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

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

Faculty of Pharmaceutical Sciences, Fukuoka Univerrsity,[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 them, the structures of cayaponosides A, A_3, A_4, A_6, B, B_2, B_3, B_4, C, C_2, C_{5a}, D and D_1 were determined based mainly on spectral analyses. They are all glucosides of 29-nor-1,2,3,4,5,10-hexadehydrocucurbitacins, different only in side chain structure.

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

Cayaponia tayuya (VELL.) COGN. is a cucurbitaceous vine distributed in South America. The roots are used as laxatives, diuretics and antirheumatics in traditional Brazilian medicine. The root powder tastes bitter and stimulates the nasal mucosa, and the powder foams when vigorously shaken in water. These properties suggested that the root contains saponins and cucurbitacins which are common bitter components in cucurbitaceous plants.

Bauer *et al.* reported the isolation and identification of cucurbitacin R and its glucoside, 23,24-dihydrocucurbitacin B and its glucoside, 23,24-dihydroisocucurbitacin B, and cucurbitacin B and its 2-*O*-glucoside from the $CHCl_3$ extract of the root.[2)]

The authors started to investigate the saponin constituents in the root. However, the MeOH extract obtained after $CHCl_3$ extraction also tastes bitter, and the glycoside fraction showed on TLC several spots which absorb UV. These observations suggested that the glycoside fraction contains some cucurbitacins more polar than those reported by Bauer *et al.* The glycoside fraction was fractionated by column chromatography on silica gel into the less polar fraction (fr. I), which tastes bitter, and the more polar fraction (fr. II), which has a foaming property. Fraction I was further fractionated into four fractions (frs. A—D) by repeated column chromatography on silica gel. Each fraction was again subjected to preparative HPLC on the octadecyl silica (ODS) column using two solvent systems, MeOH–H_2O and acetonitrile–H_2O systems, to give 24 chromatographically homogeneous compounds: from fr. A, cayaponosides A, A_1, A_3, A_4, A_5 and A_6; from fr. B, cayaponosides B, B_2, B_3, B_4, B_5, B_{6a} and B_{6b}; from fr. C, cayaponosides C, C_2, C_3, C_4, C_{5a} and C_{5b}; from fr. D, cayaponosides D, D_1, D_2, D_{3a} and D_{3b}.

These compounds absorb UV, and they were divided into three groups according to their UV absorption patterns. The first group, cayaponosides A, A_3, A_4, A_6, B, B_2, B_3, B_4, C, C_2, C_{5a}, D and D_1, showed absorption maxima at 206, 220 (shoulder) and 280 nm. The second group, cayaponosides A_5, B_5, C_4, C_{5b} and D_2, showed UV absorption maxima at 205, 231, 282 and 315 nm. And the third group, cayaponosides A_1, B_{6a}, B_{6b}, C_3, D_{3a} and D_{3b}, showed absorption maxima at 204, 230 and 297 nm (Fig. 1).

This paper deals with the structures of these cayaponosides which belong to the first group.

Cayaponoside A was obtained as an amorphous powder. It showed an $[M+Na]^+$ ion at *m/z* 729.3466 in the positive ion FAB-MS, which gave the molecular formula $C_{37}H_{54}O_{13}$. The negative ion FAB-MS showed an $[M-H]^-$ ion at *m/z* 705, and fragment ions at *m/z* 543 ($[M-H-162]^-$), *m/z* 483 (543−60) and *m/z* 385. These mass data suggested that cayaponoside A is a monohexoside of an aglycone ($C_{31}H_{44}O_8$), and that the aglycone has an acetyl group in the molecule. The UV absorption pattern of cayaponoside A indicated the presence of a substituted phenyl group in the aglycone.

Its ^{1}H-NMR spectrum showed the signals of seven tertiary methyl groups (δ 0.95, 0.99, 1.31, 1.35, 1.42 (6H) and 2.09), an acetyl methyl group (δ 1.92 s), one phenyl

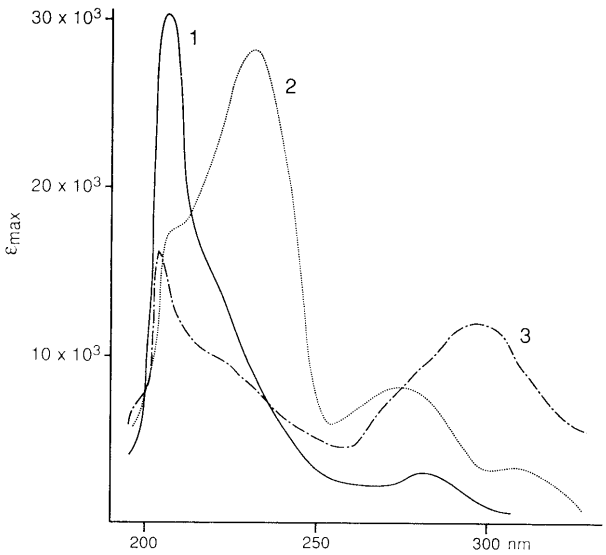

Fig. 1. UV Spectra of Cayaponosides A_3 (1), A_4 (2) and A_1 (3)

proton (δ 6.67 s), an anomeric proton of the sugar moiety (δ 4.57, d, $J=7$ Hz) and a hydroxymethine proton (δ 4.43, br dd, $J=7$, 9 Hz), in addition to the hexosyl oxymethine protons.

