



One new cucurbitane triterpenoid from the fruits of *Momordica charantia*

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ABSTRACT

One new cucurbitane triterpenoid commonly named neokuguagluconide, together with three known compounds momordicoside M, momordicoside N, and momordicoside A were isolated from the fresh fruits of *Momordica charantia*. The new one's structure with an interesting sugar-like groups attached to the side chain was elucidated by spectroscopic analysis and semiempirical (AM1) quantum chemical method.

1. Introduction

The plant *Momordica charantia* L. is cultivated in Asian countries. Its fruit was called "kugua" in China and bitter melon in western country. It is a favorable vegetable in China and becoming increasingly popular as a food supplement to lower blood glucose in western country [1]. Recently, studies discovered that the triterpenes from this genus showed biological activities such as antidiabetic, anti-HIV, anti-cancer, and so on [2-4]. In this report, we have examined the methanolic extract of the fresh fruits of *M. charantia* and have isolated one new triterpenoid named neokuguagluconide (**1**) (Figure 1) together with three known compounds momordicoside M (**2**) [5], momordicoside N (**3**) [5], momordicoside A (**4**) [6]. Herein, we reported the isolation and structural elucidation of the new constituent with an interesting sugar-like group attached to the side chain based on spectroscopic analysis and AM1 quantum chemical method.

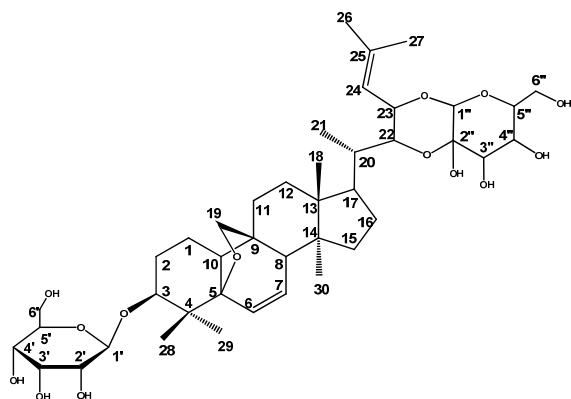


Figure 1. Structure of neokuguagluconide (**1**).

2. Experimental

2.1. General experimental procedures

Optical rotations were recorded on a HORIBA SEPA-300 digital polarimeter using a sodium lamp. IR spectra were measured using a Bio-Rad FTS-135 spectrometer. FABMS and HRESIMS were performed on a VG Auto Spec-3000 spectrometer. Column chromatography was carried out on normal phase chromatographic (Qingdao Marine Chemical, China), Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd.), RP-18 (Merck, Darmstadt, Germany). NMR spectra were recorded on Bruker AV-400 spectrometers with TMS as internal standard.

2.2. Plant material

The fresh fruits of *M. charantia* were purchased from the Chengjiang, Yuxi, Yunnan in August 2008, and identified by Prof. Shukun Chen.

2.3. Extraction and isolation

Dried and powdered fruits of *M. charantia* (35 kg) were extracted with MeOH. Removal of solvent in a vacuum gave the MeOH extract (700 g), which was partitioned in water and extracted with petroleum ether, ethyl acetate, and *n*-BuOH. The *n*-BuOH layer was concentrated and the residue (300 g) was chromatographed on a D₁₀₁ resin column, eluted with water, MeOH, and (Me)₂CO. The MeOH residue was fractionated by column chromatography on normal-phase silica gel, eluted with gradient CHCl₃/MeOH (30:1, 10:1, 5:1, 3:1, 1:1, 0:1) afforded six fractions (*Fr.1-Fr.6*). Compounds **1** (23 mg), **2** (29 mg), **3** (30 mg), **4** (250 mg) were isolated from *Fr.5* by using chromatographic column on normal-phase silica gel column

Table 1. The ^1H and ^{13}C NMR data of **1** (δ , ^1H , 400Hz, ^{13}C , 125 Hz, J in Hz, in pyridine- d_5).

