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STEROLS OF THE CUCURBITACEAE

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Key Word Index—Cucurbitaceae; sterol; configurations at C-24 of 24-alkylsterols; ^{1}H and ^{13}C NMR spectroscopy.

Abstract—The sterol constituents of 32 seed and mature plant (leaves and stems, pericarp of the fruit and roots) materials from the 12 genera of the family Cucurbitaceae were investigated, and 44 sterols were identified or newly characterized. The sterols found were those possessing the following 7 types of skeletons: saturated, and Δ^5-, Δ^7-, Δ^8-, $\Delta^{8(14)}$- and 14α-methyl-$\Delta^{9(11)}$-unsaturated, and 14α-methyl-9β,19-cyclo skeletons, with the side chains of 24-unsubstituted, Δ^{24}-, 24-methyl-, 24-methyl-Δ^{22}-, 24-methylene-, 24-ethyl-, 24-ethyl-Δ^{22}-, 24-ethyl-$\Delta^{24(25)}$-, 24-ethylidene-, 24-ethyl-Δ^{25}- and 24-ethyl-$\Delta^{22,25}$-substituted, 24-methylene-25-methylated and 24,24-dimethylated (24,24-dimethyl-, 24,24-dimethyl-Δ^{22}- and 24,24-dimethyl-Δ^{25}-substituted) structures. The configurations at C-24 of the 24-alkylsterols were examined by high resolution ^{1}H NMR and ^{13}C NMR spectroscopy. The 24-methyl- and 24-ethylsterols lacking a Δ^{25}-bond examined (i.e. 24-methyl-, 24-methyl-Δ^{22}-, 24-ethyl- and 24-ethyl-Δ^{22}-sterols) were shown to occur as the C-24 epimeric mixtures, or as a single diastereoisomer, either 24α- and 24β-epimer. The 24-ethylsterols bearing a Δ^{25}-bond (i.e. 24-ethyl-Δ^{25}- and 24-ethyl-$\Delta^{22,25}$-sterols) were, on the other hand, composed of only 24β-epimers. The possible biosynthetic sequences to the cucurbitaceous sterols are discussed.

INTRODUCTION

Plants of the family Cucurbitaceae contain 24-ethyl-Δ^7-sterols, such as 24-ethylcholest-7-enol (**3f**), 24-ethylcholesta-7,22-dienol (**3g**), 24-ethylcholesta-7,25-dienol (**3j**) and 24-ethylcholesta-7,22,25-trienol (**3k**), as the major sterol components [1–13] which constituted the characteristic feature of the plants of this family since the great majority of higher plants contain predominantly sterols bearing a Δ^5-bond with a 24α-alkyl substituted side chain (24*R* if a saturated or Δ^{25}-unsaturated side chain, 24*S* if the Δ^{22} derivative) represented by sitosterol (24α-**2f**) [14–16]. The configuration at C-24 of **3j** and **3k** isolated from the seeds of *Cucurbita pepo* was established as 24β by high resolution ^{1}H NMR spectroscopy [5] and further by stereospecific synthesis of the two C-24 epimers of **3k** [17, 18], whereas **3g** isolated from the seeds was shown to be the 24α-epimer, i.e. spinasterol (24α-**3g**), by ^{1}H NMR spectroscopy [5]. This constituted the first demonstration of the occurrence of 24α- (24α-**3g**) and 24β- (**3j** and **3k**) ethylsterols in a higher plant which prompted us to undertake an extensive investigation of the sterol constituents of the plants of the Cucurbitaceae with special reference to the configurations at C-24 of 24-alkylsterols. This paper summarizes our recent [19–31] and present studies on the sterol constituents of 32 seed and mature plant materials from the 12 genera of Cucurbitaceae. 24-Alkylsterols from the mature plants of *C. pepo* [8] and *Ecballium elaterium* [10] and the seeds of *C. maxima* [12, 13] have recently been studied stereochemically.

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RESULTS

Table 1 lists the 32 seed and mature plant materials from the 12 genera *Apodanthera* (**I**), *Benincasa* (**II**), *Citrullus* (**III, XXI**), *Coccinea* (**XXII**), *Cucumis* (**IV, V, XXIII**), *Cucurbita* (**VI–IX**), *Gynostemma* (**XXIV**), *Lagenaria* (**X–XII, XXV**), *Luffa* (**XIII, XIV**), *Momordica* (**XV–XVII, XXVI, XXVII**), *Sechium* (**XXVIII, XXIX**), *Sicyos* (**XXX**) and *Trichosanthes* (**XVIII–XX, XXXI, XXXII**) of the family Cucurbitaceae investigated for the sterol constituents [19–31]. Compositions of the sterol fractions determined for these plant materials were described previously [27, 28, 31].

