10-Carboxyloganin, Normonoterpenoid Gluosides and Dinormonoterpenoid Glucosides from the Leaves of *Cerbera manghas* (Studies on *Cerbera*. 10)[1)]

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10-Carboxyloganin was isolated along with β-D-glucosides of cyclopentano-normonoterpenoids and dinormonoterpenoids from the frozen fresh leaves of *Cerbera manghas*. Their structures were characterized by means of MS and NMR spectroscopy. The correlation of drying conditions of the leaves and yields of glucosides or allosides of cyclocerberidol and epoxycerberidol was studied. The dinormonoterpenoids were similar to those obtained from *Thevetia peruviana*.

Key words *Cerbera manghas*; Apocynaceae; 10-carboxyloganin; iridoid; cyclopentano-normonoterpenoid glucoside; dinormonoterpenoid glucoside

Cerbera and *Thevetia* are indigenous to South-East Asia and tropical America, respectively, and they are closely related genera, containing the same cardenolide glycosides and iridoids. Recently we have investigated the polar constituents of *Thevetia*, and described iridoid biosides composed of theviridoside (**1**) and fructose or glucose,[2,3)] and polyhydroxy-dinormonoterpenoid biosides.[4)] 10-*O*-Benzoyltheviridoside (**2**) was previously obtained from *C. manghas*,[1)] along with **1** and theveside (**3**) as common iridoids in the two species[5,6)] and loganin (**4**). In order to compare the polar glycosides in the two plants, the MeOH extract from the leaves of *C. manghas* was re-investigated. This paper deals with the isolation of 10-carboxyloganin (**5**), cyclopentano-normonoterpenoid-β-D-glucosides (**6**, **7**), of which only D-allosides were previously isolated[7)] from the air-dried leaves, and dinormonoterpenoid-β-D-glucosides (**9**—**12**). Co-occurrence of the glucosides and allosides of cyclopentano-normonoterpenoids in the partially dried leaves was examined.

When the frozen fresh leaves were percolated with cold MeOH and the MeOH percolate was chromatographed on reversed-phase columns, **5** was isolated after **3**. After successive chromatographies of the polar fraction containing **1** on normal-phase and reversed-phase columns and HPLC, six compounds (**6**, **7**, **9**—**12**) were obtained besides **4**.

In FAB-MS of **5**, quasi-molecular ion peaks of $[M+Na]^+$ and $[M-1+2Na]^+$ were observed at *m/z* 443.1166 ($C_{17}H_{24}NaO_{12}$) and 465.0987 ($C_{17}H_{23}Na_2O_{12}$), respectively. In the ^{1}H-NMR spectrum, characteristic signals assignable to an iridoid, H-1 (δ 5.43, d, J=4 Hz), H-3 (δ 7.40, d, J=1 Hz), H-5 (δ 3.16, br q, J=8 Hz), H-9 (δ 2.75, td, J=10, 4 Hz), and carbomethoxyl protons (δ 3.69, s), along with an anomeric proton (δ 4.62, d, J=8 Hz) of glucose, were observed. A signal at δ 4.33 with triplet-of-doublets pattern (J=5, 2 Hz), was assignable to H-7 coupled with H-8 (δ 2.56, dd, J=10, 5 Hz) in the ^{1}H–^{1}H COSY spectrum, and **5** was considered to retain an iridoid structure with a secondary hydroxyl group at C-7 of the cyclopentane ring as in **4**, although the methyl group at

