Iodine-Catalyzed Etherification of Morroniside

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In this study, we describe a highly selective etherification procedure of unprotected morroniside catalyzed by molecular iodine in acetone. The etherification reaction furnished 7-*O*-alkyl ether derivatives in reasonable yields within few hours under neutral conditions. Studies of the obtained products on cytotoxicity activity in colon 26-L5 cell line were examined. Among the tested compounds, 7-*O*-dodecylmorroniside showed moderate cytotoxic activity, having IC_{50} values equal to 20.9 μM.

Key words etherification; morroniside; cytotoxicity

The use of natural iridoid glycosides as starting materials for the synthesis of biological active compounds received considerable attention. The importance of these compounds has been demonstrated in various synthetic approaches.[1—10] Recent synthetic applications have resulted into conversion of aucibin to aminoside antibiotic analogues.[11]

Morroniside (**1**) has been found to have some applications in pharmacology as well as in cosmetics. The compound can be used as α-glucosidase inhibitor in drugs and healthy food for preventing impaired glucose tolerance, diabetes and obesity.[12] Penta-acetate derivatives of **1** possessing melanin inhibitory effect can be used as one of the component in skin-whitening agent.[13] So far little is known about other biologically important derivatives derived from morroniside (**1**).

The use of iodine as a catalyst in organic synthesis is well known. Iodine has been employed in carbohydrate chemistry, as a catalyst for the isopropylidenation of some simple monosaccharides and sugar alcohol.[14] It is now found that iodine can be used efficiently as catalyst in various syntheses of carbohydrate derivatives.

Results and Discussion

Morroniside (**1**) was isolated from methanolic root extract of the plant *Strychnos cocculoides* by repeated silica gel vacuum liquid chromatography using methanol/dichloromethane (20 : 80) as solvents. Morroniside (**1**) was isolated as the major compound and observed in ^{1}H-NMR spectrum as a mixture of anomeric forms.[15,16] This compound has been isolated for the first time from plant of the genus *Strychnos*.

It was found that some alditol long-chain alkyl ether derivatives are interesting class of carbohydrate with important biological functions.[17] Therefore, we found worthwhile to study possibility of obtaining similar derivatives from compound **1** for biological evaluations.

When morroniside (**1**) was treated with few drops of methanol in the presence of iodine and acetone at room temperature, compound **3a** was obtained (Chart 1). The control experiments revealed that no formation of compound **3a** occurred, when iodine was omitted during the reaction or when acetone was replaced completely with methanol. The position of alkyl ethers formed was confirmed by heteronuclear multiple bond correlation (HMBC) experiments on compound **3a**. It was confirmed by HMBC experiments that the one methoxy group at δ 3.31 was attached to C-7 at δ 95.6. In regard to these observations more attentions were therefore directed towards this reaction so as to obtain long chain alkyl ether derivatives.

To determine the scope and selectivity of this reaction, different alcohols (including long chain aliphatic alcohols) were tested under the same conditions. As shown in Table 1, most

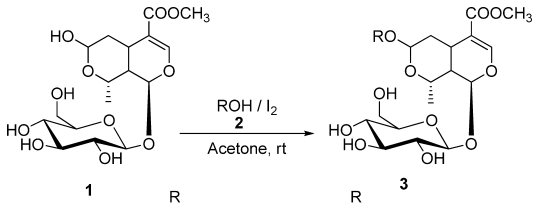

Chart 1

Table 1. Synthesis of Morroniside Derivatives **3**

Entry	ROH **2**	Time (h)	Yield (%) **3**	Entry	ROH **2**	Time (h)	Yield (%) **3**
1	Methanol	4	84.2 (**3a**)	8	Benzyl alcohol	0.5	53.2 (**3h**)
2	Ethanol	4	88.0 (**3b**)	9	*cis*-2-Hexenol	1	74.7 (**3i**)
3	Propanol	4	74.4 (**3c**)	10	1-Octanol	4	50.4 (**3j**)
4	Isopropanol	4	78.5 (**3d**)	11	1-Dodecanol	1	68.5 (**3k**)
5	*t*-Butanol	4	21.0 (**3e**)	12	1-Hexadecanol	4	56.2 (**3l**)
6	*n*-Butanol	4	85.9 (**3f**)	13	1-Octadecanol	4	54.4 (**3m**)[a]
7	Cyclohexanol	4	70.3 (**3g**)				

a) Reaction at 40 °C.

