STRUCTURES OF CAYAPONOSIDES A, B, C AND D, GLUCOSIDES OF NEW *NOR*-CUCURBITACINS IN THE ROOTS OF *CAYAPONIA TAYUYA*

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Four glucosides of new *nor*-cucurbitacins having an aromatized A ring were isolated from the roots of *Cayaponia tayuya*. Elucidation of their structures is described.

KEYWORDS *Cayaponia tayuya*; Cucurbitaceae; triterpene glucoside; *nor*-cucurbitacin glucoside; cayaponoside

The roots of *Cayaponia tayuya* (VELL.) COGN. (Cucurbitaceae) are used in Brazilian traditional medicine as laxatives, diuretics and antirheumatics. The roots taste bitter and have a foaming property when shaken in water. These properties suggest the presence of cucurbitacins and saponins in the roots.

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, cucurbitacin B and its 2-*O*-glucoside from the $CHCl_3$ extract of the roots.[1)]

With the purpose of investigating the constituents more polar than the reported cucurbitacins, we examined the MeOH extract of the roots obtained after $CHCl_3$ extraction, and found that the MeOH extract is still bitter and has a foaming property. The MeOH extract was roughly fractionated by silica gel column chromatography (AcOEt-MeOH-H_2O, 12:1:0.3) into the less polar glycoside fraction (Fr. I) which has a bitter taste and the polar glycoside fraction (Fr. II) which contains saponins.

Fr. I was fractionated into 4 fractions (Fr. I-A to D) by silica gel column chromatography ($CHCl_3$-MeOH-H_2O, 15:4:0.5). Each fraction was further subjected to preparative HPLC (Shiseido Capcell Pak C18, 30-32% acetonitrile) , and bitter compounds named cayaponosides A, B, C and D were isolated together with several minor compounds in a chromatographically homogeneous state from the corresponding fractions. This communication deals with the structures of major cayaponosides.

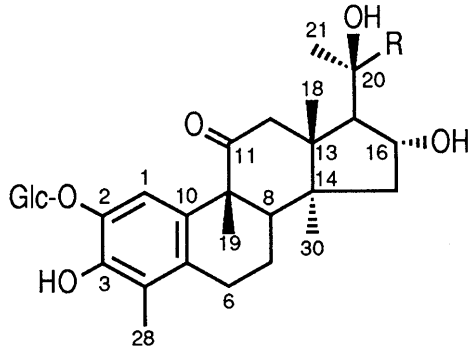
Cayaponoside A, an amorphous powder, showed an $[M+Na]^+$ ion at *m/z* 729.3455 in the positive ion FAB-MS, which gave the molecular formula $C_{37}H_{54}O_{13}$. The negative ion FAB-MS showed an $[M-H]^-$ ion at *m/z* 705 and a fragment ion at *m/z* 543 and 483. These fragment ions suggested the presence of a hexosyl group and an acetyl group in the molecule. On acid hydrolysis, it gave D-glucose as the component sugar. Cayaponoside A showed a UV absorption (MeOH) at 206 nm (log ε 4.40) and 284 nm (log ε 3.28), indicating the presence of a substituted phenyl group in the molecule. 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), one phenyl proton (δ 6.67, singlet) and an anomeric proton of the sugar moiety (δ 4.57, d, *J*=7 Hz) . The ^{13}C-NMR spectrum showed the carbon signals of 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), two carbonyl carbons (δ 217.5 and 217.9), and phenyl carbons at δ 114.3, 125.7, 130.8, 132.2, 145.5 and 145.8; the last two seemed to be phenolic carbon signals.

