New Carbazole Alkaloids from *Murraya euchrestifolia* HAYATA

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Five new monomeric carbazoles, named eustifoline-A (1), -B (2), -C (3), and -D (5) and furostifoline (6), and one dimeric carbazole alkaloid, murrastifoline-E (7), were isolated from the root bark of *Murraya euchrestifolia* collected in Taiwan in December, and their structures were elucidated by spectrometric means. Eustifoline-A (1) and -B (2) have a substituted pyran ring, and eustifoline-C (3) having a geranyl side chain is considered to be a biogenetic precursor of 2. Eustifoline-D (5) and furostifoline (6) both have a furan ring system and were shown to be structural isomers. The new dimeric carbazole murrastifoline-E (7) was found to have the structure corresponding to deoxygenated murrastifoline-D (8) and to be an adduct of murrayafoline-A (9) with girinimbine (10).

Keywords *Murraya*; Rutaceae; carbazole; alkaloid; dimer; eustifoline; furostifoline; murrastifoline; murrayafoline; girinimbine

Murraya euchrestifolia HAYATA is a shrub growing up to 4—5 m high in the central and southern parts of Taiwan.[1] Previously, we showed that this species of *Murraya* contains many kinds of monomeric and dimeric carbazole alkaloids.[2] The plant collected in December also contained carbazolequinones as well as monomeric and dimeric carbazoles.[3,4] Further studies of the constituents of the root bark of the plant collected in December gave five new monomeric and one dimeric carbazole alkaloids. This paper deals with the structural elucidation of these new carbazole alkaloids, named eustifoline-A (**1**), -B (**2**), -C (**3**), and -D (**5**) and furostifoline (**6**), and murrastifoline-E (**7**).

Results and Discussion

Structure of Eustifolines Eustifoline-A (**1**), -B (**2**), -C (**3**), and -D (**5**) showed analogous ultraviolet (UV) spectra having typical high-, medium-, and low-intensity absorption bands at λ_{max} 224—232, 300—324, and 354—378 nm, respectively, accompanied with some minor bands suggesting the presence of the carbazole chromophore in all these molecules.[5] Further, in the proton nuclear magnetic resonance (^{1}H-NMR) spectra (Table I), each eustifoline revealed a lower-field 1H singlet at δ_H 7.92—7.97 assignable to H-4,[5] two pairs of *ortho*-coupled aromatic 1H doublets at δ_H 7.29—7.40 and 7.19—7.26 (each J=8.3—8.4 Hz) and at δ_H 7.14—7.35 and 6.90—7.58 (each J=8.3—8.7 Hz), and a 3H singlet due to an aryl methyl at δ_H 2.51—2.58. This common feature in the ^{1}H-NMR spectra of the four new eustifolines (**1**, **2**, **3**, and **5**), together with the observation of nuclear Overhauser effect (NOE) enhancements between the aryl methyl signal and the lower-field 1H singlet (H-4) (see Experimental) and the lack of another lower field signal assignable to H-5 suggested a 3-methyl-5,6-substituted carbazole structure for these four eustifolines.

Eustifoline-A (**1**) was obtained as a colorless oil, and the molecular formula was determined to be $C_{18}H_{17}NO$ by high-resolution mass spectrometry (HR-MS). The presence of a dimethylpyran ring system in the molecule was suggested by the appearance of additional AB-type signals at δ_H 7.28 and 5.82 (each J=10.0 Hz, doublet) and two tertiary methyls at δ_H 1.49 (6H, singlet) linked with an oxygen atom, besides common signals in the spectra of the eustifolines described above. In the differential NOE experiment, irradiation of the singlet at δ_H 7.92 (H-4) caused a 12% enhancement of the doublet at δ_H 7.28 (H-4′), indicating the direction of the dimethylpyran ring to be as shown in formula **1**. These results led us to conclude that eustifoline-A should be represented by formula **1**.

