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Isolation and Characterization of Two Cucurbitane Type Triterpenoid Glycocide from 1-Butanol Soluble Part of *Momordica charantia* Fruit Pulp Juice

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Abstract

Momordica charantia is a member of the Cucurbitaceae family having diverse biological activities. Two cucurbitane type triterpene glycoside have been isolated from 1-butanol soluble part of clear juice of *M. charantia* fruit pulp through column chromatography and HPLC. The isolated compounds were characterized as compound MC1 (3β, 7-O-β-glucopyranosyl-25-methoxy cucurbita –5-en-19-al) and compound MC2 (5β, 19-epoxy- 3- O-glucopyranosyl-25 methoxy cucurbita-6, 23-dien) with the help of UV Spectroscopy, Thin Layer Chromatography, IR Spectroscopy, ^{1}H, ^{13}C and dept-135 NMR. The proposed structures of the compounds were further confirmed by comparing the ^{13}C-NMR values of the compounds with previous reported data.

Keywords: Momordica charantia, Fruit pulp juice, 1-butanol soluble part, Cucurbitane glycoside.

I. Introduction

Momordica charantia or bitter Melon, also known as bitter gourd, African cucumber, karela, grows in tropical areas, including parts of East Africa, Asia, the Caribbean, Middle East, Central and South America [1-2], where it is used as a vegetable as well as a medicine. Although fruit is the safest and most useful part of the plant, the seeds, leaves, and vines of bitter melon have all been used. In the literature several bioactive compounds of *M. charantia* fruit have been reported[3,7]; they are classified as proteins, lipids, carbohydrates, triterpenoids, saponins, polypeptides, flavonoids, sterols and others.

It is proved that polysaccharides from *M. charantia* fruits possess antidiabetic, antitumor, immune enhancing, antioxidant, neuroprotective and antimicrobial activities[3-9]. Proteins and peptides isolated from *M. charantia* are α-momorcharin (α-MMC), β-momorcharin (β-MMC), γ-momorcharin, ribosome inactivating proteins (RIPs), Momordica anti-HIV protein of 30 kD (MAP30), which possess RNA N-glycosidase activity, phospholipase activity, anti-tumour, anticancer and anti-microbial activity[7,8,10-12]. Saponins are a class of glycosides, found in the roots, stems, leaves and fruit of the *M. charantia*[13]. The major chemical constituents of saponins are tetracyclic triterpenoids and their glycosides, which are known as cucurbitanes. Many pharmacological investigations indicated that cucurbitanes from *M. charantia* shows both anti-diabetic and hypoglycaemic activities[14]. Cucurbitane-type compounds, isolated from the methanolic extract of *M. charantia* fruits, such as momordicosides A, C, F1, I and K; goyasaponins I, II, and III and goyaglycosides a, b, c, d, e, f, g and h are very well known[13]. β, 19-epoxy-3β, 25-dihydroxycucurbita-6, 23 (E)-diene and 3β,7β, 25-trihydroxycucurbita- 5, 23 (E)-dien-19-al are two cucurbitane type triterpenoids isolated from the methanol extract of *M. charantia*, lowered the blood sugar in diabetic mice[15]. Eight new cucurbitane-type glycosides, kugua saponins A–H were isolated by Zhang *et al*. from the directed fractionation of *M. charantia* fruits[16]. Zhang *et al*. also isolated four new cucurbitane-type triterpenes and a new glycoside 7β-hydroxy-3β-O-malonylcucurbita-5,24-diene-19-a-23-O-β-D-glucopyranoside from the rattans of wild *M. charantia*[17].

Besides bioactive compounds, unsaturated fatty acids, amino acids, minerals, vitamins are also contained in *M. charantia*[18-19]. Gallic acid, gentistic acid, t-ferulic acid, p-coumaric acid, o-coumaric acid, (+)-catechin, (−)-epicatchin and vanillic acid are important flavonoids and phenolic compounds isolated from *M. charantia*[20-21]. In the literature research on different extract of *M. charantia* fruit is reported, but almost no evidence of work with 1-butanol extract. In continuation of our search for bioactive compounds we have selected the 1-butanol extract of *M. charantia* fruit pulp juice for biological and chemical analysis.