Among the plant materials listed in Table 1, the 12 plant materials were subjected in this study for further sterol isolation, and the following sterols (see Table 2 for the name of sterols), as shown in parentheses, from individual plant materials were isolated and identified: **I** (**3f, 3g**), **IV** (**3c, 3f**), **VI** (**2g, 3c, 3f, 3g**), **VII** (**3c, 3f**), **X** (**2f**), **XIII** (**2g**), **XX** (**2g, 3f**), **XXII** (**2c**, 24α- and 24β-**2d, 2f, 2g, 2j, 2l, 3c, 3f, 3g**), **XXIV** (**1c, 2c, 2d, 2g, 3d, 3j, 3k**), **XXVII** (**3f, 3g, 4g**), **XXX** (**3c, 3d, 3f, 3g**) and **XXXI** (**3f, 3g**). Identification of these sterols was performed based on the chromatographic (GC, HPLC, and argentation TLC) and MS data, and moreover by the high resolution ^{1}H or ^{13}C NMR data, in a similar way as described previously [27]. Determination of the configurations at C-24 and the estimation of the relative proportions of diastereoisomers of C-24 epimeric mixtures of the 24-alkylsterols isolated were performed mostly by ^{1}H NMR spectroscopy [27, 32] of which results were shown in Table 3 together with our previous results [19–23, 25, 27–29]. Isolation of 24-methylcholestanol (**1c**, mixture of C-24 epimers) from **XXIV**, 24α- and 24β-methylcholesta-5,22-dienol (**2d**)

Table 1. Cucurbitaceae plant materials investigated for sterol constituents

Cucurbitaceae		References
Seeds		
I*	*Apodanthera undulata* Gray	[28]
II	*Benincasa cerifera* Savi (wax gourd)	[27, 28]
III	*Citrullus battich* Forskål (watermelon)	[22, 28]
IV*	*Cucumis melo* L. (melon)	[28]
V	*C. sativus* L. (cucumber)	[24, 25, 27, 28]
VI*	*Cucurbita digitata*	[28]
VII*	*C. foetidissima* HBK (baffalo gourd)	[28]
VIII	*C. maxima* Duschesne (squash)	[26–28]
IX	*C. pepo* L. (pumpkin)	[26–28]
X*	*Lagenaria leucantha* Rusby	[19, 20, 28]
XI*	*L. leucantha* Rusby va. *Gourda* Makino (bottle gourd)	[22, 28]
XII	*L. leucantha* Rusby var. *depressa* Makino	[28]
XIII*	*Luffa acutangula* Roxb.	[28]
XIV	*L. cylindrica* Roem. (sponge gourd)	[19, 28]
XV	*Momordica charantia* L. (balsam pear)	[28]
XVI	*M. charantia* L. var. *Pavel* Crantz	[28]
XVII	*M. cochinchinensis* Spreng	[28]
XVIII	*Trichosanthes Anguina* L. (sepent cucumber)	[28]
XIX	*T. cucumeroides* Maxim. (snake gourd)	[21]
XX*	*T. japonica* Regel.	[27, 28]
Mature plant tissues		
XXI	*Citrullus battich* (aerial parts)	[27, 28]
XXII*	*Coccinea grandis* Voigt (ivy gourd)†	[28]
XXIII	*Cucumis sativus* (aerial parts)	[27–29]
XXIV*	*Gynostemma pentaphyllum* Makino (aerial parts)	[28, 28, 30]
XXV	*Lagenaria leucantha* var. *depressa* (pericarp)	[28]
XXVI	*Momordica charantia* (aerial parts)	[28]
XXVII*	*M. cochinchinensis* (aerial parts)	[28]
XXVIII	*Sechium edule* Sw. (chayote) (aerial parts)	[28]
XXIX	*S. edule* (pericarp)	[28]
XXX*	*Sicyos angulatus* L. (bur cucumber) (aerial parts)	[31]
XXXI*	*Trichosanthes dioica* (roots)	[28]
XXXII	*T. japonica* (roots)	[23, 28]

*Investigated further in this study.

†This was erroneously indicated as 'seed material' in ref. [28].

from XXII and XXIV, and 24-methylene-25-methylcholest-5-enol (**21**) from XXII, in this study, constituted the first instance of their isolation from cucurbitaceous plants, and the ^{1}H NMR data of these sterols and sterol **4g** were given in Table 4 for which signal assignments were performed by comparison with literature data [8, 27, 32–35].

It is worth noting that although the side chain methyl signals are the usual criteria for differentiating the C-24 epimers of 24-ethyl-Δ^{22}-sterol [8, 27, 32, 33], the 22-H and 23-H olefinic signals can also be used to support the assignment since a difference was observed in the chemical shift of the olefinic signals between the C-24 epimers of **4g**, i.e. 24α-epimer, δ5.021 and 5.157 (each 1H); 24β-epimer, δ5.026 and 5.163 (each 1H). Analogously, the differentiation of the C-24 epimers of the 24-methyl-Δ^{22}-sterol could be supported by the 22-H and 23-H olefinic signals since 24α-**2d** displayed the multiplet signals centered at δ5.16 whereas the 24β-epimer showed them at δ5.18 (Table 4).

