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Novel Synthesis Strategy for the Preparation of Individual Phytosterol Oxides

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ABSTRACT: Sterols (cholesterol and phytosterols) are important structural components of cell membranes and major constituents of lipid metabolism. Research on their oxides, such as the factors affecting oxidation, oxides' structures, and qualitative and quantitative analysis, aroused more attention in this decade. However, the biological roles of individual phytosterol oxides are still unclear because no commercial individual phytosterol oxide standards are available. Different from the traditional chemical synthesis, in the present study, chemical synthesis from a starting phytosterol mixture followed with a semipreparative HPLC separation produced individual oxides. TLC and analytical HPLC were used here to not only monitor the reaction process but also specifically analyze the synthetic intermediates and oxides. The chromatographic results exhibited strict rules and similar characteristics. Finally, for the first time, four individual phytosterol oxides were successfully separated and collected by a semipreparative HPLC system, thus providing a novel strategy for the preparation of individual phytosterol oxides.

KEYWORDS: *phytosterols, individual phytosterol, chemical synthesis, semipreparative HPLC, isolation*

INTRODUCTION

Phytosterol is a general name for more than 100 plant sterols with similar structures related to cholesterol.[1] Phytosterol-enriched foods (vegetable oils, margarine, yogurt, etc.) have experienced a great increase in the market, due to the asserted cholesterol-lowering effect of plant sterols.[2−4] Owing to the existence of double bonds in their chemical structures, phytosterols may through the same process as cholesterol, including nonenzymatic (such as heating treatment, UV radiation, or catalytic reactions etc.) and enzymatic pathways, produce a wide variety of oxides[2,3,5,6] with possible controversial biological effects. This becomes an important issue for human health.

It was known that cholesterol oxides had shown cytotoxic, apoptotic, and pro-inflammatory effects.[7−9] However, in the case of phytosterol oxides, research is still limited and their biological roles are unclear,[10,11] because no commercial phytosterol oxide standards are available. In 2003, Maguire[12] first pointed out that it would be extremely interesting to extend the toxicological analysis to individual phytosterol oxides rather than mixtures of these compounds. To better understand and define the single contribution of each phytosterol oxide on consumers' health,[13] it is valuable and urgent to develop some efficient methods to prepare the individual oxides.

Phytosterol oxides are a quite complex mixture, which may involve dozens to hundreds of different structural compounds in natural existence or produced by artificial generation.[2,3,5,6] Therefore, it is a challenge to perform all individual phytosterol oxides. Two strategies are mainly explored in this area so far. One is to thermo-oxidize or phyto-oxidize the sterols to produce the mixture of sterol oxides and then separate the blend to different structural compounds.[14−16] However, although this method opens the door to obtaining oxides, the formation of a variety of different oxides together, even dimeric and polymeric oxides,[17] made the separation and isolation difficult and challenging. Another is to chemically synthesize the high-purity target oxides from a commercial sterol at specific oxidation sites.[18,19] Because no phytosterols are available except stigmasterol and β-sitosterol, this also limits the formation of other phytosterol oxides.

Most papers available so far on phytosterol oxides were focused on their qualitative and quantitative analysis in a food matrix or in human plasma by gas or liquid chromatography in combination with mass spectrometry.[20,21] However, to consider the preparation and purification purpose, a milder and nondestructive process, such as crystallization or liquid chromatography, should be better than other techniques.

The main aim of the present study was to develop a simple and effective method for the preparation of the individual phytosterol oxides. Different from the traditional chemical synthesis mentioned above, in the present study, a phytosterol mixture was used as the starting material to synthesize two series of phytosterol oxide mixtures (7-keto- and 7-hydroxyphytosterols). Thin-layer chromatography (TLC) and analytical high-performance liquid chromatography (HPLC) were used to analyze the reaction process. On the basis of the results in analytical HPLC, a semipreparative HPLC system was first applied to the separation of individual oxides.

MATERIALS AND METHODS

Materials and Reagents. Generol 95R, a commercial industrial phytosterol mixture extracted from soybean oil, was provided by Cognis Co. (Saint-Fargeau-Ponthierry, France). The blend of Generol 95R contained 3% unknown sterol, 9% avenasterol, 10% brassicasterol, 32%

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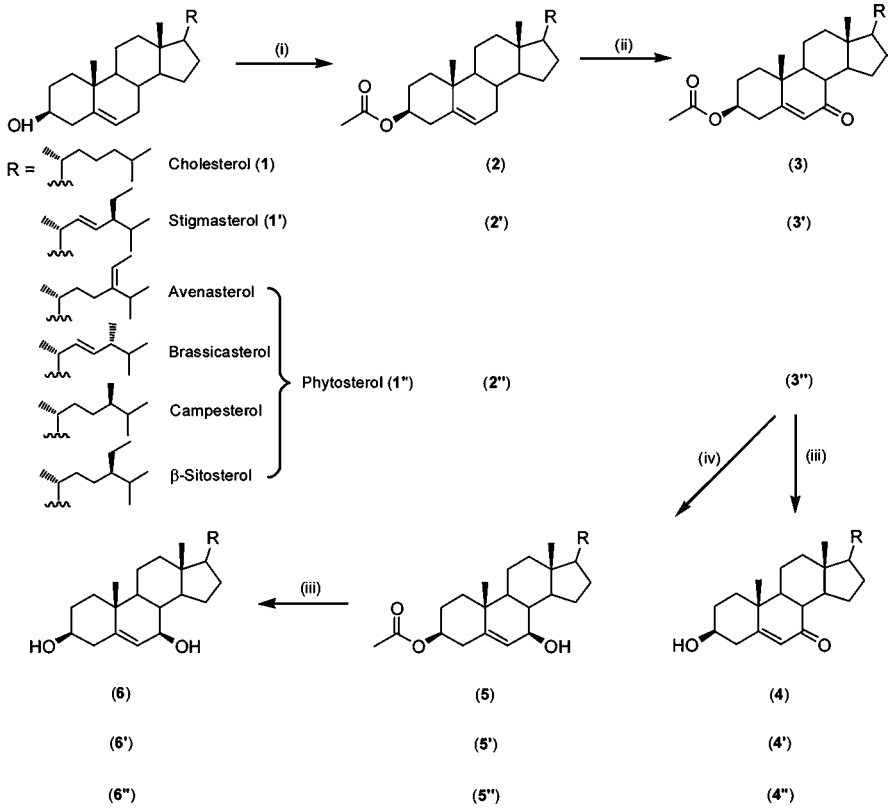
Scheme 1. Chemical Synthesis Pathway of Cholesterol Oxides (4 and 6), Stigmasterol Oxides (4′ and 6′), and Phytosterol Oxides (4″ and 6″)[a]



