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MALONIC ACID: THE ACYL MOIETY OF THE *MIMULUS LUTEUS* ANTHOCYANIN

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Key Word Index—*Mimulus luteus* complex, Scrophulariaceae, acylated anthocyanin, malonic acid, aliphatic acylation

Abstract—Malonic acid has been identified as the acylating agent of the anthocyanin produced by the *Mimulus luteus* complex

INTRODUCTION

Acylation of anthocyanins is commonly by aromatic phenolic acids.[1] Aliphatic acylation is apparently rare Its occurrence is suggested in *Sorghum*,[2] in *Vitis* acetylation has been reported [3] Acyl groups are invariably linked to the pigment via the sugar attached to the 3-hydroxyl group[1] and spectral tests[4] indicate that this is true for the *Mimulus* anthocyanin

Previous work on the *M. luteus* complex established the sole anthocyanin as an acylated cyanidin 3-glucoside and suggested the possibility of a non-cinnamic acid substituent [5] This was based on the pigment's chromatographic behavior: freshly extracted, it gave a single band in HOAc–HCl–H_2O (15 3 82) on paper of R_f 0 34. Rechromatography in the same solvent yielded two additional bands with R_fs of 0·27 and 0 46 When stored in water, rather than methanol, the R_f 0 27 band did not materialize Alkaline hydrolysis of any of the three bands yields only the slowest (R_f 0 27) band, which was identified by standard techniques[4] as cyanidin 3-glucoside UV spectra of the three bands are identical,[5,6] rendering aromatic acylation unlikely. IR spectroscopy did not reveal an ester carbonyl peak,[6] although such peaks have been demonstrated for other complex anthocyanins.[3,7] IR spectroscopy, therefore, cannot be depended upon to confirm anthocyanin acylation.

RESULTS AND DISCUSSION

An ether extract of the alkali-hydrolysed *Mimulus* anthocyanin (purified by PC) was concentrated and examined by TLC in *n*-BuOH–EtOH–HCOOH–H_2O (5 1.1 1) along with malonic, citric, tartaric and ascorbic acids The *Mimulus* compound had the same R_f (0 88) as malonic acid, and these two were the only compounds to give bright blue spots with $K_3Fe(CN)_6$–$Fe(NH_4)$ $(SO_4)_2$ [8] Their R_fs by TLC in four other solvent systems were

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identical The *p*-nitrobenzyl ester of each of these two acids had identical R_fs in six solvent systems by TLC Based on these results (Tables 1 and 2), it is concluded that the *Mimulus* anthocyanin is acylated with malonic acid

TABLE 1 TLC OF MALONIC ACID AND *Mimulus* ACID

Adsorbant	Solvent	R_f (×100) Malonic acid	R_f (×100) *Mimulus* acid
Cellulose	EtOH–H_2O–NH_3, 100 12 16	2	2
Cellulose	Py–NH_3–H_2O, 6 2 1	19	19
Silica gel	C_6H_6–MeOH–AcOH, 45 8 4	21	17
Silica gel	IsoBuOH5 M formic acid, 2 3 (upper phase)	56	54

TABLE 2 SILICA GEL TLC OF *p*-NITROBENZYL ESTERS OF MALONIC AND *Mimulus* ACIDS

Solvent	R_f (×100) Malonic ester	R_f (×100) *Mimulus* ester
Benzene	58	55
Petrol–ethyl acetate, 32 1	30	30
Chloroform	70	70
Petrol–isopropyl ether, 10 1	38	38
Chloroform–petrol, 4 1	78	78
Benzene–petrol, 1 1	32	32

EXPERIMENTAL

Plant material Only *Mimulus cupreus* Dombrain flowers from seed collected in S America were used in the identification of the acyl moiety, and were obtained from plants raised in the UCLA greenhouse

Anthocyanin extraction Approx 30–40 flowers were extracted with 200 ml cold (< 0°) 0 01 N methanolic HCl at room temp for 1 hr The extract was filtered, washed with light petrol (b p 70–90°), and concentrated to dryness at 25° The pigment, in MeOH, was separated by PC as before [5]

Alkaline hydrolysis The 5 ml of extract was treated with 5 ml 10% KOH under N_2 for 20 min at room temp and the mixture was acidified with conc HCl The product was extracted with Et_2O (Soxhlet) for 48 hr

TLC Pre-coated plates (Merck) of cellulose F and silica gel F_{254} were used Visualization of the acids was accomplished by spraying with brom cresol green Localization of the esters was by fluorescence quenching The cellulose plates were pre-washed in 0 2 N KOH and 1 0 N HCl for the $K_3Fe(CN)_6$–$FeNH_4(SO_4)_2$ spray system [8]

Derivatives The Et_2O extracts from *ca* 100 flowers were pooled, the product dissolved in 50% MeOH and concentrated down to *ca* 0 1 ml 1 ml H_2O, 2 mg of *p*-nitrobenzyl chloride, and *ca* 0 1 ml of MeOH were added and the mixture refluxed for 3 hr [9] The solution was evaporated to dryness and the product taken up in EtOAc

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