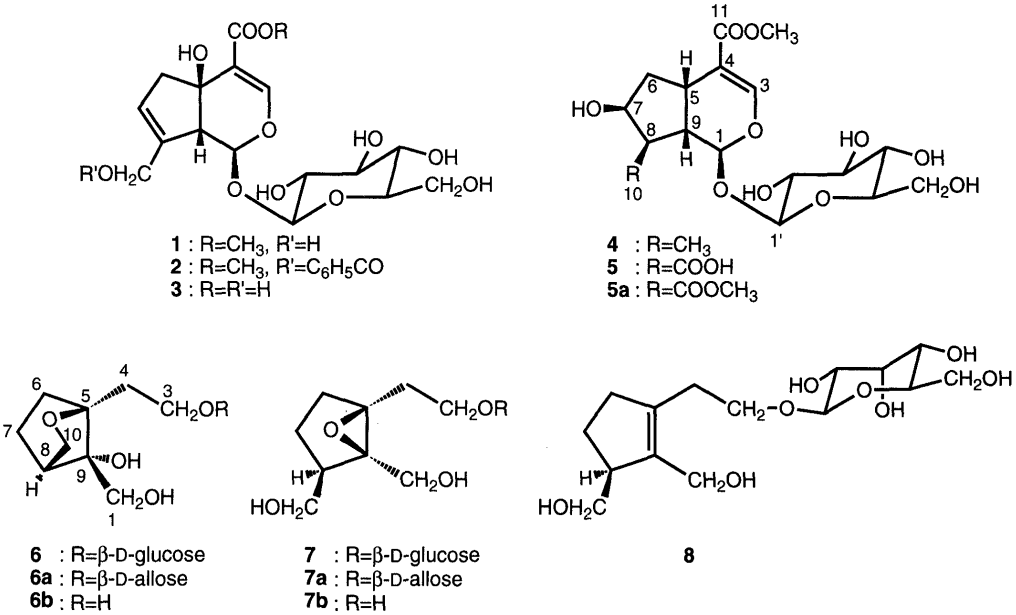

Fig. 1

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C-10 was not observed. In the ^{13}C-NMR spectrum of **5**, signals were observed at similar chemical shifts to those of **4**[8] (within 2 ppm), except for the carboxyl carbon in **5** at δ 180.2 instead of the methyl carbon in **4**. The C-8 carbon signal was also shifted by +10 ppm in **5**. While the signal at δ 170.0 corresponded to the methoxycarbonyl carbon signal at δ 167.7 in **4** and was assignable to be C-11, the signal at δ 180.2 was considered to be due to the free carboxyl group of C-10.

Upon methylation with CH_2N_2/ether, **5** gave a methylate ($C_{18}H_{26}O_{12}$) (**5a**), of which all the ^{1}H and ^{13}C signals were consistent with a 10-carbomethoxyloganin structure. When the differential NOEs were examined in order to confirm the stereochemistry at C-7 and C-8, responses were observed between H-1 and H-8, H-1′; H-7 and H-8; and H-5 and H-9. Therefore, the orientations at the hydroxyl group at C-7 and the carboxyl group at C-8 were both assigned as β.

The molecular formula of **6** was suggested to be $C_{15}H_{26}O_9$ based on the $[M+Na]^+$ peak at m/z 373.1475 in FAB-MS as in cyclocerberidol-3-*O*-β-D-allopyranoside (**6a**). In the ^{1}H- and ^{13}C-NMR spectra, the aglycone moiety of **6** showed almost the same chemical shifts as those of **6a** previously obtained from *Cerbera manghas*.[7] All the proton signals due to H-1′—H-5′ were observed in *axial* mode, suggesting that the component sugar in **6** was β-linked glucose instead of D-allose in **6a**. Therefore, **6** was assigned as cyclocerberidol-3-*O*-β-D-glucoside.

FAB-MS of **7** also suggested the same molecular formula as that of **6**, $C_{15}H_{26}O_9$, and the component sugar was assigned as glucose, based on the coupling constants of H-1′ and H-4′ and the ^{13}C signals. The aglycone of **7** was identified as epoxycerberidol (**7b**).[7] By comparison of the ^{13}C-NMR spectrum of **7** with that of epoxycerberidol-3-*O*-β-D-allopyranoside (**7a**), the linkage site of glucose was assigned as the 3-hydroxyl group of the aglycone. The structure of **7** was thus characterized as epoxycerberidol-3-*O*-β-D-glucoside.

Since allosides but no glucosides of **6b** and **7b** were obtained from the air-dried leaves,[7] and only the glucosides (**6**, **7**) from the fresh leaves, the glycosides in the partially dried leaves were examined. When three samples, air-dried to 50%, 42% and 19% of the weight of the original fresh leaves, were examined, the former two samples contained **6**, **7**, **6a** and **7a**. However, the sample, air-dried to 19% of the original weight, contained only **6a** and **7a** and a small amount of cerberidol β-D-alloside (**8**),[7] instead of **6** and **7**, suggesting that **6b** or **7b** might be converted into cerberidol, and glucosides into allosides during the air-drying procedure.

