Normonoterpenoids and Their Allopyranosides from the Leaves of *Cerbera* Species (Studies on *Cerbera*. VIII)[1)]

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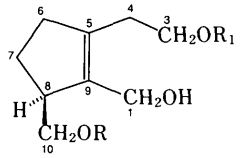
Three normonoterpenoids, cerberidol, epoxycerberidol, and cyclocerberidol, and their β-D-allopyranosides were obtained from the air-dried leaves of *Cerbera manghas* and *C. odollam*.

Keywords *Cerbera*; Apocynaceae; normonoterpenoid; cerberidol; epoxycerberidol; cyclocerberidol; allopyranoside

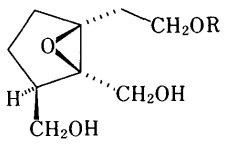
Cerbera species are indigenous to the sea-coast of South-East Asian, Oceanian, and Indian Ocean regions. In the preceding papers of this series, we described cardiac glycosides,[2-5)] yellow pigments with an irridoid framework[6)] and lignans.[1,7)] This paper deals with normonoterpenoids, cerberidol (**1**), epoxycerberidol (**2**), and cyclocerberidol (**3**), and their four β-D-allopyranosides (**4**—**7**) isolated from the air-dried leaves of *Cerbera manghas* L. and *C. odollam* GAERTN.

Seven compounds (**1**—**7**) were isolated from the BuOH soluble fraction when the air-dried leaves were percolated with MeOH and the percolate was partitioned with benzene, $CHCl_3$ and BuOH. In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, **1** showed nine signals, of which two singlet signals at δ 138.8 and 139.6 suggested the presence of a tetrasubstituted olefinic linkage. Triplet signals at δ 26.3, 33.0 and 35.2 revealed the presence of three methylene carbons and those at δ 57.5, 60.4 and 65.6, three primary carbinol groups. One doublet carbon signal due to a methine carbon was observed at δ 51.5. The molecular formula of **1** was determined to be $C_9H_{16}O_3$ from the high resolution chemical ionization (CI) mass spectrum (MS), and **1** was considered to be an unsaturated alicyclic triol.

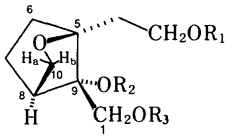
In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, signals due to the three primary carbinol groups were observed at δ 3.89 (2H, t), 3.93 (2H, d), and at δ 4.44 (1H, d, J=12 Hz) and 4.64 (1H, d, J=12 Hz) as an AB



1: $R_1=R_2=H$
4: $R_1=\beta$-D-allose, $R_2=H$
5: $R_1=R_2=\beta$-D-allose



2 (=**1b**): R=H
6: R=β-D-allose



3: $R_1=R_2=R_3=H$
3a: $R_1=R_3=COCH_3$, $R_2=H$
3b: $R_1=H$, R_2, $R_3=>C(CH_3)_2$
7: $R_1=\beta$-D-allose, $R_2=R_3=H$

Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts of Compounds **1**—**7**, δ (ppm) from TMS in Pyridine-d_5

C	1[a)]	1a	2 (=1b)	3[a)]	3a	3b	4[a)]	5	6[a)]	7[a)]
1	57.5	61.7	61.8	62.5	65.8	65.5	57.4	57.1	61.6	62.3
3	60.4	58.8	58.8	59.0	61.3	58.8	67.8	67.9	66.5	66.4
4	33.0	34.4	34.1	33.8	29.5	33.4[b)]	29.7	29.6	31.3	30.8
5	138.8[b)]	70.9[b)]	69.5[b)]	85.9	85.0	82.6	137.8[b)]	137.7[b)]	69.3[b)]	85.1
6	35.2	29.7	30.1	27.2	27.2	26.6	35.1	34.8	30.1	27.1
7	26.3	23.2	23.8	33.7	33.8	33.1[b)]	26.3	26.5	23.3	33.9
8	51.5	45.0	44.4	43.5	43.7	43.9	51.0	47.7	44.6	43.4
9	139.6[b)]	71.5[b)]	72.2[b)]	83.5	82.0	90.7	139.5[b)]	139.6[b)]	72.4[b)]	83.8
10	65.6	62.6	63.5	71.7	71.5	71.2	65.4	73.4	63.0	71.4
1′ (1″)							101.9	102.1, 102.3	102.0	101.8
2′ (2″)							72.4	72.4 (×2)	72.3	72.4
3′ (3″)							72.9	72.8, 72.9	72.9	72.8
4′ (4″)							69.0	69.0 (×2)	69.0	68.9
5′ (5″)							75.9	75.9 (×2)	75.9	75.8
6′ (6″)							63.0	62.9 (×2)	63.0	62.9
					-OAc	Acetonide				
					20.6	25.5				
					20.8	27.0				
					170.6	109.3				
					171.0					

