Structural Elucidation of Murrafolines, Six Novel Binary Carbazole Alkaloids Isolated from *Murraya euchrestifolia*

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In previous studies on the chemical constituents of *Murraya euchrestifolia* HAYATA (Rutaceae) collected in Taiwan, we preliminarily reported the first isolation and structure of a binary carbazole alkaloid, murrafoline-A (1). Since then, the isolation and spectroscopic structural elucidation of three new binary carbazoles, murrafoline-B (2), -C (5), and -D (3), have been reported. This paper describes in detail the structural elucidation of these novel binary carbazole alkaloids and also introduces two additional ones, murrafoline-G (4) and -H (6). Treatment of a mixture of girinimbine (7) and murrayafoline-A (8), which are monomeric carbazoles and structural components of murrafolines, with Nafion 117, an acidic perfluorinated ion-exchange resin, was found to produce murrafoline-D (3), -G (4), and -H (6).

Keywords murrafoline; *Murraya*; carbazole; Rutaceae; crystal structure; Nafion

Since the first isolation of a binary carbazole alkaloid, murrafoline-A (**1**)[1] from *Murraya euchrestifolia* HAYATA (Rutaceae) collected in Taiwan, we have reported the isolation of several kinds of binary carbazoles from the same plant.[2-4] This paper describes in detail the structural elucidation of the binary carbazoles, murrafoline-A (**1**), -B (**2**), -C (**5**), and -D (**3**), which were introduced in preliminary communications.[1,2] In our continuing study of the chemical constituents of this plant, two additional binary carbazole alkaloids, murrafoline-G (**4**) and -H (**6**), have been isolated, and their structures have also been determined by spectroscopic methods.

An acetone extract of the root bark of the plant collected in Taiwan in December was fractionated using a combination of silica gel column chromatography and preparative TLC, as shown in Chart 1, to give murrafoline-A (**1**), -B (**2**), -D (**3**), -G (**4**), and -H (**6**) along with known monomeric carbazoles and carbazolequinones. Murrafoline-C (**5**) was obtained from the ethanol extract of the root bark of the plant collected in February, along with murrafoline -A (**1**), -B (**2**), and -D (**3**),

The reaction of girinimbine (**7**)[5,6] and murrayafoline-A (**8**),[6] both of which are monomeric carbazoles and structural components of murrafolines, in the presence of Nafion 117,[7] an acidic perfluorinated ion-exchange resin, was found to produce murrafoline-D (**3**), -G (**4**), and -H (**6**).

Results and Discussion

Structure of Murrafoline-A[1] (1) This alkaloid was obtained as colorless needles, mp 260—262 °C, $[\alpha]_D$ ±0° in chloroform and showed no circular dichroism (CD) in the range of 200—350 nm. The molecular formula was determined as $C_{41}H_{42}N_2O_2$ by high-resolution mass spectroscopy (HR-MS). The UV spectrum (see Experimental) revealed a typical absorption of a carbazole nucleus[10-12] and the appearance of an M^{2+} ion at *m/z* 297 together with an M^+ ion at *m/z* 594 in the HR-MS suggested a dimeric structure for **1**. The ^{1}H-NMR spectrum measured in $CDCl_3$ at 60 °C showed signals attributable to two aryl (δ_H 2.41, 2.31), one vinyl (δ_H 1.54), and three

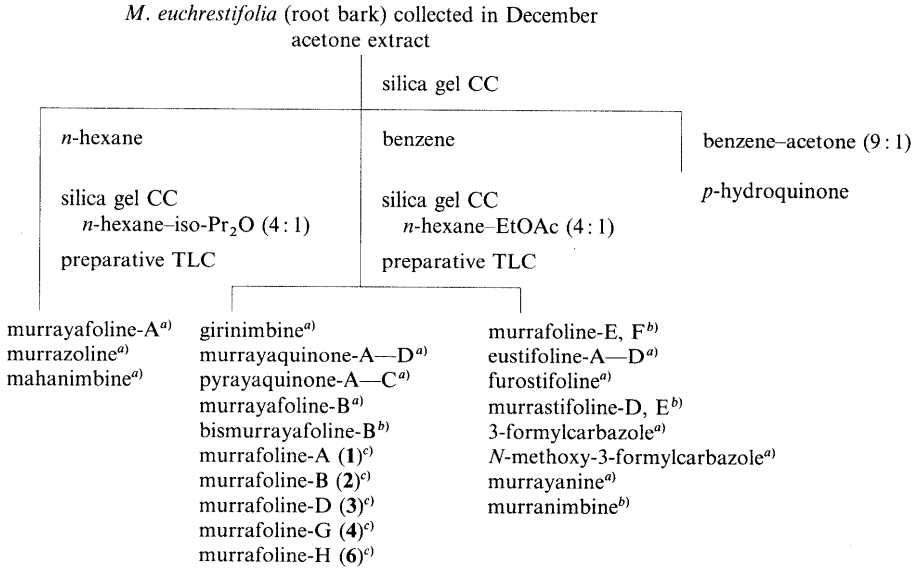

Chart 1. Isolation Procedure of Murrafolines from the Root Bark of *Murraya euchrestifolia*

a) Carbazoles or carbazolequinones. *b*) Binary carbazoles. *c*) New binary carbazoles. CC, column chromatography; TLC, thin-layer chromatography.