These data suggested that cayaponoside A is a *nor*-cucurbitacin glucoside having a structure similar to those of fevicordin A glucoside which was isolated from the seeds of *Fevillea cordifolia* (Cucurbitaceae) by Achenbach *et al.* [2)] and to those isolated

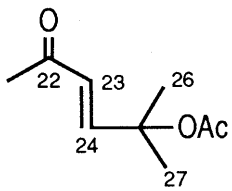
TABLE I. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Spectral Data of Cayaponosides (CD_3OD, TMS as Internal Standard)

	Cayaponoside A		Cayaponoside B		Cayaponoside C		Cayaponoside D	
	δH	δC	δH	δC	δH	δC	δH	δC
1	6.67(s)	114.3	6.64(s)	114.3	6.67(s)	114.4	6.63(s)	114.3
2		145.5		145.5		145.5		145.4
3		145.8		145.8		145.8		145.7
4		125.7		125.7		125.7		125.7
5		132.2		132.2		132.2		132.2
6	*ca* 2.65	25.6	2.66(m)	25.6	*ca* 2.68	25.6	2.65(m)	25.6
	ca 2.85		2.86(m)		*ca* 2.85		2.85(m)	
7	*ca* 1.95	21.1	1.95(m)	21.1	*ca* 1.94	21.1	1.96(m)	21.0
	2.25(m)		2.25(m)		2.25(m)		2.27(m)	
8	2.13(br.d,7)	44.9	2.15(br.d,7)	44.8	2.13(br.d,7)	44.9	2.15(br.d,7)	44.8
9		53.0		52.8		53.0		52.8
10		130.8		130.8		130.8		130.8
11		217.9		218.1		218.1		218.2
12	2.63(d,14)	52.8	2.60(d,14)	53.0	2.65(d,14)	52.8	2.59(d, 14)	52.9
	2.91(d,14)		2.76(d,14)		2.96(d,14)		2.78(d, 14)	
13		52.1		52.7		52.1		52.8
14		50.6		50.6		50.6		50.6
15	1.50(m)	47.2	1.60(d,14)	46.4	1.48(d,14)	47.2	1.59(d,14)	46.2
	ca 1.97(m)		1.98(m)		1.94(dd,9,14)		1.99(dd,10,14)	
16	4.43(br.dd)	72.3	4.63(br.dd)	73.0	4.42(br.dd)	72.3	4.62(br.dd)	73.0
17	2.48(d,7)	60.6	2.38(d,6)	57.8	2.51(d,7)	60.3	2.34(d,7)	57.4
18	0.95(s)	21.2	1.00(s)	21.1	0.96(s)	21.2	1.00(s)	21.0
19	1.31(s)	30.0	1.30(s)	29.9	1.31(s)	30.0	1.30(s)	29.9
20		81.7		78.0		81.7		77.9
21	1.35(s)	26.3	1.19(s)	24.3	1.36(s)	26.2	1.21(s)	24.8
22		217.5	4.08(dd,1,6)	82.2		217.8	3.95(br.d,5)	82.6
23	2.70-2.80(m)	33.6	5.79(dd,6,16)	130.4	2.70-2.80(m)	33.9	5.74(dd,5,16)	127.0
24	*ca* 1.95(2H)	36.6	6.36(dd,1,16)	136.5	1.70(2H,m)	38.9	5.83(dd,1,16)	142.3
25		83.9		143.8		71.6		72.0
26	1.42(s)	27.0[a)]	4.94(s,2H)	117.5	1.17(s)	30.2[a)]	1.25(s)	30.8[a)]
27	1.42(s)	27.1[a)]	1.82(s)	19.6	1.17(s)	30.0[a)]	1.25(s)	30.9[a)]
28	2.09(s)	12.3	2.09(s)	12.3	2.08(s)	12.3	2.09(s)	12.3
30	0.99(s)	20.9	0.98(s)	20.7	1.00(s)	20.9	0.98(s)	20.7
Ac	1.92(s)	23.1						
		173.2						

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



fevicordin A glucoside : R =



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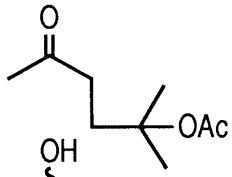
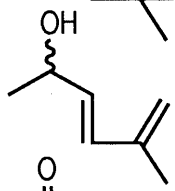
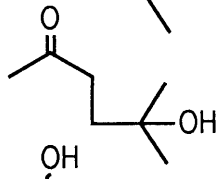


