Mass Spectrometry Characterization of the 5α-, 7α-, and 7β-Hydroxy Derivatives of β-Sitosterol, Campesterol, Stigmasterol, and Brassicasterol

Renzo Bortolomeazzi,* Michela De Zan, Lorena Pizzale, and Lanfranco S. Conte

Food Science Department, Udine University, via Marangoni 97, 33100 Udine, Italy

The 5α-hydroperoxides of β-sitosterol, campesterol, stigmasterol, and brassicasterol were obtained by photooxidation of the respective sterols in pyridine in the presence of hematoporphyrine as sensitizer. The reduction of the hydroperoxides gives the corresponding 5α-hydroxy derivatives. The 7α- and 7β-hydroperoxides of the sterols were obtained by allowing an aliquot of the 5α-hydroperoxides to isomerize to 7α-hydroperoxides, which in turn epimerize to 7β-hydroperoxides. The reduction gave the corresponding 7α- and 7β-hydroxy derivatives. The 5α-, 7α-, and 7β-hydroxy derivatives of β-sitosterol, campesterol, stigmasterol, and brassicasterol were identified by comparing thin-layer chromatography mobilities, specific color reactions, and mass spectral data with those of the corresponding hydroxy derivatives of cholesterol, which were synthesized in the same manner. The phytosterols had the same behavior to photooxidation as cholesterol and, moreover, the different phytosterols photooxidized at about the same rate. The mass spectra of the trimethylsilyl ethers of the hydroxy derivatives of the phytosterols investigated and of the corresponding hydroxy derivatives of cholesterol have the same fragmentation patterns and similar relative ion abundances.

Keywords: *Sterols; phytosterols; hydroxysterols; photooxidation; gas chromatography/mass spectrometry; GC/MS; mass spectra; trimethylsilyl ether*

INTRODUCTION

Phytosterols are among the most important components of the unsaponifiable matter of vegetable oils. Their composition is characteristic of the botanical species from which the oil is obtained, and the analysis of sterols in vegetable oils is used for identity control.

Among phytosterols, β-sitosterol (**Ib**) is the most representative and widely distributed in vegetable oils and other products of plant origin, together with other minor sterols such as campesterol (**Ic**), stigmasterol (**Id**), and brassicasterol (**Ie**) (Itoh et al., 1973).

Phytosterols can undergo transformation during the refining process of oils with the formation of isomers and dehydrated products (Niewiadomski et al., 1966; Kaufmann and Hamza, 1970; Grob et al., 1994; Mennie et al., 1994), which in turn can be analyzed to evaluate the quality of the oil and of other products (Lanzon et al., 1989; Cert et al.,1994; Grob et al., 1995; Biedermann et al., 1996; Crews et al., 1997). Little is known of the fate of sterol oxides eventually present in the oil during the refining processes (Niewiadomski et al., 1964; Kaufmann et al., 1970; Grob et al., 1994; Bortolomeazzi et al., 1996).

The molecular structures of phytosterols are strictly related to the structure of cholesterol as represented in Scheme 1. Cholesterol can oxidize by thermal (autoxidation) as well by photosensitized (photooxidation) processes, and the oxidation products have been thoroughly studied due to their biological activity (Smith, 1981).

Because of the structural similarity, phytosterols also can oxidize in a similar way to cholesterol (Smith, 1981). Among the vegetable sterols, some oxidation products of β-sitosterol and stigmasterol have been studied to verify their presence in vegetable oils and other plant-based foods because of their possible toxicity like the corresponding oxidation products of cholesterol (Lee et al., 1985; Blekas et al., 1989; Nourooz-Zadeh et al., 1992; Finocchiaro et al., 1983; Dutta et al., 1996).

In the present study, three hydroxy derivatives, namely, the 5α-, 7α-, and 7β-hydroxy of cholesterol, β-sitosterol, campesterol, stigmasterol, and brassicasterol, have been synthesized and characterized by mass spectral data of their trimethylsilyl (TMS) ether derivatives as a necessary preliminary step to analyze their degradation products during the refining process of vegetable oils, the last part being the subject of an other paper.