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of the selected alcohols gave the corresponding products in reasonable yields (50.4—88.0%). The results indicated that the reaction with benzyl alcohol was completed by the shortest reaction time as compared to other selected alcohols. Low yield of the corresponding product was obtained, when compound **1** was treated with *t*-butanol. The introduction of long-chain alkyl ether using 1-octanol and 1-dodecanol showed a further possibility of introducing other long chain aliphatic hydrocarbons. Therefore, compounds **3l** and **3m** were obtained using similar approach. Due to low solubility of 1-octadecanol in acetone, etherification reaction was performed at 40 °C.

Attempts to obtain alkyl ether derivatives using D-glucose, methyl α-D-glucoside and loganic acid as substrates under the same conditions were unsuccessful. Only starting materials and some decomposed products were recovered. This type of etherification reaction using alcohol in the presence of iodine and acetone as a solvent has not yet been reported. The mechanism of the reaction described herein is activated by the presence of iodine. The sequences are initiated by the formation of an acetone–iodine addition complex at hydroxyl group (C-7),[18] followed by attack of alcohol to form the product.[19] So far the present reaction can be unique and specific etherification on hydroxyl group (C-7) of unprotected iridoid glucoside **1**. The presence of acetone in this reaction therefore promotes etherification reaction rather than isopropylidenation reaction.[20,21]

Some of the prepared alkyl ether derivatives have also been isolated from natural sources. Compound **3b** was isolated from ethanol extract of *Fructus corni*.[22] The alkyl ether derivative **3f** was isolated from *Cornus officinalis*,[23] and its 7*R* isomer was obtained from leaves of *Hydrangea macrophylla* subsp. *serrata*.[24]

Recently, it is reported that incubation of cells with morroniside led to a significant dose-dependent elevation of cellular GSH accompanied by a marked protection against H_2O_2-mediated cytotoxicity. Morroniside (**1**) inhibited the formation of intracellular oxygen species and the activation of caspase-3 and 9.[25] This result stimulated us to prepare a series of morroniside derivatives and to test their cytotoxicity. The alkyl ether derivatives were tested for their cytotoxicity toward colon 26-L5 cell line. Among them, compound **3k** showed the strongest cytotoxic activity, having inhibitory concentration IC_{50} values equal to 20.9 μM (Table 2).

Table 2. Cytotoxic Activity of Morroniside Derivatives against the Colon 26-L5 Cell Line

Compound	R	IC_{50} (μM)
1	H	969
3a	Methyl	1707
3b	Ethyl	1248
3c	Propyl	1085
3d	Isopropyl	1031
3e	*t*-Butyl	1014
3f	*n*-Butyl	887
3g	Cyclohexyl	921
3h	Benzyl	1166
3i	*cis*-2-Hexenyl	524
3j	1-Octyl	69.3
3k	1-Dodecyl	20.9
3l	1-Hexadecyl	96.1
3m	1-Octadecyl	156

In conclusion, a convenient method based on iodine-catalyzed etherification of iridoid glycoside has been developed. The reaction featured under mild and neutral conditions, high selectivity, and reasonable yields. Further structural modifications on compound **1** to introduce other functional groups are currently under investigations.

Experimental

General Experimental Procedure IR spectra were recorded on a JASCO FTIR-460 Plus spectrophotometer. Optical rotations were determined with a JASCO P-1010 polarimeter at specified temperature and concentrations in the solvents indicated. ^{1}H- (600 MHz) and ^{13}C- (125 MHz) NMR spectra were recorded on JEOL ECA 600 spectrometer with tetramethyl silane (TMS) as an internal standard. MS were recorded using a JEOL JMS-700/GI spectrometer. Column chromatography was performed on silica gel N-60 (40—50 μm) and, TLC on pre-coated plates of silica gel 60 F_{254}.

Extraction and Isolation Morroniside (**1**) was isolated from methanol extract of root bark of *Strychnos cocculoides* using vacuum liquid chromatography followed by column chromatography (methanol/dichloromethane =2/8).

Reaction Conditions Morroniside (**1**) (0.134 mmol), iodine (1.7 mg, 0.1 mmol) and alcohol **2** (1.5 mmol) in acetone (5 ml) were stirred at room temperature for 0.5—4 h. The excess iodine was removed by addition of 5% sodium thiosulphate until colourless, concentrated *in vacuo*, and the resultant products were purified by silica gel column chromatography (methanol/dichloromethane=1/99).

