Studies on the Constituents of the Root of *Cayaponia tayuya* (VELL.) COGN. II.[1] Structures of Cayaponosides, New 29-Nor-2,11-dioxocucurbita-3,5-diene Glucosides[2]

Eiji HIMENO,[a] Tsuneatsu NAGAO,[a] Junko HONDA,[a] Hikaru OKABE,*[,a] Nobuto IRINO,[b] and Tetsuo NAKASUMI[c]

Faculty of Pharmaceutical Sciences, Fukuoka University,[a] 8–19–1, Nanakuma, Jonan-ku, Fukuoka 814–01, Japan, Nippon Mektron Ltd.,[b] 831–2, Kamisohda, Isohara-machi, Kitaibaraki, Ibaraki 319–15, Japan, and Instituto de Pesouisas de Plantas Medicinais do Brazil,[c] Rua Galvao Bueno, 218, Sao Paulo, Brazil.
Received May 30, 1994; accepted July 12, 1994

The bitter constituents in the root of *Cayaponia tayuya* (VELL.) COGN. were investigated, and 24 29-norcucurbitacin glucosides, named cayaponosides, were isolated. Among the 24 29-norcucurbitacin glucosides, named cayaponosides, isolated from the root of *Cayaponia tayuya* (VELL.) COGN., structures of cayaponosides A_1, B_{6a}, B_{6b}, C_3, D_{3a} and D_{3b} were determined based mainly on spectral analyses. They are all 3-*O*-glucosides of 29-nor-2,11-dioxocucurbita-3,5-diene, only different in side chain structure.

Keywords *Cayaponia tayuya*; Cucurbitaceae; cayaponoside; triterpene glucoside; 29-nor-2,11-dioxocucurbita-3,5-diene

In the preceding papers[1] of this series, the isolation of 24 bitter compounds, named cayaponosides, and the structures of cayaponosides which share a common 29-nor-1,2,3,4,5,10-hexadehydrocucurbitacin framework were reported. This paper deals with the structures of cayaponosides A_1, B_{6a}, B_{6b}, C_3, D_{3a} and D_{3b} which show UV absorption maxima at 204, 230 and 297 nm. The isolation of these cayaponosides was described in the previous paper.[2]

Cayaponoside A_1 was isolated as an amorphous powder and it showed in the positive ion FAB-MS an $[M+Na]^+$ ion at *m/z* 729 and fragment ions at *m/z* 669 ($[M+Na-60]^+$). The high resolution (HR) FAB-MS indicated its molecular formula to be $C_{37}H_{54}O_{13}$, the same as that of cayaponoside A reported in the previous paper. The negative ion FAB-MS showed an $[M-H]^-$ ion at *m/z* 705 and fragment ions at *m/z* 543 ($[M-H-162]^-$), 483 ($[M-H-162-60]^-$) and 385, suggesting that it is a monohexoside of an aglycone ($C_{31}H_{44}O_8$) which has an acetyl group.

The ^{1}H-NMR spectrum showed the signals of three methyl groups on the oxygenated quaternary carbons, three methyl groups on the C–C bonded quaternary carbons, one methyl group on an olefinic carbon, an acetoxyl group on a quaternary carbon, one proton on a trisubstituted olefine carbon and a hydroxymethine group in addition to the signals due to a β-D-glucopyranosyl group. The ^{13}C-NMR spectrum showed the presence of two isolated carbonyl carbons, one conjugated carbonyl carbon and one oxygenated olefinic carbon, in addition to the functional groups revealed by the ^{1}H-NMR spectrum. These spectral data indicated that cayaponoside A_1 is a norcucurbitacin glucoside having a similar structure to that of cayaponoside A. The NMR spectra of both cayaponosides were compared. The ^{1}H- and ^{13}C-NMR signals assignable to rings C, D and the side chain moieties were almost superimposable; however, signals due to rings A and B were quite different. Namely, the ^{1}H-NMR spectrum showed that A_1 does not have an isolated phenyl proton; instead, it showed the signals of an olefinic proton adjacent to a methylene group which links to a methine carbon, and of a methylene group which links to a methine carbon and a quaternary carbon. The ^{13}C-NMR spectrum revealed the presence of a conjugated carbonyl group. The combination of these functional groups led to a partial structure with a 3-hydroxy-2-oxo-3,5(6)-diene system. The NMR spectra were examined in detail using the ordinary NMR techniques, correlation spectroscopy *via* long-range

