Characterization Of Cucurbitane Glycosides From The Fruit of *Momordica grosvenori* With LCMS And Their Inhibitory Effects On Epstein-Barr Virus Activation

NAOTO SHIMIZU[1], Toshihiro Akihisa[2], Hiroki Kumagai[1], Christine Miller[1], Takashi Suzuki[2], Yumiko Kimura[2], Harukuni Tokuda[3]
[1]Agilent Technologies, Tokyo & Santa Clara, Japan & USA, [2]Nihon University, Tokyo & Chiba, Japan, [3]Kyoto Prefectural University of Medicine, Kyoto, Japan

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Introduction

The fruit of *Momordica grosvenori* Swingle (Cucurbitaceae) growing in Kwangshi, China is used as an expectorant as well as a natural sweet food in that country, from which many cucurbitane-type triterpene glycosides have been isolated and characterized. We have recently reported the isolation and characterization of some cucurbitane glycosides from *M. grosvenori* fruit, and their inhibitory effects on the induction of Epstein-Barr virus early antigen (EBV-EA) by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells. In this report, we present the characterization of eight new naturally-occurring cucurbitane glycosides in the fruit of *Momordica grosvenori* by HPLC TOF and IonTrap as well as by NMR, and their inhibitory effects on EBV-EA induction.

Experimental

1. Extraction of cucurbitae glycosides

   Powdered *M. grosvenori* fruit (2 kg) was extracted with 99% ethanol (EtOH). The extract (75.5 g) was partitioned into ethyl acetate (EtOAc)/water (1:1, v/v) mixture. The water phase was then extracted with n-butanol (n-BuOH) which gave n-BuOH and water phases. The n-BuOH- and water-soluble fractions were subjected to preparative scale HPLC for the isolation of cucurbitae glycosides

2. LCMS and NMR

   HRMS and MS^n were recorded on the Agilent 6210 TOF and Agilent 6330 Ion Trap LCMS systems. NMR spectra were recorded with a JEOL ECA-600 or a JEOL LA-500 spectrometers

3 in vitro EBV-EA (Epstein-Barr virus early antigen) activation experiment

   For the protocol for this in vitro assay, refere to a previous article (T.Akihisa, et al., J. Nat. Prod. 2006, 69, 38-42).

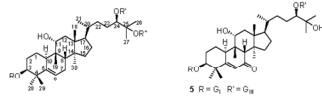
Experimental

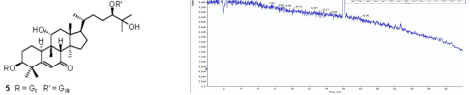
1. LCMS and NMR conditions

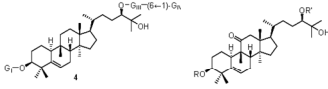
HPLC
Agilent 1200 series HPLC system
Column: ZORBAX XDB C-18 (2.1mm X 150mm, 3.5µm)
Mobile phase: A; 0.1% CH_3COOH aq., B; CH_3CN
Gradient: B(%) 10-90-90% 0-30-40min
Column temperature: 40 °C
Injection volume: 5µL
Sample concentration: each 20ng/µL

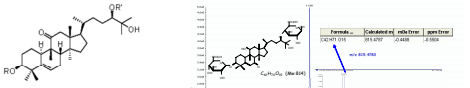
LCMS
Agilent 6330 LC Ion Trap , Agilent 6210 LC TOF
Mass range; m/z 100-1500
Ionization; ESI
Polarity; Positive
Dry gas flow; 10L/min
Dry gas temperature; 350 °C
Nebulizer; 50psi
CID voltage; 200V(IonTrap), 150V(TOF)
Mode; Ion Trap MS, MS^2, MS^3 and MS^4
Mode; TOF MS (with MS references)

NMR
JEOL ECA-600 spectrometer (^{1}H, 600MHz; ^{13}C, 150MHz)
JEOL LA-500 spectrometer (^{1}H, 500MHz; ^{13}C, 125MHz)
Solvent; C_5D_5N with tetramethylsilane as IS
Mode; ^{1}H-^{1}H COSY, ^{13}C DEPT, HMQC, HMBC and NOESY









G = β-D-glucopyranosyl

New cucurbitane glycosides
2. Mogroside II B
4. 11-Deoxymogroside III
5. 7-Oxomogroside II E
6. 7-Oxomogroside V
7. 11-Oxomogroside II A_1
8. 11-Oxomogroside IV A