Morroniside (1)[16] White amorphous solid. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 1.31 (3H, d, J=7.2 Hz, H-10β), 1.38 (3H, d, J=7.2 Hz, H-10α), 1.75 (1H, ddd, J=2.0, 4.0, 9.0 Hz, H-9β), 1.80 (1H, ddd, J=2.0, 4.0, 9.0 Hz, H-9α), 1.88 (2H, m, H-6β), 2.00 (2H, m, H-6α), 2.82 (1H, dt, J=4.0, 13.0 Hz, H-5α), 3.12 (1H, dt, J=4.0, 13.0 Hz, H-5β), 3.35—3.41 (4H, m, H-2′, H-3′, H-4′, H-5′), 3.70 (3H, s, OCH_3), 3.87 (1H, dd, J=2.0, 7.0 Hz, H-8α), 3.93 (1H, dd, J=3.0, 7.0 Hz, H-8β), 4.24 (1H, dd, J=2.0, 12.0 Hz, H-6′α), 4.44 (1H, dd, J=2.0, 12.0 Hz, H-6′β), 4.78 (1H, d, J=4.0 Hz, H-7), 4.80 (1H, d, J=8.0 Hz, H-1′), 5.82 (1H, d, J=8.6 Hz, H-1α), 5.86 (1H, d, J=9.0 Hz, H-1β), 7.51 (1H, s, H-3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 19.8 (C-10), 27.3 (C-5β), 31.9 (C-5α), 34.5 (C-6β), 37.1 (C-6α), 39.7 (C-9α), 40.4 (C-9β), 51.8 (OCH_3), 62.6 (C-6′), 65.8 (C-8β), 71.4 (C-2′), 74.0 (C-4′), 74.9 (C-8α), 77.8 (C-5′), 78.3 (C-3′), 95.5 (C-7), 96.9 (C-1), 99.9 (C-1′), 110.7 (C-4), 154.4 (C-3), 168.7 (C=O). IR (KBr) cm^{-1}: 3423, 1699, 1638. FAB-MS m/z: 429.1 $[M+Na]^+$.

7-*O*-Methylmorroniside (3a) White amorphous solid. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 1.31 (3H, d, J=7.0 Hz, H-10β), 1.36 (3H, d, J=7.0 Hz, H-10α), 1.47 (1H, ddd, J=2.0, 4.0, 9.0 Hz, H-9β), 1.76 (1H, m, H-9α), 1.88 (2H, m, H-6β), 1.96 (2H, m, H-6α), 2.78 (1H, dt, J=4.0, 13.0 Hz, H-5α), 3.00 (1H, dt, J=4.0, 13.0 Hz, H-5β), 3.26—3.45 (4H, m, H-2′, H-3′, H-4′, H-5′), 3.32 (3H, s, OCH_3), 3.65 (3H, s, OCH_3), 3.85 (1H, dd, J=2.0, 7.0 Hz, H-8α), 3.90 (1H, dd, J=3.0, 7.0 Hz, H-8β), 4.44 (1H, dd, J=2.0, 12.0 Hz, H-6′α), 4.44 (1H, dd, J=2.0, 12.0 Hz, H-6′β), 4.70 (1H, d, J=4.0 Hz, H-7), 4.75 (1H, d, J=8.0 Hz, H-1′), 5.76 (1H, d, J=9.0 Hz, H-1α), 5.84 (1H, d, J=9.0 Hz, H-1β), 7.45 (1H, s, H-3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 19.6 (C-10), 28.0 (C-5β), 31.8 (C-5α), 33.7 (C-6β), 35.6 (C-6α), 40.2 (C-9α), 40.4 (C-9β), 51.7 (OCH_3), 54.9 (OCH_3), 62.8 (C-6′), 66.3 (C-8β), 71.7 (C-2′), 74.0 (C-4′), 75.0 (C-8α), 78.0 (C-5′), 78.5 (C-3′), 95.6 (C-7), 99.5 (C-1), 100.1 (C-1′), 110.7 (C-4), 154.4 (C-3), 168.6 (C=O). IR (KBr) cm^{-1}: 3426, 1705, 1639. FAB-MS m/z: 443.2 $[M+Na]^+$, 421.2 $[M+1]^+$.

