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Cucurbitane, hexanorcucurbitane and octanorcucurbitane glycosides from fruits of *Trichosanthes tricuspidata*☆

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Abstract

From the fruits of *Trichosanthes tricuspidata*, 14 cucurbitane glycosides (khekadaengosides A–J, M–N, cucurbitacin J 2-*O*-β-glucopyranoside and cucurbitacin K 2-*O*-β-glucopyranoside), a hexanorcucurbitane glucoside (khekadaengoside K) and octanorcucurbitane (khekadaengoside L) were isolated along with two known cucurbitane glucosides (cucurbitacin 2-*O*-β-glucopyranoside and 25-*O*-acetyl-cucurbitacin 2-*O*-β-glucopyranoside). Structural elucidations were based on chemical and spectroscopic analyses.

Keywords: *Trichosanthes tricupidata*; Cucurbitaceae; Cucurbitane glycoside; Hexanorcucurbitane glycoside; Octanorcucurbitane glycoside; Khekadaengosides A–N; Khekadaengogenins I–V

1. Introduction

As part of our ongoing studies on Thai medicinal plants, we investigated the constituents of *Trichosanthes tricuspidata* Lour. (Cucurbitaceae, Thai name: Khe-Ka-Daeng) collected from Chantaburi province, eastern Thailand. *T. tricupidata* is a vine which ranges from the southern area of China through south and south-east Asia. In Thai traditional medicine, the plant is used in antifever, laxative, anthelmintic, as well as in migraine treatments. In this paper, we report the isolation and structural elucidation of 18 cucurbitane glycosides (**1**–**18**), of which 14 are new (**3**–**14**, **17**–**18**), together with a new hexanorcucurbitane glucoside (**15**) and a novel skeleton, octanorcucurbitane glycosides (**16**) from the fruits of this plant. The conformation of the parent cucurbitane, 10α-cucurbita-5,24-dien-3β-ol, was described by Nes et al. (1991).

2. Results and discussion

The methanolic extract of the fruits of *T. tricuspidata* was first suspended in H_2O and defatted with Et_2O, with the resulting aqueous layer being subjected to a column of highly porous copolymer resin of styrene and divinylbenzene, using as eluants H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH was next repeatedly subjected to silica gel, RP-18, or prep. HPLC chromatographies, to afford 18 cucurbitane glycosides.

Compounds **1** and **2** were identified as the previously known, cucurbitacin L 2-*O*-β-glucopyranoside (bryoamaride) and 25-*O*-acetyl-cucurbitacin L 2-*O*-β-glucopyranoside (25-*O*-acetyl-bryoamaride), respectively, by comparison of their physical and spectroscopic properties with those reported in the literature (Ripperger, 1976; Oobayashi et al., 1992).

Khekadaengoside A (**3**) was obtained as an amorphous powder and determined as $C_{42}H_{64}O_{16}$ by HR–FAB mass spectrometry. The ^{13}C NMR spectral data revealed the presence of two sugar moieties in addition to 30 signals for the aglycone moiety. The negative FAB mass spectrometry analysis of **3** exhibited a quasi-molecular ion peak at m/z 823 $[M–H]^-$ with significant peaks at m/z 677 $[M–Rham]^-$, which could be identified as a β-glucopyranosyl and a terminal α-rhamnopyranosyl

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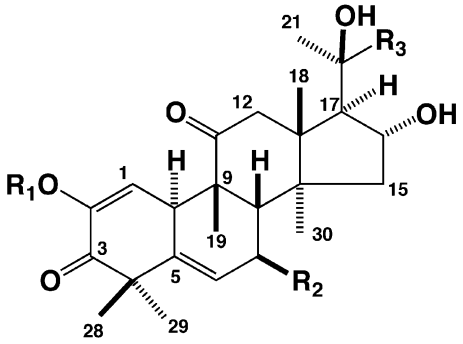

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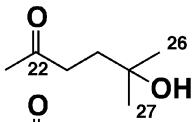
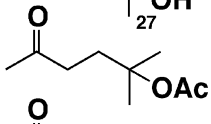
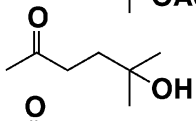
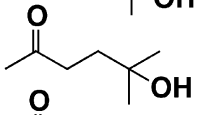
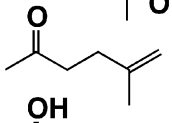
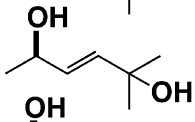
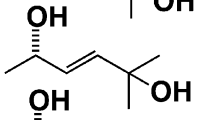
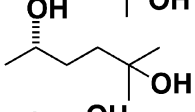
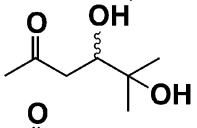
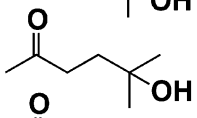
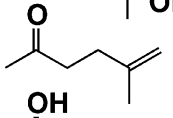
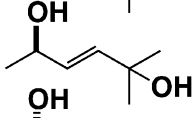
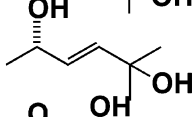
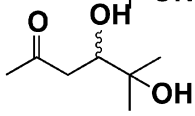
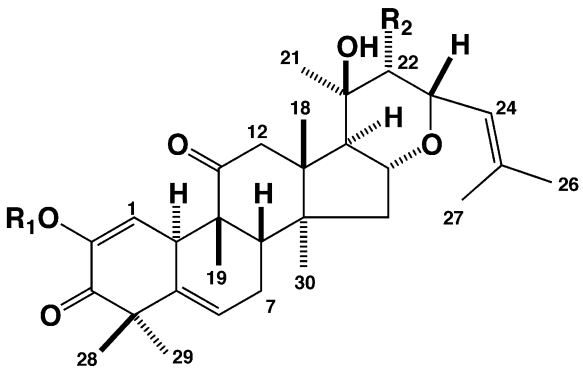
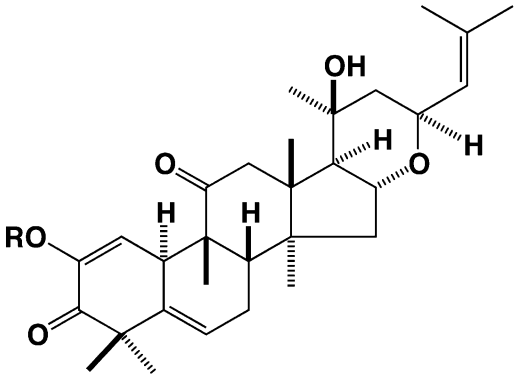
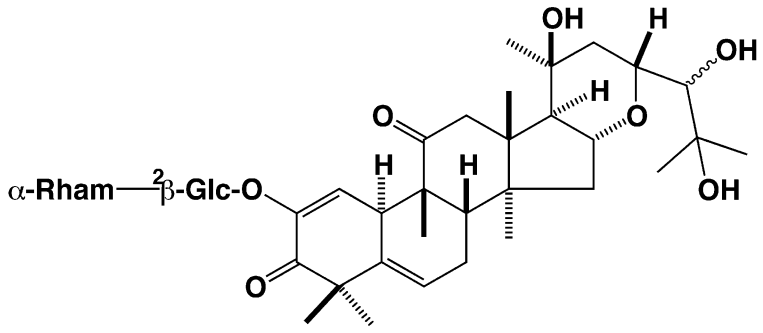
1	β-Glc	H	
2	β-Glc	H	
3	α-Rham—^{2}β-Glc	H	
4	α-Glc—^{4}β-Glc	H	
5	β-Glc	H	
6	β-Glc	H	
7	β-Glc	H	
7b	β-Glc	H	
8, 9	β-Glc	H	
10	β-Glc	OH	
5a	H	H	
6a	H	H	
7a	H	H	
8a, 9a	H	H	



	R_1	R_2
11	β-Glc	H
13	β-Glc	OH
11a	H	H



	R
12	β-Glc
12a	H



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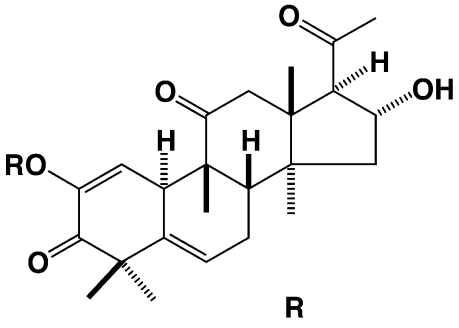
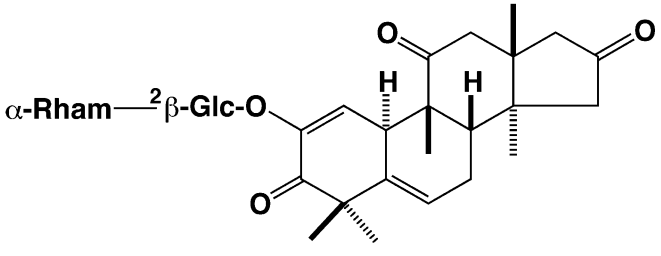


	R
15	β-Glc
15a	H



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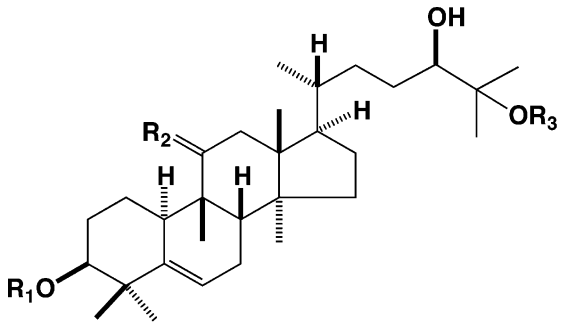

	R_1	R_2	R_3
17	β-Glc	H, OH	β-Glc^{2}—α-Rham
17a	H	H, OH	H
18	β-Glc	O	β-Glc^{2}—α-Rham

units. The chemical shifts of the aglycone moiety were in agreement with those of **1**. The downfield shift of C-2′ (+1.9 ppm) together with the upfield shift of C-1′ (-2.1 ppm) and C-3′ (-0.3 ppm) of the β-glucopyranosyl unit indicated that the α-rhamnopyranosyl unit was attached to C-2′ of the glucopyranosyl moiety. Therefore, **3** was elucidated as cucurbitacin L 2-*O*-α-rhamnopyranosyl-(1→2)-β-glucopyranoside.