TABLE I. ^{1}H-NMR Spectral Data for New Monomeric Carbazole Alkaloids

	1	2	3	4	5	6
H-1	7.30 (d, 8.4)	7.31 (d, 8.3)	7.29 (d, 8.3)	7.29 (d, 7.1)	7.40 (d, 8.4)	—
H-2	7.20 (br d, 8.4)	7.19 (br d, 8.3)	7.21 (dd, 8.3, 1.2)	7.21 (br d, 7.1)	7.26 (dd, 8.4, 1.7)	—
3-CH_3	2.52 (3H, s)	2.52 (3H, s)	2.51 (3H, s)	2.51 (3H, s)	2.58 (3H, s)	2.67 (3H, s)
H-4	7.92 (br s)	7.92 (br s)	7.92 (br s)	7.90 (br s)	7.97 (br s)	7.79 (1H, br s)
H-5	—	—	—	—	—	8.06 (d, 8.0)
H-6	—	—	—	—	—	7.25 (t, 8.0)
6-OCH_3	—	—	—	3.88 (3H, s)	—	—
H-7	6.91 (d, 8.4)	6.90 (d, 8.3)	6.96 (d, 8.6)	7.08 (d, 8.6)	7.58 (dd, 8.7, 0.7)	7.37 (t, 8.0)
H-8	7.16 (d, 8.4)	7.14 (d, 8.3)	7.15 (d, 8.6)	7.29 (d, 8.6)	7.35 (d, 8.7)	7.49 (d, 8.0)
NH	7.81	7.78	7.81	7.83	8.12	8.28
Others	7.28 (d, 10.0)	7.28 (d, 9.8)	5.41 (t, 6.4)	5.34 (t, 6.6)	7.81 (d, 2.0)	7.73 (d, 2.0)
	5.82 (d, 10.0)	5.79 (d, 9.8)	5.05 (m)	5.04 (m)	7.32 (dd, 2.0, 0.7)	7.00 (d, 2.0)
	1.49 (6H, s)	5.11 (t, 6.7)	4.90 (br, OH)	3.97 (2H, d, 6.6)		
		2.17 (2H, m)	4.00 (2H, d, 6.4)	2.09 (4H, m)		
		1.78 (2H, m)	2.09 (4H, m)	1.94 (3H, s)		
		1.65 (3H, s)	1.94 (3H, s)	1.56 (3H, s)		
		1.58 (3H, s)	1.63 (3H, s)	1.53 (3H, s)		
		1.45 (3H, s)	1.56 (3H, s)			

Spectra of **1**, **3**, and **4** were measured at 400 MHz and others at 270 MHz in $CDCl_3$. Values are in δ (ppm). Each signal corresponds to 1H, unless otherwise stated. Figures in parentheses are coupling constants (J) in Hz. s, singlet; d, doublet; t, triplet; dd, double doublets; m, multiplet; br, broad.

