Tetrahedron Letters,Vol.30,No.48,pp 6729-6732,1989
Printed in Great Britain

0040-4039/89 $3.00 + .00
Pergamon Press plc

STRUCTURE OF ANTHOCYANINS IN SCARLET, PURPLE, AND BLUE FLOWERS OF *SALVIA*

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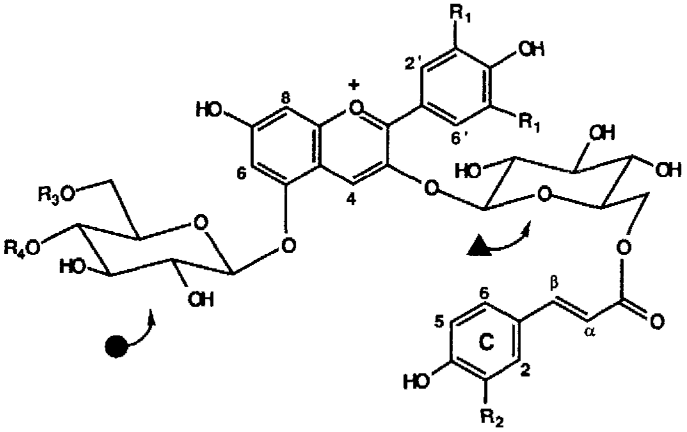
Scarlet flowers of *Salvia splendens* contain salvianin (major), monardaein (minor) and their demalonyl derivatives, whereas salviadelphin and its demalonyl derivatives as well as dimalonylawobanin are present in purple flowers of *S. splendens*. In blue flowers of *Salvia farinacea* is found salviamalvin as well as malonylawobanin; mostly the latter being found in stems. Structures of these anthocyanins have been completely determined.

In 1917 Willstätter and Bolton1) isolated from *Salvia splendens* an anthocyanin, salvianin, which was reported to contain pelargonidin diglucoside with 25% malonic acid. Later Karrer and Widmer2) isolated an anthocyanin, monardaein, from *Monarda dydima*, which was reported to be identical with salvianin. They suggested its structure to be pelargonidin diglucoside acylated with two molecules of malonic acid and a molecule of p-coumaric acid. Shibata et al.3) obtained from purple variety of *S. splendens* several acylated anthocyanins that contain delphinidin, cyanidin and pelargonidin acylated with p-coumaric acid or malonic acid, whereas mostly caffeic acid derivatives were reported by Asen4) in the same flower.

When we reported5) a complete structure of monardaein (**8**) isolated from *M. didyma*, we also preliminary reported that fresh petals of scarlet *S. splendens* contain monardaein as a minor component; a major anthocyanin being different from monardaein and its demalonylated products. Recently Harborne et al.6) reported that the pigments of scarlet *S. splendens* consist of pelargonidin 3-caffeylglucoside-5-dimalonylglucoside and monardaein, although structure of salvianin had been assigned by Birkofer et al.7) to be pelargonidin-3-(6-caffeylglucoside)-5-glucoside without malonic acid.

Now we report here structures of new anthocyanins isolated from purple flowers of *S. splendens* and blue flowers of *S. farinacea* as well as the complete structure of salvianin obtained from scarlet flowers of S. splendens.

Fresh scarlet petals of S. splendens (5.7 kg) stored at -20 °C was pulverized in the presence of liq. N_2 and extracted with 0.5% aq trifluoroacetic acid (TFA) at room temp. The extract (22 L) was poured on an XAD-7 column to adsorb pigments. Stepwise elution with aqueous methanol (20, 30 and 80%) containing 0.1% TFA gave, after evaporation below 10 °C, a pigment mixture, which was further chromatographed on an ODS column by gradient elution with a mixture of water and solvent A (30 to 100%) containing 3% phosphoric acid [solv. A = $CH_3COOH:CH_3CN:H_2O$ (4:5:11)]. The phosphate anions in the fractions containing pigments were replaced with trifluoroacetate anions by passing through an ODS column with 0.01% TFA-50% solv. A, and the fractions were evaporated under vacuum to give as their TFA salt salvianin (**1**) (79.9 mg), monodemalonylsalvianin (**2**) (40.3 mg), bisdemalonylsalvianin (**3**) (3.4 mg), monardaein (**8**) (21.4 mg), and monodemalonylmonardaein (**9**) (11.4 mg).