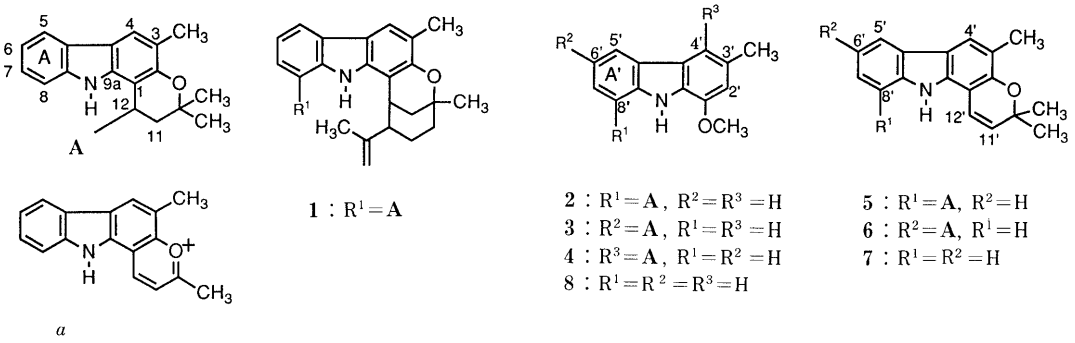

Chart 2

TABLE I. Crystallographic Data[a] for Murrafoline-A (**1**)

Molecular formula	$C_{41}H_{42}N_2O_2$
Formula weight	594.80
Crystal system	Monoclinic
Space group	$P2_1/c(C_{2h}^5)$–No. 14
a (Å)	11.949 (3)
b (Å)	12.475 (3)
c (Å)	21.678 (5)
β (°)	93.70 (1)
V (Å^3)	3225 (2)
Z	4
D_{calcd} (g cm^{-3})	1.225
μ(CuK_α radiation, $\lambda = 1.5418$ Å)	5.5
Temp. (°C)	25
Crystal dimensions (mm)	$0.08 \times 0.15 \times 0.50$
Scan type	θ–2θ
Scan width (°)	$1.10 + 0.14 \tan\theta$
θ_{max} (°)	75
Intensity control refls.;	124, 131, 11$\bar{5}$, 14$\bar{1}$;
Variation; repeat time (h)	<1%; 2
Total no. of refls. ($+h$, $+k$, $\pm l$) recorded	6944
No. of non-equiv. refls. recorded	6621
R_{merge} (on I)	0.024
No. of refls. retained [$I > 3.0\sigma(I)$]	4010
No. of parameters refined	575
Extinction correction	$7(1) \times 10^{-7}$
R (R_w)[b]	0.041 (0.054)
Goodness-of-fit[c]	1.25
Max. shift:esd in final least-squares cycle	0.03
Final $\Delta\rho$ (e/Å^3) max.; min.	0.18; −0.19

a) An Enraf–Nonius CAD-4 diffractometer (CuK_α radiation, graphite monochromator) was used for all measurements. Refined unit-cell parameters were derived from the diffractometer setting angles for 25 reflections ($35° < \theta < 40°$) widely separated in reciprocal space. Intensity data were corrected for the usual Lorentz and polarization effects. Crystallographic calculations were performed on PDP11/44 and Micro VAX computers by use of the Enraf–Nonius Structure Determination Package (SDP). For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from "International Tables for X-Ray Crystallography," Vol. IV, The Kynoch Press, Birmingham, England, 1974. *b*) $R = \Sigma \| F_o | - | F_c \| / \Sigma | F_o |$; $R_w = [\Sigma w(| F_o | - | F_c |)^2 / \Sigma w | F_o |^2]^{1/2}$; $\Sigma w \Delta^2 [w = 1/\sigma^2(| F_o |)$, $\Delta = (| F_o | - | F_c |)]$ was minimized. *c*) Goodness-of-fit = $[\Sigma w \Delta^2 / (N_{observations} - N_{parameters})]^{1/2}$.

TABLE II. Non-hydrogen Atom Fractional Coordinates and Equivalent Isotropic Thermal Parameters for Murrafoline-A (**1**), with Estimated Standard Deviations in Parentheses

