4-Demethylsterols and Triterpene Alcohols from Two *Vanilla* Bean Species: *Vanilla fragrans* and *V. tahitensis*

Béatrice Ramaroson-Raonizafinimanana[1], Emile M. Gaydou*, and Isabelle Bombarda

Laboratoire de Phytochimie de Marseille, Faculté des Sciences et Techniques de Saint Jérôme, Université d'Aix-Marseille III, Marseille Cedex 20, France

ABSTRACT: 4-Demethylsterol and triterpene alcohol compositions of two *Vanilla* bean species (*V. fragrans* and *V. tahitensis*) were investigated. From retention times and gas chromatography–mass spectrometry, nine 4-demethylsterols were identified in *V. fragrans* and seven in *V. tahitensis*. The 4-demethylsterol fraction of *V. fragrans* was characterized by a high content of 24-methylene cholesterol (27–40%) and of β-sitosterol (35–46%). The 4-demethylsterol fraction of *V. tahitensis* was characterized by a high content of stigmasterol (27%) and of β-sitosterol (57.5%), and a lower amount of 24-methylene cholesterol (5%). *Vanilla tahitensis* was also characterized by the presence of ergosta-5,25-dien-3β-ol (2%) and the absence of campesterol, stigmasta-5,22,25-trien-3β-ol, and ergosta-7,24(28)-dien-3β-ol. The beans' age modified the ratio 24-methylene cholesterol/β-sitosterol in *V. fragrans*. Combining liquid chromatography and gas chromatography allowed the identification of four other demethylsterols in *V. fragrans* (brassicasterol, 0.02%; stigmasta-5,23-dien-3β-ol, 1.43%; stigmasten-22-ol, 0.1%; and fucosterol, 0.5%) from the 4-demethylsterol fraction. 24-Methylene cholesterol and β-sitosterol were isolated, and their structures were confirmed by ^{1}H and ^{13}C nuclear magnetic resonance. Four triterpene alcohols were identified in *V. fragrans,* including cycloartenol (0.9–1.6%) from the triterpene alcohol fraction, 24-dihydrotirucallol (17–23%) from the triterpene alcohol fraction, tirucall-7-en-3β-ol (6–7.5%) from the triterpene alcohol fraction, and in a higher content cyclosadol (66–69%) from the triterpene alcohol fraction. The content ranges were studied as a function of the beans' age. Demethylsterol and triterpene alcohols profile could be used for origin differentiation.

JAOCS 75, 51–55 (1998).

KEY WORDS: 4-Demethylsterols, GC–MS, NMR, triterpene alcohols, *Vanilla* beans, *Vanilla fragrans, Vanilla tahitensis*.

Vanilla is undoubtedly the most used flavoring in the world. Its flavor is delicate and intriguing. Vanilla is principally used in sweets, chocolate and biscuit factories and in the manufacture of yogurts and ice creams. Other industries, such as perfumery and pharmaceuticals, use vanilla to flavor their products or as a synthetic intermediate for drugs against Parkinson's disease. The characteristic odor of vanilla bean develops as the result of a fermentation process, which changes unripe and odorless fruits into brown and sweet-smelling beans. Vanilla is, after saffron, the most expensive spice. The major flavor constituent of vanilla extract is vanillin. Synthetic vanillin is of interest because natural vanillin is about 300-fold more expensive. Volatile compounds, generally associated with superior vanilla aroma from natural beans, have been compared with synthetic materials (1,2). This information is used to detect adulteration (3,4) or to distinguish vanilla species (5). It is well known that qualitative and quantitative analysis of sterols and triterpene alcohols from unsaponifiable matter of vegetable oil gives useful information for genuine oil characterization. The balance between nonaromatic compounds, such as lipids that contain sterolic compounds, and the vanillin content should distinguish natural, adulterated vanilla products, or vanilla species. Because the composition of 4-demethylsterols and triterpene alcohols from *Vanilla* has not been reported to our knowledge, we present data for two commercial *Vanilla* species: *V. fragrans,* originating from the Indian Ocean area (Réunion, Madagascar, Comores), Hawaii and Indonesia, and *V. tahitensis* from Tahiti.

EXPERIMENTAL PROCEDURES

Vanilla bean materials. Vanilla fragrans species were collected in Réunion, Madagascar, Comores, Hawaii and Indonesia, and *V. tahitensis* in the Tahiti islands. Mature vanilla beans were collected during the ninth month after pollination. Immature vanilla beans were collected between the fifth and the seventh month after pollination. Commercial vanilla beans have undergone drying and curing processes during which time their characteristic aroma and flavor are developed.

