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CUCURBITACIN GLYCOSIDES FROM *CAPUT NIGRI*

KIMIKO NAKANO, YUU KANAI, KŌTARO MURAKAMI and YOSHIHISA TAKAISHI

Faculty of Pharmaceutical Sciences, Tokushima University, Shomachi 1-78, Tokushima, 770, Japan

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Abstract—The chemical structures of cabenosides I–L were investigated. These are four of the 12 cucurbitacin glycosides isolated from 'Cabeça-de-negro', the roots of *Caput nigri*. By means of chemical analyses the structures of cabenosides I–L were elucidated as 10α-cucurbit-5-ene-3β,11α,20*S*,24ξ,25-pentaol [3, 25-di-*O*-β-D-glucopyranoside, and 3-*O*-β-D-glucopyranosyl-(1-2)-β-D-glucopyranosyl (sophorosyl), 25-*O*-β-D-glucopyranoside], and 10α-cucurbit-5,20,24-triene-11-oxo-3β,26-diol [3-*O*-β-D-glucopyranosyl-(1-6)-β-D-glucopyranosyl (gentiobiosyl), 26-*O*-β-D-glucopyranoside and 3,26-di-*O*-gentiobioside], respectively.

INTRODUCTION

In one of the two previous papers [1], we reported the structural elucidation of three new nor-cucurbitacin glucosides (cabenosides A–C) isolated from 'Cabeça-de-negro', the roots of *Caput nigri*. In the preceding paper [2], we described the isolation of nine cucurbitacin glycosides (cabenosides D–L) from 'Cabeça-de-negro' and the structural elucidation of cabenosides D–H. The present paper reports the structural elucidation of cabenosides I(**1**), J(**2**), K(**3**) and L (**4**).

RESULTS AND DISCUSSION

Cabenoside I(**1**), obtained as powder, showed a $[M + Na]^+$ ion peak at *m/z* 839 in the FAB-mass spectrum. The ^1H and ^{13}C NMR spectra indicated the presence of two β-D-glucopyranosyl units [H-1′: δ4.88 (*d*, *J* = 7.32 Hz), C-1′: δ107.3 and H-1″: δ5.14 (*d*, *J* = 7.82 Hz), C-1″: δ97.5]. A total of 42 carbon signals observed in the ^{13}C NMR spectrum of **1** was separated into Me × 8, CH_2 × 8, CH × 7, C × 7 and D-glucose × 2 with the help of DEPT spectra. The ^{13}C NMR signals at δ118.5 (CH) and 144.3 (C) and five carbinyl carbon signals at δ76.3 (CH), 77.8 (CH), 87.9 (CH), 74.6 (C) and 80.6 (C), indicated that the aglycone of **1** should be triterpenoid, having the molecular formula $C_{30}H_{52}O_5$. Comparison of the ^{13}C NMR spectra between **1** and authentic sample (cabenosides D and E) [2], suggested that **1** should be 10α-cucurbit-5-ene-3β, 11α, 20*S*, 24ξ, 25-pentaol. The linkage position between each D-glucose and the aglycone was established by observations of the ^1H–^{13}C long-range COSY spectrum of **1**. The carbon signals at δ107.3 and 80.5 showed correlation with the proton signals at δ3.69 (*br s*, 3-H) and 5.14 (*d*, 1″-H), respectively. Accordingly, the structure of **1** was elucidated to be 10α-cucurbit-5-ene-3β, 11α, 20*S*, 24ξ, 25-pentaol 3, 25-di-*O*-β-D-glucopyranoside.

Cabenoside J (**2**) obtained as powder, revealed a $[M + H + Na]^+$ ion peak at *m/z* 1002, 162 mass units more than that of **1** in the FAB-mass spectrum. In the ^1H and ^{13}C NMR spectral data of **2**, signals owing to aglycone moieties were in good agreement with those of **1**, while signals owing to sugar moieties were identical with 3-*O*-sophorosyl, 25-*O*-glucopyranoside. These were also supported by the ^1H–^1H COSY, ^1H–^{13}C COSY and ^1H–^{13}C long-range COSY spectra. From the above evidence the structure of **2** was determined as shown and was concluded to be 10α-cucurbit-5-ene-3β,11α,20*S*,24ξ, 25-pentaol 3-*O*-β-D-glucopyranosyl-(1-2)-β-D-glucopyranosyl, 25-*O*-β-D-glucopyranoside.

