3300 (H-bonded hydroxyl), 718, 728 cm^{-1} (methylene chain) (Found C, 79·28; H, 13·26. Calc. for $C_{28}H_{56}O_2$: C, 79·24; H, 13·20%). The acid on esterification with CH_2N_2 yielded its methyl ester; IR 1740 (ester carbonyl), 719, 729 cm^{-1} (methylene chain) (Found: C, 79·48; H, 13·26. Calc. for $C_{29}H_{58}O_2$: C, 79·45; H, 13·24%).

Fraction 2 (4·6 g) was separated into fractions 2a and 2b by rechromatography over $AgNO_3$ impregnated silica gel. Fraction 2a on crystallization from $CHCl_3$–MeOH yielded β-amyrin acetate in needles (700 mg), m.p. and m.m.p 234–236°; $[\alpha]_D^{27}$ +79° ($CHCl_3$). Fraction 2b on crystallization from $CHCl_3$–MeOH afforded lupeol acetate (2·8 g), m.p. and m.m.p. 216–218°; $[\alpha]_D^{27}$ +42° ($CHCl_3$).

Fraction 3 (2·7 g) on rechromatography on $AgNO_3$ impregnated silica gel yielded α-amyrin and lupeol (characterized by m.p., m.m.p., co-TLC, IR). *Fraction* 4 on rechromatography over alumina yielded β-amyrin and sitosterol (characterized by m.p., m.m.p., co-TLC, IR). *Fraction* 5 on further purification by chromatography over alumina afforded more sitosterol (characterized by m.p., m.m.p., co-TLC, IR).

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BERBERIDACEAE

ANTHOCYANINS IN FRUITS OF *BERBERIS BUXIFOLIA*

A. B. POMILIO

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, Buenos Aires, Argentina

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Key Word Index—*Berberis buxifolia*; Berberidaceae; petunidin; peonidin; malvidin and delphinidin plycosides.

The genus *Berberis* (Berberidaceae) has not been fully investigated although nearly 150 species are distributed all over the world. Recently Mamaev and Semkina[1,2] identified the anthocyanins of *Berberis thunbergii* and *B. vulgaris* (common barberry) and observed a maximum pigment concentration in spring. The leaves of the purple-leaf and green-leaf forms of barberry contained five anthocyanin pigments, the main ones being 3-monoglycosides of peonidin, cyanidin and delphinidin. No reports on isolation of anthocyanins from *Berberis* fruits are known.

The present work describes the identification of ten anthocyanins isolated from *Berberis buxifolia* Lam. fruits, which is indigenous to Argentina and south of Chile. Chromatography of the crude extract yielded six coloured bands, in amounts decreasing in the order IV > III ⩾ II > I > V > VI, the latter band being feint. Each band was rechromatographed in 15% HOAc to give complete purification. R_fs are shown in Table 1. Only pigments IIIa, IIIb, IVb, IVc, Va and Vb changed to blue with 1% ethanolic $Pb(OAc)_2$.[3]

The visible and UV spectra of pigments IVa and Va were characteristic of 3,5-diglycosides and only differed by substitution in the B-ring.[4] The other spectra corresponded to 3-glyco-

[1] S. A. MAMAEV and L. A. SEMKINA, *Rast. Resvr.* 7, 280 (1971); *Chem. Abs.* 75, 95377p (1971).
[2] L. A. SEMKINA, *Ekologika* 45 (1971); *Chem. Abs.* 75, 85143v (1971).
[3] T. FULEKI and F. J. FRANCIS, *Phytochem.* 6, 1161 (1967).
[4] J. B. HARBORNE, *Biochem. J.* 70, 22 (1958).

sides and all lacked the distinguishing features of 3,7-diglycosides or acylation.[5] Some pigments (IIIa, IIIb, IVb, IVc, Va and Vb) gave a positive wavelength shift on the addition of $AlCl_3$ (Table 1).

TABLE 1. ANTHOCYANINS FROM *Berberis buxifolia*

Pigments	Absorption spectra* λ_{max} (nm)	$\Delta\lambda$† $AlCl_3$ (nm)	Acid hydrolysis Aglycone	Sugar	Oxidation products	BAW	R_f (×100)‡ Bu-HCl	1% HCl	HOAc-HCl
(I)	279; 528	0	Pn	Glu	Glu	39	31	7	32
(IIa)	273; 535	0	Mv	Glu Rham	Rut	34	18	17	47
(IIb)	278; 532	0	Mv	Glu	Glu	36	17	5	29
(IIIa)	272; 535	30	Pt	Glu Rham	Rut	33	16	13	41
(IIIb)	274; 535	45	Pt	Glu	Glu	34	15	4	22
(IVa)	273; 526	0	Pn	Glu Rham	Rut	28	10	32	56
(IVb)	272; 535	34	Dp	Glu Rham	Rut	27	15	10	35
(IVc)	272; 536	36	Dp	Glu	Glu	27	13	2	18
(Va)	271; 534	25	Pt	Glu Rham	Rut	20	10	36	63
(Vb)	277; 534	32	Pt	Glu	Gent	22	9	14	41

* In MeOH containing 0·01% conc. HCl.

† Three drops of a solution of $AlCl_3$ in EtOH (5% w/v) added to 2·5 ml solution.

