oleanolic acid is an artefact produced during hydrolysis of swartziasaponins with Kiliani's reagent and work is in progress to settle this point.

The flavone, $C_{15}H_{10}O_6$, m.p. 276–279° ($M^+ = 286$), ν_{max} 3330, 3100, 1650 and 1605, had strong ions at $m/e = 258$, 152 and 134 in its mass spectrum, which were formed by elision of carbon monoxide and retro-Diels-Alder fragmentation of the molecular ion. The spectroscopic data and the m.p. suggested that the compound was kaempferol, and this was confirmed by preparation of the tetramethyl ether and the tetra-acetate whose physical constants and spectroscopic data were in agreement with recorded values.

EXPERIMENTAL

Isolation of oleanolic acid and kaempferol from seed pods. Ground seed pods of *Swartzia madagascariensis* (940 g) were exhaustively extracted with ether, acetone, and methanol. Evaporation of the extracts gave 13, 53 and 315 g of solid respectively. Hydrolysis of a portion of the methanol extract (87 g) in aqueous ethanol (47%; 1 l.) with 10 N HCl (250 ml), afforded a mixture of aglycones which were chromatographed on kieselgel. Elution with petrol–ether (2:3) gave oleanic acid, $C_{30}H_{48}O_3$, (1·7 g; m.p., IR, MS, TLC; methyl ester, $C_{31}H_{50}O_3$, m.p., IR, MS, TLC), and ether furnished kaempferol, $C_{15}H_{10}O_6$ (1·6 g; m.p., IR, MS; trimethyl ether, m.p., IR, MS, NMR; tetraacetate, m.p., IR, MS).

Separation of swartziagenin by PLC. Swartziagenin (334 mg) was chromatographed on 3 × 20 × 40 cm plates coated with 0·75 mm layers of kieselgel using $CHCl_3$–MeOH (9:1) as eluant. Extraction of the band R_f *ca.* 0·75 with $CHCl_3$ gave oleanolic acid (232 mg; m.p., IR, MS, TLC), and the band R_f *ca.* 0·95 with $CHCl_3$ afforded *O*-acetyloleanolic acid, $C_{32}H_{50}O_4$ (60 mg; IR, MS, TLC).

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ROSACEAE

ANTHOCYANINS IN PETALS OF *CHAENOMELES SPECIOSA*

C. F. TIMBERLAKE and P. BRIDLE
Research Station, Long Ashton, Bristol

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Abstract—*Chaenomeles speciosa* petals are pigmented with previously unrecorded diglycosides of pelargonidin and cyanidin containing glucose and galactose, together with cyanidin 3-galactoside and 3-glucoside, pelargonidin 3-galactoside and traces of pelargonidin 3-glucoside.

INTRODUCTION

ALTHOUGH anthocyanins of the Rosaceae have been identified,[1] those of the genus *Chaenomeles* (sub-family Pomoideae) have been studied only once, by Hayashi,[2] who found that petals of *Chaenomeles lagenaria* (= *Chaenomeles speciosa*) contained a cyanidin 3-diglucoside.

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[1] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, pp. 130, 154, 236, Academic Press, London (1967).

[2] K. HAYASHI, *Acta Phytochim.* **14**, 47 (1944).

This work was carried out before the advent of paper chromatography. Using this technique we have re-investigated the pigment composition of petals of this common shrub which may vary in colour from orange-red to pink-red.

RESULTS AND DISCUSSION

In addition to cyanidin 3-galactoside and 3-glucoside, pelargonidin 3-galactoside and traces of pelargonidin 3-glucoside, petals of *Chaenomeles speciosa* were found to contain previously unrecorded diglycosides of cyanidin and pelargonidin containing glucose and galactose. The anthocyanin diglycosides possessed R_f values identical to those of pelargonidin and cyanidin 3-sophorosides in aqueous-acid solvents (HOAc, HOAc–HCl, HCl) and were inseparable from them on co-chromatography. However, in butanol-based solvents (BAW, BuHCl, BuHClW, BBWF) their R_f values were slightly lower than those of their corresponding 3-sophorosides and separation occurred during co-chromatography after prolonged solvent development. Their spectra were characteristic of 3-glycosides non-acylated with phenolic acids and without substituents in the 5-position. Moreover, they lacked the distinguishing features of 3,7-diglycosides.[3,4] A positive wavelength shift on the addition of aluminium chloride to the cyanidin derivative suggested that its 3′ and 4′ positions were free. Controlled acid hydrolysis of each extract gave only one intermediate anthocyanin band. The anthocyanins were probably 3-biosides therefore, but cannot be unequivocally designated as such, since we have been unable, as yet, to isolate the free disaccharides, presumably because the inter-sugar link is unusually labile. After hydrolysis with reagents usually employed for this purpose (H_2O_2, 10% HOAc),[5] only galactose and glucose could be identified with certainty. A further complication was that the monoside fraction obtained by partial hydrolysis of each diglycoside contained glucose and galactose. Although they behaved chromatographically as single entities, each isolated diglycoside fraction may have contained more than one bioside of differing structures.