7-*O*-Ethylmorroniside (3b) White amorphous solid. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 1.22 (3H, t, J=7.0 Hz, CH_3), 1.32 (3H, d, J=7.0 Hz, H-10β), 1.38 (3H, d, J=7.0 Hz, H-10α), 1.51 (1H, ddd, J=2.0, 4.0, 9.0 Hz, H-9β), 1.81 (1H, m, H-9α), 1.91 (2H, m, H-6β), 2.00 (2H, m, H-6α), 2.78 (1H, dt, J=4.0, 13.0 Hz, H-5α), 3.00 (1H, dt, J=4.0, 13.0 Hz, H-5β), 3.20—3.47 (4H, m, H-2′, H-3′, H-4′, H-5′), 3.69 (3H, s, OCH_3), 3.89 (1H, m, H-8α), 4.34 (1H, dd, J=2.0, 12.0 Hz, H-6′α), 4.58 (1H, dd, J=2.0, 12.0 Hz, H-6′β), 4.78 (1H, d, J=4.0 Hz, H-7), 4.86 (1H, d, J=8.0 Hz, H-1′), 5.80 (1H, d, J=9.0 Hz, H-1α), 5.88 (1H, d, J=9.0 Hz, H-1β), 7.51 (1H, s, H-3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 15.4 (CH_3), 19.6 (C-10), 28.0 (C-5β), 31.8 (C-5α), 33.9 (C-6β), 35.8 (C-6α), 40.1 (C-9α), 40.4 (C-9β), 51.7 (OCH_3), 62.9 (C-6′), 63.8 (OCH_2), 66.3 (C-8β), 71.6 (C-2′), 74.1 (C-4′), 74.9 (C-8α), 77.9 (C-5′), 78.4 (C-3′), 95.6 (C-7), 98.1 (C-1), 100.0 (C-1′), 111.6 (C-4), 154.4 (C-3), 168.7 (C=O). IR (KBr) cm^{-1}: 3428, 1707, 1637. FAB-MS m/z:

457.1 $[M+Na]^+$.

7-*O*-Propylmorroniside (3c) White amorphous solid. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 0.99 (3H, t, J=7.0 Hz, CH_3), 1.29 (3H, d, J=7.0 Hz, H-10β), 1.35 (3H, d, J=7.0 Hz, H-10α), 1.48 (1H, ddd, J=2.0, 4.0, 9.0 Hz, H-9β), 1.66—1.53 (4H, m, 2×CH_2), 1.78 (1H, m, H-9α), 1.88 (2H, m, H-6β), 2.00 (2H, m, H-6α), 2.77 (1H, dt, J=4.0, 13.0 Hz, H-5α), 3.04 (1H, dt, J=4.0, 13.0 Hz, H-5β), 3.16—3.36 (4H, m, H-2′, H-3′, H-4′, H-5′), 3.65 (3H, s, OCH_3), 3.83 (1H, m, H-8), 4.30 (1H, dd, J=2.0, 12.0 Hz, H-6′α), 4.54 (1H, dd, J=2.0, 12.0 Hz, H-6′β), 4.75 (1H, d, J=4.0 Hz, H-7), 4.80 (1H, d, J=8.0 Hz, H-1′), 5.75 (1H, d, J=9.0 Hz, H-1α), 5.83 (1H, d, J=9.0 Hz, H-1β), 7.47 (1H, s, H-3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 15.4 (CH_3), 18.3 (C-10), 22.5 (CH_2), 26.7 (C-5β), 30.5 (C-5α), 32.6 (C-6β), 34.4 (C-6α), 38.8 (C-9α), 39.9 (C-9β), 51.7 (OCH_3), 62.8 (C-6′), 66.3 (C-8β), 70.1 (OCH_2), 71.7 (C-2′), 74.2 (C-4′), 75.0 (C-8α), 78.0 (C-5′), 78.5 (C-3′), 95.6 (C-7), 98.3 (C-1), 100.1 (C-1′), 111.7 (C-4), 154.5 (C-3), 168.7 (C=O). IR (KBr) cm^{-1}: 3423, 1707, 1638. FAB-MS *m/z* 471.3 $[M+Na]^+$, 449.3 $[M+1]^+$.

7-*O*-Isopropylmorroniside (3d) White amorphous solid. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 1.12 (3H, d, J=7.0 Hz, CH_3), 1.18 (3H, d, J=7.0 Hz, CH_3), 1.29 (3H, d, J=7.0 Hz, H-10β), 1.35 (3H, d, J=7.0 Hz, H-10α), 1.48 (1H, ddd, J=2.0, 4.0, 9.0 Hz, H-9β), 1.66—1.53 (1H, m, CH), 1.78 (1H, m, H-9α), 1.83 (2H, m, H-6β), 2.00 (2H, m, H-6α), 2.78 (1H, dt, J=4.0, 13.0 Hz, H-5α), 3.03 (1H, dt, J=4.0, 13.0 Hz, H-5β), 3.16—3.35 (4H, m, H-2′, H-3′, H-4′, H-5′), 3.65 (3H, s, OCH_3), 3.84 (1H, m, H-8), 4.30 (1H, dd, J=2.0, 12.0 Hz, H-6′α), 4.53 (1H, dd, J=2.0, 12.0 Hz, H-6′β), 4.73 (1H, d, J=4.0 Hz, H-7), 4.80 (1H, d, J=8.0 Hz, H-1′), 5.76 (1H, d, J=9.0 Hz, H-1α), 5.84 (1H, d, J=9.0 Hz, H-1β), 7.48 (1H, s, H-3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 18.3 (C-10), 20.5, 22.4 [$(CH_3)_2$], 26.8 (C-5β), 30.7 (C-5α), 33.1 (C-6), 39.3 (C-9), 51.7 (OCH_3), 62.6 (C-6′), 65.2 (C-8β), 68.8 (OCH), 70.4 (C-2′), 70.5 (C-4′), 73.8 (C-8α), 76.8 (C-5′), 77.3 (C-3′), 94.4 (C-7), 95.1 (C-1), 98.9 (C-1′), 110.5 (C-4), 153.3 (C-3), 167.5 (C=O). IR (KBr) cm^{-1}: 3406, 1707, 1638. FAB-MS *m/z*: 471.2 $[M+Na]^+$, 449.3 $[M+1]^+$.

