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CUCURBITACIN GLYCOSIDES FROM CABEÇA-DE-NEGRO

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Key Word Index—*Caput nigri*; Cucurbitaceae; Cabeça-de-negro; roots; cucurbitacin glycoside; bryodulcosigenin; cabenoside D–H.

Abstract—Five cucurbitacin glycosides named cabenosides D–L were isolated from 'Cabeça-de-negro', the roots of *Caput nigri*. Among them, the structures of cabenosides D–H were elucidated as 10α-cucurbit-5-en-11-oxo-3β,24R,25-triol-3-*O*-β-D-glucopyranoside, 10α-cucurbit-5-en-24-oxo-3β,11α,25-triol-25-*O*-β-D-glucopyranosyl, [3,*O*-β-D-glucopyranoside, 3-*O*-β-D-glucopyranosyl-(1-2)-β-D-glucopyranoside, 3-*O*-β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside] and 10α-cucurbit-5-en-11,24-dioxo-3β,25-diol-25-*O*-β-D-glucopyranosyl, 3-*O*-β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside, respectively, on the basis of chemical and spectral evidence.

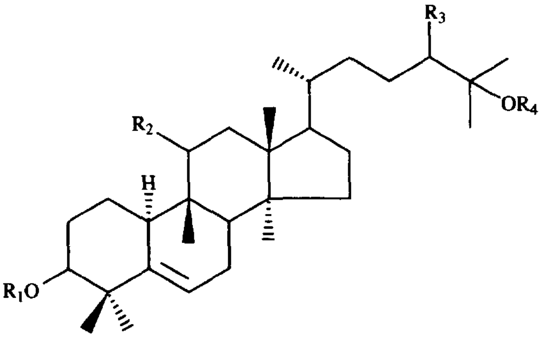
INTRODUCTION

In the preceding paper [1], we reported the isolation and structure elucidation of three new nor-cucurbitacin glucosides named cabenosides A–C from ’Cabeça-de-negro’ the roots of *Caput nigri*. In the continuing study on glucosidic constituents, we obtained nine additional cucurbitacin glucosides, named cabenosides D(**1**), E(**2**), F(**3**), G(**4**), H(**5**), I, J, K and L. This paper reports the structural characterization of **1**–**5**.

RESULTS AND DISCUSSION

Cabenoside D(**1**), amorphous powder, $[\alpha]_D + 80.0°$ (MeOH), showed a $[M + Na]^+$ peak at *m/z* 659 in the FAB-mass spectrum. The ^{1}H NMR spectrum of **1** exhibited signals due to seven tertiary methyl groups (δ0.73, 0.95, 1.12, 1.17, 1.54, 1.57 and 1.61) and one secondary methyl group (δ0.94, *d*, $J = 6.35$ Hz), those due to methylene groups (δ2.49 and 2.91, each 1H, *d*, $J = 14.16$ Hz) adjacent to a carbonyl group, those due to two hydroxy methine groups (δ3.69, *br s* and 3.79, *br d*, $J = 7$ Hz) and an olefin (δ5.53, *d*, $J = 5.37$ Hz). It also gave doublet signal at δ4.88 ($J = 7.32$ Hz) ascribable to an anomeric proton. These results, combined with ^{13}C NMR data, suggested **1** should be bryodulcosigenin [2, 3] glucoside. This was supported by the 2D-NMR experiments; the H-3 at δ3.79 showed a long range correlation with the anomeric carbon at δ107.3, which was confirmed by direct comparison with an authentic sample mogroside IE$_2$ [4]. Thus, the structure of **1** was concluded to be 10α-cucurbit-5-en-11-oxo-3β,24R,25-triol-3-*O*-β-D-glucopyranoside.

Cabenoside E (**2**) obtained as a powder, $[\alpha]_D + 7.7°$ (pyridine), showed a peak *m/z* 821 $[M + Na]^+$ in the FAB-mass spectrum. The ^{1}H NMR spectrum of **2** displayed seven tertiary methyl signals, one secondary methyl signal, one olefinic proton signal and two anomeric proton signals. The ^{13}C NMR spectrum revealed the presence of five quaternary carbon signals at δ40.1, 42.4, 47.3, 49.7 and 82.8, a set of olefinic carbon signals at δ118.4 and 144.2, one carbonyl carbon signal at δ214.3 and two anomeric carbon signals at δ99.6 and 107.4.