EXPERIMENTAL PROCEDURES

Materials. All solvents were of analytical grade. Hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS), the silylating mixture Fluka II according to Horning, and 7-dehydrocholesterol were from Fluka (Buchs, Switzerland). Cholest-5-en-3β-ol (**Ia**), cholest-5-ene-3β,7α-diol (7α-OH-cholesterol) (**VIa**), cholest-5-ene-3β,7β-diol (7β-OH-cholesterol) (**VIIa**), and cholest-4,6-dien-3β-ol were supplied by Nu-Chek-Prep, Inc. (Elysian, MI); [24*R*]-24-ethylcholest-5-en-3β-ol (**Ib**) and [24*S*]-24-ethylcholest-5,22-dien-3β-ol (**Id**) by were provided by Merck (Bracco, Milan, Italy).

The gas chromatography (GC) and GC/mass spectrometry (MS) analyses of β-sitosterol standard as TMS ether revealed the following composition: β-sitosterol, 76%; [24*R*]-24-methylcholest-5-en-3β-ol (campesterol), 8%; [24*R*]-24-methylcholest-3β-ol (campestanol), 1.4%; [24*R*]-24-ethylcholest-3β-ol (sitostanol), 14.6%.

* Author to whom correspondence should be addressed (fax +39-0432590719; e-mail Renzo.Bortolomeazzi@DSA.uniud.it).

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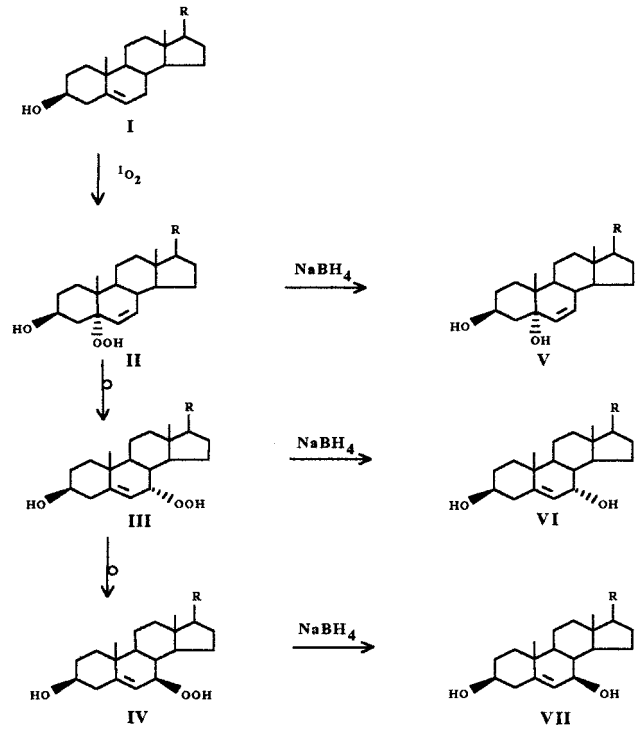
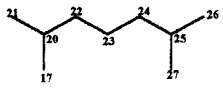
Scheme 1

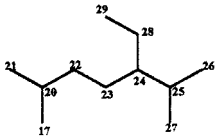


R=

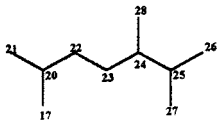


Ia, IIa, IIIa, IVa, Va, VIa, VIIa

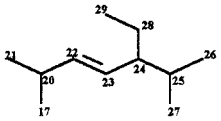
R=



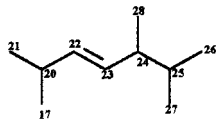
Ib, IIb, IIIb, IVb, Vb, VIb, VIIb



Ic, IIc, IIIc, IVc, Vc, VIc, VIIc



Id, IId, IIId, IVd, Vd, VId, VIId



Ie, IIe, IIIe, IVe, Ve, VIe, VIIe

The composition of the stigmasterol standard was as follows: stigmasterol, 91%; β-sitosterol, 4.8%; campesterol, 2.6%; [24*S*]-24-methylcholest-5,22-en-3β-ol (brassicasterol), 1.5%.

The melting points were determined on a Gallen Kamp MFB-595 (England) melting apparatus.

GC and GC/MS. A Carlo Erba Mega 5160 gas chromatograph equipped with a split−splitless injector and a flame ionization detector was used. The fused silica column was an SPB5, 30 m × 0.32 mm i.d., 0.25 μm film thickness (Supelco, Bellefonte, PA). The column temperature was 280 °C isothermal, and the detector and injector temperatures were 300 °C. The carrier gas (helium) flow rate was 1.3 mL/min and the split ratio 1:40 (v/v).