7-*O*-*t*-Butylmorroniside (3e) White amorphous solid. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 1.27 [9H, s, $(CH_3)_3$], 1.29 (3H, d, J=7.0 Hz, H-10β), 1.35 (3H, d, J=7.0 Hz, H-10α), 1.52 (1H, ddd, J=2.0, 4.0, 9.0 Hz, H-9β), 1.81 (1H, m, H-9α), 1.83 (2H, m, H-6β), 2.00 (2H, m, H-6α), 2.83 (1H, dt, J=4.0, 13.0 Hz, H-5α), 3.08 (1H, dt, J=4.0, 13.0 Hz, H-5β), 3.21—3.40 (4H, m, H-2′, H-3′, H-4′, H-5′), 3.70 (3H, s, OCH_3), 3.87 (1H, m, H-8), 4.30 (1H, dd, J=2.0, 12.0 Hz, H-6′α) 4.54 (1H, dd, J=2.0, 12.0 Hz, H-6′β), 4.77 (1H, d, J=4.0 Hz, H-7), 4.79 (1H, d, J=8.0 Hz, H-1′), 5.84 (1H, d, J=9.0 Hz, H-1α), 5.88 (1H, d, J=9.0 Hz, H-1β), 7.50 (1H, s, H-3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 15.4 [$(CH_3)_3$], 18.3 (C-10), 26.7 (C-5β), 27.5 [$\underline{C}(CH_3)_3$], 30.8 (C-5α), 32.5 (C-6), 39.0 (C-9), 50.4 (OCH_3), 61.6 (C-6′), 65.0 (C-8β), 70.4 (C-2′), 73.7 (C-4′), 74.1 (C-8α), 76.6 (C-5′), 77.2 (C-3′), 94.3 (C-7), 98.2 (C-1), 98.8 (C-1′), 110.5 (C-4), 153.1 (C-3), 167.3 (C=O). IR (KBr) cm^{-1}: 3440, 1704, 1639. FAB-MS *m/z*: 485.3 $[M+Na]^+$, 463.3 $[M+1]^+$.

7-*O*-*n*-Butylmorroniside (3f) White amorphous solid. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 0.91 (3H, d, J=7.0 Hz, CH_3), 1.29 (3H, d, J=7.0 Hz, H-10β), 1.35 (3H, d, J=7.0 Hz, H-10α), 1.37—1.58 (5H, m, aliphatic H, H-9β), 1.78 (1H, m, H-9α), 1.88 (2H, m, H-6β), 2.00 (2H, m, H-6α), 2.79 (1H, dt, J=4.0, 13.0 Hz, H-5α), 3.03 (1H, dt, J=4.0, 13.0 Hz, H-5β), 3.36—3.16 (4H, m, H-2′, H-3′, H-4′, H-5′), 3.65 (3H, s, OCH_3), 3.85 (1H, m, H-8), 4.30 (1H, dd, J=2.0, 12.0 Hz, H-6′α), 4.54 (1H, dd, J=2.0, 12.0 Hz, H-6′β), 4.75 (1H, d, J=4.0 Hz, H-7), 4.80 (1H, d, J=8.0 Hz, H-1′), 5.75 (1H, d, J=9.0 Hz, H-1α), 5.84 (1H, d, J=9.0 Hz, H-1β), 7.47 (1H, s, H-3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 13.1 (CH_3), 18.5 (C-10), 19.2 (CH_2), 26.9 (C-5β), 30.7 (C-5α), 31.7 (CH_2), 32.8 (C-6), 39.1 (C-9), 51.4 (OCH_3), 61.7 (C-6′), 65.2 (C-8β), 67.0 (OCH_2), 70.4 (C-2′), 73.1 (C-4′), 73.9 (C-8α), 76.8 (C-5′), 77.4 (C-3′), 94.5 (C-7), 97.2 (C-1), 99.0 (C-1′), 110.5 (C-4), 154.4 (C-3), 167.6 (C=O). IR (KBr) cm^{-1}: 3424, 1708, 1638. FAB-MS *m/z*: 485.3 $[M+Na]^+$, 463.3 $[M+1]^+$.