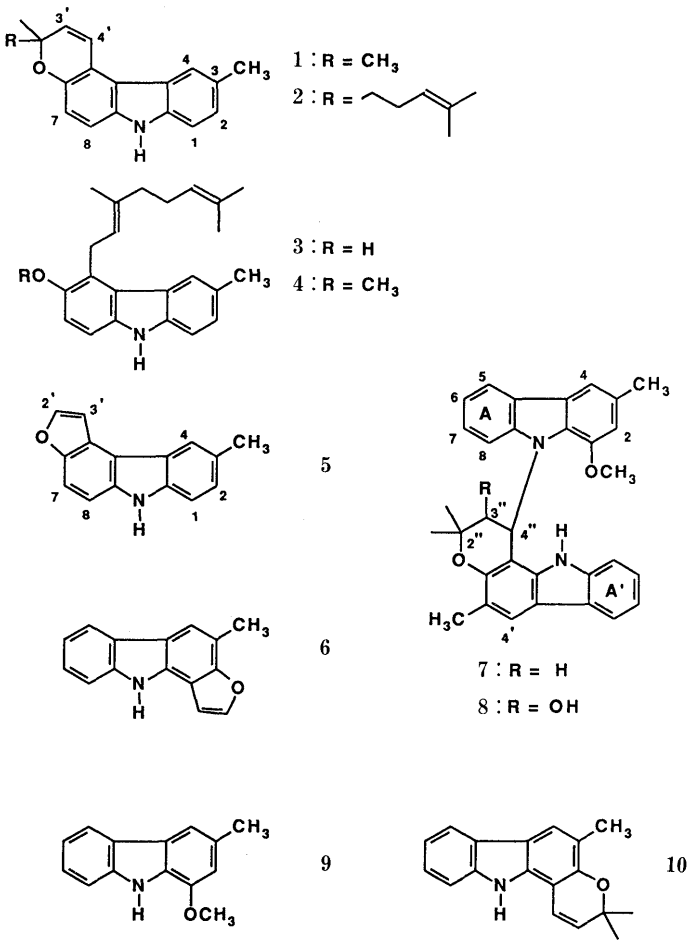

Chart 1

Eustifoline-B (**2**) was isolated as a colorless oil and gave the molecular formula $C_{23}H_{25}NO$ from the HR-MS. The signal pattern of the ^{1}H-NMR spectrum of eustifoline-B (**2**) was similar to that of **1**, except for the appearance of signals at δ_H 5.11 (1H, triplet, J=6.7 Hz), 2.17 (2H, multiplet), 1.78 (2H, multiplet), 1.65 (3H, singlet), and 1.58 (3H, singlet) instead of a methyl signal at δ_H 1.49 in the spectrum of **1**. The electron impact mass spectrum (EI-MS) showed a characteristic fragment peak at *m/z* 248, the same as that of **1**, which was ascribed to the fragment corresponding to the loss of CH_3 or C_6H_{11} radical from the molecular ion at *m/z* 263 for **1** and at *m/z* 331 for eustifoline-B, respectively. These spectral findings indicated the presence of the side chain [$-CH_2CH_2-CH=C(CH_3)_2$] in the molecule, and the structure of eustifoline-B was proposed to be as shown by formula **2**.

Eustifoline-C (**3**) was obtained as a colorless amorphous powder and gave the molecular ion at *m/z* 333 which analyzed for $C_{23}H_{27}NO$, a difference of H_2 compared with **2**. Besides the common ^{1}H-NMR signals of eustifolines, eustifoline-C (**3**) showed signals at δ_H 5.41 (1H, triplet, J=6.4 Hz), 5.05 (1H, multiplet), 4.00 (2H, doublet, J=6.4 Hz), 2.09 (4H, multiplet), 1.94 (3H, singlet), 1.63 (3H, singlet), and 1.56 (3H, singlet), which were assignable to protons of a geranyl moiety, [$-CH_2CH=C(CH_3)-CH_2CH_2CH=C(CH_3)_2$]. The observation of a base fragment peak at *m/z* 210 in the EI-MS, arising by homolytic fission at the benzylic position in the molecular ion, also supported the presence of this side chain in the molecule. The location of the side chain at C-5 was indicated by NOE experiments on *O*-methyleustifoline-C (**4**) which was prepared by treatment of **3** with diazomethane. Irradiation of the benzylic methylene protons at δ_H 3.97 (2H, doublet) gave a 17% enhancement of a signal at δ_H 7.90 (H-4). On irradiation of the methoxy signal at δ_H 3.88 (3H, singlet), a 5% increase of a doublet at δ_H 7.08 (H-7) was observed. On the basis of these spectrometric results, eustifoline-C was assigned the structure **3**, and considered to be a biogenetic precursor of eustifoline-B (**2**).

Eustifoline-D (**5**) was obtained as a colorless oil, $C_{15}H_{11}NO$. In the MS, the molecular ion at *m/z* 221 appeared as a base peak and the relative abundances of other fragments were lower than 10%. As additional ^{1}H-NMR signals, two 1H signals coupled with each other (J=2.0 Hz) at δ_H 7.81 (doublet) and 7.32 (double doublet) were assignable to α and β protons on a furan ring system, respectively, and the β-proton was found to couple further (J=0.7 Hz) with H-7 at δ_H 7.58 (1H, double doublets, J=8.7 and 0.7 Hz). On the basis of these results, together with the observation of an NOE enhancement of β-H of the furan ring at δ_H 7.32 on irradiation of the H-4 signal at δ_H 7.97, the structure **5** was proposed for eustifoline-D.

