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Lignans Related to Olivil from Genus *Cerbera* (*Cerbera*. VI)¹⁾

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Three olivil dimers, 5',5'''-bis-olivil, 5',5''-bis-olivil, and 5,5''-bis-olivil, were isolated from the stems of *Cerbera manghas* and *C. odollam* together with (–)-olivil and (+)-cycloolivil, and their structures were determined on the basis of spectral evidence. Olivil 4-*O*-glucoside and 4'-*O*-glucoside were also obtained from the leaves of *C. manghas*.

Keywords—Apocynaceae; *Cerbera manghas*; *Cerbera odollam*; lignan; olivil; cycloolivil; olivil-*O*-glucoside; olivil dimer

During our studies on the constituents of Apocynaceae plants, we have described the isolation and structure determinations of the cardenolide glycosides from *Cerbera manghas* L.^{2a,b)} and *C. odollam*.^{2b)} This paper deals with olivil (1), cycloolivil (2), and dimers of olivil (5, 6, and 7) from the stems of *C. manghas* and *C. odollam*, and olivil glucosides (3 and 4) from the leaves of *C. manghas*. Lignans were transferred into the BuOH layer when the stems and leaves were percolated with MeOH, and the MeOH percolates were extracted with benzene, CHCl₃, and then BuOH. The BuOH extract was subjected to chromatography on a reversed-phase column and a silica gel column, and high-performance liquid chromatography (HPLC) in some cases.

Lignan 1, mp 106–111 °C, $[\alpha]_D -48.5^\circ$, showed the M⁺ peak at *m/z* 374, and ultraviolet (UV) absorptions at 229 and 280 nm, and was identified as (–)-olivil by direct comparison with an authentic sample^{3a)} and from proton nuclear magnetic resonance (¹H-NMR) and carbon-13 nuclear magnetic resonance (¹³C-NMR) considerations. Similarly, 2, mp 171–173 °C, $[\alpha]_D +74.5^\circ$, was identified as (+)-cycloolivil by comparison of the ¹H- and ¹³C-NMR spectra with those already published.^{3b)} Lignans 3 and 4 showed a homogeneous spot on thin layer chromatography (TLC) and were finally separated from each other by HPLC. On the basis of the M⁺ + Na peaks at *m/z* 561.193 (3) and *m/z* 561.198 (4) and the NMR spectra, 3

- 1: R₁ = R₂ = H
3: R₁ = β-D-glucosyl, R₂ = H
4: R₁ = H, R₂ = β-D-glucosyl

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Chart 1

TABLE I. ^1H Chemical Shifts of the Lignans from Genus *Cerbera*, δ (ppm) from TMS in Pyridine- d_5 (J/Hz in Parentheses)

	1 ^{a)}	3	4	5	7	6	
H-2(2'')	7.62 (d, 2)	7.63 (d, 2)	7.62 (d, 2)	7.63 (d, 2)	7.61 (d, 1)	m -Coup. 7.29 7.48 7.53 7.61 7.63 7.68 o -Coup. 7.21 (2H) o,m -Coup. 7.34 7.36	
H-5(5'')	7.22 (d, 8)	7.56 (d, 8)	7.22 (d, 8)	7.22 (d, 8)			
H-6(6'')	7.36 (dd, 8, 2)	7.32 (dd, 8, 2)	7.36 (dd, 8, 2)	7.36 (dd, 8, 2)	7.60 (d, 1)		
H-2'(2''')	7.32 (d, 2)	7.31 (d, 2)	7.32 (d, 2)	7.29 (d, 1)	7.33 (d, 1)		
H-5'(5''')	7.22 (d, 8)	7.21 (d, 8)	7.55 (d, 8)		7.22 ^{b)} (2H)		
H-6'(6''')	7.19 (dd, 8, 2)	7.18 (dd, 8, 2)	7.15 (dd, 8, 2)	7.54 (d, 1)			
H-7(7'')	5.33 (d, 7)	5.32 (d, 7)	5.31 (d, 7)	5.31 (d, 7)	5.31 (d, 8)	5.30, (d, 7)	5.35 (d, 7)
H-8(8'')	3.02 (m)	2.95 (m)	3.09 (m)	3.03 (m)	3.05 (m)	3.01, (m)	3.07 (m)
H-7'(7''')	3.41 3.57 (d, 14)	3.37 3.53 (d, 14)	3.38 3.55 (d, 14)	3.44 3.59 (d, 14)	3.42 3.59 (d, 14)	3.40, 3.56, (d, 14)	3.42 3.58
H-9'(9''')	4.24 4.35 (d, 9)			4.30 4.41 (d, 9)	4.24 4.35 (d, 9)		
-OMe	3.67 3.72	3.65 3.71	3.67 3.69	3.67 3.71	3.65 3.72	3.64, 3.70,	3.67 3.71
Others		5.64 (d, 7, H _{glc} -1)	5.62 (d, 7, H _{glc} -1)				