7-*O*-Cylohexylmorroniside (3g) White amorphous solid. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 1.31 (3H, d, J=7.0 Hz, H-10), 1.52 (2H, m, CH_2), 1.79 (2H, m, CH_2), 1.81 (1H, m, H-9α), 1.87 (2H, m, CH_2), 1.92 (2H, m, H-6β), 2.00 (2H, m, H-6α), 2.82 (1H, dt, J=4.0, 13.0 Hz, H-5α), 3.05 (1H, dt, J=4.0, 13.0 Hz, H-5β), 3.20—3.40 (4H, m, H-2′, H-3′, H-4′, H-5′), 3.55 (2H, m, OCH), 3.69 (3H, s, OCH_3), 3.87 (1H, m, H-8), 4.40 (1H, dd, J=2.0, 12.0 Hz, H-6′α), 4.64 (1H, dd, J=2.0, 12.0 Hz, H-6′b), 4.72 (1H, d, J=4.0 Hz, H-7), 4.79 (1H, d, J=8.0 Hz, H-1′), 5.80 (1H, d, J=9.0 Hz, H-1α), 5.86 (1H, d, J=9.0 Hz, H-1β), 7.50 (1H, s, H-3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 18.4 (C-10), 24.0 (CH_2), 24.2 (CH_2), 25.7 (2×CH_2), 26.9 (C-5β), 30.7 (C-5α), 31.6 (C-6), 33.3 (CH_2), 39.3 (C-9), 50.6 (OCH_3), 61.7 (C-6′), 65.2 (C-8β), 65.3 (OCH), 70.5 (C-2′), 73.9 (C-4′), 75.1 (C-8α), 76.8 (C-5′), 77.3 (C-3′), 94.5 (C-7), 95.2 (C-1), 99.0 (C-1′), 110.6 (C-4), 153.4 (C-3), 167.6 (C=O). IR (KBr) cm^{-1}: 3420, 1708, 1639. FAB-MS *m/z*: 511.2 $[M+Na]^+$, 489.3 $[M+1]^+$.

7-*O*-Benzylmorroniside (3h) White amorphous solid. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 1.26 (3H, d, J=7.0 Hz, H-10), 1.80 (1H, m, H-9), 1.94 (2H, m, H-6), 3.09 (1H, dt, J=4.0, 13.0 Hz, H-5β), 3.18—3.30 (6H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 3.64 (3H, s, OCH_3), 3.83 (1H, m, H-8), 4.33 (1H, dd, J=2.0, 12.0 Hz, H-6′α), 4.50 (1H, d, J=12.0 Hz, CH_2Ph), 4.64 (1H, d, J=12.0 Hz, CH_2Ph), 4.75 (1H, d, J=4.0 Hz, H-7), 4.80 (1H, d, J=8.0 Hz, H-1′), 5.80 (1H, d, J=9.0 Hz, H-1), 7.24—7.34 (5H, d, aromatic H), 7.48 (1H, s, H-3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 19.5 (C-10), 28.0 (C-5β), 29.3 (C-5α), 31.8 (C-6β), 33.8 (C-6α), 40.4 (C-9), 51.7 (OCH_3), 62.8 (C-6′), 66.6 (C-8β), 70.0 (OCH_2), 71.7 (C-2′), 75.0 (C-4′), 78.0 (C-3′), 78.5 (C-5′), 95.6 (C-7), 97.6 (C-1), 100.0 (C-1′), 111.6 (C-4), 128.7—129.3 (aromatic C), 139.3 (aromatic C), 154.5 (C-3), 168.7 (C=O). IR (KBr) cm^{-1}: 3419, 1706, 1638. FAB-MS *m/z*: 517.2 $[M+Na]^+$, 495.2 $[M+1]^+$.