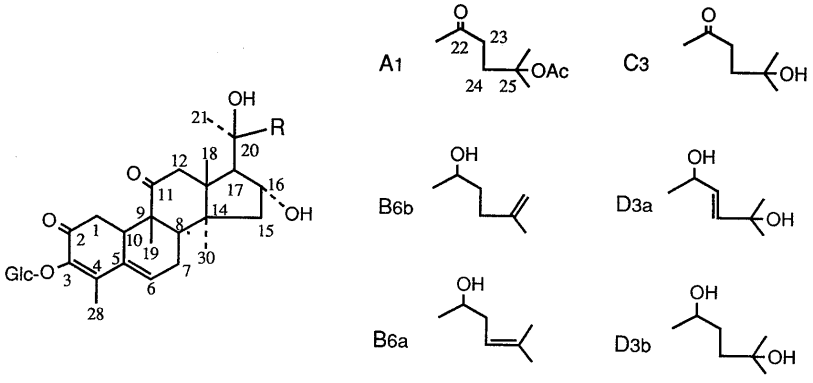

Chart 1

TABLE I. ^{1}H-NMR Chemical Shifts of the Aglycone Moieties of Cayaponosides

	A_1	B_{6a}	B_{6b}	C_3	D_{3a}	D_{3b}
1	2.22 (dd, 4, 15)	2.21 (dd, 5, 15)	2.20 (dd, 5, 14)	2.23 (dd, 5, 14)	2.19 (dd, 5, 14)	2.21 (dd, 5, 15)
	2.38 (dd, 14, 15)	2.38 (t, 15)	2.37 (t, 14)	2.38 (t, 14)	2.38 (t, 14)	2.38 (t, 15)
6	6.48 (m)	6.49 (m)	6.48 (m)	6.48 (m)	6.48 (m)	6.49 (m)
7	2.28 (ddd, 2, 6, 20)	2.30 (m)	2.27 (m)	2.29 (br dd, 8, 22)	2.31 (br dd, 6, 20)	2.31 (br dd, 5, 21)
	ca. 2.6	2.60 (m)	2.60 (m)	2.60 (m)	2.61 (m)	2.62 (m)
8	2.10 (d, 8)	2.12 (d, 8)	2.12 (d, 8)	2.10 (d, 8)	2.11 (d, 8)	2.12 (d, 9)
10	3.02 (br d, 14)	3.02 (br d, 14)	3.02 (br d, 14)	3.02 (br d, 14)	3.00 (br d, 14)	3.10 (br d, 14)
12	2.57 (d, 15)	2.56 (d, 14)	2.56 (d, 14)	2.58 (d, 15)	2.53 (d, 14)	2.56 (d, 14)
	3.27 (d, 15)	3.18 (d, 14)	3.19 (d, 14)	3.27 (d, 15)	3.15 (d, 14)	3.19 (d, 14)
15	*ca.* 1.43	1.55 (d, 13)	1.54 (d, 13)	1.43 (d, 13)	1.54 (d, 14)	1.54 (d, 13)
	1.87 (dd, 9, 13)	1.93 (dd, 9, 13)	1.92 (dd, 9, 13)	1.88 (dd, 9, 13)	1.93 (dd, 10, 14)	1.92 (dd, 9, 13)
16	4.44 (dd, 7, 9)	4.60 (dd, 7, 9)	4.60 (dd, 7, 9)	4.44 (dd, 7, 9)	4.64 (dd, 7, 10)	4.60 (dd, 7, 9)
17	2.53 (d, 7)	2.38 (d, 7)	2.36 (d, 7)	2.55 (d, 7)	2.37 (d, 7)	2.39 (d, 7)
18	0.92 (s)	0.97 (s)	0.97 (s)	0.93 (s)	0.96 (s)	0.97 (s)
19	1.11 (s)	1.11 (s)	1.11 (s)	1.11 (s)	1.11 (s)	1.11 (s)
21	1.37 (s)	1.23 (s)	1.21 (s)	1.37 (s)	1.22 (s)	1.22 (s)
22	—	*ca.* 3.36	*ca.* 3.38	—	3.96 (d, 6)	*ca.* 3.30
23	2.69 (ddd, 6, 10, 18)	2.13 (m)	*ca.* 1.5 (m)	2.72 (ddd, 6, 10, 18)	5.77 (dd, 6, 16)	1.45 (m)
	2.84 (ddd, 6, 10, 18)	2.28 (m)	*ca.* 1.75 (m)	2.85 (ddd, 6, 10, 18)		
24	*ca.* 2.0 (2H, m)	5.25 (t, 7)	2.05 (m)	1.72 (2H, m)	5.85 (d, 16)	1.45 (m)
			ca. 2.77 (m)			1.80 (m)
26	1.44 (s)	1.70 (s)[a]	4.70 (2H, br s)	1.18 (s)	1.27 (s)	1.18 (s)[a]
27	1.44 (s)	1.62 (s)[a]	1.72 (s)	1.18 (s)	1.27 (s)	1.19 (s)[a]
28	2.14 (s)	2.15 (s)	2.15 (s)	2.15 (s)	2.14 (s)	2.15 (s)
30	1.30 (s)	1.31 (s)	1.30 (s)	1.30 (s)	1.28 (s)	1.31 (s)
Ac	1.94 (s)					

a) Assignment of the signals in the same vertical column may be interchanged.