a) Signal assignments were confirmed based on the two-dimensional (2D) NMR (^1H - ^1H COSY, NOESY) spectra. b) Signals of H-5'(5''') and H-6'(6''') overlapped one of the proton signals of pyridine.

and **4** were both considered to be olivil monoglucoside. Upon hydrolysis with cellulase, olivil was detected on TLC. Since downfield shifts of the signals were observed at C-1 in **3** (+3.2 ppm) and at C-1' in **4** (+3.2 ppm) in comparison with those of **1**, the structures of **3** and **4** were determined to be olivil 4-*O*- β -D-glucoside⁴⁾ and olivil 4'-*O*- β -D-glucoside,^{3a,5)} respectively.

Lignans **5**, **6**, and **7** showed a homogeneous spot on TLC, and the three lignans were separated preparatively by HPLC. In the ^{13}C -NMR spectra, signals in the aliphatic regions were observed at almost the same chemical shifts as those of **1**. The molecular peaks of the three lignans were seen at m/z 773 ($\text{M}^+ + \text{Na}$), suggesting the molecular formula to be $\text{C}_{40}\text{H}_{46}\text{O}_{14}$. Based on the similarity of the ^1H - and ^{13}C -NMR spectra to those of **1**, as well as the levorotatory values of specific rotation (**5**, -76.0° ; **6**, -75.4° ; and **7**, -57.3°), **5**, **6**, and **7**, were considered to be dilignans composed of 2 mol of **1**.

Of the three lignans, **5** and **7** showed twenty signals in the ^{13}C -NMR spectra, suggesting that two olivil units are linked in a magnetically symmetric mode. Carbon signals due to ring A (A') in **1** were assignable in the ^{13}C -NMR spectrum of **5**. In the ^1H -NMR spectrum of **5**, the ABX pattern due to ring A (A') was retained, whilst that due to ring B (B') was transformed to a pair of m -coupled proton doublets (Table I). Therefore, the linkage between the two olivil moieties of **5** was determined to be at C-5' and C-5''' as shown in Chart 2. The fact that

TABLE II. ^{13}C Chemical Shifts of Olivil and Olivil Dimers, δ (ppm)
 from TMS in Pyridine- d_5

