# New Carbazole Alkaloids from *Murraya euchrestifolia*

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**Two new monomeric and one dimeric carbazole alkaloids were isolated from root bark of *Murraya euchrestifolia* HAYATA collected in Taiwan. Their structures were elucidated by spectrometric and synthetic studies. The structures of the monomeric carbazoles were assigned as 3-formyl-7-hydroxy-9*H*-carbazole (1) and *N*-methoxy-3-hydroxymethyl-9*H*-carbazole (2). The dimeric carbazole, named chrestifoline-D (9), was found to be identical with the oxidation product of bismurrayafoline-A (10).**

**Keywords** *Murraya euchrestifolia*; Rutaceae; alkaloid; carbazole; 3-formyl-7-hydroxy-9*H*-carbazole; *N*-methoxy-3-hydroxymethyl-9*H*-carbazole; chrestifoline-D; bismurrayafoline-A; dimer

*Murraya euchrestifolia* HAYATA (Rutaceae) is a rich source of carbazole alkaloids. Many kinds of monomeric and dimeric carbazole alkaloids have been isolated from this plant.[1)] In a continuation of our study of alkaloidal components from *M. euchrestifolia*,[1)] we have isolated two new monomeric and a new dimeric carbazole alkaloid, and their structures were determined. The acetone extract of root bark of the plant was subjected repeatedly to silica gel column and preparative thin layer chromatographies (TLC) to obtain three new carbazoles as well as known carbazole alkaloids as shown in Fig. 1.

## Results and Discussion

**Structure of 3-Formyl-7-hydroxy-9*H*-carbazole (1)** This compound was obtained as a colorless powder from acetone. The molecular formula $C_{13}H_{19}NO_2$ was confirmed by high-resolution mass spectrometry (HR-MS). The ultraviolet (UV) spectrum suggested the presence of a carbazole nucleus.[2)] The infrared (IR) spectrum showed an absorption band due to an OH and/or NH group at $\nu_{max}$ 3336 $cm^{-1}$. A lower-field 1H singlet at $\delta$ 10.10 in the proton nuclear magnetic resonance ($^1$H-NMR) spectrum and an IR band at $\nu_{max}$ 1673 $cm^{-1}$ suggested the presence of a formyl group in the molecule. The remaining $^1$H-NMR signals were observed as two sets of ABC-type signals. One of them appeared at $\delta$ 7.53 (1H, d, $J$=8.5 Hz), 7.98 (1H, dd, $J$=0.7, 8.5 Hz), and 8.62 (1H, d, $J$=0.7 Hz), and they were assignable to H-1, H-2, and H-4, respectively, because the most deshielded *meta*-coupled proton signal at $\delta$ 8.62 was due to H-4, located *ortho* to the formyl group.[2,3)] The signals of another three-spin system at $\delta$ 7.33 (1H, dd, $J$=8.0, 4.5 Hz), 7.48 (1H, d, $J$=4.5 Hz) and 8.15 (1H, d, $J$=8.0 Hz) were assigned to H-6, H-8, and deshielded H-5, respectively. These spectral data indicated this alkaloid to be 3-formyl-7-hydroxy-9*H*-carbazole (**1**).

**Structure of *N*-Methoxy-3-hydroxymethyl-9*H*-carbazole (2)** This compound was isolated as a colorless oil. The HR-MS showed the molecular formula $C_{14}H_{13}NO_2$ for this alkaloid. The UV spectrum was similar to *N*-methoxy-3-formyl-9*H*-carbazole (**3**), which had been isolated from root bark of the same plant,[3)] except for some bathochromic shifts of the bands in the spectrum of **3**. In the $^1$H-NMR spectrum, a 3H singlet was seen at $\delta$ 4.13 and a 2H singlet at $\delta$ 4.86, which were assignable to methoxy and hydroxymethyl protons, respectively. In the aromatic proton region, deshielded proton signals at $\delta$ 8.06 (1H, s) and 8.04 (1H, d, $J$=8.8 Hz), overlapped signals corresponding to four protons at $\delta$ 7.50, and a 1H signal at $\delta$