Structure of Furostifoline (6) Furostifoline (**6**) was also obtained as a colorless oil, and the molecular formula was determined as $C_{15}H_{11}NO$, isomeric with eustifoline-D (**5**). In the MS, the molecular ion appeared as a base peak at *m/z* 221, the same as that of **5**. The UV bands at λ_{max} 238, 274, and 334 nm are typical of a 2-oxygenated carbazole nucleus.[5] The ^{1}H-NMR spectrum showed an N-H, an aryl methyl, and a lower-field H-4 signal at δ_H 8.28, 2.67, and 7.79, respectively. The signals of the four-spin system at δ_H 8.06 (1H, doublet, J=8.0 Hz), 7.25 (1H, triplet, J=8.0 Hz), 7.37 (1H, triplet, J=8.0 Hz), and 7.49 (1H, doublet, J=8.0 Hz) were assigned to H-5, -6, -7, and -8, respectively. The remaining two 1H doublets (J=2.0 Hz) at δ_H 7.73 and 7.00 were deduced to be due to the α and β protons on the furan ring. The location of the furan ring as shown in formula **6** was proposed based on the observation of an NOE enhancement only at H-4 (δ_H 7.79) on irradiation of the aryl methyl proton (δ_H 2.67). From the above spectral data, the structure of furostifoline was confirmed to be as shown by formula **6**.

Structure of Murrastifoline-E (7) Murrastifoline-E (**7**) was obtained as a colorless oil, $[\alpha]_D$ −5.7° (chloroform), and the molecular formula was established as $C_{32}H_{30}N_2O_2$ by HR-MS. This alkaloid gave UV spectral data typical of a carbazole nucleus (see Experimental).[5] The binary carbazole structure of **7** was suggested by the observation of two fragments at *m/z* 211 and 264 (a base peak) in the MS due to the fragments corresponding to the upper and lower halves of the molecule, respectively. The ^{1}H- and ^{13}C-NMR spectra in acetone-d_6 showed signals attributable to an N-H (δ_H 8.94), a methoxy (δ_H 4.11; δ_C 56.89), and two aryl methyl groups (δ_H 2.56 and 2.42; δ_C 17.56 and 22.11). Among three broad 1H singlets at δ_H 7.02, 7.58 and 7.89, the lower two signals are characteristic of H-4 and H-4′ in carbazoles. From the results of proton–proton decoupling experiments in the aromatic proton region, the observation of two pairs of four-spin proton systems at δ_H 6.63 (1H, doublet, J=8.1 Hz), 6.89 (1H, triplet, J=8.1 Hz), and 6.97

(1H, triplet, $J=8.1$ Hz) and at δ_H 6.92 (1H, doublet, $J=8.1$ Hz) and 7.01 (2H, overlapped triplets, $J=8.1$ Hz) including lower-field doublets ($J=8.1$ Hz) at δ_H 7.98 and 7.90, respectively, indicated the lack of substituents on the A and A′ rings in the carbazole nuclei. Further observations of ^{1}H signals at δ_H 7.42 (1H, double doublet, $J=7.3$ and 11.0 Hz), 2.23 (2H, multiplet), 1.50 (3H, singlet), and 1.48 (3H, singlet) and ^{13}C signals at δ_C 77.01 (singlet), 50.59 (doublet), 38.88 (triplet), and 24.35 (quartet)[6] suggested the presence of a 4-substituted 2,2-dimethyldihydropyran ring system in the molecule. The chemical shift value of the 1H double doublet at δ_H 7.42 and the appearance of eleven sp^2 carbon signals as doublets in the ^{13}C-NMR spectrum indicated that the two carbazole nuclei are linked through a benzylic carbon (C-4″) and a nitrogen atom. In NOE experiments, a lower field 1H singlet at δ_H 7.89 (H-4′) showed a 6% NOE enhancement on irradiation of the aryl methyl at δ_H 2.42, and irradiation of another aryl methyl at δ_H 2.56 gave 6 and 8% enhancements of the signals at δ_H 7.58 (H-4) and 7.02 (H-2). Further, irradiation of the methoxy signal at δ_H 4.11 gave an 8% increase of the signal at δ_H 7.02 (H-2). Based on these results, murrastifoline-E was concluded to have structure **7**,[7] corresponding to deoxygenated murrastifoline-D (**8**), and to be an adduct of murrayafoline-A (**9**) and girinimbine (**10**), both of which occur in the same plant.[3]

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 δ values (ppm) with tetramethylsilane (TMS) as an internal reference. EI- and HR-MS were taken with an M-52, M-80 (Hitachi) or JMS-HX-110 (JEOL) mass spectrometer having a direct inlet system. UV spectra were recorded on a Jasco UVIDEC-610C double-beam spectrophotometer in methanol, infrared (IR) spectra on a Jasco IR-810 in $CHCl_3$, and an optical rotation on a Jasco DIP-181 in $CHCl_3$. The thin layer chromatography (TLC) and preparative TLC were done on Kieselgel 60 F_{254} (Merck).

