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STRUCTURE OF CYANODELPHIN, A TETRA-*p*-HYDROXYBENZOATED ANTHOCYANIN FROM BLUE FLOWER OF *DELPHINIUM HYBRIDUM*

Tadao KONDO,+* Kanako SUZUKI, Kumi YOSHIDA,++ Kaori OKI,
Minoru UEDA, Minoru ISOBE and Toshio GOTO.

+Chemical Instrument Center, Nagoya University and Laboratory of Organic Chemistry, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya 464-01, Japan

++School of Life Studies, Sugiyama Jogakuen University, Hoshigaoka, Chikusa, Nagoya 464, Japan

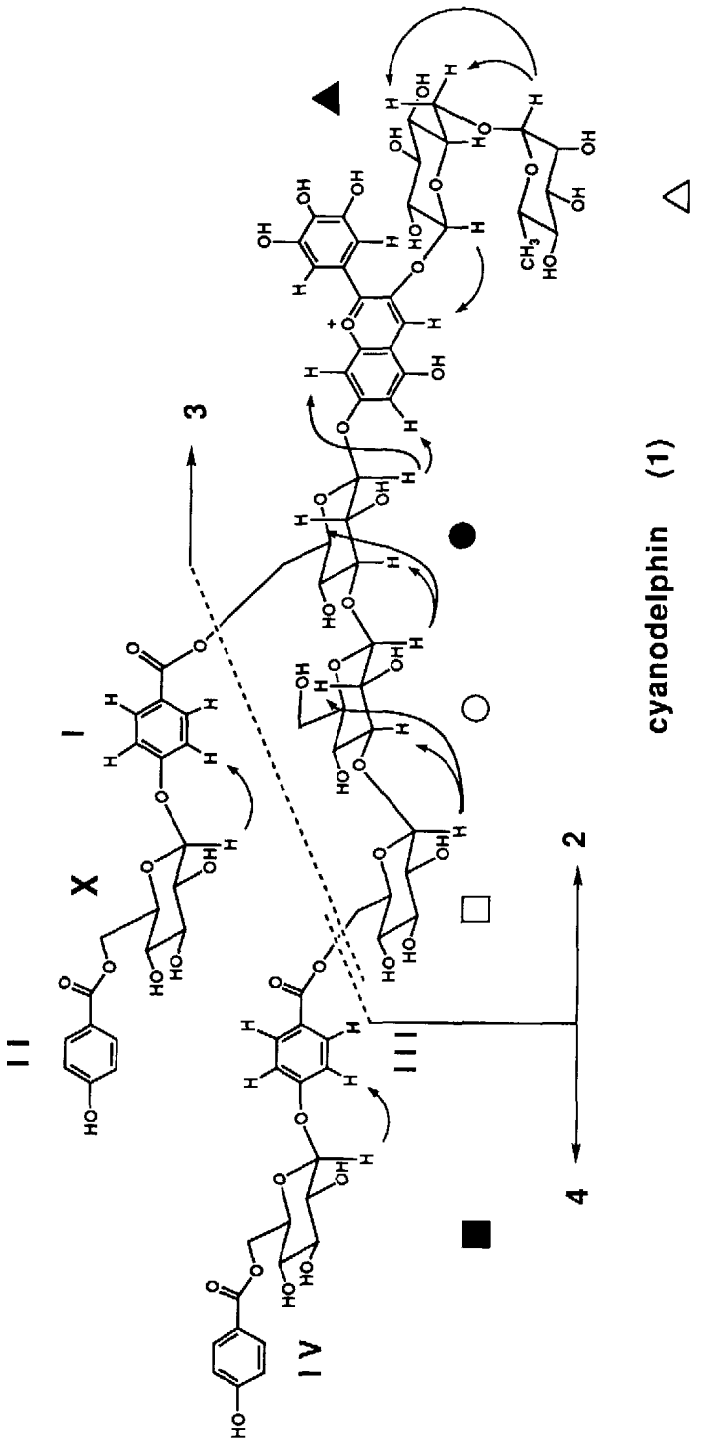
Abstract: Structure of cyanodelphin from blue petals of *Delphinium hybridum* was determined. It contains delphinidin nucleus, four molecules of *p*-hydroxybenzoic acid and seven molecules of hexoses.