DISCUSSION

Sterol occurrence

Through our recent [19–31] and present studies, 44 sterols were identified or newly characterized in the Cucurbitaceae as listed in Table 2 together with their chromatographic data (GC, HPLC and argentation TLC). Among these sterols, the Δ^7-sterols (**3c–3k**) were found to be the major sterols of most of the Cucurbitaceae investigated which were accompanied by small amounts of the sterols with saturated (**1c, 1f, 1g**) and Δ^5-(**2a–2g, 2i–2k**) and Δ^8-(**4g, 4j, 4k**) skeletons [21, 27, 28]. The 24-ethyl-$\Delta^{7,22}$-(**3g**), $\Delta^{7,25}$-(**3j**) and $\Delta^{7,22,25}$-(**3k**) sterols constituted the predominant sterols for the seed materials, whereas the 24-ethyl-Δ^7-sterol (**3f** and **3g**) were the major ones for the mature plant tissues [27, 28]. Several new or uncommon sterols as described below were detected in the following Cucurbitaceae as the minor or trace constituents: 24-methylene-25-methylcholest-7-enol (**3l**) in

Table 2. Chromatographic data of some sterols found in the Cucurbitaceae

Sterol*		*RR*,† GC	*RR*,† HPLC	R_s†
1c(24α)	24α-Methylcholestanol (campestanol)	1.33	1.11	1.18
1c(24β)	24β-Methylcholestanol	1.33	1.11	1.18
1f(24α)	24α-Ethylcholestanol	1.65	1.37	1.18
1g(24ξ)	24ξ-Ethylcholest-22-enol	1.45	—	1.18
2a	Cholest-5-enol (cholesterol)	1.00	1.00	1.00
2b	Cholesta-5,24-dienol (desmosterol)	1.21	—	0.60
2c(24α)	24α-Methylcholest-5-enol (campesterol)	1.31	1.07	1.00
2c(24β)	24β-Methylcholest-5-enol	1.31	1.07	1.00
2d(24α)	24α-Methylcholesta-5,22-dienol	1.14	0.86	0.65
2d(24β)	24β-Methylcholesta-5,22-dienol (brassicasterol)	1.14	0.91	0.65
2e	24-Methylenecholest-5-enol	1.35	0.80	0.18
2f(24α)	24α-Ethylcholest-5-enol (sitosterol)	1.63	1.18	1.00
2f(24β)	24β-Ethylcholest-5-enol (clionasterol)	1.63	1.18	1.00
2g(24α)	24α-Ethylcholesta-5,22-dienol (stigmasterol)	1.43	1.04	0.96
2g(24β)	24β-Ethylcholesta-5,22-dienol (poriferasterol)	1.14	1.04	0.96
2i(24*Z*)	24*Z*-Ethylidenecholest-5-enol (isofucosterol)	1.81	1.00	0.53
2j	24β-Ethylcholesta-5,25-dienol (clerosterol)	1.64	0.86	0.36
2k	24β-Ethylcholesta-5,22,25-trienol	1.52	0.80	0.14
2l	24-Methylene-25-methylcholest-5-enol	1.68	0.97	0.12
3c(24α)	24α-Methylcholest-7-enol (stellasterol)	1.55	1.07	1.00
3c(24β)	24β-Methylcholest-7-enol	1.55	1.07	1.00
3d(24α)	24α-Methylcholesta-7,22-dienol	1.36	0.86	0.63
3d(24β)	24β-Methylcholesta-7,22-dienol	1.36	0.91	0.63
3e	24-Methylenecholest-7-enol (episterol)	1.61	0.80	0.16
3f(24α)	24α-Ethylcholest-7-enol	1.94	1.18	1.00
3f(24β)	24β-Ethylcholest-7-enol	1.93	1.18	1.00
3g(24α)	24α-Ethylcholesta-7,22-dienol (spinasterol)	1.70	1.04	0.95
3g(24β)	24β-Ethylcholesta-7,22-dienol (chondrillasterol)	1.71	1.04	0.95
3h	24-Ethylcholesta-7,24(25)-dienol (peposterol)	2.31	1.02	0.64
3i(24*E*)	24*E*-Ethylidenecholest-7-enol (isoavenasterol)	2.04	0.98	0.53
3i(24*Z*)	24*Z*-Ethylidenecholest-7-enol (avenasterol)	2.15	1.00	0.53
3j	24β-Ethylcholesta-7,25-dienol	1.95	0.88	0.41
3k	24β-Ethylcholesta-7,22,25-trienol	1.80	0.76	0.14
3l	24-Methylene-25-methylcholest-7-enol	1.98	0.85	0.18
3m	24,24-Dimethylcholest-7-enol	2.02	1.22	0.94
3n	24,24-Dimethylcholesta-7,22-dienol	1.66	0.91	0.65
3o	24,24-Dimethylcholesta-7,25-dienol	2.00	0.84	0.27
4g(24α)	24α-Ethylcholesta-8,22-dienol	1.54	0.95	0.89
4g(24β)	24β-Ethylcholesta-8,22-dienol	1.54	0.95	0.89
4j	24β-Ethylcholesta-8,25-dienol	1.74	0.80	0.40
4k	24β-Ethylcholesta-8,22,25-trienol	1.63	0.69	0.18
5g(24α)	24α-Ethylcholesta-8(14),22-dienol	1.49	0.93	1.00
6e	14α-Methyl-24-methylene-9β,19-cyclocholestanol (24-methylenepollinastanol)	1.62	0.98	0.39
7f(24α)	14α-Methyl-24α-ethylcholest-9(11)-enol	1.82	0.88	0.89