	R$_1$	R$_2$	R$_3$	R$_4$
Cabenoside D (**1**) :	glc	O	OH	H
Cabenoside E (**2**) :	glc	αOH	O	glc
Cabenoside F (**3**) :	glc(1 → 2)glc	αOH	O	glc
Cabenoside G (**4**) :	glc(1 → 6)glc	αOH	O	glc
Cabenoside H (**5**) :	glc(1 → 6)glc	O	O	glc

On acid hydrolysis, **2** afforded D-glucose and an aglycone (**2a**). In the ^{1}H NMR spectrum of **2a**, an hydroxyl methine proton appeared at δ4.20 as a double doublet signal ($J = 4.88$, 11.23 Hz). Furthermore, the ^{13}C NMR signals of **2a** arising from C-1, C-10 and C-19 were displaced downfield, while the signals from C-12 and C-13 moved upfield as compared with those of **1**. These results suggested cabenoside E should be a 24-oxo-cucurbit-5-en type triterpenoid having 3*β*,11*α*,25-trihydroxyl groups. Comparison of the ^{13}C NMR spectrum of **2** with that of **2a** showed glycosylation shifts [5] for the C-3 and C-25 signals of the aglycone. These were also supported by the ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and ^{1}H–^{13}C long range COSY spectra. From the above evidence the structure of **2** was concluded to be 10*α*-cucurbit-5-en-24-oxo-3*β*,11*α*,25-triol-3,25-di-*O*-*β*-D-glucopyranoside.

Cabenoside F (**3**) and G (**4**), obtained as powder, $[\alpha]_D$ + 19.5° (pyridine) and + 3.8° (pyridine), exhibited a $[M + Na]^+$ peak at *m/z* 983 and a $[M + H + Na]^+$ peak at *m/z* 984 in their FAB-mass spectra. In the ^{1}H and ^{13}C NMR spectra of **3** and **4**, signals due to the aglycone moieties were in good agreement with those of **2**, while signals due to the sugar moieties were identical with 3-O-*β*-sophorosyl, 25-*O*-*β*-D-glucopyranoside and 3-*O*-*β*-gentiobiosyl, 25-*O*-*β*-D-glucopyranoside, respectively. From the above evidence, the structures of **3** and **4** were deduced for these compounds.

Cabenoside H (**5**), obtained as powder, $[\alpha]_D$ + 42.5° (pyridine), exhibited a $[M + Na]^+$ peak at *m/z* 981 in the FAB-mass spectrum. The ^{1}H NMR spectrum showed seven singlet methyl signals and one doublet methyl signal, one olefinic proton signal and three anomeric proton signals. The ^{13}C NMR spectrum revealed the presence of five quaternary carbon signals at δ41.4, 49.0, 49.0, 49.6 and 82.8, a pair of olefinic carbon signals at δ118.4 and 141.3, two carbonyl carbon signals at δ213.3 and 214.3 and three anomeric carbon signals at δ99.6, 105.4 and 106.9. From the analysis of ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and ^{1}H–^{13}C long range COSY spectra, the structure of **5** was determined as shown and it was concluded to be 10*α*-cucurbit-5-en-11,24-di-oxo-3*β*,25-diol-3-*O*-*β*-sophorosyl,25-*O*-*β*-D-glucopyranoside.

EXPERIMENTAL

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in the preceding paper [1].

Isolation of compounds **1**–**5**. Fr. 2 (13.8 g), Fr. 5 (5.7 g), Fr. 7 (5.24 g) and Fr. 8 (13 g) [1] were repeatedly chromatographed on silica gel, Sephadex LH-20 and ODS column with $CHCl_3$–MeOH–EtOAc–H_2O (4:4:10:1, 6:6:8:1), $CHCl_3$–MeOH (1:1), MeOH–H_2O (1:1, 3:2) respectively, to afford cabenoside D (**1**, 166 mg), E (**2**, 349 mg), F (**3**, 222 mg), G (**4**, 180 mg), H (**5**, 69 mg), I (134 mg), J (12 mg), K (69 mg) and L (15 mg).