The ^{13}C-NMR spectrum showed 37 carbon signals, and among them the signals of the sugar carbons, methyl groups (δ 12.3, 20.9, 21.2, 26.3, 27.0, 27.1 and 30.0), an acetyl group (δ 23.1 and 173.2), the C–C bonded quaternary carbons (δ 50.6, 52.1 and 53.0), the oxygenated quaternary carbons (δ 81.7 and 83.9), carbonyl carbons (δ 217.5 and 217.9), and phenyl carbons (δ 114.3, 125.7, 130.8, 132.2, 145.5 and 145.8) were identified from the chemical shifts and data obtained from distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{13}C correlation spectroscopy (COSY) and selective insentive nuclei enhanced by polarization transfer (INEPT) spectra. The chemical shifts of the sugar carbons indicated that the sugar moiety is D-glucopyranose linked in the β-configuration, and the chemical shifts of two phenyl carbons (δ 145.5 and 145.8) suggested that these are phenolic carbons.

Cayaponoside A was suggested to be a β-D-glucopyranoside of a tetracyclic nortriterpene which has a pentasubstituted aromatic ring, two carbonyl groups, one acetyl group linked to a quaternary carbon, one hydroxyl group linked to a quaternary carbon, one hydroxyl group linked to a methine carbon and two phenolic hydroxyl groups. From these data, cayaponoside A was considered to be a 29-norcucurbitacin glucoside having a structure similar to fevicordin A glucoside, which was isolated from the seeds of *Fevillea cordifolia* (Cucurbitaceae) by Achenbach *et al.*[3a)]

^{1}H-NMR and ^{13}C-NMR spectra of cayaponoside A were compared with the reported data for fevicordin A glucoside. The only difference between the two compounds was the absence of the signals of protons and carbons of a disubstituted olefine group in the spectra of cayaponoside A; therefore, cayaponoside A was concluded to be 23,24-dihydrofevicordin A glucoside. The negative ion FAB-MS signal at m/z 385 is considered to be originated from the aglycone by fission of the bond between C_{20} and C_{22}.

Cayaponosides A_6, C and C_{5a} also have two carbonyl groups and showed in the negative ion FAB-MS a fragment ion at m/z 385, and their NMR spectra were similar to those of cayaponoside A. These data indicated that all have the same tetracyclic framework as that of cayaponoside A, but differ in the structures of the side chain moieties.

Cayaponoside C ($C_{35}H_{52}O_{12}$) showed the signals of two hydroxylated quaternary carbons in the side chain, and no signal of an acetyl group was observed. The overall features of the ^{1}H-NMR and ^{13}C-NMR spectra were almost the same as those of cayaponoside A, although some deviations were observed in the chemical shifts of C_{24}-protons and C_{24}—C_{27} carbons. From these spectral data, cayaponoside C was concluded to be desacetyl cayaponoside A.

Cayaponoside C_{5a} ($C_{35}H_{50}O_{12}$) showed similar spectra to those of cayaponoside C; however, it depicted the signals of a disubstituted olefine group located between a carbonyl carbon and hydroxylated quaternary carbon in the side chain, indicating that cayaponoside C_{5a} is 23,24-didehydrocayaponoside C, which is desacetylfevicordin A glucoside.

Cayaponoside A_6 ($C_{35}H_{50}O_{11}$) showed the signals of one methyl group (^{1}H-NMR: δ 1.27 s; ^{13}C-NMR: δ 23.5) and an exomethylene group (^{1}H-NMR: δ 4.68; ^{13}C-NMR:

