Sterol Compositions of Seeds and Mature Plants of Family Cucurbitaceae

Toshihiro Akihisa (ne Itoh)*,[a], P. Ghosh[b], Swapnadip Thakur[b], Fumiko U. Rosenstein[c] and Taro Matsumoto[a]
[a]College of Science and Technology, Nihon University; [b]Department of Chemistry, University of Burdwan, Burdwan, India, and [c]Department of Nutrition and Food Science, College of Agriculture, University of Arizona, Tucson, Arizona.

The sterol fractions of the unsaponifiable lipids obtained from 32 seed and mature plant (leaves and stems, pericarp of the fruit, and roots) materials from the 12 genera *Apodanthera, Benincasa, Citrullus, Coccinea, Cucumis, Cucurbita, Gynostemma, Lagenaria, Luffa, Momordica, Sechium* and *Trichosanthes*, of the family Cucurbitaceae were investigated by gas liquid chromatography (GLC) on an OV-17 glass capillary column. Among the 23 sterols with Δ^5-, Δ^7- and Δ^8-skeletons identified by GLC, the Δ^7-sterols were found to be the major sterols of most of the Cucurbitaceae investigated. The seed materials contained 24-ethyl-Δ^7-sterols possessing Δ^{25}-bonds, i.e. 24-ethylcholesta-7,25-dienol and 24-ethylcholesta-7,22,25-trienol, whereas the mature plant materials contained 24-ethyl-Δ^7sterols without a Δ^{25}-bond, i.e. 24-ethylcholest-7-enol and 24-ethylcholesta-7,22-dienol, as the most predominant sterols, with a few exceptions. The isolation and identification of 24α-ethylcholesta-8(14),22-dienol from the aerial parts of *Cucumis sativus* also is described.

Previous studies on the sterol constituents of seeds (1–12) and mature plants (13–17) of the family Cucurbitaceae have shown the occurrence of 24-ethyl-Δ^7-sterols, such as 24-ethylcholest-7-enol (19), 24-ethylcholesta-7,22-dienol (15), 24-ethylcholesta-7,25-dienol (20), and 24-ethylcholesta-7,22,25-trienol (17), as the major components accompanied with other 24-methyl-Δ^7-sterols. Several Δ^5-sterols (2,4–6,10,11) and saturated sterols (2,9) also have been detected as the minor constituents. The stereochemistry at C-24 of the 24-ethyl-Δ^7-sterols from Cucurbitaceae has been studied, and sterols 17 and 20, which contain Δ^{25}-bonds, have been established to be the 24β-epimers (3,8,10,11,17), whereas the occurrence either of 24α- (3,11,15,16) or 24β-epimers (7), or the co-occurrence of both of the C-24 epimers (8,10,12,17) have been demonstrated for sterols 15 and 19, which lack a Δ^{25}-bond. Recently we have undertaken a further investigation on the sterols of some Cucurbitaceae seeds and mature plants which revealed the presence of several Δ^8-sterols besides saturated-, Δ^5, and Δ^7-sterols (18). This demonstrated, moreover, that most of the 24-alkylsterols possessing 24-methyl-, 24-methyl-Δ^{22}-, 24-ethyl-, and 24-ethyl-Δ^{22}-side chains, which lack a Δ^{25}-bond, occur as the C-24 epimeric mixtures, whereas 24-ethylsterols, which contain a Δ^{25}-bond, are, consistent with previous observations (3,8,10, 11,17), constituted only of the 24β-epimers (18).

The predominance of Δ^7-sterols, the presence of Δ^8-sterols (lacking a 4-methyl group) and Δ^{25}-sterols together with the occurrence of significant amounts of 24β-alkylsterols constituted the characteristic features of the plants of the family Cucurbitaceae since the great majority of higher plants contain predominantly 24α-alkyl-Δ^5sterols and no other higher plant has been shown so far to contain $\Delta^{8(9)}$-sterols (19–21). Thus, it was worthwhile to examine more extensively the sterol constituents of the members of this unique family. This study presents the compositions of sterol fractions obtained from 32 seed and mature plant materials from species of 12 genera of Cucurbitaceae by GLC on an OV-17 glass capillary column.