Atom	x	y	z	B_{eq} (Å^2)
C(1)	0.3411 (2)	0.4253 (1)	−0.0400 (1)	2.71 (3)
C(2)	0.3820 (2)	0.4705 (1)	−0.0927 (1)	2.75 (3)
C(3)	0.3957 (2)	0.5826 (2)	−0.1003 (1)	3.03 (4)
C(4)	0.3628 (2)	0.6503 (2)	−0.0544 (1)	3.05 (4)
C(4a)	0.3194 (2)	0.6088 (2)	−0.0010 (1)	2.83 (3)
C(4b)	0.2793 (2)	0.6556 (2)	0.0547 (1)	2.88 (3)
C(5)	0.2647 (2)	0.7603 (2)	0.0750 (1)	3.49 (4)
C(6)	0.2239 (2)	0.7763 (2)	0.1327 (1)	3.89 (4)
C(7)	0.1987 (2)	0.6898 (2)	0.1701 (1)	3.57 (4)
C(8)	0.2111 (2)	0.5840 (2)	0.1514 (1)	2.91 (3)
C(8a)	0.2524 (2)	0.5691 (2)	0.0931 (1)	2.77 (3)
N(9)	0.2730 (1)	0.4739 (1)	0.0631 (1)	2.96 (3)
C(9a)	0.3119 (2)	0.4973 (2)	0.0055 (1)	2.75 (3)
C(10)	0.4461 (2)	0.6269 (2)	−0.1569 (1)	4.22 (5)
C(11)	0.3317 (2)	0.3051 (1)	−0.0329 (1)	2.76 (3)
C(12)	0.4194 (2)	0.2548 (2)	−0.0725 (1)	3.18 (4)
C(13)	0.3979 (2)	0.2926 (2)	−0.1389 (1)	3.12 (4)
C(14)	0.4833 (2)	0.2471 (2)	−0.1807 (1)	4.28 (5)
C(15)	0.2793 (2)	0.2635 (2)	−0.1635 (1)	3.67 (4)
C(16)	0.1868 (2)	0.2953 (2)	−0.1219 (1)	3.59 (4)
C(17)	0.2142 (2)	0.2602 (2)	−0.0549 (1)	3.10 (4)
C(18)	0.1227 (2)	0.2856 (2)	−0.0115 (1)	3.50 (4)
C(19)	0.1171 (2)	0.2272 (2)	0.0412 (1)	6.14 (6)
C(20)	0.0423 (2)	0.3703 (3)	−0.0263 (1)	6.01 (6)
O(21)	0.4130 (1)	0.4093 (1)	−0.1417 (1)	3.28 (3)
C(1′)	0.1863 (2)	0.5046 (2)	0.2579 (1)	3.03 (4)
C(2′)	0.0998 (2)	0.4871 (2)	0.2966 (1)	3.36 (4)
C(3′)	0.1112 (2)	0.5015 (2)	0.3614 (1)	3.62 (4)
C(4′)	0.2144 (2)	0.5296 (2)	0.3884 (1)	3.64 (4)
C(4a′)	0.3067 (2)	0.5415 (2)	0.3521 (1)	3.17 (4)
C(4b′)	0.4253 (2)	0.5567 (2)	0.3661 (1)	3.26 (4)
C(5′)	0.4919 (2)	0.5672 (2)	0.4211 (1)	4.02 (5)
C(6′)	0.6065 (2)	0.5744 (2)	0.4181 (1)	4.87 (5)
C(7′)	0.6552 (2)	0.5726 (2)	0.3612 (1)	4.91 (5)
C(8′)	0.5922 (2)	0.5624 (2)	0.3059 (1)	4.10 (5)
C(8a′)	0.4761 (2)	0.5546 (2)	0.3093 (1)	3.24 (4)
N(9′)	0.3936 (1)	0.5398 (1)	0.2621 (1)	3.21 (3)
C(9a′)	0.2902 (2)	0.5298 (2)	0.2878 (1)	2.88 (3)
C(10′)	0.0124 (2)	0.4815 (2)	0.3999 (1)	5.09 (5)
C(11′)	0.1706 (2)	0.4900 (2)	0.1885 (1)	3.06 (4)
C(12′)	0.0457 (2)	0.4728 (2)	0.1697 (1)	3.88 (4)
C(13′)	−0.0137 (2)	0.4017 (2)	0.2141 (1)	4.12 (5)
C(14′)	0.0358 (2)	0.2896 (2)	0.2198 (1)	4.84 (5)
C(15′)	−0.1395 (2)	0.3984 (2)	0.1969 (1)	5.99 (6)
O(21′)	−0.0051 (1)	0.4532 (1)	0.2747 (1)	4.39 (3)

oxygen-linked tertiary methyl groups [δ_H 1.40, 1.44 (6H)] as well as a broad triplet [δ_H 4.63 (1H)] and several aromatic protons (see Experimental). Further structural assignment of **1** using NMR techniques was not possible due to the broadening of signals in the spectrum.

The complete structure and relative stereochemistry of **1** were obtained from a single-crystal X-ray analysis. The crystal structure was solved by direct methods[8] using data recorded on an Enraf–Nonius CAD-3 diffractometer (Ni-filtered CuK_α radiation), and atomic parameters were refined to $R = 0.045$ over 3280 statistically significant reflections.[1] For the present work, intensity data were recorded on an Enraf–Nonius CAD-4 diffractometer. Crystal data and data collection parameters are summarized in Table I. Atomic coordinates derived from our earlier analysis were used as initial input into the structure-factor