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

No.	Cayaponoside						
	A	C	C_{5a}	A_6	D	A_3	B_4
1	6.67 (s)	6.67 (s)	6.67 (s)	6.67 (s)	6.63 (s)	6.66 (s)	6.63 (s)
6	*ca.* 2.65	*ca.* 2.68 (m)	*ca.* 2.66 (m)	*ca.* 2.65 (m)	*ca.* 2.65 (m)	*ca.* 2.65 (m)	*ca.* 2.65 (m)
	ca. 2.85 (m)	*ca.* 2.85 (m)	*ca.* 2.85 (m)	*ca.* 2.83 (m)	*ca.* 2.85 (m)	*ca.* 2.80 (m)	*ca.* 2.85 (m)
7	*ca.* 1.95 (m)	*ca.* 1.94 (m)	*ca.* 1.95 (m)	*ca.* 1.95 (m)	*ca.* 1.96 (m)	*ca.* 1.95 (m)	*ca.* 1.98 (m)
	ca. 2.25 (m)	*ca.* 2.25 (m)	*ca.* 2.25 (m)	*ca.* 2.75 (m)	*ca.* 2.27 (m)	*ca.* 2.25 (m)	*ca.* 2.25 (m)
8	2.13 (br d, 7)	2.13 (br d, 7)	2.13 (br d, 7)	2.13 (br d, 7)	2.15 (br d, 7)	2.15 (br d, 7)	2.15 (d, 7)
12	2.63 (d, 14)	2.65 (d, 14)	2.65 (d, 14)	2.64 (d, 14)	2.59 (d, 14)	2.62 (d, 14)	2.60 (d, 14)
	2.91 (d, 14)	2.96 (d, 14)	2.90 (d, 14)	2.91 (d, 14)	2.78 (d, 14)	2.78 (d, 14)	2.75 (d, 14)
15	1.50 (d, 13)	1.48 (d, 14)	1.49 (br d, 13)	1.48 (d, 13)	1.59 (d, 14)	1.60 (d, 13)	1.60 (d, 13)
	ca. 1.97 (m)	1.94 (dd, 9, 14)	1.95 (m)	1.94 (dd, 9, 13)	1.99 (dd, 10, 14)	1.95 (m)	1.99 (dd, 8, 13)
16	4.43 (br dd, 7, 9)	4.42 (t-like, 7)	4.46 (t, 8)	4.42 (t-like, 7)	4.62 (t-like, 7)	4.57 (t-like, 7)	4.63 (t-like, 8)
17	2.48 (d, 7)	2.51 (d, 7)	2.54 (d, 7)	2.50 (d, 7)	2.34 (d, 7)	2.31 (d, 7)	2.33 (d, 7)
18	0.95 (s)	0.96 (s)	0.95 (s)	0.96 (s)	1.00 (s)	0.96 (s)	1.00 (s)
19	1.31 (s)	1.31 (s)	1.31 (s)	1.31 (s)	1.30 (s)	1.30 (s)	1.30 (s)
21	1.35 (s)	1.36 (s)	1.37 (s)	1.35 (s)	1.21 (s)	1.21 (s)	1.22 (s)
22	—	—	—	—	3.95 (br d, 5)	4.05 (t, 7)	4.01 (d, 5)
23	*ca.* 2.70 (m)	*ca.* 2.70 (m)	6.79 (d, 16)	*ca.* 2.75 (m)	5.74 (dd, 5, 16)	1.90 (m, 2H)	5.76 (dd, 5, 16)
	ca. 2.80 (m)	*ca.* 2.80 (m)		*ca.* 2.85 (m)			
24	*ca.* 1.95 (m, 2H)	*ca.* 1.70 (m, 2H)	6.946 (d, 16)	*ca.* 2.22 (m, 2H)	5.83 (dd, 1, 16)	*ca.* 1.70 (m, 2H)	5.68 (d, 16)
26	1.42 (s)	1.17 (s)	1.30 (s)	4.68 (br d, 5, 2H)	1.25 (s)	1.23 (s)	1.24 (s)
27	1.42 (s)	1.17 (s)	1.30 (s)	1.27 (s)	1.25 (s)	1.23 (s)	1.24 (s)
28	2.09 (s)	2.08 (s)	2.09 (s)	2.09 (s)	2.09 (s)	2.09 (s)	2.09 (s)
30	0.99	1.00 (s)	1.01 (s)	0.99 (s)	0.98 (s)	1.01 (s)	0.96 (s)
	1.92 (Ac)					0.84 (t, 7) } (OBu)	3.13 (s) (OMe)
						ca. 1.4 (m, 2H) } (OBu)	
						ca. 3.28 (m) } (OBu)	

TABLE II. ^{1}H-NMR Chemical Shifts of Cayaponosides

No.	Cayaponoside						
	D_1	B_2	B_3	B	A_4	C_2	Fevi.[a]
1	6.65 (s)	6.65 (s)	6.65 (s)	6.64 (s)	6.66 (s)	6.66 (s)	6.66 (s)
6	2.65 (ddd, 1, 9, 18)	*ca.* 2.65 (m)	*ca.* 2.65 (m)	*ca.* 2.66 (m)	*ca.* 2.65 (m)	2.66 (ddd, 1, 10, 18)	2.64 (br dd, 9, 18)
	2.85 (dd, 9, 18)	*ca.* 2.85 (m)	*ca.* 2.86 (m)	*ca.* 2.86 (m)	*ca.* 2.8 (m)	2.85 (dd, 10, 18)	*ca.* 2.84 (ddd, 9, 9, 18)
7	*ca.* 1.98 (m)	*ca.* 1.95 (m)	*ca.* 1.95 (m)	*ca.* 1.95 (m)	*ca.* 1.95 (m)	*ca.* 1.96 (m)	2.26 (dddd, 7, 9, 9, 1)
	ca. 2.26 (m)	*ca.* 2.25 (m)	*ca.* 2.25 (m)	*ca.* 2.25 (m)	*ca.* 2.25 (m)	*ca.* 2.28 (m)	♯
8	2.16 (br d, 7)	2.15 (d, 7)	2.15 (d, 8)	2.15 (br d, 7)	2.15 (d, 7)	2.13 (br d, 7)	2.12 (br d, 7)
12	2.62 (d, 14)	2.21 (d, 14)	2.62 (d, 14)	2.60 (d, 14)	2.62 (d, 14)	2.48 (d, 14)	2.62 (d, 15)
	2.82 (d, 14)	2.62 (d, 14)	2.81 (d, 14)	2.76 (d, 14)	2.78 (d, 14)	2.98 (d, 14)	2.88 (br d, 15)
15	1.59 (d, 13)	1.59 (d, 14)	1.60 (d, 14)	1.60 (d, 14)	1.60 (d, 13)	1.59 (dd, 1, 13)	♯
	1.97 (m)	1.98 (m)	1.98 (m)	1.95 (m)	2.05 (br dd, 9, 13)	1.99 (dd, 8, 13)	1.49 (br d, 14)
16	4.57 (br t, 7)	4.57 (t, 8)	4.58 (t, 8)	4.63 (t-like, 8)	4.57 (br t, 7)	4.83 (ddd, 1, 7, 9)	4.53 (br dd, 7, 8)
17	2.36 (d, 7)	2.34 (d, 7)	2.36 (d, 7)	2.38 (d, 6)	2.31 (d, 7)	3.14 (d, 7)	2.49 (d, 7)
18	1.00 (s)	1.00 (s)	1.00 (s)	1.00 (s)	0.96 (s)	0.69 (s)	0.91 (s)
19	1.30 (s)	1.30 (s)	1.31 (s)	1.30 (s)	1.30 (s)	1.31 (s)	1.30 (s)
21	1.21 (s)	1.19 (s)	1.21 (s)	1.19 (s)	1.21 (s)	2.14 (s)	1.36 (s)
22	*ca.* 3.30	*ca.* 3.32	3.38 (dd, 2, 10)	4.08 (dd, 1, 6)	4.05 (t, 7)	—	—
23	*ca.* 1.40 (m)	*ca.* 2.05 (m)	*ca.* 2.08 (m)	5.79 (dd, 6, 16)	1.90 (s, 2H)	—	6.80 (d, 16)
	ca. 1.60 (m)	*ca.* 2.25 (m)	*ca.* 2.25 (m)				
24	*ca.* 1.40, 1.80	*ca.* 1.48, 1.70	6.36 (br t, 7)	6.36 (dd, 1, 16)	*ca.* 1.7 (m, 2H)	—	6.98 (d, 16)
26	1.17 (s)[b]	4.69 (br s, 2H)	1.60 (s)[b]	4.94 (s, 2H)	1.23 (s)	—	1.53 (s)
27	1.18 (s)[b]	1.71 (s)	1.68 (s)[b]	1.82 (s)	1.23 (s)	—	1.53 (s)
28	2.09 (s)	2.09 (s)	2.09 (s)	2.09 (s)	2.09 (s)	2.09 (s)	2.08 (s)
30	1.00 (s)	0.99 (s)	1.00 (s)	0.98 (s)	1.01 (s)	1.02 (s)	0.99 (s)
							1.98 (s) (AC)