a) Signal assignments were made based on the two-dimensional (2D) NMR (^{1}H-^{13}C COSY) spectra. *b*) Signal assignments marked *b*) may be reversed. TMS=tetramethylsilane.

TABLE II. ^{1}H Chemical Shifts of Compounds **1**—**7**, δ (ppm) from TMS in Pyridine-d_5 (J/Hz in Parentheses)

H	1	1a	2 (=1b)	3	3b	4	5	6	7
1	4.44 (d, 12, H_a) 4.64 (d, 12, H_b)	4.20 (d, 12, H_a) 4.31 (d, 12, H_b)	4.18 (d, 13, H_a) 4.53 (d, 13, H_b)	4.32 (d, 12) 4.36 (d, 12)	4.04 (d, 9, H_a) 4.22 (d, 9, H_b)	4.42 (d, 13) 4.55 (d, 13)	4.47 (br s)	4.06 (d, 12) 4.47 (d, 12)	4.27 (s)
3	3.89 (t, 7)	4.02 (t, 6)	4.00—4.04 (m)	4.02—4.15 (m)	4.04—4.17 (m)	3.92 (t, 6)			
4	2.54—2.68 (m)	2.18 (m)	2.10—2.30 (m)	2.17 (dt, 14, 6) 2.32—2.39 (m)	2.10—2.21 (m)	2.50—2.62 (m)	2.20—2.30 (m) 2.64 (dt, 14, 7)	2.19 (br t, 7)	2.21 (ddd, 14, 7, 6) 2.27—2.38 m)
6	2.38 (ddd, 15, 7, 6) 2.50 (m)	1.88 (m) 2.02 (dd, 14, 8)	1.98 (dd, 13, 8) 2.10—2.30 (m)	1.51 (ddd, 12, 10, 4) 2.32—2.39 (m)	1.44 (t, 10, H_a) 2.00—2.05 (m)	2.28 (m) 2.42 (m)	2.20—2.30 (m) 2.45 (m)	1.92 (dd, 13, 8) 2.12 (dt, 13, 9)	1.45 (m) 2.27—2.38 (m)
7	1.79 (m) 2.01 (m)	1.20 (m) 1.67 (dd, 13, 9)	1.67 (dd, 13, 9) 1.80 (m)	1.84 (ddd, 12, 9, 4) 2.47 (td, 12, 3)	1.93 (t, 8) 2.00—2.05 (m)	1.76 (m) 1.97 (m)	1.67 (m) 1.92 (m)	1.60—1.75 (m)	1.79 (m) 2.27—2.38 (m)
8	3.22 (m)	2.73 (m)	2.91 (m)	2.50 (br s)	2.02 (br s)	3.21 (m)	3.35 (m)	2.89 (q, 7)	2.44 (br s)
10	3.99 (d, 6)	3.91 (dd, 11, 5, H_a) 4.13 (dd, 11, 9, H_b)	4.05 (dd, 11, 5, H_a) 4.16 (dd, 11, 5, H_b)	3.64 (d, 7) 4.11 (dt, 7, 3)	3.51 (d, 8, H_a) 3.66 (dt, 8, 3, H_b)	3.92 (d, 5)		4.00—4.10 (m)	3.58 (d, 7) 4.05 (dt, 7, 3)
1′(1″)						5.30 (d, 8)	5.28 (d, 8) 5.24 (d, 8)	5.26 (d, 8)	5.29 (d, 8)
2′(2″)						3.92 (dd, 8, 3)	3.91 (dd, 8, 3) 3.93 (dd, 8, 3)	3.91 (dd, 8, 3)	3.91 (dd, 8, 3)
3′(3″)						4.67 (t, 3)	4.66 (t, 3) 4.67 (t, 3)	4.67 (t, 3)	4.68 (t, 3)
4′(4″)						4.17 (dd, 10, 3)		4.16 (dd, 10, 3)	4.19 (dd, 10, 3)
6′(6″)						4.32 (dd, 11, 5) 4.45 (dd, 11, 2)			4.31 (dd, 11, 4) 4.44 (dd, 11, 2)

