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Studies on the Constituents of the Root of *Cayaponia tayuya* (VELL.) COGN. III. Structures of Cayaponosides, 29-Nor-1,2,3,4,5,10-hexadehydrocucurbit-6-ene Glucosides[1)]

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The bitter constituents in the root of *Cayaponia tayuya* (VELL.) COGN. were investigated. Among 24 29-norcucurbitacin glucosides, named cayaponosides, the structures of cayaponosides A_5, B_5, C_4, C_{5b} and D_2 were determined based mainly on spectral analyses. They are all glucosides of 29-nor-1,2,3,4,5,10-hexadehydrocucurbit-6-enes different only in side chain structure.

Keywords *Cayaponia tayuya*; Cucurbitaceae; cayaponoside; bitter triterpene glucoside; 29-nor-1,2,3,4,5,10-hexadehydrocucurbit-6-ene glucoside

In the first paper[1)] of this series, the isolation of 24 cayaponosides and the structures of 13 cayaponosides having 29-nor-1,2,3,4,5,10-hexadehydrocucurbitacin structures were reported, and the previous paper described the structures of 6 cayaponosides which have a common 29-nor-2,11-dioxo-cucurbita-3,5(6)-diene framework. This paper deals with the structures of cayaponosides A_5, B_5, C_4, C_{5b} and D_2 which have a common 29-nor-1,2,3,4,5,10-hexadehydrocucurbit-6-ene structure.

The isolation of these cayaponosides were described in the first paper.

Cayaponoside A_5 was obtained as an amorphous powder and it showed in the positive ion FAB-MS an $[M+Na]^+$ ion at m/z 727 and fragment ions at m/z 667 ($[M+Na-60]^+$), m/z 645 ($[M+H-60]^+$), and m/z 482 ($[M-162-60]^+$). The high resolution (HR) FAB-MS indicated the molecular formula of cayaponoside A_5 to be $C_{37}H_{52}O_{13}$. The negative ion FAB-MS showed an $[M-H]^-$ ion at m/z 703 and fragment ions at m/z 541 ($[M-H-162]^-$), m/z 481 ($[M-H-162-60]^-$) and m/z 383.

The ^{1}H-NMR spectra showed the presence of six methyl groups on quaternary carbons and one methyl group which attaches to an olefine carbon, one olefine proton, two olefine protons and an acetyl group in addition to the signals of the β-D-glucopyranosyl group. The ^{13}C-NMR spectrum of the aglycone moiety showed the signals of seven methyl carbons, three C–C bonded quaternary carbons, two oxygenated quaternary carbons, two carbonyl carbons, eight olefinic carbons, one hydroxymethine carbon and acetyl carbons.

These spectral data indicated that cayaponoside A_5 is a monoglucoside of a tetracyclic nortriterpene ($C_{31}H_{42}O_8$), having an aromatic ring and an acetyl group in the molecule similar to the aglycone of cayaponoside A which was reported in the first paper of this series. The NMR spectra were compared with those of cayaponoside A. The spectra of both compounds are very similar, the only difference being that cayaponoside A_5 has signals of a disubstituted olefine, while cayaponoside A does not. Judging from the fragment ion signal at m/z 383 in the negative ion FAB-MS, the double bond should be located in the tetracyclic frame work. Closer examination of the NMR spectra revealed the structure of cayaponoside A_5 to be 6-dehydro cayaponoside A. This compound seems to be the same as that isolated from the root of a South American cucurbitaceous plant, *Wilbrandia ebracteata* COGN.[2)]

Cayaponoside C_{5b}, $C_{35}H_{50}O_{12}$, showed similar NMR spectra to those of cayaponoside A_5. Cayaponoside C_{5b} has no acetyl group and spectral differences include the deviation of chemical shifts of protons on C_{24}, C_{26} and C_{27}, and carbons C_{24}—C_{27}.