Khekadaengoside B (**4**) was obtained as an amorphous powder with a molecular formula of $C_{42}H_{64}O_{17}$, deduced from HR–FAB mass spectrometry. The ^{1}H and ^{13}C NMR spectra showed the presence of two sugar moieties, these being identified as a β-glucopyranosyl and a terminal α-glucopyranosyl unit, together with compound **1**. Enzymatic hydrolysis of **4** with α-amylase gave **1**, confirming that the α-glucopyranosyl unit is a terminal sugar connected to a β-glucopyranosyl unit at C-4′. Furthermore, the ^{13}C NMR spectrum also revealed the attachment of a terminal sugar to C-4′ of a β-glucopyranosyl unit due to the downfield shift of this atom (+9.4 ppm) and upfield shift of C-3′ (−0.9 ppm) and C-5′ (−1.7 ppm). Consequently, the structure of **4** was identified as cucurbitacin L 2-*O*-α-glucopyranosyl-(1→4)-β-glucopyranoside.

The molecular formula of khekadaengosides C (**5**), D (**6**) and E (**7**) were determined as $C_{36}H_{52}O_{11}$, $C_{36}H_{54}O_{12}$ and $C_{36}H_{54}O_{12}$, respectively by HR–FAB mass spectrometry. On enzymatic hydrolysis with crude hesperidinase (Kohda and Tanaka, 1975) of **5**, **6** and **7** gave new aglycones **5a**, **6a** and **7a** named khekadaengogenin I ($C_{30}H_{42}O_6$), II ($C_{30}H_{44}O_7$) and III ($C_{30}H_{44}O_7$), respectively. The ^{13}C NMR spectral data of **5**, **6** and **7** were very similar to those of **1** except for different chemical shifts of the side chains.

Khekadaengoside C (**5**) showed signals corresponding to one methyl group (δ 22.7, C-27) and an exomethylene group (δ 110.3, C-26) in the side chain instead of two methyl group (δ 29.8 and 30.0) signals on C-25, compared to **1**, indicating that khekadaengoside **5** was the 25-ene derivative of **1**. Accordingly, the structure of **5** was assigned as shown.

Khekadaengoside D (**6**) showed signals corresponding to a disubstituted olefin group (δ 126.0, C-23 and δ 141.7, C-24) located between a hydroxymethine group (δ 81.6, C-22) and a hydroxylated quaternary carbon (δ 69.9, C-25) in the side chain. The ^{13}C NMR spectral data of the side chain were almost the same to those reported for kinonin A, a cucurbitacin with *S*- configuration at C-22 (Achenbach et al., 1993). Consequently, the relative configuration at C-22 of **6** was concluded to be *S*, and the structure of **6** was assigned as shown.

Khekadaengoside E (**7**) also displayed signals corresponding to a disubstituted olefin group (δ 126.8, C-23 and δ 142.1, C-24) located between a hydroxymethine group (δ 76.5, C-22) and a hydroxylated quaternary carbon (δ 69.8, C-25) in the side chain. Comparison of its ^{13}C NMR spectrum with those of **6** revealed significant differences in chemical shifts at C-21 and C-22 (Table 2), indicating that **7** is a 22-epimer of **6** with a *R*-configuration. Moreover, the relative configuration at C-22 was confirmed by comparing the chemical shifts of the side chain of 23,24-dihydrokhekadaengoside E (**7b**), which were in agreement with those of 22(*R*)-23,24-dihydroderivative of kinonin B (Achenbach et al., 1993). The structure of **7** was, therefore, elucidated as shown in the formula.

Compounds **8** and **9** have the same molecular formula, $C_{36}H_{54}O_{13}$, based on HR–FAB mass spectrometry. Each compound revealed the presence of one β-glucopyranosyl unit together with a cucurbitacin skeleton, assigned from the NMR spectral data. The ^{13}C NMR spectra of both **8** and **9** were virtually indistinguishable from each other. However, their HPLC retention times were different (37.2 and 43.5 min, respectively). Therefore, the two compounds were considered to have the same skeleton with a different relative configuration. Enzymatic hydrolysis of **8** and **9** with crude hesperidinase afforded cucurbitacin J (**8a**, $C_{30}H_{44}O_8$) and cucurbitacin K (**9a**, $C_{30}H_{44}O_8$). The structures of the aglycones were elucidated by comparison of both physical and spectroscopic data with those reported in the literature (Enslin and Norton, 1964; Gamlath et al., 1988). Consequently, the structure of compounds **8** and **9** were elucidated as cucurbitacin J 2-*O*-β-glucopyranoside and cucurbitacin K 2-*O*-β-glucopyranoside, respectively.

Khekadaengoside F (**10**) was obtained as an amorphous powder and determined as $C_{36}H_{54}O_{13}$ by HR–FAB mass spectrometry. Signals due to a β-glucopyranosyl unit were observed in the ^{1}H and ^{13}C NMR spectra.

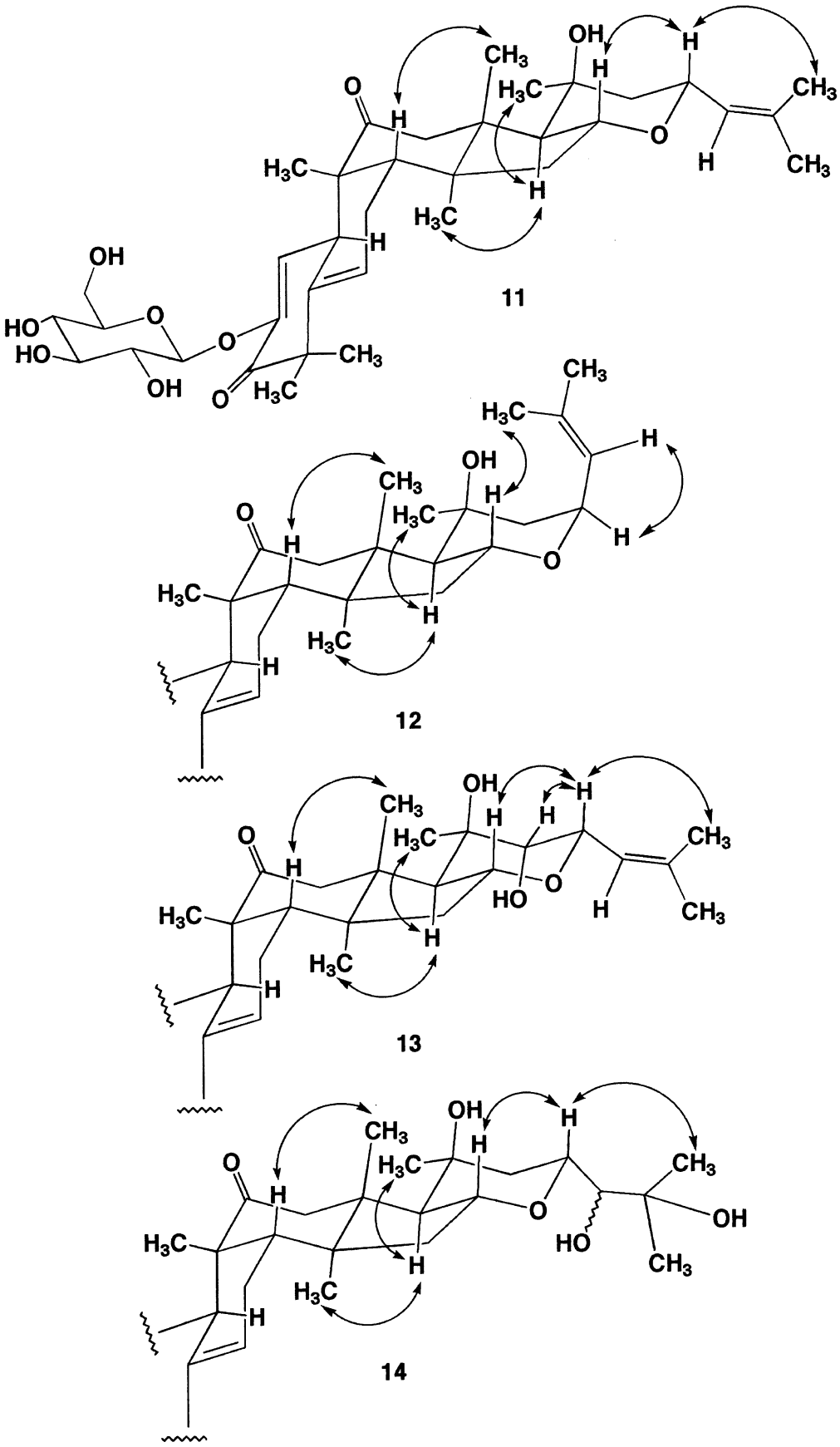

Fig. 1. The significant NOE correlations of khekadaengosides G, H, I and J.