quartet pattern. In the ^{1}H–^{1}H shift correlation spectroscopy (COSY) measurement of **1**, one methine proton signal at δ 3.22 coupled to the 2H doublet signal at δ 3.93 and the methylene proton signals at δ 1.79 and 2.01, which further showed a connection to the neighboring methylene proton signals at δ 2.38 and 2.50. The evidence based on the NMR spectra led to the structure of **1** as a cyclopentene having one hydroxyethyl and two hydroxymethyl residues. The methine carbon bearing one of the hydroxymethyl groups and the methylene carbon was assigned as C-8. The hydroxyethyl and the other hydroxymethyl groups were allocated to C-5 and C-9, respectively, based on the differential nuclear Overhauser effect (NOE) measurements between H-8/H-1a, and H-10/H-1b. Since **1** showed the positive circular dichroism (CD) maximum at 200 nm,[8] the configuration at C-8 is tentatively assigned as β, by the application of the olefin octant rule according to Scott and Wrixon[8] (Chart 2).

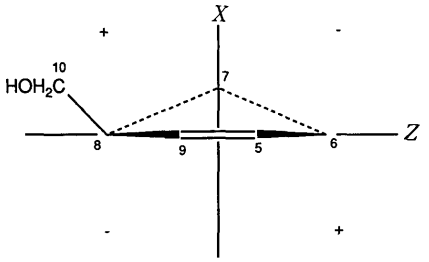
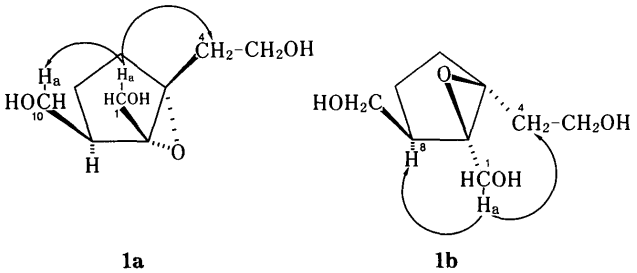


Chart 2



1a **1b**

Chart 3

Compound **2** showed the $M^+ + Na$ peak at m/z 211.0946, 16 mass units more than **1**, suggesting the molecular formula to be $C_9H_{16}O_4$. In the ^{13}C-NMR spectrum of **2**, signals of carbons having an oxygen function were observed at δ 69.5 and 72.2, instead of the two olefinic carbon signals in **1**, suggesting **2** to be an epoxide of **1**. The other seven carbon signals were characterized similarly to those of **1**. In the ^{1}H-NMR spectrum of **2**, a pair of doublets was observed at δ 4.18 and 4.53 with similar coupling constants (J=13 Hz), as seen in **1** at δ 4.44 and 4.64. Two doublets of doublets corresponding to H-10 in **1** appeared at δ 4.05 and 4.16, which showed couplings to a 1H multiplet signal at δ 2.91, and the evidence in the ^{1}H-NMR spectrum was consistent with the epoxide structure.

