Tetrahedron Letters,Vol.26,No.48,pp 5879-5882,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

STRUCTURE OF MONARDAEIN, A BIS-MALONYLATED ANTHOCYANIN ISOLATED FROM GOLDEN BALM, MONARDA DIDYMA¶

Tadao Kondo[†*], Yukari Nakane, Hirotoshi Tamura, and Toshio Goto[*]

Laboratory of Organic Chemistry, Faculty of Agriculture, Nagoya University, and
[†] Chemical Instruments Center, Nagoya University
Chikusa, Nagoya 464, Japan

C. H. Eugster

Organisch-Chemisches Institut, Universität Zürich
CH-8057 Zürich, Switzerland

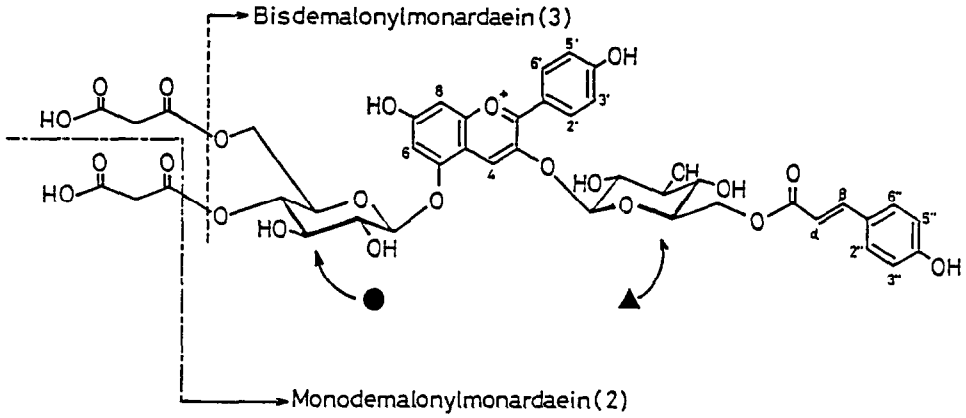
Structure of monardaein was determined to be 3-O-(6-O-trans-p-coumaryl-β-D-glucopyranosyl)-5-O-(4,6-di-O-malonyl-β-D-glucopyranosyl)pelargonidin (1).

Monardaein is an anthocyanin isolated from red petals of golden balm, Monarda didyma.[1] In 1929 Karrer and Widmer[2] proposed its structure to be pelargonidin diglucoside acylated with two molecules of malonic acid and one molecule of p-coumaric acid. In 1964 Harbone[3] reported, however, that it was erroneous and that no anthocyanins having malonic acid moiety had been found in nature. Birkofer et al.[4] determined the structure of the monardaein they obtained to be 3-O-(p-coumarylglucosyl)-5-O-glucosylpelargonidin. Using a new extraction method, we have recently isolated malonylated anthocyanins, which had been believed not to have malonic acid in their molecule.[5] There also have been isolated recently a few malonylated anthocyanins by others.[6] Here we report the complete structure of monardaein, which indeed has two molecules of malonic acid. Apparently, Harbone[3] and Birkofer et al.[4] had obtained demalonylated products by extraction with methanolic hydrochloric acid.

Dried flower petals (80 g) of golden balm, Monarda didyma, were carefully extracted three times with 3% aq trifluoroacetic acid (TFA) at 5 °C for 24 hrs. The extracts were applied on a column of Amberlite XAD-7, which was eluted stepwise with a mixture of water and methanol both containing 0.5% TFA. A main anthocyanin fraction eluted with the mixture (MeOH:H_2O = 1:1) was concentrated under vacuum below 5 °C, and diluted with ether to precipitate a crude pigment. It was purified by preparative ODS-HPLC using H_3PO_4-AcOH-CH_3CN-H_2O (3:8:10:79) as the eluent to give three fractions of pigments (III, II and I in the order of elution). TFA,

¶ Dedicated to Professor Harry H. Wasserman on the occasion of his 65th birthday.

instead of H_3PO_4, was not suitable in this separation. In order to remove H_3PO_4, each fraction was poured on an ODS (30-60 μ) column, and the adsorbed pigment was then eluted with TFA-AcOH-CH_3CN-H_2O (1:20:25:54). The eluates were dried up to give pure pigments as their TFA salt: pigment I [monardaein (1)] red powder[7] (73.6 mg), pigment II [monodemalonylmonardaein (2)] red powder[8] (154.0 mg), and pigment III [bisdemalonylmonardaein (3)] red powder[9] (65.5 mg).



