STRUCTURES OF CACHINESIDE III, IV AND V, IRIDOID GLUCOSIDES FROM CAMPSIS CHINENSIS VOSS.

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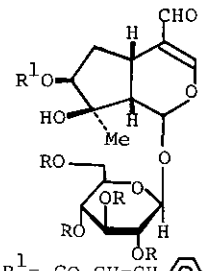
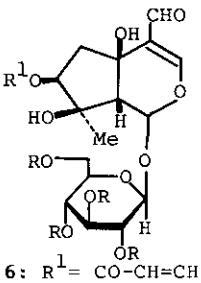
Abstract —— Three new iridoid glucosides, cachineside III, IV and V have been isolated from the dried leaves of Campsis Chinensis Voss., and established to have the structures **8**, **9** and **10**, respectively, on the basis of spectral data and chemical transformations.

We recently reported the structural determinations of six iridoid glucosides, campenoside (**1**),[1] 5-hydroxycampenoside (**2**),[2,3] cachineside I (**3**),[3] tecomoside (**4**),[3] campsiside (**5**)[2] and 5-hydroxycampsiside (**6**),[2] and of three phenylpropanoid glycosides, acetoside (**7**),[4] campneoside I (**7a**),[4] and campneoside II (**7b**),[4] which were isolated from a methanol extract of fresh leaves of Campsis chinensis Voss. (Bignoniaceae). Further investigations on the iridoid components of the methanol extract of dried leaves of the same plant have now led to the isolation of three new iridoid glucosides named cachineside III (**8**), cachineside IV (**9**) and cachineside V (**10**) by the procedure described in the Experimental.

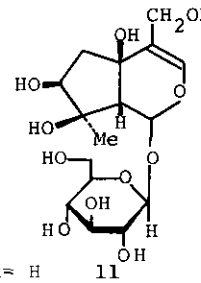
We report herein the structural elucidations of these compounds.



5: R^1= CO-CH=CH-Ph, R= H
5a: R^1= CO-CH=CH-Ph, R= Ac
10: R^1= R= H
10a: R^1= R= Ac



6: R^1= CO-CH=CH-Ph, R= H
6a: R^1= CO-CH=CH-Ph, R= Ac
8: R^1= CO-CH=CH-Ph-OH, R= H
8a: R^1= CO-CH=CH-Ph-OAc, R= Ac
9: R^1= R= H
9a: R^1= R= Ac



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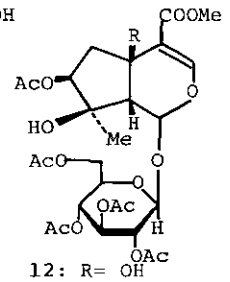

12: R= OH
13: R= H

Cachineside III (**8**) was isolated as an amorphous powder, $[\alpha]_D$ -103.2°(MeOH), which gave methyl glucopyranoside on acid methanolysis. The ^{1}H-NMR and IR spectra of **8** showed the presence of a p-hydroxy-trans-cinnamoyl moiety[1715, 1640 cm^{-1}; δ 6.81, 7.48(each 2H, d, J= 8.6), and 6.41, 7.70(each 1H, d, J= 16.0)], a conjugated aldehyde group [1690, 1630 cm^{-1}; δ 9.28 and 7.39(each 1H, s)], a tertiary methyl group [δ 1.16(3H, s)] and a β-D-glucopyranosyl moiety[3400 cm^{-1}; δ 4.66(1H, d, J= 7.7, H-1']. Acetylation of **8** with acetic anhydride and pyridine gave a pentaacetyl cachineside III (**8a**), $C_{33}H_{38}O_{17}$, mp 231–232 °C, $[\alpha]_D$ -120°($CHCl_3$). As shown in Tables I and II, ^{1}H and ^{13}C-NMR spectral data, such as chemical shifts and the coupling patterns of **8** and **8a**, were quite similar to those of **6** and **6a**, respectively, except for the presence of a p-hydroxycinnamoyl moiety in the former compounds. Furthermore, the presence of a p-hydroxycinnamoyl moiety at C-7 in **8** was demonstrated by the fact that the signals of H-7 in **8**(δ 4.83) and **8a**(δ 4.86) were similar to those in **6**(δ 4.85) and **6a**(δ 4.84), and the signals of C-9 and C-10 in **8** (δ 58.22 and 21.46) and **8a**(δ 57.20 and 21.29) were similar to those in **6**(δ 58.28 and 21.43) and **6a**(δ 57.23 and 21.20), respectively. From these findings, the structure of cachineside III was deduced to be **8**.

