[Chem. Pharm. Bull. 36(1) 234—243 (1988)]

Glycosides from Chinese Medicinal Plant, *Hemsleya panacis-scandens*, and Structure–Taste Relationship of Cucurbitane Glycosides

RYOJI KASAI,[a] KAZUHIRO MATSUMOTO,[a] RUI-LIN NIE,[b] JUN ZHOU[b] and OSAMU TANAKA*,[a]

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[a] Kasumi, Minami-ku, Hiroshima 734, Japan and Kunming Institute of Botany, Academia Sinica,[b] Kunming, China

(Received June 15, 1987)

From rhizomes of *Hemsleya panacis-scandens* collected in Yunnan, China, eight new cucurbitane glycosides named scandenosides R1—R7 and dihydrocucurbitacin F glucoside (2-*O*-β-D-glucopyranoside of 23,24-dihydrocucurbitacin F) were isolated, along with six known cucurbitane glycosides. The structures of these new glycosides were determined on the basis of chemical and spectral data except for the configuration at C-20. Of these new glycosides, scandenoside R6 tastes sweet and R5 and dihydrocucurbitacin F glucoside taste bitter, while the others are tasteless. The structure–taste relationships of cucurbitane glycosides are discussed.

Keywords——*Hemsleya panacis-scandens*; Cucurbitaceae; Chinese folk medicine; cucurbitane glycoside; scandenosides R1, R2, R3, R4, R5, R6, R7; dihydrocucurbitacin F glucoside; scandenogenin A, B, C; sweet natural glycoside; bitter natural glycoside; structure–taste relationship

A number of plants of *Hemsleya* spp. (Cucurbitaceae) grow abundantly in Yunnan and Sichusan, China. Rhizomes of these plants have been used as a folk medicine (*e.g.*, as an anti-inflammatory agent) in China. In our serial studies on Chinese cucurbitaceous plants, isolation and structure elucidation of saponins of oleanane-type triterpenes from *H. macrosperma* C. Y. WU and *H. chinensis* COGN. *ex* FORBES *et* HEMSL were reported.[1] Further, six cucurbitane glycosides named carnosiflosides I—VI (**1**—**6**) were recently isolated from *H. carnosiflora* C. Y. WU *et* Z. L. CHEN[2] and of these glycosides, **2**—**4** taste bitter and **5** and **6** taste sweet, while **1** is tasteless. The present paper reports the isolation and structural determination of cucurbitane glycosides from the rhizomes of *H. panacis-scandens* C. Y. WU *et* Z. L. CHEN (Chinese name "teng san chi xue dan" 藤三七雪胆) which is closely related to *H. carnosiflora* in the taxonomical viewpoint.

Isolation and Structure Elucidation

An ethanolic extract of the rhizomes, harvested in Yunnan, was suspended in water and the suspension was extracted with ether, ethyl acetate and 1-butanol, successively. From the ethyl acetate fraction, seven known cucurbitacins, cucurbitacins B, E and F, isocucurbitacin B, 23,24-dihydrocucurbitacins B and F, and 25-*O*-acetyl-23,24-dihydrocucurbitacin F, have already been isolated and identified by Nie *et al.*[3]

The butanol fraction was subjected to column chromatography on highly porous polymer and the fraction eluted with 95% ethanol was repeatedly chromatographed on columns of silica gel and silanized silica gel, affording eight new compounds named scandenosides R1 (**7**), R2 (**8**), R3 (**9**), R4 (**10**), R5 (**11**), R6 (**12**) and R7 (**13**) as well as dihydrocucurbitacin F glucoside (**14**) together with known glycosides, **1**, **2**, **3**, **5**, **6** and cucurbitacin II glucoside (=2-*O*-β-D-glucopyranoside of 25-*O*-acetyl-23,24-dihydrocucurbitacin F) (**15**). Compounds **1**—**3**, **5** and **6** were identified by direct comparison of ^{1}H and

^{13}C nuclear magnetic resonance (^1H- and ^{13}C-NMR) spectra, electron impact mass spectra (EI-MS) of their acetates and optical rotations ($[\alpha]_D$) with those of authentic samples[2] and the identification of **15** was established by comparison of the ^1H- and ^{13}C-NMR spectra and $[\alpha]_D$ with reference data.[4]

Hydrolysis of **7** and **9** with crude pectinase[5] gave a common aglycone named scandenogenin A (**16**), which showed infrared (IR) bands due to hydroxyl and carbonyl groups at 3450 and 1690 cm^{-1}, respectively. On acetylation with acetic anhydride and pyridine at room temperature, **16** gave a diacetate (**17**) which still has an unacetylated hydroxyl group (IR: 3500 cm^{-1}). The ^1H-NMR spectrum of **16** (Table I) exhibited signals due to $-CH_2OH$ on an olefinic carbon, a carbinyl proton, a methyl group on an olefinic carbon and two olefinic protons at almost the same positions as those of carnosiflogenin A (**18**),[2] the common aglycone of **1**—**3**. However, the ^1H-NMR spectrum of **16** exhibited six singlet signals due to methyl groups on sp^3-carbons, lacking a doublet methyl signal which was observed in the spectrum of **18**, (assigned to the 20-secondary methyl group). In the ^{13}C-NMR spectra (Table II), on going from **18** to **16**, the signal due to C-20 (at δ 35.9, d) was replaced by a signal due to a tertiary alcoholic group (at δ 73.9, s) and the signals due to the carbons around the C-20 position were somewhat displaced; signals due to C-16 and -23 were shifted upfield and those due to C-17 and -22 (and probably C-18 and -21 though definite assignments of these signals have not been made) were displaced downfield, while other carbon signals remained almost unshifted. It follows that **16** can be formulated as 20-hydroxy-carnosiflogenin A. The configuration of C-20 is left undetermined.

Acid hydrolysis of **7** and **9** gave D-glucose. The ^1H- and ^{13}C-NMR spectra indicated the presence of one β-glucopyranosyl unit in **7** [anomeric proton: δ 4.76 (1H, d, $J=7$ Hz), anomeric carbon: δ 106.9] and two in **9** [δ 4.79 (1H, d, $J=8$ Hz), and 4.82 (1H, d, $J=7$ Hz), δ 107.2 and 103.4]. Comparison of the ^{13}C-NMR spectrum of **7** with that of **16** (Table II) showed glycosylation shifts[6] for C-2 and C-3, demonstrating that a β-glucopyranosyl group is located at the 3-hydroxy group in **7**. In the case of **9**, the glycosylation shifts were observed for the C-2 and C-3 signals as well as the C-24, C-25 and C-26 signals, indicating that both the 3- and 26-hydroxyl groups in **9** are glycosylated. Based on these results, **7** and **9** are formulated as the 3-*O*-β-D-glucopyranoside and 3,26-di-*O*-β-D-glucopyranoside of **16**, respectively.