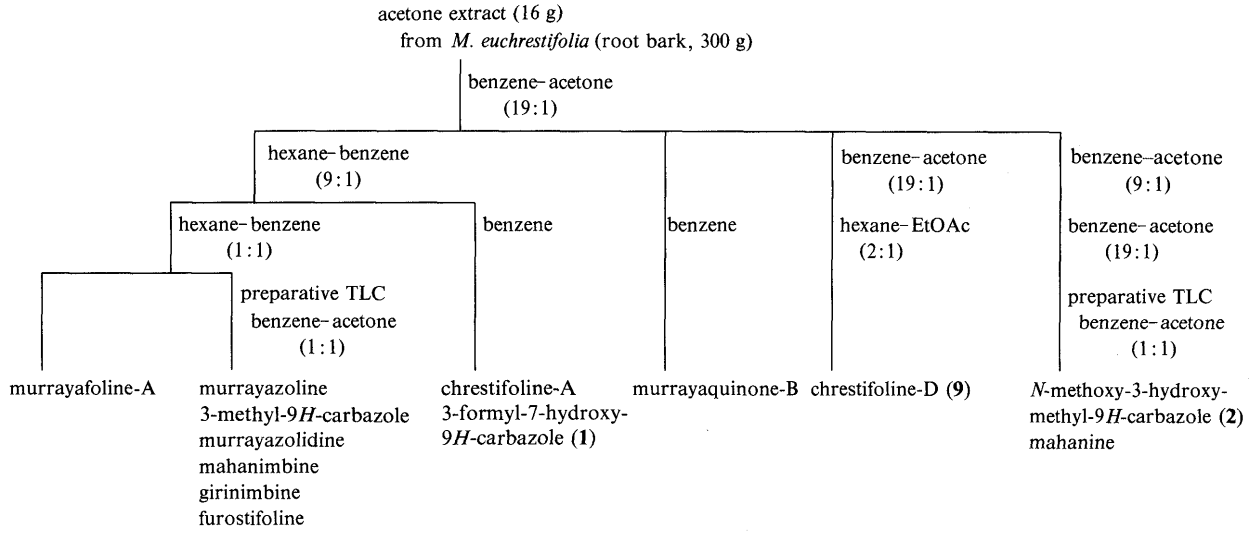


Fig. 1. Isolation Procedure of Carbazole Alkaloids by Silica Gel Column Chromatography

7.25 (t, $J=8.8$ Hz) were observed. The mass spectrum (MS) showed the significant fragment peak at $m/z$ 167 due to the loss of methoxy and hydroxymethyl radicals from the molecular ion with transfer of two hydrogens. In nuclear Overhauser effect (NOE) experiments, irradiation of the 2H singlet at $\delta$ 4.86 showed 15 and 11% enhancements of the signals at $\delta$ 8.06 (H-4) and one of the overlapped signals at $\delta$ 7.50, respectively. However, on irradiation of the methoxy proton at $\delta$ 4.13, no NOE enhancement was observed on any proton signal. Catalytic hydrogenation of this alkaloid in the presence of palladium–carbon (Pd–C) gave **4**, which was found to be identical with the synthetic 3-hydroxymethyl-9*H*-carbazole (**4**) derived from a commercially available carbazole (**5**) (Experimental). Further, the $NaBH_4$ reduction of **3**[3] gave **2**, which was also identical with the natural specimen isolated at this time. On the basis of these data, the structure of the alkaloid was determined as *N*-methoxy-3-hydroxymethyl-9*H*-carbazole (**2**).