*All sterols have a hydroxyl group at C-3β. All sterols, with the exception of Δ^5-unsaturated sterols, are 5α-sterols. All C22–C23 double bonds are *trans* (*E*) oriented.

†*RR*, on GC and HPLC, and R_s-values on argentation TLC were measured for the acetyl derivatives and were expressed relative to **2a** acetate.

XXX [31], 24,24-dimethylcholest-7-enol (**3m**), 24,24-dimethylcholesta-7,22-dienol (**3n**) and 24,24-dimethylcholesta-7,25-dienol (**3o**) in **XXIV** [30], 24α-ethylcholesta-8(14),22-dienol (24α-**5g**) [28] and 24-methylenepollinastanol (**6e**) and 14α-methyl-24α-ethylcholest-9(11)-enol (24α-**7f**) [29] in **XXIII**.

Although four 24-ethyl-Δ^8-sterols, i.e. 24α- and 24β-**4g**, **4j** and **4k**, were detected in the Cucurbitaceae, the occurrence in nature of Δ^8-sterols (lacking a 4-methyl group) is quite rare, and they so far have been detected only in some lower organisms [14, 15, 36–38]. The structures of two 24-methylene-25-methylsterols **2l** and **3l**, of which the former has hitherto been detected in some Cruciferae plants [35], and three 24,24-dimethylated sterols **3m**, **3n** and **3o** are unusual because they contain an acyclic, quaternary carbon in the side chain. Only some

Table 3. Relative proportions of C-24 epimers of some 24-alkylsterols from the Cucurbitaceae estimated by ^1H and ^{13}C NMR*

Side chain	24-Me(c)			24-Me-Δ^{22}(d)		24-Et(f)			24-Et-Δ^{22}(g)		
Ring system	Δ^0(1)	Δ^5(2)	Δ^7(3)	Δ^5(2)	Δ^7(3)	Δ^0(1)	Δ^5(2)	Δ^7(3)	Δ^5(2)	Δ^7(3)	Δ^8(4)
Configuration at C-24	$\alpha:\beta$	$\alpha:\beta$	$\alpha:\beta$	$\alpha:\beta$	$\alpha:\beta$	$\alpha:\beta$	$\alpha:\beta$	$\alpha:\beta$	$\alpha:\beta$	$\alpha:\beta$	$\alpha:\beta$
Seeds											
I†								8:2		10:0	
II (refs. [25, 27])			2:8		8:2			6:4‡	10:0‡	6:4‡	
III (ref. [22])										5:5‡	
IV†			8:2					8:2			
V (ref. [27])		8:2	7:3		8:2		9:1‡	7:3‡	8:2‡	3:7‡	4:6
VI			8:2					10:0	10:0	7:3	
VII†			8:2					10:0			
VIII (ref. [27])								7:3‡		9:1‡	10:0
IX (ref. [27])			8:2					9:1‡		9:1‡	
X (ref. [20])							10:0†			0:10	
XI (ref. [22])										5:5	
XIII†									8:2		
XIV (ref. [19])										0:10‡	
XIX (ref. [21])						10:0‡					
XX (ref. [27])							4:6‡	3:7†	0:10†	1 < 9‡	
Mature plant tissues											
XXI (ref. [27])			7:3					9:1‡		6:4‡	
XXII†		7:3	8:2	2:8			8:2	9 > 1‡	9:1‡	9:1	
XXIII (ref. [27])		7:3	8:2		8:2		9:1‡	9:1‡	10:0	7:3‡	
XXIV (ref. [27])	7:3†	8:2†	8:2	2:8†	4:6†				10:0	1 < 9‡	
XXVII†							1:9			0:10‡	0:10
XXX†			8:2		4:6		2:8			0:10	
XXXI†							7:3			7:3	
XXXII (ref. [23])							8:2‡			8:2‡	