To confirm this assignment, cachineside III (**8**) was converted to an alcohol (**11**): reduction of **8a** with $LiAlH_4$ gave the alcohol (**11**), which was identical to authentic **11**[2] obtained previously by $LiAlH_4$ reduction of **6a**. Thus, the stereostructure of cachineside III was established as **8**. This establishment was supported by the mass spectral data on **8a**, as shown in Chart 1.

Cachineside IV (**9**) was isolated as a pentaacetyl cachineside IV (**9a**),[5] $C_{26}H_{34}O_{16}$, mp 118–120°C, $[\alpha]_D$ -138°($CHCl_3$). The ^{1}H and ^{13}C-NMR spectral data (Tables I and II) indicated the presence of five acetoxy groups[δ1.94, 2.01, 2.04, 2.12 and 2.14 (each 3H, s)], two tertiary hydroxy groups, a tertiary methyl group, a conjugated aldehyde group and a tetraacetyl-β-D-glucopyranosyl moiety (see Experimental). From the similarities of ^{1}H-NMR[the chemical shifts and coupling constants in Table I, and the NOE increments of H-1(12.7 %) and H-7(15.4 %) from Me-8] and ^{13}C-NMR (Table II) data on **9a** to those on **6a**, except for the signals of the trans-cinnamoyl, the pentaacetyl cachineside IV (**9a**) was concluded to have the same functional groups as those of **6a** in the same positions but also to have an acetoxy group at C-7. Thus, the structure of pentaacetyl cachineside IV was deduced to be **9a**.

To confirm this assignment, **9a** was related chemically to a pentaacetyl lamiide (**12**). Oxidation of **9a** with sodium chlorite,[6] followed by methylation with diazomethane gave a methyl ester (**12**), mp 187–188°C, $[\alpha]_D$ -93.1°($CHCl_3$), which was found

to be identical to authentic pentaacetyl lamiide (**12**)[2] by comparison of spectral data and by the mixed melting point. Thus, the stereostructure of cachineside IV was established as **9**. This assignment was supported by the mass spectral data on **9a**, as shown in Chart 1.

Table I. ^{1}H-NMR Spectral data for **6**, **8**, **6a**, **8a**, **9a**, **5a**, and **10a** (δ Values)[a)]

Compd.	H-1	H-3	H-5	αH-6	βH-6	H-7	H-9	Me-8	CHO-4	H-1'
6	5.95d (0.7)	7.39	——	2.48dd (15.5, 4.4)	2.38dd (15.5, 2.4)	4.85m	2.90d (0.7)	1.17	9.28	4.65d (8.0)
8	5.95d (0.7)	7.38	——	2.46dd (16.0, 6.0)	2.35dd (16.0, 2.0)	4.83dd (6.0, 2.0)	2.89d (0.7)	1.16	9.28	4.66d (7.7)
6a	5.80d (1.0)	7.12	——	2.42dd (15.9, 5.1)	2.54dd (15.9, 2.7)	4.86dd (5.1, 2.7)	2.95d (1.0)	1.21	9.35	4.87d (8.1)
8a	5.79d (1.0)	7.11	——	2.43dd (16.4, 5.6)	2.54dd (16.4, 2.7)	4.86dd (5.6, 2.7)	2.94d (1.0)	1.21	9.40	4.89d (8.0)
9a	5.77d (1.2)	7.09	——	2.34dd (16.6, 5.1)	2.46dd (16.6, 2.7)	4.69dd (5.1, 2.7)	2.87d (1.2)	1.16	9.28	4.86d (7.8)
5a	5.63d (1.4)	7.09	3.11ddd (10.0, 9.0, 5.5)	1.80dt (15.0, 5.5)	2.47ddd (15.0, 9.0, 2.5)	4.90dd (6.0, 2.5)	2.73dd (10.0, 1.4)	1.29	9.28	4.88d (7.5)
10a	5.60d (1.5)	7.08	3.09ddd (10.3, 9.8, 5.4)	1.74dt (15.7, 5.4)	2.41ddd (15.7, 9.8, 2.7)	4.74dd (5.4, 2.7)	2.66dd (10.3, 1.5)	1.24	9.26	4.88d (7.8)

a) Measured in $CDCl_3$ except for those of **6** and **8**(CD_3OD), with TMS as an internal standard. d, doublet; dd, double doublet; ddd, double double doublet; dt, double triplet; unmarked, singlet. Numbers in parentheses are coupling constants J(Hz).