Cabenoside K (**3**), obtained as powder, exhibited a $[M + H + Na]^+$ ion peak at *m/z* 964 in the FAB-mass spectrum. The ^1H NMR spectrum of **3** showed signals for six tertiary methyl protons at δ0.68, 1.08, 1.09, 1.18, 1.54 and 1.80, two olefinic protons at δ5.53 (*br s*) and 5.65 (*br t*), three anomeric protons at δ4.83 (*d*, *J* = 7.82 Hz), 4.91 (*d*, *J* = 7.82 Hz) and 5.16 (*d*, *J* = 7.81 Hz) and two protons at δ4.93 and 5.05 (each 1H, *s*).

A total of 48 carbon signals observed in the ^{13}C NMR spectrum of **3** were separated into Me × 6, CH_2 × 13, CH × 21 and C × 8 with the help of DEPT spectra. The ^{13}C NMR spectrum indicated that it had the same nucleus as authentic samples (cabenosides D and H) [2] and differed in the structure of the side chain. Signals from the sugar moieties were almost superimposable on those of cabenosides G and H (gentiobiose and glucose) [2].

The ^1H–^{13}C COSY and long-range COSY (Fig. 1) showed that the terminal methylene group placed at C-21 and hydroxy methylene and olefinic methyl groups were placed at C-26 and C-27. To determine the position of the hydroxy methylene group, difference NOE spectra were

measured. When the olefinic proton (δ5.65, *br t*) was irradiated, NOE was observed on the signal of hydroxy methylene group (δ4.28) and methylene (δ2.24). Furthermore, long-range correlations were seen between the anomeric carbon (δ106.9) and H-3 (δ3.68), the anomeric carbon (δ103.2) and H_2-26 (δ4.28 and 4.51), the carbinol carbon (δ74.9) and anomeric proton (δ4.91) and the carbinol carbon (δ70.3) of glucose C-6 and anomeric proton (δ5.16) in the ^1H–^{13}C long-range COSY spectrum.

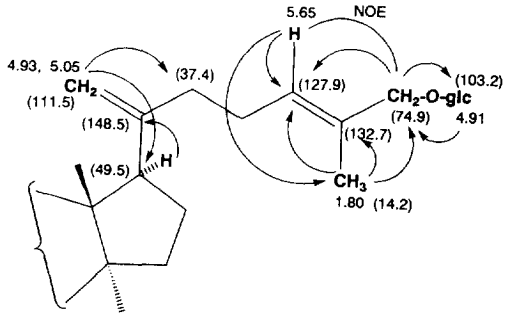
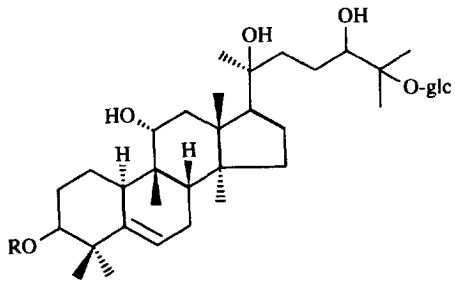
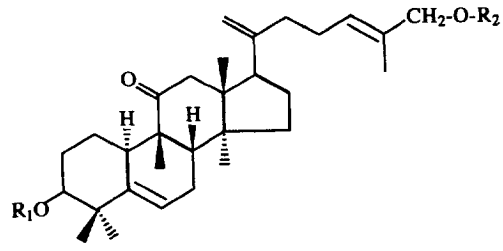


Fig 1. ↷ ^1H–^{13}C long-range correlations of side chain of cabenoside K.



Cabenoside I (1) : R = glc
Cabenoside J (2) : R = glc (1→2) glc –



Cabenoside K (3) : R_1 = glc (1→6) glc –
R_2 = glc

Cabenoside L (4) : R_1 = R_2 = glc (1→6) glc –

Fig. 2.