Cabenoside D (**1**). Amorphous powder, $[\alpha]_D$ + 80° (MeOH; *c* 0.5), IR ν_{max}^{KBr} cm^{-1}: 3400, 1687, 1656. FAB-MS *m/z*: 659 $[M + Ma]^+$. ^{1}H NMR (d_5-pyridine): δ0.73 (3H, *s*, Me-18), 0.90 (3H, *d*, J = 6.35 Hz, Me-21), 0.95 (3H, *s*, Me-30), 1.12 (3H, *s*, Me-28), 1.17 (3H, *s*, Me-19), 1.54, 1.57, 1.61 (each 3H, *s*, Me-26, Me-27, Me-29), 2.49 (1H, *d*, J = 14.16 Hz, H-12), 2.92 (1H, *d*, J = 14.16 Hz, H-12), 3.62 (1H, *br s*, H-3), 3.79 (1H, *br d*, H-24), 4.89 (1H, *d*,

Table 1. ^{13}C NMR spectral data of aglycone moieties of cabenosides D, E and H (d_5-pyridine, δ values)

C	D (**1**)	E (**2**)	H (**5**)
1	22.1	26.8	22.2
2	28.5	29.5	28.6
3	87.2	87.9	86.5
4	42.0	42.4	41.4
5	141.2	144.2	141.3
6	118.5	118.4	118.4
7	24.1	24.5	24.1
8	43.9	43.5	43.9
9	49.0	40.1	49.0
10	35.9	36.8	35.9
11	213.8	77.8	213.3
12	48.7	41.0	48.7
13	49.6	47.3	49.6
14	49.1	49.7	49.0
15	34.5	34.5	34.5
16	28.1	28.2	28.0
17	49.9	50.9	49.8
18	16.9	16.8	16.9
19	20.3	26.2	20.2
20	36.0	36.1	35.9
21	18.2	18.8	18.2
22	34.0	30.8	30.5
23	28.7	33.8	33.6
24	79.0	214.3	214.3
25	72.8	82.8	82.8
26	25.9	24.6	24.6
27	26.0	23.6	23.7
28	28.3	27.7	28.3
29	26.1	26.2	25.7
30	18.2	19.2	18.5

Table 2. ^{13}C NMR spectral data of sugar moieties of cabenosides D–H (d_5–pyridine, δ values)

C	D (**1**)	E (**2**)	F (**3**)	G (**4**)	H (**5**)
Glc-1′	107.3	107.4	104.8	106.9	106.9
2′	75.5	75.5	82.0	75.1	75.2
3′	78.3	78.3	77.1	78.2	78.2
4′	71.7	71.8	71.7	71.5	71.2
5′	78.7	78.7	78.4	77.2	77.3
6′	63.0	63.0	62.8	70.2	70.4
1″			105.2	105.3	105.1
2″			75.5	75.3	75.3
3″			78.3	78.4	78.4
4″			71.6	71.6	71.7
5″			78.6	78.3	78.6
6″			62.7	62.7	62.7
1‴		99.6	99.6	99.5	99.6
2‴		75.3	75.3	75.3	75.3
3‴		78.1	78.2	78.2	78.4
4‴		71.7	71.9	71.8	71.8
5″		78.6	78.4	78.6	78.7
6″		63.0	62.9	62.9	62.9

J = 7.32 Hz, H-1′), 5.52 (1H, *br d*, H-6). ^{13}C NMR data in Tables 1 and 2.