On hydrolysis with crude pectinase, **8** and **10** gave a common aglycone named scandenogenin B (**19**), which exhibited IR bands due to hydroxyl and carbonyl groups at 3450 and 1690 cm^{-1}, respectively. On going from **16** to **19**, the carbon signals due to 24-olefinic carbon and the methyl carbon on an olefinic carbon were displaced downfield and the signal due to $-CH_2OH$ was shifted upfield, while other signals remained almost unshifted (Table II). This indicated that **19** is the *z*-isomer of **16** with respect to the double bond of the side chain. This formulation was also supported by the comparison of the ^{13}C-NMR spectrum of **19** with that of carnosiflogenin B (**20**),[2] an aglycone of **4**; hydroxylation shifts were observed for the signals due to carbons around the C-20 position as in the case of **16** and **18** described above.

On mineral acid hydrolysis, **8** and **10** yielded D-glucose. The ^1H- and ^{13}C-NMR spectra of **8** and **10** showed the presence of one β-glucopyranosyl group in **8** [anomeric proton: δ 4.68 (1H, d, $J=7$ Hz), anomeric carbon: δ 107.1] and two in **10** [δ 4.52 (1H, d, $J=8$ Hz) and δ 4.72 (1H, d, $J=8$ Hz), δ 107.1 and 102.4]. Glycosylation shifts were observed for the signals due to carbons around the 3-hydroxyl group in the spectrum of **8** and for those around both the 3- and 26-hydroxyl groups in that of **10** (Table II). Consequently, **8** and **10** were formulated as the 3-*O*-β-D-glucopyranoside and 3,26-di-*O*-β-D-glucopyranoside of **19**, respectively.

Acid hydrolysis of **11** produced D-glucose, while hydrolysis of **11** with crude pectinase afforded **20** as an aglycone. In the EI-MS of peracetylated **11**, the fragment ions associated with terminal glucosyl (*m/z* 331) and glucobiosyl (*m/z* 619) units were observed. Comparison of the ^{13}C-NMR spectrum of **11** with that of **20** indicated that both the 3- and 26-hydroxyl