Unsaponifiable extraction. Neutral lipids were obtained from crushed beans by Soxhlet extraction with pentane (150 mL, 16 h). The unsaponifiable fraction was obtained by using

[1]Present address: Département Industries Agricoles et Alimentaires, Ecole Supérieure des Sciences Agronomiques, Université d'Antananarivo, Madagascar.

*To whom correspondence should be addressed at Laboratoire de Phytochimie de Marseille, Faculté des Sciences et Techniques de Saint Jérôme, Université d'Aix-Marseille III, ave. Escadrille Normandie Niemen, 13397 Marseille Cedex 20, France. E-mail: emile.gaydou @ iut-chimie.u-3mrs.fr.

TABLE 1
4-Demethylsterol Composition of Two Commercial Vanilla Bean Species[a]

Species	RRT[b]	RRT[c]	*Vanilla fragrans*	*V. tahitensis*
Cholesterol	1.00	1.00	0.46	trace
Brassicasterol[d]	1.13	—		0.02
Ergosta-5,25-dien-3β-ol—	1.24		—	2.4
Campesterol1.32	1.29		5.22	—[e]
24-Methylene cholesterol1.36	1.27		38.08	5.1
Stigmasterol1.44	1.39		10.74	26.7
Stigmasten-22-ol[e]	1.46	—	0.10	
Stigmasta-5,22,25-trien-3β-ol	1.52	1.40	1.13	—
Ergosta-7,24(28)-dien-3β-ol	1.60	1.44	1.20	—
Stigmasta-5,23-dien-3β-ol[e]	1.62	—	1.43	
β-Sitosterol	1.63	1.60	35.30	57.5
Fucosterol[e]	1.74	—	0.50	
Δ^5-Avenasterol	1.80	1.67	4.70	8.1
Δ^7-Avenasterol	2.14	1.95	0.30	trace

[a]Determined on OV-17 and OV-1 capillary columns at 260°C.
[b]Relative retention time expressed against cholesterol acetate on OV-17 glass capillary column at 260°C.
[c]Relative retention time expressed against cholesterol acetate on OV-1 glass capillary column at 260°C.
[d]Identified in *V. fragrans* by both liquid chromatography (LC) and gas chromatography–mass spectrometry (GC–MS).
[e]Not detected.

the Itoh *et al.* procedure (6). Water (40 mL) was added to the reaction mixture (10 mL), and unsaponifiable matter was extracted with diisopropyl ether (three 30-mL volumes). The organic layer was washed with a 10% aqueous carbonate solution (three 4-mL volumes), then washed with distilled water to neutrality, dried, and evaporated (19.5 and 31.9% yield for *V. tahitensis* and *V. fragrans,* respectively).

Column chromatography (CC). The unsaponifiable extract (0.425 g from *V. fragrans* beans) was fractionated by CC (46 × 3 cm) over alumina gel (Aluminoxid 90 II-III, Merck 1097, 70–230 mesh A.S.T.M., 3–6% hydration, 200 g; Darmstadt, Germany) with hexane, hexane–benzene and hexane–diethylether. Fractions were collected, and each was checked by thin-layer chromatography (TLC) on precoated plates (5 × 10 cm, silica gel 60 F_{254}, 0.25 mm, Merck), with $CHCl_3$-Et_2O (90:10, vol/vol). Spots were visualized by examination under phosphomolybdic acid spray reagent. 4,4-Dimethylsterols (R_f 0.47–0.54), and 4-demethylsterols (R_f 0.32–0.41) were separated. For *V. fragrans* from Réunion, the 4,4-dimethylsterol fraction represented 1.9, 0.6, and 0.05% with regard to unsaponifiable, lipid, and bean weights, respectively, and the 4-demethylsterol fraction represented 4.4, 1.4, and 0.12%. For *V. fragrans* from Madagascar, the 4,4-dimethylsterol fraction represented 3.3, 1.1, and 0.08%, and the 4-demethylsterol fraction represented 3.9, 1.3, and 0.10%, respectively.