In order to confirm the structure, the oxidation of **1** was carried out. When **1** was reacted with *m*-chloroperbenzoic acid in $CHCl_3$, two products (**1a** and **1b**) were obtained, and considered to be epoxides based on the $M^+ + Na$ peaks at m/z 211, as well as the signals in the ^{1}H- and ^{13}C-NMR spectra. Differential NOE measurements of **1a** showed the proximity of H-1a and H-10a, and H-1a, b and H-4, respectively, being consistent with the assignment of **1a** as an epoxide having *trans* configuration of C-10 and the epoxide ring. In **1b**, NOE was observed between H-8/H-1a as well as H-1a/H-4, indicating **1b** to be an epoxide having *cis* configuration. The reaction mechanism of the epoxidation on **1** also suggested the major product to be the *trans* epoxide. The ^{1}H- and ^{13}C-NMR spectra of **1b** were in good agreement with those of **2**, and the structure of **2** was confirmed.

Compound **3** showed the $M^+ + 1$ peak at m/z 189.1123, suggesting the molecular formula to be $C_9H_{16}O_4$, the same as for **2**. The presence of nine carbon signals was confirmed in the ^{13}C-NMR spectrum, and **3** seemed to be a homologous compound with **1** and **2**. Upon usual acetylation, **3**

gave a diacetate (**3a**), although three triplet signals (δ 59.0, 62.5, and 71.7) and two singlet signals (δ 83.5 and 85.9) were observed in the region of carbinyl carbons. In a comparison of **3** with **3a**, the triplet signals at δ 59.0 and 62.5 in **3** showed downfield shifts to δ 61.3 and 65.8, respectively, while the signal at δ 71.7 retained almost the same chemical shift (δ 71.5).

In the ^{1}H-NMR spectrum, the signals due to four protons (1H: δ 4.32, 1H: 4.36, 2H: δ 4.02—4.15) in **3** were shifted downfield in **3a** but two signals at δ 3.64 (d) and 4.11 (dt) in **3** retained the same chemical shifts in **3a**, corresponding to the evidence in the ^{13}C-NMR spectrum. Since the 2H multiplet signal at δ 4.02—4.15 showed cross peaks with two signals at δ 2.17 (1H, dt) and 2.32—2.39 (1H, m) in the ^{1}H–^{1}H COSY, they were assignable to the protons on the hydroxyethyl residue at C-5, as observed in **1** and **2**. The broad singlet signal at δ 2.50, showing a coupling to the signal at δ 4.11, and a carbon signal at δ 43.5 were assignable to H-8 and C-8, respectively. Since the signals at δ 3.64 and 4.11 showed no acetylation shifts, the C-10 side chain seemed to be linked to another carbon, possibly to C-5, forming an ether linkage, and the two singlet carbon signals having an oxygen function at δ 83.5 and 85.9 can be assigned to C-5 and C-9 based on the biogenetic consideration that **3** is formed by the epoxide ring opening of **1a**. Two doublet signals at δ 4.32 and 4.36, showing a cross peak with each other in the ^{1}H–^{1}H COSY, were assignable to H-1a, b. The glycol system at C-1 and C-9 was confirmed by the formation of **3**-acetonide (**3b**). In the two dimensional NOE spectrum (NOESY) of **3b**, cross peaks were observed between H-1a, b/H-10b, H-10a/H-6a, and H-8/H-1a, showing the same orientations of C-1 and C-10 on the cyclopentane ring.

Compounds **4**, **6**, and **7** showed an anomeric proton signal at δ 5.26—5.30 (d, J=8 Hz) and six carbon signals due to a hexose moiety with almost the same chemical shifts, besides the signals due to **1**, **2**, and **3**, respectively, suggesting **4**, **6** and **7** to be the glycosides of **1**, **2** and **3**, containing the same component sugar. The $M^+ + 1$ peaks in the fast atom bombardment (FAB)-MS were consistent with the proposed structures. Since glycosylation shifts were observed at C-3, a glycosyl group was considered to be linked at the 3-hydroxyl group in these compounds. In the spectra of **5**, two anomeric protons and carbon signals were observed at similar chemical shifts (δ 5.24, 5.28; δ 102.1, 102.3). Other carbon signals due to the sugar and the terpenoid moieties were observed at nearly the same chemical shifts as in **4** except for the downfield shift of C-10, suggesting **5** to be a 3,10-bis-*O*-desmosidic glycoside of **1**, composed of the same sugar as the others.