TABLE II. ^{13}C-NMR Chemical Shifts of the Aglycone Moieties of Cayaponosides

	A_1	B_{6a}	B_{6b}	C_3	D_{3a}	D_{3b}
1	40.7	40.7	40.6	40.7	40.6	40.6
2	197.1	197.1	197.1	197.1	197.1	197.1
3	147.1	147.1	147.1	147.1	147.1	147.1
4	134.3	134.4	134.3	134.3	134.3	134.3
5	145.8	145.8	145.9	145.8	145.8	145.8
6	132.6	132.7	132.6	132.6	132.7	132.6
7	26.7	26.8	26.8	26.7	26.8	26.8
8	44.5	44.5	44.5	44.6	44.5	44.5
9	50.8	50.4	50.4	50.6	50.4	50.4
10	37.8	37.8	37.7	37.8	37.8	37.8
11	215.8	216.2	216.2	215.8	216.3	216.2
12	50.6	51.2	51.2	50.8	51.0	51.2
13	52.4	53.0	53.0	52.4	53.1	53.0
14	50.3	50.4	50.4	50.4	50.1	49.9
15	47.6	46.7	46.8	47.7	46.6	46.7
16	72.1	72.9	72.8	72.2	73.0	72.9
17	60.4	57.4	57.5	60.2	57.4	57.5
18	21.4	21.2	21.2	21.4	21.1	21.3
19	20.6	20.6	20.6	20.7	20.5	20.6
20	81.6	77.7	77.7	81.6	77.9	77.9
21	26.4	24.5	24.3	26.4	25.1	24.3
22	217.3	82.9	81.8	218.0	82.7	82.9
23	33.6	32.0	31.3	33.9	127.0	27.8
24	36.6	124.2	37.1	39.0	142.4	43.3
25	83.9	134.3	147.1	71.6	72.0	72.1
26	27.0[a]	26.8[a]	111.4	30.2[a]	30.9[a]	30.0[a]
27	27.1[a]	18.9[a]	23.4	30.0[a]	30.7[a]	30.3[a]
28	14.4	14.4	14.4	14.4	14.4	14.4
30	19.6	19.5	19.5	19.7	19.5	19.5
Ac	23.1					
	173.2					

a) Assignment of the signals in the same vertical column may be interchanged.

coupling (COLOC) and selective insentive nuclei enhanced by polarization transfer (INEPT) techniques (Fig. 1), and the structure of cayaponoside A_1 was concluded to be as shown in Chart 1.

Cayaponoside C_3 ($C_{35}H_{52}O_{12}$) showed almost the same NMR spectra as those of cayaponoside A_1. The only differences were the absence of the signals of an acetyl group and the deviation of the chemical shifts of protons and carbons of the side chain moiety; therefore, cayaponoside C_3 was concluded to be desacetyl cayaponoside A_1.

Cayaponosides B_{6a} and B_{6b} have the same molecular formula, $C_{35}H_{52}O_{11}$. Both showed similar NMR spectra to those of C_3 except that they have one more hydroxyl group instead of one carbonyl group. They showed in the negative FAB-MS a fragment ion at m/z 385 which is originated by the C_{20}–C_{22} bond fission, thus indicating that the frameworks of both cayaponosides are the same as that of cayaponoside C_3, and cayaponosides B_{6a} and B_{6b} have two hydroxyl groups and one double bond in their side chains. The NMR spectra of the side chain moieties of cayaponosides B_{6a} and B_{6b} were almost superimposable on those of cayaponosides B_3 and B_2, respectively, and therefore, structures of cayaponosides B_{6a} and B_{6b} were concluded to be as shown.

The NMR data of cayaponoside D_{3a} ($C_{35}H_{52}O_{12}$) showed that it has the same framework as that of cayaponoside C_3, and the side chain moiety has two hydroxyl groups linked to the quaternary carbons, one hydroxymethine group and a disubstituted double bond. The NMR spectrum of the side chain moiety was the same as that of cayaponoside D. Therefore, the structure of cayaponoside D_{3a} was concluded to be as shown.