For the GC/MS analysis a Varian 3400 gas chromatograph coupled to a Varian Saturn ion trap detector was used. The fused silica column was a DB-5 (J&W, Folsom, CA), 30 m × 0.25 mm i.d., 0.25 μm film thickness, operating at 280 °C. The injection was in split mode (split ratio = 1:40) with helium as carrier gas at a flow rate of 1 mL/min. The injector, transfer line, and ion trap temperatures were 300, 300, and 170 °C, respectively. The electron impact (70 eV) spectra were recorded at 1 s/scan with a filament emission current of 10 μA.

The sterols and their hydroxy derivatives were derivatized to TMS ethers prior to GC and GC/MS analyses. The derivatization was carried out with a mixture of pyridine, HMDS, and TMCS (5:2:1 v/v/v) (Sweeley et al., 1963) for ~1 h at room temperature. In these conditions the 5-hydroxy sterols were only partially silanized, probably due to steric hindrance, with the formation of different products. For these compounds the silanization was obtained by using a more powerful silylating mixture formed by *N*,*O*-bis(trimethylsilyl)acetamide, 1-(trimethylsilyl)imidazole, and trimethylchlorosilane (3:3:2 v/v/v) (silylating mixture Fluka II) for ~2 h at room temperature.

Synthesis of 5-α-OH-, 7α-OH-, and 7β-OH-cholesterol. Cholesterol (1.0795 g) and hematoporphyrin (15 mg) were dissolved in 20 mL of pyridine in a three-neck, double-wall glass container equipped with a bulb condenser. The container was then placed in the center of four cross-disposed 100 W tungsten lamps, at ~2 cm from each lamp. A flow of ~300 mL/min of air, supplied by a gas cylinder, was bubbled into the solution, ensuring a vigorous stirring, and the temperature of the mixture was maintained at 10 °C by external circulation with a water cooling bath.

The reaction was carried out for ~5 h, and then the pyridine was removed in a Rotavapor at 35 °C. The residue, dissolved in chloroform, was mixed with silica and then absorbed by removing the chloroform under reduced pressure at 35 °C. The sample was then loaded into a silica chromatography column (14 cm length × 3 cm i. d.) and eluted with diethyl ether/*n*-hexane (6:4 v/v). The separation was monitored by spotting the eluate from the column on a thin-layer chromatography (TLC) silica plate sprayed with 80% sulfuric acid, and warming with hot air cholesterol gave a red color while the hydroperoxides gave a blue-green color and were, moreover, positive to the KI test, producing a yellow-brown spot on a silica plate impregnated with a KI solution. The hydroperoxides fraction, evaporated to dryness under reduced pressure, gave 850 mg of product. The hydroperoxides were dissolved in 50 mL of chloroform and then separated in two aliquots of, respectively, 10 and 40 mL.

The 10 mL aliquot was evaporated to dryness, dissolved in methanol/diethyl ether, and immediately reduced with $NaBH_4$ to yield 170 mg of the corresponding hydroxy compounds. A TLC silica analysis (eluent diethyl ether) of the reduced product revealed four spots with R_f 0.42 (predominant), blue color with 80% sulfuric acid; R_f 0.27, blue color; R_f 0.23, light blue-gray color; R_f 0.21, yellow color.

The mixture was absorbed on silica and then loaded into a silica flash chromatography column (25 cm length × 2 cm i.d.) and eluted with a mixture of diethyl ether/*n*-hexane starting from a 7:3 ratio to 100% ether and then methanol. The main fraction, 84 mg, gave after crystallization from aqueous methanol 68 mg of pure cholest-6-ene-3β,5α-diol (5-α-OH-cholesterol) (**Va**), R_f 0.42 (silica gel, eluent diethyl ether), immediate blue color with 80% H_2SO_4, mp 176−179 °C [lit. mp 148−149, 147−150, 170−175, 166−171, 134−135, 181 °C) (Teng et al., 1973; Smith et al., 1978)]. Calcd for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 79.51; H, 11.51.