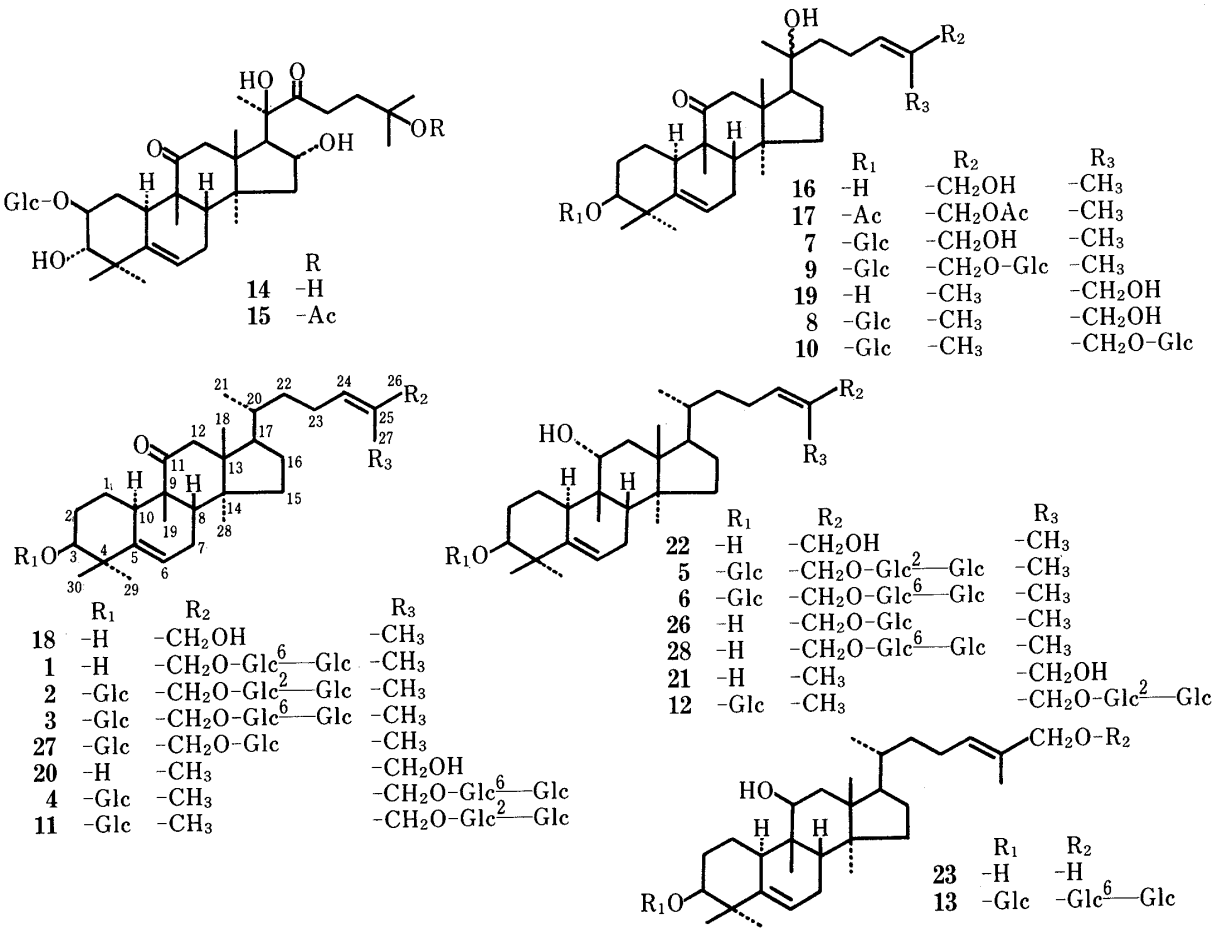

Chart 1

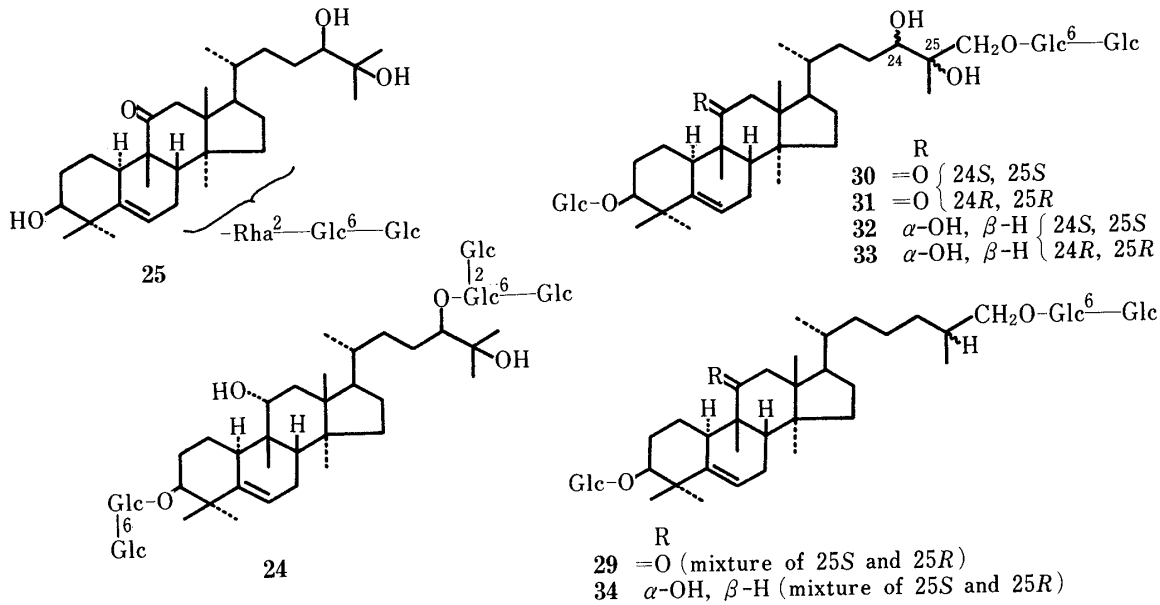

Chart 2

groups were glycosylated (Table II). Anomeric proton signals of **11** at δ 4.82 (1H, d, $J=7$ Hz), 4.90 (1H, d, $J=7$ Hz) and 5.28 (1H, d, $J=8$ Hz) indicated the presence of three β-glucopyranosyl residues. In the ^{13}C-NMR spectrum of **11**, signals attributable to the sugar moiety appeared at almost the same positions as those of **2** and **5** (Table III). Consequently,

compound **11** was formulated as the 3-*O*-*β*-D-glucopyranosido-27-*O*-*β*-sophoroside of **20**.

Acid hydrolysis of **12** yielded D-glucose, while hydrolysis of **12** with crude pectinase afforded an aglycone named scandenogenin C (**21**), which showed a hydroxyl group IR band at 3450 cm^{-1}. In the ^{13}C-NMR spectrum of **21**, carbon signals due to the A-D'ring were almost superimposable on those of carnosiflogenin C (**22**),[2] a common aglycone of **5** and **6**, while those associated with the side chain appeared at almost the same positions as those of **20** (Table II). This indicated that **21** is the *z*-isomer of **22**, leading to the formulation of **21** as shown in Chart 1.

In the ^{13}C-NMR spectrum of **12** (Table II), glycosylation shifts was observed for the signals due to carbons around both the 3- and 27-hydroxy groups. The EI-MS of peracetylated **12** showed the same fragment ions due to the sugar moiety as in the case of **11**. The ^{13}C-NMR signals of **12** due to the sugar moiety are almost superimposable on those of **11** (Table III). Based on these results, the structure of **12** was established as the 3-*O*-*β*-D-glucopyranosido-27-*O*-*β*-sophoroside of **21**.

Scandenoside R7 (**13**) was identified as the 11*β*-hydroxy epimer of **6**, and was obtained from **3** along with **6** by reduction with sodium borohydride.[2] The aglycone of **13** was designated as 11-*epi*-carnosiflogenin C (**23**).

Acid hydrolysis of **14** afforded D-glucose. The ^{1}H- and ^{13}C-NMR spectra of **14** are essentially similar to those of **15** except for the absence of signals due to an acetyl group [(proton signal at δ 1.80 (3H, s) and carbon signals at δ 22.2 and 170.1)] of **15** (Table IV), suggesting that **14** is a deacetylated derivative of **15**. On treatment with 5% KOH–MeOH, **15** afforded a deacetylated compound, which was proved to be identical with **14**. Therefore, **14** was formulated as 2-*O*-*β*-D-glucopyranoside of 23,24-dihydrocucurbitacin F.

Structure–Sweetness Relationship

Table V summarizes the taste of glycosides obtained in our studies in comparison with the sweet glycosides previously isolated from cucurbitaceous plants (Chart 2); mogroside V (**24**) from fruits of *Momordica grosvenori* SWINGL.[7] and bryodulcoside (**25**) from roots of *Bryonia dioica* JACQ.[8] As for the sweet compounds, the relative sweetness with respect to

TABLE I. ^{1}H-NMR Chemical Shifts for **16**, **18**, **19**, **21** and **23** in $CDCl_3$[a]

	18	**16**	**19**	**21**	**23**
H-3	3.48 (t-like, $W_{1/2}=6$)	3.47 (t-like, $W_{1/2}=6$)	3.47 (t-like, $W_{1/2}=6$)	3.46 (t-like, $W_{1/2}=6$)	3.48 (t-like, $W_{1/2}=6$)
H-6	5.66 (br d, $J=5.7$)	5.67 (br d, $J=6$)	5.67 (br d, $J=6$)	5.56 (br d, $J=6$)	5.62 (br d, $J=4.8$)
H-11				3.93 (dd, $J=7$, 7)	3.89 (br s, $W_{1/2}=5$)
H-12	2.45 (d, $J=14.3$)	2.54 (d, $J=14$)	2.52 (d, $J=14$)		
	2.94 (d, $J=14.3$)	2.96 (d, $J=14$)	2.96 (d, $J=14$)		
H-21	0.91 (d, $J=6.5$)	1.29 (s)	1.27 (s)	0.93 (d, $J=6$)	0.95 (d, $J=5.8$)
H-24	5.38 (t, $J=6.5$)	5.39 (t, $J=7$)	5.28 (t, $J=7$)	5.28 (t, $J=7$)	5.39 (t, $J=7$)
H-26	3.99 (s)	3.99 (s)	1.78 (s)	1.79 (br s)	3.99 (s)
H-27	1.66 (s)	1.67 (s)	4.08 (d, $J=12$)	4.13 (br s)	1.66 (s)
			4.16 (d, $J=12$)		
H-18	0.74 (s)	0.93 (s)	0.90 (s)	0.82 (s)	0.81 (s)
H-19	1.03 (s)	1.03 (s)	1.02 (s)	0.87 (s)	1.01 (s)
H-28	1.03 (s)	1.05 (s)	1.04 (s)	1.06 (s)	1.03 (s)
H-29	1.12 (s)	1.12 (s)	1.11 (s)	1.12 (s)	1.03 (s)
H-30	1.16 (s)	1.17 (s)	1.16 (s)	1.15 (s)	1.14 (s)