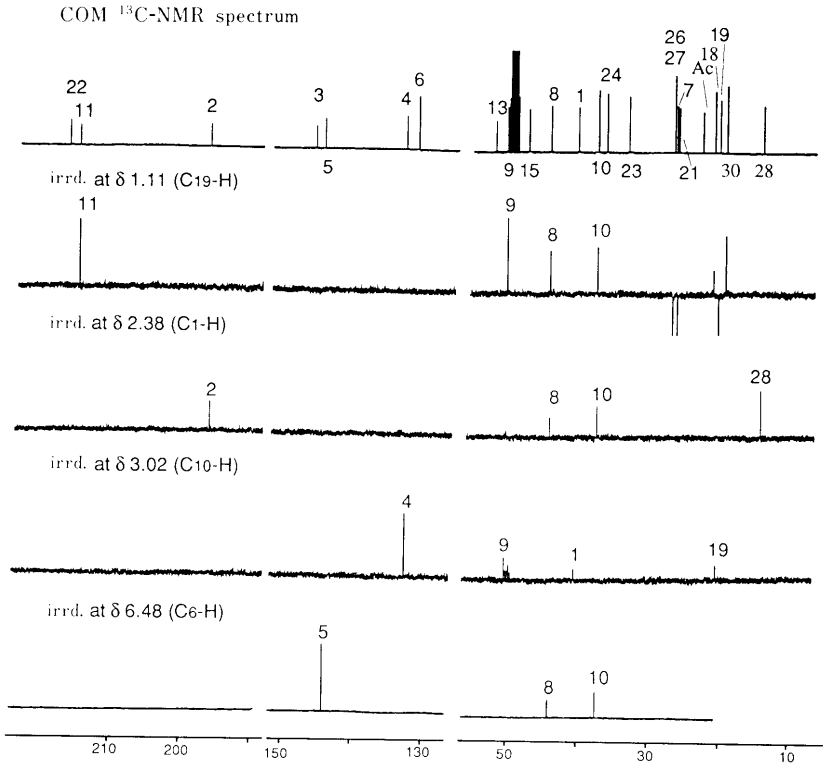

Fig. 1. Selective INEPT ($J=10$ Hz) Spectra of Cayaponoside A_1

Cayaponoside D_{3b} ($C_{35}H_{54}O_{12}$) exhibited almost the same NMR spectra as those of cayaponoside D_{3a}. The only differences were the absence of the signals of a double bond in the side chain and a deviation of the chemical shifts of protons and carbons in the side chain. The NMR spectra were compared with those of cayaponosides C_3 and D_1, and D_{3b} was concluded to be 23,24-dihydrocayaponoside D_{3a}.

Cayaponosides reported in this paper are the 29-norcucurbitacin glucosides having a non-aromatized ring A, first isolated from the plant belonging to Cucurbitaceae.

Some of cayaponosides isolated from this plant were tested for several biological activities. They exhibit no cytotoxic or anti-human immunodeficiency virus (anti-HIV) activity; however, some cayaponosides exhibited significant inhibitory effects on Epstein–Barr virus (EBV) activation induced by the tumor promoter, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA). The results will be reported later by the collaborators.

Experimental[3]

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

Cayaponoside A_1: An amorphous powder, mp 148—155 °C, $[\alpha]_D^{26}$ +19.5° ($c=1.19$, MeOH). Positive ion high resolution (HR) FAB-MS *m/z*: 729.3466 ($[M+Na]^+$). $C_{37}H_{54}NaO_{13}$ requires 729.3463. *m/z*: 669.3254 ($[M+Na-AcOH]^+$). $C_{35}H_{50}NaO_{11}$ requires 669.3251. Negative ion FAB-MS *m/z*: 705 ($[M-H]^-$), 543 ($[M-H-162]^-$), 483 ($[M-H-162-AcOH]^-$), 385. UV λ_{max}^{MeOH} nm (log ε): 206 (4.35), 300 (4.12). ^{1}H-NMR δ: aglycone moiety: shown in Table I; sugar moiety: 4.72 (C'_1-H, d, $J=7$ Hz), 3.31—3.38 ($C'_{2,3,4}$-H), 3.19 (C'_5-H, m), 3.64 (C'_6-Ha, dd, $J=6$, 12 Hz), 3.81 (C'_6-Hb, dd, $J=2$, 12 Hz). ^{13}C-NMR δ: aglycone moiety: shown in Table II; sugar moiety: 104.8 (C'_1), 76.5 (C'_2), 79.0 (C'_3), 72.1 (C'_4), 79.1 (C'_5), 63.5 (C'_6).