	R_1	R_2	R_3	R_4
1	H	OH	malonyl	malonyl
2	H	OH	malonyl	H
3	H	OH	H	H
4	OH	OH	malonyl	malonyl
5	OH	OH	malonyl	H
6	OH	OH	H	H
7	OCH_3	H	malonyl	H
8	H	H	malonyl	malonyl
9	H	H	malonyl	H
10	OH	H	malonyl	malonyl
11	OH	H	malonyl	H

Salvianin (**1**), on treatment with 0.1M HCl at 80 °C, was transformed into **3** via **2**. FABMS of **1**, **2** and **3** gave molecular ions at m/z 929, 843, and 757, respectively. Presence of one and two molecules of malonic acid in **2** and **1**, respectively, was shown by acid methanolysis to produce dimethyl malonate, which was quantitatively analyzed by gas chromatography.5)

The structure of bisdemalonylsalvianin (**3**) was determined to be 3-O-(6-O-E-caffeyl-β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl)pelargonidin as follows: in the ^1H NMR spectrum (500 MHz) all of the proton signals assigned by ^1H spin-spin decoupling and HOHAHA experiments showed the presence of pelargonidin, caffeic acid, and two molecules of glucose in β-pyranoside form ($J_{1,2}$ = 7.5 Hz and $J_{2,3}$ = $J_{3,4}$ = $J_{4,5}$ = 9 Hz). NOE (▲-1 ↷

Table 1. ^{1}H NMR, FAB mass, and visible spectra of *Salvia* anthocyanins

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(10)		
NMR^a			Chemical shift (ppm)						J (Hz)	
4	8.98	8.99	8.96	8.88	8.88	8.81	8.98	8.78	s	
6	6.98br	7.00br	7.00	6.94	6.93	6.95br	7.02br	6.86	d	2.0
8		7.01br	6.94	6.98	6.98	6.96br		6.89	d	2.0
2',6'	8.57	8.60	8.58	7.78s	7.77s	7.73s	7.97s	7.68s	d	9.0
3',5'	7.06	7.06	7.06	-	-	-	-	-	d	9.0
OMe	-	-	-	-	-	-	4.00	-	s	
●-1	5.25	5.20	5.18	5.23	5.18	5.22	5.21	5.14	d	7.5
●-2	3.90	3.77	3.78	3.87	3.81	3.80	3.73	3.78	dd	9.0 7.5
●-3	3.83	3.57	3.58	3.80	3.64	3.59	3.58	3.70	t	9.0
●-4	5.02	3.47	3.44	4.49	3.52	3.43	3.47	4.89	t	9.0
●-5	4.06	3.80	3.63	4.05	3.95	3.66	3.81	3.95	ddd	9.0 6.0 2.0
●-6a	4.43	4.57	4.03	4.42	4.54	4.07	4.55	4.28	dd	12.0 2.0
●-6b	4.24	4.26	3.75	4.21	4.44	3.76	4.24	4.08	dd	12.0 6.0
▲-1	5.44	5.38	5.39	5.42	5.45	5.48	5.47	5.33	d	7.5
▲-2	3.75	3.72	3.71	3.80	3.77	3.81	3.78	3.70	dd	9.0 7.5
▲-3	3.63	3.59	3.59	3.61	3.58	3.63	3.62	3.52	t	9.0
▲-4	3.54	3.50	3.50	3.51	3.47	3.51	3.49	3.40	t	9.0
▲-5	3.92	3.91	3.91	3.93	3.82	3.97	3.95	3.85	ddd	9.0 6.0 2.5
▲-6a	4.58	4.53	4.48	4.55	4.58	4.47	4.48m	4.45	dd	12.0 2.5
▲-6b	4.44	4.43	4.51	4.43	4.26	4.54		4.34	dd	12.0 6.0
C-a	6.17	6.19	6.19	6.20	6.21	6.23	6.23	6.16	d	16.0
C-b	7.30	7.32	7.28	7.31	7.32	7.26	7.37	7.29	d	16.0
C-2	6.82	6.84	6.94	6.82	6.84	6.84	7.24^b	7.14^b	d	2.0
C-6	6.73	6.26	6.71s	6.75	6.78	6.69s			dd	8.0 2.0
C-3	-	-	-	-	-	-	6.73^b	6.63^b		
C-5	6.70	6.21	6.71s	6.70	6.71	6.69s			d	8.0
NOE^c	+		+	+	+	+		+		
FAB^d	929	843	757	961	875	789	887	945		
Vis^e	509	509		541	541	542	539			