Cachineside V (**10**) was isolated as a pentacetyl cachineside V (**10a**),[5] $C_{26}H_{34}O_{15}$, mp 197–199°C, $[\alpha]_D$ -113°($CHCl_3$). The ^{1}H and ^{13}C-NMR spectral data (Tables I and II) on **10a** showed the presence of signals closely resembling those of **5a**,[2] as well as the typical signals of a tetraacetyl-β-D-glucopyranosyl moiety[^{1}H: see Experimental] except for the signals of a trans-cinnamoyl moiety. Furthermore, ^{1}H-NMR data on **10a** showed NOE increments of 9.0 and 10.0 % for H-7 and H-1, respectively, from Me-8, as observed for **5a**. This coincidence indicated the same stereochemistry of all chiral centers in **10a** and **5a**. Thus, the structure of pentaacetyl cachineside V was deduced to be **10a**.

To confirm this assignment, **10a** was converted to the known pentaacetyl caryptoside (**13**).[2] Oxidation of **10a** with sodium chlorite, followed by methylation with diazo-

methane afforded a pentaacetyl caryptoside, mp 139–140°C, $[\alpha]_D$ -110°($CHCl_3$), which was confirmed to be identical with authentic **13**[2] by comparison of spectral data and the mixed melting point. Thus, the stereostructure of cachineside V was established as **10**. This establishement was supported by the mass spectral data on **10a**, as shown in Chart 2.

Table II. ^{13}C-NMR Spectral data for **6**, **8**, **6a**, **8a**, **9a**, **5a**, and **10a** (δ Values)[a)]

Carbon	**6**	**8**	**6a**	**8a**	**9a**	**5a**	**10a**
Aglycone moiety							
1	95.19 d	95.19 d	94.31 d	94.28 d	94.26 d	94.17 d	94.14 d
3	162.23 d	162.17 d	156.24 d	156.22 d	156.27 d	158.32 d	158.32 d
4	127.10 s	127.02 s	126.46 s	126.46 s	126.40 s	125.70 s	125.64 s
5	68.27 s	68.24 s	67.68 s	67.71 s	67.60 s	24.38 d	24.35 d
6	44.59 t	44.56 t	43.07 t	43.10 t	42.95 t	34.63 t	34.51 t
7	80.50 d	80.18 d	79.02 d	79.28 d	79.22 d	80.56 d	80.53 d
8	78.66 s	78.63 s	78.02 s	78.05 s	77.76 s	78.46 s	78.25 s
9	58.28 d	58.22 d	57.23 d	57.20 d	57.14 d	47.45 d	47.36 d
10	21.43 q	21.46 q	21.20 q	21.19 q	21.20 q	21.61 q	21.52 q
11	192.19 d	192.16 d	189.39 d	189.36 d	189.36 d	189.50 d	189.47 d
Glucose moiety							
1'	99.89 d	99.86 d	95.95 d	95.98 d	95.98 d	95.60 d	95.63 d
2'	74.46 d	74.37 d	71.01 d	71.01 d	71.01 d	70.78 d	70.78 d
3'	77.47 d[b)]	77.44 d[b)]	71.98 d[b)]	71.98 d[b)]	71.98 d[b)]	72.38 d[b)]	72.37 d[b)]
4'	71.65 d	71.63 d	68.21 d	68.21 d	68.24 d	68.24 d	68.30 d
5'	78.43 d[b)]	78.34 d[b)]	72.38 d[b)]	72.41 d[b)]	72.41 d[b)]	72.47 d[b)]	72.47 d[b)]
6'	62.81 t	62.75 t	61.64 t	61.61 t	61.64 t	61.73 t	61.76 t
trans-Cinnamoyl or p-hydroxy-trans-Cinnamoyl moiety							
1"	167.98 s	168.56 s	166.49 s	166.38 s		166.44 s	
2"	119.19 d	115.48 d	117.38 d	117.53 d		117.38 d	
3"	146.43 d	146.70 d	146.11 d	144.97 d		145.94 d	
4"	135.89 s	127.28 s	134.20 s	131.86 s		134.17 s	
5"	129.99 d[c)]	131.08 d	128.94 d[c)]	129.41 d		128.97 d[c)]	
6"	129.24 d[c)]	116.80 s	128.27 d[c)]	122.17 d		128.24 d[c)]	
7"	131.45 d	161.12 s	130.58 d	152.42 d		130.61 d	
8"	129.24 d	116.80 d	128.27 d	122.17 d		128.24 d	
9"	129.99 d	131.08 d	128.94 d	129.41 d		128.94 d	