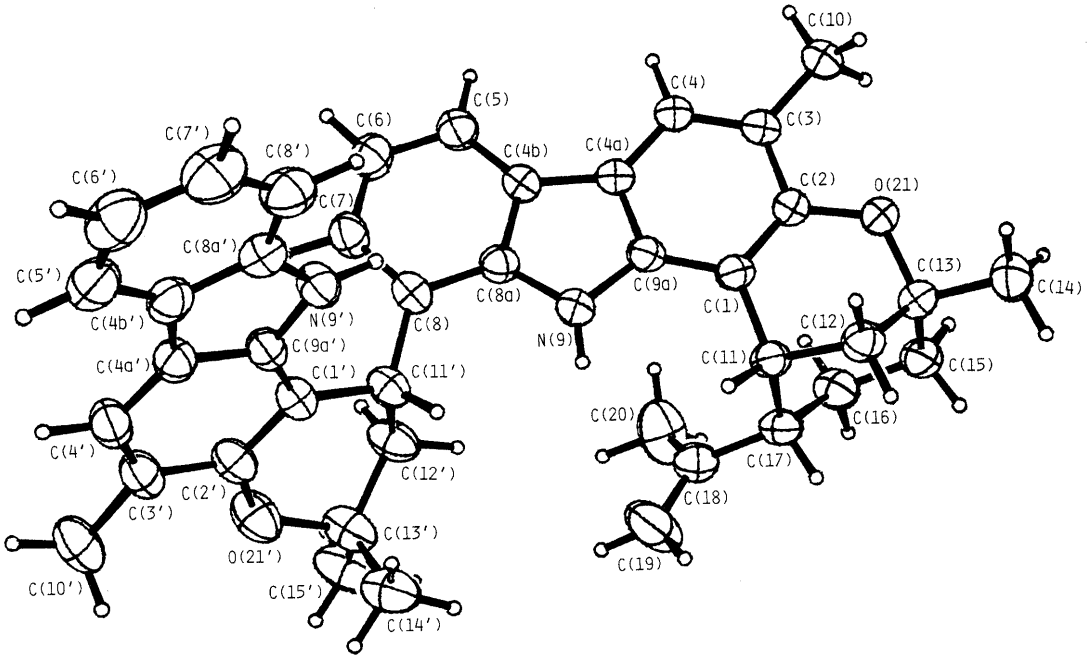

Fig. 1. ORTEP Diagram (50% Probability Ellipsoids) Showing the Structure and Solid-State Conformation of One Enantiomer of Murrafoline-A (**1**)
Small circles represent hydrogen atoms.

TABLE III. ^{1}H-NMR Data for Murrafoline-B (**2**), -C (**5**), -D (**3**), -G (**4**), and -H (**6**)

	2	**5**	**3**	**4**	**6**
3-Me	2.35 (3H, s)	2.39 (3H, s)	2.38 (3H, s)	2.39 (3H, s)	2.38 (3H, s)
H-4	7.77 (s)	7.82 (s)	7.79 (s)	7.77 (s)	7.78 (s)
H-5	7.86 (d, 7.8)	7.92 (d, 7.8)	7.89 (d, 8.6)	7.89 (d, 8.6)	7.89 (d, 8.1)
H-6	6.95 (t, 7.8)	7.01 (t, 7.8)	6.96—7.04	6.99 (t, 8.6)	6.97—7.05
H-7	6.99 (t, 7.8)	7.04 (t, 7.8)	(3H, overlapped)	7.00 (t, 8.6)	(3H, overlapped)
H-8	6.92 (t, 7.8)	6.97 (d, 7.8)		6.89 (d, 8.6)	
N–H	8.75 (br s)	8.97 (br s)	8.33 (br s)	8.04 (br s)	8.26 (br s)
10-Me	1.37 (3H, s)	1.41 (6H, s)	1.41 (3H, s)	1.58 (3H, s)	1.41 (3H, s)
	1.38 (3H, s)		1.46 (3H, s)	1.60 (3H, s)	1.47 (3H, s)
H-11	2.00 (m)	2.00 (m)	2.05 (m)	2.24 (dd, 12.2, 14.0)	2.00 (m)
	2.61 (dd, 7.3, 13.7)	2.62 (dd, 7.7, 13.7)	2.38 (m)	2.46 (dd, 6.7, 14.0)	2.38 (m)
H-12	5.16 (t, 7.8)	5.09 (t, 7.7)	4.62 (dd, 6.7, 11.0)	5.79 (dd, 6.7, 12.2)	4.60 (dd, 6.6, 11.0)
1′-OMe	3.93 (3H, s)	—	3.97 (3H, s)	3.96 (3H, s)	—
H-2′	6.80 (s)	—	6.81 (s)	6.66 (s)	—
3′-Me	2.46 (3H, s)	2.33 (3H, s)	2.45 (3H, s)	1.73 (3H, s)	2.26 (3H, s)
H-4′	7.47 (s)	7.77 (s)	7.39 (s)	—	7.64 (s)
H-5′	7.84 (d, 7.8)	7.81 (d, 7.8)	8.00 (d, 1.8)	8.35 (d, 8.6)	7.93 (d, 1.8)
H-6′	6.91 (t, 7.8)	6.91 (t, 7.8)	—	7.21 (t, 8.6)	—
H-7′	6.86 (d, 7.8)	6.75 (d, 7.8)	7.17 (dd, 1.8, 8.6)	7.43 (t, 8.6)	7.09 (dd, 1.8, 8.1)
H-8′	—	—	7.43 (d, 8.6)	7.69 (d, 8.6)	7.30 (d, 8.1)
N–H	9.96 (br)	10.11 (br)	10.19 (br)	10.48 (br)	10.25 (br)
10′-Me	—	1.48 (6H, s)	—	—	1.46 (6H, s)
H-11′	—	5.78 (d, 9.5)	—	—	5.77 (d, 9.5)
H-12′	—	6.92 (d, 9.5)	—	—	6.91 (d, 9.5)

Values are in δ ppm. Figures in parentheses are coupling constant (J) in Hz. All proton signals appeared as 1H, unless otherwise stated.

calculations. Full-matrix least-squares refinement of positional and thermal parameters (anisotropic C, N, O; isotropic H) converged at $R=0.041$ over 4010 reflections. A view of the solid-state conformation, with the atom numbering scheme, is presented in Fig. 1. Atomic parameters are listed in Table II.[9] Corresponding bond lengths in the carbazole moieties agree well, and all bonded distances are in accord with expected values.[10]

Structures of Other Murrafolines Murrafolines (**2**—**6**) showed common features in their UV and ^{1}H-NMR spectra and contained a 12-substituted dihydrogirinimbine (**A**) skeleton as a common structural unit. This fact was suggested by the following spectral data. (a) In the UV spectra, a strong absorption appeared in the range of 239 to 243 nm and a broad band with fine structure appeared in the range of 290 to 360 nm, suggesting the presence of the carbazole nucleus[11-13] in these alkaloid molecules. (b) The ^{1}H-NMR spectra (Table III) showed singlets due to an aryl methyl and a lone aromatic proton, which is characteristic of a deshielded H-4 in the carbazole