The 40 mL aliquot was allowed to isomerize for 5 days at room temperature on the laboratory bench. After isomerization, the 40 mL aliquot was reduced with $NaBH_4$ in methanol/diethyl ether to yield 608 mg of hydroxy compounds. A TLC silica analysis revealed four principal bands with R_f 0.38, blue color, with 80% sulfuric acid; R_f 0.27 (predominant), blue color; R_f 0.23, light blue-gray color; and R_f 0.21, yellow color. The mixture of hydroxides was absorbed on silica and then loaded into a silica flash chromatography column (30 cm length × 2 cm i.d.) and eluted with diethyl ether/*n*-hexane from an initial 7:3 ratio to 100% diethyl ether followed by methanol. The first fraction eluted, 71 mg, gave after crystallization from aqueous methanol 40 mg of pure cholest-5-ene-3β,7β-diol (7β-OH-cholesterol) (**VIIa**), R_f 0.38 (silica gel, eluent diethyl ether),

Table 1. Mass Spectrometric Data of the 7α- and 7β-Hydroxy TMS Derivatives of Cholesterol and Phytosterols

ion	*m/z* (%)									
	VIa[a]	**VIIa**[a]	**VIb**[a]	**VIIb**[a]	**VIc**[a]	**VIIc**[a]	**VId**[a]	**VIId**[a]	**VIe**[a]	**VIIe**[a]
M	546 (1)	546 (2)	574 (2)	574 (1)	560 (2)	560 (1)	572 (2)	572 (2)	558 (2)	558 (0.8)
M − CH_3	531 (1)	531 (0.3)	559 (1)	559 (0.3)	545 (1)	545 (−)	557 (1)	557 (0.2)	543 (0.3)	543 (−)
M − (TMSOH)	456 (100)	456 (100)	484 (100)	484 (100)	470 (100)	470 (100)	482 (100)	482 (100)	468 (100)	468 (100)
M − (TMSOH + CH_3)	441 (2)	441 (2)	469 (1)	469 (2)	455 (2)	455 (3)	467 (1)	467 (1)	453 (2)	453 (2)
M − (TMSOH + C_3H_7)	−	−	−	−	−	−	439 (0.4)	439 (0.5)	425 (0.2)	425 (−)
M − (2TMSOH)	366 (1)	366 (0.5)	394 (1)	394 (1)	380 (1)	380 (−)	392 (0.3)	392 (0.1)	378 (−)	378 (−)
M − (2TMSOH + CH_3)	351 (2)	351 (2)	379 (1)	379 (1)	365 (2)	365 (2)	377 (1)	377 (0.5)	363 (1)	363 (−)
M − (R^b + TMSOH)	343 (0.3)	343 (0.2)	343 (0.2)	343 (0.3)	343 (1)	343 (−)	343 (1)	343 (1)	343 (1)	343 (0.3)
M − (R^b + 2TMSOH)	253 (2)	253 (1)	253 (2)	253 (1)	253 (2)	253 (1)	253 (2)	253 (1)	253 (2)	253 (2)
M − (2TMSOH + R^b + C_3H_6)	211 (1)	211 (1)	211 (1)	211 (1)	211 (1)	211 (2)	211 (1)	211 (1)	211 (1)	211 (−)

[a] Analyzed as TMS derivatives. [b] R = side chain.

immediate blue color with 80% H_2SO_4 (Smith et al., 1967), mp 172−175 °C [lit. mp 175−178, 174−178 °C) (Smith et al., 1973, 1978]. Calcd for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 79.64; H, 11.48. GC retention time and MS spectrum of the TMS derivative were in agreement with those of an authentic sample. The second fraction eluted, 428 mg, gave after crystallization from aqueous methanol 364 mg of pure cholest-5-ene-3β,7α-diol (7α-OH-cholesterol) (**VIa**), R_f 0.27 (silica gel, eluent diethyl ether), immediate blue color with 80% H_2SO_4 (Smith et al., 1967), mp 181−184 °C [lit. mp 181−183, 183−186 °C) (Smith et al., 1973, 1978]. Calcd for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 79.80; H, 11.43. GC retention time and MS spectrum of the TMS derivative were in agreement with those of an authentic sample.

The third fraction, 42 mg, consisted of a mixture of **VIa** and of the two epimers cholest-4-ene-3β,6β-diol (6β-OH-cholesterol), R_f 0.23, light blue-gray color with 80% H_2SO_4, and cholest-4-ene-3β,6α-diol (6α-OH-cholesterol), R_f 0.21, yellow color. The two latter compounds were identified on the basis of the R_f values, which are lower than those of the other diols, on the color developed with H_2SO_4, (Kulig and Smith, 1973), and by computer matching of the mass spectra with the NIST library. The two 6α and 6β diols derived from the photooxidation of cholesterol (Kulig and Smith, 1973) and were present also in the aliquot of hydroperoxides reduced immediately after the photooxidation. The presence also in this aliquot of the 7α-diol derivative was probably due to isomerization during reduction with $NaBH_4$.