a) Measured in $CDCl_3$ except for those of **6** and **10** (CD_3OD), with TMS as an internal standard. s, singlet; d, doublet; t, triplet; q, quartet. The signals of acetyl groups are not given. b, c) Assignments in each column may be interchanged.

EXPERIMENTAL

All melting points are uncorrected. Infrared(IR) and mass(MS) spectra were measured with Hitachi IR-215 and JEOL JMS-D-300 spectrometers, respectively. NMR spectra were taken with FX-200(^{1}H: 200 MHz, ^{13}C: 50.10 MHz) NMR spectrometer with tetramethylsilane(TMS) as an internal standard. For column chromatography, Merck Kieselgel 60(70-230 mesh) and Sephadex LH-20 were used. Thin-layer chromatography(TLC) and preparative thin-layer chromatography(P-TLC) were performed with Merck Kieselgel GF_{254} and PF_{254}, respectively. Spots were detected under ultraviolet(UV) light or by spraying with 1% $Ce(SO_4)_2$-10% H_2SO_4 and then heating.

Isolation of iridoid glucosides

Leaves of Campsis chinensis Voss.(590 g) collected at the Botanic Garden of the Faculty of Pharmaceutical Sciences, University of Tokushima in September 1985 were dried and extracted with $CHCl_3$(5 l) and MeOH(10 l). The MeOH extract was evaporated in vacuo to give a residue(130 g), 36 g of which was washed three times with 30 ml of $CHCl_3$ to give a residue(21.4 g). The residue(20 g) was subjected to column chromatography on active charcoal(50 g)-Celite 535(50 g) with water(200 ml, fr. 1), water-MeOH(1:1, until fr. 13, 300 ml each), and then MeOH(from fr. 14, 300 ml each). Fractions 16-17 were combined (1.21 g) and subjected to column chromatography on Kieselgel(70 g) in $CHCl_3$-MeOH-H_2O(7:3:0.3) and fr. 2-5(708 mg) and fr. 6-7(105 mg)(20-30 ml each) were collected. Then, fr. 2-5(708 mg) was subjected to gel filtration on a Sephadex LH-20 column(50 g) with 50% MeOH as solvent to obtain fr. 4-10(286 mg, a mixture of iridoid compounds) and fr. 12-15(300 mg, acteoside) (10 ml each). Column chromatography on Kieselgel(30 g) of fr. 4-10(286 mg) in $CHCl_3$-MeOH-H_2O(50:15:3, lower layer) gave fr. 10-12[35 mg, 5-hydroxycampsiside (**6**)] and fr. 14-15 [81 mg, a mixture of tecomoside (**4**), cachineside IV (**9**) and V (**10**)], together with fr. 19-27 [29.3 mg, cachineside III (**8**)] and fr. 33[23 mg, acteoside (**7**)] (20-30 ml each). The ^{1}H-NMR spectrum(CD_3OD) of fr. 14-15 showed the presence of conjugated aldehyde groups[δ9.19, 9.22 and 9.28(each s), and 7.32, 7.38 and 7.39 (each s)] and methyl groups[δ1.10(d, J= 7.0), 1.14 and 1.29(each s)] but the absence of an acetyl group. A solution of fr. 14-15(81 mg) in acetic anhydride(3 ml) and pyridine(3 ml) was stirred at 25°C for 10 h. After the usual work-up, the crude acetate was purified by P-TLC with $CHCl_3$-acetone(10:1) as developer(three developments) to give a pentaacetyl tecomoside[3](**4**, 41 mg, R_f=0.35), a pentaacetyl cachineside IV (**9a**, 7 mg, R_f=0.09) and a pentaacetyl cachineside V (**10a**, 23 mg, R_f=0.24).