*To whom correspondence should be addressed at College of Science and Technology, Nihon University, 1-8, Kanda Surugadai, Chiyoda-ku, Tokyo, 101, Japan.

EXPERIMENTAL

Materials, extraction and separation of sterol fraction. Thirty-two seed and mature plant materials obtained from 22 species of 12 genera of the family Cucurbitaceae used in this study are shown in Table 1. The plant materials were obtained from the following eight sources: A, B, and C, Sakata Seeds Co. (Yokohama, Japan), Y. Ohshima (Tochigi, Japan), and S. Miyake (Okinawa, Japan), respectively; D and E, Kinokuniya Kan-yaku Kyoku Co. (Tokyo, Japan) and Tochigi Seeds Co. (Tochigi, Japan), respectively; F and G, collected locally in the U.S. (22) and India, respectively; and H, purchased locally in Japan. Twenty-four sterols (1–24), as shown in Table 2, were used as the authentic compounds, of which 23 (1–23) were those identified and characterized recently in Cucurbitaceae (18). The origin of sterol 24 was described in our previous article (23).

Extraction of lipid from dried materials was performed by either of the following four methods: (a), extracted with $CHCl_3$-MeOH (2:1) at room temperature under stirring; (b) and (c), with CH_2Cl_2 and $CHCl_3$ in a Soxhlet extractor, respectively; and (d), with MeOH under reflux, and the extract was then treated with cold acetone to remove insoluble phospholipid; the acetone soluble portion was subjected to subsequent work. The extracted lipid was saponified with 5% KOH in EtOH solution under reflux, and the unsaponifiable lipid was extracted with isopropyl ether. The sterol fraction was separated from the unsaponifiable lipid by preparative thin layer chromatography (TLC) and then acetylated in Ac_2O-pyridine at room temperature overnight. The acetylated sterol fraction was subjected to GLC analysis.

Chromatography, spectroscopy and hydrogenation. Preparative TLC on silica gel (20 × 20 cm, 0.5 mm thick) was developed three times using *n*-hexane-EtOAc (6:1), and argentic ($AgNO_3$-silica gel, 1:4) preparative TLC (20 × 20 cm, 0.5 mm thick) was developed four times with CCl_4-CH_2Cl_2 (5:1). GLC was performed with a Shimadzu GC-4CM instrument on an OV-17 SCOT glass capillary column (30 m × 0.3 mm id, column temp 260 C) under the conditions already described (23). HPLC was carried out on a Partisil 5 ODS-2 column (25 cm× 10 mm id) with MeOH as the mobile phase. The RRts (relative retention times) in the GLC and HPLC were expressed relative to cholesterol acetate (1.00). Table 2 shows the RRts in GLC of the authentic sterol (1–24) acetates. Mass spectra (EI-MS, 70 eV) were recorded by means of a probe injec-

TABLE 1

Cucurbitaceae Plants Investigated and the Sterol Contents of Dried Plant Materials

Cucurbitaceae	Plant material	Source of plant material[a]	Lipid extraction[a]	Content of sterol mixture[b]
Apodanthera undulata Gray	seeds	F	(a)	(339)
Benincasa cerifera Savi (wax gourd)	seeds	A	(b)	38
Citrullus battich Forskål (watermelon)	seeds	A	(b)	111
	aerial parts	H	(d)	33
Coccinea grandis Voigt (ivy gourd)	seeds	G	(c)	
Cucumis melo L. (melon) (I)	seeds	E	(b)	91
(II)	seeds	G	(c)	(663)
C. sativus L. (cucumber)	seeds	A	(b)	63
	aerial parts	H	(d)	63
Cucurbita digitata	seeds	F	(a)	(187)
C. foetidissima HBK (buffalo gourd)	seeds	F	(a)	(127)
C. maxima Duschesne (squash)	seeds	A	(b)	52
C. pepo L. (pumpkin)	seeds	A	(b)	83
Gynostemma pentaphyllum Makino	aerial parts	D	(d)	43
Lagenaria leucantha Rusby var. *Gourda* Makino (bottle gourd)	seeds	A	(b)	105
L. leucantha Rusby var. *depressa* Makino	seeds	E	(b)	51
	pericarp	B	(b)	22
L. leucantha Rusby	seeds	G	(c)	(887)
Luffa acutangula Roxb.	seeds	G	(c)	(654)
L. cylindrica Roem. (sponge gourd) (I)	seeds	A	(b)	53
(II)	seeds	G	(c)	(316)
Momordica charantia L. (balsam pear)	seeds	A	(b)	73
	aerial parts	G	(c)	
M. charantia L. var. *Pavel* Crantz	seeds	C	(b)	35
M. cochinchinensis	seeds	G	(c)	(218)
	aerial parts	G	(c)	
Sechium edule Sw. (chayote)	aerial parts	H	(d)	16
	pericarp	H	(b)	38
Trichosanthes anguina L. (serpent cucumber)	seeds	G	(c)	(344)
T. dioica	roots	G	(c)	
T. japonica Regel	seeds	A	(b)	83
	roots	D	(d)	8