Preparative TLC. The relative contents of 4,4-dimethylsterols in the unsaponifiable lipid of *V. fragrans* and the relative content of 4-demethylsterols in *V. tahitensis* were also determined by preparative TLC on precoated plates (10 × 20 cm, silica gel 60 F_{254}, 0.25 mm; Merck). The unsaponifiable lipid (10 mg) was dissolved in CCl_4 and applied as a streak to the Si gel chromoplate. Cholesterol was also spotted as marker. After development with $CHCl_3$-Et_2O (90:10, vol/vol), spots were visualized with Rhodamine B (250 mg in ethanol, 150 mL) under ultraviolet light at 254 nm. The corresponding bands of 4-demethylsterols and 4,4-dimethylsterols were scraped off the chromatoplate and extracted with CH_2Cl_2 (ten 1-mL volumes). Mass percentages of *V. tahitensis* 4-demethylsterols represented 1.6, 0.3, and 0.02% of unsaponifiable, lipid, and bean weights, respectively. For *V. fragrans*, mass percentages of 4,4-dimethylsterols were 2–3, 0.5–1, and 0.05–0.1%, respectively.

Liquid chromatography (LC) analysis. The sterolic fraction (20 µL) was fractionated by LC. The liquid chromatograph was equipped with an SPD-2A Shimadzu spectrophotometer and a Analprep 93 pump (Thouzart et Matignon, Paris, France). A 4 × 250 mm Lichrosorb RP8, 5 µm column was used for fractionation of the 4-demethylsterol fraction. The elution was performed with a mixture of acetonitrile–water (90:10, vol/vol) at a flow rate of 1 mL/min.

Gas chromatography (GC). The sterol fraction (1 mg) was acetylated with acetic anhydride (0.1 mL) and pyridine (0.1 mL) according to NFT 60-232 Norms (7) before GC analysis. A Girdel 30 gas chromatograph, equipped with a flame-ionization detector (FID), was used for compound separations with an OV-1 glass capillary column (25 m × 0.31 mm i.d.) (phase thickness, 0.15 µm; column temperature, 260°C). Detector and inlet temperatures were 290 and 280°C. Hydrogen was used as carrier gas at an inner pressure of 0.5 bar. We also used an OV-17 glass capillary column (25 m × 0.36 mm i.d.) (phase thickness, 0.30 µm; column temperature, 260°C). Hydrogen was used as carrier gas at an inner pressure of 0.9 bar. The injections averaged 1 µL of a 0.5% solution of crude mixtures in hexane.

GC–mass spectrometry (MS). Combined GC–MS was recorded on a Girdel gas chromatograph, linked to a Ribermag R-10-10B mass spectrometer with a quadrupole mass analyzer [15.6 mm (i.d.) × 350 mm, 10^{-6} mm Hg] and coupled with a Sidar data computer. The GC column was an OV-1701 fused capillary column (50 m × 0.36 mm, 0.30 µm phase thickness). The column temperature was 280°C; carrier gas was helium (2 bars); ion source, 270°C; ionizing voltage, 70 eV.

Nuclear magnetic resonance (NMR) spectroscopy. All spectra were recorded on a Bruker AM-200 spectrometer. The NMR spectra were measured as solutions in chloroform-*d* in 5-mm o.d. tubes for ^{13}C and ^{1}H. Tetramethylsilane was used as internal standard in both measurements.

RESULTS AND DISCUSSION

The neutral lipid content was 10.8 and 9.3% in commercial beans for *V. fragrans* and *V. tahitensis,* respectively. The unsaponifiable matter in the neutral lipid fraction is relatively important and represented 31.9 and 19.5% of the lipid frac-

TABLE 2
^{13}C NMR Chemical Shifts of β-Sitosterol Acetate and 24-Methylene Cholesterol Acetate