Cachineside III (8)

Amorphous powder, $[\alpha]_D$ -103.2°(c= 0.2, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3400, 1720, 1680, 1640, 1630; ^1H and ^{13}C-NMR: see Tables I and II.

Acid methanolysis of 8

A solution of **8**(8 mg) in dry 15% HCl-MeOH(2 ml) was refluxed for 1 h, neutralized with silver carbonate and filtered to remove the powder. The powder was washed with dry MeOH. The combined filtrate and the washings were evaporated in vacuo to give methyl glucopyranoside, which was confirmed to be identical with authentic methyl glucopyranoside by HPTLC [Merck, Kieselgel 60 F_{254}, R_f=0.31, developed with $CHCl_3$-MeOH-H_2O(65:35:10, lower layer)].

Acetylation of 8

Compound (**8**)(10 mg) was acetylated with acetic anhydride(1 ml) and pyridine(1 ml) to give a pentaacetyl cachineside III (**8a**, 7.8 mg), which was crystallized from EtOH-H_2O.

Colorless needles, mp 231–232 °C, $[\alpha]_D$ -120 °(c= 0.3, $CHCl_3$); ^1H-NMR($CDCl_3$):δ 1.95, 2.01, 2.04, 2.11, 2.32(each 3H, s, OAc X 5), tetraacetyl-β-D-glucopyranosyl moiety [δ 4.89(1H, d, J= 8.0, H-1'), 5.00(1H, dd, J= 8.0, 9.3, H-2'), 5.27(1H, t, J= 9.3, H-3'), 5.10(1H, t, J= 9.3, H-4'), 3.79(1H, ddd, J= 2.4, 4.4, 12.6, H-5'), 4.15(1H, dd, J= 2.4, 12.6, H-6'), 4.33(1H, dd, J= 4.4, 12.6, H-6')]. See Table I for other signals; ^{13}C-NMR: see Table II; FABMS: m/z 771(M^+ + Na, 100 %), $C_{35}H_{40}O_{18}Na$; High-resolution and EI-MS: see Chart 1; Anal. Calcd. for $C_{35}H_{40}O_{18}$: C, 56.15; H, 5.39. Found: C, 56.00; H, 5.31 %.

Reduction of 8a

A solution of $LiAlH_4$(40 mg) in dry tetrahydrofuran(THF, 1 ml) was added to a solution of **8a**(15 mg) in dry THF(1 ml) under ice-cooling. The resulting mixture was stirred at 25–27°C for 2 h, treated with wet Et_2O and then water to decompose excess $LiAlH_4$, and neutralized with 5% HCl. The Et_2O and THF were evaporated under reduced pressure, and the residue was washed with AcOEt. The AcOEt-insoluble portion was chromatographed on active charcoal-Celite 535(4:1, 5 g) with water(30 ml) and MeOH(50 ml) as solvents. The MeOH fraction was concentrated in vacuo to give **11**(8 mg), which was identical with lamiidol (**11**) prepared from lamiide as judged by TLC and ^1H-NMR examinations.

Pentaacetyl cachineside IV (9a)

Colorless needles(from EtOH), mp 118–120°C, $[\alpha]_D$ -138°(c= 0.5, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 1.94, 2.01, 2.04, 2.12 and 2.14(each 3H, s, OAc X 5), tetraacetyl-β-D-glucopyranosyl moiety[δ 4.86(1H, d, J= 7.8, H-1'), 4.98(1H, dd, J= 7.8, 9.3, H-2'), 5.27(1H, t, J= 9.3, H-3'), 5.10(1H, t, J= 9.3, H-4'), 3.77(1H, ddd, J= 2.4, 4.4, 9.3, H-5'), 4.16(1H, dd, J= 2.4, 12.5, H-6') and 4.32(1H, dd, J= 4.4, 12.5, H-6')]. See Table I for other signals ; ^{13}C-NMR: see Table II.; FABS: m/z 625(M^+ + Na, 100 %), $C_{26}H_{34}O_{16}Na$; High-resolution MS and EI-MS: see Chart 1 ; Anal. Calcd. for $C_{26}H_{34}O_{16}$: C, 51.82; H, 5.69. Found: C, 51.58; H, 5.70 %.