Table 1
^{1}H NMR spectral data for compounds **1**–**16** (400 MHz, C_5D_5N)

H	1	2	3	4	5	6	7	7b	8	9	10	11	12	13	14	15	16
1	6.45, *brs*	6.33, *d*, 2.2	6.38, *d*, 2.4	6.35, *d*, 2.2	6.45, *d*, 2.4	6.44, *d*, 2.4	6.45, *d*, 2.4	6.46, *d*, 2.4	6.45, *d*, 2.4	6.44, *d*, 2.4	6.57, *d*, 2.7	6.44, *d*, 2.4	6.44, *d*, 2.2	6.48, *d*, 2.5	6.37, *d*, 2.4	6.38, *d*, 2.4	6.30, *brs*
6	5.64, *brs*	5.61, *brs*	5.63, *brs*	5.63, *brs*	5.64, *brs*	5.63, *brs*	5.64, *brs*	5.63, *brs*	5.63, *brs*	5.63, *brs*	6.18, *brs*	5.62, *brs*	5.62, *brs*	5.59, *brs*	5.63, *brs*	5.64, *brs*	5.61, *brs*
7	2.12, *m*	2.11, *m*	2.12, *m*	2.14, *m*	2.14, *m*	2.14, *m*	2.14, *m*	2.11, *m*	2.13, *m*	2.13, *m*	4.46, *d*, 3.4	2.10, *m*	2.06, *m*	2.10, *m*	2.07, *m*	2.12, *m*	2.12, *m*
	1.86, *m*	1.88, *m*	1.86, *m*	1.86, *m*	1.88, *m*	1.96, *m*	1.98, *m*	1.88, *m*	1.88, *m*	1.88, *m*		1.85, *m*	1.85, *m*	1.78, *m*	1.85, *m*	1.80, *d*, 12.9	1.84, *m*
8	1.92, *m*	1.89, *m*	1.92, *m*	1.92, *m*	1.92, *m*	1.96, *m*	1.98, *m*	1.98, *m*	1.92, *m*	1.92, *m*	2.56, *brs*	1.96, *m*	1.95, *m*	1.95, *m*	1.95, *m*	1.93, *m*	2.05, *m*
10	3.70, *brs*	3.63, *brs*	3.68, *brs*	3.67, *brs*	3.71, *brs*	3.66, *brs*	3.69, *brs*	3.72, *brs*	3.68, *brs*	3.68, *brs*	3.81, *brs*	3.65, *brs*	3.65, *brs*	3.65, *brs*	3.63, *brs*	3.73, *brs*	3.70, *brs*
12	3.32, *d*, 14.7	3.34, *d*, 14.7	3.40, *d*, 15.0	3.34, *d*, 14.7	3.39, *d*, 14.2	3.17, *d*, 14.4	3.23, *d*, 14.7	3.30, *d*, 14.4	3.29, *d*, 14.9	3.29, *d*, 14.7	3.35, *d*, 14.9	3.25, *d*, 14.9	3.26, *d*, 14.9	3.32, *d*, 14.7	3.34, *d*, 14.9	3.44, *d*, 15.1	3.40, *d*, 14.6
	2.89, *d*, 14.4	2.85, *d*, 14.4	2.94, *d*, 15.0	2.90, *d*, 14.7	2.93, *d*, 14.4	2.85, *d*, 14.4	2.89, *d*, 14.7	2.93, *d*, 14.4	2.87, *d*, 14.6	2.88, *d*, 14.7	2.97, *d*, 14.7	2.77, *d*, 14.9	2.16, *d*, 14.9	2.80, *d*, 14.7	2.80, *d*, 14.7	2.70, *d*, 14.4	2.48, *d*, 14.6
15	1.85, *m*	1.88, *m*	1.86, *m*	1.86, *m*	1.88, *m*	1.96, *m*	1.98, *m*	1.98, *m*	1.92, *m*	1.92, *m*	2.19, *m*	1.85, *m*	1.88, *m*	1.90, *m*	1.92, *m*	1.93, *m*	2.55, *d*, 17.0
	1.69, *d*, 12.9	1.63, *d*, 12.7	1.69, *d*, 12.9	1.69, *d*, 12.9	1.67, *d*, 12.2	1.84, *d*, 12.7	1.82, *d*, 12.7	1.80, *d*, 12.7	1.69, *d*, 12.7	1.69, *d*, 12.7	1.90, *d*, 12.9	1.67, *dd*, 14.2, 3.2	1.63, *dd*, 14.2, 2.9	1.74, *dd*, 13.4, 3.2	1.60, *dd*, 12.7, 3.1	1.38, *dd*, 14.6, 3.5	1.92, *d*, 17.8
16	4.92, *t*, 7.8	4.86, *t*, 7.6	4.96, *t*, 7.8	4.92, *t*, 7.6	4.93, *t*, 7.6	5.25, *t*, 7.5	5.31, *t*, 7.3	5.19, *t*, 7.3	5.04, *t*, 7.6	4.96, *t*, 7.6	4.94, *t*, 7.8	4.84, *td*, 10.3, 3.5	5.07, *td*, 9.5, 2.5	4.96, *td*, 10.3, 3.2	4.57, *m*	5.31, *t*, 7.4	–
17	2.95, *d*, 7.1	2.85, *d*, 7.3	2.96, *d*, 6.8	2.90, *d*, 7.1	2.91, *d*, 7.1	2.83, *d*, 6.4	2.85, *d*, 6.6	2.77, *d*, 6.6	3.02, *d*, 7.1	2.94, *d*, 7.1	2.97, *d*, 7.3	2.12, *d*, 9.5	2.16, *d*, 9.3	2.99, *d*, 9.8	2.20, *d*, 9.5	3.45, *d*, 6.8	2.24, *d*, 17.7
18	1.40, *s*	1.34, *s*	1.38, *s*	1.38, *s*	1.41, *s*	1.40, *s*	1.39, *s*	1.39, *s*	1.40, *s*	1.39, *s*	1.44, *s*	1.24, *s*	1.25, *s*	1.30, *s*	1.21, *s*		2.10, *d*, 17.7
19	1.51, *s*	1.51, *s*	1.54, *s*	1.51, *s*	1.54, *s*	1.54, *s*	1.55, *s*	1.55, *s*	1.52, *s*	1.50, *s*	1.54, *s*	1.64, *s*	1.66, *s*	1.68, *s*	1.39, *s*	0.99, *s*	0.98, *s*
21	1.62, *s*	1.58, s	1.62, s	1.63, s	1.62, s	1.56, *s*	1.60, *s*	1.58, *s*	1.63, *s*	1.70, *s*	1.64, *s*	1.49, *s*	1.48, *s*	1.78, *s*	1.48, *s*	1.56, *s*	1.18, *s*
22	–	–	–	–	–	4.61, *d*, 5.1	5.15, *d*, 5.6	4.42, *d*, 10.3	–	–	–	1.96, *m*	1.95, *m*	3.53, *brs*	2.44, *d*, 13.9	2.18, *s*	–
												1.63*	1.73, *d*, 13.7		2.00, *m*		
23	3.47, *ddd*, 16.4, 10.8, 5.1	3.22, *ddd*, 15.9, 11.0, 4.9	3.47, *ddd*, 15.6, 10.3, 5.1	3.46, *ddd*, 16.1, 10.5, 5.1	3.32, *m*	6.52, *dd*, 15.6, 5.4	6.50, *dd*, 15.6, 5.6	2.34, *m*	3.64, *dd*, 15.9, 9.5	3.65, *dd*, 14.7, 2.5	3.47, *ddd*, 15.6, 10.3, 5.1	4.95, *m*	4.89, *dd*, 7.6, 7.3	5.22, *d*, 8.3	4.98, *m*	–	–
	3.27, *ddd*, 16.4, 10.8, 5.4	3.01, *ddd*, 15.9, 10.5, 5.1	3.01, *ddd*, 15.6, 10.3, 5.4	3.26, *ddd*, 16.1, 10.3, 5.1	3.09, *m*				3.36, *dd*, 15.9, 1.5	3.62, *dd*, 14.7, 9.0	3.27, *ddd*, 15.6, 10.3, 5.4						
24	2.18, *m*	2.26, *m*	2.18, *m*	2.18, *m*	2.55, *t*, 8.1	6.38, *d*, 15.6	6.57, *d*, 15.6	1.98, m	4.55, *dd*, 9.5, 1.5	4.63, *dd*, 9.0, 2.5	2.19, *m*	5.42, *d*, 9.3	6.53, *d*, 8.3	6.02, *d*, 8.0	4.40, *m*	–	–
26	1.36, *s*	1.43, *s*	1.36, *s*	1.35, *s*	4.82, s 4.76, s	1.50, *s*	1.50, *s*	1.42, *s*	1.48, *s*	1.50, *s*	1.37, *s*	1.60, *s*	1.65, *s*	1.62, *s*	1.58, *s*	–	–
27	1.36, *s*	1.42, *s*	1.36, *s*	1.35, *s*	1.68, *s*	1.52, *s*	1.52, *s*	1.42, *s*	1.50, *s*	1.50, *s*	1.37, *s*	1.39, *s*	1.40, *s*	1.37, *s*	1.54, *s*	–	–
28	1.18, *s*	1.11, *s*	1.18, *s*	1.19, *s*	1.20, *s*	1.25, *s*	1.24, *s*	1.23, *s*	1.19, *s*	1.20, *s*	1.54, *s*	1.24, *s*	1.24, *s*	1.20, *s*	1.38, *s*	1.24, *s*	1.30, *s*
29	1.27, *s*	1.20, *s*	1.36, *s*	1.24, *s*	1.27, *s*	1.27, *s*	1.24, *s*	1.27, *s*	1.27, *s*	1.29, *s*	1.34, *s*	1.39, *s*	1.41, *s*	1.45, *s*	1.39, *s*	1.40, *s*	1.38, *s*
30	1.00, *s*	0.93, *s*	0.96, *s*	0.94, *s*	1.02, *s*	1.00, *s*	1.00, *s*	1.01, *s*	1.00, *s*	0.99, *s*	1.26, *s*	1.01, *s*	0.99, *s*	1.03, *s*	0.95, *s*	0.79, *s*	0.88, *s*
1′	5.46, *d*, 7.6	5.30, *d*, 7.8	5.51, *d*, 7.8	5.34, *d*, 7.6	5.46, *d*, 7.3	5.51, *d*, 7.8	5.50, *d*, 7.8	5.47, *d*, 7.8	5.47, *d*, 7.6	5.45, *d*, 7.6	5.50, *d*, 7.6	5.44, *d*, 7.8	5.45, *d*, 7.6	5.47, *d*, 7.6	5.50, *d*, 7.8	5.44, *d*, 7.6	5.48, *d*, 7.8
2′	4.21, *dd*, 8.3, 8.3	4.07, *dd*, 8.8, 7.8	4.40[a]		4.22, *dd*, 8.8, 7.8	4.22, *dd*, 9.0, 7.6	4.21, *dd*, 8.5, 8.1	4.21, *dd*, 8.8, 7.8	4.21, *dd*, 9.0, 7.8	4.20, *dd*, 8.8, 7.8	4.21, *dd*, 9.0, 8.0	4.20, *dd*, 8.3, 8.3	4.21, *dd*, 8.5, 8.1	4.22, *dd*, 8.8, 7.8	4.34, *dd*, 9.3, 9.0	4.20, *dd*, 9.0, 7.6	4.41[a]