*3j and 3k isolated from **II** [27], **III** [22], **V** [27], **VIII** [27], **XI** [22], **XXII** [27] and **XXIV** were shown to be 24β-epimers on the basis of the ^{13}C NMR data. Compounds **2j**, **2k**, **4j** and **4k** isolated from **V** [27], and **2j** isolated from **XXII** also were shown to be 24β-epimers on the basis of the ^1H NMR data. Compounds **5j** [28] and **7f** [29] isolated from **XXIII** were shown to be 24α-epimers.

†Examined and estimated in this study.

‡Estimate based on the ^{13}C NMR data. All others were based on the ^1H NMR data.

Table 4. ^1H NMR chemical shifts ($CDCl_3$)*

Sterol	origin	Substituent at C-3		18-H_3 (*s*)	19-H_3 (*s*)	21-H_3 (*d*)	26-H_3 (*d*)	27-H_3 (*d*)
			(24α)			0.893 (6.4)	0.848 (6.7)	0.800 (7.6)
1c†	**XXIV**	OAc		0.646	0.815			
			(24β)			0.901 (6.6)	0.854 (6.8)	0.780 (7.7)
2d‡	**XXIV**	OH	(24α)	0.693	1.011	1.002 (6.7)	0.836 (6.7)	0.819 (7.3)
2d‡	**XXIV**	OH	(24β)	0.694	1.011	1.012 (6.7)	0.835 (6.3)	0.819 (6.7)
2l	**IV**	OAc		0.687	1.021	0.963 (6.4)	1.057 (*s*)	
4g	**XXVII**	OAc	(24β)	0.621	0.964	1.031 (6.7)	0.844 (6.4)	0.792 (6.4)
4g	**IX** (ref. [27])	OAc	(24α)	0.624	0.963	1.029 (6.6)	0.847 (6.6)	0.799 (5.9)

*Chemical shifts given in δ values from TMS; figures in parentheses denote coupling constants multiplet signals.

†Mixture of C-24 epimers.

‡Determined at 400 MHz; others were determined at 250 MHz.

sterols from marine sponges [39–41], and tetracyclic triterpenoids from *Neolitsea* species (Lauraceae) [42, 43] and from *Gyrinops walla* (Thymelacaceae) [44] are known to possess side chains with the quaternary centre at C-24 or C-25. $\Delta^{8(14)}$-Sterols are known to be formed from Δ^7-sterols by isomerization under certain chemical conditions [22]. 24α-Ethyl-$\Delta^{8(14),22}$-sterol (24α-**5g**) thus detected in **XXIII** [28] was, however, considered not to be an artefact formed from its Δ^7-isomer (**3g**) since **3g** occurred as the C-24 epimeric mixture in **XXIII** (Table 3). We have further detected two 14α-methylsterols, **6e** and 24α-**7f**, in **XXIII** as the minor sterols [29]; the occurrence of such sterols is not very common. Other examples of 14α-methylsterols are those possessing $\Delta^{9(11)}$-[45, 46], 5β,19-cyclo-$\Delta^{9(11)}$-[47], 9β,19-cyclo- [48–51] and Δ^8- [49, 52, 53] skeletons. The occurrence of 24β-methyl-Δ^{25}-sterols with side chain **p** was not confirmed in this study although sterols **2p** and **3p** have been detected in *Cucurbita maxima* seeds [12, 13] and the presence of these sterols is highly probable in our cucurbitaceous plant materials.

Configurations at C-24 of 24-alkylsterols

Table 3 shows the configurations at C-24 and the relative proportions of the C-24 epimers of the 24-alkylsterols lacking a Δ^{25}-bond of the Cucurbitaceae examined by ^{1}H NMR and ^{13}C NMR spectroscopy in this and in our recent studies [19–23, 25, 27–29]. All of the sterols with a 24-methyl (**c**) and 24-methyl-Δ^{22} (**d**) side chains were shown to be mixtures of 24α- and 24β-epimers in which the 24α-epimers were mostly predominant in the cases of 24-methylsterols (**1c**, **2c**, **3c**). The coexistence of C-24 epimers and the predominance of the 24α-epimers in 24-methyl-Δ^5-sterols (**2c**) were consistent with those for **2c** found in many higher plants [8, 15, 54, 55], whereas the predominance of the 24β-epimers as for 24-methyl-$\Delta^{5,22}$-sterols (**2d**) was closely correlated to that of **2d** found in some Cruciferae species [34, 56]. The co-occurrence of both 24α- and 24β-epimers was observed further in the 24-ethylsterols possessing Δ^5-(**2f**) and Δ^7-(**3f**) skeletons, and, moreover in the 24-ethyl-Δ^{22}-sterols bearing Δ^5-(**2g**), Δ^7-(**3g**) and Δ^8-(**4g**) skeletons. As with these 24-ethyl substituted sterols, some were found as a single diastereoisomer, either 24α- or 24β-epimer.