Table 1. ^{13}C NMR spectral data of cabensoides I–L (pyridine-d_5, δ values)

C	I (1)	J (2)	K (3)	L (4)	C	I (1)	J (2)	K (3)	L (4)
1	26.8	26.4	22.1	22.2	g-1′	107.3	104.7	106.9	106.9
2	29.5	29.9	28.5	28.5	2′	75.5	82.1	75.3	75.3
3	87.9	87.1	86.6	86.6	3′	78.1	77.1	78.3	78.3
4	42.4	42.1	41.9	42.0	4′	71.7	71.6	71.6	71.4
5	144.3	144.1	141.3	141.4	5′	78.7	78.4	77.2	77.2
6	118.5	118.8	118.3	118.4	6′	62.7	62.8	70.3	70.3
7	24.5	24.5	24.6	24.7	1″		105.2	105.3	105.2
8	42.7	42.7	44.3	44.3	2″		75.5	75.1	75.1
9	40.1	40.1	48.7	48.7	3″		77.8	78.3	78.4
10	36.9	36.7	35.8	35.8	4″		71.6	71.6	71.7
11	77.8	77.8	213.1	213.4	5″		78.8	78.4	78.5
12	41.6	41.6	47.6	47.6	6″		62.8	62.7	62.7
13	48.3	48.2	49.3	49.4	25-g				
14	50.0	50.0	50.0	50.1	1‴	97.5	97.5	103.2	103.2
15	34.2	34.2	34.8	34.9	2‴	75.5	75.5	75.1	74.9
16	29.9	29.9	24.1	24.1	3‴	78.5	78.2	78.3	78.3
17	53.7	53.7	49.5	49.5	4‴	71.8	71.6	71.6	71.5
18	18.7	18.7	18.5	18.6	5‴	78.9	78.9	78.4	77.1
19	26.4	26.2	20.3	20.3	6‴	63.0	62.8	62.7	69.9
20	74.6	74.5	148.5	148.6	1⁗				105.3
21	26.3	26.3	111.5	111.5	2⁗				75.1
22	43.1	43.1	37.4	37.5	3⁗				78.4
23	26.5	26.4	27.0	27.1	4⁗				71.7
24	76.4	76.2	127.9	128.0	5⁗				78.5
25	80.6	80.5	132.7	132.7	6⁗				62.7
26	22.6	22.6	74.9	74.9					
27	22.9	22.9	14.2	14.3					
28	26.3	26.4	28.3	28.3					
29	27.7	27.6	25.8	25.8					
30	19.5	19.4	18.4	18.4					

From the above evidence, the structure of **3** was revealed to be 10α-cucurbit-5,20,24-triene-11-oxo-3β,26-diol 3-*O*-β-D-glucopyranosyl-(1-6)-β-D-glucopyranosyl, 26-*O*-β-D-glucopyranoside.

Cabenoside L (**4**), obtained as a powder, exhibited a $[M + H + Na]^+$ ion peak at *m/z* 1126, 162 mass units more than that of **3** in the FAB-mass spectrum. In the ^1H and ^{13}CNMR spectral data of **4**, signals owing to aglycone moieties were in good agreement with those of **3**, while signals from the sugar moieties were identical with 3,26-di-*O*-gentiobioside. These were also supported by the ^1H–^1H, ^1H–^{13}C and ^1H-^{13}C long-range COSY spectra. From the above evidence the structure of **4** was determined as shown in Fig. 2, and it was concluded to be 10α-cucurbit-5,20,24-triene-11-oxo-3β,26-diol, 3,26-di-*O*-[β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside].

EXPERIMENTAL

General experimental procedures were as described in ref. [2].