| Position | δ_c | δ_H | Position | δ_c | δ_H |
|----------|------------|--|----------|------------|--------------------------------|
| 1 | 18.9 (t) | 1.29, (2H, m) | 22 | 72.8 (d) | 4.39, 1H, m |
| 2 | 27.6 (t) | 2.30, 1.70, (2H, m) | 23 | 75.3 (d) | 4.71, 1H, m |
| 3 | 85.1 (d) | 3.62, 1H, overlapped | 24 | 122.7 (d) | 5.43 (1H, d, $J = 9.4$) |
| 4 | 39.1(s) | - | 25 | 137.4 (s) | - |
| 5 | 86.0(s) | - | 26 | 26.1 (q) | 1.66, 3H, s |
| 6 | 134.2 (d) | 6.18 (1H, d, $J = 9.3$) | 27 | 18.9 (q) | 1.83, 3H, s |
| 7 | 130.0 (d) | 5.53 (1H, dd, $J = 9.6, 3.3$) | 28 | 21.1 (q) | 1.45, 3H, s |
| 8 | 52.2 (d) | 2.26, 1H, m | 29 | 25.6 (q) | 0.86, 3H, s |
| 9 | 45.3(s) | - | 30 | 20.1 (q) | 0.79, 3H, s |
| 10 | 40.0 (d) | 2.23, 1H, m | 1' | 103.9 (d) | 5.39 (1H, d, $J = 7.7$) |
| 11 | 23.9 (t) | 1.61, 1.29, 2H, overlapped | 2' | 73.1 (d) | 3.95 (1H, dd, $J = 7.6, 2.2$) |
| 12 | 31.2 (t) | 1.51, 1.44, 2H, overlapped | 3' | 72.5 (d) | 4.69, 1H, m |
| 13 | 45.6(s) | - | 4' | 69.3 (d) | 4.17 1H, m |
| 14 | 48.6(s) | - | 5' | 76.2 (d) | 4.45, 1H, m |
| 15 | 33.5 (t) | 1.14, 1.03, 2H, overlapped | 6' | 63.3 (t) | 4.33, 4.48, 2H, 2m |
| 16 | 27.9 (t) | 1.13, 2H, m | 1'' | 99.5 (d) | 4.93, 1H, s |
| 17 | 46.9 (d) | 1.69, 1H, m | 2'' | 93.3(s) | - |
| 18 | 14.4 (q) | 0.60 3H, s | 3'' | 77.6 (d) | 4.29 (1H, d, $J = 8.7$) |
| 19 | 80.2 (t) | 3.76 (1H, d, $J = 7.8$), 3.57 (1H, d, $J = 7.8$) | 4'' | 71.4 (d) | 4.49, 1H, m |
| 20 | 40.1 (d) | 1.83, 1H, m | 5'' | 80.9 (d) | 4.18, 1H, m |
| 21 | 15.0 (q) | 1.08 (3 H, d, $J = 6.5$) | 6'' | 63.2 (t) | 4.48, 4.60, 2H, 2m |

eluted with *n*-BuOH/AcOEt/H₂O (4:4:1 upper layer) and on reversed-phase silica gel column eluted with MeOH/H₂O (45%) as well as on Sephadex LH-20 column using MeOH as fluent.

2.4. Spectral data

Neokuguagluside (**1**): C₄₂H₆₆O₁₄; White powder; $[\alpha]_{\text{D}}^{25} = -80.8^\circ$ ($c=0.1$, MeOH); negative FABMS: m/z 793 $[\text{M}-\text{H}]^-$; HRESIMS: m/z 829.4129 $[\text{M}+\text{Cl}]^-$ (calcd. 829.4141); IR (KBr, cm^{-1}) ν_{max} : 3410, 2929, 1630, 1452, 1371, 1072, 998; ^1H -NMR (C₅D₅N, 400 MHz) and ^{13}C -NMR (C₅D₅N, 125 MHz): See Table 1.

3. Results and discussion

Compound **1** was obtained as a white powder. $[\alpha]_{\text{D}}^{25} = -80.8^\circ$ ($c=0.1$, MeOH). In the negative-ion FAB-MS of **1**, quasi-molecular ion peaks were observed at m/z 793 $[\text{M}-\text{H}]^-$. HRESIMS afforded a possible molecular formula of **1** as C₄₂H₆₆O₁₄ $[\text{M}+\text{Cl}]^-$, m/z 829.4129, calcd. 829.4141). The IR spectrum exhibited the presence of hydroxyl groups (3402, 3315, 3267 cm^{-1}). The ^1H -NMR (Table 1) spectrum of **1** showed signals for six tertiary methyls, a secondary methyl at δ 1.08 (3H, d, $J = 6.5$ Hz), two vinyl methyls at δ 1.66, 1.83, and three olefinic protons at δ 6.18 (1H, d, $J = 9.3$ Hz), 5.53 (1H, dd, $J = 9.6, 3.3$ Hz), 5.43 (1H, d, $J = 9.4$ Hz) as well as one anomeric protons at δ 5.39 (1H, d, $J = 7.7$ Hz).

The ^{13}C -NMR (Table 1) of **1** showed 42 carbon signals including 30 of aglycone, six signals of one sugar, and six signals of one sugar-like group, which indicated that **1** was a cucurtriterpene saponin. Comparison of the ^{13}C -NMR data of **1** with karaviloside X [7] showed that they have the same aglycone with an allopyranose attached to C-3 of aglycone. Furthermore, the HMBC (Figure 2) permitted the above deduction.