From these spectral data, the structure of cayaponoside C_{5b} was determined to be desacetyl cayaponoside A_5, and this compound seems to be the same as the compound isolated from the root of *Wilbrandia* sp. by Matos *et al.*[3)]

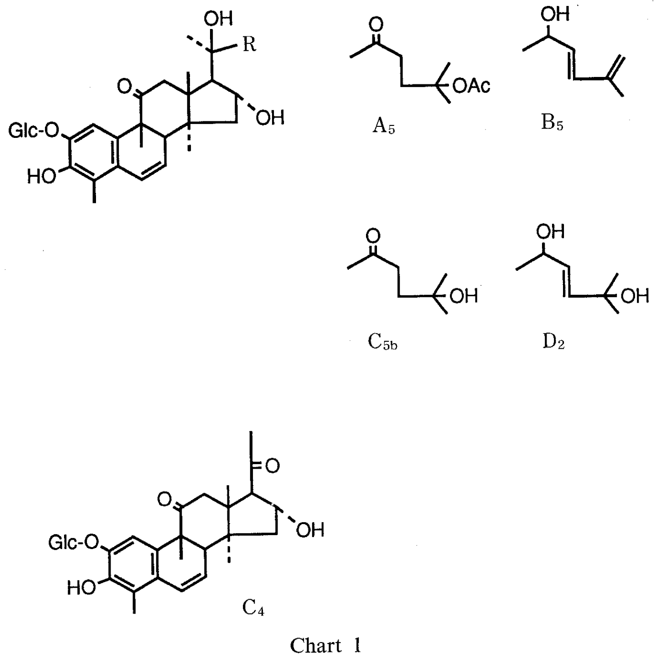

Chart 1

Table I. ^{1}H-NMR Chemical Schifts of Cayaponosides

No.	Cayaponoside				
	A_5	C_{5b}	C_4	B_5	D_2
1	6.56 (s)	6.57 (s)	6.56 (s)	6.53 (s)	6.53 (s)
6	6.87 (d, 10)	6.87 (d, 10)	6.89 (d, 10)	6.88 (d, 10)	6.88 (d, 10)
7	5.79 (dd, 6, 10)	5.80 (dd, 6, 10)	5.82 (dd, 6, 10)	5.82 (dd, 6, 10)	5.82 (dd, 6, 10)
8	2.55 (d, 6)	2.56 (d, 6)	2.56 (d, 6)	2.57 (d, 6)	2.57 (d, 6)
12	2.70 (d, 14)	2.72 (d, 15)	2.55 (d, 14)	2.67 (d, 15)	2.67 (d, 15)
	3.01 (d, 14)	3.02 (d, 15)	3.07 (d, 14)	2.87 (d, 15)	2.88 (d, 15)
15	*ca.* 1.35	1.32 (d, 14)	1.45 (dd, 2, 14)	1.48 (d, 14)	1.47 (d, 14)
	ca. 2.00	2.03 (dd, 9, 14)	2.14 (dd, 10, 14)	2.08 (dd, 8, 14)	2.08 (dd, 9, 14)
16	4.44 (br t, 8)	4.43 (t-like, 7)	4.84 (ddd, 2, 7, 10)	4.65 (dd, 6, 8)	4.64 (dd, 7, 9)
17	2.45 (d, 8)	2.47 (d, 7)	3.10 (d, 7)	2.36 (d, 6)	2.29 (d, 7)
18	0.94 (s)	0.94 (s)	0.74 (s)	1.05 (s)	0.92 (s)
19	1.22 (s)	1.22 (s)	1.23 (s)	1.21 (s)	1.22 (s)
21	1.39 (s)	1.38 (s)	2.16 (s)	1.21 (s)	1.21 (s)
22	—	—	—	4.08 (dd, 1, 6)	3.96 (dd, 1, 6)
23	2.7—2.8	2.7—2.8 (m)	—	5.80 (dd, 6, 10)	5.75 (dd, 6, 15)
24	*ca.* 2.0 (2H)	*ca.* 1.7 (m) (2H)	—	6.36 (dd, 1, 16)	5.83 (dd, 1, 15)
26	1.42 (s)$^{a)}$	1.17 (s)	—	4.95 (s)	1.25 (s)
27	1.43 (s)$^{a)}$	1.17 (s)	—	1.82 (s)	1.25 (s)
28	2.22 (s)	2.22 (s)	2.23 (s)	2.23 (s)	2.23 (s)
30	1.00 (s)	1.01 (s)	0.96 (s)	0.92 (s)	1.05 (s)
Ac	1.92 (s)				

a) Assignment of the signals may be interchanged.