[a]See Experimental Section.
[b]Contents of sterols (mg) from dried plant material (seeds or mature plants) (100 g). Figures in parentheses denote contents of sterols (mg) from the extracted lipid (100 g).

tion, and ^{1}H NMR spectra (250 MHz) were determined in $CDCl_3$ with tetramethylsilane as internal standard. Hydrogenation was performed in EtOH over PtO_2 at atmospheric pressure and temperature overnight.

RESULTS AND DISCUSSION

Table 1 shows the contents of sterols of the dried plant materials (seeds and mature plants) or of the extracted lipids. The compositions of the sterol fractions of the seed and mature plant [aerial parts (leaves and stems), pericarp of the fruits, and roots] materials of Cucurbitaceae were indicated in Tables 3 and 4, respectively, which were determined by GLC on an OV-17 glass capillary column. Identification of 23 sterols (**1–23**, cf. Table 2) for the following plant materials: the seeds of *Benincasa cerifera, Cucumis sativus, Cucurbita maxima, C. pepo,* and *Trichosanthes japonica;* and the aerial parts of *Citrullus battich, Cucumis sativus,* and *Gynostemma pentaphyllum,* as shown in Tables 3 and 4, already has been performed by GLC, argentic TLC, combined gas chromatography-mass spectrometry (GC-MS), and further by high-resolution MS and ^{1}H NMR, and ^{13}C NMR spectroscopy for some isolated sterols, in our recent study (18). The major sterols in the seeds (8) of *Citrullus battich* and *Lagenaria leucantha* var. *Gourda* and the roots (17) of *Trichosanthes japonica* also were fully characterized in our previous study. Identification of the sterols from other plant materials is tentative (by GLC) in this study because it can be expected that the sterol constituents among the members within the same plant family will be similar (21). Although GLC on an OV-17 glass capillary column showed excellent separation among the component peaks of the cucurbitacious sterol mixtures, the components of each of the following three sets of sterols, 24-methylenecholesterol (**5**) and 24-methylcholesta-7,22-dienol (**6**); 24-ethylcholesterol (**12**), 24-ethylcholsta-8,22,25-tirenol (**13**), and 24-ethylcholesta-5,25-dienol (**14**); and 24-ethylcholest-7-enol (**19**) and 24-ethylcholesta-7,25-dienol (**20**), remain unresolved in this study.

Most of the cucurbitacious seeds investigated contained Δ^7-sterols (**6,10,11,15,17,19–23**) in consequential