Sterol biogenesis

The Δ^8- and $\Delta^{8(14)}$-sterols, formed as a consequence of the C-14 demethylation step from 14α-methylsterols, play a role as biosynthetic intermediates for Δ^7-sterols which are then metabolized to other sterols through the sequence $\Delta^7 \rightarrow \Delta^{5,7} \rightarrow \Delta^5$ [14, 15]. Taking into account this and that Δ^5- and Δ^7-sterols are the component sterols of Cucurbitaceae species, it is highly probable that the Δ^8- and $\Delta^{8(14)}$-sterols detected in our study are metabolized (at the C-4, C-14 demethylated sterol level) into other sterols in the Cucurbitaceae.

Based on the considerations of sterol biogenesis and the present detection of alkylsterols, the possible biosynthetic pathways to 24α- and 24β-alkylsterols [8, 15, 57–59] and to 24-methylene-25-methyl- and 24,24-dimethylsterols [39, 41] can be postulated, as shown in Fig. 2. The 24β-methylsterols with side chain 24β-**c** are considered to be formed via 24β-methyl-Δ^{25}-sterols with side chain **p** [15, 57–59]. The 24β-methyl-Δ^{22}-sterols with side chain 24β-**d** may arise from those with 24β-**c** by dehydrogenation analogous to the 24α-ethyl pathway as described below. The 24α-methyl pathway leading to sterols with side chain 24α-**c**, through those with side chain **q**, has already been discussed [8, 15, 57, 59]. The detection of further 24α-methyl-Δ^{22}-sterols with side chain 24α-**d** may be explained by the presence of a 24α-**c** → 24α-**d** dehydrogenation route analogous to the 24α-ethyl pathway. The 24α-ethyl pathway, through side chain **k**, and the 24α-ethyl pathway (**24Z-i** → **h** → 24α-**g** → 24α-**g**) have been proposed already for tracheophyte sterols [8, 15, 58]. The presence of additional sterols with side chain 24β-**f** may

of some sterols isolated from Cucurbitaceae

28-H_3 (*d*)	29-H_3 (*t*)	3β-OAc (*s*)	3α-H (*m*)	6-H (*m*)	22-H (*dd*)	23-H (*dd*)
0.770 (7.0)	—	2.018	4.70 (25)	—	—	—
0.910 (7.3)	—	—	3.52 (23)	5.35 (10)	5.16) (*m*, 10)	
0.912 (7.1)	—	—	3.52 (23)	5.35 (10)	5.18 (*m*, 14)	
4.660 (1H, *d*, 1.2) 4.834 (1H, *s*)	1.057 (*s*)	2.032	4.62 (25)	5.38 (9)	—	—
—	0.812 (7.3)	2.026	4.70 (26)	—	5.026 (8.2, 15.3)	5.163 (8.2, 15.3)
—	0.805 (7.3)	2.029	4.70 (26)	—	5.021 (8.2, 15.3)	5.157 (8.2, 15.3)

(*J* values) as for doublet and triplet signals, whereas half-width ($W_{1/2}$) values as for