Flowers of *Delphinium*, which have various colored petals *i. e.* white, red, violet and blue, are widely cultivated in the world. In 1915 Willstätter isolated a pigment, delphinin, from reddish purple petals of *Delphinium consolida* and proposed the structure to be di-(*p*-hydroxybenzoyl)delphin,[1] but, in 1964, Harborne doubted existence of *p*-hydroxybenzoyl gruop in its molecule.[2] Asen re-examined the pigment of blue larkspur cv "Dark blue Supreme" in 1975 and reported the structure to be delphinidin 3-di-(*p*-hydroxybenzoyl)-glucosylglucoside and bluing in the flower by copigmentation.[3] Recently we isolated violdelphin[4] from violet flower of *Delpinium* cv "Black Night" and determined the structure to be delphinidin 3-*O*-rutinoside-7-*O*-(6-*O*-(4-*O*-(6-*O*-*p*-hydroxybenzoylglucosyl)-*p*-hydroxylbenzoyl) glucoside.[5] In this paper we will describe the complete structure of cyanodelphin isolated from blue flower, *Delphinium hybridum* cv "Blue Springs", which contains four *p*-hydroxybenzoic acids in the molecule. The structure of cyanodelphinin is different from those pigments in purple petals reported previously.

Fresh blue petals of *Delphinium hybridum* (3.1 Kg) was frozen with liq. N_2 and pulverized by a blender. The powder was extracted with aq. 70% CH_3CN containing 3% trifluoroacetic acid (TFA). The extract was condensed and chromatographed on an Amberlite XAD-7 column using stepwise elution from aq. 0.5% TFA to aq. CH_3CN containing 0.5% TFA. The crude pigment was purified by repeated precipitation as follows; to the crude pigment aq. 10% TFA was added and ultrasonicated, then precipitates formed were collected by centrifugation to give pure cyanodelphin (**1**)[6] as TFA salts (4.63 g).

^1H NMR of **1**[7] (*m/z* 1901)[8] in TFA-*d*-CD_3OD showed existence of delphinidin nucleus, assigned by means of ^1H-^1H COSY from basis on the signal at 8.69 ppm as H-4,[9] four molecules of *p*-hydroxybenzoic acid and seven molecules of hexoses. Partial hydrolysis of **1** with 1N NaOH-CH_3OH at 0 °C gave bisdeacylcyanodelphin (**2**, m/z 1499),[6,7] tetrakisdeacylcyanodelphin (**3**, *m/z* 1097),[6,7] 4-*O*-(6-*O*-*p*-hydroxybenzoyl-β-D-glucopyranosyl)-*p*-hydroxybenzoic acid (**4**), 4-*O*-β-D-glucopyranosyl-*p*-hydroxybenzoic acid (**5**) and *p*-hydroxybenzoic acid (**6**).

^1H NMR of the tetrakisdeacylate **3** in 10% TFA*d*-CD_3OD showed delphinidin and five hexoses in the molecule. One of five hexoses was determined to be α-L-rhamunoside by comparison with the signals of sugar moieties of violdelphin.[5] In order to separate the highly overlapping signals at sugar region, **3** was derived to


I
II
III
IV
X
2
3
4
cyanodelphin (1)
: NOE's by irradiating the anomeric protons

the pertrifluoroacetate (**7**) by treatment with trifluoroacetic anhydride (TFAA).[10] By ^{1}H NMR of 1D-HOHAHA spectra of **7** irradiating at each anomeric proton, four hexoses were determined to be β-glucopyranosides from the vicinal couplings ($J_{1,2}$=7.5Hz, and $J_{2,3}$=$J_{3,4}$=$J_{4,5}$=9.0Hz). The sugar linkages of ▲, △ and ● could be clarified by NOE difference spectra (Scheme). Irradiation of the anomeric proton of □ and ○ enhanced the signals of H-2 and 3 of ○ and ● simultaneously, so the glycosyl position of both □ and ○ -sugar was obscure whether 2-OH or 3-OH. Since the chemical shifts of signals of H-3 of ● (4.42 ppm) and ○ (4.26 ppm) are observed about 0.8 ppm higher field than that of H-2 of ● (5.41 ppm) and ○ (5.13 ppm), the glycosyl position of □ and ○ -glucose was determined to be the OH-3 of ○ and ● -glucose, respectively. Thus, the structure of **3** must be 3-*O*-(6-*O*-(α-L-rhamunosyl)-β-D-glucopyranosyl)-7-*O*-(3-*O*-(3-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)delphinidin.