Known cucurbitane glycosides
1. Mogroside II A_1
3. Mogroside III A_2

Chemical structures of cucurbitane glycosides

Results and Discussion

Fig.1-6 are LC TOF TIIC, mass spectra and calculated results of cucurbitane glycosides. As the mobile phase were 0.1% CH_3COOH aq. and CH_3CN, the formula calculation was done as MH^+ or $(M+Na)^+$

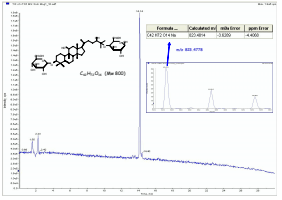


Fig.1 TIC and MS spectrum of Mogroside II B (2)

Fig.2 TIC and MS spectrum of 11-Deoxymogroside III (4)

Fig.3 TIC and MS spectrum of 7-Oxomogroside II E (5)

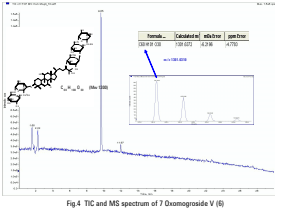


Fig.4 TIC and MS spectrum of 7 Oxomogroside V (6)

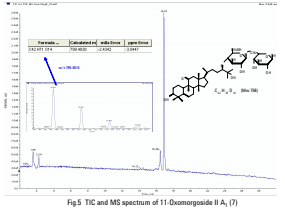


Fig.5 TIC and MS spectrum of 11-Oxomorgoside II A_1 (7)

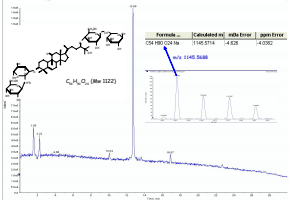


Fig.6 TIC and MS spectrum of 11-Oxomogroside IV A (8)

Results and Discussion

Fig.7-9 are LCMS^n of Morgroside II B. Other new compounds are analyzed same as Morgroside II B.

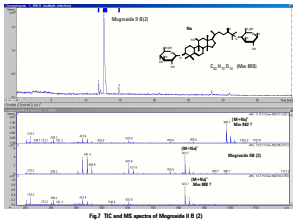


Fig.7 TIC and MS spectra of Mogroside II B (2)

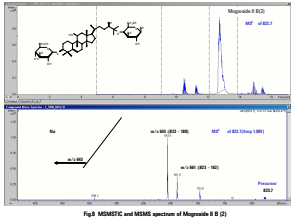


Fig.8 MSMSTIC and MSMS spectrum of Mogroside II B (2)

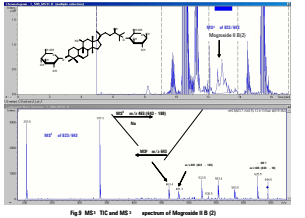


Fig.9 MS^3 TIC and MS^3 spectrum of Mogroside II B (2)

Table 1: ^{13}C NMR Spectroscopic Data (6 values; 150 MHz, C5D5N) for Six Cucurbitane Glycosides from the Fruit of *Momordica grosvenori*