a) Data for fevicordin A glucoside reported by Achenbach *et al.*[3a] *b*) Assignment of the signals in the same vertical column may be interchanged. ♯: overlapped signal at about 1.95 ppm.

TABLE III. ^{13}C-NMR Chemical Shifts of Cayaponosides

No.	Cayaponoside													
	A	C	C_{5a}	A_6	D	A_3	B_4	D_1	B_2	B_3	B	A_4	C_2	Fev.[a]
1	114.3	114.4	114.3	114.4	114.3	114.2	114.3	114.3	114.3	114.4	114.3	114.4	114.4	113.3
2	145.5	145.4	145.5	145.5	145.4	145.5	145.5	145.5	145.5	145.5	145.5	145.5	145.5	144.7
3	145.8	145.8	145.8	145.8	145.7	145.8	145.8	145.8	145.8	145.8	145.8	145.8	145.9	144.8
4	125.7	125.7	125.7	125.7	125.7	125.7	125.7	125.7	125.7	125.7	125.7	125.7	125.8	124.8
5	132.2	132.2	132.2	132.2	132.2	132.2	132.2	132.2	132.2	132.2	132.2	132.3	132.1	131.3
6	25.6	25.6	25.6	25.6	25.6	25.6	23.6	25.6	25.6	25.6	25.6	25.7	25.5	24.8
7	21.1	21.1	21.1	21.1	21.0	21.0	21.0	21.1	21.1	21.1	21.1	21.1	21.0	20.3
8	44.9	44.9	44.9	44.9	44.8	44.8	44.8	44.8	44.8	44.8	44.8	44.9	45.1	44.1
9	53.0	53.0	53.0	53.0	52.8	52.8	52.8	52.8	52.8	52.7	52.8	52.8	53.3	52.1
10	130.8	130.8	130.8	130.8	130.8	130.7	130.8	130.8	130.8	130.8	130.8	130.8	130.6	130.0
11	217.9	218.1	217.9	217.3	218.2	218.0	218.0	218.2	218.2	218.2	218.1	218.1	216.1	217.2
12	52.8	52.8	52.7	52.8	52.9	52.9	52.9	53.2	53.2	53.1	53.0	52.1	51.0	51.8
13	52.1	52.1	52.2	52.1	52.8	52.8	52.8	52.6	52.6	52.8	52.7	52.7	51.8	51.3
14	50.6	50.6	50.6	50.6	50.6	50.6	50.6	50.7	50.7	50.6	50.6	50.8	51.2	49.7
15	47.2	47.2	47.2	47.3	46.2	46.2	46.3	46.4	46.5	46.4	46.4	46.2	46.7	46.3
16	72.3	72.3	72.6	72.3	73.0	73.0	73.1	72.9	72.9	72.9	73.0	73.8	73.2	71.8
17	60.6	60.3	60.6	60.1	57.4	57.6	57.6	57.5	57.3	57.4	57.8	60.3	69.0	60.4
18	21.2	21.2	21.4	21.2	21.0	20.9	21.0	21.0	21.0	20.8	21.1	21.2	20.9	20.7
19	30.0	30.0	30.0	30.0	29.9	29.9	29.9	30.0	30.0	30.0	29.9	30.0	29.9	29.2
20	81.7	81.7	80.8	81.6	77.9	77.8	77.8	78.0	77.8	77.8	78.0	77.3	211.6	80.3
21	26.3	26.2	26.1	26.2	24.8	24.9	24.6	23.8	23.8	21.0	24.3	22.2	32.5	25.5
22	217.5	217.8	205.9	217.9	82.6	82.5	82.1	82.7	81.5	82.6	82.2	85.9	—	205.4
23	33.6	33.9	122.1	37.3	127.0	130.1	130.8	27.7	37.1	31.9	130.4	27.9	—	122.6
24	36.6	38.9	156.2	33.4	142.3	139.9	139.0	43.3	31.1	124.3	136.5	40.0	—	151.6
25	83.9	71.6	72.3	147.0	72.0	76.7	77.2	72.2	147.8	134.3	143.8	83.5	—	81.0
26	27.0[b]	30.2[b]	30.0	111.3	30.8[b]	28.0[b]	27.3[b]	30.2[b]	111.4	26.8[b]	117.5	28.5[b]	—	26.6[b]
27	27.1[b]	30.0[b]	30.0	23.5	30.9[b]	27.4[b]	26.9[b]	29.9[b]	23.4	18.9[b]	19.6	29.6[b]	—	26.7[b]
28	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	11.5
30	20.9	20.9	20.9	20.9	20.7	20.8	20.7	20.7	20.7	18.9	20.7	20.8	20.8	20.1
	23.1 } Ac					15.1 } OBc	51.6 (OMe)						21.9 } Ac	
	173.2 }					34.6							171.9	
						34.6								
						64.4								