TABLE 2

RRts in GLC of the Acetyl Derivatives of Authentic Sterols

	Sterol[a] acetate	RRt
1	Cholesterol (cholest-5-enol)	1.00
2	24-Methylcholesta-5,22-dienol	1.14
3	Desmosterol (cholesta-5,24-dienol)	1.21
4	24-Methylcholesterol	1.31
5	24-Methylenecholesterol	1.35
6	24-Methylcholesta-7,22-dienol	1.36
7	24-Ethylcholesta-5,22-dienol	1.43
8	24-Ethylcholesta-5,22,25-trienol	1.52
9	24-Ethylcholesta-8,22-dienol	1.54
10	24-Methylcholest-7-enol	1.55
11	24-Methylenecholest-7-enol	1.61
12	24-Ethylcholesterol	1.63
13	24-Ethylcholesta-8,22,25-trienol	1.63
14	24-Ethylcholesta-5,25-dienol	1.64
15	24-Ethylcholesta-7,22-dienol	1.70
16	24-Ethylcholesta-8,25-dienol	1.74
17	24-Ethylcholesta-7,22,25-trienol	1.80
18	28-Isofucosterol (24Z-ethylidenecholesterol)	1.82
19	24-Ethylcholest-7-enol	1.94
20	24-Ethylcholesta-7,25-dienol	1.95
21	28-Isoavenasterol (24*E*-ethylidenecholest-7-enol)	2.04
22	Avenasterol (24Z-ethylidenecholest-7-enol)	2.15
23	Peposterol (24-ethylcholesta-7,24-dienol)	2.31
24	24-Ethylcholest-8(14)-enol	1.67

[a]The hydroxyl group at C-3 of all sterols is β-oriented. Sterols with a Δ^7-, Δ^8-, or a $\Delta^{8(14)}$-unsaturated ring system are 5α-sterols. All C-22–C-23 double bonds are *trans (E)* oriented.

amounts (over 70%) in the sterol fractions. Among these, two 24-ethylsterols possessing Δ^{25}-bonds, 24-ethylcholesta-7,22,25-trienol (**17**) and 24-ethylcholesta-7,25-dienol (**20**), and 24-ethylcholesta-7,22-dienol (**15**) and 24-ethylcholest-7-enol (**19**) were the major sterols, a result consistent with previous observations (2,4–6,10,11,18). Although sterols **19** and **20** were not distinguished in this study, previous studies (4,5,10,11,18) have suggested that sterol **20** predominates. Thus, the seeds of Cucurbitaceae contain 24-ethyl-Δ^7-sterols with Δ^{25}-bonds, **17** and **20**, as the principal sterols. There are a few exceptions, such as have been observed for the seeds of *Coccinea grandis* and *Momordica cochinchinensis,* that contained an unusually high proportion (ca. 30%) of sterols with RRt 1.63 (**12**,**13**,**14**) and among which 24-ethylcholesterol (**12**) is considered to be the major sterol (18). In addition to the Δ^7-sterols, this study demonstrated the widespread occurrence of several Δ^5- (**1–5**,**7**,**8**,**12**,**14**,**18**) and Δ^8- (**9**,**13**,**18**) sterols as the minor sterol components of cucurbitacious seeds. The occurrence of the Δ^8-sterols (lacking a 4-methyl group) in higher plants was demonstrated in some Cucurbitaceae species, for the first time, by our recent study (18).

The mature plant materials of Cucurbitaceae studied also contained Δ^7-sterols as the major sterols accompanied with minor amounts of Δ^5- and Δ^8-sterols as was observed with the seed materials. The most predominant components were, however, 24-ethylcholesta-7,22-dienol (**15**) and the mixture of sterols **19** and **20** (RRt = 1.94 in GLC) of which the former, 24-ethylcholest-7-enol (**19**), is considered to be predominant (17,18). Therefore, 24-ethyl-Δ^7-sterols which lack a Δ^{25}-bond, **15** and **19**, are the principal sterols of most of the mature plant materials of Cucurbitaceae, again consistent with previous observations (15,17,18). Thus, the present work has demonstrated the existence of significant differences in the sterol compositions between seeds and mature plant tissues of Cucurbitaceae, with 24-ethyl-Δ^7-sterols which possess Δ^{25}-bonds, **17** and **20**, occurring in the seeds, whereas 24-ethyl-Δ^7-sterols which lacked a Δ^{25}-bond, **15** and **19**, were found in the mature plant tissues, as the principal sterols (15). The stereochemical assignment at C-24 of the major 24-alkylsterols of some Cucurbitaceae, other than those previously investigated (18), is underway.