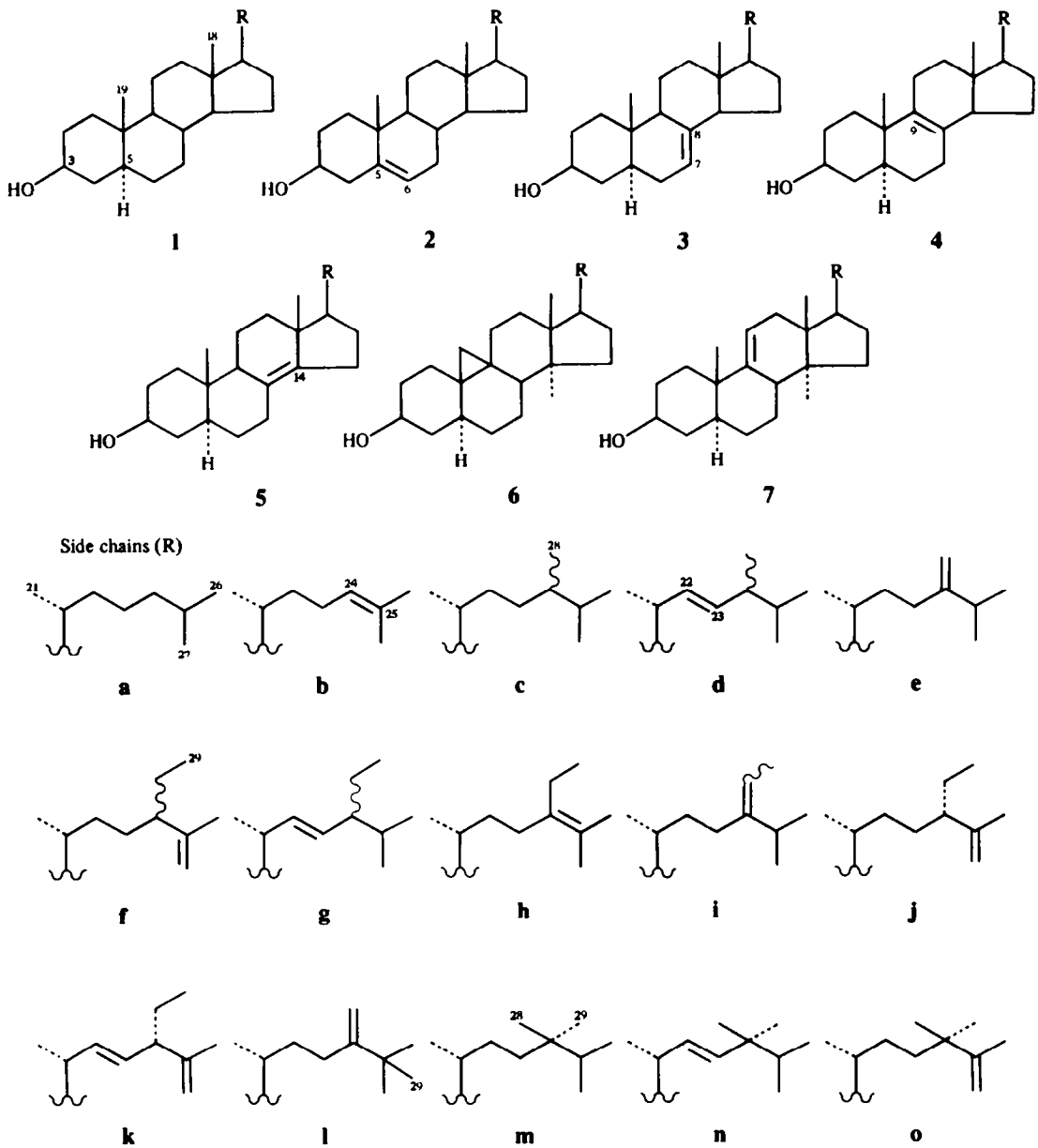

Fig. 1. Structures of sterols found in the Cucurbitaceae. All Δ^{22}-double bonds are *trans* (*E*).

suggest the presence of an alternative pathway, **j** → 24β-**f** → 24β-**g**, which is one of the possible sequences to 24β-ethylsterols in algae [15, 57]. The most plausible sequences to 24-methylene-25-methylsterols with side chain **l** and 24,24-dimethyl-Δ^{25}-sterols with side chain **o** are via biomethylation at C-25 and C-24, respectively, of 24-methyl-$\Delta^{24(25)}$-sterols with side chain **q** [39, 41]. Operation of the hydrogenation–dehydrogenation route (**o** → **m** → **n**), analogous to one of the two proposed routes to the 24β-ethyl pathway, may afford the 24,24-dimethylsterols with side chains **m** and subsequently **n**.

EXPERIMENTAL

Argentation TLC (Si gel–$AgNO_3$, 4:1) was developed with CCl_4–CH_2Cl_2 (5:1). HPLC was carried out on a Partisil 5 ODS-2 column (Whatman; 25 cm × 10 mm i.d.) with MeOH as a mobile phase (flow rate, 4 ml/min) which was monitored by an RI detector. GC on OV-17 SCOT glass capillary column (30 m × 0.3 mm i.d., column temp. 260°) was under the conditions already described [60]. RR_t on HPLC and GC and R_c-values on argentation TLC of the sterol acetates were expressed relative to cholesterol (**2a**) acetate. EI-MS (70 eV) were recorded by means of a probe injection. ^1H (250 or 400 MHz) and ^{13}C (62.9 MHz) NMR spectra were determined in $CDCl_3$ with TMS as internal standard.