Table II. ^{13}C-NMR Chemical Shifts of Cayaponosides

No.	Cayaponoside				
	A_5	C_{5b}	C_4	B_5	D_2
1	113.6	113.6	113.6	113.5	113.5
2	146.1$^{a)}$	146.1$^{a)}$	146.2$^{a)}$	146.1$^{a)}$	146.1$^{a)}$
3	146.5$^{a)}$	146.5$^{a)}$	146.6$^{a)}$	146.5$^{a)}$	146.4$^{a)}$
4	124.1	124.1	124.3	124.1	124.1
5	130.6$^{b)}$	130.6$^{b)}$	130.4$^{b)}$	130.6$^{b)}$	130.6$^{b)}$
6	126.9	126.9	127.0	126.9	126.9
7	128.1	128.0	127.7	130.4	130.6
8	49.0	49.0	49.1	48.9	48.9
9	52.8	52.9	53.2	52.6	52.5
10	129.1$^{b)}$	129.1$^{b)}$	129.1$^{b)}$	129.1$^{b)}$	129.1$^{b)}$
11	216.9	216.8	215.2	217.1	217.2
12	52.9	52.7	51.0	53.2	53.1
13	51.3	51.3	51.0	51.9	52.0
14	50.4	50.4	51.0	50.6	50.4
15	45.6	45.6	45.2	44.8	44.6
16	72.7	72.6	73.7	73.3	73.3
17	60.7	60.5	69.0	58.2	57.8
18	21.1	21.1	20.7	21.0	20.9
19	27.4	27.4	27.3	27.4	27.4
20	81.6	81.6	211.4	77.9	77.8
21	26.3	26.2	32.6	24.4	24.8
22	217.4	218.0	—	82.2	82.5
23	33.6	33.9	—	128.1	128.1
24	36.6	38.9	—	136.6	142.4
25	83.9	71.6	—	143.8	72.0
26	27.0$^{c)}$	30.2$^{c)}$	—	117.5	30.7$^{c)}$
27	27.1$^{c)}$	29.9$^{c)}$	—	19.6	30.8$^{c)}$
28	12.0	12.0	12.1	12.0	12.0
30	18.9	18.9	18.9	18.7	18.7
Ac	23.3				
	173.2				

Assignment of the signals having the same superscript in the same vertical column may be interchanged.

Cayaponoside C_4, $C_{29}H_{38}O_{10}$, showed in the positive ion FAB-MS an $[M+Na]^+$ ion at m/z 569 and a fragment ion at m/z 384 ($[M-162]^+$), and the negative ion FAB-MS showed an $[M-H]^-$ ion at m/z 545 and a fragment ion at m/z 383. These MS data indicated that the molecular formula of the aglycone is $C_{23}H_{28}O_5$. The NMR spectra of cayaponoside C_4 are similar to those of cayaponoside A_5; however, the signals of the side chain moiety of A_5 were not observed in the spectra of C_4, and instead, a signal of methylketone was observed. These MS and NMR data suggested that cayaponoside C_4 is 22,23,24,25,26,29-hexanorcucurbitacin glucoside, similar to cayaponoside C_2. The NMR spectra of both cayaponosides were compared, and the structure of cayaponoside C_4 was concluded to be 6-dehydro cayaponoside C_2. This compound seems to be the same as compound **1** isolated by Tomimatsu *et al.* from "cabeça-de-negro" (*Caput nigri*).[4)]

Cayaponosides B_5 ($C_{35}H_{48}O_{11}$) and D_2 ($C_{35}H_{50}O_{12}$) showed NMR spectra similar to those of cayaponosides B and D, respectively. The difference is the presence of a disubstituted double bond conjugated to the aromatic ring in cayaponosides B_5 and D_2. Therefore, B_5 and D_2 were concluded to be 6-dehydro derivatives of cayaponosides B and D, respectively.

Some of cayaponosides shown in this paper were tested for several biological activities. None exhibited any cytotoxic or anti-human immunodeficiency virus (anti-HIV) activities; however, some cayaponosides exhibited significant inhibitory effects on Epstein-Barr virus (EBV) activation induced by the tumor promoter, 12-*O*-tetradecanoylphorbol 13-acetate (TPA). The results will be reported later by the collaborators.

Experimental[5)]

Extraction and Isolation of Cayaponosides The procedures for the isolation of cayaponosides are described in the first paper.[1)]