Compound	$\delta^{13}C$[a] of β-sitosterol acetate			$\delta^{13}C$[a] of 24-methylene cholesterol acetate		
Assignment[b]	This work	Reference (9)	$\Delta\delta$[c]	This work	Reference (10)	$\Delta\delta$[c]
C-1	37.32	36.99	0.33	37.28	37.30	–0.02
C-2	28.06	27.78	0.28	30.17	31.68	–1.51
C-3	74.21	73.96	0.25	74.17	71.84	2.33
C-4	38.42	38.12	0.30	38.38	42.38	–4.00
C-5	140.04	139.60	0.44	140.01	140.78	–0.77
C-6	122.73	122.59	0.14	122.70	212.73	0.97
C-7	32.16	31.88	0.28	32.20	31.94	0.26
C-8	32.24	31.88	0.36	32.11	31.94	0.17
C-9	50.49	50.03	0.46	50.44	50.17	0.27
C-10	36.90	36.59	0.31	36.87	36.52	0.35
C-11	21.32	21.03	0.29	21.28	21.11	0.17
C-12	40.01	39.72	0.29	40.06	39.82	0.24
C-13	42.65	42.31	0.34	42.65	42.31	0.34
C-14	57.05	56.67	0.39	57.00	56.80	0.20
C-15	24.51	24.30	0.21	24.46	24.31	0.15
C-16	28.38	28.23	0.15	28.30	28.23	0.07
C-17	56.51	56.01	0.50	56.41	56.04	0.37
C-18	12.06	11.87	0.19	12.04	11.88	0.16
C-19	19.45	19.31	0.14	19.43	19.41	0.02
C-20	36.21	36.15	0.06	35.92	35.79	0.13
C-21	19.00	18.79	0.21	18.91	18.74	0.17
C-22	34.16	33.91	0.25	35.11	34.74	0.37
C-23	26.21	26.10	0.11	31.33	31.02	0.31
C-24	45.92	45.84	0.08	156.98	156.86	0.12
C-25	29.48	29.16	0.32	34.10	33.84	0.26
C-26	19.90	19.81	0.09	22.00	21.89	0.11
C-27	19.32	19.04	0.28	22.12	22.01	0.11
C-28	23.48	23.08	0.40	106.21	105.99	0.22
C-29	12.15	11.99	0.16			
*CH_3*COO-	21.39	21.43	–0.04	21.36	—	—
CH_3*C*OO-	170.40	170.46	–0.06	170.37	—	—

[a]In ppm with regard to TMS.
[b]Determined from DEPT analyses.
[c]Difference between δ of this work and δ found in the References.

tions of *V. fragrans* and *V. tahitensis,* respectively. Among the compound families contained in the unsaponifiable matter, we have studied the 4-demethylsterol fraction of *V. fragrans* (4, 1.3, and 0.1% of unsaponifiables, lipids and bean, respectively) and *V. tahitensis* (1.6, 0.3, and 0.02% of unsaponifiables, lipids, and bean, respectively). Nine 4-demethylsterols

TABLE 3
4-Demethylsterol Composition of *Vanilla fragrans* Commercial Vanilla Beans of Different Origin and Stages of Maturity[a]

Maturity	Very immature[b]	Immature[c]			Mature[d]				
Origin	Réunion	Madagascar	Réunion	Comores	Madagascar	Réunion	Comores	Hawaii	Indonesia
4-Demethylsterol									
Cholesterol + brassicasterol	0.6	0.7	0.5	0.7	0.6	0.4	0.4	0.5	0.5
Campesterol	4.2	4.5	4.5	4.6	5.2	5.0	5.0	4.9	4.8
24-Methylene cholesterol	40.6	38.2	39.5	38.4	29.4	31.4	29.8	30.2	27.3
Stigmasterol + stigmasten-22-ol	10.9	11.9	11.8	11.9	13.9	13.6	14.1	14.0	14.4
Stigmasta-5,22,25trien-3β-ol	trace	trace	0.1	0.1	0.1	0.1	0.1	0.1	trace
Ergosta-7,24(28)-dien-3β-ol + stigmasta-5,23-dien-3β-ol	1.6	1.7	1.4	1.4	1.4	1.2	1.3	1.3	1.3
β-Sitosterol + fucosterol	35.5	35.9	35.8	36.2	43.8	43.0	44.2	43.6	46.4
Δ^5-Avenasterol	6.3	6.6	6.1	6.2	5.1	4.8	4.7	4.9	4.9
Δ^7-Avenasterol	0.3	0.5	0.3	0.5	0.5	0.5	0.4	0.5	0.4

[a]Determined on OV-17 and OV-1 capillary columns at 260°C.
[b]Beans were collected between the fifth and the sixth month after pollination.
[c]Beans were collected during the seventh month after pollination.
[d]Mature beans were collected during the ninth month after pollination.