a) Run at 270 MHz. Chemical shifts are given on the δ (ppm) scale, and half-band width ($W_{1/2}$) and coupling constants are given in Hz (s, singlet; d, doublet; t, triplet; br, broad).

TABLE II. ^{13}C-NMR Chemical Shifts for **16**, **18**—**23** and Aglycone Moieties of **7**—**13**, **30**—**33** in C_5D_5N

Carbon No.	**18**[a]	**16**	**7**	**9**	**20**[b]	**19**	**8**	**10**	**11**
1	21.2	21.2	21.9	22.1	21.2	21.1	22.0	21.9	21.8
2	29.7	29.8	28.3	28.3	29.8	29.7	28.3	28.3	28.3
3	75.5	75.6	87.3	87.3	75.5	75.5	87.1	87.1	87.1
4	41.9	41.9	41.8	41.9	41.9	41.8	41.9	41.9	41.9
5	141.1	141.4	141.1	141.2	141.1	141.2	141.1	141.1	141.2
6	118.9	119.1	118.5	118.5	118.9	118.9	118.5	118.5	118.4
7	24.2[c]	24.2[c]	24.2[c]	24.0[c]	24.2[c]	24.0[c]	24.0[c]	23.7[c]	24.0[c]
8	44.1	43.5	43.2	43.3	44.1	43.4	43.2	43.2	43.9
9	49.1[d]	49.0[d]	48.9[d]	48.8[d]	49.1[d]	49.9[d]	49.8[d]	48.7[d]	49.0[d]
10	35.9	36.0	36.0	35.9	35.9	35.9	35.9	35.9	35.9
11	213.8	214.1	214.1	214.0	213.7	214.1	214.0	214.0	213.7
12	48.7	49.4	49.1	49.0	48.7	49.2	49.2	49.1	48.8
13	49.1[d]	50.3[d]	50.1[d]	50.2[d]	49.6[d]	50.2[d]	50.1[d]	50.1[d]	49.0[d]
14	49.4[d]	49.4[d]	49.1[d]	49.3[d]	49.6[d]	49.2[d]	49.2[d]	49.1[d]	49.6[d]
15	34.5	34.3	34.5	34.4	34.5	34.2	34.2	34.4	34.5
16	28.0	22.3	21.9	22.1	28.0	22.1	22.0	21.9	28.0
17	49.6	51.5	51.0	51.4	49.6	51.2	51.9	51.5	49.6
18	16.8[e]	19.2[e]	19.0[e]	19.0[e]	16.9[e]	19.0[e]	19.1[e]	19.1[e]	16.9[e]
19	20.1	20.2	20.2	20.2	20.2	20.1	20.2	20.2	20.2
20	35.9	73.9	73.8	73.8	36.0	73.8	73.8	73.8	35.9
21	18.2[e]	26.3[e]	25.8[e]	25.9[e]	18.3[e]	26.2[e]	25.8[e]	25.8[e]	18.1[e]
22	36.3	45.0	45.0	44.6	36.9	45.3	45.3	45.3	36.7
23	24.6[c]	23.2[c]	23.1[c]	23.1[c]	24.6[c]	23.0[c]	23.1[c]	23.0[c]	24.8[c]
24	124.9	125.0	125.0	128.6	127.1	127.2	127.2	130.9	130.0
25	136.1	136.2	135.8	132.2	136.4	136.0	136.1	131.8	132.2
26	68.0	68.0	67.9	75.4	21.8	21.8	20.2	20.2	21.8
27	13.9	13.9	13.8	14.2	60.8	60.7	60.7	66.8	67.2
28	18.4[e]	18.5[e]	18.3[e]	18.3[e]	18.5[e]	18.4[e]	18.4[e]	18.3[e]	18.4[e]
29	27.9[e]	28.0[e]	28.3[e]	28.3[e]	28.0[e]	27.9[e]	28.3[e]	28.3[e]	28.3[e]
30	26.3[e]	26.4[e]	26.3[e]	26.3[e]	26.3[e]	26.2[e]	26.3[e]	26.1[e]	25.8[e]

Carbon No.	**22**	**21**	**12**	**23**	**13**	**30**	**31**	**32**	**33**
1	25.8	25.7	26.2	24.7	23.9	22.0	22.1	26.3	26.3
2	30.8	30.7	28.3	30.4	29.3	28.3	28.3	29.5	29.5
3	76.3	76.2	87.8	76.0	87.5	87.1	87.1	87.9	87.9
4	42.3	42.3	42.3	41.8	41.8	41.9	41.9	42.3	42.3
5	144.2	144.2	144.2	142.9	142.9	141.2	141.2	144.2	144.2
6	119.2	119.1	118.4	119.8	119.3	118.4	118.4	118.4	118.4
7	24.6[c]	24.6[c]	25.0[c]	24.7	25.0[c]	24.1	24.0	24.5	24.5
8	43.6	43.6	43.5	41.8	41.9	43.9	43.9	43.5	43.5
9	40.2	40.1	40.0	40.8	40.7	49.0	49.0	40.1	40.1
10	36.6	36.8	36.7	39.9	40.0	35.9	35.9	36.3	36.3
11	77.8	77.8	77.9	72.1	72.1	213.7	213.7	77.8	77.8
12	41.1	41.1	41.0	39.3	39.3	48.7	48.7	41.1	41.1
13	47.4	47.3	47.3	45.7	45.7	49.5	49.5	47.3	47.3
14	49.8	49.7	49.7	49.9	49.9	49.8	49.8	49.7	49.7
15	34.5	34.5	34.5	35.2	35.2	34.5	34.5	34.5	34.5
16	28.4	28.3	28.3	28.1	28.2	28.3	28.3	28.3	28.3
17	50.7	50.5	50.7	51.2	51.2	49.0	49.0	51.0	51.0
18	17.0[d]	17.0[d]	16.9[d]	18.0[c]	18.0[d]	16.9[c]	16.9[c]	17.0[c]	17.0[c]