a) Data for fevicordin A glucoside reported by Achenbach *et al.*[3a] *b*) Assignment of the signals in the same vertical column may be interchanged.

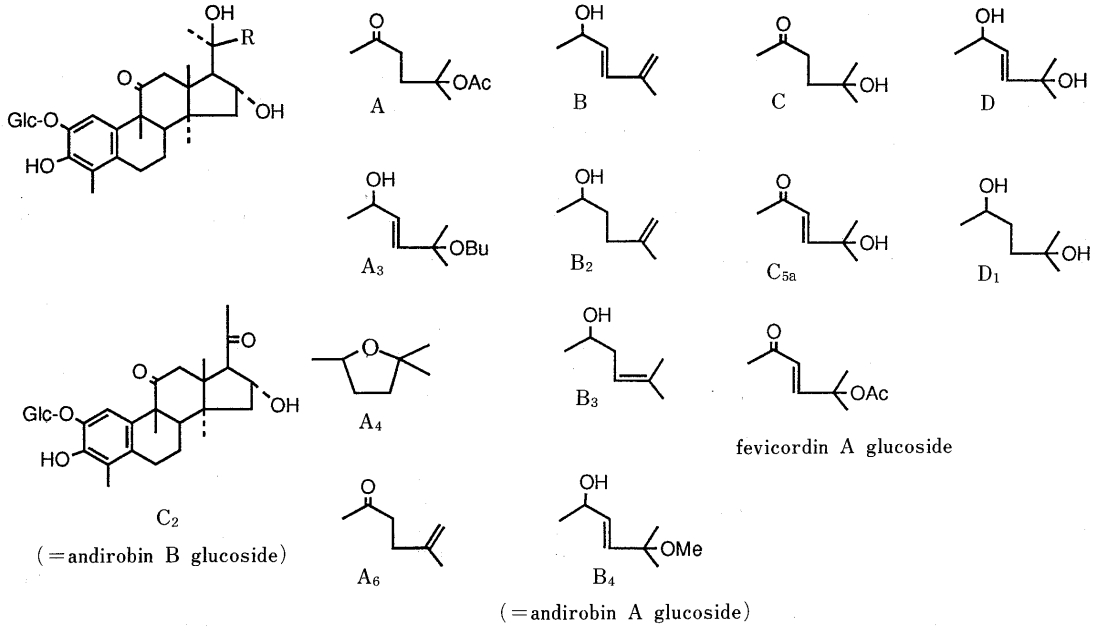

Chart 1

δ 111.3 and 147.0) instead of the signals of two methyl groups (^{1}H-NMR: δ 1.42 s (6H); ^{13}C-NMR: δ 27.0 and 27.1) on C_{25} of cayaponoside A. These functional groups should be placed on the side chain, and therefore, cayaponoside A_6 was concluded to be the 25-ene derivative which was formed by the elimination of acetic acid from cayaponoside A.

Cayaponosides A_3, A_4, B, B_2, B_3, B_4, D and D_1 showed a fragment ion at *m/z* 385 in the negative ion FAB-MS, and all showed almost the same NMR spectral signals corresponding to those of the tetracyclic framework of cayaponoside A, indicating that they have the same framework as that of cayaponoside A and differ in the structures of the side chains. All showed signals of one carbonyl carbon, indicating the absence of a carbonyl group in the side chain.

Cayaponoside D ($C_{35}H_{52}O_{12}$), the major cayaponoside, showed the signals of a disubstituted olefine group (^{1}H-NMR: δ 5.74 dd, J=5, 16 Hz; δ 5.83 dd, J=1, 16 Hz; ^{13}C-NMR: δ 127.0 and 142.3) placed between a hydroxymethine group (^{1}H-NMR: δ 3.95 br d, J=5 Hz; ^{13}C-NMR: δ 82.6) and a hydroxylated quaternary carbon (^{13}C-NMR: δ 72.0) in the side chain, and therefore, the structure was concluded to be as shown in the chart.

Cayaponosides A_3 ($C_{39}H_{60}O_{12}$) and B_4 ($C_{36}H_{54}O_{12}$) showed almost the same NMR spectra as those of cayaponoside D. The differences are the presence of the signals of a butoxyl group in A_3 and a methoxyl group in B_4, and some deviations of the chemical shifts of C_{24}-protons and C_{24}—C_{27} carbons. From these observations, cayaponosides A_3 and B_4 were concluded to be cayaponoside D 25-butyl ether and methyl ether, respectively.