Synthesis of the Hydroxy Derivatives of Phytosterols. The synthesis of the 5α-, 7α-, and 7β-hydroxy derivatives of β-sitosterol and stigmasterol was carried out under the same experimental conditions used for the hydroxy derivatives of cholesterol.

From β-sitosterol: 5α-OH-β-sitosterol (**Vb**), R_f 0.42, blue color with 80% H_2SO_4; 7α-OH-β-sitosterol (**VIb**), R_f 0.27, blue color; 7β-OH-β-sitosterol (**VIIb**), R_f 0.38, blue color.

The GC/MS analysis of the β-sitosterol hydroxides as TMS ethers revealed also the presence of the corresponding hydroxy derivatives of the campesterol, which were not separated on TLC.

From stigmasterol: 5α-OH-stigmasterol (**Vd**), R_f 0.42, blue color with H_2SO_4; 7α-OH-stigmasterol (**VId**), R_f 0.27, blue color; 7β-OH-stigmasterol (**VIId**), R_f 0.38, blue color.

The GC/MS analysis of the stigmasterol hydroxides as TMS ethers revealed also the presence of the corresponding hydroxy derivatives of campesterol, β-sitosterol, and brassicasterol, which were not separated on TLC.

RESULTS AND DISCUSSION

Photooxidation was used as a synthetic route for the preparation of the hydroxy derivatives of sterols because of the lower number of oxidation products formed with respect to autoxidation. The 5α-hydroperoxide (**IIa**) is the main product of photooxidation of cholesterol, together with small amounts of 6α- and 6β-hydroperoxides (Kulig and Smith, 1973). **IIa** can then isomerize to 7α-hydroperoxide (**IIIa**), which in turn isomerizes to 7β-hydroperoxide (**IVa**) (Beckwith et al., 1989); in this way, the three hydroperoxides and their corresponding hydroxides **Va**, **VIa**, and **VIIa**, by reduction, can be prepared in a relatively simple and clean way as shown in Scheme 1.

Identification of the hydroxy derivatives of β-sitosterol and campesterol present as impurities of the β-sitosterol, stigmasterol, and brassicasterol present as impurities of stigmasterol was carried out by comparing them with the corresponding oxidation products of cholesterol. The latter, contrary to the oxidation products of phytosterols, have been, in fact, widely studied and well characterized.

The presence of campesterol as a relatively abundant impurity (8%) in the β-sitosterol standard (ratio β-sitosterol/campesterol = 9:1) allowed the characterization of the oxidation products of the campesterol itself. The same was valid also for the hydroxides of brassicasterol, which was present as 1.5% impurity in the stigmasterol standard.

The GC analyses showed that the ratio between the corresponding hydroxy derivatives was about the same as between β-sitosterol and campesterol, suggesting that the two sterols oxidized at about the same rate.

The same was observed also for the hydroxides of stigmasterol and the other sterols present as impurities in the stigmasterol standard.

The R_f values of the 5α-, 7α-, and 7β-hydroxy derivatives of phytosterols **Vb**−**VIb**−**VIIb**, **Vc**−**VIc**−**VIIc**, **Vd**−**VId**−**VIId**, and **Ve**−**VIe**−**VIIe** were 0.42, 0.27, and 0.38, respectively, on TLC (silica gel, eluent diethyl ether). In the case of the derivatives of β-sitosterol and campesterol, a single spot was visible and only the GC/MS analysis revealed the presence of the hydroxides of the two sterols. In a similar way, the hydroxides of stigmasterol presented a single spot, with the GC/MS analysis revealing, instead, the presence also of the corresponding hydroxides of the other sterols present as impurities. The same R_f values were obtained for the corresponding hydroxy derivatives of cholesterol; moreover, all of the hydroxy derivatives of phytosterols as well as those of cholesterol developed an intense blue color after spraying with 80% H_2SO_4. The order of migration and the color developed in the case of the 7-OH diols **VIb** and **VIIb** were in agreement with literature data (Daly et al., 1983; Yanishlieva et al., 1980, 1983).

