Direct Observation of a Small-Molecule Associated Supramolecular Pigment, Commelinin, by Electrospray Ionization Mass Spectroscopy

Tadao Kondo,* Minoru Ueda,† Kumi Yoshida,*,‡
Koiti Titani,§ Minoru Isobe,† and Toshio Goto†

Chemical Instrument Center, Nagoya University
Chikusa, Nagoya 464-01, Japan
Laboratory of Organic Chemistry
School of Agriculture, Nagoya University
Chikusa, Nagoya 464-01, Japan
School of Life Studies
Sugiyama Jogakuen University
Chikusa, Nagoya 464, Japan
Division of Biomedical Polymer Science
Institute for Comprehensive Medical Science
School of Medicine, Fujita Health University
Toyoake, Aichi 470-11, Japan

Received October 25, 1993
Revised Manuscript Received June 14, 1994

Commelinin (**1**)[1] is a supramolecular pigment isolated from the blue flower petals of *Commelina communis*.[2] In 1992, we determined the complete structure of commelinin by X-ray crystallographic analysis[3] and ended the controversy of pH theory[4] and metal-chelation theory[5] on blue flower color development. This is the first example of the structural determination of intact anthocyanin in neutral, physiological conditions. This study revealed that commelinin is composed of six molecules of anthocyanin, malonylawobanin (**M**, **2**), six molecules of flavone, flavocommelin (**F**, **3**), and two magnesium ions, $[\mathbf{M}_6\mathbf{F}_6Mg_2]^{6-}$. All the components associated with weak interactions and no covalent bonds, such as the metal chelation between **M** and Mg^{2+}, and hydrophobic stacking between **M** and **M**, **M** and **F**, and **F** and **F** (Figure 1).[3]

The molecular weight of **1** had been measured by sedimentation equilibrium ultracentrifugation to be about 9000,[2b] which was the only successful method of obtaining the molecular weight of **1**. Traditional mass spectrometrical methods, *e.g.* electron ionization (EI), field desorption (FD), and fast atom bombardment (FAB), ended in failure, and only the molecular ion of the monomer components was obtained, indicating that the energy for ionization was too high to keep the supramolecule, after which dissociation occurred. The newly developed matrix-assisted laser desorption ionization time-of-flight (MALD-TOF)[6] could not give the molecular weight, either.

Recently, electrospray ionization mass spectrometry (ESI-MS) has proven efficient for the measurement of macromolecular complexes.[7] This MS technique enables the direct observation of noncovalent complexes,[8] such as biomacromolecule–small-molecule complexes,[8b] biomolecular associates,[8c] and metal complexes.[8d] However, measurement of the molecular ion of a supramolecule associated with more than one dozen small molecular components has never been accomplished. We applied ESI-MS for commelinin and succeeded in measuring the molecular ion. This is the first example of direct observation of the small-molecule associated supramolecule.

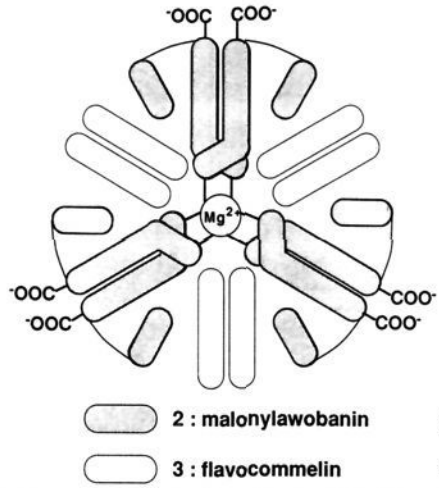
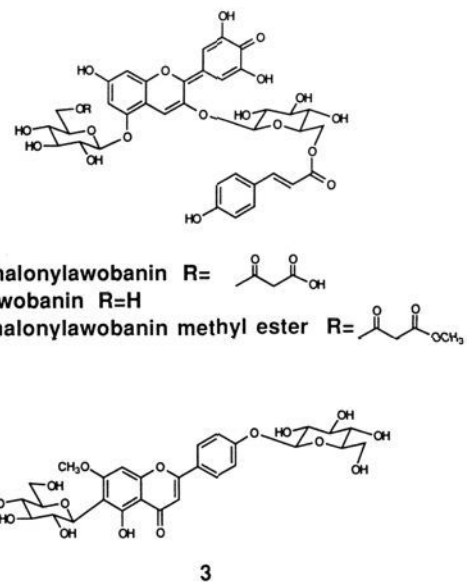


Figure 1. Supramolecular structure of commelinin. Six molecules of **M** and six molecules of **F** surround two magnesium ions. (There exists another magnesium ion under the one shown.) Each magnesium ion is coordinated with three keto anions of **M**. Six carboxylates of the **M** could be located around the molecular surface.

