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

FUMIKO ABE,[a] TATSUO YAMAUCHI,*[,a] and ALFRED S. C. WAN[b]

Faculty of Pharmaceutical Sciences, Fukuoka University,[a] 8–19–1 Nanakuma, Jonan-ku, Fukuoka 814–01, Japan and Department of Pharmacy, Faculty of Science, National University of Singapore,[b] 10 Kent Ridge Crescent, Singapore 0511

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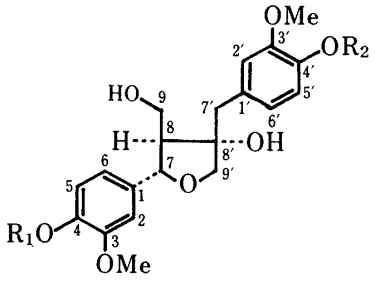
Three olivil dimers, 5′,5‴-bis-olivil, 5′,5″-bis-olivil, and 5,5″-bis-olivil, were isolated from the stems of *Cerbera manghas* and *C. odollam* together with (−)-olivil and (+)-cycloolivil, and their structures were determined on the basis of spectral evidence. Olivil 4-*O*-glucoside and 4′-*O*-glucoside 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, $CHCl_3$, 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°, 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 (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) considerations. Similarly, **2**, mp 171—173 °C, $[\alpha]_D$ +74.5°, was identified as (+)-cycloolivil by comparison of the ^{1}H- and ^{13}C-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: $R_1=R_2=H$
3: $R_1=\beta$-D-glucosyl, $R_2=H$
4: $R_1=H$, $R_2=\beta$-D-glucosyl

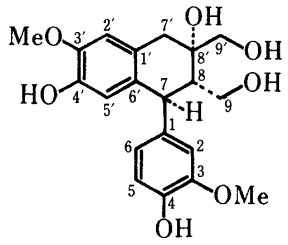


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Chart 1

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

a) Signal assignments were confirmed based on the two-dimensional (2D) NMR (^{1}H–^{1}H 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-glucoside[4] and olivil 4′-*O*-*β*-D-glucoside,[3a,5] respectively.

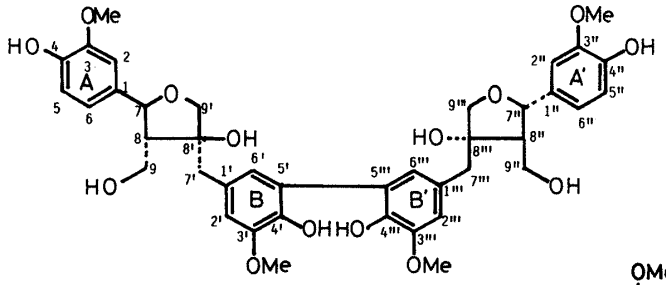
Lignans **5**, **6**, and **7** showed a homogeneous spot on TLC, and the three lignans were separated preparatively by HPLC. In the ^{13}C-NMR spectra, signals in the aliphatic regions were observed at almost the same chemical shifts as those of **1**. The molecular peaks of the three lignans were seen at *m/z* 773 ($M^+ + Na$), suggesting the molecular formula to be $C_{40}H_{46}O_{14}$. Based on the similarity of the ^{1}H- 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 ^{1}H-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.



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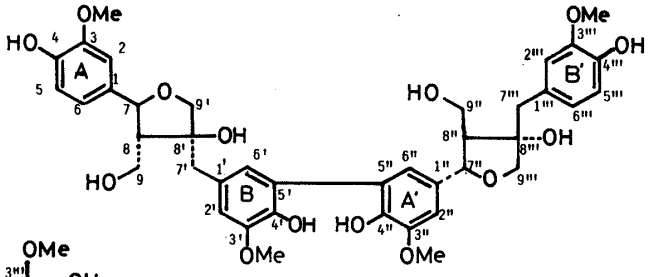