Cayaponoside D_1 ($C_{35}H_{54}O_{12}$) has two more hydrogen atoms than cayaponoside D, and the NMR spectra showed the signals of two adjacent methylene groups located between a hydroxymethine group and a hydroxylated quaternary carbon. Thus, cayaponoside D_1 is 23,24-dihydrocayaponoside D.

Cayaponosides B_2 and B_3 have the same molecular formula $C_{35}H_{52}O_{11}$, H_2O less than cayaponoside D_1. They have a hydroxyl group and a double bond in their side chains. Cayaponoside B_2 has an exomethylene group, and B_3 has a trisubstituted double bond. Thus, their structures were concluded to be as shown in the chart. Both are dehydration products derived from cayaponoside D_1.

Cayaponoside B has the molecular formula $C_{35}H_{50}O_{11}$, 2H less than cayaponoside D_1. It showed the signals of a disubstituted olefine conjugated to an exomethylene group. Thus, the structure of cayaponoside B was deduced to be 23,24-didehydrocayaponoside B_2.

Cayaponoside A_4 has the molecular formula $C_{35}H_{52}O_{11}$. Negative ion FAB-MS showed a fragment ion at *m/z* 385, indicating that this compound also has the same tetracyclic framework as those of other cayaponosides. The unsaturation of the molecular formula indicated that the side chain has a ring structure. The side chain has one oxygen atom, however the ^{13}C-NMR spectra of the side chain moiety showed two oxygenated carbon signals (δ 83.5 and 85.9). The ^{1}H-NMR spectrum showed the signals of two adjacent methylene groups linked to an oxymethine carbon (δ 85.9) and an oxygenated quaternary carbon (δ 83.5). From these spectral data, the structure of cayaponoside A_4 was concluded to be as shown in the chart.

Cayaponoside C_2, the last one of the first group showed in the positive ion FAB-MS an $[M+Na]^+$ ion at *m/z* 571 and a fragment ion at *m/z* 386 ($[M-162]^+$). From the high-resolution FAB-MS data, the molecular formulae of cayaponoside C_2 and its aglycone were determined to be $C_{29}H_{40}O_{10}$ and $C_{23}H_{30}O_5$, respectively. The NMR showed spectra similar to those of other cayaponosides; however, the signals of the side chain moiety were not

observed, and instead, the signals of one acetyl group were depicted in the spectra. These data indicated that cayaponoside C_2 is a corresponding 22,23,24,25,26,27,29-heptanorcucurbitacin glucoside, and the structure was concluded to be as shown in the chart.

The absolute configuration of C_{22}, having a hydroxyl group or an ether oxygen atom, remains undetermined.

For several years, the cucurbitacins in South American cucurbitaceous plants have been extensively investigated by several groups, and some 29-norcucurbitacin glycosides having an aromatized A ring were reported.[3] Among these cayaponosides, A, C and C_{5a} seem to be identical, respectively, with fevicordin B glucoside, fevicordin D glucoside and fevicordin C glucoside, which have been isolated from the seed of *Fevillea cordifolia* L.,[3a] and B_4, C_2 with andirobin A glucoside and andirobin B glucoside, respectively, which were isolated from the seed of *Fevillea trilobata* L.[3b] Cayaponosides B and D were also reported to be isolated from the last plant.

Experimental[4]

Extraction and Isolation of Cayaponosides The powder (1.7 kg) of the dried root of *Cayaponia tayuya* sent from Brazil was percolated first with $CHCl_3$ (10 l). The residue was successively percolated with MeOH (20 l) and then with 50% MeOH (20 l). Both solutions were combined and the MeOH was evaporated. The remaining aqueous solution was passed through a column of polystyrene resin Diaion HP-20 (3 l). The column was washed with water and then eluted with MeOH (5 l). The MeOH eluate was evaporated to dryness to give a glycoside fraction. This fraction was repeatedly chromatographed on silica gel (10—200 times the weights of the materials) using AcOEt–MeOH–H_2O (12 : 1 : 0.3) to give the less polar fraction (fr. I) (50.7 g) and the more polar fraction (fr. II) (7.8 g). Fraction I was repeatedly chromatographed on silica gel and fractionated into four fractions (fr.-IA; 3.59 g, fr.-IB; 4.28 g, fr-IC; 7.67 g and fr. ID; 30.12 g). Fraction IA was subjected to preparative high-resolution liquid chromatography (HPLC) on an ODS column using 35% acetonitrile and 66% MeOH as solvents to give cayaponosides A_1 (502 mg), A (1.758 g), A_3 (288 mg), A_4 (121 mg), A_5 (86 mg) and A_6 (138 mg). Fraction IB was subjected to preparative HPLC using 32% acetonitrile and 62.5% MeOH to give cayaponosides B (2.028 g), B_2 (287 mg), B_3 (241 mg), B_4 (546 mg), B_5 (81 mg), B_{6a} (40 mg) and B_{6b} (75 mg). From fr. IC, cayaponosides C (3.395 g), C_2 (1.230 g), C_3 (641 mg), C_4 (105 mg), C_{5a} (88 mg) and C_{5b} (150 mg) were isolated by using 30% acetonitrile and 62.5% MeOH in preparative HPLC. Fraction ID (10 g) was chromatographed in the same manner using 21—25% acetonitrile and 55% MeOH to give cayaponoside D (6.01 g), D_1 (512 mg), D_2 (370 mg) D_{3a} (42 mg) and D_{3b} (122 mg).