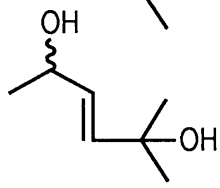
cayaponoside B : R =



cayaponoside C : R =



cayaponoside D : R =



from a Brazilian species of *Wilbrandia*.[3] The NMR spectra of cayaponoside A were examined in detail, and all proton and carbon signals were assigned using such NMR techniques as ^1H-^1H COSY, ^1H-^{13}C COSY, NOESY and COLOC. The results are summarized in Table I. The structure of cayaponoside A was determined as shown in the figure, and it was concluded to be 23,24-dihydrofevicordin A glucoside.

Cayaponoside B, a crystalline powder, UV λmax (MeOH): 208 nm (log ε 4.42), 225 nm (log ε 4.44) and 283 nm (log ε 3.20), showed in high resolution FAB-MS an $[M+Na]^+$ ion at *m/z* 669.3251, from which the molecular formula $C_{35}H_{50}O_{11}$ was deduced. The general feature of NMR spectra was similar to those of cayaponoside A except for the absence of the signals of an acetyl group, one carbonyl group and one tertiary methyl group and the presence of the signals of a terminal conjugated diene system and a hydroxymethylene group adjacent to the diene system. These NMR data indicated that cayaponoside B has the same nucleus as that of A and only differs in the structure of the side chain. The detailed investigation of the NMR spectra has led to the structure of B as shown in the figure. The absolute configuration of C_{22} remains uncharacterized.

Cayaponoside C, an amorphous powder, UV λ max (MeOH): 208 nm (log ε 4.25) and 283 nm (log ε 3.30), $C_{35}H_{52}O_{12}$, showed a quite similar NMR spectra to those of cayaponoside A except that it lacks the signals of the acetyl group, and the chemical shifts of ^1H and ^{13}C of the side chain moiety are slightly different. From these data, cayaponoside C was concluded to be desacetyl cayaponoside A.

Cayaponoside D, an amorphous powder, UV λ max (MeOH): 208 nm (log ε 4.31) and 284 nm (log ε 3.25), $C_{35}H_{52}O_{12}$, showed similar NMR spectra to those of above-mentioned cayaponosides, and the NMR spectra indicated that it has the same nucleus as other cayaponosides and differs in the structure of the side chain. It has a disubstituted double bond adjacent to a hydroxymethylene group at one end and to a quaternary carbon at the other end. There are two alternate possibilities for the positions of the hydroxyl group and double bond: one, a 22-hydroxy-23-ene, and the other, a 22-ene-24-hydroxy. To determine the position of the hydroxyl group, ROE difference spectra were measured. When the hydroxymethylene proton (δ 3.95, br. d, *J*=5 Hz) was irradiated, NOE was observed on the signal of one methyl group (δ 1.21, 3H, s), and irradiation on the oleffinic proton (δ 5.83, H, dd, *J*=1, 16 Hz) showed NOE on the signal of two methyl groups (δ 1.25, 6H, s). These data clearly indicate that the hydroxyl group is located at C_{22}. Therefore the structure of cayaponoside D was determined as shown in the figure.

The position of glucopyranosyl groups in cayaponosides was determined at all C_2-OH of the aglycones by measurement of ROE difference spectra. Irradiation at the anomeric protons caused NOE at the phenyl protons, and irradiation on the phenyl protons in turn brought NOE on the anomeric protons.

Cucurbitacins are generally known for their cytotoxicity; however, cayaponoside D showed no notable cytotoxicity for mouse tumor cells YAC-1 and EL-4.

The MeOH extract of the root contains several other minor cucurbitacins. Their isolation and structure elucidation are now in progress.

ACKNOWLEDGEMENT The authors are grateful to Mr. H. Hanazono for measurement of mass spectra.

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(Received August 14, 1992)