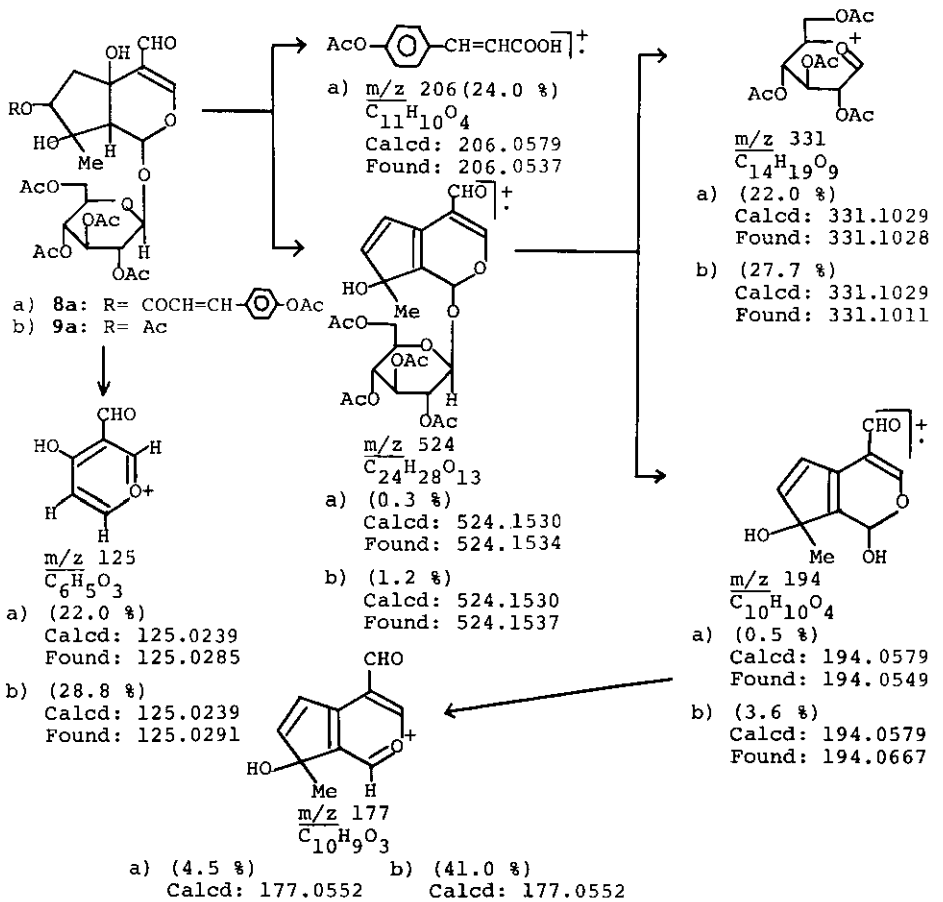

Chart 1 Mass Spectra of **8a** and **9a**

Oxidation followed by methylation of 9a

A solution of **9a**(39 mg) in tert-BuOH and 2-methyl-2-butene(0.8 ml) was treated with a solution of $NaClO_2$(140 mg) and NaH_2PO_4(140 mg) in water(1.5 ml), and the mixture was stirred at 25–27 °C for 3 h. The mixture was then diluted with ice-water(30 ml)

and extrated with AcOEt (40 ml X 3). The AcOEt extract was concentrated in vacuo to give a residue(25 mg). A solution of CH_2N_2 in Et_2O(2 ml) was added to a solution of the residue(25 mg) in Et_2O(3 ml) and MeOH(0.5 ml), and the mixture was stood for 30 min at 25–27°C. The mixture was concentrated and the residue was purified by P-TLC with $CHCl_3$-acetone(10:1) as solvent to give a pentaacetyl lamiide (**12**, 20 mg, R_f= 0.15), mp 187–188 °C(from EtOH), $[\alpha]_D$ -93.1°(c= 0.25, $CHCl_3$), which was identical with an authentic sample.[2]

Pentaacetyl cachineside V (10a)