(*continued on next page*)

Table 1 (*continued*)

H	1	2	3	4	5	6	7	7b	8	9	10	11	12	13	14	15	16
3′	4.28, *dd*, 9.0, 8.8	4.15, *dd*, 9.0, 8.8	4.25[a]		4.29, *dd*, 8.8, 8.5	4.30, *dd*, 9.0, 8.8	4.30, *dd*, 9.0, 8.8	4.29, *dd*, 9.0, 8.8	4.28, *dd*, 8.8, 8.8	4.28, *dd*, 9.0, 8.8	4.30, *dd*, 8.8, 8.8	4.28, *dd*, 8.8, 8.8	4.28, *dd*, 9.0, 8.8	4.29, *dd*, 9.0, 8.8	4.42[a]	4.28, *dd*, 9.0, 8.8	4.39[a]
4′	4.62, *dd*, 9.0, 9.0	4.32, *dd*, 9.3, 9.0	4.25[a]		4.47, *dd*, 9.3, 9.0	4.47, *dd*, 9.3, 9.3	4.46, *dd*, 9.3, 9.0	4.46, *dd*, 9.3, 9.0	4.46, *dd*, 9.0, 9.0	4.45, *dd*, 9.3, 9.0	4.47, *dd*, 9.3,9.0	4.46, *dd*, 9.0, 9.0	4.46, *dd*, 9.3, 9.0	4.48, *dd*, 9.3, 9.0	4.26[a]	4.46, *dd*, 9.3, 9.1	4.34, *dd*, 9.5, 9.0
5′	4.04, *m*	3.90, *m*	3.92, *m*	3.84, *m*	4.05, *m*	4.06, *m*	4.05, *m*	4.04, *m*	4.04, *m*	4.03, *m*	4.07, *m*	4.04, *m*	4.04, *m*	4.05, *m*	3.93, *m*	4.02, *m*	3.93, *m*
6′	4.69, *brd*, 12.0	4.56, *dd*, 12.0, 2.2	4.57[a]		4.70, *dd*, 12.2, 1.2	4.70, *dd*, 12.0, 2.0	4.69, *dd*, 12.0, 2.2	4.69, *dd*, 12.0, 2.5	4.69, *dd*, 12.0, 2.4	4.69, *dd*, 12.2, 2.4	4.73, *dd*, 12.0, 2.4	4.68, *brd*, 12.0	4.68, *dd*, 12.2, 2.2	4.70, *dd*, 12.2, 2.2	4.57[a]	4.67, *dd*, 12.2, 2.4	4.56, *brd*, 11.5
1″	4.56, *dd*, 12.0, 2.9	4.43, *dd*, 12.0, 3.4	4.45[a]	6.30, *brs*	4.58, *dd*, 11.1, 3.4	4.56, *dd*, 12.0, 3.7	4.55, *dd*, 12.0, 3.4	4.56, *dd*, 12.0, 3.4	4.56, *dd*, 12.0, 3.4	4.55, *dd*, 12.0, 3.7	4.57, *dd*, 12.0, 3.4	4.56, *dd*, 12.0, 3.2	4.55, *dd*, 12.0, 3.2	4.57, *dd*, 12.2, 3.4	4.44[a]	4.55, *dd*, 12.0, 3.4	4.45[a]
2″			4.74, *brd*, 2.0	5.91, *d* 3.7											6.29, *brs*		6.30, *brs*
3″			4.40[a]												4.76, *brd*, 2.0		4.77, *brs*
4″			4.27[a]												4.42[a]		4.45[a]
5″			4.65, *m*												4.26[a]		4.27[a]
6″			1.78, *d*, 6.1												4.63, *m*		4.63, *m*
CH_3CO		1.86, *s*													1.79, *d*, 6.1		1.78, *d*, 5.8

[a] Chemical shift obtained approximately from HSQC.

The ^{13}C NMR spectral data were very similar to those of **1**. However, the downfield shift of C-7 from δ 23.8 to δ 64.8 together with the downfield shift of C-6 from δ 120.8 to δ 124.7, as well as the downfield shift of C-8 from δ 41.8 to δ 52.9 indicated the presence of an additional hydroxy group at C-7. Furthermore, the appearance of H-8 (δ 2.56) as a broad singlet provided a small coupling constant between H-7 and H-8 ($J < 1.0$ Hz) corresponding to a dihedral angle of approximately 90°, led us to conclude that the hydroxy group at C-7 in the β-epimer form. Thus, the structure of **10** was determined as shown.

The molecular formula of khekadaengoside G (**11**) was determined as $C_{36}H_{52}O_{10}$ by HR–FAB mass spectrometry. Additionally, the ^1H and ^{13}C NMR spectral data indicated that **11** is a curcurbitacin β-glucopyranoside, and its enzymatic hydrolysis with crude hesperidinase gave it a new aglycone **11a**, named khekadaengogenin IV ($C_{30}H_{42}O_5$). The carbon signals of **11** in rings A, B and C closely matched those of compound **1**, and the remaining carbon signals were in agreement with those reported for a 16α,23-epoxy-cucurbitane type triterpene (Schenkel et al., 1992; Kubo et al., 1996). The detailed analyses of COSY, HSQC data confirmed the formulation of **11**. The stereochemistry of the remaining C- and D-rings and the side chain was determined by an NOE experiment as shown in Fig. 1, in which a significant cross peak was found between H-16 (δ 4.84) and H-23 (δ 4.95). On the basis of these results, the structure of **11** was characterized as 2,20(*S*) - dihydroxy - 16α,23(*R*) - epoxycucurbita - 5,24 - diene-3,11-dione 2-*O*-β-glucopyranoside. This compound has independently been isolated from Thai medicinal plant, *Gymnopetalum integrifolium* (Kurihara et al., 2001).

Khekadaengoside H (**12**) had the same molecular formula, $C_{36}H_{52}O_{10}$, as **11**, as deduced from its HR–FAB mass spectrometry. Enzymatic hydrolysis of **12** with crude hesperidinase afforded a new aglycone **12a**, named khekadaengogenin V ($C_{30}H_{42}O_5$). Although the ^1H and ^{13}C NMR spectral data of **12** were very similar to those of **11**, significant differences of the chemical shifts at C-16, C-22 and C-23 (Table 2) were observed from the spectra, suggesting that **12** has the same skeleton with a different relative configuration. The complete assignments for the stereochemistry were based on an NOE experiment as shown in Fig. 1, in which a significant cross peak was found between H-16 (δ 5.07) and H-26 (δ 1.65). Consequently, the structure of **12** was elucidated as 2,20(*S*)-dihydroxy-16α,23(*S*)-epoxycucurbita-5,24-diene-3,11-dione 2-*O*-β-glucopyranoside.

Khekadaengoside I (**13**) has the molecular formula, $C_{36}H_{52}O_{11}$, as determined by HR–FAB mass spectrometry, and the ^1H and ^{13}C NMR spectra revealed the presence of a β-glucopyranosyl unit in addition to 30 carbon signals for an aglycone moiety. Although the ^{13}C NMR spectral data were very similar to those of khekadaengoside G (**11**), the downfield shift of a

methylene signal at C-22 from δ 49.6 in **11** to δ 78.2 together with the different chemical shifts at C-17, C-20, C-21 and C-23 indicated the presence of a hydroxy group at C-22. The configuration was concluded from the coupling constant of H-22 ($J<1.0$ Hz), which corresponded to a dihedral angle of approximately 90°. This led us to conclude the configuration of hydroxy group at C-22 as being α. Also, the NOE experiment confirmed this assignment as shown in Fig. 1. Therefore **13** was determined to be 2,20(*S*),22-trihydroxy-16α,23(*S*)-epoxy-cucurbita-5,24-diene-3,11-dione 2-*O*-β-glucopyranoside.