**The Structure of Chrestifoline-D (9)** Chrestifoline-D (**9**) was also isolated as a colorless oil. The molecular formula $C_{28}H_{22}N_2O_3$ was determined by HR-MS. The UV spectrum exhibited bands at $\lambda_{max}$ 223, 241, 250, 275, 291, and 335 nm indicative of the presence of the carbazole nucleus.[2] The IR spectrum showed typical bands due to carbonyl and NH groups at $\nu_{max}$ 1680 and 3370 $cm^{-1}$, respectively. The $^1$H-NMR spectrum of chrestifoline-D (**9**) indicated the presence of a formyl group, two methoxyls, an isolated methylene, two sets of four aromatic proton systems, and two pairs of *meta*-coupled aromatic protons (see Experimental). This $^1$H-NMR signal pattern was similar to that of bismurrayafoline-A[4] (**10**) except for the presence of a lower field 1H singlet due to a formyl group instead of an aryl methyl signal in the spectrum of **10**. In the MS, the significant base fragment ion at $m/z$ 210 represented the lower portion of formula **9**. The same ion was prominent in the MS of **10**.[4] These spectral data together with the results of NOE experiments (see Experimental) suggested that the structure of chrestifoline-D was represented by formula **9**.

Oxidation of bismurrayafoline-A (**10**) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in benzene solution afforded **9** as a colorless oil, which was shown to be identical with natural chrestifoline-D by $^1$H-NMR, IR, UV, and MS comparisons. These data led to the structure **9** for chrestifoline-D.

Known carbazole alkaloids, murrayazoline,[5] 3-methylcarbazole,[6] murrayazolidine,[7] mahanimbine,[8] murrayafoline-A,[9] girinimbine,[9,10] furostifoline,[11] chrestifoline-A,[12] murrayaquinone-B,[9] and mahanine[13] were also isolated and characterized.

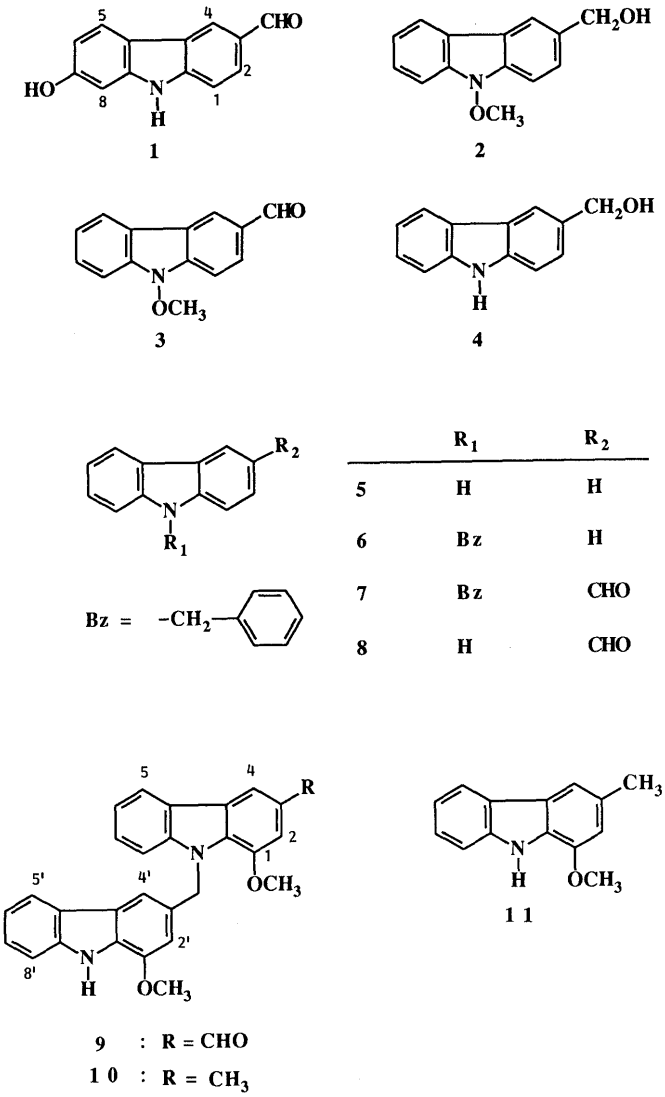


Chart 1

## Experimental

$^1$H- and $^{13}$C-NMR spectra were recorded on a GX-270 (JEOL) or GX-400 (JEOL) spectrometer in $CDCl_3$, unless otherwise stated. Chemical shifts are shown in $\delta$ values (ppm) with tetramethylsilane (TMS) as an internal reference. All MS and HR-MS were taken under electron impact (EI) conditions using a Hitachi M-80 or JMS-HX-110 (JEOL) mass spectrometer having a direct inlet system. IR spectra were recorded on a JASCO IR-810 IR spectrophotometer in $CHCl_3$, unless otherwise stated and UV spectra on a JASCO UVIDEC-610C double beam spectrophotometer in MeOH.