Cabenoside I (**1**). Amorphous powder, $[\alpha]_D^{21}$ + 2.0° (pyridine; *c* 0.49), IR ν_{max} cm^{-1}: 3470, 1687, 1560, 1480, 1380, 1078. FAB-MS *m/z*: 839 $[M + Na]^+$. ^1H NMR (pyridine-d_5): δ1.04 (3H, *s*, Me-30), 1.18 (3H, *s*, Me-29), 1.32 (3H, *s*, Me-19), 1.37 (3H, *s*, Me-18), 1.43 (3H, *s*, Me-27), 1.51 (3H, *s*, Me-26), 1.55 (3H, *s*, Me-28), 1.57 (3H, *s*, Me-21), 3.69 (1H, *br s*, H-3), 4.89 (1H, *d*, *J* = 7.32 Hz, H-1′), 5.14 (1H, *d*, *J* = 7.82 Hz, H-1″), 5.52 (1H, *br d*, *J* = 5.37 Hz, H-6). ^{13}C NMR data: Table 1.

Cabenoside J (**2**). Amorphous powder, $[\alpha]_D^{23}$ − 1.9° (pyridine; *c* 0.53), IR ν_{max} cm^{-1}: 3400, 1686, 1562, 1460, 1389, 1078. FAB-MS *m/z*: 1002 $[M + H + Na]^+$. ^1H NMR (pyridine-d_5): δ1.06 (3H, *s*, Me-30), 1.15 (3H, *s*, Me-29), 1.34 (3H, *s*, Me-19), 1.37 (3H, *s*, Me-18), 1.40 (3H, *s*, Me-27), 1.52 (3H, *s*, Me-26), 1.55 (3H, *s*, Me-28), 1.60 (3H, *s*, Me-21), 3.66 (1H, *br s*, H-3), 4.91 (1H, *d*, *J* = 6.83 Hz, H-1′), 5.17 (1H, *d*, *J* = 7.81 Hz, H-1‴), 5.41 (1H, *d*, *J* = 7.81 Hz, H-1″), 5.84 (1H, *br d*, *J* = 5.21 Hz, H-6). ^{13}C NMR data: Table 1.

Cabenoside K (**3**). Amorphous powder, $[\alpha]_D^{21}$ + 34.1° (pyridine; *c* 0.50), IR ν_{max} cm^{-1}: 3400, 1689, 1460, 1385, 1078. FAB-MS *m/z*: 964 $[M + H + Na]^+$. ^1H NMR (pyridine-d_5): δ0.68 (3H, *s*, Me-18), 1.08 (3H, *s*, Me-28), 1.09 (3H, *s*, Me-30), 1.18 (3H, *s*, Me-19),1.54 (3H, *s*, Me-29), 1.80 (3H, *s*, Me-27), 2.33 (1H, *d*, *J* = 14.16 Hz, H-12), 2.78 (1H, *t*, *J* = 9.28 Hz, H-17), 3.03 (1H, *d*, *J* = 13.68 Hz, H-12), 3.68 (1H, *br s*, H-3), 4.28, 4.51 (each 1H, AB *q*, *J* = 12.69 Hz, H_2-26), 4.83, 4.91 (each 1H, *d*, *J* = 7.82 Hz, H-1′ and H-1‴), 4.93, 5.05 (each 1H, *s*, H_2-21), 5.16 (1H, *d*, *J* = 7.81 Hz, H-1″), 5.53 (1H, *br s*, H-6), 5.65 (1H, *t*-like, *J* = 6.34 Hz, H-24). ^{13}C NMR data: Table 1.

Cabensoide L (**4**). Amorphous powder, $[\alpha]_D^{21}$ + 27.3° (pyridine; *c* 0.55), IR ν_{max} cm^{-1}: 3400, 1678, 1655, 1562, 1460, 1383, 1078. FAB-MS *m/z*: 1126 $[M + H + Na]^+$. ^1H NMR (pyridine-d_5): δ0.67 (3H, *s*, Me-18), 1.08 (3H, *s*, Me-28), 1.09 (3H, *s*, Me-30), 1.18 (3H, *s*, Me-19), 1.54 (3H, *s*, Me-29), 1.80 (3H, *s*, Me-27), 3.69 (1H, *br s*, H-3), 4.84, 4.87, 5.18, 5.33 (each 1H, *d*, *J* = 7.32–7.82 Hz, anomeric H), 4.92, 5.04 (each 1H, *s*, H_2-21), 5.53 (1H, *br s*, H-6), 5.68 (1H, *t*-like, H-24). ^{13}C NMR data: Table 1.

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