	1 ^{a)}	5	7	6	
C-1(1'')	135.6	135.6	134.7	135.7	134.7
C-2(2'')	111.6	111.7	110.0	111.7	110.0
C-3(3'')	148.7	148.7 ^{b)}	149.2	148.7 ^{b)}	149.3
C-4(4'')	147.6	147.5	146.7	147.5	146.7
C-5(5'')	116.1 ^{b)}	116.0	127.2	116.0	126.5
C-6(6'')	120.5	120.4	123.0	120.4	123.0
C-7(7'')	84.8	84.7	84.7	84.8	
C-8(8'')	62.1	62.1	62.1	62.1	
C-9(9'')	60.5	60.5	60.3	60.5	60.3
C-1'(1''')	130.1	129.5	130.1	129.3	130.1
C-2'(2''')	115.4	114.1	115.5	114.0	115.4
C-3'(3''')	148.2	148.6 ^{b)}	148.2	148.6 ^{b)}	148.1 ^{b)}
C-4'(4''')	146.7	144.0	146.7	144.2	146.7
C-5'(5''')	116.0 ^{b)}	127.2	116.1	127.3	116.1
C-6'(6''')	123.7	126.6	123.8	126.4	123.8
C-7'(7''')	40.7	40.8	40.7	40.8	40.7
C-8'(8''')	82.0	82.0	82.0	82.0	
C-9'(9''')	78.1	78.1	78.0	78.1	
-OMe	55.8	55.8	55.8	55.8	55.7
	55.9	56.0	55.9	56.0	55.9

a) Assignment was done based on the two-dimensional (2D) NMR (^1H - ^{13}C , long-range ^1H - ^{13}C) spectra. b) Assignments marked b) in each column may be reversed.

Chart 2

carbon signal at δ 116.0 (d) in **1** was shifted downfield (+11.2 ppm) and was transformed into singlet peak, is consistent with the 5'-5''' linkage. Similarly, **7** showed a collapse of the ABX pattern due to ring A (A') and a downfield shift (+11.1 ppm) of C-5 (and C-5'), indicating linkage between C-5 and C-5''.

In the ^{13}C -NMR spectrum of **6**, common signals in **5** and **7** were observed, suggesting linkage between rings A' and B. On the basis of the presence of two quaternary carbon signals at δ 127.3 (C-5') and δ 126.5 (C-5''), and also six *m*-coupled aromatic protons instead of three in **5** and **7**, the linkage was concluded to be between C-5' and C-5''.

No lignans from Apocynaceae plants have been investigated except for those from *Trachelospermum asiaticum*⁶⁾ and from *Allamanda neriifolia*.⁷⁾ This is the first reported isolation of lignans from genus *Cerbera*, including the novel olivil dimers, **5**, **6**, and **7**.

Experimental

Physical constants and spectral data were obtained as described in Part III^{2b)} of this series. For silica gel column chromatography and TLC, the following solvent systems were applied: solv.1, CHCl_3 -MeOH- H_2O (bottom layer); solv.2, EtOAc-MeOH- H_2O (top layer). For HPLC, a Waters ALC-200 equipped with a Radial Pack C_{18} column was used.

Extraction and Isolation of Lignans from Stems—Air-dried stems of *Cerbera manghas* L., cultivated in the greenhouse of Fukuoka University and harvested in Sept. 1986, were powdered and percolated with MeOH. The MeOH extract was concentrated *in vacuo* to 2 l and diluted with H_2O to 4 l. The mixture was filtered and the filtrate was extracted with benzene and then with CHCl_3 (ext. 17.0 g). The H_2O layer was concentrated *in vacuo* in order to remove MeOH and then extracted with BuOH (ext. 128.1 g). The CHCl_3 and BuOH extracts were combined and passed through a polystyrene column (MCI gel, CHP-20P, Mitsubishi Chem. Co.). The fraction eluted with 40–50% MeOH contained **1** (ext. 17.3 g). After chromatography on a silica gel column with solv.1 (7:2:2), **1** (1.8 g) was isolated. The 60–90% MeOH eluate (ext. 45.0 g) was chromatographed on silica gel columns with solv.1 (7:2:2—7:2:1—7:3:1), and then with solv.2 (4:1:5—4:1:4). The fraction containing each lignan was further purified on a reversed phase column (ODS-column, RQ-1, Fuji-gel) with 20–30% CH_3CN to isolate **1** (1.41 g) and **2** (110 mg). The fraction containing **5**, **6**, and **7** was subjected to HPLC and eluted with 26% CH_3CN (0.6 ml/min) to give **5** (t_R 16.0 min, 70 mg), **6** (t_R 17.0 min, 230 mg) and **7** (t_R 18.4 min, 90 mg).