The mass spectra of **VIa**, **VIIa**, and **Va** as TMS derivatives are represented in Figure 1. The mass spectra of the two 7-OH-diols **VIa** and **VIIa** show the

Table 2. Mass Spectrometric Data of the 5α-Hydroxy TMS Derivatives of Cholesterol and Phytosterols

	m/z (%)				
ion	**Va**[a]	**Vb**[a]	**Vc**[a]	**Vd**[a]	**Ve**[a]
M	546 (0.5)	574 (1)	560 (−)	572 (1)	558 (−)
M − CH_3	531 (5)	559 (3)	545 (2)	557 (4)	543 (1)
M − (TMSOH)	456 (100)	484 (100)	470 (100)	482 (100)	468 (100)
M − (TMSOH + CH_3)	441 (17)	469 (15)	455 (17)	467 (12)	453 (10)
M − (TMSOH + C_3H_7)	−	−	−	439 (1)	425 (−)
M − (2TMSOH)	366 (13)	394 (12)	380 (12)	392 (10)	378 (11)
M − (2TMSOH + CH_3)	351 (25)	379 (22)	365 (25)	377 (17)	363 (19)
M − (R^b + TMSOH)	343 (3)	343 (3)	343 (5)	343 (8)	343 (3)
M − (R^b + 2TMSOH)	253 (7)	253 (7)	253 (9)	253 (11)	253 (12)
M − (2TMSOH + R^b + C_3H_6)	211 (5)	211 (5)	211 (7)	211 (5)	211 (2)

[a] Analyzed as TMS derivatives. [b] R = side chain.

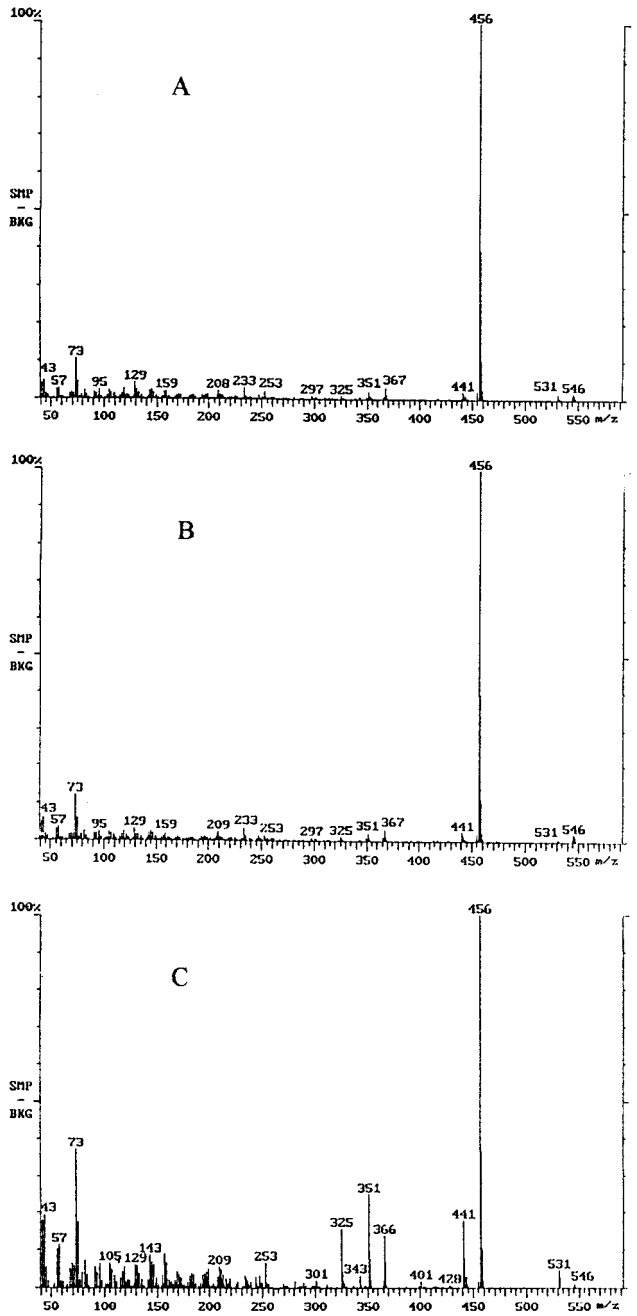


Figure 1. Mass spectra of (A) 7α-OH-cholesterol TMS, (B) 7β-OH-cholesterol TMS, and (C) 5α-OH-cholesterol TMS.

same fragmentation patterns, which are characterized by an intense fragment at *m/z* 456 (M − TMSOH) with all of the other high-mass ions lower than 5%. The fragments, *m/z* values, and relative abundances of all 7-OH-diol TMS derivatives are given in Table 1.