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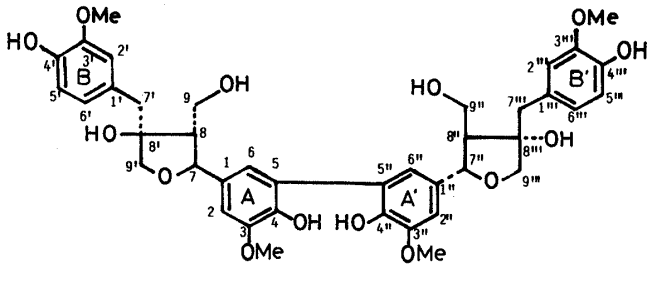

7

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 chromatogrphed 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° (c=1.15, MeOH). On admixture with authentic (−)-olivil (mp 107—112 °C),[3a] no melting point depression was observed and the ^{1}H- 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 ($C_{20}H_{24}O_7$), UV λ_{max}^{MeOH} nm(ε): 229 (15000), 280 (7200).

Lignan 2 ((+)-Cycloolivil)——mp 171—173 °C from EtOAc–hexane, $[\alpha]_D^{28}$ +74.5° (c=1.25, MeOH) (ref. mp 170—171 °C, $[\alpha]_D$ +61.9°).[3b] EI-MS *m/z*: 376 ($C_{20}H_{24}O_7$). ^{1}H-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, 2Hz, 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 ^{1}H- 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° (c=0.48, MeOH), fast atom bombardment MS (FAB-MS) *m/z*: 561.193 (Calcd for $C_{26}H_{34}O_{12}$ + 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 *Rf* values as **1** on TLC [solv.1 (7 : 2 : 1), *Rf* 0.35; solv.2 (9 : 1 : 0.5), *Rf* 0.60].

Lignan 4 (Olivil 4′-*O*-Glucoside)——A. solid, $[\alpha]_D^{24}$ −76.0° (c=0.73, MeOH), FAB-MS m/z: 561.198 (Calcd for $C_{26}H_{34}O_{12}$+Na: 561.195). ^{13}C-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 *Rf* values as **1**.

Lignan 5——A solid, $[\alpha]_D^{29}$ −76.0° (c=0.55, MeOH), FAB-MS m/z: 773 ($C_{40}H_{46}O_{14}$+Na), UV λ_{max}^{MeOH} (ε): 222 (39800), 282 (11500).

Lignan 6——A solid, $[\alpha]_D^{28}$ −75.4° (c=0.35, MeOH), FAB-MS m/z: 773 ($C_{40}H_{46}O_{14}$+Na), λ_{max}^{MeOH} (ε): 223 (46000), 282 (12000).

Lignan 7——A solid, $[\alpha]_D^{27}$ −57.3° (c=0.20, MeOH), FAB-MS m/z: 773.278 (Calcd for $C_{40}H_{46}O_{14}$+Na: 773.278), UV λ_{max}^{MeOH} (ε): 223 (49000), 282 (12000).

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References

1) Part V: T. Yamauchi, F. Abe, and A. S. C. Wan, *Chem. Pharm. Bull.*, **35**, 4993 (1987).
2) *a*) F. Abe and T. Yamauchi, *Chem. Pharm. Bull.*, **25**, 2744 (1977); *b*) T. Yamauchi, F. Abe, and A. S. C. Wan, *ibid.*, **35**, 2744 (1987).
3) *a*) K. Kudo, T. Nohara, T. Komori, T. Kawasaki, and H. R. Schulten, *Planta Medica*, **40**, 250 (1980); *b*) R. Ghogomu-Tih, B. Bodo, B. Nyasse, and B. L. Sondengam, *ibid.*, **45**, 464 (1985).
4) Y. Tokuoka, K. Daigo, and T. Takemoto, *Yakugaku Zasshi*, **95**, 557 (1975); T. Deyama, T. Ikawa, S. Kitagawa, and S. Nishibe, *Chem. Pharm. Bull.*, **34**, 4933 (1986).
5) M. Kikuchi and Y. Yamauchi, *Yakugaku Zasshi*, **104**, 390 (1984).
6) S. Nishibe, S. Hisada, and I. Inagaki, *Chem. Pharm. Bull.*, **21**, 674 (1973); F. Abe and T. Yamauchi, *ibid.*, **34**, 4340 (1986).
7) F. Abe and T. Yamauchi, *Phytochemistry*, in press (1988).