Chart 1



1 was dissolved in $EtOH:H_2O$ (1:1) and was infused at 3.0 mL/min through the ion-spray interface. By means of negative charge accelerating ESI-MS, the multiply-charged peaks were observed at m/z = 2216.5, 1768.7, 1474.1, and 1262.5, corresponding to the $[M-4H]^{4-}$, $[M-5H]^{5-}$, $[M-6H]^{6-}$, and $[M-7H]^{7-}$ charge states, respectively (Figure 2). By deconvolution of the Fenn method,[9] the molecular weight was measured to be 8849 (calculated average molecular weight for **1** as $[\mathbf{M}_6\mathbf{F}_6Mg_2]-6H$ was 8846.2).[10] In a positive accelerating experiment, no multiply-charged ions for **1** appeared, but only the molecular ion peaks of each component, **M** and **F**, were detected.

* Author to whom correspondence should be addressed. Chemical Instrument Center, Nagoya University.
† School of Agriculture, Nagoya University.
‡ School of Life Studies, Sugiyama Jogakuen University.
§ School of Medicine, Fujita Health University.

(1) Hayashi, K.; Abe, Y.; Mitsui, S. *Proc. Jpn. Acad.* **1958**, *34*, 373. Mitsui, S.; Hayashi, K.; Hattori, S. *Proc. Jpn. Acad.* **1959**, *35*, 169. Hayashi, K.; Takeda, K. *Proc. Jpn. Acad.* **1970**, *46*, 535.
(2) (a) Goto, T.; Kondo, T. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 17. (b) Tamura, H.; Kondo, T.; Goto, T. *Tetrahedron Lett.* **1986**, *27*, 1801.
(3) Kondo, T.; Yoshida, K.; Nakagawa, A.; Kawai, T.; Tamura, H.; Goto, T. *Nature* **1992**, *358*, 515.
(4) Willstätter, R.; Mallison, H. *Justus Liebigs Ann. Chem.* **1915**, *408*, 147.
(5) Shibata, K.; Shibata, Y.; Kashiwagi, I. *J. Am. Chem. Soc.* **1919**, *41*, 208.
(6) Hillenkamp, F.; Karas, M.; Beavis, R. C.; Chait, B. T. *Anal. Chem.* **1991**, *63*, 1193A.
(7) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64. Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Mass Spectrom. Rev.* **1990**, *9*, 37. Mann, M. *Org. Mass. Spectrom.* **1990**, *25*, 575. Smith, R. D.; Loo, J. A.; Edmonds, C. G.; Barinaga, C. J.; Udseth, H. R. *Anal. Chem.* **1990**, *62*, 882. Smith, R. D.; Loo, J. A.; Ogorzalek Loo, R. R.; Bushman, M.; Udseth, H. R. *Mass Spectrom. Rev* **1991**, *10*, 359.

0002-7863/94/1516-7457$04.50/0

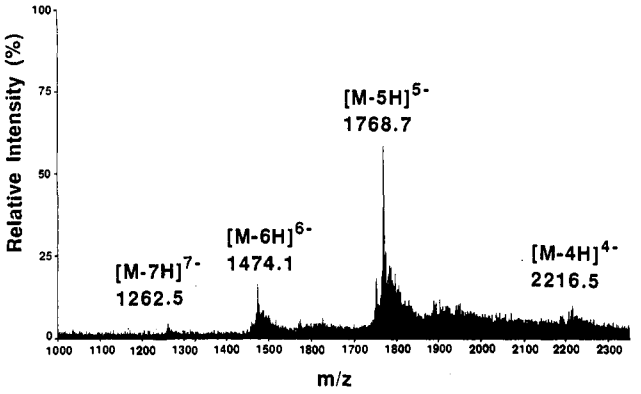


Figure 2. Negative-mode ESI mass spectrum of commelinin recorded on a Sciex API–III triple quadrupole mass spectrometer. Solution of **1** was infused through the electrospray interface. Capillary skimmer voltage, –105 V. The spectrum is an averaged sum of 50 scans from m/z 300 to 2350.