Isolation of 24α-ethylcholesta-8(14),22-dienol from the aerial parts of Cucumis sativus. The sterol mixture (3.08 g) obtained from MeOH extraction (528 g) of the dried aerial parts of *C. sativus* (5.3 Kg) was separated by silica gel TLC into two fractions, a fraction contained mainly Δ^5-sterols (74 mg) and that consisted mainly of Δ^7-sterols (2.50 g) (18). The Δ^7-sterol fraction was, after acetylation, crystallized to give crystalline (1.54 g) and filtrate (695 mg) portions. The latter was subjected to argentic TLC which afforded five bands, and a fraction (185 mg) recovered from the least polar band (Rf = 0.63) contained, in addition to **10**- and **19**-acetates, a sterol acetate (RRt = 1.49 in GLC) which was then isolated (2.2 mg; RRt = 0.93 in HPLC) by repetitive HPLC fractionation. The MS of the sterol acetate showed M^+ at m/z 454 ($C_{31}H_{50}O_2$, relative intensity 43%), with the fragmentation ions at m/z 439 (M^+ − Me, 5%), 411 ($M^+ - C_3H_7$, 9%), 394 (M^+ − HOAc, 3%), 351 ($M^+ - C_3H_7$ − HOAc, 2%), 315 [$M^+ - C_{10}H_{19}$ (side chain), 19%], 313 ($M^+ - C_{10}H_{19}$ − 2H, 23%), 266 ($M^+ - C_{10}H_{19}$ − HOAc, 34%), 229 (45%), and 81 (100%), indicating that it was an acetate of a C_{29}-sterol with two double bonds, one of which is in the skeleton and the other in the C_{10} side chain. The ion $M^+ - C_3H_7$ is typical for the Δ^{22}-unsaturation (24). The ^{1}H NMR spectrum of the sterol acetate displayed the signals at δ 0.855 (3H, *s*, 18-H_3), 0.704 (3H, *s*, 19-H_3), 1.033 (3H, *d*, *J* = 6.7 Hz, 21-H_3), 0.843 (3H, *d*, *J* = 6.7 Hz, 26-H_3), 0.800 (3H, *d*, *J* = 6.7 Hz, 27-H_3), 0.805 (3H, *t*, *J* = 6.7 Hz, 29-H_3), 2.022 (3H, *s*, 3β-OAc), 4.74 (1H, *m*, 3α-H), and 5.12 (2H, *m*, 22-H, 23-H), which suggested the 3β-acetoxy-5α-$\Delta^{8(14)}$-sterol skeleton (25) and 24α-ethyl-*trans*-Δ^{22}-side chain (18). Hydrogenation of the sterol acetate gave the dihydro derivative (RRt = 1.67 in GLC, M^+ = m/z 456 in MS) which showed the ^{1}H NMR signals at δ 0.840 (3H, *s*, 18-H_3), 0.705 (3H, *s*, 19-H_3), 0.935 (3H, *d*, *J* = 6.4 Hz, 21-H_3), 0.836 (3H, *d*, *J* = 6.7 Hz, 26-H_3), 0.814 (3H, *d*, *J* = 6.7 Hz, 27-H_3), 0.843 (3H, *t*, *J* = 8.3 Hz, 29-H_3), 2.022 (3H, *s*, 3β-OAc), and 4.71 (1H, *m*, 3α-H). The chromatographic and spectroscopic data of the dihydro derivative were indistinguishable from those of authentic 24α-ethyl-5α-cholest-8(14)-en-3β-ol (**24**) acetate, and hence, the sterol isolated from the aerial parts of *C. sativus* was 24α-ethyl-5α-cholesta-8(14),*trans*-22-dien-3β-ol. This sterol has so far been isolated only from *Aplopappus heterophyllus* (26). $\Delta^{8(14)}$-Sterols have been suggested as intermediates arising during sterol biosynthesis as a consequence of the C-14 demethylation step (20).