The molecular formula of khekadaengoside J (**14**) was determined as being $C_{42}H_{64}O_{16}$ by HR–FAB mass spectrometry and the ^{1}H and ^{13}C NMR spectral data showed the presence of a α-rhamnopyranosyl-(1→2)-β-glucopyranosyl unit, compared to **3**, and a 16α,23-epoxy-cucurbitane type triterpene for its aglycone moiety. The carbon signals of **14** were almost the same as those of khekadaengoside G (**11**). However, the signal of a methine carbon (δ 78.6, C-24) was observed instead of a olefin signal (C-24) in **11**, indicating the presence of a hydroxy group at this position. While the stereochemistry of ring D was confirmed by an NOE experiment as shown in Fig. 1, the absolute configuration at C-24 could not be determined. On the basis of these results, the structure of **14** was elucidated as 2,20

Table 2
^{13}C NMR spectral data for compounds **1**–**16** (100 MHz, C_5D_5N)

C	1	2	3	4	5	6	7	7b	8	9	10	11	12	13	14	15	16
1	120.9	120.8	120.0	120.0	120.9	120.6	120.9	120.9	120.8	120.8	121.2	120.8	120.9	120.8	119.8	120.4	119.1
2	146.8	146.5	146.9	146.6	146.8	146.8	146.8	146.8	146.8	146.8	147.0	146.8	146.8	146.8	146.9	146.8	146.9
3	197.1	197.0	196.5	196.9	197.0	197.1	197.1	197.1	197.1	197.1	196.9	197.0	197.0	197.1	196.5	196.9	196.2
4	49.4	49.3	49.5	49.4	49.4	49.5	49.4	49.4	49.4	49.4	49.0	49.4	49.4	49.4	49.5	49.4	49.5
5	137.0	136.7	137.0	136.9	137.0	137.0	137.0	137.0	137.0	136.9	139.3	137.0	137.0	137.0	137.1	137.0	137.0
6	120.8	120.7	120.8	120.9	120.7	121.1	121.2	121.0	120.8	120.8	124.7	120.7	120.7	120.8	120.8	120.7	120.3
7	23.8	23.7	23.9	23.8	23.9	23.9	23.9	23.9	23.8	23.8	64.8	23.9	23.9	23.9	24.0	23.7	24.1
8	41.8	41.7	41.8	41.8	41.8	41.8	41.9	41.9	41.8	41.7	52.9	41.5	41.6	41.7	41.5	42.1	42.0
9	50.9	50.7	50.9	50.8	50.9	51.1	51.0	51.1	51.0	51.0	50.3	49.7	49.7	49.8	49.6	50.2	50.5
10	35.5	35.4	35.5	35.5	35.6	35.5	35.6	35.6	35.5	35.5	36.3	35.3	35.3	35.4	35.5	35.5	35.5
11	214.0	213.9	213.8	213.9	213.9	214.4	214.5	214.5	213.9	214.0	214.5	213.8	213.9	214.0	213.6	212.8	211.8
12	49.6	49.5	49.7	49.6	49.6	50.0	49.8	49.9	49.5	49.2	49.6	49.3	49.3	49.3	49.3	47.8	49.4
13	49.3	49.1	49.1	49.2	49.1	49.1	49.2	49.2	49.2	49.2	49.2	48.7	48.7	48.9	48.8	49.6	45.3
14	48.6	48.4	48.5	48.6	48.7	48.4	48.5	48.5	48.7	48.5	47.9	48.3	48.6	48.0	48.6	49.0	44.4
15	46.5	46.3	46.5	46.4	46.5	45.6	46.8	46.8	46.2	46.4	46.5	41.6	42.0	41.7	41.8	46.1	46.5
16	70.3	70.4	70.3	70.3	70.4	71.4	71.4	71.3	70.6	70.4	70.3	76.5	70.4	76.7	76.2	71.4	215.5
17	58.9	59.0	59.0	58.9	59.1	56.9	57.2	57.3	57.7	58.8	59.0	56.1	56.3	49.9	56.1	67.8	50.3
18	20.1	20.0	20.1	20.1	20.2	20.1	20.2	20.2	20.1	20.1	20.3	20.2	20.1	20.4	20.3	20.1	20.8
19	18.3	18.1	18.3	18.3	18.3	18.1	18.0	18.1	18.3	18.2	19.0	18.3	17.8	18.6	20.0	18.3	18.3
20	80.1	80.0	80.1	80.1	80.0	76.3	76.8	76.8	80.3	80.2	80.1	71.3	72.4	74.8	71.5	208.4	
21	25.5	25.5	25.5	25.5	25.5	24.7	21.8	21.5	24.8	25.4	25.5	29.5	30.2	26.3	29.7	32.6	
22	216.1	215.1	216.1	216.1	214.8	81.6	76.5	77.5	216.0	215.5	216.1	49.6	46.3	78.2	42.2		
23	32.7	32.1	32.8	32.7	32.2	126.0	126.8	27.3	41.0	40.8	32.8	73.6	71.6	76.4	71.6		
24	38.4	35.2	38.4	38.3	35.8	141.7	142.1	42.0	75.8	74.7	38.4	127.3	127.6	125.3	78.6		
25	69.1	81.5	69.1	69.1	145.5	69.9	69.8	69.8	72.0	72.2	69.1	133.8	133.6	139.9	73.0		
26	29.8	25.8	29.8	29.7	110.3	30.7	30.7	30.0	24.7	27.0	29.8	25.6	25.9	25.8	26.4		
27	30.0	25.9	30.0	30.0	22.7	30.8	30.7	30.2	27.4	25.1	30.0	20.5	20.4	20.6	26.4		
28	20.3	20.2	20.4	20.3	20.4	20.3	20.4	20.4	20.3	20.4	21.5	20.4	20.3	20.6	20.4	20.8	
29	27.5	27.4	27.3	27.5	27.5	27.6	27.5	27.6	27.5	27.5	28.0	27.4	27.4	27.3	27.2	27.4	
30	20.8	20.6	20.7	20.7	20.8	20.8	20.8	20.8	20.8	20.7	20.7	20.8	20.8	20.7	20.8	19.9	
1′	100.6	100.3	98.5	100.1	100.6	100.7	100.7	100.6	100.6	100.6	100.8	100.6	100.6	100.6	98.6	100.5	98.6
2′	74.4	74.1	79.3	75.4	74.4	74.4	74.4	74.4	74.4	74.4	74.4	74.4	74.4	74.4	79.4	74.3	79.3
3′	78.4	78.0	78.1	77.5	78.4	78.4	78.4	78.4	78.4	78.3	78.4	78.4	78.4	78.4	78.1	78.4	78.1
4′	70.7	70.2	70.8	80.1	70.7	70.7	70.7	70.7	70.7	70.7	70.8	70.7	70.7	70.7	70.8	70.6	70.8
5′	78.6	78.3	78.5	76.9	78.6	78.7	78.7	78.6	78.6	78.6	78.7	78.6	78.7	78.7	78.7	78.6	78.6
6′	61.9	61.9	61.8	61.0	62.0	62.0	20.0	62.0	61.9	61.9	62.1	61.9	61.9	62.0	61.8	61.9	61.7
1″			102.3	103.0											102.3		102.3
2″			72.7	73.8											72.8		72.8
3″			72.4	75.2											72.4		72.4
4″			74.2	71.8											74.3		74.3
5″			69.8	74.4											69.9		69.8
6″			18.8	62.7											18.8		18.8
$\underline{C}H_3CO$		22.1															
$C\underline{H_3}CO$		170.1															

(*S*),24(ξ)-trihydroxy-16α,23(*R*)-epoxycucurbita-5-ene-3,11-dione 2-*O*-α-rhamnopyranosyl-(1→2)-β-glucopyranoside.

Khekadaengoside K (**15**) has the molecular formula, $C_{30}H_{42}O_{10}$, based on HR–FAB mass spectrometry. The ^{13}C NMR spectra indicated the presence of a β-glucopyranosyl unit in addition to 24 carbon signals for the aglycone moiety, which suggested a hexanorcucurbitacin skeleton. The chemical shifts of rings A, B and C were in agreement with those of **1**. Enzymatic hydrolysis of **15** with crude hesperidinase yielded an aglycone **15a**, which identical with 2,16-dihydroxy-22,23,24,25,26,27-hexanorcucurbit-5-ene-11,20-dione (hexanorcucurbitacin I, $C_{24}H_{32}O_5$) (Rao et al., 1974), by analyses of the spectral data. On the basis of these data, the structure of **15** was determined as 2,16-dihydroxy-22,23,24,25,26,27-hexanorcucurbit-5-ene-11,20-dione 2-*O*-β-glucopyranoside.

The molecular formula of khekadaengoside L (**16**) was determined as $C_{34}H_{48}O_{13}$ by HR–FAB mass spectrometry. The ^{13}C NMR spectrum indicated the presence of α-rhamnopyranosyl-(1→2)-β-glucopyranosyl unit, compared to khekadaengoside A (**3**), in addition to 22 carbon signals for the aglycone moiety. DEPT experiments revealed the presence of five tertiary methyl groups, four methylenes, four methines, as well as nine quaternary carbons. Comparison of the chemical shifts from the ^{13}C NMR spectral data of **16** with those of **3**, helped us to establish the structures of rings A, B and C. The lack of eight signals for the side chain together with the presence of an additional methylene (δ 50.3) group in ring D led us to conclude that the structure of **16** was an octanorcucurbitacin glycoside, which structurally differed from known cucurbitacins by loss of the side chain at C-17. The downfield shift of a methine signal (C-16, δ 70.3) in **3** to δ 215.5 indicated the presence of an additional carbonyl group at C-16. The position of this carbonyl group was confirmed by the coupling constants of the adjacent protons (H-15 and H-17), in which each proton showed only geminal coupling as shown in Table 1. Consequently, **16** was elucidated as 2-hydroxy-20,21,22,23,24,25,26,27-octanorcucurbit-5-ene-11,16,20-trione 2-*O*-α-rhamnopyranosyl-(1→2)-β-glucopyranoside. This is the first report of the octanorcucurbitane skeleton.