**Extraction and Isolation** The acetone extract (16 g) of dried root bark (300 g) of *Murraya euchrestifolia* HAYATA collected at Kuantaochi, Nantou Hsien, Taiwan, in August, 1987 was treated in the manner shown in Fig. 1 to give murrayafoline-A (4.5 g), murrayazoline (1 mg), 3-methylcarbazole (1 mg), murrayazolidine (1 mg), mahanimbine (30 mg), girinimbine (7 mg), furostifoline (5 mg), chrestifoline-A (2.6 mg), 3-formyl-7-hydroxy-9*H*-carbazole (**1**) (1.5 mg), murrayaquinone-B (3 mg), chrestifoline-D (**9**) (6 mg), *N*-methoxy-3-hydroxymethyl-9*H*-carbazole (**2**) (2.5 mg), and mahanine (30 mg). The known carbazoles were characterized by $^1$H-NMR, MS, UV, and/or IR spectra.

**3-Formyl-7-hydroxy-9*H*-carbazole (1)** A colorless powder from acetone. UV $\lambda_{max}$ nm: 233, 245 (sh), 273, 288, 326. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3336 (OH, NH), 1673 (C=O), 1600. $^1$H-NMR $\delta$: 7.33 (1H, dd, $J=8.0$, 4.5 Hz, H-6), 7.48 (1H, d, $J=4.5$ Hz, H-8), 7.53 (1H, d, $J=8.5$ Hz, H-1), 7.98 (1H, dd, $J=0.7$, 8.5 Hz, H-2), 8.15 (1H, d, $J=8.0$ Hz, H-5), 8.41 (1H, br s, NH), 8.62 (1H, d, $J=0.7$ Hz, H-4), 10.10 (1H, s, CHO). MS $m/z$ (%): 211 ($M^+$, 5), 196 (20), 195 (100), 194 (84), 167 (14), 166 (57), 139 (35). HR-MS: Calcd for $C_{13}H_9NO_2$: 211.0633. Found: 211.0633.

***N*-Methoxy-3-hydroxymethyl-9*H*-carbazole (2)** A colorless oil. UV $\lambda_{max}$ nm: 237, 263, 283, 287, 293, 334, 350. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3600, 3450, (OH), 1610. $^1$H-NMR $\delta$: 4.13 (3H, s, $OCH_3$), 4.86 (2H, s, $CH_2OH$), 7.25 (1H, t, $J=8.8$ Hz, H-6), 7.50 (4H, m), 8.04 (1H, d, $J=8.8$ Hz, H-5), 8.06 (1H, br s, H-4). $^1$H-NMR (acetone-$d_6$) $\delta$: 4.17 (3H, s, $OCH_3$), 4.78 (2H, s, $CH_2OH$), 5.15 (1H, br s, OH), 7.25 (1H, t, $J=8.8$ Hz, H-6), 7.46—7.58 (4H, m), 8.11 (2H, m). MS $m/z$ (%): 227 ($M^+$, 100), 212 (47), 196 (27), 167 (84). NOE: irradiation of $CH_2$ ($\delta$ 4.86), 15% and 11% enhancements of H-4 ($\delta$ 8.06) and H-2 ($\delta$ 7.50), respectively; irradiation of $OCH_3$ ($\delta$ 4.13), no NOE. $^{13}$C-NMR $\delta$: 137.95 (s), 137.31 (s), 130.14 (s), 126.68 (d), 126.16 (d), 120.52 (d), 120.49 (d), 120.11 (d), 120.01 (s), 119.90 (s), 108.35 (d), 108.25 (d), 72.43 (t), 63.46 (q). HR-MS: Calcd for

$C_{14}H_{13}NO_2$: 227.0944. Found: 227.0895.