In the ^{1}H-NMR spectra of **9**—**12**, an anomeric proton signal was observed at δ 4.79—4.90. Coupling patterns from H-1′ to H-6′ as well as signals in the ^{13}C-NMR spectra showed the component sugar of **9**—**12** to be β-linked glucose and the aglycones to be composed of eight carbons. FAB-MS of **9** afforded the $[M+Na]^+$ peak at m/z 347.1681, suggesting the molecular formula to be $C_{14}H_{28}O_8$. NMR considerations, as well as the result of FAB-MS, indicated that **9** is a saturated compound which retains a hydroxyisopropyl group and a terminal methyl group linked to a hydroxymethine (H-4: δ 4.20, C-4: δ 70.2). The aglycone was considered to have the same branched-chain carbon array as those obtained from *Thevetia peruviana*.[4] The glucosidic linkage to the primary carbinol (C-1: δ 70.5) was determined by the HMBC cross peak between the anomeric proton and C-1. Thus, **9** was

Table 1. ^{1}H- and ^{13}C-NMR Data for **5**, **6** and **7** (δ ppm from TMS in C_5D_5N, J=Hz in Parentheses)

No. of C or H	**5**[a]		**6**		**7**	
	C	H	C	H	C	H
1	97.8	5.43 (d, 4)	62.2	4.28 (2H, s)	61.6	4.08 (d, 12) 4.47 (d, 12)
3	152.3	7.40 (d, 1)	66.5	4.43 (ddd, 9, 8, 5) 3.95—4.04	66.6	
4	113.7		30.8	2.24 (ddd, 14, 8, 6) 2.30—2.40	31.3	2.23 (2H, br t, 7)
5	31.7	3.16 (br q, 8)	85.0[b]		69.3[b]	
6	42.3	1.57 (ddd, 14, 7, 5) 2.28 (ddd, 14, 8, 2)	27.2	1.45 (m) 2.30—2.40	30.2	1.94 (dd, 14, 7) 2.14 (dt, 14, 9)
7	73.5	4.33 (td, 5, 2)	33.9	1.82 (m) 2.30—2.40	23.4	1.60—1.75 (m)
8	54.6	2.56 (dd, 10, 5)	43.4	2.44 (t, 3)	44.6	2.89 (br q, 6)
9	43.4	2.75 (td, 10, 4)	83.8[b]		72.4[b]	
10	180.2		71.4	3.58 (d, 7) 4.05 (dt, 7, 3)	63.0	
11	170.0					
OCH_3	52.0	3.69 (s)				
1′	100.0	4.62 (d, 8)	104.4	4.85 (d, 8)	104.5	4.83 (d, 8)
2′	74.3	3.20 (dd, 9, 8)	75.1	3.98 (dd, 8, 9)	75.1	
3′	77.5	3.37 (t, 9)	78.4[c]	4.22 (t, 9)	78.4[c]	
4′	71.2	3.31 (t, 9)	71.6	4.19 (t, 9)	71.6	4.18 (t, 9)
5′	77.9	3.30 (m)	78.5[c]	3.90 (m)	78.5[c]	3.90 (m)
6′	62.3	3.67 (dd, 12, 5) 3.85 (dd, 12, 2)	62.7	4.35 (dd, 12, 5) 4.49 (dd, 12, 2)	62.8	4.35 (dd, 11, 5) 4.49 (dd, 11, 2)

a) Dissolved in CD_3OD–D_2O. *b*) or *c*) Signal assignments may be interchangeable in each column.

Table 2. ^{1}H- and ^{13}C-NMR Data for **9**—**11** and **12** (δ ppm from TMS in C_5D_5N, J=Hz in Parentheses)