‡ On Whatman No. 1 paper. *Abbreviations:* BAW (*n*-BuOH–HOAc–H_2O; 4:1:5); Bu–HCl (*n*-BuOH–2N HCl; 1:1); 1% HCl (conc.HCl–H_2O; 3:97); HOAc–HCl (HOAc–conc.HCl–H_2O; 15:3:82). Pn = peonidin; Mv = malvidin; Pt = petunidin; Dp = delphinidin; Glu: glucose; Rham = rhamnose; Rut: rutinose; Gent: gentiobiose.

On complete acid hydrolysis the pigments gave peonidin, malvidin, petunidin and delphinidin, which were identified by conventional chromatographic and spectral methods.[6] The sugar moieties were determined by PC in EtOAc–pyridine–H_2O (10:4:3) and BuOH–pyridine–H_2O (9:5:8). Pigments I, IIb, IIIb, IVc and Vb yielded only glucose and the others contained both glucose and rhamnose. On H_2O_2 degradative oxidation[7] pigments IIa, IIIa, IVa, IVb and Va gave rutinose, Vb provided gentiobiose, and the rest of the anthocyanins gave glucose.

According to the data, the fruit pigments were identified as follows: (I), peonidin-3-glucoside; (IIa), malvidin-3-rutinoside; (IIb), malvidin-3-glucoside; (IIIa), petunidin-3-rutinoside; (IIIb), petunidin-3-glucoside; (IVa), peonidin-3-rutinoside-5-glucoside; (IVb), delphinidin-3-rutinoside; (IVc), delphinidin-3-glucoside; (Va), petunidin-3-rutinoside-5-glucoside; and, (Vb), petunidin-3-gentiobioside.

It should be noted that band V decomposed upon prolonged storage or upon concentration to give reaction products of unknown nature. During PC in 15% HOAc it was also observed that the anthocyanins obtained from this band, which behaved initially as single components, were accompanied by faint additional bands, which were disregarded. However, when HCl was added to band V eluates, the minor bands decreased in intensity and at higher acid concentration were entirely absent. Albach *et al.*[8] suggested that the additional bands might be due to the existence of different pH regions or gradients on freshly developed

[5] J. B. HARBORNE, *Phytochem.* **3**, 151 (1964).
[6] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, London (1967).
[7] B. V. CHANDLER and K. A. HARPER, *Austral. J. Chem.* **14**, 586 (1961).
[8] R. F. ALBACH, R. E. KEPNER and A. D. WEBB, *J. Food Sci.* **30**, 69 (1965).

chromatograms, whereas Timberlake *et al.*[9] supposed they were produced by the combined action of acetic and hydrochloric acids (e.g. 1% conc. HCl in HOAc) during concentration of components eluted from the paper. Although these new bands have not been fully identified they behave as if they contain sugars acylated with one or more acetate groups.[9]

EXPERIMENTAL

Plant material. Ripe fruits were harvested in the region of Lago Argentino (Calafate, Santa Cruz Province, Argentina) during February.

Analysis of anthocyanins. Fresh fruits were macerated several times with 0·1% HCl–MeOH, at 0° in the darkness under N_2. The concentrated combined extracts were streaked on Whatman No. 3MM paper and irrigated with BAW allowing to run off the paper for nearly 40 hr. Purification was carried out with 15% HOAc. Preliminary tests for acylation were negative. H_2O_2 oxidation and hydrolysis products were identified by the methods earlier described.

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[9] C. F. TIMBERLAKE, P. BRIDLE and S. S. TANCHEV, *Phytochem.* **10,** 165 (1971).

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BIGNONIACEAE

HYDROQUINONE FROM THE LEAVES OF *JACARANDA MIMOSAEFOLIA*

S. SANKARA SUBRAMANIAN, S. NAGARAJAN and N. SULOCHANA

Department of Chemistry, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry-6, India

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Plant. Jacaranda mimosaefolia D. Don. (Syn. *J. ovalifolia* R. Br.) (voucher specimen No. 6/72 deposited at JIPMER). *Source.* Annamalai University Campus, South India. *Uses.* Medicinal.[1] *Previous work.* Wood,[2] leaves (flavonoid).[3]

Present work. Fresh leaves extracted with hot 80% alcohol and the aq. concentrate fractionated into petrol (40–60°), Et_2O and EtOAc. *Petrol fraction.* A triterpenoid, yield, 0·01%, m.p. 257–259° (Me_2CO–MeOH). *Ether fraction.* Hydroquinone, yield, 0·1%, colourless prismatic needles, m.p. 171–172° (MeOH), λ_{max} (EtOH) 225, 294 nm, no shift with $AlCl_3$ or NaOAc. IR (KBr) bands at 755, 828, 1092, 1195, 1250, 1360, 1460, 1510, 3150 cm^{-1}. NMR: 4 aromatic protons (*s*, 7·2 ppm), the acetate 6 acetyl protons (*s*, 2·3 ppm). MS: parent peak at *m/e* 110 (M^+) and fragmentation at *m/e* 108 (M^+-2H) and 81

[1] *Wealth of India, Raw Materials*, Vol. V, p. 277, C.S.I.R., New Delhi (1959).
[2] J. M. WATT and M. G. BREYER-BRANDWIJK, *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd Edn, p. 142, Livingstone, London (1962).
[3] S. S. SUBRAMANIAN, S. NAGARAJAN and N. SULOCHANA, *Phytochem.* **11,** 1499 (1972).