II. Materials and Methods

General Methods

Analytical or laboratory grade solvents and chemicals used in the research were collected from E. Merck (Germany), BDH (England) and RDH (Sweden). Commercial grade methanol, ethanol, chloroform and DCM were distilled in a glass distillation apparatus before use. All evaporations were carried out under reduced pressure using rotary vacuum evaporator at bath temperature not exceeding 40°C. All freeze-drying were carried out by Hetosic CD 52 (Hetolab Equipments, Denmark) freeze dryer. The samples were pre-frozen in round bottom flasks in a methanol freezer (Hetofrig CB 5, Heto Birkero, Denmark) at -30 °C to -40 °C. For TLC pre-coated (0.2 mm thin coating of silica-gel on aluminum sheets) plate were used. For application of samples capillary tubes were used. TLC was done by the ascending technique in glass jars or tanks. The plates were examined under uv light at two different wave lengths (254 nm and 350 nm). Irrigated plates were developed by spraying with 1% vanillin in concentrated sulphuric acid followed by heating in an oven at 120 °C for 10 minutes. Glass column of different sizes varying from

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(90 cm x 10 cm) large glass tubes fitted with a rota-flow to small (30 cm x1 cm) burette like tube fitted with teflon flow control unit were used. For stationary phase column grade silica gel (230-400 mesh, ASTM) was used. High-performance liquid chromatography (HPLC) was carried out by a Shimadzu HPLC system using a Shim-pack C_{18} analytical column of serial No-0125, associated with two LC-10 AT VP pump and a UV detector (SPD-10 AVP, Shimadzu). For ultraviolet spectrum a Shimadzu UV-160 recording spectrophotometer was used. A shimadzu IR-470 spectrometer was used to obtain IR spectrum by making KBr pellets. The ^{1}H, ^{13}C and Dept-135 NMR spectra of different extracts, fractions and compounds were recorded by using a Bruker 400 MHz machine. In all cases tetramethylsilane (TMS) was used as internal reference.

Collection of Momordica charantia fruits

Fresh fruits (40kg) of *Momordica charantia* were bought from a local market of Dhaka city. The fruits were washed with water and the adhering water was removed by air-drying. Each of the fruit was divided into two halves with a sharp knife and the seeds were removed using a tea spoon. The seed free fresh fruit was sliced, dried in the air followed by drying in an oven at 40°C.

M. charantia fruit juice

The dried fruit pulp was crushed in a kitchen blender (Philips) and the juice was squeezed out with pre-cleaned cloth filter. The residual pulp was further blended with an ultra-turrax adding some fresh juice to the pulp and more juice was squeezed with cloth filter. All the juice was centrifuged and the clear juice was immediately stored in a deep freezer (-20 °C).

Liquid –liquid extraction (partition) of pure juice

The centrifuged clear juice was partitioned with 1-butanol using a separating funnel (1000 ml). Both 1-butanol part and aqueous part was separately collected and freeze-dried. Finally 1-butanol part, aqueous part, pure centrifuged freeze-dried juice were selected for biological testing and after biological study 1-butanol part was also selected for chemical analysis.

Preparation of Sephadex LH-20 (reverse phase) column

The required amount (200 cm^3 column volume) of Sephadex LH-20 gel (particle size 25-100 μm) was suspended in water for two hours containing 2, 2, 2-trichloro-2, 2-dimethyl ethanol as a preservative. The gel was de-gassed for one hour with occasional shaking and the glass column was packed with the gel as a stationary phase. The column was conditioned with H_2O (200 mL x3) following CH_3OH (200 mL x3) and again with H_2O (200 mL x3). Finally the column was equilibrated with 25% MeOH (200 mL x3) in water.

Application of sample into the column

The 1-butanol part (8.0 g) was suspended in 25% MeOH and applied to the column. The sample was then eluted by 25% MeOH in water (200 cm^3) and the polarity of the solvent was gradually increased from 35, 45, 60, 80 to 100% MeOH in water (200 cm^3 each) followed by 100% acetone at the end. The eluted sample was collected in 63 test tubes (25 cm^3 each). All the samples collected were monitored by TLC. The eluted samples were combined according to their R_f values and three different fractions BP1 (2.45 g), BP2 (2.5 g) and BP3 (0.2 g) were obtained.