TABLE 4
4,4-Dimethylsterol Composition of *Vanilla fragrans* Commercial Beans of Different Origin and Stages of Maturity[a]

Maturity		Immature[b]				Mature[c]				
Origin		Madagascar		Réunion	Comores	Madagascar		Comores	Hawaii	Indonesia
Sample		1	2	3	4	5	6	7	8	9
RRT[d]	4,4-Dimethylsterol									
1.23	24-Dihydrotirucallol	22.9	22.8	22.7	22.6	17.6	17.4	17.3	17.6	17.5
1.60	Tirucall-7-en-3β-ol	5.9	6.1	5.9	6.2	7.3	7.4	7.4	7.2	7.2
1.86	Cycloartenol	0.9	1.0	0.9	0.9	1.6	1.6	1.5	1.5	1.4
2.06	Cyclosadol	66.6	66.4	66.8	66.3	69.0	69.2	69.4	69.2	69.3
2.73	X1[e]	0.7	0.7	0.8	0.8	2.2	2.0	2.1	2.1	2.2
3.53	X2	3.0	3.0	2.9	3.2	2.3	2.4	2.3	2.4	2.4

[a]Determined on OV-17 and OV-1 capillary columns at 260°C.
[b]Beans were collected during the seventh month after pollination.
[c]Mature beans were collected during the ninth month after pollination.
[d]Retention indices expressed against cholesterol acetate on OV-17 glass capillary column at 260°C.
[e]Not identified.

were discovered in *V. fragrans* with the OV-17 glass capillary column and seven in *V. tahitensis* with the OV-1. The compositions of the demethylsterol fractions of the two vanilla species are given in Table 1. Identifications were made with the help of mixtures of known sterols as standards and GC–MS of sterol acetate. The more important demethylsterols were 24-methylene cholesterol (38%) and β-sitosterol (35%) for *V. fragrans*, and β-sitosterol (57%) and stigmasterol (27%) for *V. tahitensis.* The 4-demethylsterol fraction was also fractionated by LC on RP-8 by the Bianchini *et al.* procedure (8). Four other demethylsterols [brassicasterol (0.02%), stigmasten-22-ol (0.1%), stigmasta-5,23-dien-3β-ol (1.43%), and fucosterol (0.5%)] were identified, and their structures were confirmed by GC–MS. 24-Methylene cholesterol and β-sitosterol were isolated with up to 98% purity by GC, and their structures were confirmed by NMR analysis, particularly the configuration at the C-24 of β-sitosterol. The ^{13}C chemical shifts of these two compounds are given in Table 2 (9,10). Matsumoto *et al.* (11) found that the difference between the two configurations (ethyl-24*R*/α-sterol or β-sitosterol and ethyl-24*S*/β-sterol or clionasterol) was the ^{1}H NMR chemical shift at C-21 with $\delta_{R/\alpha} = 0.921$ ppm and $\delta_{S/\beta} = 0.925$ ppm. Our results are in agreement with the 24*R*/α configuration or β-sitosterol ($\delta_{H\text{-}21} = 0.919$ ppm). Akihisa *et al.* (9) established the 24*R*/α-sterol configuration from the ^{13}C chemical shifts at C-20,21,23-29 at 36.20, 19.00, 26.21, 45.92, 29.48, 19.90, 19.32, 23.48, and 12.15 ppm, respectively.

Vanilla tahitensis was characterized by the presence of ergosta-5,25-dien-3β-ol (2%) and the absence of campesterol, stigmasta-5,22,25-trien-3βol and ergosta-7,24(28)-dien-3β-ol, which were found in *V. fragrans*. As shown in Table 3, the demethylsterol composition did not vary with the country of origin. However, it varied with the stage of maturity. 24-Methylene cholesterol, ergosta-7,24(28)-dien-3β-ol and Δ^5-avenasterol contents decreased with maturity, whereas β-sitosterol, stigmasterol, and campesterol contents increased.

The 4,4-dimethylsterol fraction of *V. fragrans* representing 2–3, 0.5–1, and 0.05–0.1% of the unsaponifiable matter, lipid and bean weights, respectively, was also investigated. The composition of this compound family of triterpene alcohols from *Vanilla fragrans* commercial beans of different origins and different stages of maturity is reported in Table 4. Tentative identification was based on their relative retention times (RRT), expressed against cholesterol, acetate, with those found in the literature (12,13) and on GC–MS of sterol acetates. *Vanilla fragrans* was characterized by the presence of six 4,4-dimethylsterols, including 24-dihydrotirucallol, tirucall-7-en-3β-ol, cycloartenol, cyclosadol, and two other compounds not identified (RRT 2.73 and 3.53). Composition did not vary with the country of origin but varied with the stage of bean development. Dihydro-24-tirucallol and an unknown substance (X2) were higher in immature (samples 1–4) than in mature beans (samples 5–9) and ranged from 22.9–17.3% and from 3.2–2.3%, respectively. Tirucall-7-en-3β-ol, X1, cycloartenol, and cyclosadol contents were lower in immature than in mature beans and ranged from 5.9–7.4%, 0.7–2.2%, 0.9–1.6%, and 66.3–69.3%, respectively.