Khekadaengoside M (**17**) has the molecular formula, $C_{48}H_{82}O_{18}$, as deduced from HR–FAB mass spectrometry. The ^1H and ^{13}C NMR spectra exhibited three sugar moieties, which could be determined as two β-glucopyranosyl units, one of which was substituted at C-2, and a terminal α-rhamnopyranosyl unit in addition to 30 carbon signals for the aglycone moiety. On enzymatic hydrolysis with crude hesperidinase, glycoside **17** gave mogrol (**17a**, $C_{30}H_{52}O_4$) by analyses of both physical and spectral data (Takemoto et al., 1983; Kasai et al., 1989). The glycosylation shifts were observed at signals due to C-3 and C-25 of **17** (Table 4), indicating that **17** was a bisdesmosidic cucurbitacin glycoside. The position of sugar moieties were assigned by a HMBC experiment, in which significant three bond correlations were found from H-1″ to C-25 and C-2″, as well as in the correlations from H-1‴ to C-2″ and from H-1′ to C-3 as shown in Fig. 2. Consequently, **17** was determined

Table 3
^1H NMR spectral data for compounds **5a**–**9a**, **11a**, **12a** and **15a** (400 MHz, C_5D_5N)

H	5a	6a	7a	8a[a]	9a[a]	11a	12a	15a
1	6.32, *d*, 2.6	6.32, *d*, 2.7	6.33, *d*, 2.7	5.94, *d*, 2.7	5.94, *d*, 2.7	6.34, *d*, 2.7	6.34, *d*, 2.7	6.27, *d*, 2.7
6	5.69, *brs*	5.68, *brs*	5.68, *brs*	5.76, *brs*	5.76, *brs*	5.66, *brs*	5.66, *brs*	5.68, *brs*
7	2.23, *m*	2.23, *m*	2.23, *m*	2.02, *m*	2.02, *m*	2.23, *m*	2.20, *m*	2.20, *m*
	1.90, *m*	1.96, *m*	2.02, *m*	2.02, *m*	2.02, *m*	1.95, *m*	1.89, *m*	1.96, *m*
8	1.90, *m*	1.96, *m*	2.02, *m*	2.38, *m*	2.38, *m*	2.00, *m*	1.95, *m*	1.96, *m*
10	3.77, *brs*	3.74, *brs*	3.77, *brs*	3.51, *brs*	3.50, *brs*	3.72, *brs*	3.72, *brs*	3.80, *brs*
12	3.38, *d*, 13.2	3.19, *d*, 14.4	3.25, *d*, 14.6	3.23, *d*, 14.4	3.21, *d*, 14.2	3.26, *d*, 14.9	3.26, *d*, 14.6	3.42, *d*, 14.6
	2.90, *d*, 13.2	2.85, *d*, 13.2	2.88, *d*, 14.4	2.72, *d*, 14.4	2.70, *d*, 14.4	2.76, *d*, 14.9	2.76, *d*, 14.6	2.69, *d*, 14.6
15	1.90, *m*	1.96, *m*	2.00, *m*	1.90, *m*	1.87, *m*	1.95, *m*	1.95, *m*	1.96, *m*
	1.71, *d*, 12.7	1.87, *d*, 12.7	1.85, *d*, 12.7	1.90, *m*	1.87, *m*	1.70, *dd*, 14.2, 3.5	1.70, *dd*, 14.2, 2.9	1.82, *dd*, 14.2, 2.9
16	4.93, *t*, 7.6	5.26, *t*, 7.3	5.34, *t*, 7.1	4.47, *t*, 7.6	4.39, *t*, 7.6	4.87, *td*, 10.2, 3.2	5.10, *td*, 9.5, 2.5	5.33, *t*, 7.1
17	2.91, *d*, 7.8	2.84, *d*, 7.1	2.86, *d*, 6.4	2.62, *d*, 7.1	2.55, *d*, 7.1	2.14, *d*, 9.5	2.17, *d*, 9.5	3.48, *d*, 6.6
18	1.45, *s*	1.45, *s*	1.45, *s*	1.02, *s*	1.02, *s*	1.27, *s*	1.28, *s*	1.14, *s*
19	1.57, *s*	1.55, *s*	1.53, *s*	1.44, *s*	1.42, *s*	1.65, *s*	1.67, *s*	1.60, *s*
21	1.60, *s*	1.59, *s*	1.65, *s*	1.39, s	1.39, s	1.49, *s*	1.48, *s*	2.17, *s*
22		4.61, *d*, 5.4	5.17, *d*, 5.4	–	–	–	–	–
23	3.32, *m*	6.53, *dd*, 15.6, 5.6	6.51, *dd*, 15.6, 5.6	2.99, *dd*, 16.6, 9.5	2.98, *dd*, 16.1, 1.1	4.98, *m*	4.89, *m*	–
	3.09, *m*			2.57, *dd*, 16.6, 1.2	2.68, *dd*, 16.1, 9.8			
24	2.55, *t*, 8.1	6.39, *d*, 15.8	6.58, *d*, 15.6	3.97, *dd*, 9.5, 1.2	3.91, *dd*, 9.5, 1.7	5.44, *d*, 9.3	6.54, *d*, 8.6	–
26	4.83, *brs*	1.51, *s*	1.51, *s*	1.20, *s*	1.19, *s*	1.61, *s*	1.65, *s*	
	4.77, *brs*							
27	1.68, *s*	1.52, *s*	1.53, *s*	1.24, *s*	1.22, *s*	1.29, *s*	1.29, *s*	
28	1.22, *s*	1.30, *s*	1.27, *s*	1.35, *s*	1.35, *s*	1.43, *s*	1.45, *s*	1.31, *s*
29	1.33, *s*	1.30, *s*	1.30, *s*	1.24, *s*	1.25, *s*	1.44, *s*	1.45, *s*	1.45, *s*
30	1.13, *s*	1.13, *s*	1.13, *s*	1.00, *s*	0.99, *s*	1.14, *s*	1.11, *s*	0.81, *s*

[a] Measured in CD_3Cl.

to be mogrol 3-*O*-β-glucopyranosyl-26-*O*-α-rhamnopyranosyl (1→2)-*O*-β-glucopyranoside.

The molecular formula of khekadaengoside N (**18**) was determined as $C_{48}H_{80}O_{18}$ by HR–FAB mass spectrometry.

Table 4
^{13}C NMR spectral data for compounds **5a**–**9a, 11a, 12a** and **15a** (100 MHz, C_5D_5N)

C	5a	6a	7a	8a[a]	9a[a]	11a	12a	15a
1	115.8	116.0	116.1	114.8	114.9	116.0	116.0	115.7
2	147.4	147.3	147.3	144.6	144.6	147.3	147.3	147.4
3	198.9	198.9	198.9	198.7	198.7	198.9	198.9	198.8
4	48.6	48.6	48.6	47.6	47.6	48.5	48.7	48.6
5	137.7	137.7	137.7	136.9	136.9	137.7	137.7	137.7
6	120.5	120.6	120.6	120.6	120.7	120.5	120.5	120.3
7	23.9	24.0	24.0	23.6	23.6	24.0	23.9	23.9
8	42.0	42.0	42.1	41.6	41.6	41.8	41.7	42.3
9	51.0	51.7	51.2	50.8	50.7	49.7	49.7	50.3
10	35.2	35.1	35.2	34.7	34.7	35.0	35.0	35.1
11	213.7	214.1	214.3	212.7	212.9	213.5	213.6	212.5
12	49.5	49.9	49.7	48.8	48.8	49.7	49.2	47.7
13	49.3	49.1	49.2	48.8	48.8	48.8	48.8	49.6
14	48.7	48.6	48.6	48.4	48.4	48.6	48.6	49.1
15	46.7	45.6	46.9	45.5	45.7	41.7	42.0	46.2
16	70.3	71.4	71.5	71.6	71.1	76.5	70.5	71.4
17	59.0	56.6	57.3	55.9	57.5	56.2	56.4	67.9
18	20.2	20.2	20.2	19.8	19.8	20.2	20.1	19.9
19	18.4	18.2	18.1	18.4	18.3	18.3	17.8	18.4
20	80.1	76.4	76.8	79.3	79.4	71.3	72.4	208.4
21	25.4	24.7	21.9	23.6	24.3	29.6	30.2	31.6
22	214.8	81.7	76.5	214.0	215.5	49.2	46.3	
23	32.2	126.0	126.8	39.3	38.3	73.6	71.6	
24	35.8	141.8	142.1	74.4	74.3	127.4	127.6	
25	145.5	69.9	69.9	72.2	72.3	133.8	133.6	
26	110.3	30.8	30.8	25.7	25.7	25.6	25.9	
27	22.7	30.8	30.8	24.8	24.7	20.6	20.5	
28	20.4	20.4	20.4	20.1	20.1	20.4	20.3	20.1
29	28.0	28.1	28.1	27.9	27.9	27.9	27.7	27.9
30	20.8	20.8	20.8	20.2	20.2	20.8	20.8	20.8

[a] Measured in CD_3Cl.

The ^1H and ^{13}C NMR spectra showed the presence of a β-glucopyranosyl unit and an α-rhamnopyranosyl (1→2)-*O*-β-glucopyranosyl unit, compared to khekadaengoside M (**17**), together with 30 carbon signals for a cucurbitacin skeleton. The chemical shifts of **18** were almost the same as those of **17** except for significant different chemical shifts at C-12. The downfield shift from δ 77.8 in **17** to δ 213.8 in **18**, indicating the presence of a carbonyl group at C-12. The chemical shifts of the aglycone moiety were in agreement with those reported for bryodulcosigenin (Oobayashi et al., 1992). Therefore, the structure of **18** was elucidated as bryodulcosigenin 3-*O*-β-glucopyranosyl-26-*O*-α-rhamnopyranosyl-(1→2)-*O*-β-glucopyranoside.