The mass spectra of the hydroxides of phytosterols **VIb**−**VIIb**, **VIc**−**VIIc**, **VId**−**VIId**, and **VIe**−**VIIe** as TMS derivatives present the same fragmentation patterns and have similar relative ion abundances of the corresponding hydroxides of cholesterol with the base peaks at, respectively, *m/z* 484, 470, 482, and 468. The differences of 28, 14, 26, and 12 amu with respect to the base peak (*m/z* 456) of the 7-OH-diols **VIa** and **VIIa** reflect the differences of the side-chain structure in the original sterols (Scheme 1). The ions at *m/z* 343, 253, and 211 do not contain the side chain and are common to all of the hydroxy derivatives of phytosterols as well of cholesterol. These ions originate, respectively, from the loss of one TMSOH group plus the side chain for *m/z* 343, the loss of two TMSOH groups, and the side chain for *m/z* 253 and the loss of two TMSOH groups and the side chain plus ring D for *m/z* 211. These fragmentations are characteristic of sterolic structures (Dumazer et al., 1986).

The mass spectral data of the two epimeric 7-OH-diols **VIb**−**VIIb** are in accord with the data reported by Nourooz-Zadeh and Appelqvist (1992).

The ion at *m/z* 439 in the spectra of the 7-OH-diols **VId**−**VIId** and the ion at *m/z* 425 in the spectra of the 7-OH-diols **VIe**−**VIIe** derived from the loss of one TMSOH plus isopropyl (C_{25}, C_{27}) group at the end of the side chain and are characteristic of a Δ^{22} double bond in the side chain, although with a much lower relative abundance with respect to the corresponding sterols TMS (Dumazer et al., 1986; Pizzoferrato et al., 1993). The presence of a second hydroxy group at C7 results in a very close similarity of the mass spectra of all the hydroxy sterols analyzed, keeping into account the different molecular weights.

The fragments, *m/z*, and relative abundances of the 5α-OH-diols **Va**, **Vb**, **Vc**, **Vd**, and **Ve** as TMS derivatives are shown in Table 2. The mass spectra of the 5-hydroxy derivatives of phytosterols present the same fragmentation patterns and similar relative ions abundances of the corresponding 5α-OH-cholesterol TMS. The considerations made previously about the ions with differences of 28, 14, 26, and 12 amu with respect to the corresponding fragments of the hydroxy derivative of cholesterol are valid also in the analysis of the spectra of the hydroxy derivatives of phytosterols.

The mass spectrum of the 5α-OH **Va** has a quite different appearance with respect to the spectrum of the 7-OH-diols **VIa**−**VIIa**, although the fragmentation patterns are pratically the same. In all spectra the base peak is the *m/z* 456 ion due to the loss of one TMSOH group, but in the case of **Va**, all of the other high-mass

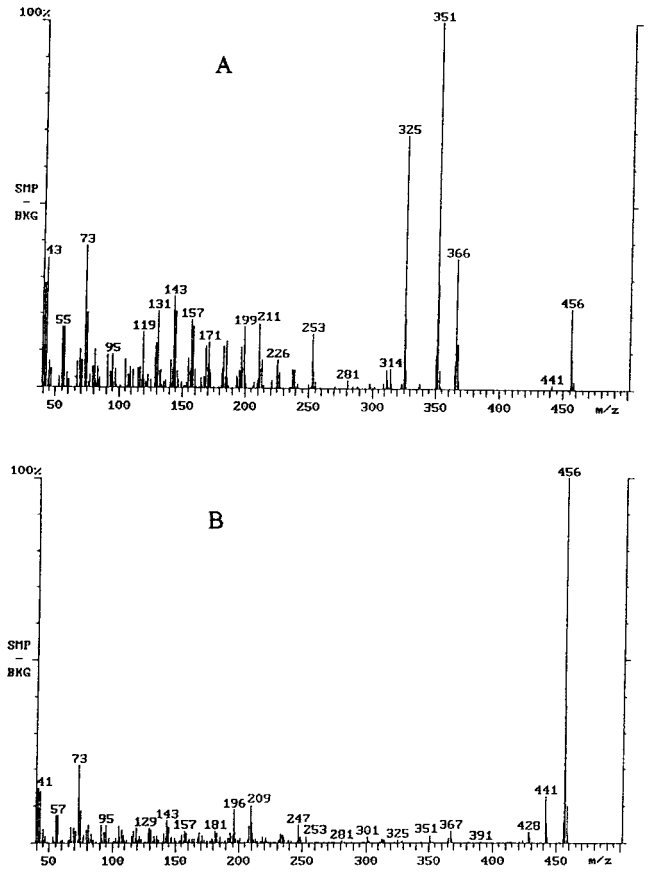


Figure 2. Mass spectra of (A) 7-dehydrocholesterol TMS and (B) 4,6-cholestadien-3β-ol TMS.