The sterolic fraction of the neutral lipid extract of vanilla beans has a characteristic pattern with a high content of 24-methylene cholesterol, particularly in *V. fragrans* (27–40%), and of β-sitosterol (35–46%). *Vanilla tahitensis* is characterized by a high content of stigmasterol (27%) and of β-sitosterol (57.5%), and a lower content of 24-methylene cholesterol (5%). Maturity modifies the ratio of 24-methylene cholesterol/β-sitosterol in *V. fragrans*. The 4,4-dimethylsterol fraction of *V. fragrans* was characterized by a high content of cyclosadol (66–69%).

ACKNOWLEDGMENT

The authors gratefully acknowledge Michel Derbesy for providing us some authentic vanilla bean samples from Comores, Hawaii, and Indonesia.

REFERENCES

1. Belay, M.T., and C.F. Poole, Determination of Vanillin and Related Flavor Compounds in Natural Vanilla Extracts and Vanilla Flavored Foods by Thin-Layer Chromatography and Automated Multiple Development, *Chromatographia 37*:365 (1993).
2. Hartman, T.G., K. Karmas, J. Chen, A. Shevade, M. Deagio, and H. Hwang, Determination of Vanillin, Other Phenolic Compounds, and Flavors in Vanilla Beans. Direct Thermal Desorption–Gas Chromatography and Gas Chromatography–Mass Spectrometric Analysis, *ACS Symp. Ser. 506*:60 (1992).
3. Ehlers, D., M. Pfister, and S. Bartholomae, HPLC Analysis of Natural and Artificial Vanilla Flavorings, *GIT Fachz. Lab. 39*:765 (1995).
4. Riley, K.A., and D.H. Kleyn, Fundamental Principles of Vanilla/Vanilla Extract Processing and Methods of Detecting Adulteration in Vanilla Extract, *Food Technol. 43*:64 (1989).
5. Ehlers, D., M. Pfister, and S. Bartholomae, Analysis of Tahiti Vanilla by High-Performance Liquid Chromatography, *Z. Lebensm-Unters. Forsch. 199*:38 (1994).
6. Itoh, T., T. Tamura, and T. Matsumoto, Methyl Sterol Composition of 19 Vegetable Oils, *J. Am. Oil Chem. Soc. 50*:300 (1973).
7. Afnor, *Recueil des Normes Françaises des Corps Gras, Graines Oléagineuses, Produits dérivés*, Afnor, Paris, 1992.
8. Bianchini, J.P., E.M. Gaydou, J.C. Sigoillot, and G. Terrom, Determination of Sterol and Triterpene Alcohol Acetates in Natural Products by Reversed Phase Liquid Chromatography and Gas Chromatography–Mass Spectrometry, *J. Chromatogr. 329*:231 (1985).
9. Akihisa, T., S. Thakur, F.U. Rosenstein, and T. Matsumoto, Sterols of *Cucurbitaceae*: The Configuration at C-24 of 24-alkyl-Δ^5-,Δ^7- and Δ^8-Sterols, *Lipids 21*:39 (1986).
10. McInnes, A.G., J.A. Walter, and J.L.C. Wright, Carbon-13 NMR Spectra of $\Delta^{24(28)}$-Phytosterols, *Org. Magn. Reson. 13*:302 (1980).
11. Matsumoto, T., T. Sighemoto, and T. Itoh, (22E,24S)-5α-Ergosta-7,22-dien-3β-ol from the Seeds of *Cucumis satiris*, *Phytochemistry 22*:2622 (1983).
12. Itoh, T., T. Tamura, and T. Matsumoto, Triterpene Alcohols and Sterols in the Seeds of *Brassica napus, Fette Seifen. Anstrichm. 80*:382 (1978).
13. Itoh, T., H. Tani, K. Fukushima, T. Tamura, and T. Matsumoto, Structure Retention Relationship of Sterols and Triterpene Alcohols in Gas Chromatography on a Glass Capillary Column, *J. Chromatogr. 234*:65 (1982).

[Received February 20, 1997; accepted August 11, 1997]