7-*O*-*cis*-2-Hexenylmorroniside (3i) White amorphous solid. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 0.89 (3H, t, J=7.0 Hz, CH_3), 1.35 (3H, d, J=7.0 Hz, H-10), 1.40—1.37 (4H, m, 2×CH_2), 1.78 (1H, m, H-9α), 2.04 (2H, m, H-6), 2.79 (1H, dt, J=4.0, 13.0 Hz, H-5α), 3.05 (1H, dt, J=4.0, 13.0 Hz, H-5β), 3.18—3.35 (4H, m, H-2′, H-3′, H-4′, H-5′), 3.69 (3H, s, OCH_3), 3.83 (1H, m, H-8), 4.31 (1H, dd, J=2.0, 12.0 Hz, H-6′α), 4.58 (1H, dd, J=2.0, 12.0 Hz, H-6′β), 4.74 (1H, d, J=4.1 Hz, H-7), 4.80 (1H, d, J=8.0 Hz, H-1′), 5.55 (2H, m, CH=CH), 5.77 (1H, d, J=9.0 Hz, H-1α), 5.85 (1H, d, J=9.0 Hz, H-1β), 7.47 (1H, s, H-3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 14.0 (CH_3), 19.6 (C-10), 23.7 (2×CH_2), 28.0 (C-5β), 30.5 (C-5α), 31.8 (C-6), 40.4 (C-9), 51.7 (OCH_3), 62.8 (C-6′), 65.3 (OCH_2), 66.4 (C-8β), 71.6 (C-2′), 74.2 (C-4′), 75.0 (C-8α), 77.9 (C-3′), 78.5 (C-5′), 95.7 (C-7), 97.5 (C-1), 100.0 (C-1′), 111.6 (C-4), 126.9, 134.6 (CH=CH), 154.5 (C-3), 168.7 (C=O). IR (KBr) cm^{-1}: 3422, 1703, 1638. FAB-MS *m/z* 511.2 $[M+Na]^+$, 489.3 $[M+1]^+$.

7-*O*-Octylmorroniside (3j) White amorphous solid. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 0.85 (3H, t, J=7.0 Hz, CH_3), 1.26—1.61 (15H, m, 6×CH_2, H-10), 1.78 (1H, m, H-9α), 1.89 (2H, m, H-6), 3.00 (1H, dt, J=4.0, 13.0 Hz, H-5α), 3.05 (1H, dt, J=4.0, 13.0 Hz, H-5β), 3.18—3.60 (6H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 3.65 (3H, s, OCH_3), 3.90 (1H, m, H-8), 4.28 (1H, dd, J=2.0, 12.0 Hz, H-6′α), 4.53 (1H, dd, J=2.0, 12.0 Hz, H-6′β), 4.75 (1H, d, J=4.1 Hz, H-7), 4.81 (1H, d, J=8.0 Hz, H-1′), 5.84 (1H, d, J=9.0 Hz, H-1), 7.48 (1H, s, H-3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 13.3 (CH_3), 18.5 (C-10), 22.6—29.5 (aliphatic CH_2), 26.7 (C-5β), 29.5 (C-5α), 31.9 (C-6), 39.3 (C-9), 50.6 (OCH_3), 61.7 (C-6′), 65.2 (C-8β), 67.3 (OCH_2), 70.5 (C-2′), 73.9 (C-4′), 76.8 (C-3′), 77.4 (C-5′), 94.5 (C-7), 97.2 (C-1), 99.0 (C-1′), 110.5 (C-4), 153.4 (C-3), 167.6 (C=O). IR (KBr) cm^{-1}: 3428, 1708, 1638. FAB-MS *m/z*: 541.4 $[M+Na]^+$, 517.3 $[M+1]^+$.

7-*O*-Dodecylmorroniside (3k) White amorphous solid. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 0.86 (3H, t, J=7.0 Hz, CH_3), 1.25—1.41 (23H, m, aliphatic H, H-10), 1.46 (1H, m, H-6), 1.57 (2H, m, OCH_2), 1.77 (1H, m, H-9α), 1.88 (1H, m, H-6), 3.00 (1H, m, H-5β), 3.24—3.65 (6H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 3.66 (3H, s, OCH_3), 3.85 (1H, m, H-8), 4.30 (1H, dd, J=2.0, 12.0 Hz, H-6′α), 4.53 (1H, dd, J=2.0, 12.0 Hz, H-6′β), 4.74 (1H, d, J=4.0 Hz, H-7), 4.80 (1H, d, J=8.0 Hz, H-1′), 5.85 (1H, d, J=9.0 Hz, H-1), 7.48 (1H, s, H-3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 13.2 (CH_3), 18.4 (C-10), 22.5—31.8 (aliphatic C), 26.2 (C-5β), 29.4 (C-5α), 32.7 (C-6), 39.2 (C-9), 50.5 (OCH_3), 61.6 (C-6′), 65.1 (C-8β), 67.2 (OCH_2), 70.4 (C-2′), 73.8 (C-4′), 76.7 (C-3′), 77.3 (C-5′), 94.4 (C-7), 97.2 (C-1), 98.9 (C-1′), 110.4 (C-4), 153.3 (C-3), 167.5 (C=O). IR (KBr) cm^{-1}: 3416, 1708, 1638. FAB-MS *m/z*: 597.3 $[M+Na]^+$, 575.3 $[M+1]^+$.