Fractionation of BP3 by HPLC

The gummy colored solid BP3 (150 mg) obtained from Sephadex LH-20 was further purified by preparative high performance liquid chromatography. Solvent system was chosen by passing the sample in an analytical C_{18} column. Separation of the sample was performed on a shim-pack RP-18 column using 50% aqueous CH_3CN (acetonitrile) as the mobile phase at a flow rate of 1.5 ml/min in a Shimadzu HPLC system. Three fractions were separated as F1 (4.6 mg), F2 (1.6 mg) and F3 (5.0 mg).

Purification of the fraction F1

The fraction F1 (4.6 mg) was washed with n-hexane and fatty materials was removed and dried. The dried solid material was resolubilised in methanol and kept for crystallization. But no crystal was found except solid material. The solid material was collected and labeled as MC1 (4.5 mg).

Purification of the fraction F2

The fraction F2 (1.6 mg) was washed with n-hexane and fatty materials was removed and dried. The dried solid material was resolubilised in methanol and kept for crystallization. But no crystal was found except solid material. The solid material was collected and labeled as MC2 (1.5 mg).

III. Results and Discussion

Characterization of the compound MC1

The compound MC1 was obtained as amorphous solid with characteristics reddish colour. It was completely soluble in methanol. Melting point of the sample was recorded and found 112-115 °C. It gave brown colour with vanillin–sulphuric acid reagent indicating that MC1 might be a triterpenoid glycoside. The compound MC1 had an absorption maximum at 215 nm in CH_3OH.

The IR spectrum of MC1 had absorption band at 3400 cm^{-1} due to –OH stretching. Absorption bands at 1700 cm^{-1} and 1600 cm^{-1} due to C=O and C=C stretching vibration respectively. The band at 1075 cm^{-1} was due to the isopropyl group.

^{1}H-NMR spectroscopy of the compound MC1

The ^{1}H-NMR spectrum of the compound MC1 had a broad singlet at δ= 5.58 that may be assigned to the olefinic proton (H-6) coupled with the methine proton of (H-7). The signal at 4.47 (J= 7.8 Hz) which is a doublet indicate the anomeric proton as well as the β-linkage of the glucose to the aglycon. The signals at 3.83, 3.77, 3.65 and 3.54 for

different protons of the glucose moiety. The signal at 9.84 was due to the aldehydic proton.

The broad singlet at 3.14 was due to the methoxy group. The signals between 1.31-1.93 were due to methylene and methine protons. Seven singlets at 0.81, 0.90, 0.93, 0.96, 1.08, 1.24 and 1.28 were assigned for seven angular methyl groups present in the compound MC1.

^{13}C-NMR and Dept-135 spectroscopy of the compound MC1

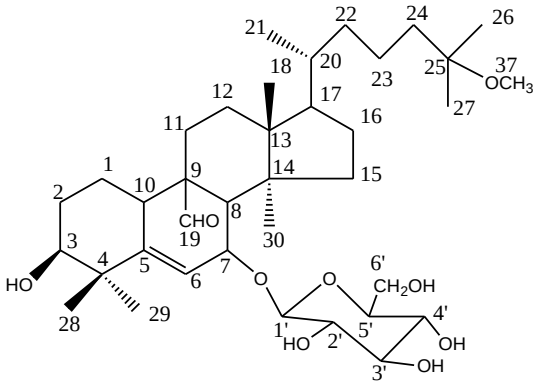
The ^{13}C and Dept-135 NMR spectra of the compound MC1 had 37 signals indicating that the compound contained 37 carbons. By ^{13}C-NMR spectrum and Dept-135 and their expansions and with the help of previously reported data all the methyl, methylene and methine carbons were distinguished (Table 1).