Monardaein (1), on treatment with 1N HCl at 80 °C, was transformed into 3 via 2. FAB-MS of 1, 2 and 3 gave molecular ions at m/z 913, 827, and 741, respectively. The differences between the m/z of 1 and 2 (86), and 2 and 3 (86) indicated loss of a malonic acid moiety in each of the transformation, suggesting that monardaein (1) consists of 3 and two molecules of malonic acid. 1 and 2 were treated with 5% HCl-MeOH at 70 °C for 3.5 hrs and the resulting dimethyl malonate was analyzed by means of GLC (5% polyethylene glycol on 20μ Chromosorb W). Yields of dimethyl malonate were 140% from 1 and 90% from 2, respectively, thus confirming the above assumption.

The structure of bisdemalonylmonardaein (3) was determined to be 3-O-(6-O-trans-p-coumaryl-β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl) pelargonidin as follows: In the ^{1}H-NMR (500 MHz)[9] all of the proton signals were able to be assigned by ^{1}H spin-spin decoupling and pseudo-INDOR experiments. The spectrum showed that the pigment consists of pelargonidin, p-coumaric acid, and two molecules of hexoses, of which the ^{1}H coupling constants ($J_{1,2}$ = 8 Hz and $J_{2,3} = J_{3,4} = J_{4,5}$ = 9 Hz) proved the hexoses to be glucoses in β-pyranoside form. NOE between the anomer proton of ▲-glucose and H-4 of the anthocyanidin indicated that ▲-glucose is attached to 3-position of the pelargonidin nucleus with a glycosidic linkage. 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 p-coumaric acid, of which double bond geometry is E configuration ($J_{\alpha,\beta}$ =16 Hz).

In ^{1}H-NMR of monodemalonylmonardaein (2), the $-CH_2O-$ protons of ●-glucose shifted about

0.5 ppm toward low field, by comparison with the corresponding protons of 3, indicating that 2 is malonylated at 6-position of ●-glucose. In ^{1}H-NMR of monardaein (1), the signal assigned to ●-4 appeared at 4.98 ppm, which was at about 1 ppm lower than that of the corresponding ●-4 of 2. Thus, structure of monardaein (1) was determined to be 3-O-(6-O-trans-p-coumaryl-β-D-glucopyranosyl)-5-O-(4,6-di-O-malonyl-β-D-glucopyranosyl)pelargonidin.