fragments have a much higher relative abundance. Particularly interesting is the ion at *m*/*z* 325, the formation of which can be explained by the loss of a 131 amu fragment, which comprises the TMS group and C1−C3, from the *m*/*z* 456 ion. This elimination is characteristic of $\Delta^{5,7}$ di-unsatered sterols as reported by Brooks et al. (1968) for the TMS derivatives of 7-dehydrocholesterol and ergosterol. The spectrum of **Va** (Figure 1C) is, in fact, similar to that of 7-dehydrocholesterol TMS in the *m*/*z* 200−500 range (Figure 2A), with the difference that in the first the *m*/*z* 456 ion is the base peak. In **Va** the 1,4 elimination between the TMSO group at C5 and the H at C8 with rearrangement of double bonds can lead to the formation of a structure, stabilized by double-bond conjugation, like that of the molecular ion of 7-dehydrocholesterol TMS. Another more probable fragmentation pattern with the formation of a species stabilized by double-bond conjugation is the 1,2 elimination between the TMSO group at C5 and the H at C4 with the formation of a structure like that of 4,6-cholestadien-3β-ol TMS, the spectrum of which (Figure 2B) is characterized by the base peak at *m*/*z* 456 and all of the other fragments of low intensity. This can explain the base peak at *m*/*z* 456 and the low intensity of the ions under 200 amu in the spectrum of **Va**. On the bases of these considerations and by comparison with the spectra of Figure 2, we can hypothesize that in the **Va** spectrum, the ion at *m*/*z* 456 is due mainly to two species, one with a structure like the molecular ion of 7-dehydrocholesterol TMS and one, probably predominant, because of the *m*/*z* 456 ion as base peak, with a structure like the molecular ion of 4,6-cholestadien-3β-ol TMS. The possible losses of the TMSO group at C3 should not lead to the formation of structures stabilized by double-bond conjugation.

In the case of the spectra of the two diol epimers **VIa** and **VIIa** it seems there is no formation of the ion at *m*/*z* 456 with a structure like that of 7-dehydrocholesterol TMS. Among the possible losses of the TMSO group, the 1,2 elimination between the TMSO group at C3 and H at C4 with the formation of a structure like 3,5-cholestadien-7-ol TMS, stabilized by double-bond conjugation, seems the more probable.

As reported under Experimental Procedures, all of the hydroxy derivatives of sterols were analyzed by GC/MS as TMS ethers. The silylation with the mixture of HMDS and TMCS in pyridine at room temperature, normally utilized for the sterols, works well also with the 7-OH-sterols, whereas in the case of the 5α-OH-sterols, the GC/MS analysis reveals the presence of three principal products. The more abundant one has a spectrum that is compatible with the structure of a mono-TMS derivative with the TMS group bonded to C(3), characterized by the base peak at *m*/*z* 456 (M − 18), the (M − 90) ion at *m*/*z* 384, and the rest of the spectrum like that of 7-dehydrocholesterol TMS. The second has the retention time and the spectrum in agreement with the di-TMS derivative; the third, which is characterized by a pronounced tail, has a retention time and the spectrum in agreement with that of 4,6-cholestadien-3β-ol TMS. This last compound is likely to be the decomposition product of the mono-TMS derivative, formed by the thermal loss of a molecule of water from C5 in the injection port of the gas chromatograph. This behavior reflects the difficulty, probably due to steric hindrance, of introducing the second TMS group at C5. A higher temperature would probably lead the reaction to completness but with a high risk of decomposition by dehydration. The 5α-OH derivatives of sterols have been successfully silylated at room temperature by using a more powerful silanizing mixture as reported under Experimental Procedures.

CONCLUSIONS

β-Sitosterol, campesterol, stigmasterol, and brassicasterol have the same behavior to photooxidation as cholesterol, resembling in this the similarity already seen in the case of autoxidation. Moreover, the different phytosterols photooxidized at about the same rate as evidenced by the similar percent composition of the sterols and of the corresponding sterol oxides. The mass spectra of the corresponding hydroxy derivatives of the phytosterols investigated and of the cholesterol have the same fragmentation patterns and similar relative ion abundances. In particular, the two 7-OH epimers of each sterol have practically the same spectra, which are instead quite different from the spectra of the 5α-OH derivative.

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