Colorless needles(from EtOH), mp 197–199°C, $[\alpha]_D$ -113°(c= 0.3, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 1.90, 2.00 and 2.03(each 3H, s, OAc X 3), and 2.11(6H, s, OAc X 2), tetraacetyl-β-D-glucopyranosyl moiety [δ4.88(1H, d, J= 7.8, H-1'), 4.97(1H, dd, J= 7.6, 9.0, H-2'), 5.23(1H, dd, J= 9.0, 9.5, H-3'), 5.09(1H, t, J= 9.5, H-4'), 3.74 (1H, ddd, J= 2.4, 4.4, 9.5, H-5'), 4.16(1H, dd, J= 2.4, 14.5, H-6') and 4.31(1H, dd, J= 4.4, 14.5, H-6')]. See Table I for other signals ; ^{13}C-NMR: see Table II ; FABS: m/z 609(M^+ + Na, 100 %), $C_{26}H_{34}O_{15}Na$; High-resolution MS and EI-MS: see Chart 2 ; Anal. Calcd. for $C_{26}H_{34}O_{15}$: C, 53.24; H, 5.84. Found: C, 53.10; H, 5.95 %.

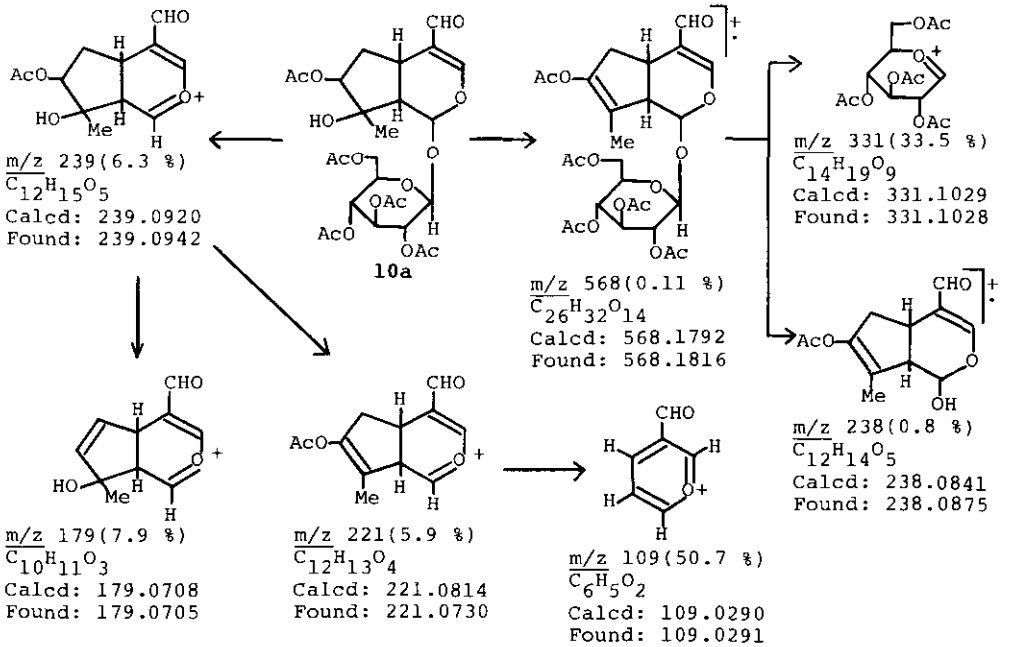

Chart 2 Mass Spectrum of **10a**

Oxidation followed by methylation of 10a

A solution of **10a**(24 mg) in tert-BuOH(2.5 ml) and 2-methyl-2-butene(0.6 ml) was treated with a solution of $NaClO_2$(120 mg) and NaH_2PO_4(120 mg) in water(1 ml) and the reaction mixture was stirred at 25–27°C for 2 h. The mixture was then treated as described for **9a**, and the crude product was methylated with CH_2N_2 in Et_2O. The crude methylated compound was purified by P-TLC with AcOEt-Et_2O(1:2) as solvent to give a pentaacetyl caryptoside(**13**, 15 mg, R_f=0.31), mp 139–140°C, $[\alpha]_D$ -110°(c= 0.1, $CHCl_3$), which was recrystallized from EtOH, and confirmed to be identical with an authentic sample.[2]

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