Table 1. Exact Molecular Weight of Commelinin (**1**) and the Analogues **4**, **5**, **6**, **7**, **8**, and **10** Obtained by ESI MS

metal complex pigments	formula	molecular weight calcd (av)	molecular weight obsd
commelinin (**1**)	$C_{402}H_{414}O_{222}Mg_2$	8846	8849
Mn–commelinin (**4**)	$C_{402}H_{414}O_{222}Mn_2$	8908	8909
Co–commelinin (**5**)	$C_{402}H_{414}O_{222}Co_2$	8915	8914
Ni–commelinin (**6**)	$C_{402}H_{414}O_{222}Ni_2$	8915	8916
Zn–commelinin (**7**)	$C_{402}H_{414}O_{222}Zn_2$	8928	8931
Cd–commelini (**8**)	$C_{402}H_{414}O_{222}Cd_2$	9022	9023
Aw–commelinin (**10**)	$C_{384}H_{402}O_{204}Mg_2$	8330	n.d.[a]

[a] Not determined.

Previously we reported the reconstruction of various commelinin-like pigments through replacement of the metal ion and anthocyanin residue.[3,11] Replacing the chelating metal ion from Mg^{2+} to the divalent ions such as Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}, multiply-charged ions corresponding to $[M - 4H]^{4-}$, $[M - 5H]^{5-}$, $[M - 6H]^{6-}$, and $[M - 7H]^{7-}$ were also detected. The deconvolution process gave the exact molecular weight for each metal-replacing commelinin, respectively (Table 1). Thus, we succeeded in the direct detection of the molecular weight of a small-molecule associated supramolecular pigment by ESI-MS. Concurrently, the structural identities of those commelinin-like pigments were confirmed.

By the ESI-MS experiments of anthocyanin-replacing commelinin, the origin of the appearance of multiply-negative-charged ions was clarified. Commelinin-like pigment constructed from demalonated anthocyanin, awobanin(**A**, **9**), **F**, and Mg^{2+} gave no ions corresponding to the molecular weight. When the supramolecule constructed from malonylawobanin methyl ester (**11**), **F**, and Mg^{2+} was measured, no molecular ion was detected, either. Therefore, the negative charge of commelinin and commelinin-like pigments would be generated at the malonic acid residue. A mixture of seven commelinin-like pigments was obtained from the reconstruction of **2**, **9**, **3**, and Mg^{2+}. Their composition was determined to be $[M_{6-n}A_nF_6Mg_2]$ (n = 0–6) by electrophoresis followed by HPLC analysis.[2b] Recording the ESI-MS of this mixture, peaks corresponding to $[M - 4H]^{4-}$ and $[M - 5H]^{5-}$ of $[M_5AF_6Mg_2]$, $[M_4A_2F_6Mg_2]$, and $[M_3A_3F_6Mg_2]$ appeared, though the S/N of the spectrum was low (Figure 3).[12] These results confirmed that the carboxylic acid moieties were essential for ESI-MS detection, just as the carboxylate should be in the supramolecule. Its plural anion charges might retain the structure of the supramolecule and prevent dissociation during the ionization process.

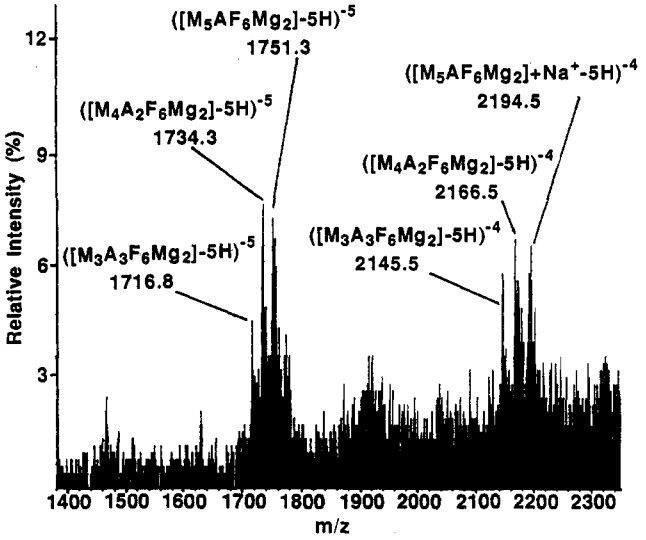


Figure 3. Negative-mode ESI mass spectrum of a mixture of commelinin-like pigments constructed from **2**, **9**, **3**, and Mg^{2+}. This spectrum was recorded on the same conditions as in Figure 2.