Cayaponoside A: An amorphous powder, mp 142—146 °C, $[\alpha]_D^{26}$ −26° (c=1.30, 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:669.3261 ($[M+Na-AcOH]^+$). $C_{35}H_{50}NaO_{11}$ requires 669.3251. Negative ion FAB-MS m/z: 705 ($[M-H]^-$), 543 ($[M-H-162]^-$), 483 ($[M-H-162-AcOH]^-$), 385. $UV\lambda_{max}^{MeOH}$ nm (log ε): 205 (4.40), 220 (shoulder, 3.91), 284 (3.28). ^{1}H-NMR δ: aglycone moiety: shown in the Table I, sugar moiety: 4.57 (C'_1-H, d, J=7 Hz), 3.45—3.49 (C'_2-H, C'_3-H, C'_4-H), 3.31 (C'_5-H, m), 3.84 (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 III; sugar moiety: 106.1 (C'_1), 75.6 (C'_2), 78.6 (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 were almost the same as those of cayaponoside A, so a description of their chemical shifts is omitted.

Cayaponoside C: An amorphous powder, mp 160—165 °C, $[\alpha]_D^{24}$ −28.1° (c=1.40, MeOH). Positive ion HR FAB-MS m/z: 687.3374 ($[M+Na]^+$).$C_{35}H_{52}NaO_{12}$ requires 687.3356. Negative ion FAB-MS m/z: 663 ($[M-H]^-$), 501 ($[M-H-162]^-$), 385. $UV\lambda_{max}^{MeOH}$ nm (log ε): 208 (4.41), 220 (shoulder, 4.00), 283 (3.31). ^{1}H- and ^{13}C-NMR data are shown in Tables I and III.

Cayaponoisde C_{5a}: An amorphous powder, mp 173—175 °C, $[\alpha]_D^{26}$ −40.4° (c=1.08, MeOH). Positive ion HR FAB-MS m/z: 685.3209 ($[M+Na]^+$). $C_{35}H_{50}NaO_{12}$ requires 685.3213. Negative ion FAB-MS m/z: 661 ($[M-H]^-$), 499 ($[M-H-162]^-$), 385. $UV\lambda_{max}^{MeOH}$ nm (log ε): 206 (4.46), 223 (shoulder, 4.26), 280 (3.41). ^{1}H- and ^{13}C-NMR data are shown in Tables I and III.

Cayaponoside A_6: An amorphous powder, mp 150—154 °C, $[\alpha]_D^{26}$ −27.2° (c=0.95, MeOH). Positive ion HR FAB-MS m/z: 669.3248 ($[M+Na]^+$). $C_{35}H_{50}NaO_{11}$ requires 669.3251. Negative ion FAB-MS m/z: 645 ($[M-H]^-$), 483 ($[M-H-162]^-$), 385. $UV\lambda_{max}^{MeOH}$ nm (log ε): 206 (4.48), 220 (shoulder, 4.12), 281 (3.36). ^{1}H- and ^{13}C-NMR data are shown in Tables I and III.

Cayaponoside D: An amorphous powder, mp 170—173 °C, $[\alpha]_D^{26}$ −7.6° (c=2.23, MeOH). Positive ion HR FAB-MS m/z: 687.3374 ($[M+Na]^+$). $C_{35}H_{52}NaO_{12}$ requires 687.3356. Negative ion FAB-MS m/z: 663 ($[M-H]^-$), 501 ($[M-H-162]^-$), 385. $UV\lambda_{max}^{MeOH}$ nm (log ε): 207 (4.43), 220 (shoulder, 4.06), 282 (3.31). ^{1}H- and ^{13}C-NMR data are shown in Tables I and III.

Cayaponoside A_3: Colorless needles, mp 138—140 °C, $[\alpha]_D^{26}$ −8.9° (c=0.74, MeOH). Positive ion HR FAB-MS m/z: 743.3983 ($[M+Na]^+$). $C_{39}H_{60}NaO_{12}$ requires 743.3983. Negative ion FAB-MS m/z: 719 ($[M-H]^-$), 557 ($[M-H-162]^-$), 385. $UV\lambda_{max}^{MeOH}$ nm (log ε): 205 (4.54), 220 (shoulder, 4.14), 281 (3.34). ^{1}H- and ^{13}C-NMR data are shown in Tables I and III.

Cayaponoside B_4: An amorphous powder, mp 160—164 °C, $[\alpha]_D^{26}$ −2.6° (c=1.00, MeOH). Positive ion HR FAB-MS m/z: 701.3530 ($[M+Na]^+$). $C_{36}H_{54}NaO_{12}$ requires 701.3513. Negative ion FAB-MS m/z: 677 ($[M-H]^-$), 515 ($[M-H-162]^-$), 385. $UV\lambda_{max}^{MeOH}$ nm (log ε): 207 (4.42), 220 (shoulder, 4.11), 282 (3.37). ^{1}H- and ^{13}C-NMR data are shown in Tables I and III.

Cayaponoside D_1: An amorphous powder, mp 169—173 °C, $[\alpha]_D^{27}$ −2.7° (c=1.21, MeOH). Positive ion HR FAB-MS m/z: 689.3516 ($[M+Na]^+$). $C_{35}H_{54}NaO_{12}$ requires 689.3513. Negative ion FAB-MS m/z: 665 ($[M-H]^-$), 503 ($[M-H-162]^-$), 385. $UV\lambda_{max}^{MeOH}$ nm (log ε): 206 (4.43), 220 (shoulder, 3.99), 280 (3.20). ^{1}H- and ^{13}C-NMR data are shown in Tables II and III.

