS hardwood soda lignins from Dr. Conrad Schuerch, sitka spruce cellulase and aspen cellulase lignins from Dr. T. Kent Kirk, marasperse hardwood lignosulfonate lignin from American Can Co., and reax 31 and indulin AT Kraft pine lignins from Westvaco Co. The benzoylated dialysis membranes were prepared according to Pearse [10]. The kinetic measurements as a function of pH were made with a spectrophotometer in cells of 1 cm light path at 25°. The double reciprocal plots were based on measurements made in cells of 10 cm light path at room temp. All *A* changes were recorded on a 25 cm scale representing 0.1 *A* units.

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AN ACYLATED DELPHINIDIN 3-RUTINOSIDE-5,3′,5′-TRIGLUCOSIDE FROM *LOBELIA ERINUS*

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Key Word Index—*Lobelia erinus*; Campanulaceae; acylated anthocyanin; caffeylferulyl-*p*-coumaryl delphinidin 3-rutinoside-5,3′,5′-triglucoside.

A blue pigment was isolated from blue petals of garden *Lobelia*. The absorption peaks of this pigment in H_2O were at 316, 530, 568 and 612 nm, and the R_fs were 0.80, 0.90 and 0.92 in 50% EtOH, 2PW and H_2O, respectively. The absorption peaks in MeOH–HCl (0.01%) were at 302, 320 and 544 nm; the values of $E_{440}/E_{vis\ max}$ and $E_{acid\ max}/E_{vis\ max}$ being 19% and 142%, respectively. No bathochromic shift of the visible max. occurred upon addition of $AlCl_3$ (5% in EtOH), indicating the absence of an *o*-dihydroxyl grouping. The R_fs of the chloride were 0.24, 0.15, 0.60 and 0.30 in BAW, BuN, AAH and 1% HCl, respectively. Upon acid hydrolysis, the pigment gave delphinidin, glucose, rhamnose, and *p*-coumaric, ferulic and caffeic acids, the molar ratio of delphinidin, glucose and rhamnose being 1:4:1 respectively.

The deacylated anthocyanin (**1**) had an orange red colour and fluoresced light orange in UV light on the chromatogram. The R_fs were 0.05, 0.00, 0.77 and 0.71 in BAW, BuN, AAH and 1% HCl, respectively. That **1** is pentaglycoside follows from its higher R_f values in aqueous solvents and lower in alcoholic solvents than those of related di- and tri-glycosides of delphinidin. **1** showed λ_{max} at 522, 275nm in 0.01% MeOH–HCl, and the values of $E_{440}/E_{vis\ max}$ and $E_{UV\ max}/E_{vis\ max}$ were 17% and 60%, respectively. The 3′- and/or 5′-hydroxyls must be glycosylated, because the visible max. was at a lower wavelength than that of delphinidin glycosides having sugars at the 3- and/or 5-positions [1]. **1** furnished rutinose upon H_2O_2 degradation. Upon partial hydrolysis, **1** gave delphinidin and the 3-rutinoside-5,3′-glucoside, 3-rutinoside-5-glucoside, 3,5,3′-triglucoside, 3,5-diglucoside and 3-monoglucoside of delphinidin.

The absence of substitution at the 7- and 4′-positions

was confirmed by alkaline degradation of the methylated glycoside, which gave phoroglucinol monomethyl ether from the A-ring and 3,5-dihydroxy-4-methoxybenzoic acid from the B-ring [2].

Thus the deacylated product of the *Lobelia* anthocyanin is the 3-rutinoside -5,3',5'-triglucoside of delphinidin. This is the first report in Nature of an anthocyanidin pentaglycoside.

Although the amount of organic acids was not determined precisely, there appear to be four molecules since the $E_{\text{acid max}}/E_{\text{vis max}}$ ratio was 142%; the molar ratio of caffeic, ferulic and *p*-coumalic acid seems to be 2:1:1, respectively, from the absorption peaks of acid in UV region [3, 4]. The acylated pigment exhibits a stable blue colour in neutral solution even as the chloride, and stabilization may be due to an intermolecular interaction between the anthocyanin moiety and attached organic acids, as in the case of blue flowers of cineraria [1, 5] and bellflower [6].

EXPERIMENTAL

Isolation and purification of the Lobelia *anthocyanin.* A blue pigment was extracted from fresh blue petals (375 g) with a mixture of Me_2CO–EtOH–H_2O (4:1:4), and purified by the same procedure as described for cineraria [5]. The original pigment was obtained in the form of blue homogenous granules (97 mg).

Delphinidin 3,5,3'-triglucoside. The glucoside (**2**) was obtained by partial hydrolysis of **1**, and purified by PC. The absorption peaks of **2** in MeOH–HCl (0.01%) were at 270, 530 nm, and the value of $E_{440}/E_{\text{vis max}}$ and $E_{\text{UV max}}/E_{\text{vis max}}$ were 18% and 90%, respectively. It is presumed that the glycosylation of **2** lies at the 3' (or 5')-OH, because of the position of the visible max. and the high value of $E_{440}/E_{\text{vis max}}$ when compared with these values for delphinidin glycosides, substituted with sugar at the 3-, 3,5- and/or 7-hydroxyls [1]. The positive shift on addition of $AlCl_3$ indicated the presence of an *o*-dihydroxyl grouping. On partial hydrolysis, **2** gave the following intermediate products: delphinidin 3,5-diglucoside, delphinidin 3-glucoside, and delphinidin. Accordingly, **2** is a delphinidin 3,5,3'-triglucoside.

Delphinidin 3-rutinoside-5,3'-glucoside. Another glycoside (**3**) was also obtained by partial hydrolysis of **1**. **3** showed the $\lambda_{\max}$ at 270 and 531 nm in 0.01% MeOH–HCl, the value of $E_{440}/E_{\text{vis max}}$ and $E_{\text{UV max}}/E_{\text{vis max}}$ being 16% and 110%, respectively. A bathochromic shift occurred upon addition of $AlCl_3$, indicating the presence of an *o*-dihydroxyl grouping. The position of the visible max. indicated that **3** is glycosylated at the 3' (or 5')-hydroxyl [1]. On partial hydrolysis, **3** gave delphinidin 3-rutinoside-5-glucoside, 3,5,3'-triglucoside, 3,5-diglucoside, 3-glucoside and delphinidin. Consequently, the anthocyanin must be delphinidin 3-rutinoside-3',5-glucoside.

Chromatography. Avicel plates and Tôyô No. 51 filter paper were used for chromatography. The solvent mixtures were: BAW (*n*-BuOH–HOAc–H_2O, 4:1:5), AAH (HOAc–HCl–H_2O, 15:3:82), BuN (*n*-BuOH–2N HCl, 1:1) and 2PW (*n*-PrOH–H_2O, 22:78).

Quantitative analysis of delphinidin, glucose and rhamnose. *Lobelia*-anthocyanin was dissolved in 6N HCl and the soln boiled for 3 min. The components, delphinidin and sugars, were partitioned as usual into *iso*-amyl alcohol and H_2O, respectively. Delphinidin was determined spectrophotometrically using $\lambda_{\max}$ 555 nm [5]. The aq. soln was evapd to dryness *in vacuo*; the residue was dissolved in a small amount of H_2O, and the soln was used for chromatographic measurement. The ratio of sugar components was determined by the method of ref. [7] with slight modification.

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