X-ray crystallographic analysis of commelinin revealed that **1** contains more than 30% water in crystal. Some water might contact very strongly with commelinin, and the complete removal of it was difficult, therefore; the quantitative metal analysis was fairly dispersed. The molecular ion recorded by ESI-MS is the exact molecular weight without any solvent. This suggests that desolvation from commelinin occurred completely during ionization in a chamber without the dissociation into components. ESI-MS showed a great ability to directly observe such an assembled supramolecule.

Acknowledgment. We thank Mr. K. Nojima, JEOL LTD., for measurement of ESI–MS and MALD–TOF MS. This research was financially supported by The Ministry of Education, Science and Culture of Japan (Grant-Aid for Scientific Research No. 02250103 to T.K. and No. 05760100 to K.Y.) and Towa Food Foundation Urakami Foundation to K.Y.

Supplementary Material Available: Negative MALD-TOF MS spectrum of commelin, deconvolution spectrum of commelin, and negative ESI-MS spectra of Mn^-, Co^-, Ni^-, Zn^-, and Cd-commelinin (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(8) (a) Ganem, B.; Henion, J. D. *Chemtracts—Org. Chem.* **1993**, *6*, 1. (b) Ganem, B.; Li, Y.-T.; Henion, J. *J. Am. Chem. Soc.* **1991**, *113*, 6294. Ganem, B.; Li, Y-T.; Henion, J. D. *J. Am. Chem. Soc.* **1991**, *113*, 7818. Katta, V.; Chait, B. T. *J. Am. Chem. Soc.* **1991**, *113*, 8534. Ganguly, A. K.; Pramanik, B. N.; Tsarbopoulos, A. T.; Convey, T. R.; Huang, E.; Fuhrman, S. A. *J. Am. Chem. Soc.* **1992**, *114*, 6559. Ogorzalek Loo, R. R.; Goodlett, D. R.; Smith, R. D.; Loo, J. A. *J. Am. Chem. Soc.* **1993**, *115*, 4391. (c) Baca, M.; Kent, S. B. H. *J. Am. Chem. Soc.* **1992**, *114*, 3992. Smith, R. D.; Light-Wahl, K. J.; Winger, B. E.; Loo, J. A. *Org. Mass. Spectrom.* **1992**, *27*, 811. Light-Wahl, K. J.; Winger, B. E.; Smith, R. D. *J. Am. Chem. Soc.* **1993**, *115*, 5869. Light-Wahl, K. J.; Springer, D. L.; Winger, B. E.; Edmonds, C. G.; Camp, D. G., II; Thrall, B. D.; Smith, R. D. *J. Am. Chem. Soc.* **1993**, *115*, 803. Ganem, B.; Li, Y.-T.; Henion, J. D. *Tetrahedron Lett.* **1993**, *34*, 1445. Siuzdak, G.; Ichikawa, Y.; Caulfield, T. J.; Munoz, B.; Wong, C.-H.; Nicolaou, K. C. *J. Am. Chem. Soc.* **1993**, *115*, 2877. Li, Y.-T.; Hsieh, Y.-L.; Henion, J. D.; Senko, M. W.; McLafferty, F. W.; Ganem, B. *J. Am. Chem. Soc.* **1993**, *115*, 8409. (d) Katta, V.; Chowdhury, S. K.; Chait, B. T. *J. Am. Chem. Soc.* **1990**, *112*, 5348. Wilson, S. R.; Yasmin A.; Wu, Y. *J. Org. Chem.* **1992**, *57*, 6941. Allen, M. H.; Hutchens, T. W. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 308. Curtis, J. M.; Derrick, P. J.; Schnell, A.; Constantin, E.; Gallagher, R. T.; Chapman, J. R. *Org. Mass Spectrom.* **1992**, *27*, 1176. Leize, E.; Van Dorsselaer, A.; Krämer, R.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1993**, 990. Jaquinod, M.; Leize, E.; Potier, N.; Albrecht, A.-M.; Shanzer, A.; Van Dorsselaer, A. *Tetrahedron Lett.* **1993**, *34*, 2771.

(9) Mann, M.; Meng, C. K.; Fenn, J. B. *Anal. Chem.* **1989**, *61*, 1702.

(10) In commelinin, M takes a keto-anion form, see ref 3.

(11) Takeda, K. *Proc. Jpn. Acad.* **1977**, *53*, 257B.

(12) **10** has potentially two negative charges, since it is a metal complex coordinating six keto anions of 9 to two Mg^{2+}. Thus, $[M_3A_3F_6Mg_2]$ could have five negative charges: two negative charges arising from the complexation and three from three malonate groups of M.