TABLE 3

Sterol Compositions (%) of the Seed Materials of Cucurbitaceae

	Compositions (%)																			
RRt (acetate)	1.00	1.14	1.21	1.31	1.35	1.43	1.52	1.54	1.55	1.61	1.63	1.70	1.74	1.80	1.82	1.94	2.04	2.15	2.31	Others
Cucurbitaceae / Estimated compd.	**1**	**2**	**3**	**4**	**5,6**	**7**	**8**	**9**	**10**	**11**	**12–14**	**15**	**16**	**17**	**18**	**19,20**	**21**	**22**	**23**	
Apodanthera undulata			tr	tr	0.5	0.4		0.2	0.5	0.6	1.1	34.0		36.4		16.4	2.6	3.1	0.7	3.5
Benincasa cerifera[a]	tr			1.6	0.5	1.5		0.7	1.8	2.1	7.2	17.4	0.8	12.4		44.0	1.9	5.0		3.1
Citrullus battich	0.4		0.3	0.2	0.2	2.2		0.8	0.6	0.5	2.8	22.9	0.4	34.4	0.5	30.3	0.5	1.6		1.4
Coccinea grandis	1.9	0.3	0.8	7.9	0.5	4.5		tr	3.0		29.5	7.7	1.4	2.0	0.3	32.0	0.5	0.9		6.8
Cucumis melo (I)				0.2		0.5		0.7	0.7		1.8	12.9		54.7	0.4	27.0	tr	0.6		0.5
(II)			tr	tr	tr	0.6	0.5	1.5	1.4	0.8	1.1	8.7	0.6	44.4	1.7	38.7				
C. sativus[a,b]	tr		tr	0.4	0.6	1.5		0.9	1.3	tr	5.0	11.3	0.3	54.9	tr	20.6	0.3	2.1		0.8
Cucurbita digitata				tr	0.3			0.2	1.6		2.2	30.2	1.0	20.5	0.3	36.1	0.8	5.6		1.2
C. foetidissima	0.2	0.1		0.4	0.5		0.2	1.4	tr		3.7	29.8		17.5	1.4	29.9	1.3	12.0		1.6
C. maxima[a]	tr		tr	0.6	1.1	0.6		0.2	0.4	tr	2.2	27.1	0.4	32.1		19.0	1.7	10.9		3.7
C. pepo[a]	tr			2.2	2.7	0.3	0.1	0.7	0.4	0.9	6.1	22.2	0.2	17.6	0.1	31.5	0.8	9.7		4.5
Lagenaria leucantha var. *Gourda*				2.4	1.2	7.5	0.7	0.6	0.9	0.6	8.3	14.6	1.8	18.8	1.4	40.5	tr	0.7		
L. leucantha var. *depressa*	0.2			1.8	0.4	7.4	0.3	0.9	0.3	0.7	8.7	11.0	2.0	20.3	0.4	42.8	0.4	1.3		1.1
L. leucantha		tr		tr	tr	0.6	0.5	1.5	1.4	0.8	1.1	8.7	0.6	44.4	1.7	38.7				
Luffa acutangula			tr	0.4	1.4	0.2	0.5	0.6	0.4	2.0		13.6		62.8	1.6	15.4				1.1
L. cylidrica (I)		0.2	0.1		0.1			0.2	0.3	1.0	0.6	17.9		67.4	2.1	10.1				
(II)	0.1			0.2	0.3	tr	0.5	0.2	0.7	0.7	0.4	21.4	1.6	61.7	1.7	10.5				
Momordica charantia	0.4			0.8	0.9	3.2	tr	tr		1.0	3.9	23.6	0.3	37.7	0.8	25.5	0.6	tr		1.3
M. charantia var. *Pavel*	0.3			1.6	0.5	8.1	0.2	0.2	tr		13.8	19.2	tr	30.2	0.6	21.7	0.2	0.3		3.1
M. cochinchinensis	5.4		0.3	4.5	2.2	1.6	0.6	0.5	1.8	tr	31.7	26.3		9.7	1.5	8.4		0.5		4.0
Trichosanthes anguina	1.2	tr	tr	2.7	0.6	2.7	0.1	0.1	0.4	tr	14.5	21.5		40.9	1.5	13.1		0.7		
T. japonica[a]	0.1	tr		0.3	0.2	0.7		1.1	1.7	tr	9.5	34.3	0.4	35.8	tr	11.2	tr	tr	tr	4.7

[a]Cf. ref. 18.

[b]Contained a trace amount of 24α-ethylcholesta-8(14),22-dienol (acetate, RRt = 1.49 in GLC).