**Reduction of 3** $NaBH_4$ (10 mg) was added to a methanolic solution (5 ml) of *N*-methoxy-3-formyl-9*H*-carbazole (**3**) (4 mg) isolated from stem bark of *M. euchrestifolia* at room temperature. After 2 h, the solvent was evaporated off *in vacuo* and the residue was subjected to preparative TLC to give **2** (3.2 mg), which was identical with a natural specimen of *N*-methoxy-3-hydroxymethyl-9*H*-carbazole (**2**) by $^1$H-NMR, IR, UV, and MS comparisons.

**Catalytic Reduction of 2** Natural **2** (3 mg) in dry THF (10 ml) was stirred in $H_2$ gas in the presence of Pd-C (5 mg). After the reaction, the catalyst was filtered off and the filtrate was concentrated to dryness. The residue was subjected to preparative TLC (acetone–hexane = 1 : 3) to give **4** (1.5 mg), which was found to be identical with synthetic 3-hydroxymethyl-9*H*-carbazole (**4**) by $^1$H-NMR, IR, UV, and MS comparisons.

**Synthesis of 4** A mixture of a commercially available carbazole (**5**) (1.0 g), KOH (fine powder, 1.6 g), and benzyl chloride (2.3 g) in THF (10 ml) was refluxed for 2 d. The solvent was evaporated off *in vacuo* and the residue was dissolved in water. The solution was extracted with $CHCl_3$. The extract was dried with anhydrous $MgSO_4$ and concentrated to dryness. The residue was subjected to silica gel column chromatography with hexane–ethyl acetate (10 : 1) to give **6** as colorless prisms from hexane in quantitative yield. **6**: mp 108—111 °C. UV $\lambda_{max}$ nm: 210, 236, 260, 290 (sh), 292, 328, 341. IR $\nu_{max}$ cm$^{-1}$: 1630, 1600. $^1$H-NMR $\delta$: 5.52 (2H, s), 7.14 (2H, m), 7.21—7.30 (5H, m), 7.30—7.46 (4H, m), 8.13 (2H, d, $J$=7.7 Hz). MS *m/z* (%): 257 ($M^+$, 83), 180 (7), 167 (5), 166 (13), 140 (6).

**6** (100 mg) was added to a mixture of *N*-methylformanilide (63 mg) and $POCl_3$ (71 mg) in a flask with cooling. The mixture was stirred for 16 h at room temperature and then for 2 h at 60 °C. The reaction mixture was diluted with ice water and extracted with EtOAc. The organic layer was dried with anhydrous $MgSO_4$ and concentrated to dryness. The residue was subjected to preparative TLC with $CHCl_3$–hexane (1 : 1) to give **7** (82 mg) as colorless prisms from EtOAc–MeOH solution. **7**: mp 129—132 °C. UV $\lambda_{max}$ nm: 210, 235, 242 (sh), 273, 289, 330, 342 (sh), 352 (sh). IR $\nu_{max}$ cm$^{-1}$: 1685, 1630, 1600. $^1$H-NMR $\delta$: 5.57 (2H, s), 7.14 (2H, m), 7.23—7.53 (7H, m), 7.98 (1H, dd, $J$=8.4, 1.4 Hz), 8.20 (1H, d, $J$=7.1 Hz), 8.65 (1H, d, $J$=1.4 Hz), 10.11 (1H, s). MS *m/z* (%): 285 ($M^+$, 100), 254 (8), 208 (5), 195 (9), 194 (9), 166 (28), 165 (17), 164 (15). *Anal.* Calcd for $C_{20}H_{15}NO$: C, 84.19; H, 5.30; N, 4.19. Found: C, 83.89; H, 5.35; N, 4.67.