Comparing the ^{13}C-NMR signals and the Dept-135 signals, the signals at 34.9, 29.7, 30.4, 30.3, 29.4, 28.6, 32.74, 27.3 and 30.1 were assigned nine methylene carbons at C-1, C-2, C-11, C-12, C-15, C-16, C-22, C-23 and C-24, respectively. The signals at 15.1, 19.4, 25.6, 18.9, 26.1, 25.7 and 17.8 ppm were due to methyl groups at C-18, C-21, C-26, C-27, C-28, C-29 and C-30, respectively. The signals at 76.7, 45.9, 37.2, 51.2 and 37.1 were due to methine carbons at C-7, C-8, C-17 and C-20, respectively.

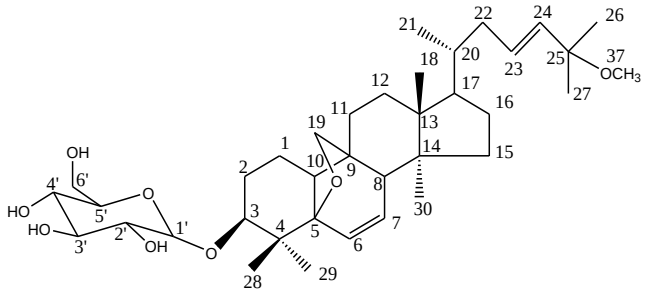
Table 1. Comparison between ^{13}C-NMR data of compound MC1 and previously reported data.

Carbon number	Type of carbon	Chemical shift of MC1 (ppm)	Chemical shift of published data[22-25] (ppm)	Carbon number	Type of carbon	Chemical shift of MC1 (ppm)	Chemical shift of published data[22-25] (ppm)
1	$-CH_2-$	34.9	34.8	21	$-CH_3$	19.4	21.3
2	$-CH_2-$	29.7	29.5	22	$-CH_2-$	32.74	36.2
3	>CH-OH	71.4	70.5	23	$-CH_2-$	27.3	27.7
4	>C<	42.5	41.5	24	$-CH_2-$	30.1	30.5
5	>C<(olefinic)	147.0	145.6	25	>C-O-	74.5	75.1
6	>CH(olefinic)	123.0	124.0	26	$-CH_3$	25.6	25.5
7	>CH-O	76.7	76.0	27	$-CH_3$	18.9	18.8
8	>CH-	45.9	45.1	28	$-CH_3$	26.1	27.5
9	>C<	48.9	47.6	29	$-CH_3$	25.7	27.2
10	>CH-	37.2	39.1	30	$-CH_3$	17.8	18.0
11	$-CH_2-$	30.4	30.1	1'	>CH(anomeric)	101.8	101.8
12	$-CH_2-$	30.3	30.0	2'	-CHOH	73.5	74.1
13	>C<	50.3	50.1	3'	-CH-OH	77.6	78.0
14	>C<	50.8	50.2	4'	-CH-OH	71.5	71.7
15	$-CH_2-$	29.4	29.1	5'	-CH-OH	77.7	78.1
16	$-CH_2-$	28.6	28.5	6'	$-CH_2-$	62.5	63.0
17	>CH-	51.2	55.6	37	$-OCH_3$	50.5	49.9
18	$-CH_3$	15.1	15.1				
19	-CHO	209.9	207.7				
20	>CH-	37.1	36.7				

From the ^{1}H-NMR, ^{13}C-NMR, Dept-135, IR and UV spectral data and also from chemical evidences the compound MC1 might be 3β, 7-O-β-glucopyranosyl-25-methoxy cucurbita –5 -en-19-al (Fig. 1).



MC1



MC2

Fig. 1. Proposed structure of the compound MC1 and MC2

Characterization of the compound MC2

The compound MC2 was obtained as amorphous solid with characteristics reddish colour. The solid compound was completely soluble in methanol. Melting point of the sample was recorded and found 135-138 $^{\circ}$C. It gave brown colour with vanillin–sulphuric acid reagent indicating that MC2 might be a triterpenoid glycoside. The compound MC2 had an absorption maximum at 215 nm in CH_3OH. The IR spectrum of MC2 had absorption band at 1620 cm^{-1} due to C=C stretching vibration.