No. of C or H	9		10		11		12	
	C	H	C	H	C	H	C	H
1	70.5	3.91 (m) 4.28 (m)	70.4	3.94 (m) 4.31 (m)	65.5	3.78 (m) 4.15—4.25	69.7	3.87 (dt, 10, 7) 4.26 (dt, 10, 7)
2	29.5	1.68 (m) 1.86 (m)	31.3	1.64—1.74 2.28 (m)	28.9	2.05 (m) 2.22 (m)	31.4	2.44 (2H, br t, 7)
3	52.3	1.77 (m)	48.8	1.50 (m)	64.5		142.3	
4	70.2	4.20 (m)	24.3	1.17 (m) 1.64—1.74	63.6	3.62 (dd, 6, 4)	126.5	5.67 (t, 6)
5	22.6	1.40 (d, 7)	13.8	0.95 (t, 7)	60.3	4.01 (dd, 12, 6) 4.08 (dd, 12, 4)	58.1	4.87 (2H, d, 6)
6	73.9		72.5		31.4	1.73 (qui, 7)	29.3	2.84 (qui, 7)
7, 8	25.8 29.8	1.39 (s) 1.41 (s)	26.9 28.6	1.26 (s) 1.32 (s)	17.7 18.7	0.93 (d, 7) 0.97 (d, 7)	21.0 (×2)	0.89 (6H, d, 7)
1′	104.7	4.90 (d, 8)	104.9	4.88 (d, 8)	104.6	4.79 (d, 8)	104.7	4.87 (d, 8)
2′	75.2	4.05 (dd, 8, 9)	75.2	4.04 (dd, 8, 9)	75.1	4.00 (dd, 8, 9)	75.2	4.03 (dd, 8, 9)
3′	78.6	4.25 (t, 9)	78.6	4.22 (t, 9)	78.5	4.15—4.25	78.5	4.25 (t, 9)
4′	71.7	4.22 (t, 9)	71.7	4.20 (t, 9)	71.6	4.15—4.25	71.7	4.22 (t, 9)
5′	78.4	3.95 (m)	78.3	3.92 (m)	78.3	3.90 (m)	78.4	3.95 (m)
6′	62.8	4.37 (dd, 12, 5) 4.53 (br d, 12)	62.8	4.35 (dd, 12, 5) 4.51 (dd, 12, 2)	62.7	4.36 (dd, 12, 5) 4.50 (dd, 12, 2)	62.8	4.38 (dd, 12, 5) 4.53 (dd, 12, 2)

assigned as 3-(hydroxyisopropyl)pentane-1,4-diol-1-*O*-*β*-D-glucoside.

Based on the quasi-molecular peak at *m/z* 331.1734, **10** appeared to have the molecular formula $C_{14}H_{28}O_7$, one oxygen less than **9**. The aglycone moiety retained one tertiary and one primary hydroxyl groups, which were assignable to the hydroxyisopropyl group and a carbinol at C-1, respectively, based on the ^{1}H- and ^{13}C-NMR spectra. Since a terminal methyl group was observed as a triplet signal, **10** was characterized as 3-(hydroxyisopropyl)pentan-1-ol-1-*O*-*β*-D-glucoside.

Compound **11** showed the quasi-molecular peak at *m/z* 345.1522, suggesting the molecular formula $C_{14}H_{26}O_8$, 2 mass units smaller than **9**. Among four oxygen-linked carbon signals at δ 60.3—65.5, two of them at δ 60.3 and 65.5 were due to primary carbinol carbons (C-1, C-5). The other two signals (δ 63.6, 64.5) were observed at rather higher field than those of usual carbinols and suggest the presence of an epoxide as in some of the homologous glycosides from *Thevetia*.[4] The molecular formula was also consistent with the 3,4-epoxide structure, as shown in Fig. 2. The steric relation of the isopropyl and the terminal hydroxymethylene groups was confirmed to be *cis* based on NOEs between H-5/H-6 and H-2/H-4. In the HMBC, a cross peak was observed between H-1′ and C-1, suggesting the glycosidic linkage at the C-1 hydroxyl. Thus, the structure of **11** was characterized as (3*ξ*,4*ξ*)-3-isopropyl-3,4-epoxypentane-1,5-diol-1-*O*-*β*-D-glucoside.

Compound **12**, $C_{14}H_{26}O_7$, seemed to retain the same carbon array as **8**—**11**, with one trisubstituted olefinic linkage. The presence of an isopropyl group at C-3 was assigned on the basis of the NMR spectra. No terminal methyl protons, but instead hydroxymethylene protons, were observed at δ 4.87 as a doublet pattern (J=6 Hz) coupled to the olefinic proton, indicating the location of the olefinic linkage at C-3(4). Since NOEs between H-2/H-4 and H-5/H-6 were observed, the isopropyl and the terminal carbinol groups were considered to be in a *cis*-relation. Thus, the structure of **12** was established as (*Z*)-3-isopropyl-3-pentene-1,5-diol-1-*O*-*β*-D-glucoside.