a) ^{1}H NMR spectra in CD_3OD containing CF_3COOD: (1) 5% TFA at 25°C; (2) 10% TFA at 19 °C; (3) 10% TFA at 20 °C; (4) 5% TFA at 25°C; (5) 10% TFA at 27 °C; (6) 10% TFA at 0 °C; (7) 5% TFA at 23 °C; (10) 5% TFA at 25 °C

b) d, J = 8.5 Hz c) NOE: ▲-1 → H-4; ●-1 → H-6

d) FAB-MS spectra were measured in 1M HCl/glycerol

e) Vis spectra in MeOH contg. 3% TFA, except 7, which in 0.1M HCl-MeOH

H-4; ●-1 $\curvearrowright$ H-6) indicated the position of ▲- and ●-glucose attached to the pelargonidin nucleus. Since the $-CH_2O-$ signals of ▲-glucose are deshielded about 0.5 ppm more than the corresponding signals of ●-glucose, the former $-CH_2O-$ group is acylated with caffeic acid, of which double bond geometry is *E* configuration ($J_{\alpha,\beta}$ = 16 Hz).

In ^{1}H NMR of monodemalonylsalvianin (2), the $-CH_2O-$ protons of ●-glucose shifted about 0.5 ppm toward lower field by comparison with the corresponding protons of 3, indicating that 2 is malonylated at 6 position of ●-glucose. In ^{1}H NMR of salvianin (1), the signal assigned to ●-4 appeared at 5.02 ppm, which was about 1.5 ppm lower than that of the corresponding ●-4 of 2. Thus, structure of salvianin (1) was determined to be 3-O-(6-O-*E*-caffeyl-β-D-glucopyranosyl)-5-O-(4,6-di-O-malonyl-β-D-glucopyranosyl)pelargonidin.

Purple petals of *S. splendens* (1100 g) on extraction by the procedure similar to above gave salviadelphin (4) (25.2 mg), monodemalonylsalviadelphin (5) (20.5 mg), bisdemalonylsalviadelphin (6) (5.8 mg) and dimalonylawobanin (10) (8.5 mg). Structure of these pigments was determined similarly from their FABMS and ^{1}H NMR spectra (Table 1).

Similarly, blue petals of *S. farinacea* (150 g) gave pigment A (16 mg) and B (8.4 mg) as their TFA salt. Pigment A was identified as malonylawobanin (11) by comparison of HPLC retention time and ^{1}H NMR spectra with those of an authentic sample.[8)]

Pigment B, which was named salviamalvin (7), gave a molecular ion at m/z 887 in its FABMS spectrum; 28 mass unit being larger than malonylawobanin (11), which corresponded to two methyl groups. This was verified from analysis of its ^{1}H NMR, in which a six-proton singlet signal (CH_3 x 2) was observed at 4.00 ppm. Complete analysis of the spectrum in comparison with that of malonylawobanin (11)[8)] concluded that the pigment is 3',5'-O-dimethylmalonylawobanin (7).

Stems of *S. farinacea* (1600 g) contain mostly malonylawobanin (11) (28 mg isolated yield) and a trace of salviamalvin (7).

We thank the Ministry of Education, Science and Culture for financial support.

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(Received in Japan 8 September 1989)