TABLE IV. ^{13}C-NMR Data for Murrafoline-B (**2**), -C (**5**), -D (**3**), and -G (**4**)

Carbon No.	2	5	3	4
1	106.76	106.55	107.48	108.57
2	153.38	153.43	152.64	152.14
3	120.08	119.23	119.52	120.24
3-Me	17.60	17.60	17.16	17.57
4	121.20	122.22	120.59	120.56
4a	117.13	117.07	116.67	117.03
4b	124.73	125.55	124.21	124.61
5	119.75	120.00	119.39	119.84
6	119.83	119.82	119.46	119.93
7	124.73	125.55	124.21	124.72
8	111.84	111.83	111.38	111.99
8a	141.14	141.17	140.48	140.90
9a	139.17	139.15	138.89	138.93
10	75.88	75.79	75.25	75.85
10-Me	25.35	25.70	24.46	24.51
	30.30	30.03	30.00	30.30
11	43.68	43.61	46.71	40.82
12	34.04	33.72	38.62	34.10
1′	147.18	106.24	146.65	145.65
1′-OMe	56.32	—	55.84	56.34
2′	109.09	151.12	108.53	111.89
3′	130.48	119.82	129.61	128.76
3′-Me	22.40	16.74	21.85	20.73
4′	113.57	121.23	113.26	128.21
4′a	125.32	118.67	124.54	124.16
4′b	126.12	125.97	124.26	124.61
5′	120.00	118.67	120.43	123.72
6′	120.72	120.94	135.40	120.31
7′	125.32	124.78	126.03	126.22
8′	128.16	127.58	112.65	113.00
8′a	139.97	140.12	140.09	141.97
9′a	129.84	136.98	129.61	130.82
10′-Me		28.34		
		28.34		
10′		76.99		
11′		130.38		
12′		119.23		

Assignments were established by ^{1}H–^{13}C COSY and/or HMBC techniques.

nucleus.[12,13] (c) Observation of a nuclear Overhauser effect (NOE) between these protons suggested that the aryl methyl was located at C-3. (d) In the studies of the ^{1}H-NMR spectra using ^{1}H–^{1}H correlation spectroscopy (COSY), each alkaloid showed a pair of four-spin proton systems (H-5, 6, 7, and 8) assigned to protons on the non-substituted carbazole A-ring. (e) Signals of a three-spin proton system (H_2-11 and H-12) accompanied by two tertiary methyls attached to an oxygenated carbon suggested the presence of a 2,2-dimethyl-4-substituted dihydrobenzopyran ring. (f) In the MS, an intense peak at *m/z* 248 corresponding to fragment *a*, due to cleavage at the benzylic position followed by the loss of hydrogen and methyl radicals from the molecular ion, supported the presence of structural unit **A**, corresponding to the dihydro derivative of girinimbine (**7**),[5,6] in each of the murrafoline molecules.

Therefore, structural elucidations of these five murrafolines require determination of the substituents at C(12) on the common structural unit **A**.

(a) Structures of Murrafoline-B (**2**), -D (**3**), and -G (**4**): These three murrafolines were obtained as racemates and showed the same molecular formula, $C_{32}H_{30}N_2O_2$, by HR-MS. Moreover, in the ^{1}H- and ^{13}C-NMR spectra (Tables III and IV, respectively), each alkaloid showed the presence of an additional aryl methyl (δ_H 1.73 or 2.45—2.46, δ_C 20.73—22.44), a methoxy (δ_H 3.93—3.97, δ_C 55.84—56.34), a lone aromatic proton (H-2′) (δ_H 6.66—6.81), and an N–H group (δ_H 9.96—10.48) as common moieties in addition to signals due to structural unit **A**. In difference NOE experiments, irradiation of the methoxy signal gave an enhancement at the lone aromatic proton (H-2′), which also showed an area increase upon irradiation of the aryl methyl signal, indicating that the methoxy and aryl methyl are located at C-1 and C-3, respectively, in the carbazole nucleus. Therefore, another carbazole unit was assigned to the substituted murrayafoline-A (**8**), which also occurred in the same plant.[6] Determination of the location of the linkage on **8** to **A** was easily established by analysis of the characteristic signal patterns in the aromatic proton region of each murrafoline using proton decoupling and/or ^{1}H–^{1}H COSY experiments.

Murrafoline-B (**2**)[2] was obtained as colorless needles from methanol, mp 234—237 °C. In the ^{1}H-NMR spectrum of **2**, a lower-field singlet, characteristic of H-4′ (δ_H 7.47) in the carbazole nucleus,[12,13] and ABC-type signals *ortho*-coupled with each other at δ_H 6.86 (d, J=7.8 Hz), 6.91 (t, J=7.8 Hz), and 7.84 (d, J=7.8 Hz), including a deshielded H-5′ proton,[12,13] were observed in addition to the signals described above. These data together with NOE enhancements between H-12 (δ_H 5.16) and both N–H protons (δ_H 8.75, 9.96) suggested that the linkage of **8** was located at C-8′. Therefore, murrafoline-B was proposed to have structure **2**.