Origins of the 12 cucurbitaceous plant materials (**I, IV, VI, VII, VIII, X, XIII, XX, XXII, XXIV, XXVII, XXX** and **XXXI**; see Table 1 for the names of plant materials) were described previously [27, 28, 31]. Sterol isolation from the above plant materials was performed in the same way as has been described previously [27]. The following sterols: **1c, 2c, 2d, 2f, 2g, 2j, 3c, 3d,**

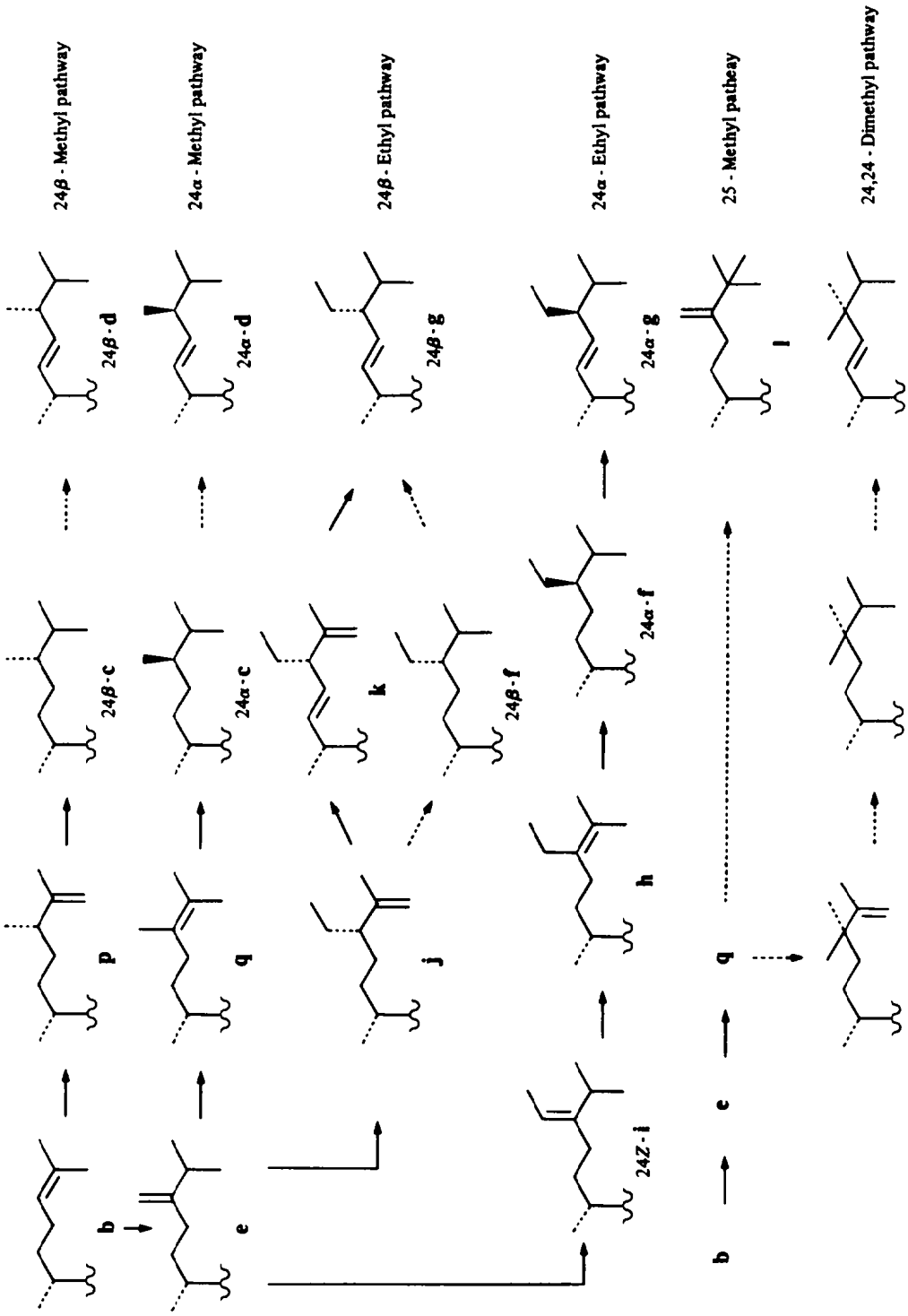

Fig. 2. Possible biogenetic sequences to 24-alkyl- and 25-alkylsterols in Cucurbitaceae. Dotted arrows denote that the routes have not been proposed thus far in higher plants.

3f, **3g**, **3j**, **3k** and **4j** [27] and **2l** [35] (see Table 2 for the names of sterols), were used as the reference specimens. The ^{1}H NMR and ^{13}C NMR spectra for the sterols identified in this study, with the exception for those listed in Table 4, were almost indistinguishable from those of the corresponding sterols described previously [27]. Determination of the configurations at C-24 and the estimation of the relative proportions of diastereoisomers of C-24 epimeric mixtures of the 24-alkylsterols were performed by ^{1}H NMR and, in some cases, by ^{13}C NMR spectroscopy in the same way as previously described [27]. Estimation of the relative proportions of the C-24 epimers of 24-methyl-$\Delta^{5,22}$-sterol (**2d**) also was performed by the ^{1}H NMR data prior to separation from each other by HPLC.

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