Extraction and Separation The acetone extract of the dried, powdered root bark (900 g) of *Murraya euchrestifolia* HAYATA, collected at Kuantaochi, Nantou Hsien, Taiwan, in December as described in the previous paper,[4] was further subjected to silica gel column chromatographies and preparative TLC to afford five new monomeric carbazoles, eustifoline-A (**1**), -B (**2**), -C (**3**), and -D (**5**), and furostifoline (**6**) (2, 2, 10, 3, and 2 mg, respectively) and one dimeric carbazole, murrastifoline-E (**7**) (2 mg).

Eustifoline-A (1) Colorless oil. HR-MS: Calcd for $C_{18}H_{17}NO$: 263.1308. Found: 263.1302. UV λ_{max} nm: 218, 232, 262, 273, 286, 314, 324, 378. IR ν_{max}: 3470 cm^{-1}. ^{1}H-NMR (270 MHz, acetone-d_6) δ: 10.06 (1H, NH), 7.99 (1H, d, $J=0.7$ Hz, H-4), 7.36 (1H, d, $J=9.7$ Hz H-3′), 7.36 (1H, d, $J=8.4$ Hz, H-1), 7.25 (1H, d, $J=8.4$ Hz, H-8), 7.19 (1H, dd, $J=0.7$, 8.4 Hz, H-2), 6.84 (1H, d, $J=8.4$ Hz, H-7), 5.90 (1H, d, $J=9.7$ Hz, H-2′), 2.48 (3H, s), 1.45 (6H, s). EI-MS m/z (%): 263 (M^+, 32), 249 (18), 248 (100), 149 (18). Differential NOE: Irradiation of H-4 (δ_H 7.92)—12 and 4% enhancements of a doublet at δ_H 7.28 (H-4′) and a 3H singlet at δ_H 2.52 (3-CH_3), respectively.

Eustifoline-B (2) Colorless oil. HR-MS: Calcd for $C_{23}H_{25}NO$: 331.1934. Found: 331.1967. UV λ_{max} nm: 220, 230, 268, 304, 324, 362. IR ν_{max}: 3475 cm^{-1}. EI-MS m/z (%): 331 (M^+, 11), 249 (20), 248 (100), 247 (13), 246 (6), 234 (6), 233 (5), 232 (4), 221 (4), 220 (6), 219 (6), 218 (10), 217 (6), 205 (5), 204 (12).

Eustifoline-C (3) Colorless amorphous powder. HR-MS: Calcd for $C_{23}H_{27}NO$: 333.2092. Found: 333.2068. UV λ_{max} nm: 205, 231, 258, 268, 290, 300, 325, 357. IR ν_{max}: 3457 cm^{-1}. EI-MS m/z (%): 333 (M^+, 78), 248 (67), 210 (100), 209 (70).

Methylation of 3 A methanolic solution (5 ml) of **3** (2 mg) was treated with CH_2N_2 in ether overnight at room temperature. The solution was evaporated, and the residue was subjected to preparative silica gel TLC (hexane–acetone, 4 : 1) to give *O*-methyleustifoline-C (**4**) as a colorless oil (2 mg). HR-MS: Calcd for $C_{24}H_{29}NO$: 347.2249. Found: 347.2286. UV λ_{max} nm: 230, 267, 288, 290, 350. IR ν_{max}: 3475 cm^{-1}. EI-MS m/z (%): 347 (M^+, 38), 278 (60), 263 (15), 262 (12), 248 (100), 247 (41), 246 (34), 232 (42), 231 (45), 220 (29), 218 (29), 217 (25), 204 (30), 194 (51), 180 (50). Differential NOE: Irradiation of 3-CH_3 (δ_H 2.51)—4 and 5% enhancements of H-4 (δ_H 7.90) and H-2 (δ_H 7.21), respectively; irradiation of 5-CH_2 (δ_H 3.97)—17% enhancement of H-4 (δ_H 7.90); irradiation of OCH_3 (δ_H 3.88)—5% enhancement of H-7 (δ_H 7.08).