Murrafoline-D (**3**)[2] was isolated as a colorless amorphous powder. The ^{1}H-NMR spectrum of **3** showed a lower-field H-4′ singlet at δ_H 7.39 and ABC-type signals at δ_H 8.00 (d, J=1.8 Hz), 7.17 (dd, J=1.8, 8.6 Hz), and 7.43 (d, J=8.6 Hz). Among these signals, the deshielded H-5′ signal[12,13] appeared as a *meta*-coupled doublet, suggesting that the linkage of **8** was located at C-6′. NOE increments of the signals of H-5′ (δ_H 8.00) and H-7′ (δ_H 7.17) upon irradiation of H-12 (δ_H 4.62), and three-bond correlations between H-12 and both C(5′) (δ_C 120.43) and C(7′) (δ_C 126.03) in the ^{1}H detected heteronuclear multiple bond connectivity (HMBC) spectrum supported the presence of the linkage of **8** at C-6′. On the basis of these data coupled with other ^{13}C–^{1}H three-bond correlations as shown by arrows in Fig. 2, murrafoline-D was determined to have structure **3**.

Murrafoline-G (**4**) was obtained as a colorless oil. In the ^{1}H-NMR spectrum, an additional four-spin system due to a non-substituted A′-ring of the murrayafoline-A (**8**) unit and the lack of the deshielded H-4′ signal[12,13] suggested that the linkage of **8** was located at C-4′. Based on these observations, murrafoline-G was determined to have structure **4**.

Notable spectral features of this structure include an abnormal higher-field shift of the 3′-methyl signal (δ_H 1.73) compared to the 3-methyl signal (δ_H 2.39) and a 24% NOE enhancement of H-5′ (δ_H 8.35) upon irradiation of H-12 (δ_H 5.79).

(b) Structures of Murrafoline-C (**5**) and -H (**6**): These two murrafolines were also obtained as racemates, and found to have the same molecular formula, $C_{36}H_{34}N_2O_2$, by HR-MS. In ^{1}H-NMR spectra, both alkaloids showed common signals due to an aryl methyl (δ_H 2.33, 2.26),

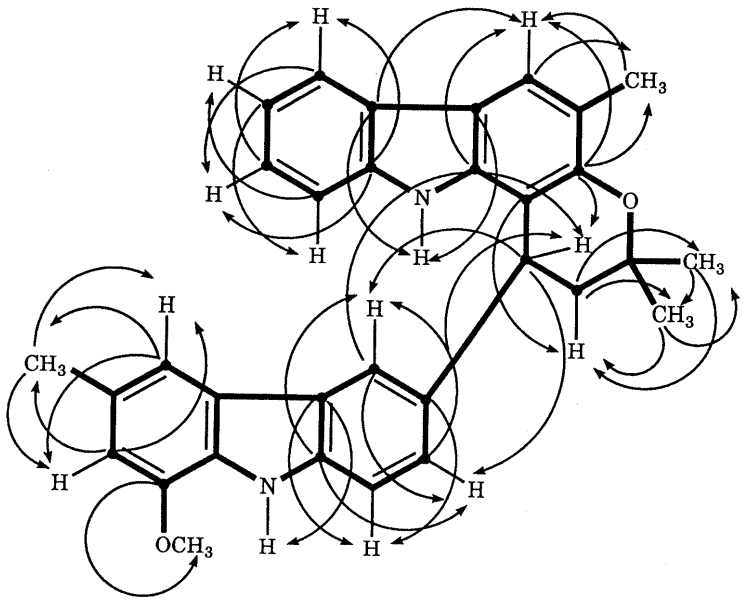

Fig. 2. ^{13}C–^{1}H Correlations (^{3}J) from the HMBC Spectrum of Murrafoline-D (**3**)

2,2-dimethylpyran ring [δ_H 1.48, 1.46; 5.78, 5.77 (d, J=9.5 Hz); 6.92, 6.91 (d, J=9.5 Hz)], and a lower-field characteristic of H-4′ (δ_H 7.77, 7.64),[12,13] respectively, in addition to signals due to the skeleton **A**, suggesting the presence of another carbazole unit: girinimbine (**7**).[5,6] Therefore, the structure establishments of **5** and **6** required determination of the location of the substituent in the girinimbine (**7**) unit.

Murrafoline-C (**5**)[2] was isolated as a colorless oil from the plant collected in February. The ^{1}H-NMR spectrum revealed an ABC-type signal in the aromatic proton region at δ_H 7.81 (d, J=7.8 Hz), 6.91 (t, J=7.8 Hz), and 6.75 (d, J=7.8 Hz). The presence of a lower-field signal (δ_H 7.81) assignable to H-5′ in the carbazole nucleus[12,13] and the large coupling constant values (7.8 Hz) due to three *ortho*-located protons indicated that the **A**-moiety on **7** is located at C-8′. This was supported by long-range correlations through three bonds from H-12 (δ_H 5.09) to C(7′) (δ_C 124.78) and C(8′a) (δ_C 140.12) in the HMBC spectrum, and a 5% NOE enhancement of N–H (δ_H 10.11) upon irradiation of H-12. These results led us to conclude that murrafoline-C had structure **5**.

Murrafoline-H (**6**) was isolated as a colorless oil. The linkage of **7** to **A** was suggested to be at C-6′ by the following ^{1}H-NMR data. (a) Among the ABC-type signals due to protons on the A′-ring in the carbazole nucleus at δ_H 7.93 (d, J=1.8 Hz), 7.09 (dd, J=1.8, 8.1 Hz), and 7.30 (d, J=8.1 Hz), the lower-field signal at δ_H 7.93, which is characteristic of H-5′,[12,13] appeared as a doublet coupled with only a *meta*-located proton. (b) In the difference NOE experiment, irradiation of H-12 (δ_H 4.60) produced an 8% enhancement of H-5′ at δ_H 7.93. These data led us to propose that murrafoline-H had structure **6**.