(continued)

Carbon No.	22	21	12	23	13	30	31	32	33
19	26.7[d]	26.7[d]	26.7[d]	22.8	22.9	20.2	20.2	26.8[c]	26.8[c]
20	36.2	36.0	36.1	36.3	36.3	36.6	35.9	36.8	36.8
21	18.8[d]	18.7[d]	18.7[d]	18.8[c]	18.8[d]	18.8[c]	18.5[c]	19.2[c]	19.2[c]
22	36.9	36.8	36.9	36.7	36.3	28.1	28.0	28.3	27.7
23	24.9[c]	24.7[c]	25.0[c]	24.7	24.7[c]	34.1	33.6	34.5	33.9
24	125.1	127.2	130.2	125.2	129.1	75.1	75.2	75.1	75.1
25	136.1	136.5	132.0	136.0	132.1	77.5	77.3	77.3	77.2
26	68.1	21.8	21.9	21.8	75.4	78.6	78.6	78.5	78.5
27	14.0	60.8	67.3	67.3	14.2	22.3	21.4	22.2	21.5
28	19.3[d]	19.3[d]	19.3[d]	18.0[c]	18.0[d]	18.2[c]	18.1[c]	19.2[c]	19.2[c]
29	27.3[d]	27.2[d]	27.6[d]	27.8[c]	28.2[d]	28.0[c]	28.0[c]	27.7[c]	27.7[c]
30	26.2[d]	26.1[d]	26.2[d]	26.3[c]	25.9[d]	25.9[c]	25.8[c]	26.3[c]	26.3[c]

a) The assignment was made based on comparison with the reference data on 4,4,14α-trimethyl-19(10→9β)abeo-10α-pregn-5-en-11-one, cucurbitacin C[11] and cycloart-24-ene-3β,26-diol.[12] *b*) The assignment of carbon signals due to the side chain was performed by reference to the carbon signal difference between 1-hydroxylinalool and 9-hydroxylinalool.[13] *c*—*e*) These assignments may be interchanged in each column.

TABLE III. ^{13}C-NMR Chemical Shifts for Sugar Moieties of **2**, **7**—**13**, **30**—**33** in C_5D_5N

Carbon No.	7	9	8	10	2	11	12	13	30	31	32	33
3-Sugar												
Glc-1	106.9	107.2	107.1	107.1	107.1	107.2	107.1	107.3	107.2	107.2	107.2	107.2
Glc-2	75.2	75.1	75.3	75.2	74.9	75.4	75.4	75.0	74.9[a]	74.8[a]	74.9	74.9
Glc-3	78.2[a]	78.5[a]	78.5[a]	78.4[a]	78.5[a]	78.4[a]	78.4[a]	78.4[a]	78.3[b]	78.3[b]	78.3[a]	78.3[a]
Glc-4	71.4	71.7	71.5	71.7	71.5[b]	71.5[b]	71.4[b]	71.6	71.4[c]	71.7[c]	71.4[b]	71.4
Glc-5	77.8[a]	78.1[a]	78.0[a]	78.1[a]	78.2[a]	78.1[a]	78.0[a]	78.1[a]	78.1[b]	78.1[b]	78.1[a]	78.1[a]
Glc-6	62.5	62.8	62.8	62.8	62.8[c]	62.7	62.9[c]	63.1[b]	62.9[d]	62.9[d]	62.9[c]	62.6
26- or 27-Sugar												
Inner												
Glc-1		103.4		102.4	101.7	101.3	101.2	103.4	105.9	105.8	105.8	105.8
Glc-2		75.1		74.9	83.7	83.7	83.4	75.4	74.9[a]	75.2[a]	74.9	74.9
Glc-3		78.5[a]		78.4[a]	78.0[a]	78.4[a]	78.4[a]	78.4[a]	78.3[b]	78.1[b]	78.3[a]	78.3[a]
Glc-4		71.7		71.7	71.6[b]	71.3[b]	71.7[b]	71.6	71.6[c]	71.7[c]	71.7[b]	71.4
Glc-5		78.3[a]		78.1[a]	78.0[a]	78.1[a]	78.0[a]	77.2	76.8	76.7	76.8	76.8
Glc-6		62.8		62.8	62.9[c]	62.7	62.6[c]	70.0	70.1	70.1	70.1	70.1
Terminal												
Glc-1′					106.1	106.2	106.0	105.4	105.1	105.0	105.1	105.0
Glc-2′					76.6	76.7	76.6	75.0	75.4[a]	74.8[a]	74.9	74.9
Glc-3′					78.2[a]	78.1[a]	78.4[a]	78.4[a]	78.1[b]	78.3[b]	78.3[a]	78.3[a]
Glc-4′					71.6[b]	71.5[b]	71.2[b]	71.6	71.6[c]	71.4[c]	71.7[b]	71.4
Glc-5′					77.9[a]	78.1[a]	78.0[a]	78.4[a]	78.1[b]	78.1[b]	78.3[a]	78.3[a]
Glc-6′					62.5[c]	62.7	62.4[c]	62.7[b]	62.5[d]	62.5[d]	62.6[c]	62.6

a—*d*) Assignments may be interchanged in each column.

sucrose is also given in Table V except for **25**. From these results, the following structure–taste relationships were proposed for glycosides of 3β-hydroxy-cucurbit-5-ene derivatives.

The presence of at least three sugar units in the molecule is essential for the occurrence of taste. Monoglucosides **7**, **8** and **26**, diglucosides **9**, **10** and **27** and glucobiosides **1** and **28** are all tasteless.