Air-dried stems of *C. odollam*, collected in Singapore in January 1987 (1.1 kg), were percolated as described above. The following lignans were obtained: **1** (6 mg), **2** (4 mg), **5** (8 mg), **6** (14 mg), and **7** (4 mg).

Isolation of 3 and 4 from the Fresh Leaves of C. manghas—The fresh leaves of *C. manghas* collected in Singapore in January 1986 (2.6 kg) were stored in a refrigerator for 2 d and then at room temperature for 2 d. The leaves were then homogenized with MeOH. The MeOH solution was concentrated *in vacuo* to 1 l, and extracted with BuOH. The BuOH extract was treated with benzene in order to remove the benzene-soluble substances. The benzene-insoluble BuOH extract (22.8 g) was passed through MCI-gel. The eluate with 10–20% MeOH was chromatographed on an ODS column with 10–20% CH_3CN - H_2O . The fraction showing a homogeneous spot on TLC (solv.1) (200 mg) was purified by droplet countercurrent chromatography with solv.1 (4:6:5, ascending) to afford a mixture of **3** and **4** (70 mg). The mixture was separated by preparative HPLC (16% CH_3CN - H_2O , 0.8 ml/min) to give **3** (t_R 12.2 min, 10 mg) and **4** (t_R 9.8 min, 20 mg).

Lignan 1 ((-)-Olivil)—mp 106–111 °C from MeOH, $[\alpha]_D^{26} -48.5^\circ$ ($c=1.15$, MeOH). On admixture with authentic (-)-olivil (mp 107–112 °C),^{3a)} no melting point depression was observed and the ^1H - and ^{13}C -NMR spectra, as well as *Rf* values on TLC (solv.1, 7:3:1, *Rf* 0.35; solv.2, 9:1:0.5, *Rf* 0.60), of the two samples were in good agreement. Electron impact MS (EI-MS) *m/z*: 376 ($\text{C}_{20}\text{H}_{24}\text{O}_7$), UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 229 (15000), 280 (7200).

Lignan 2 ((+)-Cycloolivil)—mp 171–173 °C from EtOAc-hexane, $[\alpha]_D^{28} +74.5^\circ$ ($c=1.25$, MeOH) (ref. mp 170–171 °C, $[\alpha]_D +61.9^\circ$).^{3b)} EI-MS *m/z*: 376 ($\text{C}_{20}\text{H}_{24}\text{O}_7$). ^1H -NMR (pyridine- d_5) δ (ppm): 7.08 (1H, d, $J=2$ Hz, H-2), 7.16 (1H, d, $J=8$ Hz, H-5), 6.98 (1H, dd, $J=8, 2$ Hz, H-6), 4.71 (1H, d, $J=12$ Hz, H-7), 2.75 (1H, m, H-8), 4.22 (1H, dd, $J=11, 2$ Hz, H-9a), 4.45 (1H, dd, $J=11, 4$ Hz, H-9b), 6.85 (1H, s, H-2'), 6.97 (1H, s, H-5'), 3.14, 3.81 (1H each, d, $J=14$ Hz, H-7' a,b), 4.25, 4.49 (1H each, d, $J=12$ Hz, H-9' a,b). ^{13}C -NMR (pyridine- d_5) δ (ppm): 133.9 (C-1), 113.9 (C-2), 146.1, 146.6, 147.3, 148.7 (C-3, C-4, C-3', C-4'), 116.4 (C-5), 123.3 (C-6), 44.8 (C-7), 47.8 (C-8), 60.6 (C-9), 137.9 (C-1'), 113.4 (C-2'), 117.9 (C-5'), 126.2 (C-6'), 40.4 (C-7'), 74.3 (C-8'), 69.8 (C-9'). The ^1H - and ^{13}C -NMR spectra measured in CD_3OD were in good agreement with those given in the literature.^{3b)}