7-*O*-Hexadecylmorroniside (3l) White amorphous solid. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 0.87 (3H, t, J=7.0 Hz, CH_3), 1.25—1.43 (33H, m, aliphatic H, H-10), 1.43 (1H, m, H-6), 1.57 (2H, m, OCH_2), 1.75 (1H, m, H-9α), 1.88 (1H, m, H-6), 3.00 (1H, m, H-5β), 3.24—3.65 (6H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 3.69 (3H, s, OCH_3), 3.85 (1H, m, H-8), 4.25 (1H, m, H-6′), 4.53 (1H, m, H-6′), 4.74 (1H, br d, H-7), 4.80 (1H, br d, H-1′), 5.75 (1H, br d, H-1), 7.47 (1H, s, H-3); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 14.0 (CH_3), 19.1 (C-10), 22.6—31.8 (aliphatic C), 26.3 (C-5), 32.6 (C-6), 38.9 (C-9), 51.4 (OCH_3), 61.5 (C-6′), 64.7 (C-8β), 67.6 (OCH_2), 69.8 (C-8α), 72.9 (C-4′), 73.5 (C-2′), 76.0 (C-3′), 76.2 (C-5′), 95.2 (C-7), 97.9 (C-1), 99.8 (C-1′), 110.6 (C-4), 153.3 (C-3), 166.9 (C=O). IR (KBr) cm^{-1}: 3426, 1707, 1637. FAB-MS *m/z*: 653.4 $[M+Na]^+$, 631.4 $[M+1]^+$.

7-*O*-Octadecylmorroniside (3m) White amorphous solid. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 0.87 (3H, t, J=7.0 Hz, CH_3), 1.25—1.41 (37H, m, aliphatic H, H-10), 1.43 (1H, m, H-6), 1.57 (2H, m, OCH_2), 1.75 (1H, m, H-9α), 1.95 (1H, m, H-6), 3.08 (1H, m, H-5β), 3.33—3.70 (6H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 3.71 (3H, s, OCH_3), 3.89 (1H, m, H-8), 4.27 (1H, m, H-

6′), 4.50 (1H, m, H-6′), 4.74 (1H, br d, H-7), 4.83 (1H, br d, H-1′), 5.75 (1H, br d, H-1), 7.46 (1H, s, H-3); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 14.0 (CH_3), 19.0 (C-10), 22.6—31.8 (aliphatic C), 26.3 (C-5), 32.8 (C-6), 39.0 (C-9), 51.3 (OCH_3), 62.0 (C-6′), 64.6 (C-8β), 67.6 (OCH_2), 69.8 (C-8α), 72.9 (C-4′), 74.4 (C-2′), 76.0 (C-3′), 76.2 (C-5′), 95.1 (C-7), 97.9 (C-1), 99.8 (C-1′), 110.8 (C-4), 152.8 (C-3), 166.9 (C=O). IR (KBr) cm^{-1}: 3441, 1707, 1639. FAB-MS *m/z*: 681.4 $[M+Na]^+$, 659.5 $[M+1]^+$.

Cytotoxicity Bioassay Murine metastatic colon carcinoma cells (colon 26-L5) were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA, U.S.A.) supplemented with 10% heat-inactivated fetal bovine serum (FBS), and 2 mM of L-glutamine, 100 units/ml of penicillin and 100 μg/ml of streptomycin. Cultures were kept at 37 °C in a humidified atmosphere of 5% CO_2/95% air. Cells were seeded into 96-well plates (1×10^4 cells/ml, 100 μl/well), and cultured with various concentrations of each compound for 24 h. WST-1 solution (DOJINDO, Kumamoto, Japan) was added to each well (10 μl/well) for 2 h before the end of the incubation. The activity was assessed by measuring the absorbance of the culture at 450 nm using a plate reader (Tecan Group Ltd., Männedorf, Germany).

Statistical Analysis Data were expressed as mean S.D., and analyzed by one-way ANOVA. Whenever ANOVA was significant, further comparison between vehicle- and drug treatment groups were made using Dunnett's *t*-test, *p*-values <0.05 were considered as significant.

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