3. Experimental

3.1. General

NMR spectra were recorded in C_5D_5N using a Jeol JNM A-400 spectrometer (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) with tetramethylsilane (TMS) as internal standard. MS were recorded on a Jeol JMS-SX 102 spectrometer. Preparative HPLC was carried out on columns of ODS (150×20 mm i.d., YMC), Polyamine II (250×20 mm i.d., YMC) and Diol 120A (300×8 i.d., YMC) equipped with a Tosoh refraction index (RI-8) detector; the flow rate was 6 ml/min for ODS and Polyamine II, and 3 ml/min for Diol 120A. For CC, silica gel G 60 (Merck), RP-18 (50 mm, YMC) and highly porous copolymer of styrene and divinylbenzene (Mitsubishi Chem. Ind. Co. Ltd) were used. The solvent systems were: (I) EtOAc–MeOH (9:1), (II) EtOAc–MeOH–H_2O (4:1:0.1), (III) EtOAc–MeOH–H_2O (7:3:0.3), (IV) EtOAc–MeOH–H_2O (6:4:1), (V) 50–80% MeOH, (VI) 45% MeCN, (VII) 20% MeCN, (VIII) 90% MeCN, (VIII) 85% MeCN, (IX) 25% MeCN, (X) 30% MeCN

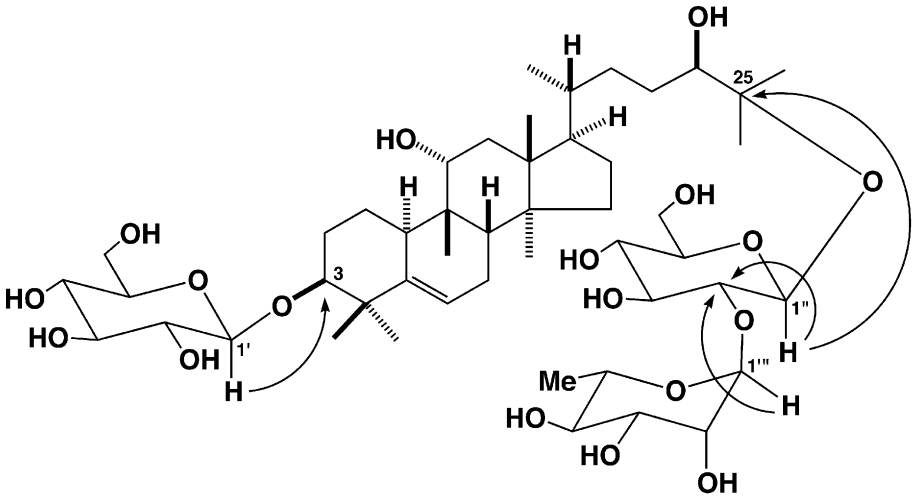

Fig. 2. The significant HMBC correlations of khekadaengoside M (**17**).

and (XI) MeCN. The spray reagent used was 10% H_2SO_4 in ethanol.

3.2. Plant material

The fruits of *Trichosanthes tricupidata* Lour. were collected from Chantaburi province, east of Thailand, in February 2000. The identification of the plant was confirmed by Professor Vichiara Jirawongse, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. A voucher sample (KKU-0023) is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

3.3. Extraction and isolation

The fruits (1.2 kg) of *T. tricuspidata* were extracted with hot MeOH. After removal of the solvent by evaporation, the residue (224.0 g) was defatted with Et_2O. The aqueous layer was subjected to a column of highly porous copolymer of styrene and divinylbenzene, using H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH (47.0 g) was subjected to a column of silica gel (systems I, II, III and IV, respectively) affording eight fractions, together with compound **1** (6.2 g) from fraction 3. Fraction 2 (4.2 g) was applied to a column of RP-18 using system V to provide compounds **2** (1.1 g) and **5** (100 mg). Fraction 2-9 was purified by prep. HPLC-ODS (system VI) to give compounds **11** (101 mg), **12** (176 mg) and **13** (8 mg). Fraction 5 (4.2 g) was further separated on a RP-18 column (system V) to give seven fractions. Fraction 5-2 was purified by

Table 5
^{1}H NMR spectral data for compounds **17** and **18** (400 MHz, C_5D_5N)

H	17	17a	18
3	3.65, *brs*	3.73, *brs*	3.65, *brs*
6	5.47, *brd*, 4.6	5.65, *brd*, 5.6	4.49, *brd*, 4.6
18	0.89, *s*	0.94, *s*	0.72, *s*
19	1.29, *s*	1.34, *s*	1.51, *s*
21	1.00, *d*, 6.8	1.00, *d*, 6.1	0.91, d, 6.1
26	1.56, *s*	1.42, *s*	1.57, *s*
27	1.57, *s*	1.48, *s*	1.58, *s*
28	1.22, *s*	1.19, *s*	1.12, *s*
29	1.53, *s*	1.53, *s*	1.51, *s*
30	0.87, *s*	0.90, *s*	0.94, *s*
1′	4.85, *d*, 7.8		4.83, *d*, 7.8
2′	3.92[a]		3.92[a]
3′	4.20[a]		4.20[a]
4′	4.08, *dd*, 9.3, 8.8		4.09, *dd*, 9.5, 8.5
5′	3.83[a]		3.83[a]
6′	4.43, *dd*, 12.0, 2.2		4.44, *dd,* 12.0, 2.2
	4.33, *dd*, 12.0, 4.9		4.34[a]
1″	5.10, *d*, 7.1		5.10, *d*, 7.3
2″	4.20[a]		4.20[a]
3″	4.20[a]		4.20[a]
4″	4.20[a]		4.20[a]
5″	3.83[a]		3.83[a]
6″	4.48, *dd*, 12.0, 2.7		4.50, *dd*, 12.0, 2.7
	4.20[a]		4.20[a]
1‴	6.43, *d*, 1.0		6.43, *brs*
2‴	4.76, *d*, 1.7		4.76, *d*, 1.2
3‴	4.61, *dd*, 9.0, 3.4		4.61, *dd*, 9.0, 3.4
4‴	4.28[a]		4.29[a]
5‴	4.82, *m*		4.85, *m*
6‴	1.71, *d*, 6.3		1.71, *d*, 6.1

[a] Chemical shift obtained approximately from HSQC.

Table 6
^{13}C NMR spectral data for compounds **17** and **18** (100 MHz, C_5D_5N)

C	17	17a	18
1	26.7	25.8	22.1
2	29.5	30.8	28.4
3	87.8	76.2	84.1
4	42.3	42.3	41.9
5	144.2	144.2	141.2
6	118.4	119.2	118.5
7	24.5	24.6	24.1
8	43.5	43.6	43.9
9	40.1	40.2	49.0
10	36.8	36.9	35.9
11	77.8	77.9	213.8
12	41.6	41.2	48.7
13	47.4	47.4	49.6
14	49.7	49.8	48.9
15	34.5	34.5	34.5
16	28.3	28.4	28.1
17	51.2	51.0	50.1
18	17.0	17.0	17.0
19	26.3	26.7	20.3
20	36.1	36.3	35.9
21	18.9	18.9	18.7
22	33.9	34.2	33.7
23	28.9	29.0	28.6
24	77.3	79.1	77.3
25	81.6	72.7	81.6
26	23.8	26.1	23.8
27	21.8	25.9	21.9
28	27.6	27.2	28.3
29	26.2	26.1	25.8
30	19.2	19.3	18.2
1′	107.2		107.2
2′	75.4		75.5
3′	78.0		78.0
4′	71.7		71.7
5′	78.6		78.7
6′	32.7		62.7
1″	97.1		97.2
2″	79.8		79.8
3″	77.5		77.5
4″	72.1		72.1
5″	78.0		78.2
6″	63.0		62.9
1‴	101.7		101.7
2‴	72.3		72.3
3‴	72.6		72.6
4‴	74.2		74.2
5‴	69.5		69.5
6‴	18.6		18.6

prep. HPLC-ODS (system VII) to provide compound **10** (13 mg). Fraction 5-3 was purified by prep. HPLC-Polyamine II (system VIII) to afford compounds **8** (62 mg), **9** (137 mg) and **15** (36 mg). Fraction 6 (2.2 g) was subjected to a column of RP-18 (system V) to provide seven fractions. Fraction 6-3 was further purified by prep. HPLC-Polyamine II (system VIII) to give compound **16** (8 mg). Fraction 6-4 was purified by prep. HPLC-Polyamine II (system VIII) and HPLC-ODS (system IX) to afford compounds **6** (24 mg) and **7** (111 mg). Finally, fraction 7 (5.2 g) was similarly separated on a column of RP-18 to provide 11 fractions. Fraction 7-3 was purified by prep. HPLC-ODS to give compounds **3** (50 mg) and **4** (51 mg). Fraction 7-7 was purified by prep. HPLC-ODS (system X) and HPLC-Diol (system XI) to afford compound **14** (15 mg). Fractions 7-9 and 7-10 were purified by prep. HPLC-ODS (system X) to provide compounds **17** (52 mg) and **18** (10 mg), respectively.

3.4. Khekadaengoside A (*3*)

Amorphous powder, $[\alpha]_D^{23}$ −109.4° (MeOH, *c* 3.1); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) assignments, see Tables 1 and 2; negative HR–FAB–MS, *m*/*z*: 823.4108 ($C_{42}H_{63}O_{16}$ requires 823.4115).

3.5. Khekadaengoside B (*4*)

Amorphous powder, $[\alpha]_D^{23}$ −37.0° (MeOH, *c* 2.7); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 1 and 2; Negative HR–FAB–MS, *m*/*z*: 839.4053 ($C_{42}H_{63}O_{17}$ requires 839.4065).

3.6. Enzymatic hydrolysis of khekadaengoside B (*4*)

To a solution of compound **4** in MeOH (0.5 ml) was added a solution of α-amylase (Sigma, 40 mg in 2 ml of H_2O). After stirring at 37 °C for 5 days, the mixture was extracted with *n*-Butanol. The *n*-butanol extract was evaporated to provide cucurbitacin L 2-*O*-β-glucopyranoside (**1**) whose structure was identified by ^1H and ^{13}C NMR spectral analysis.

3.7. Khekadaengoside C (*5*)

Amorphous powder, $[\alpha]_D^{23}$ −42.3° (MeOH, *c* 4.9); for ^1H (C_5D_5N) ^{13}C NMR (C_5D_5N): spectral analyses, see Tables 1 and 2; negative HR–FAB–MS, *m*/*z*: 659.3451 ($C_{36}H_{51}O_{11}$ requires 659.3431).

3.8. Khekadaengoside D (*6*)

Amorphous powder, $[\alpha]_D^{23}$ −71.1° (MeOH, *c* 0.6); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 1 and 2; negative HR–FAB–MS, *m*/*z*: 677.3542 ($C_{36}H_{53}O_{12}$ requires 677.3536).