TABLE IV. ^{13}C-NMR Chemical Shifts for **14** and **15** in C_5D_5N

Carbon No.	**15**[4]	**14**	Carbon No.	**15**	**14**	Carbon No.	**15**	**14**
1	33.1	33.2	16	70.4	70.3	25-OAc	170.1	
2	82.9	83.2	17	58.7	58.6		22.2	
3	80.5	80.6	18	20.1	20.2	Glc-1	106.1	106.4
4	42.4	42.5	19	18.9	19.0	Glc-2	75.5	75.8
5	141.5	141.6	20	81.5	80.0	Glc-3	78.1	78.5
6	118.8	118.9	21	24.1[c]	25.3[c]	Glc-4	71.1	71.3
7	25.0	24.1	22	214.9	215.9	Glc-5	78.1	78.5
8	34.0	34.2	23	31.9	32.6	Glc-6	62.3	62.5
9	48.4[b]	48.8[b]	24	35.1	38.4			
10	43.0	43.0	25	79.9	71.3			
11	213.0	213.0	26	26.0	29.9[d]			
12	49.1[a]	49.1[a]	27	26.0	30.1[d]			
13	48.4[b]	48.6[b]	28	20.1[c]	22.2[c]			
14	50.8[b]	51.0[b]	29	22.2[c]	22.3[c]			
15	46.5[a]	46.3[a]	30	25.0[c]	25.3[c]			

a—*d*) Assignments may be interchanged in each column.

TABLE V. Taste and Relative Sweetness

Compound	Conc.[a] (%)	Taste	Relative sweetness[b]
1, **7**—**10**, **13**, **26**—**28**		Tasteless	
2—**4**, **11**, **29**		Bitter	
5	0.025	Sweet	51
6	0.025	Sweet	77
12	0.025	Sweet	54
24[7]	0.02	Sweet	250
25[8]		Sweet	
30	0.05	Sweet	21
31	0.05	Sweet	16
32	0.05	Sweet	24
33	0.05	Sweet	25
34	0.05	Sweet	33

a) Concentration of sample in aqueous solution (w/v %). *b*) Sucrose = 1.

The oxygen function at the 11-position of the aglycone is responsible for the occurrence of taste. The 11α-hydroxy compounds, **5**, **6**, **12** and **24** taste sweet, while the 11β-hydroxy epimer (**13**) is tasteless. On the other hand, the 11-oxo compounds, **2**, **3**, **4** and **11** as well as the dihydro derivative (**29**) (Chart 2) prepared from **3** taste bitter.

It is notable that the hydroxylation of the side chain (Chart 2) also affects the taste. 11-Oxo-24,25-dihydroxy-glycoside (**25**) was reported to be sweet[8] and the bitter 11-oxo-glycoside (**3**) became sweet on hydroxylation of the side chain double bond with osmium tetroxide (isomers **30** and **31**). In contrast to this, hydroxylation of the side chain double bond of the sweet 11α-hydroxy-glycoside (**6**) resulted in a slight decrease of sweetness (**32** and **33**). Hydrogenation of the side chain double bond of the sweet glycoside (**6**) to give the dihydro-glycoside (**34**) also led to a slight decrease of sweetness. To date, mogroside (**24**), which is a pentaglucoside of the 3β,11α,24,25-tetrahydroxy-aglycone, is the sweetest compound among glycosides of this type.

Experimental

General Procedure——Melting points were determined on a Yanaco micro hot stage and are uncorrected. Optical rotations were measured with a Union PM-101 automatic digital polarimeter. IR spectra were taken on a Shimadzu IR-408 spectrometer. NMR spectra were recorded on JEOL FX-100 and GX-270 instruments using tetramethylsilane (TMS) as an internal standard. For gas liquid chromatography (GLC), a Shimadzu GC-8A apparatus was used. MS were taken on a JEOL JMS-01-SG-2 spectrometer by the direct inlet method; ionization voltage 75 eV. For column chromatography, silica gel (Kieselgel 60, 70—230 mesh, Merck), silanized silica gel (LiChroprep RP-8, 40—63 μm, Merck), and highly porous polymer [DIAION HP-20 (Mitsubishi Chem. Ind. Co., Ltd., Tokyo) and DA-120 (made in China)] were used. All solvent systems for chromatography were homogeneous. Acid hydrolysis of glycosides followed by identification of the resulting monosaccharides (including absolute configuration)[9] was carried out as described in the previous paper.[10]

Plant material was cultivated and harvested in the Botanical Garden of Kunming Institute of Botany, Yunnan, China and authenticated by Emeritus Professor Cheng-Yih Wu of this Institute. A specimen has been deposited in the Herbarium of this Institute.

Extraction and Separation of Glycosides——Dried rhizomes of *Hemsleya panacis-scandens* were extracted with EtOH, and the EtOH extract was concentrated to dryness. The residue was suspended in H_2O and then extracted with Et_2O, AcOEt and 1-BuOH. The 1-BuOH extract was chromatographed on a column of DA-201 and eluted with H_2O, 95% EtOH and EtOH. The fraction eluted with 95% EtOH was chromatographed on silica gel ($CHCl_3$–MeOH–H_2O, 50 : 10 : 1) to give fractions I—X. Fraction VIII was subjected to a column of silica gel ($CHCl_3$–MeOH–H_2O, 10 : 5 : 1) to give **3** in a yield of 1.8% from dried rhizomes. Fractions I—VII, IX and X were each subjected to a column of silanized silica gel (63—70% MeOH) to afford **1**, **2** and **5**—**15** in yields of 0.05, 0.16, 0.03, 0.27, 0.02, 0.03, 0.15, 0.27, 0.15, 0.08, 0.03, 0.02 and 0.02%, respectively. Compounds **1**—**3**, **5**, **6** and **13** were identified by direct comparison (thin layer chromatography (TLC), $[\alpha]_D$, ^{1}H- and ^{13}C-NMR) with authentic samples. The identification of **15** was established by comparison ($[\alpha]_D$, ^{1}H- and ^{13}C-NMR) with reference data.[4]

Scandenoside R1 (**7**): A white powder, $[\alpha]_D^{24}$ +83.8° (c=0.68, MeOH). *Anal.* Calcd for $C_{36}H_{58}O_9 \cdot H_2O$: C, 66.23; H, 9.26. Found: C, 66.28; H, 9.29. IR (Nujol) cm^{-1}: 3350 (OH), 1680 (C=O). ^{1}H-NMR (100 MHz, C_5D_5N) δ: anomeric H, 4.76 (1H, d, J=7 Hz).