Cayaponoside A_5: An amorphous powder, mp 149—151 °C, $[\alpha]_D^{26}$ $-135.5°$ (c=0.96, MeOH). Positive ion HR FAB-MS *m*/*z*: 727.3318 ($[M+Na]^+$). $C_{37}H_{52}NaO_{13}$ requires 727.3305. *m*/*z*: 667.3089 ($[M+Na-AcOH]^+$). $C_{35}H_{48}NaO_{11}$ requires 667.3094. Negative ion FAB-MS *m*/*z*: 703 ($[M-H]^-$), 541 ($[M-H-162]^-$), 481 ($[M-H-162-AcOH]^-$), 383. UV λ_{max}^{MeOH} nm (log ε): 205 (4.20), 232 (4.39), 274 (3.87), 283 (sh) (3.84). ^{1}H-NMR δ: aglycone moiety: shown in Table I; sugar moiety: 4.61 (C'_1-H, d, J=7 Hz), 3.4—3.55 ($C'_{2,3,4}$-H), 3.31 (C'_5-H, m), 3.83 (C'_6-Ha, dd, J=4, 12 Hz), 3.95 (C'_6-Hb, dd, J=2, 12 Hz). ^{13}C-NMR δ: aglycone moiety: shown in Table II; sugar moiety: 105.8 (C'_1), 75.6 (C'_2), 78.5 (C'_3), 71.8 (C'_4), 79.0 (C'_5), 63.0 (C'_6).

The chemical shifts of the sugar moieties of the other cayaponosides are almost the same as those of cayaponoside A_5, so a description of their chemical shifts is omitted.

Cayaponoside C_{5b}: An amorphous powder, mp 168—170 °C, $[\alpha]_D^{26}$ $-138.2°$ (c=1.16, MeOH). Positive ion HR FAB-MS *m*/*z*: 685.3195 ($[M+Na]^+$). $C_{35}H_{50}NaO_{12}$ requires 685.320. Negative ion FAB-MS *m*/*z*: 661 ($[M-H]^-$), 499 ($[M-H-162]^-$), 383. UV λ_{max}^{MeOH} nm (log ε): 205 (4.03), 233 (4.36), 274 (3.83), 284 (3.81), 303 (3.45), 315 (3.35). ^{1}H-NMR: aglycone moiety: shown in Table I. ^{13}C-NMR: aglycone moiety: shown in Table II.

Cayaponoside C_4: An amorphous powder, mp 170—173 °C, $[\alpha]_D^{26}$ $-39.3°$ (c=1.20, MeOH). Positive ion HR FAB-MS *m*/*z*: 569.2363 ($[M+Na]^+$). $C_{29}H_{38}NaO_{10}$ requires 569.2362. *m*/*z*: 384.1935 ($[M-C_6H_{10}O_5]^+$). $C_{23}H_{28}O_5$ requires 384.1937. Negative ion FAB-MS *m*/*z*: 545 ($[M-H]^-$), 383 ($[M-H-162]^-$). UV λ_{max}^{MeOH} nm (log ε): 204 (4.08), 233 (4.45), 274 (3.98), 283 (3.92), 303 (3.55), 314 (3.44). ^{1}H-NMR: aglycone moiety: shown in Table I. ^{13}C-NMR: aglycone moiety: shown in Table II.

Cayaponoside B_5: An amorphous powder, mp 168—175 °C, $[\alpha]_D^{26}$ $-90.7°$ (c=0.98, MeOH). Positive ion HR FAB-MS *m*/*z*: 667.3087 ($[M+Na]^+$). $C_{35}H_{48}NaO_{11}$ requires 667.3094. Negative ion FAB-MS *m*/*z*: 643 ($[M-H]^-$), 481 ($[M-H-162]^-$), 383. UV λ_{max}^{MeOH} nm (log ε): 207 (4.28), 231 (4.53), 276 (3.84), 284 (3.82), 303 (3.45), 315 (3.35). ^{1}H-NMR: aglycone moiety: shown in Table I. ^{13}C-NMR: aglycone moiety: shown in Table II.

Cayaponoside D_2: An amorphous powder, mp 172—175 °C, $[\alpha]_D^{26}$ $-106.9°$ (c=1.38, MeOH). Positive ion HR FAB-MS *m*/*z*: 685.3198 ($[M+Na]^+$). $C_{35}H_{50}NaO_{12}$ requires 685.3200. Negative ion FAB-MS *m*/*z*: 661 ($[M-H]^-$), 499 ($[M-H-162]^-$), 383. UV λ_{max}^{MeOH} nm (log ε): 206 (4.11), 233 (4.36), 274 (3.85), 284 (3.82), 303 (3.47), 315 (3.37). ^{1}H-NMR: aglycone moiety: shown in Table I. ^{13}C-NMR: aglycone moiety: shown in Table II.

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References and Notes

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5) The materials and instruments used in this work are the same as those described in the previous paper. NMR spectra were measured in MeOH-d_4 and chemical shifts are expressed in the δ scale using tetramethylsilane as an internal standard.