carbon	2	4	5	6	7	8	carbon	2	4	5	6	7	8
aglycone moiety							sugar moiety						
1	39.7	22.7	27.0	27.1	21.2	22.2	Gl 1	107.3	107.4	107.1	106.8		106.9
2	29.5	29.1	29.0	28.9	29.7	28.6	Gl 2	75.5	75.5	75.4	75.4	75.5	75.4
3	87.9	87.7	87.8	87.3	75.6	86.6	Gl 3	78.7	78.6	78.7	78.5		78.6
4	42.4	41.8	43.9	43.8	41.9	42.8	Gl 4	71.8	71.9	71.8	71.7		71.8
5	144.2	143.2	178.6	178.9	141.4	141.3	Gl 5	78.1	78.2	78.5	78.3		77.3
6	118.4	118.8	125.2	125.2	119.0	118.5	Gl 6	63.1	63.2	63.0	70.4		70.5
7	24.6	24.6	200.2	200.2	24.2	24.1	Gl 1				105.5		105.5
8	43.5	43.9	60.0	60.1	44.1	44.0	Gl 2				75.3		75.3
9	40.1	34.7	40.7	40.8	45.1	45.0	Gl 3				78.1		78.6
10	36.8	38.6	39.3	39.2	36.1	36.0	Gl 4				71.8		71.8
11	77.8	72.5	76.7	76.9	212.9	212.8	Gl 5				78.5		78.6
12	41.1	39.8	40.7	40.8	48.8	48.8	Gl 6				62.8		62.8
13	47.4	46.5	47.8	47.7	49.1	49.0	Gl 1		106.3	105.9	102.7	106.3	106.3
14	49.8	49.4	48.6	48.7	49.7	49.7	Gl 2		75.1	75.4	82.5	75.1	75.1
15	34.6	35.1	34.6	34.6	34.6	34.6	Gl 3		78.5	78.2	78.7	78.5	78.5
16	28.4	28.1	28.3	28.6	28.0	28.0	Gl 4		72.1	71.8	71.5	72.1	72.1
17	51.1	51.2	50.6	50.9	50.0	50.0	Gl 5		76.4	78.6	77.4	76.4	76.4
18	17.0	15.7	16.9	16.9	17.0	17.0	Gl 6		79.5	62.7	70.2	79.4	79.4
19	26.3	28.2	26.6	26.6	20.2	20.3	Gl 1		104.8		104.9	104.9	104.8
20	36.5	36.2	36.5	36.8	36.0	36.0	Gl 2		75.5		75.3	75.5	75.5
21	18.9	18.9	18.8	19.0	18.5	18.5	Gl 3		78.1		78.5	78.1	78.1
22	34.5	33.2	33.4	33.3	33.0	33.0	Gl 4		71.5		71.8	71.5	71.5
23	29.1	29.8	29.6	29.8	29.8	29.7	Gl 5		78.6		78.4	78.6	78.6
24	75.7	92.8	90.8	92.2	92.7	92.7	Gl 6		62.6		63.6	62.6	62.6
25	80.6	72.7	72.0	72.6	72.7	72.7	Gl$_{IV}$ 1	97.6			105.7		
26	22.5	24.3	25.3	24.5	24.3	24.3	Gl$_{IV}$ 2	75.5			76.4		
27	23.0	27.0	27.0	27.1	27.0	27.0	Gl$_{IV}$ 3	78.4			78.3		
28	27.7	28.4	27.6	27.5	27.9	28.3	Gl$_{IV}$ 4	71.8			72.7		
29	26.3	26.6	25.6	25.5	26.3	25.8	Gl$_{IV}$ 5	78.9			78.5		
30	19.3	18.0	19.5	19.7	18.3	18.3	Gl$_{IV}$ 6	62.8			62.6		

Table 2 Inhibitory Effects on the Induction of Epstein-Barr Virus Early Antigen and Inhibitory Ratio (I.R.) on NOR 1 Action of Compounds **1–8** and Reference Compounds

Code		Compound	Percentage of EBV-EA induction[a] Concentration (mol ratio/32 pmol TPA) 500	100	10	IC$_{50}$[b] (mol ratio/32 pmol TPA)	I.R. of NOR 1 activation[c]
EB-767	1	Mogroside □ A_1	9.5 (70)	26.9	81.3	100	358	1.6
EB-860	2	Mogroside IIB	7.4 (70)	30.9	70.0	100	359	1.5
EB-861	3	Mogroside IIA_2	5.1 (70)	24.0	75.3	100	352	1.5
EB-765	4	11-Deoxymogroside □	10.6 (70)	25.7	81.9	100	357	1.5
EB-764	5	7-Oxomogroside □ E	7.7 (70)	24.4	80.8	100	343	1.5
EB-766	6	7-Oxomogroside V	13.7 (70)	31.0	84.7	100	400	1.4
EB-762	7	11-Oxomogroside □ A_1	8.5 (70)	25.0	80.6	100	346	1.4
EB-763	8	11-Oxomogroside □ a	10.3 (70)	27.3	82.5	100	367	1.4
Reference compound								
		β-Carotene	8.6 (70)	34.2	82.1	100	397	
		Glycyrrhizin						2.2
		Carbenoxolone						8.0

[a] Values represent percentage relative to the positive control value. TPA (32 pmol, 20 ng) = 100%. Values in parentheses are viability percentages of Raji cells. [b] IC_{50} represents the molar ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol TPA. [c] Determined at the concentration of 350 nmol. Inhibitory ratio of NOR 1 (positive control, 350 nm) was taken as 1.0.

Conclusions

Six new cucurbitane glycosides, mogroside II B , 11-deoxymogroside III, 7-oxomogroside II E , 7-oxomogroside V , 11-oxomogroside II A_1 and 11-oxomogroside IV A , and two known but new naturally-occurring cucurbitane glycosides, mogroside II A_1 and mogroside III A_2, have been isolated from the EtOH extract of the fruit of *M. grosvenori*. The characterization of these compounds was performed by the high resolution exact mass of TOF, MS^n spectra of Ion Trap, and ^{1}H and ^{13}C NMR. Upon evaluation of these compounds for inhibitory effects against the EBV-EA activation induced by TPA, all compounds exhibited potent inhibitory effects