Lignan 3 (Olivil 4-O- β -D-Glucoside)—A solid, $[\alpha]_D^{24} -66.9^\circ$ ($c=0.48$, MeOH), fast atom bombardment MS (FAB-MS) *m/z*: 561.193 (Calcd for $\text{C}_{26}\text{H}_{34}\text{O}_{12} + \text{Na}$: 561.195). ^{13}C -NMR (pyridine- d_5) δ (ppm): 138.8 (C-1), 112.1 (C-2), 147.2, 149.3 (C-3, C-4), 116.1 (C-5), 119.7 (C-6), 84.5 (C-7), 62.0 (C-8), 60.4 (C-9), 130.0 (C-1'), 115.4 (C-2'), 148.2 (C-3'), 146.7 (C-4'), 116.1 (C-5'), 123.8 (C-6'), 40.5 (C-7'), 81.9 (C-8'), 78.0 (C-9'), 55.8 (3,3'-OMe), 102.5 (C_{glc} -1),

74.9 (C_{glc}-2), 78.7, 78.5 (C_{glc}-3,5), 71.2 (C_{glc}-4), 62.3 (C_{glc}-6). Lignan **3** (3 mg) was dissolved in 25% EtOH (1 ml) and the solution was shaken with cellulase (Sigma Chem. Co., Ltd.) (5 mg) for 10 h at 38 °C. The mixture was extracted with BuOH and the BuOH extract showed the same *R_f* values as **1** on TLC [solv.1 (7:2:1), *R_f* 0.35; solv.2 (9:1:0.5), *R_f* 0.60].

Lignan 4 (Olivil 4'-O-Glucoside)—A. solid, $[\alpha]_D^{24} -76.0^\circ$ ($c=0.73$, MeOH), FAB-MS *m/z*: 561.198 (Calcd for C₂₆H₃₄O₁₂ + Na: 561.195). ¹³C-NMR (pyridine-*d*₅) δ (ppm): 135.2 (C-1), 111.6 (C-2), 148.7 (C-7), 147.6 (C-4), 116.2, 116.0 (C-5, C-2'), 120.4 (C-6), 84.7 (C-7), 62.0 (C-8), 60.4 (C-9), 133.3 (C-1'), 146.7, 148.7 (C-3', C-4'), 123.3 (C-6'), 40.6 (C-7'), 81.8 (C-8'), 77.9 (C-9'), 55.8, 56.0 (3, 3'-OMe), 102.7 (C_{glc}-1), 74.9 (C_{glc}-2), 78.7, 78.5 (C_{glc}-3, 5), 71.3 (C_{glc}-4), 62.4 (C_{glc}-6). Lignan **4** (3 mg) was treated with cellulase in the same manner as **3**, and the product showed the same *R_f* values as **1**.

Lignan 5—A solid, $[\alpha]_D^{29} -76.0^\circ$ ($c=0.55$, MeOH), FAB-MS *m/z*: 773 (C₄₀H₄₆O₁₄ + Na), UV $\lambda_{\max}^{\text{MeOH}}$ (ϵ): 222 (39800), 282 (11500).

Lignan 6—A solid, $[\alpha]_D^{28} -75.4^\circ$ ($c=0.35$, MeOH), FAB-MS *m/z*: 773 (C₄₀H₄₆O₁₄ + Na), $\lambda_{\max}^{\text{MeOH}}$ (ϵ): 223 (46000), 282 (12000).

Lignan 7—A solid, $[\alpha]_D^{27} -57.3^\circ$ ($c=0.20$, MeOH), FAB-MS *m/z*: 773.278 (Calcd for C₄₀H₄₆O₁₄ + Na: 773.278), UV $\lambda_{\max}^{\text{MeOH}}$ (ϵ): 223 (49000), 282 (12000).

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