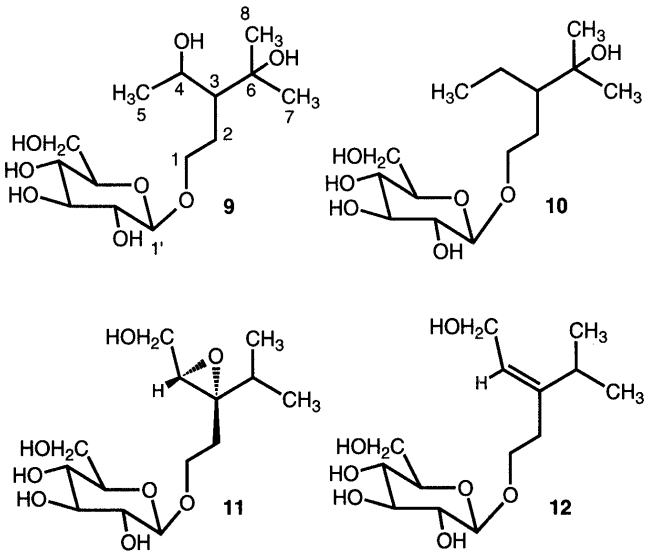

Fig. 2

It would be of interest to investigate further the occurrence of the allosides during the drying process of the leaves. Since a cardenolide glycoside with 3-oxoglucose has been isolated from *Cerbera*,[9] the possibility of conversion of glucosides into allosides through 3-oxoglucosides can not be excluded, although **6** and **7** are mostly hydrolyzed to their aglycones, **6b** and **7b**, during the drying procedure. In this work, the presence of dinormonoterpenoids with similar structures to those from *Thevetia* was disclosed. Their sugar moieties are simply glucosides in *Cerbera* and apiosylglucosides in *Thevetia*. Glucosides in this study are tentatively assigned as D-form, since cardenolides and iridoids from these plants are all *β*-D-glucopyranosides.

Experimental

^{1}H- and ^{13}C-NMR spectra were measured on a JEOL GX-400 and α 500 spectrometer in pyridine-d_5 unless otherwise mentioned. Chemical shifts are given in δ values referred to internal tetramethylsilane, and the following abbreviations are used: s=singlet, d=doublet, br=broad, t=triplet, m=multiplet, q=quartet, qui=quintet. FAB-MS were recorded on a JEOL HX 110 spectrometer. The following solvent systems were used for silica gel column chromatographies and TLC: solvent 1, $CHCl_3$–MeOH–H_2O (7:3:1.6—7:3:1.2, bottom layer); solvent 2, EtOAc–MeOH–H_2O (7:1:1.2—5:1:1.2, top layer). Detection in TLC was done by charring the plate after spraying 10% H_2SO_4. For reversed-phase column chromatographies, MCI-gel (solvent: H_2O–MeOH), Fuji-gel (H_2O–MeOH), and YMC-gel (H_2O–MeCN) were used.

Extraction and Isolation *Cerbera manghas* L. has been cultivated in a greenhouse of Fukuoka University. The leaves were frozen at −20 °C immediately after collection in November, 1994. The frozen leaves were percolated with cold MeOH without cutting or homogenizing. The MeOH percolate was concentrated *in vacuo*, and extracted with benzene to remove chlorophyl. The MeOH layer was concentrated *in vacuo* and the residue was passed through an MCI-gel column. The column was eluted with H_2O, 20%, 40%, 60% and 80% MeOH, successively. The 2nd fraction (20% MeOH eluate) was then chromatographed on a Fuji-gel column with H_2O–20% MeOH and again on a YMC-gel column with 5% MeCN to afford **1** (*ca.* 7 g) and **5** (135 mg). One-fourth of the 1st fraction (H_2O eluate) of the MCI-gel eluate was fractionated similarly using a YMC-gel column with H_2O–20% MeCN and each fraction was then subjected to silica gel column chromatography with solvent 1 and finally to HPLC (Capcell pak C_{18}, Shiseido) with 5% MeCN to give **4** (11 mg), **6** (17.7 mg), **7** (24.5 mg), **9** (8.3 mg), **10** (20.6 mg), **11** (34.6 mg), and **12** (35.0 mg).