Cabenoside E (**2**). Amorphous powder, $[\alpha]_D$ + 7.7° (pyridine; *c* 0.52), IR ν_{max}^{KBr} cm^{-1}: 3400, 1700, 1655, 1662, 1460, 1380. FAB-MS *m/z*: 821 $[M + Na]^+$. ^{1}H NMR (d_5-pyridine): δ0.87 (3H, *s*, Me-18), 0.88 (3H, *s*, Me-30), 0.94 (3H, *d*, *J* = 5.86 Hz, Me-21), 1.15 (3H, *s*, Me-28), 1.32 (3H, *s*, Me-19), 1.57 (3H, *s*, Me-29), 1.62 (6H, *s*, Me-26, Me-27), 3.69 (1H, *br s*, H-3), 4.90 (1H, *d*, *J* = 7.81 Hz, H-1′), 5.03 (1H, *d*, *J* = 7.32 Hz, H-1″), 5.50 (1H, *br d*, *J* = 5.37 Hz, H-6). ^{13}C NMR data in Tables 1 and 2.

Cabenoside F (**3**). Amorphous powder, $[\alpha]_D$ + 19.5° (pyridine; *c* 0.5). IR ν_{max}^{KBr} cm^{-1}: 3400, 1708, 1460, 1385. FAB-MS *m/z*: 983 $[M + Na]^+$. ^{1}H NMR (d_5-pyridine): δ0.85 (3H, *s*, Me-18), 0.90 (3H, *s*, Me-30), 0.91 (3H, *d*, *J* = 5.86 Hz, Me-21), 1.12 (3H, *s*, Me-28), 1.33 (3H, *s*, Me-19), 1.57 (3H, *s*, Me-29), 1.62 (6H, *s*, Me-26, Me-27), 3.65 (1H, *br s*, H-3), 4.91 (1H, *d*, *J* = 6.83 Hz, H-1′), 5.03 (1H, *d*, *J* = 7.81 Hz, H-1‴), 5.40 (1H, *d*, *J* = 7.32 Hz, H-1″), 5.86 (1H, *br d*, *J* = 5.86 Hz, H-6). ^{13}C NMR data in Tables 1 and 2.

Cabenoside G (**4**). Amorphous powder, $[\alpha]_D$ + 3.8° (pyridine; *c* 0.5). IR ν_{max}^{KBr} cm^{-1}: 3400, 1709, 1460, 1382. FAB-MS *m/z*: 984 $[M + H + Na]^+$. ^{1}H NMR (d_5-pyridine): δ0.87 (3H, *s*, Me-18), 0.88 (3H, *s*, Me-30), 0.96 (3H, *d*, *J* = 5.86 Hz, Me-21), 1.11 (3H, *s*, Me-28), 1.33 (3H, *s*, Me-19), 1.54 (3H, *s*, Me-29), 1.62 (6H, *s*, Me-26, Me-27), 3.73 (1H, *br s*, H-3), 4.84 (1H, *d*, *J* = 7.81 Hz, H-1′), 5.04 (1H, *d*, *J* = 7.74 Hz, H-1‴), 5.19 (1H, *d*, *J* = 7.77 Hz, H-1″), 5.50 (1H, *br d*, *J* = 5.86 Hz, H-6). ^{13}C NMR data in Tables 1 and 2.

Cabenoside H (**5**). Amorphous powder, $[\alpha]_D$ + 42.5° (pyridine; *c* 0.61). IR ν_{max}^{KBr} cm^{-1}: 3400, 1688, 1562, 1460, 1385. FAB-MS *m/z*: 981 $[M + Na]^+$. ^{1}H NMR (d_5-pyridine): δ0.69 (3H, *s*, Me-18), 0.88 (3H, *d*, *J* = 5.86 Hz, Me-21), 0.95 (3H, *s*, Me-30), 1.07 (3H, *s*, Me-28), 1.17 (3H, *s*, Me-19), 1.53 (3H, *s*, Me-29), 1.63 (6H, *s*, Me-26, Me-27), 2.91 (1H, *d*, *J* = 14.6 Hz, H-12), 3.73 (1H, *br s*, H-3), 4.83 (1H, *d*, *J* = 7.31 Hz, H-1′), 5.03 (1H, *d*, *J* = 7.32 Hz, H-1‴), 5.17 (1H, *d*, *J* = 7.33 Hz, H-1″), 5.52 (1H, *br d*, *J* = 4.40 Hz, H-6). ^{13}C NMR data in Tables 1 and 2.

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