Scandenoside R2 (**8**): A white powder, $[\alpha]_D^{24}$ +86.0° (c=0.8, MeOH). *Anal.* Calcd for $C_{36}H_{58}O_9 \cdot H_2O$: C, 66.23; H, 9.26. Found: C, 66.29; H, 9.36. IR (Nujol) cm^{-1}: 3400 (OH), 1680 (C=O). ^{1}H-NMR (100 MHz, C_5D_5N) δ: anomeric H, 4.68 (1H, d, J=7 Hz).

Scandenoside R3 (**9**): A white powder, $[\alpha]_D^{24}$ +48.0° (c=0.97, MeOH). *Anal.* Calcd for $C_{42}H_{68}O_{14} \cdot 3/2H_2O$: C, 61.22; H, 8.69. Found: C, 61.29; H, 8.72. IR (Nujol) cm^{-1}: 3350 (OH), 1680 (C=O). ^{1}H-NMR (100 MHz, C_5D_5N) δ: anomeric H, 4.79 (1H, d, J=8 Hz) and 4.82 (1H, d, J=7 Hz).

Scandenoside R4 (**10**): A white powder, $[\alpha]_D^{24}$ +69.7° (c=0.75, MeOH). *Anal.* Calcd for $C_{42}H_{68}O_{14} \cdot 3/2H_2O$: C, 61.22; H, 8.69. Found: C, 61.18; H, 8.61. IR (Nujol) cm^{-1}: 3350 (OH), 1680 (C=O). ^{1}H-NMR (100 MHz, C_5D_5N) δ: anomeric H, 4.72 (1H, d, J=8 Hz) and 4.52 (1H, d, J=8 Hz).

Scandenoside R5 (**11**): A white powder, $[\alpha]_D^{23}$ +59.7° (c=0.39, MeOH). *Anal.* Calcd for $C_{48}H_{78}O_{18} \cdot 2H_2O$: C, 58.88; H, 8.44. Found: C, 58.91; H, 8.52. IR (Nujol) cm^{-1}: 3400 (OH), 1680 (C=O). ^{1}H-NMR (100 MHz, C_5D_5N) δ: anomeric H, 4.82 (1H, d, J=7 Hz), 4.90 (1H, d, J=7 Hz) and 5.28 (1H, d, J=8 Hz).

Scandenoside R6 (**12**): A white powder, $[\alpha]_D^{22}$ +15.9° (c=0.76, MeOH). *Anal.* Calcd for $C_{48}H_{80}O_{18} \cdot 3/2H_2O$: C, 59.30; H, 8.61. Found: C, 59.12; H, 8.54. IR (Nujol) cm^{-1}: 3400 (OH), 1640, 1165 (C=C). ^{1}H-NMR (100 MHz, C_5D_5N) δ: anomeric H, 4.77, 4.84 and 5.22 (each 1H, d, J=8 Hz). The ^{13}C-NMR data of **7**—**12** are given in Tables II and III.

23,24-Dihydrocucurbitacin F glucoside (**14**): A white powder, $[\alpha]_D^{22}$ +41.2° (c=0.57, MeOH). ^{1}H-NMR (100 Hz, C_5D_5N) δ: anomeric H, 5.30 (1H, d, J=7 Hz), which was identified by direct comparison (TLC, $[\alpha]_D$, ^{1}H- and ^{13}C-NMR) with deacetylated **15**. The ^{13}C-NMR data are given in Table IV.

Enzymatic Hydrolysis of Glycosides[5]——A solution of **7** (100 mg) and crude pectinase (200 mg, Tanabe Pharm. Ind. Co., Ltd., Osaka, Japan) in H_2O (10 ml) was incubated at 37 °C for 24 h. The hydrolysate was chromatographed on Diaion HP-20 (H_2O and then MeOH). The MeOH eluate was further purified by column chromatography on silica gel ($CHCl_3$–MeOH, 10 : 1), affording **16** (35 mg). Enzymatic hydrolysis of **9** (130 mg) under the same conditions as used for **7** also afforded **16** (35 mg): A white powder, $[\alpha]_D^{22}$ +125.7° (c=0.35, MeOH). EI-MS m/z: 454 ($M^+ - H_2O$). *Anal.* Calcd for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 75.83; H, 10.39. IR ($CHCl_3$) cm^{-1}: 3450 (OH), 1690 (C=O). Compound **16** (10 mg) was acetylated with acetic anhydride and pyridine at room temperature to give **17** (13 mg): A white powder, $[\alpha]_D^{22}$ +13.3° (c=0.09, $CHCl_3$). IR (CCl_4) cm^{-1}: 3500 (OH), 1720 (C=O), 1690 (C=O). ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 2.02 and 2.07 (each 3H, s, CH_3COO–).

Hydrolysis of **8** (150 mg) and **10** (260 mg) with crude pectinase under the same conditions as used for **7** gave a common aglycone (**19**) (57 and 100 mg, respectively): Colorless needles (from EtOAc–n-C_6H_{14}), mp 148—149 °C, $[\alpha]_D^{22}$ +137.8° (c=0.45, MeOH). EI-MS m/z: 454 ($M^+ - H_2O$). *Anal.* Calcd for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.10; H, 10.50. IR ($CHCl_3$) cm^{-1}: 3450 (OH), 1690 (C=O).

On enzymatic hydrolysis under the same conditions as used for **7**, **11** (100 mg), **12** (100 mg) and **13** (100 mg) afforded **20** (42 mg), **21** (45 mg) and **23** (44 mg), respectively. The identification of **20** was established by mixed melting point determination and $[\alpha]_D$, ^{1}H- and ^{13}C-NMR comparisons with an authentic sample. Compound **21**: Colorless plates (from C_6H_6–Me_2CO), mp 120—122 °C, $[\alpha]_D^{22}$ +50.0° (c=0.44, MeOH). EI-MS m/z: 458 (M^+), 440 (M^+-H_2O), 425 (440−CH_3). *Anal.* Calcd for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 78.13; H, 10.81. IR ($CHCl_3$) cm^{-1}: 3450 (OH), 1640, 1165 (C=C). Compound **23**: Colorless plates (from EtOAc–isopropyl ether), mp 152—154 °C, $[\alpha]_D^{22}$ +50.0° (c=0.38, MeOH). EI-MS m/z: 458 (M^+), 440 (M^+-H_2O), 425 (440−CH_3). *Anal.* Calcd for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 78.29; H, 10.86. IR ($CHCl_3$) cm^{-1}: 3450 (OH), 1640, 1165 (C=C).

The ^{1}H- and ^{13}C-NMR data of **16**, **19**, **21** and **23** are given in Tables I and II, respectively.