Compound 5 (10-Carboxyloganin) Solid, $[\alpha]_D^{22}$ −52.9° (c=1.35, MeOH). FAB-MS m/z: 443.1166. Calcd for $C_{17}H_{24}NaO_{12}$: 443.1166, m/z: 465.0987. Calcd for $C_{17}H_{23}Na_2O_{12}$: 465.0985. Negative FAB-MS m/z: 419.1191. Calcd for $C_{17}H_{23}O_{12}$: 419.1190. A solution of **5** (10 mg) in MeOH (1 ml) was treated with CH_2N_2 in ether. The mixture was allowed to stand for 3 h and the solvent was evaporated *in vacuo*. The residue was purified on a silica gel column (solvent 1) to give **5**-methylate (**5a**): solid, $[\alpha]_D^{22}$ −24.9° (c=0.31, MeOH). FAB-MS m/z: 457.1324. Calcd for $C_{18}H_{26}NaO_{12}$: 457.1322. ^{1}H-NMR δ: 1.81 (1H, ddd, J=14, 7, 5 Hz, H-6a), 2.66 (1H, ddd, J=14, 8, 2 Hz, H-6b), 3.30 (1H, dd, J=8, 5 Hz, H-8), 3.49 (1H, td, J=8, 5 Hz, H-9), 3.56, 3.58 (3H each, s, $COOCH_3$), 3.63 (1H, br q, J=8 Hz, H-5), 3.99 (1H, m, H-5′), 4.02 (1H, dd, J=8, 9 Hz, H-2′), 4.20 (1H, t, J=9 Hz, H-4′), 4.25 (1H, t, J=9 Hz, H-3′), 4.35 (1H, dd, J=12, 5 Hz, H-6′a), 4.52 (1H, dd, J=12, 2 Hz, H-6′b), 4.75 (1H, br t, J=4 Hz, H-7), 5.35 (1H, d, J=8 Hz, H-1′), 5.73 (1H, d, J=5 Hz, H-1), 7.67 (1H, d, J=1 Hz, H-3). In a differential NOE experiment on **5a**, NOEs were observed between H-1/H-1′, -8, H-7/H-8 and H-5/H-9.

Compound 6 (Cyclocerberidol-3-*O*-β-D-glucoside) Solid, $[\alpha]_D^{23}$ −13.6° (c=0.39, MeOH). FAB-MS m/z: 373.1475. Calcd for $C_{15}H_{26}NaO_9$: 373.1475.

Compound 7 (Epoxycerberidol-3-*O*-β-D-glucoside) Solid, $[\alpha]_D^{23}$ −18.5° (c=1.49, MeOH). FAB-MS m/z: 373.1472. Calcd for $C_{15}H_{26}NaO_9$: 373.1475.

Glucosides and Allosides of 6b and 7b (6, 7, 6a, 7a), and 8 in Partially-Dried Leaves of *Cerbera manghas* The fresh leaves collected in Sept., 1995 were mixed uniformly, divided into 3 groups (samples A, 1.3 kg; B, 1.3 kg; and C, 1.6 kg) and air-dried in the room for 7 d (A, 50% weight of the original leaves), 10 d (B, 42%), or 22 d (C, 19%). Each sample was homogenized with MeOH and percolated with MeOH. The MeOH percolate was concentrated *in vacuo* and the polar fraction was passed through MCI-gel with H_2O–MeOH. The fraction containing **6** and **7** was chromatographed on a YMC-gel column and finally fractionated by HPLC with 5% MeCN. Yields of **6**, **7**, **6a**, and **7a**, and **8** were as follows: A: **6**, 10.0 mg; **7**, 35.0 mg; **7a**, 1.4 mg; B: **6**, 7.1 mg; **7**, 36 mg; **6a**, 2.6 mg; **7a**, 2.3 mg; C: **6**, none; **7**, none; **6a**, 2.3 mg; **7a**, 5.0 mg; **8**, *ca.* 1 mg.

Compound 9 Solid, $[\alpha]_D^{22}$ −13.5° (c=0.45, MeOH). FAB-MS m/z: 347.1681. Calcd for $C_{14}H_{28}NaO_8$: 347.1682.

Compound 10 Solid, $[\alpha]_D^{23}$ −24.8° (c=1.35, MeOH). FAB-MS m/z: 331.1734. Calcd for $C_{14}H_{28}NaO_7$: 331.1733.

Compound 11 Solid, $[\alpha]_D^{23}$ −17.5° (c=0.18, MeOH). FAB-MS m/z: 345.1522. Calcd for $C_{14}H_{26}NaO_8$: 345.1525. In a differential NOE experiment on **10**, NOEs were observed between H-5a,b/H-6 and H-2/H-4.

Compound 12 Solid, $[\alpha]_D^{23}$ −21.5° (c=0.60, MeOH). FAB-MS m/z: 329.1578. Calcd for $C_{14}H_{26}NaO_7$: 329.1576. In a differential NOE experiment on **12**, NOEs were observed between H-2/H-4 and H-5/H-6.

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