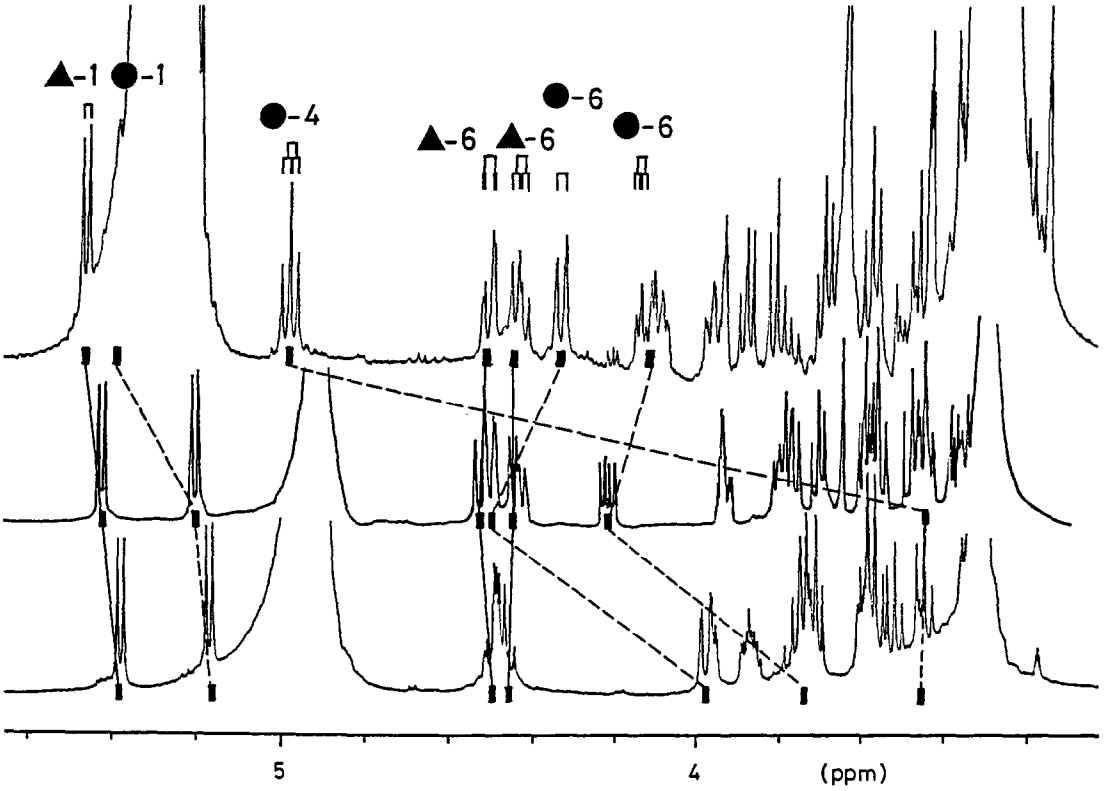


Fig. 1. ^{1}H-NMR spectra of monardaein (1) (upper)[7], monodemalonylmonardaein (2) (middle)[8], and bisdemalonylmonardaein (3) (lower)[9]

When dried flower petals of Monarda didyma were left at room temp., contents of 1 decreased and that of 2 and 3 increased by loss of malonic acid. Although we did not have fresh petals of the flowers, this observation strongly suggests that natural anthocyanin in flower petals is monardaein (1), whereas 2 and 3 could be artifacts. Salvianin, which was isolated by Willstätter and Bolton, was believed identical with monardaein by Karrer and Widmer.[2] Preliminary experiments showed that fresh flower petals of Salvia splendens contain monardaein (1) as a minor component, a major anthocyanin being different from 1 and its demalonylated products, 2 and 3.

Acknowledgement —— The auther (T. G.) thanks the Ministry of Education, Science and Culture (Japan) for the Grants-in-Aid for Scientific Research.