OsO_4 Oxidation of 3 and 6——A solution of OsO_4 (100 mg) in dry C_5H_5N (1 ml) was added to a solution of acetylated **3** (400 mg) in dry C_5H_5N (6 ml) and the mixture was allowed to stand overnight with stirring at room temperature. Then, a solution of Na_2SO_3 (700 mg) in H_2O (4 ml) was added, and the whole was refluxed for 3 h. After cooling, the reaction mixture was diluted with EtOH (10 ml), and the precipitate was filtered off. The filtrate was extracted with $CHCl_3$, and the extract was evaporated. The residue was dissolved in 2% KOH–MeOH (20 ml), and the solution was allowed to stand for 6 h with stirring at room temperature. Then, the solution was neutralized with Amberlite MB-3 and evaporated to give a crude product, which was purified by preparative high-performance liquid chromatography (HPLC) on a TSK-GEL ODS-120T column with 53% MeOH as the eluent to give **30** (55 mg) and **31** (48 mg). Compound **30**: A white powder, $[\alpha]_D^{18}$ +42.0° (c=0.88, MeOH). *Anal.* Calcd for $C_{42}H_{70}O_{16}\cdot 5/2H_2O$: C, 56.40; H, 8.38. Found: C, 56.22; H, 8.32. Compound **31**: A white powder, $[\alpha]_D^{18}$ +54.9° (c=1.02, MeOH). *Anal.* Calcd for $C_{42}H_{70}O_{16}\cdot 5/2H_2O$: C, 56.40; H, 8.38. Found: C, 56.29; H, 8.41.

Oxidation of acetylated **6** (300 mg) with OsO_4 under the same conditions as used for **3** afforded **32** (75 mg) and **33** (60 mg). Compound **32**: A white powder, $[\alpha]_D^{22}$ −3.0° (c=0.67, MeOH). *Anal.* Calcd for $C_{42}H_{72}O_{16}\cdot H_2O$: C, 57.82; H, 8.49. Found: C, 57.57; H, 8.61. Compound **33**: A white powder, $[\alpha]_D^{22}$ +13.2° (c=0.38, MeOH). *Anal.* Calcd for $C_{42}H_{72}O_{16}\cdot H_2O$: C, 57.82; H, 8.49. Found: C, 57.63; H, 8.66. The ^{13}C-NMR data of **30**—**33** are given in Tables II and III.

Catalytic Hydrogenation of 3 and 6——A solution of **6** in EtOH (15 ml) containing Pd-black (10 mg) was stirred under an H_2 atmosphere at room temperature overnight. Work-up afforded the crude product, which was purified by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (10 : 5 : 1) to give **34** (50 mg) (mixture of 25-epimers) as a white powder.

Hydrogenation of **3** (50 mg) under the same conditions as used for **6** gave **29** (75 mg) (mixture of 25-epimers) as a white powder.

Sensory Evaluation of Sweetness——Sweetness relative to sucrose was evaluated by a human sensory panel. All samples were dissolved in H_2O to make a 0.025% (w/v) solution for **5**, **6** and **12**, and a 0.05% (w/v) solution for **30**—**34**. Sucrose solutions were prepared at graduated concentrations from 0.8 to 3.2% (w/v) with intervals of 0.4% (w/v). The panelists were asked to taste a sucrose solution and estimate its total taste intensity relative to that of the sample solution. In this way, **5**, **6** and **12** were tested twice, and **30**—**34** were tested once. From the above results, a point of subjective equality (PSE) was calculated according to the following formula;

$$L_u=\Sigma X_u/k\,,\qquad L_l=\Sigma X_l/k\,,\qquad \mathrm{PSE}=L_u-L_l/2$$

X_u: upper differential limen of each evaluation
X_l: lower differential limen of each evaluation
L_u: upper differential limen
L_l: lower differential limen
k: number of tests

The sweetness of each sample relative to sucrose was calculated by means of the following formula;

$$B/A\times 100$$

A: concentration (%) of sample solution
B: concentration (%) of sucrose with the same sweetness as sample solution (PSE)

Acknowledgment We are grateful to Dr. H. Matsuura, Wakunaga Pharmaceutical Co., Ltd., for determination of NMR spectra. The authors thanks are also due to Mr. S. Ogawa, Maruzen Kasei Co., Ltd., for evaluation of sweetness. This study was supported by a Grant-in-Aid for scientific Research (to. O. Tanaka, No. 62570940, 1987—1988) from the Ministry of Education, Science and Culture of Japan.

References

1) R.-L. Nie, T. Morita, R. Kasai, J. Zhou, C.-Y. Wu and O. Tanaka, *Planta Medica*, **50**, 322 (1984).
2) R. Kasai, K. Matsumoto, R.-L. Nie, T. Morita, A. Awazu, J. Zhou and O. Tanaka, *Phytochemistry*, **26**, 1371

(1987).
3) R.-L. Nie, Q. Wang and J. Zhou, *Acta Botanica Yunnanica*, **7**, 355 (1985).
4) H. Rui, M. Yuan, Q. Yu, X. Ye, G. Qain and K. Wang, *Acta Pharmaceutica Sinica*, **16**, 445 (1981).
5) H. Kohda and O. Tanaka, *Yakugaku Zasshi*, **95**, 246 (1975).
6) R. Kasai, M. Suzuo, J. Asakawa and O. Tanaka, *Tetrahedron Lett.*, **1977**, 175.
7) T. Takemoto, S. Arihara, T. Nakajima and M. Okuhira, *Yakugaku Zasshi*, **103**, 1015 (1983).
8) P. Tunmann, W. Gerner and G. Stapel, *Arch. Pharm.*, **299**, 597 (1966).
9) R. Oshima, J. Kumanotani and C. Watanabe, *J. Chromatogr.*, **259**, 159 (1983).
10) K. Matsumoto, R. Kasai, F. Kanamaru, H. Kohda and O. Tanaka, *Chem. Pharm. Bull.*, **35**, 413 (1987).
11) J. R. Bull, A. A. Chalmers and P. C. Coleman, *S. Afr. J. Chem.*, **32**, 27 (1979).
12) V. Anjaneyulu, K. H. Prasad, K. Ravi and J. D. Connolly, *Phytochemistry*, **24**, 2359 (1985).
13) R. Tcheshe, F. Ciper and E. Breitmaier, *Chem. Ber.*, **110**, 3111 (1977).