TABLE 4

Sterol Compositions (%) of the Mature Plant Materials of Cucurbitaceae

Cucurbitaceae	Compositions (%)																			
RRt (acetate)	1.00	1.14	1.21	1.31	1.35	1.43	1.52	1.54	1.55	1.61	1.63	1.70	1.74	1.80	1.82	1.94	2.04	2.15	2.31	Others
Estimated compd.	1	2	3	4	5,6	7	8	9	10	11	12–14	15	16	17	18	19,20	21	22	23	
Citrullus battich (aerial parts)[a]	0.3			tr	0.6			0.5	tr	0.9	2.6	31.2	0.2	1.7	tr	46.4	2.4	8.6	0.1	4.5
Cucumis sativus (aerial parts)[a]	0.1	tr	tr	0.2	0.6	0.3	tr	0.2	3.1	0.4	1.6	43.9	tr	tr	tr	45.4	0.9	1.0	tr	2.3
Gynostemma pentaphyllum (aerial parts)[a]	0.9	tr	tr	0.6	4.4	tr		0.6	2.3	0.9	3.8	61.9	0.1	0.8	tr	3.8				19.9
Lagenaria leucantha var. *depressa* (aerial parts)				0.1	1.1	0.1	0.4	6.3			3.1	53.8		3.5	1.8	24.9	1.4			3.5
Momordica charantia (aerial parts)	0.5			1.9	1.0	17.1	0.3	0.3			7.8	15.2		32.5	0.4	21.8				1.2
M. cochinchinensis (aerial parts)	0.9	0.3	1.3	2.5	2.9	0.5		17.8		1.6	3.9	50.8	1.9	1.8	0.4	13.6		0.4		0.2
Sechium edule (aerial parts)	0.3	0.1	0.3	0.2	0.5	0.6		0.8	2.9	0.3	1.5	59.5	1.0	6.7	0.2	22.8		1.4		0.9
(pericarp)	0.5		0.1	0.1	0.1	0.1	0.1	1.1	4.3	0.5	1.4	23.0	2.4	15.2	1.7	46.2	0.4	1.1	0.2	1.5
Trichosanthes dioica (roots)				0.1	0.4	0.1	0.7	1.1		0.5	2.8	66.5		0.8	0.4	24.5	0.3	0.5		1.3
T. japonica (roots)	0.9			0.2	0.2	0.3		0.6	0.9	1.1	6.4	42.5	3.4	3.4	2.2	29.2	1.7	0.6	2.9	3.6

[a]Cf. ref. 18.

ACKNOWLEDGMENTS

N. Shimizu and M. Aimi assisted in the ^{1}H NMR and mass spectral measurements, and K. Cho provided technical assistance. J. W. Berry, University of Arizona, provided encouragement.

REFERENCES

1. Sucrow, W., *Tetrahedron Lett.* 2443 (1968).
2. Kintia, P.K., and Z.A. Wojciechowski, *Phytochemistry 13:*2235 (1974).
3. Sucrow, W., M. Slopianka and H.W. Kircher, *Ibid. 15:*1533 (1976).
4. Homberg, E.E., and A. Seher, *Ibid. 16:*288 (1977).
5. Bastić, M., Lj. Bastić, J.A. Jovanović and G. Spiteller, *J. Am. Oil Chem. Soc. 54:*525 (1977).
6. Jeong, T.M., M.S. Yang and T. Matsumoto, *J. Korean Chem. Soc. 21:*193 (1977).
7. Iida, T., T.M. Jeong, T. Tamura and T. Matsumoto, *Lipids 15:*66 (1980).
8. Itoh, T., Y. Kikuchi, T. Tamura and T. Matsumoto, *Phytochemistry 20:*761 (1981).
9. Iida, T., T. Tamura and T. Matsumoto, *Ibid. 20:*857 (1981).
10. Matsumoto, T., T. Shigemoto and T. Itoh, *Ibid. 22:*1300, 2622 (1983).
11. Garg, V.K., and W.R. Nes, *Ibid. 23:*2919, 2925 (1984).
12. Matsumoto, T., J. Hasegawa, N. Shimizu and T. Akihisa, *Yukagaku 34:*476 (1985).
13. Sucrow, W., *Chem. Ber. 99:*3559 (1966).
14. Sucrow, W., and A. Reimerdes, *Z. Naturforsch. 23b:*42 (1968).
15. Nes, W.R., K. Krevitz, J. Joseph, W.D. Nes, B. Harris, G.F. Gibbons and G.W. Patterson, *Lipids 12:*511 (1977).
16. Cattel, L., G. Balliano and O. Caputo, *Phytochemistry 18:*861 (1979).
17. Itoh, T., K. Yoshida, T. Tamura and T. Matsumoto, *Ibid. 21:*727 (1982).
18. Akihisa, T., S. Thakur, F.U. Rosenstein and T. Matsumoto, *Lipids 21:*39 (1986).
19. Goad, L.J., and T.W. Goodwin, *Progr. Phytochem. 3:*113 (1972).
20. Nes, W.R., and M.L. McKean, in *Biochemistry of Steroids and Other Isopentenoids,* University Park Press, Baltimore, MD, 1977.
21. Itoh, T., and T. Matsumoto, *Yukagaku 27:*745 (1978).
22. Vasconcellos, J.A., W.P. Bemis, J.W. Berry and C.W. Weber, in *New Sources of Fats and Oils,* edited by E.H. Pryde, L.H. Princen and K.D. Mukherjee, American Oil Chemists' Society, Champaign, IL, 1981, pp. 55, 319.
23. Itoh, T., H. Tani, K. Fukushima, T. Tamura and T. Matsumoto, *J. Chromatogr. 234:*65 (1982).
24. Knights, B.A., *J. Gas Chromatogr. 5:*273 (1967).
25. Iida, T., M. Kikuchi, T. Tamura and T. Matsumoto, *J. Lipid Res. 20:*279 (1979).
26. Zalkow, L.H., G.A. Gabat, G.L. Chetty, M. Ghosal and G. Keen, *Tetrahedron Lett.* 5727 (1968).