^{1}H-NMR spectroscopy of the compound MC2

The ^{1}H-NMR spectrum of the compound MC2 had a broad singlet at δ= 5.58 that may be assigned to the olefinic proton (H-6) coupled with the proton of (H-7). The signal at δ= 5.96, which is a multiplet indicate another olefinic proton of (H-23). The sinals at δ= 5.42 (d) and 5.11 (d) were for the olefinic protons of H-7 and H-24, respectively.

The region from δ= 3.50 to 4.50 indicate the presence of glucose moiety as well as the β-linkage of the glucose to the aglycon. The broad singlets at 3.14 were due to the methoxy group. The signals between 1.34-1.93 were due to methylene and methine protons. Seven singlets at 0.82, 0.90, 0.93, 0.96, 1.08, 1.24 and 1.29 were assigned for seven angular methyl groups present in the compound MC2.

^{13}C-NMR and Dept-135-spectroscopy of the compound MC2

The ^{13}C-NMR spectrum of the compound MC2 had 37 signals indicating that the compound contained 37 carbons. By ^{13}C-NMR-spectrum and Dept-135 and their expansions and with the help of previously reported compounds from fruits of *Momordica charantia* by Okabe *et al.* all the methyl, methylene, methine carbons were distinguished (Table 2).

Comparing the ^{13}C-NMR signals and the Dept-135 signals, the signals at 33.6, 39.9, 47.9, 34.3, 28.3, 27.9, 64.1 and 28.8 were assigned eight methylene carbons at C-1, C-2, C-11, C-12, C-15, C-16, C-19 and C-22 respectively. The signals at 13.9, 17.7, 24.7, 24.5 26.3, 25.0 and 17.3 ppm were due to methyl groups at C-18, C-21, C-26, C-27, C-28, C-29 and C-30 respectively. The signals at 55.2, 38.0, 49.1, 37.2 were due to methine carbons at C-8, C-10, C-17 and C-20, respectively. The signal at 49.9 is due to the methoxy proton.

From the ^{1}H-NMR, ^{13}C-NMR, Dept-135, IR and UV spectral data and also from chemical evidences, the compound MC2 might be 5β, 19-epoxy-3-O-glucopyranosyl-25-methoxy cucurbita–5, 23–dien (Fig. 1).

Table 2. Comparison between ^{13}C NMR data of compound MC2 and previously reported data.

Carbon number	Type of carbon	Chemical shift of MC2 (ppm)	Chemical shift of published data [22-25] (ppm)	Carbon number	Type of carbon	Chemical shift of MC2 (ppm)	Chemical shift of published data [22-25] (ppm)
1	-CH2-	33.6	33.9	20	>CH-	37.1	36.7
2	-CH2-	39.9	39.5	21	-CH3	17.7	17.3
3	>CH-O-	73.5	74.1	22	-CH2-	28.8	29.2
4	>C<	41.5	41.5	23	>CH(olefinic)	131.4	130.9
5	>C<	87.5	87.9	24	>CH(olefinic)	139.4	139.6
6	>CH(olefinic)	124.3	124.0	25	>C-O-	74.5	75.1
7	>CH(olefinic)	121.9	122.3	26	-CH3	24.7	24.9
8	>CH-	45.9	45.1	27	-CH3	24.5	24.6
9	>C<	47.5	47.6	28	-CH3	26.3	26.5
10	>CH-	38.2	39.1	29	-CH3	25.0	25.3
11	-CH2-	47.9	47.1	30	-CH3	17.3	17.7
12	-CH2-	34.3	33.9	1'	>CH(anomeric)	101.0	101.8
13	>C<	50.1	50.1	2'	-CHOH	72.4	72.1
14	>C<	50.2	50.2	3'	-CH-OH	76.6	76.5
15	-CH2-	28.4	28.1	4'	-CH-OH	68.9	69.3
16	-CH2-	27.9	28.1	5'	-CH-OH	76.5	76.1
17	>CH-	55.2	55.6	6'	-CH2-	61.8	62.0
18	-CH3	13.9	14.1	37	-OCH3	49.9	49.9
19	-CH2-	64.1	64.9				

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