Based on the chemical shifts in the ^{13}C-NMR and the coupling constants of H-2′ (dd, J=8, 3 Hz), H-3′ (t, J=3 Hz) and H-4′ (dd, J=10, 3 Hz), the component sugar was considered to be β-allopyranose.[9] Upon acid hydrolysis of **3**, the isolated sugar was identified as allose by comparison with authentic D-allose in thin layer chromatography (TLC), and a positive optical rotation value indicated **4**—**7** to be D-allopyranosides.

The presence of **3** in this plant can be explained biogenetically in terms of a pathway from **1**, although the attempted chemical conversion of **1a** into **3** under acidic conditions has been unsuccessful so far. Recently, similar normonoterpenoids have been isolated from *Eucommia ulmoides*,[10] *Rehmannia glutinosa*[11] and *Veronica linariaefolia*.[12] The absolute configurations of **1**, **2**, and **3** are to be confirmed separately.

Experimental

^{1}H- and ^{13}C-NMR spectra were measured on a JEOL GX-400 spectrometer in pyridine-d_5. Chemical shifts are given in δ values referred to internal tetramethylsilane, and the following abbreviations are used: s=singlet, br s=broad singlet, d=doublet, t=triplet, m=multiplet, dd=doublet of doublets. MS were recorded on a JEOL D-300 FD spectrometer. Circular dichroism was recorded on a JASCO DP 501N. Specific rotation was recorded with a JASCO DIP 360 polarimeter. The following solvent systems were used for silica gel column chromatographies and TLC: solvent 1, $CHCl_3$–MeOH–H_2O (bottom layer); solvent 2, EtOAc–MeOH–H_2O (top layer or homogeneous layer). Detection in TLC was done by charring the plate after spraying with 10% H_2SO_4.

Extraction From *Cerbera manghas* L.: The leaves of *C. manghas*, cultivated in the greenhouse of Fukuoka University, were harvested in September 1988, and air-dried in the shade. The powdered leaves (1.45 kg) were percolated with MeOH and the MeOH solution was concentrated *in vacuo* to 1 l. To this solution, 1 l of H_2O was added and the mixture was filtered. The filtrate was partitioned with benzene and then $CHCl_3$. The H_2O layer was concentrated *in vacuo* to 300 ml and then passed through a MCI-gel (Mitsubishi Chem. Co., CHP-20P) column, which was eluted stepwise with H_2O containing MeOH. The eluate with H_2O and 20% MeOH was separately chromatographed on an RQ-1 (Fuji-gel) column with H_2O–MeCN and on a silica gel column with solvent 1 (7:3:1→7:3:1.2) to afford **1** (75 mg), **2** (10 mg), **3** (160 mg), **4** (135 mg), **5** (20 mg), **6** (27 mg) and **7** (250 mg).

From *C. odollam* GAERTN.: Air-dried leaves (700 g), collected in Kent Ridge, Singapore, in November 1987, were treated in the same manner as in *C. manghas*, and **1** (15 mg), **3** (48 mg), **4** (28 mg) and **7** (14 mg) were obtained.

Cerberidol (1) A solid, $[\alpha]_D^{23}$ +11.0° (c=1.21, MeOH). CI-MS (isobutane) m/z: 173.1170 (Calcd for $C_9H_{16}O_3$+H: 173.1177). EI-MS m/z: 172, 154, 123, 103, 93. CD (c=0.009, MeOH) $[\theta]^{18}$ (nm): +3700 (200) (positive maximum).