Treatment of **7** and **8**, both of which are structural components of murrafolines, with Nafion 117[7] in refluxing aqueous methanol solution for 27 h gave three dimeric products, which were found to be natural murrafoline-D (**3**), -G (**4**), and -H (**6**) by comparisons of their ^{1}H-NMR spectra with those of authentic samples.

Experimental

Melting points were measured on a micromelting point hot-stage apparatus (Yanagimoto). ^{1}H- and ^{13}C-NMR spectra were recorded on GX-270 (JEOL) and GX-400 (JEOL) spectrometers, respectively, in acetone-d_6, unless otherwise stated. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. NOE and NOESY spectra were measured on the GX-400. ^{1}H–^{13}C long-range COSY and HMBC spectra were measured at J=5 and 8 Hz, respectively, on the GX-400. MS were recorded under electron impact (EI) conditions using an M-80 (Hitachi) or a JMS-HX-110 (JEOL) spectrometer with a direct inlet system. UV spectra were recorded on a UVIDEC-610C double-beam spectrophotometer (JASCO) in ethanol or methanol, IR spectra were recorded on an IR-810 (JASCO) in $CHCl_3$, and CD spectra were recorded on a J-600 (JASCO) in ethanol or methanol. Preparative TLC was performed on Kieselgel 60 F_{254} (Merck).

Extraction and Isolation The dried root bark (900 g) of *Murraya euchrestifolia* HAYATA collected at Kuantaochi (Nantou Hsien), Taiwan, in December was extracted with acetone at room temperature. The acetone extract was treated according to Chart 1 to give murrafoline-A (**1**) 30 mg, -B (**2**) 20 mg, -D (**3**) 10 mg, -G (**4**) 11 mg, and -H (**6**) 3 mg, as well as other components shown in Chart 1 and described in previous papers.[4] The ethanol extract of the dried root bark (2700 g) of the plant collected from the same area in February was treated in the manner described in the previous paper[14] to give murrafoline-C (**5**) 6 mg, -A (**1**) 70 mg, and -B (**2**) 18 mg, along with other known carbazoles.

Murrafoline-A (1)[1] Colorless needles from MeOH–CH_2Cl_2, mp 260—262 °C, $[\alpha]_D$ ±0 °C (c=1.0, $CHCl_3$). CD (EtOH, 200—350 nm): no absorption; UV λ_{max}^{MeOH} nm (log ε): 218 (4.66), 243 (4.86), 260 (sh, 4.66), 307 (4.45), 332 (sh, 3.91). IR ν_{max} cm^{-1}: 3450, 1630, 1610. ^{1}H-NMR δ_H (400 MHz, $CDCl_3$, 60 °C): 7.88 (1H, br d, J=7.3 Hz), 7.83 (1H, d, J=7.8 Hz), 7.73 (1H, s), 7.64 (1H, s), 7.16 (1H, br s), 7.07 (1H, t, J=7.3 Hz), 7.02 (1H, d, J=7.3 Hz), 6.87 (1H, d, J=7.3 Hz), 4.63 (1H, br t), 3.32 (1H, br s), 2.41 (3H, s), 2.31 (3H, s), 2.17 (1H, br), 2.00 (1H, br), 1.86 (1H, br), 1.54 (3H, s), 1.44 (6H, s), 1.40 (6H, s). ^{1}H-NMR δ_H (400 MHz, $CDCl_3$, 25 °C): 7.75 (1H, s), 6.90 (1H, br d), 2.41 (3H, s), 1.55 (3H, br s), 1.44 (3H, br s). Other unresolvable broad 1H signals appeared at δ_H 7.90, 7.85, 7.65, 7.53, 7.08—7.05 (2H), 4.83, 4.64, 3.95, 3.52, 3.17 (2H), 2.65, 2.30, 2.05, 1.95. ^{13}C-NMR δ_C (100 MHz, $CDCl_3$, 25 °C): 153.98, 138.47, 137.55, 123.67, 118.96, 118.85, 118.28, 116.22, 110.33, 105.74, 73.94, 48.76, 39.88, 37.40, 28.94, 24.43, 23.06, 16.92, 16.72. Other carbon signals could not be detected because of their broadening. EI-MS m/z (%): 594 (M^+, 46), 511 (13), 331 (13), 297 (M^{2+}, 8), 248 (19), 227 (14). HR-MS Calcd for $C_{41}H_{42}N_2O_2$: 594.3243. Found: 594.3218.

Murrafoline-B (2)[2] Colorless needles from MeOH, mp 234—237 °C. CD (MeOH, 200—350 nm): no absorption. UV λ_{max}^{MeOH} nm: 208, 226, 240, 292, 304, 330. IR ν_{max} cm^{-1}: 3450, 1630, 1615. EI-MS m/z (%): 474 (M^+, 100), 419 (30), 417 (32), 278 (14), 263 (9), 248 (31), 237 (M^{2+}, 24), 211 (3). HR-MS Calcd for $C_{32}H_{30}N_2O_2$: 474.2305. Found: 474.2325.

Difference NOE: irradiation of H-12, 10 and 4% enhancements of N–H protons at δ_H 9.96 and 8.75, respectively; irradiation of 3-Me, 14% enhancement of H-4; irradiation of 3′-Me, 8 and 11% enhancements of H-2′ and H-4′, respectively; irradiation of 1′-OMe, 21% enhancement of H-2′. HMBC (acetone-d_6): three-bond correlations: C(8′a)–H-12, C(7′)–H-12, C(12)–H-7′; two-bond correlation: C(8′)–H-12. Other long-range correlations in the HMBC spectrum were also consistent with structure **2**.