[Received October 18, 1985]

Soy Protein Gelation

A.-M. Hermansson
SIK - The Swedish Food Institute, P.O. Box 5401, S-402 29 Gothenburg, Sweden

Heat-induced protein gels are of importance for the structure and properties of many food products. Gel formation is a complex process which often involves several reactions such as denaturation, dissociation-association, and aggregation. The kinetics of the reactions involved will determine the type of structure formed. Protein gels can be divided into two types: gels formed by random aggregation and gels formed by association of molecules into strands in a more ordered way.

The two soy proteins glycinin and conglycinin both have the ability to form ordered structures consisting of strands 10–15 nm thick. The glycinin gel strands formed in distilled water are regular, and cross sections of strands showed a hollow cylindrical structure. In the presence of sodium chloride, glycinin forms an aggregated gel structure at 85 C, but at 95 C a regular structure similar to that found in distilled water was formed. The aggregated structure was interpreted as a transient state similar to the soluble aggregate formed on heating dilute solutions prior to dissociation into subunits.

Conglycinin gels are more irregular and more cross-linked than gels of glycinin. Also, the strands of conglycinin showed a complex mode of aggregation possibly in the form of double spirals. The addition of salt does not affect the microstructure of conglycinin gels as dramatically as in the case of glycinin gels.

Commercially produced soy protein isolates may behave quite differently from native soy proteins, due to processing conditions causing denaturation and various states of aggregation.

Heat-induced protein gels are important to the structure and properties of many food products. Heating of proteins may give rise to several reactions such as denaturation, association, dissociation and aggregation. Gel formation is a complex process involving several different reactions. The degree of random aggregation determines the type of gel structure formed. Association and dissociation reactions are of importance for the onset of gelation as well as for the orientation of molecules into strands. Denaturation is often required for gel formation to take place, but gels can form from already denatured proteins or spontaneously from native proteins under special conditions. An absolute prerequisite for gel formation is the interaction between protein molecules, strands or aggregates in such a way that some kind of a three-dimensional network is formed. As illustrated in Table 1, some of these reactions are induced by protein-water and some by protein-protein interactions (1).

The kinetics of the reactions involved in gel formation will determine the type of structure formed as well as the properties of the structure, e.g. water holding and rheological properties.

AGGREGATED AND ORDERED PROTEIN GELS

Protein gels can be divided roughly into two types, gels formed by "random" aggregation and gels formed by association of molecules into strands in a more ordered way. Due to small changes in the repulsive balance, gels of both types can be formed from one protein and the transfer from one type of gel structure to another can take