Intriguingly, there were six sugar-like carbon signals at δ 99.5 (d), 93.3 (s), 77.6 (d), 71.4 (d), 80.9 (d), 63.2 (t). The HMBC spectrum of **1** displayed the following correlations: H-21 (δ 1.08, 1H, d, $J = 6.5$ Hz) with C-20, C-22; H-22 (δ 4.39, 1H, m) with C-2'' (δ 93.3); H-23 (δ 4.71, 1H, m) with C-1'' (δ 99.5); H-24 (δ 5.43, 1H, d, $J = 9.4$ Hz) with C-23, C-25; H-26 (δ 1.66, 3H, s) with C-27, C-25, C-24; H-1'' (δ 4.93, 1H, s) with C-5'' (δ 80.9); H-5'' (δ 4.18, 1H, m) with C-4'', C-3''; H-6'' (δ 4.48, 4.60, 2H, 2m) with C-4''. The above evidence, along with two proton spin systems observed from the ^1H - ^1H COSY spectrum, H-17, H-21/H-20/H-22/H-23/H-24, and H-3''/H-4''/H-5''/H-6'', showed the existence of fragment **1b** (Figure 2).

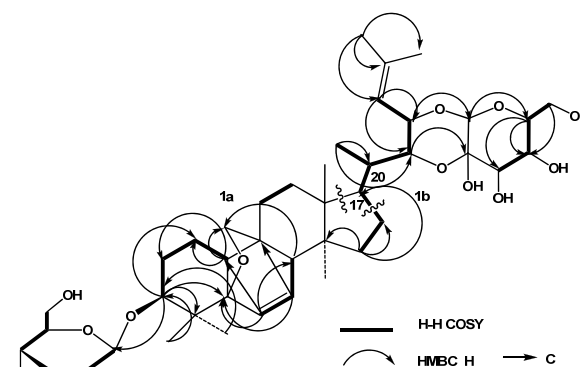


Figure 2. The Key HMBC and ^1H - ^1H COSY correlations of **1**

The stereochemistry of **1b** was characterized by ROESY experiment (Figure 3) and theoretical calculations using a semiempirical (AM1) quantum chemical method (Table 2). The usual H-20 β -configuration of cucurbitane derivatives was established for **1** by the NOESY correlations $18\beta\text{-Me}/20\beta\text{-H}$ and $21\alpha\text{-Me}/17\alpha\text{-H}$.

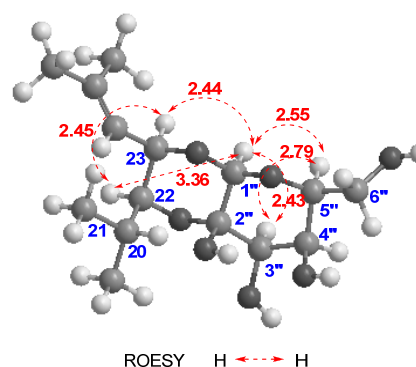


Figure 3. Key ROESY correlations of **1b** and corresponding interatomic distance (Å).

The usual H-20 β -configuration of cucurbitane derivatives was established for **1** by the NOESY correlations $18\beta\text{-Me}/20\beta\text{-H}$ and $21\alpha\text{-Me}/17\alpha\text{-H}$. Correlation contours were exhibited in the NOESY spectrum of **1b** between H-22 and H-23, H-1''; H-1'' and H-3'', H-5''; H-3'' and H-5''. In the NOESY spectrum of **1**, there was no correlation of $20\beta\text{-H}$ with 22-H, therefore, H-22, H-23, H-1'', H-3'' and H-5'' were α -configuration in **1b**. Moreover, in order to deduce the configuration of 2''-OH, we hypothesis four

Table 2. Main NOESY correlations and semiempirical calculations results of 1.

| No | The minimum spatial distance between restrict protons (Å) | | | |
|----|---|-----------------------------|--|-------------------------------|
| | H-20 with H-22, 23 ^a | H-22 with H-23 ^b | H-1'' with H-22, 23, 3'', 5'' ^b | H-3'' with H-5'' ^b |
| 1 | 3.51, 3.60 | 2.45 | 3.70, 4.01, 2.84, 2.61 | 2.52 |
| 2 | 3.51, 3.59 | 2.45 | 2.26, 3.36, 2.44, 2.43 | 2.79 |
| 3 | 2.37, 3.82 | 2.44 | 2.27, 3.44, 2.57, 2.41 | 2.96 |
| 4 | 2.41, 3.88 | 2.44 | 3.99, 3.57, 3.69, 2.55 | 4.22 |

^a Without NOESY contours.

^b Exhibited NOESY contours.

molecular modeling: 1) the configurations of H-22, H-23, H-1'', H-3'' and H-5'' were α , the configuration of 2''-OH was α ; 2) the configurations of H-22, H-23, H-1'', H-3'' and H-5'' were α , the configuration of 2''-OH was β ; 3) the configurations of H-22, H-23, H-1'', H-3'' and H-5'' were β , the configuration of 2''-OH was α ; 4) the configurations of H-22, H-23, H-1'', H-3'' and H-5'' were β , the configuration of 2''-OH was β . Theoretical calculations using AM1 quantum chemical method for above modeling yield very interesting information to support the configurations of H-22, H-23, H-1'', H-3'', H-5'' and 2''-OH. According to the distance between two protons with NOESY correlations less than 3.5 Å, only the second modeling was agreement with the NOESY experiment (Table 2). Thus, the configurations of H-22, H-23, H-1'', H-3'' and H-5'' were α , and the configuration of 2''-OH was β .

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