The chemical shifts of the sugar moieties of the other cayaponosides are almost the same as those of cayaponoside A_1, so a description of their chemical shifts is omitted.

Cayaponoside C_3: An amorphous powder, mp 163—168 °C. $[\alpha]_D^{25}$ +26.5° ($c=1.35$, MeOH). Positive ion HR FAB-MS *m/z*: 687.3352 ($[M+Na]^+$). $C_{35}H_{52}NaO_{12}$ requires 687.3356. Negative ion FAB-MS *m/z*: 663 ($[M-H]^-$), 501 ($[M-H-162]^-$), 385. UV λ_{max}^{MeOH} nm (log ε): 204 (3.97), 300 (4.06). ^{1}H-NMR: aglycone moiety: shown in Table I. ^{13}C-NMR: aglycone moiety: shown in Table II.

Cayaponoside B_{6a}: An amorphous powder, mp 167—172 °C. $[\alpha]_D^{26}$ +43.9° ($c=1.01$, MeOH). Positive ion HR FAB-MS *m/z*: 649.3594 ($[M+H]^+$). $C_{35}H_{53}O_{11}$ requires 649.3588. *m/z*: 487.3060 ($[M+H-162]^+$). $C_{29}H_{43}O_6$ requires 487.3060. Negative ion FAB-MS *m/z*: 648 ($[M]^-$), 485 ($[M-H-162]^-$). UV λ_{max}^{MeOH} nm (log ε): 204 (4.16), 300 (4.09). ^{1}H-NMR: aglycone moiety: shown in Table I. ^{13}C-NMR: aglycone moiety: shown in Table II.

Cayaponoside B_{6b}: An amorphous powder, mp 165—170 °C, $[\alpha]_D^{26}$ +50.3° ($c=1.16$, MeOH). Positive ion HR FAB-MS *m/z*: 671.3400 ($[M+Na]^+$). $C_{35}H_{52}NaO_{11}$ requires 671.3407. *m/z*: 649.3587 ($[M+H]^+$). $C_{35}H_{53}O_{11}$ requires 649.3588. *m/z*: 487.3048 ($[M+H-C_6H_{10}O_5]^+$). $C_{29}H_{43}O_6$ requires 487.3060. Negative ion FAB-MS *m/z*: 648 ($[M]^-$), 485 ($[M-H-162]^-$), 385. UV λ_{max}^{MeOH} nm (log ε): 204 (4.07), 300 (4.12). ^{1}H-NMR: aglycone moiety: shown in Table I. ^{13}C-NMR: aglycone moiety: shown in Table II.

Cayaponoside D_{3a}: An amorphous powder, mp 170—173 °C, $[\alpha]_D^{26}$ +30.3° ($c=1.25$, MeOH). Positive ion FAB-MS *m/z*: 687.3357 ($[M+Na]^+$). $C_{35}H_{52}NaO_{12}$ requires 687.3357. Negative ion FAB-MS *m/z*: 664 ($[M]^-$), 501 ($[M-H-162]^-$), 385. UV λ_{max}^{MeOH} nm (log ε): 203 (3.93), 300 (4.04). ^{1}H-NMR: aglycone moiety: shown in Table I. ^{13}C-NMR: aglycone moiety: shown in Table II.

Cayaponoside D_{3b}: An amorphous powder, mp 171—175 °C. $[\alpha]_D^{26}$ +36.9° ($c=1.53$, MeOH). Positive ion FAB-MS *m/z*: 689.3529 ($[M+Na]^+$). $C_{35}H_{54}NaO_{12}$ requires 689.3513. *m/z*: 667.3691 ($[M+H]^+$). $C_{35}H_{55}O_{12}$ requires 667.3693. Negative ion FAB-MS *m/z*: 666 ($[M]^-$), 503 ($[M-H-162]^-$), 385. UV λ_{max}^{MeOH} nm (log ε): 205 (3.88), 300 (4.07). ^{1}H-NMR: aglycone moiety: shown in Table I. ^{13}C-NMR: aglycone moiety: shown in Table II.

Acknowledgement The authors are grateful to Ms. Y. Iwase and Mr.

H. Hanazono for their measurement of NMR spectra and MS.

References and Notes

1) Part I: H. Himino, T. Nagao, J. Honda, H. Okabe, N. Irino, T. Nakasumi, *Chem. Pharm. Bull.*, **42**, 2295 (1994).
2) H. Himino, T. Nagao, J. Honda, H. Okabe, N. Irino, T. Nakasumi, *Chem. Pharm. Bull.*, **41**, 986 (1993).
3) 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.