Eustifoline-D (5) Colorless oil. HR-MS: Calcd for $C_{15}H_{11}NO$: 221.0839. Found: 221.0814. UV λ_{max} nm: 206, 224, 250, 268, 298, 310, 340, 354. IR ν_{max}: 3470 cm^{-1}. EI-MS m/z (%): 221 (M^+, 100), 220 (43), 211 (8), 196 (7), 192 (7), 191 (7), 190 (5), 149 (7). Differential NOE: Irradiation of H-4 (δ_H 7.97)—1 and 2% enhancements of H-3′ (δ_H 7.32) and 3-CH_3 (δ_H 2.58), respectively.

Frostifoline (6) Colorless oil. HR-MS: Calcd for $C_{15}H_{11}NO$: 221.0840. Found: 221.0845. UV λ_{max} nm: 214, 227, 238, 260, 274, 297, 320, 334. IR ν_{max}: 3470 cm^{-1}. EI-MS m/z (%): 221 (M^+, 100), 220 (64), 191 (11). Differential NOE: Irradiation of 3-CH_3 (δ_H 2.67)—2% enhancement of H-4 (δ_H 7.79).

Murrastifoline-E (7) Colorless oil. $[\alpha]_D$ −5.7° ($c=0.035$, $CHCl_3$). HR-MS: Calcd for $C_{32}H_{30}N_2O_2$: 474.2304. Found: 474.2280. UV λ_{max} nm: 214, 230, 239, 255, 281, 291, 303, 331, 348. IR ν_{max}: 3450 cm^{-1}. ^{1}H-NMR (acetone-d_6) δ_H: 8.94 (1H, br s, NH), 7.98 (1H, d, $J=8.1$ Hz, H-5), 7.90 (1H, d, $J=8.1$ Hz, H-5′), 7.89 (1H, s, H-4′), 7.58 (1H, s, H-4), 7.42 (1H, dd, $J=7.3$, 11.0 Hz, H-4″), 7.02 (1H, s, H-2), 7.01 (2H, overlapped t, $J=8.1$ Hz), 6.97 (1H, t, $J=8.1$ Hz), 6.92 (1H, d, $J=8.1$ Hz), 6.89 (1H, t, $J=8.1$ Hz), 6.63 (1H, d, $J=8.1$ Hz), 4.11 (3H, s, OCH_3), 2.56 (3H, s, 3-CH_3), 2.42 (3H, s, 3′-CH_3), 2.23 (2H, m, H-3″), 1.50 (3H, s, 2″-CH_3), 1.48 (3H, s, 2″-CH_3). ^{13}C-NMR (acetone-d_6) δ_C: 152.39 (s), 148.62 (s), 141.75 (s), 141.17 (s), 138.68 (s), 131.43 (s), 129.93 (s), 127.58 (s), 126.47 (d), 126.28 (s), 124.99 (d), 124.43 (s), 122.24 (d), 121.39 (d), 120.66 (d), 120.02 (2d), 114.12 (d), 113.25 (d), 112.03 (d), 111.21 (d), 105.39 (s), 77.01 (s), 56.89 (q), 50.59 (d), 38.88 (t), 24.35 (q), 22.11 (q), 17.56 (q). One quartet may be overlapped with the solvent signal (acetone-methyl) and the chemical shifts of two singlets could not be determined. EI-MS m/z (%): 474 (M^+, 21), 264 (100), 248 (38), 211 (17), HR-MS: Calcd for $C_{18}H_{18}NO$: 264.1386. Found: 264.1361. Calcd for $C_{14}H_{13}NO$: 211.0996. Found: 211.0961. Differential NOE: Irradiation of 3′-CH_3 (δ_H 2.42)—6% enhancement of H-4′ (δ_H 7.89); irradiation of OCH_3 (δ_H 4.11)—8% enhancement of H-2 (δ_H 7.02); irradiation of 3-CH_3 (δ_H 2.56)—8 and 6% enhancements of H-2 (δ_H 7.02) and H-4 (δ_H 7.58), respectively.

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6) One more quartet may be overlapped with the solvent (acetone-methyl) signal.
7) Determination of the absolute stereochemistry remains to be done.