3.9. Khekadaengoside E (*7*)

Amorphous powder, $[\alpha]_D^{23}$ −43.8° (MeOH, *c* 3.7); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 1 and 2; negative HR–FAB–MS, *m*/*z*: 677.3530 ($C_{36}H_{53}O_{12}$ requires 677.3536).

3.10. Enzymatic hydrolysis of khekadaengosides C (*5*), D (*6*) and E (*7*)

A sample of each compound **5**, **6** and **7** was individually dissolved in 0.5 ml MeOH. A solution of crude hesperidinase (100 mg in 20 ml of H_2O) was then added for each experiment. After stirring at 37 °C for 2 days, the individual mixtures were extracted with EtOAc, and concentrated to dryness, affording khegadaengenins I (**5a**), II (**6a**) and (**7a**), respectively, whose structures were identified by ^1H and ^{13}C NMR spectral analyses.

3.11. Khekadaengenin I (*5a*)

Amorphous powder, $[\alpha_D^{19}]$ −10.7° (MeOH, *c* 0.8); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral analyses, see Tables 3 and 4; negative HR–FAB–MS, *m*/*z*: 497.2941 ($C_{30}H_{41}O_6$ requires 497.2903).

3.12. Khekadaengenin II (*6a*)

Amorphous powder, $[\alpha]_D^{23}$ −14.6° (MeOH, *c* 0.8); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 3 and 4; negative HR–FAB–MS, *m*/*z*: 515.3024 ($C_{30}H_{43}O_7$ requires 515.3008).

3.13. Khekadaengenin III (*7a*)

Amorphous powder, $[\alpha]_D^{23}$ −12.2° (MeOH, *c* 0.9); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 3 and 4; negative HR–FAB–MS, *m*/*z*: 515.3025 ($C_{30}H_{43}O_7$ requires 515.3008).

3.14. Hydrogenation of khekadaengoside E (*7*)

Compound **7** (30 mg) was dissolved in EtOH (5 ml) and 8 mg Pd-C was added. After stirring under a H_2 for 2 h and subsequent filtration, the solvent was concentrated to provide 23,24-dihydrokhekadaengoside E (**7b**) (28 mg) whose structure was elucidated by ^1H and ^{13}C NMR spectral analysis.

3.15. 23,24-dihydrokhekadaengoside E (*7b*)

Amorphous powder, $[\alpha]_D^{23}$ −123.3° (MeOH, *c* 1.9); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral

assignments, see Tables 1 and 2; Negative HR–FAB–MS, *m/z*: 679.3680 ($C_{36}H_{55}O_{12}$ requires 679.3693).

3.16. Cucurbitacin J 2-O-β-glucopyranoside (**8**)

Amorphous powder, $[\alpha]_D^{23}$ −55.8° (MeOH, *c* 2.3); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 1 and 2. negative HR–FAB–MS, *m/z*: 693.3447 ($C_{36}H_{53}O_{13}$ requires 693.3486).

3.17. Cucurbitacin K 2-O-β-glucopyranoside (**9**)

Amorphous powder, $[\alpha]_D^{23}$ −64.4° (MeOH, *c* 0.8); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 1 and 2. negative HR-FAB-MS, *m/z*: 693.3477 ($C_{36}H_{53}O_{13}$ requires 693.3486).

3.18. Ezymatic hydrolysis of cucurbitacin J 2-O-β-glucopyranoside (**8**) and cucurbitacin K 2-O-β-glucopyranoside (**9**)

Individual samples of compounds **8** and **9** MeOH (0.5 ml) were treated with crude hesperidinase (100 mg in 20 ml H_2O), and worked-up as descripted in Section *3.10*, to afford cucurbitacin J (**8a**) and cucurbitacin K (**9a**), respectively. The structures were identified by comparison of both physical and spectroscopic data with those reported in the literature (Enslin and Norton, 1964; Gamlath et al., 1988).

3.19. Cucurbitacin J (**8a**)

Amorphous powder, $[\alpha]_D^{21}$ −34.0° ($CHCl_3$, *c* 0.8); for ^1H ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectral assignments, see Tables 3 and 4; negative HR–FAB–MS, *m/z*: 531.2986 ($C_{30}H_{43}O_8$ requires 531.2957).

3.20. Cucurbitacin K (**9a**)

Amorphous powder, $[\alpha]_D^{21}$ −61.2° ($CHCl_3$, *c* 1.0); for ^1H ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectral assignments, see Tables 3 and 4; negative HR–FAB–MS, *m/z*: 531.2909 ($C_{30}H_{43}O_8$ requires 531.2957).

3.21. Khekadaengoside F (**10**)

Amorphous powder, $[\alpha]_D^{23}$ −12.7° (MeOH, *c* 0.8); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 1 and 2; negative HR–FAB–MS, *m/z*: 693.3434 ($C_{36}H_{53}O_{13}$ requires 693.3486).

3.22. Khekadaengoside G (**11**)

Amorphous powder, $[\alpha]_D^{23}$ −31.2° (MeOH, *c* 2.5); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 1 and 2; negative HR–FAB–MS, *m/z*: 643.3513 ($C_{36}H_{51}O_{10}$ requires 643.3482).

3.23. Khekadaengoside H (**12**)

Amorphous powder, $[\alpha]_D^{23}$ +5.4° (MeOH, *c* 1.0); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 1 and 2; negative HR–FAB–MS, *m/z*: 643.3474 ($C_{36}H_{51}O_{10}$ requires 643.3482).

3.24. Enzymatic hydrolysis of khekadaengosides G (**11**) and H (**12**)

Samples of compounds **11** and **12** were individually dissolved in 0.5 ml MeOH and treated with crude hesperidinase (100 mg in 20 ml H_2O); these were then worked-up as descripted in Section 3.10 to give khekadaengenins IV (**11a**) and V (**12a**), whose structures were elucidated by ^1H and ^{13}C NMR spectral analyses.

3.25. Khekadaengenin IV (**11a**)

Amorphous powder, $[\alpha]_D^{23}$ +30.9° (MeOH, *c* 0.4); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 3 and 4; negative HR–FAB–MS, *m/z*: 481.2987 ($C_{30}H_{41}O_5$ requires 481.2953).

3.26. Khekadaengenin V (**12a**)

Amorphous powder, $[\alpha]_D^{23}$ +78.9° (MeOH, *c* 0.3); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 3 and 4; negative HR—FAB–MS, *m/z*: 481.2987 ($C_{30}H_{41}O_5$ requires 481.2953).

3.27. Khekadaengoside I (**13**)

Amorphous powder, $[\alpha]_D^{23}$ −10.5° (MeOH, *c* 0.5); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 3 and 4; negative HR–FAB–MS, *m/z*: 659.3420 ($C_{36}H_{51}O_{11}$ requires 659.3431).

3.28. Khekadaengoside J (**14**)

Amorphous powder, $[\alpha]_D^{23}$ −38.9° (MeOH, *c* 1.0); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 1 and 2; negative HR–FAB–MS, *m/z*: 823.4118 ($C_{42}H_{63}O_{16}$ requires 823.4115).

3.29. Khekadaengoside K (**15**)

Amorphous powder, $[\alpha]_D^{23}$ −11.9° (MeOH, *c* 1.6); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 1 and 2. Negative HR–FAB–MS, *m/z*: 561.2698 ($C_{30}H_{41}O_{10}$ requires 561.2699).

3.30. Enzymatic hydrolysis of khekadaengoside K (**15**)

Compound **15** was dissolved in 0.5 ml MeOH and treated with crude hesperidinase (100 mg in 20 ml H_2O), and worked-up as described in Section 3.10, to give hexanorcucurbitacin I (**15a**), whose structure was elucidated by ^1H and ^{13}C NMR spectral analysis, and comparison of both physical and spectroscopic data to those reported in the literature (Rao et al., 1974).

3.31. Hexanorcucurbitacin I (**15a**)

Amorphous powder, $[\alpha]_D^{23}$ +23.5° (MeOH, *c* 0.8); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 3 and 4; negative HR–FAB–MS, *m*/*z*: 399.2203 ($C_{24}H_{32}O_5$ requires 399.2171).

3.32. Khekadaengoside L (**16**)

Amorphous powder, $[\alpha]_D^{23}$ −148.2° (MeOH, *c* 0.6); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 1 and 2; negative HR—FAB–MS, *m*/*z*: 663.2986 ($C_{34}H_{47}O_{13}$ requires 663.3016).

3.33. Khekadaengoside M (**17**)

Amorphous powder, $[\alpha]_D^{23}$ +6.4° (MeOH, *c* 1.7) for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 5 and 6; negative HR–FAB–MS, *m*/*z*: 945.5419 ($C_{48}H_{81}O_{18}$ requires 945.5422).

3.34. Enzymatic hydrolysis of khekadaengoside M (**17**)

To a solution of compound **17** in 0.5 ml MeOH was added crude hesperidinase (100 mg in 20 ml H_2O); the whole was then worked-up as described in Section 3.10, to afford mogrol (**17a**), whose structure was identified by comparison of both physical and spectroscopic data to those reported in the literature (Takemoto et al., 1983; Kasai et al., 1989).

3.35. Mogrol (**17a**)

Amorphous powder, $[\alpha]_D^{23}$ +37.5° (MeOH, *c* 0.6); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 5 and 6; negative HR–FAB–MS, *m*/*z*: 475.3732 ($C_{48}H_{51}O_4$ requires 475.3787).

3.36. Khekadaengoside M (**18**)

Amorphous powder, $[\alpha]_D^{23}$ +26.1° (MeOH, *c* 2.8); for ^1H (C_5D_5N) and ^{13}C NMR (C_5D_5N) spectral assignments, see Tables 5 and 6; negative HR–FAB–MS, *m*/*z*: 943.5252 ($C_{48}H_{79}O_{18}$ requires 943.5266).

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