^{1}H NMR analysis revealed that bisdeacylcyanodelphin (**2**) is composed with tetrakisdeacylcyanodelphin (**3**), one newly observed hexose (β-glucopyranoside, X), and two *p*-hydroxybenzoic acid. Partial alkaline hydrolysis of **2** gave **3** and **4**. The -CH_2- signals of ● -glucose of **2** appeared at 0.6-0.9 ppm lower field than that of tetrakisdeacylcyanodelphin (**3**), indicating that 6-OH of ● is acylated with **4**. Thus, the structure of **2** was determined as shown in Scheme. By complete assignment of ^{1}H NMR of **1** using 1D and 2D HOHAHA and NOE spectra, the composition of **1** was clarified to be **2**, two *p*-hydroxybenzoic aicd (**6**) and one glucose (■). The sugar ■ also has the same pattern linkage to two *p*-hydroxybenzoic acids as component **4**. Comparing the chemical shifts of the -CH_2- signals of □ -glucose of **1** with those of **2**, the -CH_2- of □ -sugar appeared at lower field (4.80 ppm and 4.39 ppm), indicating that the *p*-hydroxybenzoyl group of **4** is attached to 6-OH of □. Thus, the strucrue of **1** is 3-*O*-(6-*O*-(α-L-rhamnosyl)-β-D-glucopyranosyl-7-*O*-(3-*O*-(3-*O*-(6-*O*-(4-*O*-(6-*O*-*p*-hydroxybonzoyl-β-D-glucopyranosyl)-*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-6-*O*-(4-*O*-(6-*O*-phydroxybenzoyl-β-D-glucopyranosyl)-*p*-hydroxybenzoyl)-β-D-glucopyranosyldelphinidin.

1 was very stable in a neutral aq. solution (ca 2x10^{-5}M), and the color was remaining for more than one month, while the diacylate **2** was as unstable as violdelphin and **3** was more unstable than **2**.

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References and Notes

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2. Harborne, J.B. *Phytochemistry*, **1964**, *3*, 151.
3. Asen S; Stewart, R. N.; Norris, K. H. *Phytochemistry*, **1975**, *14*, 2677.
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5. Kondo, T; Oki, K.; Yoshida, K. ; Goto, T. *Chem. Lett.*, **1990**, 137.
6. Electron absorption, λ_{max} nm (ε), of **1**, **2**, and **3** in 0.1% HCl-MeOH
   1: 548 nm (26100), 250 (54000); **2**: 548 (23000), 250 (24000), **3**: 540 (30900) 282 (14800)
7. Assignments of the ^{1}H NMR Spectra of **1**, **2**, and **3** (in 10% TFA*d*-CD_3OD at 30°C, 500MHz)

	1			**2**			**7*****		
A-4	8.69	s		8.68	s		8.86	br.s	
A-6	6.71	br.s		6.69	br.s		7.75	s	
A-8	7.12	s		7.18	s		7.77	br.s	
A-2',6'	7.89	s	(2H)	7.83	s	(2H)	8.50	s	(2H)