Cayaponoside B_2: A crystalline powder, mp 175—178 °C, $[\alpha]_D^{26}$ +1.0° (c=1.00, MeOH). Positive ion HR FAB-MS m/z: 671.3416 ($[M+Na]^+$). $C_{35}H_{52}NaO_{11}$ requires 671.3407. Negative ion FAB-MS m/z: 647 ($[M-H]^-$), 485 ($[M-H-162]^-$), 385. $UV\lambda_{max}^{MeOH}$ nm (log ε): 206 (4.48), 220 (shoulder, 4.12), 283 (3.33). ^{1}H- and ^{13}C-NMR data are shown in Tables II and III.

Cayaponoside **B_3**: An amorphous powder, mp 158—163 °C, $[\alpha]_D^{26}$ −3.6° (c=1.04, MeOH). Positive ion HR FAB-MS m/z: 671.3412 ($[M+Na]^+$). $C_{35}H_{55}NaO_{11}$ requires 671.3407. Negative ion FAB-MS m/z: 647 ($[M-H]^-$), 485 ($[M-H-162]^-$), 385. $UV\lambda_{max}^{MeOH}$ nm (log ε): 207 (4.44), 220 (shoulder, 4.11), 281 (3.40). ^{1}H- and ^{13}C-NMR data are shown in Tables II and III.

Cayaponoside B: Fine needles, mp 172—174 °C, $[\alpha]_D^{26}$ −8.3° (c=1.16, MeOH). Positive ion HR FAB-MS m/z: 669.3257 ($[M+Na]^+$). $C_{35}H_{50}NaO_{11}$ requires 669.3250. Negative ion FAB-MS m/z: 645 ($[M-H]^-$), 483 ($[M-H-162]^-$), 385. $UV\lambda_{max}^{MeOH}$ nm (log ε): 206 (4.46), 220 (4.13), 282 (3.37). ^{1}H- and ^{13}C-NMR data are shown in Tables II and III.

Cayaponoside A_4: An amorphous powder, mp 155—160 °C, $[\alpha]_D^{26}$ +17.7° (c=0.97, MeOH). Positive ion HR FAB-MS m/z: 671.3410 ($[M+Na]^+$). $C_{35}H_{52}NaO_{11}$ requires 671.3407. Negative ion FAB-MS m/z: 647 ($[M-H]^-$), 485 ($[M-H-162]^-$), 385. $UV\lambda_{max}^{MeOH}$ nm (log ε): 206 (4.49), 220 (shoulder, 4.16), 282 (3.41). ^{1}H- and ^{13}C-NMR data are shown in Tables II and III.

Cayaponoside C_2: An amorphous powder, mp 164—170 °C, $[\alpha]_D^{24}$ +47.5° (c=1.33, MeOH). Positive ion HR FAB-MS m/z: 571.2517 ($[M+Na]^+$). $C_{29}H_{40}NaO_{10}$ requires 571.2519. m/z: 386.2087 ($[M-C_6H_{10}O_5]^+$). $C_{23}H_{30}O_5$ requires 386.2093. Negative ion FAB-MS m/z: 547 ($[M-H]^-$), 385 ($[M-H-162]^-$). $UV\lambda_{max}^{MeOH}$ nm (log ε): 206 (4.30), 220 (shoulder, 3.91), 280 (3.16). ^{1}H-NMR and ^{13}C-NMR data are shown in Tables II and III.

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) Structures of cayaponosides A, B, C and D were reported as a communication. H. Himeno, T. Nagao, J. Honda, H. Okabe, N. Irino, T. Nakasumi, *Chem. Pharm. Bull.*, **40**, 2885 (1992).
2) R. Bauer, L. H. Berganza, O. Seligmann, H. Wagner, *Phyto-*

chemistry, **24**, 1587 (1985).
3) *a*) H. Achenbach, U. Hefter-Bübl, M. A. Constenla, *J. Chem. Soc., Chem. Commun.*, **1987**, 441; H. Achenbach, R. Waibel, U. Hefter-Bübl, M. A. Constenla, *J. Nat. Prod.*, **56**, 1506 (1993); *b*) L. M. M. Valente, A. A. L. Gunatilaka, T. E. Glass, D. G. I. Kingston, *ibid.*, **56**, 1772 (1993); *c*) M. E. O. Matos, M. I. L. Machado, A. A. Craveiro, F. J. A. Matos, R. Braz-Filho, *Phytochemistry*, **30**, 1020 (1991); *d*) M. R. Farias, E. P. Schenkel, R. Mayer, G. Rücker, *Planta Medica*, **59**, 272 (1993).
4) The instruments and materials used in this work were as follows: Yanaco micro melting apparatus (melting points), JASCO DIP-360 digital polarimeter (specific rotation), JEOL JNM GX-400 spectrometer (^{1}H- and ^{13}C-NMR spectra), JEOL JMS HX-110 spectrometer (MS), Shimadzu UV-200S (UV spectra), Kiesel gel 60 (70—230 mesh, E. Merck), Diaion HP-20 (Mitsubishi Chemical Industries, Ltd.), Capcell pak C_{18}-AG 120 Å (250 mm × 4.6 mm i.d. for analytical HPLC, 250 mm × 20 mm i.d. for preparative purpose). Kiesel gel $60F_{254}$ and RP-18 $WF_{254}S$ (E. Merck) (TLC). All melting points are uncorrected. NMR spectra were measured in MeOH-d_4, and chemical shifts are expressed in the δ scale using tetramethylsilane as an internal standard.