Cyanidin 3-galactoside has been found previously in the fruit skin of two Pomoideae viz *Malus* (apple) and *Pyrus* (pear), but is absent from the other sub-families of Rosaceae.[1]

EXPERIMENTAL

Chromatography. The following solvents were used:—BAW, *n*-BuOH–HOAc–H_2O (4:1:5, v/v); BuHCl, *n*-BuOH–2 N HCl (1:1, v/v); BuHClW, *n*-BuOH–conc. HCl–H_2O (7 2·5, v/v), BBFW, *n*-BuOH–benzene–HCO_2H–H_2O (100·19:10·25, v/v); HOAc, H_2O–HOAc (98 2, v/v), HOAc-HCl, HOAc–conc HCl–H_2O (15:3:82, v/v), HCl, H_2O–conc. HCl (97:3, v/v).

Purification and analysis of anthocyanins A concentrated MeOH–1% HCl extract of petals was chromatographed on Whatman No 3 paper in BAW with good separation of four bands, numbered I to IV from the origin. Each band was further purified by chromatography in HOAc–HCl, followed by BAW, intermediate elution being made with MAW (methanol–acetic acid–water, 94.3·3, v/v) by cutting each band into small pieces and soaking several times with the solvent, followed by concentration of the combined extracts. In the final solvent (BAW) each band was accompanied by a small amount of an additional component of higher R_f Aglycones and sugars were identified by the usual chromatographic methods after total acid hydrolysis[6]—I and III contained cyanidin, II and IV contained pelargonidin, I, II and III contained both glucose and galactose; IV contained largely galactose with only traces of glucose Controlled hydrolysis of I gave in addition to cyanidin an intermediate corresponding to III, II likewise gave in addition to pelargonidin an intermediate corresponding to IV, III and IV were hydrolysed directly to cyanidin and pelargonidin respectively. I and II were therefore probably 3-biosides and III and IV were mixtures of 3-galactosides and 3-glucosides of similar R_f. Since band IV contained largely galactose with very little glucose, we fully expected the intermediate hydrolysis product of II to be of similar composition The fact that an appreciable

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[3] J B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, pp 17, 20, Academic Press, London (1967).

[4] S. S. TANCHEV and C F. TIMBERLAKE, *Phytochem.* **8**, 2367 (1969)

[5] B. V. CHANDLER and K A. HARPER, *Australian J Chem* **14**, 586 (1961).

[6] J. B. HARBORNE, *Biochem. J* **74**, 262 (1960).

amount of pelargonidin 3-glucoside was formed in addition to pelargonidin 3-galactoside on hydrolysis of II, suggested that very little of the latter was hydrolysed during extraction and purification.

Hydrolysis of I and II with 10% HOAc or H_2O_2[5] (including a mild treatment for only 2 hr without the final ammonia addition) gave galactose and glucose. Since I and II appeared to contain biosides which were unstable when subjected to these treatments, tests were made with α- and β-linked biosides containing glucose and galactose. Lactose (β) solabiose (β), lycobiose (β) and melibiose (α) were unaffected by H_2O_2 omitting the final treatment with NH_4OH, but some breakdown to glucose and galactose was observed when this treatment was given.

The minor components observed during final solvent development in BAW were artifacts[7] produced by the combined action of HCl (carried over on the paper from the preceding development in HOAc–HCl) and acetic acid (from the eluting solvent MAW). They contained identical aglycone and sugar components to their corresponding major anthocyanins and were reconverted to them by cold HCl. Thus, although the artifact of I formed a single spot (R_f 0·40) in BAW without HCl in the applied solution, a small amount of I (R_f 0·25) separated when HCl (1%) was present in the applied solution. With increase in acid concentration (up to 10%) the amount of I increased; at the same time the R_f value of the artifact became less and approached I.

Six bushes were sampled including an authenticated bush from Bristol University Botany Department. Those with large red-pink petals contained more of the cyanidin than the pelargonidin glycosides; the smaller orange-pink petals contained relatively more of the pelargonidin derivates. Authentic samples of pelargonidin and cyanidin 3-sophorosides for comparison purposes were obtained from *Papaver rhoeas* petals.

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

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MONOCOTYLEDONAE

ORCHIDACEAE

NONSAPONIFIABLE CONSTITUENTS OF ORCHIDS: *ARUNDINA* AND *CATTLEYA*

A. S. C. WAN, R. T. AEXEL and H. J. NICHOLAS

School of Pharmacy, University of Singapore, Singapore; Institute of Medical Education and Research, St. Louis, Missouri, and Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri 63104, U.S.A.

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Abstract—The nonsaponifiable fraction of flowers and stems of the terrestrial orchid *Arundina* and leaves and stems of mixed *Cattleya* species were examined for nonsaponifiable constituents. Common to both, in the organs examined, were campesterol, stigmasterol, and β-sitosterol. In *Arundina* these sterols occurred largely as the mixed glycosides. A more detailed study of *Cattleya*, mainly the leaves, indicated a series of saturated long chain alcohols as the major constituents, and cycloeucalenol, cycloartenol, and 24-methylene cycloartanol as minor triterpenoid constituents.

INTRODUCTION

THE NONSAPONIFIABLE constituents of Orchidaceae have not, to the best of our knowledge, been subjected to chemical investigation. Consequently we undertook an investigation of such constituents of two genera of this large family: *Arundina* and *Cattleya*. According to