Murrafoline-C (5)[2] A colorless oil. CD (EtOH, 200—350 nm): no absorption. UV λ_{max}^{EtOH} nm: 224, 242, 251, 291, 328, 342. IR ν_{max} cm^{-1}: 3460, 1610. EI-MS *m/z* (%): 526 (M^+, 72), 511 (100), 469 (12), 263 (11), 256 (M^{2+}, 19), 248 (22), 227 (40). HR-MS Calcd for $C_{36}H_{34}N_2O_2$: 526.2618. Found: 526.2611. Difference NOE: irradiation of H-12, 3 and 5% enhancements of N–H protons at δ_H 8.97 and 10.11, respectively; irradiation of 3-Me, 7% enhancement of H-4; irradiation of 3′-Me, 7% enhancement of H-4′. HMBC (acetone-d_6): significant correlations, C(12)–H-7′; C(8a)–H-12 and H-5; C(7)–H-12.

Murrafoline-D (3) An amorphous powder. CD (MeOH, 200—350 nm): no absorption. UV λ_{max}^{MeOH} nm: 228, 240, 256 (sh.), 296, 332, 345. IR ν_{max} cm^{-1}: 3450. EI-MS *m/z* (%): 474 (M^+, 100), 420 (28), 419 (31), 418 (27), 417 (37), 403 (12), 402 (11), 248 (25), 237 (M^{2+}, 21), 211 (14), 210 (27), 209 (10). HR-MS Calcd for $C_{32}H_{30}N_2O_2$: 474.2305. Found: 474.2288; Calcd for $C_{17}H_{14}NO$: 248.1074. Found: 248.1041; Calcd for $C_{14}H_{13}NO$: 211.0996. Found: 211.0967. Difference NOE: irradiation of H-12, 8 and 4% enhancements of H-5′ and H-7′. NOESY: cross peaks were observed between 3-Me–H-4, 3′-Me–H-2′, 1′-OMe–H-2′. ^1H–^{13}C long-range COSY: three- and two-bond correlations between H-12–C(5′) and C(6′), respectively, were observed. HMBC: the results of ^{13}C–^1H three-bond long-range correlations are shown by arrows in Fig. 2.

Murrafoline-G (4) A colorless oil. CD (MeOH, 200—350 nm): no absorption. UV λ_{max}^{MeOH} nm: 229, 243, 254 (sh.), 284, 293, 302, 318 (sh.), 332, 343 (sh.), 348. IR ν_{max} cm^{-1}: 3450. EI-MS *m/z* (%): 474 (M^+, 100), 419 (14), 403 (41), 264 (41), 263 (20), 249 (17), 248 (72), 237 (M^{2+}, 15), 211 (28), 202 (14). HR-MS Calcd for $C_{32}H_{30}N_2O_2$: 474.2305. Found: 474.2280; Calcd for $C_{18}H_{18}NO$: 264.1387. Found: 264.1379; Calcd for $C_{17}H_{14}NO$: 248.1074. Found: 248.1071; Calcd for $C_{14}H_{13}NO$: 211.0996. Found: 211.0967. Difference NOE: irradiation of H-12, 24% enhancement of H-5′; irradiation of 3-Me, 7% enhancement of H-4; irradiation of 3′-Me, 10% enhancement of H-2′; irradiation of 1′-OMe, 12% enhancement of H-2′. HMBC: significant cross peaks appeared at H-12–C-4′ and H-12–C-4′a.

Murrafoline-H (6) A colorless oil. CD (MeOH, 200—350 nm): no absorption. UV λ_{max}^{MeOH} nm: 216, 239, 256 (sh.), 281, 292, 304, 331, 345, 359. IR ν_{max} cm^{-1}: 3450. EI-MS *m/z* (%): 526 (M^+, 100), 511 (19), 471 (16), 470 (13), 469 (16), 455 (18), 256 (21), 248 (29), 228 (25), 227 (19). HR-MS Calcd for $C_{36}H_{34}N_2O_2$: 526.2618. Found: 526.2607; Calcd for $C_{17}H_{14}NO$: 248.1074. Found: 248.1095. Difference NOE: irradiation of H-12, 8% enhancement of H-5′; irradiation of 3-Me, 7% enhancement of H-4; irradiation of 3′-Me, 8% enhancement of H-4′.

Dimerization Reaction of 7 and 8 A mixture of a methanolic solution (1 ml) of **7**[5,6] (20 mg) and **8**[6] (20 mg) and an aqueous solution of Nafion 117 (Aldrich)[7] (1 ml) was refluxed for 27 h. The reaction mixture was filtered and the filtrate was subjected to preparative silica gel TLC using a mixture of CH_2Cl_2 and *n*-hexane (1 : 1) as a developing solvent to obtain three reaction products as colorless oils, along with the starting materials, **7** (10 mg) and **8** (16 mg). The product obtained from the most polar zone (Rf=0.26, 4.0 mg) was found to be identical with natural murrafoline-H (**6**) by ^1H-NMR comparison. Two other products were isolated from TLC zones of Rf=0.30 (4.0 mg) and Rf=0.40 (1.5 mg) and found to be identical with **3** and **4**, respectively, by comparisons of the ^1H-NMR spectra with those of natural specimens. When the reaction was continued, the formation of several oligomers of **7** was observed.

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