m-Chloroperbenzoic acid (90 mg) was added to the solution of **1** (90 mg) in $CHCl_3$ (2 ml), and the mixture was allowed to stand at 5 °C for 6 h. The mixture was diluted with $CHCl_3$ and washed with H_2O, then the $CHCl_3$ was evaporated off *in vacuo*. The residue was chromatographed on an RQ-1 column and eluted with H_2O to give **1a** (26 mg) and **1b** (5 mg), each as a solid. **1a**: $[\alpha]_D^{21}$ +42.7° (c=0.75, MeOH). FAB-MS m/z: 211.0958. Calcd for $C_9H_{16}NaO_4$: 211.0947. **1b**: $[\alpha]_D^{28}$ +0.50° (c=0.20, MeOH). FAB-MS m/z: 211 (M^++Na). ^{13}C-NMR δ: 23.8 (C-7), 30.1 (C-6), 34.1 (C-4), 44.4 (C-8), 58.8 (C-3), 61.8 (C-1), 63.5 (C-10), 69.5, 72.2 (C-5, 9). TLC (solvents 1 and 2) and NMR data were in good agreement with those of **2**.

Epoxycerberidol (2) A solid, $[\alpha]_D^{28}$ −0.40° (c=0.50, MeOH). FAB-MS m/z: 211.0941. Calcd for $C_9H_{16}NaO_4$: 211.0946.

Cyclocerberidol (3) A solid, $[\alpha]_D^{27}$ −16.9° (c=2.10, MeOH). CI-MS (isobutane) m/z: 189.1123. Calcd for $C_9H_{16}O_4$+H: 189.1126. **3**-Diacetate (**3a**): **3a** was obtained as a solid by the acetylation of **3** with pyridine and Ac_2O at room temperature, $[\alpha]_D^{28}$ +7.6° (c=2.57, MeOH). CI-MS (isobutane) m/z: 273.1334. Calcd for $C_{13}H_{20}O_6$+H: 273.1334). ^{1}H-NMR δ: 1.95, 2.00 (3H each, s, –OAc), 2 31 (1H, br s, H-8), 3.58 (1H, d, J=7 Hz, H-10a), 3.98 (1H, dt, J=7, 2 Hz, H-10b), 4.43 (2H, td, J=8, 2 Hz, H-3), 4.62, 4.79 (1H each, d, J=12 Hz, H-1a, b). **3**-Acetonide (**3b**): **3** (80 mg) was allowed to stand at room temperature with acetone (1 ml) and 0.05 ml of concentrated HCl. The mixture was diluted with MeOH and deacidified with IR-410. The solution was concentrated *in vacuo* to dryness, and the residue was chromatographed on a silica gel column with benzene–acetone (5:1) to give **3b** as a solid, $[\alpha]_D^{30}$ +0.66° (c=0.60, MeOH). FAB-MS m/z: 229.1443. Calcd for $C_{12}H_{20}O_4$+H: 229.1440. CI-MS (isobutane) m/z: 229, 171, 153, 140.

Cerberidol-3-*O*-β-D-allopyranoside (4) A solid, $[\alpha]_D^{24}$ −18.1° (c=1.75, MeOH). FAB-MS m/z: 357.1528. Calcd for $C_{15}H_{26}NaO_8$: 357.1526.

Cerberidol-3,10-bis-*O*-β-D-allopyranoside (5) A solid, $[\alpha]_D^{26}$ −25.7° (c=1.20, MeOH). FAB-MS m/z: 519.2044. Calcd for $C_{21}H_{36}NaO_{13}$: 519.2055.

Epoxycerberidol-3-*O*-β-D-allopyranoside (6) A solid, $[\alpha]_D^{22}$ −33.5° (c=1.85, MeOH). FAB-MS m/z: 373.1466. Calcd for $C_{15}H_{26}NaO_9$: 373.1474.

Cyclocerberidol-3-*O*-β-D-allopyranoside (7) A solid, $[\alpha]_D^{28}$ −26.6° (c=2.1, MeOH). FAB-MS m/z: 373.1478. Calcd for $C_{15}H_{26}NaO_9$: 373.1474). A solution of **3** (35 mg) in 0.2 N H_2SO_4 (2 ml) was heated at 100 °C for

3 h. The solution was deacidified with IR-410 and passed through an RQ-1 column. The eluate with H_2O gave D-allose (3.2 mg), $[\alpha]_D^{26}$ +13.8° (c=0.16, H_2O).

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