▲	-1	5.31	d	7.5	5.33	d	7.5	6.50	d	7.5
	-2	3.88	dd	9.0,7.5	3.91	dd	7.5, 9.0	5.71	dd	9.0, 7.0
	-3	3.62	t	9.0	3.63	t	9.0	5.60	t	9.0
	-4	3.43	t	9.0	3.44	t	9.0	5.80	t	9.0
	-5	3.77	dd	9.0, 7.0	3.79	ddd	9.0, 7.5, 2.0	4.42	ddd	9.0, 5.5, 2.0
	-6a	4.16	br.d	12.0	4.18	dd	12.0, 2.0	4.08	dd	12.0, 2.0
	-6b	3.69	dd	12.0, 7.0	3.70	dd	12.0, 7.5	3.78	dd	12.5, 5.5
●	-1	5.38	d	7.5	5.42	d	7.5	5.77	br.d	7.5
	-2	3.84	m		3.90	m		5.41	dd	9.0, 7.5
	-3	3.84	m		3.90	m		4.42	t	9.0
	-4	3.58	t	9.0	3.61	t	9.0	5.38	t	9.0
	-5	4.08	br.t	9.0	4.13	dt	9.0, 2.5	4.45	ddd	9.0, 6.0, 2.5
	-6a	4.99	br.d	12.0	5.04	dd	12.0, 2.5	4.65	dd	12.0, 2.5
	-6b	4.29	dd	12.0, 9.0	4.28	dd	12.0, 9.0	4.52	dd	12.0, 6.0
△	-1	4.81	s		4.86	s		4.86	d	1.5
	-2	3.92	br. s		3.96	br.s		5.38	br.d	4.0
	-3	3.73	dd	9.0, 2.5	3.73	br.d	12.0	5.48	dd	10.0, 4.0
	-4	3.41	t	9.0	3.47	t	12.0	5.21	dd	10.0, 9.0
	-5	3.63	dq	9.0, 7.0	3.64	m		4.05	dq	9.0, 7.0
	-6a	1.25	d	7.0	1.25	d	7.0	1.25	d	7.0
○	-1	4.63	d	7.5	4.77	d	7.5	4.88	d	7.5
	-2	3.53	br. d	9.0	3.58	dd	9.0, 7.5	5.13	dd	9.0, 7.5
	-3	3.47	t	9.0	3.66	t	9.0	4.26	t	9.0
	-4	3.37	t	9.0	3.48	t	9.0	5.21	t	9.0
	-5	3.25	ddd	9.0, 7.5, 2.0	3.45	ddd	9.0, 4.5, 1.5	4.05	ddd	9.0, 5.0, 3.0
	-6a	3.84	dd	12.0, 2.0	3.96	dd	12.0, 1.5	4.48	m	
	-6b	3.60	dd	12.0, 7.5	3.73	dd	12.0, 4.5	4.48	m	
□	-1	4.60	d	7.5	4.62	d	7.5	4.88	d	7.5
	-2	3.51	dd	9.0, 7.5	3.33	dd	9.0, 7.5	5.17	dd	9.0, 7.5
	-3	3.44	t	9.0	3.43	t	9.0	5.51	t	9.0
	-4	3.39	t	9.0	3.32	t	9.0	5.29	t	9.0
	-5	3.77	ddd	9.0, 7.0, 2.0	3.53	ddd	9.0, 6.0, 2.0	4.13	ddd	9.0, 7.0, 4.0
	-6a	4.80	dd	12.0, 2.0	3.92	dd	12.0, 2.0	4.51	dd	12.0, 7.0
	-6b	4.39	dd	12.0, 7.0	3.67	dd	12.0, 6.0	4.45	dd	12.0, 4.0
■	-1	5.06	d	7.5						
	-2	3.56	m							
	-3	3.56	m							
	-4	3.45	t	9.0						
	-5	3.86	ddd	9.0, 7.5, 2.0						
	-6a	4.69	dd	12.0, 2.0						
	-6b	4.36	dd	12.0, 7.5						
X	-1	4.50	br.d	7.5	4.42	d	7.5			
	-2	3.56	m		3.47	dd	9.0, 7.5			
	-3	3.56	m		3.53	t	9.0			
	-4	3.36	t	9.0	3.36	t	9.0			
	-5	3.85	dt	9.0, 2.0	3.53	dt	9.0, 2.0			
	-6a	4.64	dd	12.0, 2.0	4.46	dd	12.0, 2.0			
	-6b	4.03	dd	12.0, 9.0	3.99	dd	12.0, 9.0			
	I -2',6'	7.83*	d	8.5	7.42	d	8.5			
	I -3',5'	6.83*	d	8.5	6.49	d	8.5			
	II -2,6'	7.46**	d	8.5	7.90	d	8.5			
	II -3'5'	6.51**	d	8.5	6.84	d	8.5			
	III-2',6'	7.95*	d	8.5						
	III-3',5'	7.12*	d	8.5						
	IV-2',6'	7.89**	d	8.5						
	IV-3'5'	6.87**	d	8.5						

*: Determined by NOE's. **: determined by HMBC. ***: This sample was measured in $CDCl_3$ at 25 °C.

8. FABMS was carried out using a matrix of 1N HCl-glycerol.
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