REFERENCES AND FOOTNOTES

1. P. Karrer and R. Widmer, Helv. Chim. Acta, 10, 67 (1927).
2. P. Karrer and R. Widmer, Helv. Chim. Acta, 11, 837 (1928); 12, 292 (1929).
3. J. B. Harborne, Phytochem., 3, 151 (1964).
4. L. Birkofer, C. Kaiser, M. Donike and W. Koch, Z. Naturforsh., 20b, 424 (1965).
5. T. Goto, T. Kondo, H. Tamura and S. Takase, Tetrahedron Lett., 24, 4863 (1983); H. Tamura, T. Kondo, Y. Kato and T. Goto, Tetrahedron Lett., 24, 5749 (1983); T. Goto, T. Kondo, T. Kawai and H. Tamura, Tetrahedron Lett., 25, 6021 (1984).
6. P. Bridle, R. S. T. Loeffler, C. F. Timberlake and R. Self, Phytochem., 23, 2968 (1984).
7. Monardaein (1) trifluoroacetate: m.p. 41-48 °C (dec); UV (3% TFA-MeOH) nm (ε) 509 (31,900), 316 (19,300), 287 (22,900), E_{440}/E_{510} 0.23; ^{1}H-NMR (500 MHz, 5% CF_3COOD in CD_3OD, at -20 °C) δ(ppm) 8.95 (1H, s, H-4), 8.60 (2H, d, J = 9 Hz, H-2' & 6'), 7.37 (1H, d, J = 16 Hz, H-β), 7.22 (2H, d, J = 8.5 Hz, H- 2" & 6"), 7.05 (2H, d, J = 9 Hz, H-3' & 5'), 7.02 (1H, s, H-6), 7.01 (1H, s, H-8), 6.68 (2H, d, J = 8.5 Hz, H-3" & 5"), 6.28 (1H, d, J = 16, H-α), 5.50 (1H, d, J = 8.0 Hz, ▲-1), 5.36 (1H, d, J = 8 Hz, ●-1), 4.98 (1H, t, J = 9.0 Hz, ●-4), 4.51 (1H, dd, J = 2.5 & 12.5 Hz, ▲-6), 4.44 (1H, dd, J = 8.0 & 12.5 Hz, ▲-6), 4.33 (1H, br.d, J = 12 Hz, ●-6), 4.13 (1H, dd, J = 8.0 & 12 Hz, ●-6), 4.10 (1H, br.t, ●-5), 3.98 (1H, ddd, J = 2.5, 8, & 9 Hz, ▲-5), 3.88 (1H, dd, J = 8 & 9 Hz, ●-2), 3.82 (1H, t, J = 9 Hz, ●-3), 3.70 (1H, dd, J = 8 & 9 Hz, ▲-2), 3.60 (1H, t, J = 9 Hz, ▲-3), 3.47 (1H, t, J = 9 Hz, ▲-4); NOE (at -30 °C) ▲-1 ⌒ H-4 (-19%) (protons in $-CH_2-$ of malonic acid were exchanged with D).
8. Monodemalonylmonardaein (2) trifluoroacetate: m.p. 61-89 °C (dec); UV (3% TFA-MeOH) nm (ε) 509 (27,400), 316 (16,200), 287 (19,300); ^{1}H-NMR (500 MHz, 2% DCl in CD_3OD, at room temp.) δ(ppm) 8.99 (1H, s, H-4), 8.60 (2H, d, J = 9 Hz, H-2' & 6'), 7.38 (1H, d, J = 16 Hz, H-β), 7.24 (2H, d, J = 8.5 Hz, H-2" & 6"), 7.06 (2H, d, J = 9 Hz, H-3' & 5'), 7.01 (2H, s, H-6 & 8), 6.73 (2H, d, J = 8.5 Hz, H-3" & 5"), 6.25 (1H, d, J = 16 Hz, H-α), 5.43 (1H, d, J = 8 Hz, ▲-1), 5.21 (1H, d, J = 8 Hz, ●-1), 4.53 (1H, dd, J = 2 & 12 Hz, ●-6), 4.51 (1H, dd, J = 3 & 12 Hz, ▲-6), 4.44 (1H, dd, J = 7.5 & 12 Hz, ▲-6), 4.22 (1H, dd, J = 7.5 & 12, ●-6), 3.94 (1H, ddd, J = 3, 7.5 & 9 Hz, ▲-5), 3.80 (1H, ddd, J = 2, 7.5 & 9 Hz, ●-5), 3.78 (1H, dd, J = 8 & 9 Hz, ●-2), 3.59 (1H, t, J = 9 Hz, ▲-3), 3.57 (1H, t, J = 9 Hz, ●-3), 3.48 (1H, t, J = 9 Hz, ▲-4), 3.45 (1H, t, J = 9 Hz, ●-4); NOE (at -20 °C) ▲-1 ⌒ H-4 (-13%), ●-1 ⌒ H-6 (-12%).
9. Bisdemalonylmonardaein (3) trifluoroacetate: m.p. 50-60 °C (dec); UV (3% TFA-MeOH) nm (ε) 510 (21,700), 316 (13, 500), 287 (16,400); ^{1}H-NMR (500 MHz, 3% CF_3COOD in CD_3OD, at room temp.) δ(ppm) 9.01 (1H, s, H-4), 8.59 (2H, d, J = 9.5, H-2' & 6'), 7.39 (1H, d, J = 19 Hz, H-β), 7.29 (2H, d, J = 9 Hz, H-2" & 6"), 7.05 (2H, d, J = 9.5 Hz, H-3' & 5'), 7.02 (1H, d, J = 1.5 Hz, H-6), 6.98 (1H, br.d, J = 1.5 Hz, H-8), 6.77 (2H, d, J = 9 Hz, H-3" & 5"), 6.22 (1H, d, J = 16 Hz, H-α), 5.38 (1H, d, J = 8 Hz, ▲-1), 5.17 (1H, d, J = 8 Hz, ●-1), 4.49 (1H, dd, J 3 & 12 Hz, ▲-6), 4.46 (1H, dd, J = 7 & 12 Hz, ▲-6), 3.97 (1H, dd, J = 2 & 12 Hz, ●-6), 3.85 (1H, ddd, J = 3, 7 & 9 Hz, ▲-5), 3.74 (1H, dd, J = 8 & 9 Hz, ●-2), 3.72 (1H, dd, J = 6.5 & 12 Hz, ●-6), 3.70 (1H, dd, J = 8 & 9 Hz, ▲-2), 3.59 (1H, ddd, J = 2, 6.5 & 9 Hz, ●-5), 3.58 (1H, t, J = 9 Hz, ▲-3), 3.56 (1H, t, J = 9 Hz, ●-3), 3.51 (1H, t, J = 9 Hz, ▲-4), 3.44 (1H, t, J = 9 Hz, ●-4); NOE (at -20 °C) ▲-1 ⌒ H-4 (19%), ●-1 ⌒ H-6(-11%).

(Received in USA 3 June 1985)