An anhydrous THF solution of **7** (1.16 g) was added to a liquid $NH_3$ solution of Na (3.0 g). The solution was stirred for 2 h and a small amount of $NH_4Cl$ was added. The liquid $NH_3$ was evaporated off at room temperature and the residue was extracted with EtOAc. The EtOAc extract was dried with anhydrous $MgSO_4$ and concentrated to dryness. The residue was subjected to silica gel column chromatography to give **8** (108 mg) as a colorless oil. **8**: UV $\lambda_{max}$ nm: 233, 242 (sh), 273, 288, 326. IR $\nu_{max}$ cm$^{-1}$: 3460, 1685, 1630, 1605. $^1$H-NMR (acetone-$d_6$) $\delta$: 7.24 (1H, t, $J$=8.1 Hz), 7.43 (1H, t, $J$=8.1 Hz), 7.55 (1H, d, $J$=8.1 Hz), 7.61 (1H, d, $J$=8.4 Hz), 7.92 (1H, dd, $J$=8.4, 1.7 Hz), 8.22 (1H, d, $J$=8.1 Hz), 8.66 (1H, d, $J$=1.7 Hz), 10.05 (1H, s). MS *m/z* (%): 195 ($M^+$, 100), 194 (68), 167 (11), 166 (42), 140 (12), 139 (27).

$NaBH_4$ (1.7 mg) was added to a methanolic solution (2 ml) of **8** (7 mg) and the solution was stirred for 1 h at room temperature. The solvent was evaporated off and the residue was subjected to preparative TLC to give **4** (4.4 mg) as a colorless amorphous solid. **4**: UV $\lambda_{max}$ nm: 215 (sh), 230 (sh), 235, 246 (sh), 259, 283 (sh), 295, 325, 338. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3400 (NH), 3300 (br OH). $^1$H-NMR $\delta$: 4.86 (2H, s), 7.26 (1H, overlapped solvent), 7.43 (4H, m), 8.08 (3H, m). MS *m/z* (%): 197 ($M^+$, 100), 196 (20), 180 (56), 168 (55), 167 (28), 166 (12), 139 (11).

**Chrestifoline-D (9)** A colorless oil. UV $\lambda_{max}$ nm: 223, 241, 250, 275, 291, 335. IR $\nu_{max}$ cm$^{-1}$: 3470, 1680, 1595, 1580. $^1$H-NMR $\delta$: 3.83 (3H, s), 4.05 (3H, s), 6.08 (2H, s, *N*-$CH_2$–), 6.77 (1H, d, $J$=1.5 Hz, H-2′), 7.17 (1H, t, $J$=8.4 Hz, H-6), 7.31 (1H, t, $J$=8.4 Hz, H-6′), 7.37 (1H, t, $J$=8.4 Hz, H-7), 7.42 (1H, d, $J$=8.4 Hz, H-8′), 7.45 (1H, t, $J$=8.4 Hz, H-7′), 7.49 (1H, br s, H-4′), 7.53 (1H, d, $J$=1.2 Hz, H-2), 7.53 (1H, d, $J$=8.4 Hz, H-8), 7.92 (1H, d, $J$=8.4 Hz, H-5), 8.14 (1H, d, $J$=8.4 Hz, H-5′), 8.20 (1H, br s, NH), 8.27 (1H, d, $J$=1.1 Hz, H-4), 10.07 (1H, s, CHO). MS *m/z* (%): 434 ($M^+$, 11), 225 (7), 224 (6), 211 (19), 210 (100), 167 (9). HR-MS: Calcd for $C_{28}H_{22}N_2O_3$: 434.1630. Found: 434.1637. NOE: irradiation of 1-$OCH_3$ ($\delta$ 4.05), 16% enhancement of H-2 ($\delta$ 7.53); irradiation of 1′-$OCH_3$ ($\delta$ 3.83), 12% enhancement of H-2′ ($\delta$ 6.77); irradiation of *N*-$CH_2$ ($\delta$ 6.08), 10, 9, and 6% enhancements of H-8 ($\delta$ 7.59), H-4′ ($\delta$ 7.49), and H-2 ($\delta$ 6.77), respectively.

**DDQ Oxidation of Bismurrayafoline-A (10)** A benzene solution (5 ml) of **10** (7 mg) was stirred in the presence of DDQ (50 mg) for 3 h at room temperature. After standing overnight, the solution was subjected to preparative TLC with acetone–hexane (1 : 2) to give **9** as a colorless oil (2 mg), which was found to be identical with natural chrestifoline-D (**9**) by $^1$H-NMR, IR, UV, and MS comparisons.

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