[a](i) CH_3COCl, Py, CH_2Cl_2; (ii) PCC, Celite, toluene; (iii) CH_3OH/H_2O, Na_2CO_3, 25°C; (iv) $CeCl_3{\cdot}7H_2O$, $NaBH_4$, CH_3OH/THF, 25 °C.

campesterol, and 45% β-sitosterol, along with minor yellow industrial impurities as their trimethylsilyl (TMS) ether derivates by GC analysis.

Standard compounds, including stigmasterol (95%), cholesterol (99%), 7-ketocholesterol, and 7β-hydroxycholesterol, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Acetonitrile, isopropanol, and methanol (Chromasolv, for HPLC, ≥99.9%) used for HPLC analysis were obtained from Sigma-Aldrich. The chemicals and the organic reagents (analytical grade) used for synthesis were obtained from commercial suppliers and were used without further purification unless otherwise noted. Pyridine was distilled from KOH prior to use. Toluene was dried with Al_2O_3. THF was dried by refluxing over sodium/benzophenone immediately prior to use. All chemical reactions were carried out under an argon atmosphere under anhydrous conditions.

Chemical Synthesis of Cholesterol Oxides. Besides commercial purchase from Sigma-Aldrich, two kinds of cholesterol oxides, 7-ketocholesterol **4** and 7β-hydroxycholesterol **6**, were also chemically synthesized in the laboratory from cholesterol **1** (in Scheme 1) according to the description of a series of β-sitosterol oxides in the literature.[18] After the synthesis, they were characterized completely, and the results were consistent with the commercial standards.

Chemical Synthesis of Stigmasterol Oxides. From stigmasterol **1**′, 7-ketostigmasterol **4**′ and 7β-hydroxystigmasterol **6**′ were also chemically synthesized in the laboratory via the same pathway (in Scheme 1) as above.

Chemical Synthesis of Phytosterol Oxides. As shown in Scheme 1, from the phytosterol mixture **1**″ (crystallization from Generol 95R by acetone), the oxides were chemically synthesized as above. The average molecular weight ($\bar{M}_w$) of the starting compound was calculated as

$$\bar{M}_w = A_1 \times M_{w(\text{avenasterol})} + A_2 \times M_{w(\text{brassicasterol})} + A_3 \times M_{w(\text{campesterol})} + A_4 \times M_{w(\beta\text{-sitosterol})}$$

where A means the percentage of each sterol in the total amount of sterols (4% Δ5-avenasterol, 9% brassicasterol, 44% campesterol, and 40% β-sitosterol, which was different from the content of the original Generol 95R).

Phytosterol Oxidation Intermediates ***2″***. **1**″ (7.50 g, 18.84 mmol) and pyridine (4.47 g, 4.39 mL, 56.52 mmol) were dissolved in 120 mL of CH_2Cl_2; acetyl chloride (4.36 mg, 4.02 mL, 56.52 mmol) was then added dropwise at 4 °C. The reaction mixture was stirred under nitrogen at room temperature for 3 h. The residue was washed with water (50 mL) and extracted with CH_2Cl_2 (5 × 50 mL). The organic phase was washed sequentially with a 10% HCl solution (50 mL) and a saturated NaCl aqueous solution (50 mL) and then dried over $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure. The crude product was recrystallized from methanol to give compound **2**″ as a white solid (7.46 g, 16.96 mmol, 90% yield).

Phytosterol Oxidation Intermediates ***3″***. Celite (40 g) and PCC (20.58 g, 95.4 mmol) were added to a solution of compound **2**″ (3.50 g, 7.95 mmol) in toluene (300 mL). After being refluxed for 30 h using a Dean−Stark apparatus, the reaction mixture was allowed to cool to room temperature. The residue was filtered and washed with Et_2O, distilled under reduced pressure. The crude product was eluted with a stepwise gradient of ethyl acetate in *n*-hexane (50 mL each of 10:90, 20:80, and 40:60, v/v) in a silica gel column to give compound **3**″ as a white solid (1.98 g, 4.37 mmol, 55% yield).

7-Ketophytosterols ***4″***. Compound **3**″ (1.50 g, 2.20 mmol) in CH_3OH (100 mL) was treated with sodium carbonate (1.17 g, 11.01 mmol) and H_2O (20 mL). After being stirred under nitrogen at room temperature for 15 h, the solvent was removed under reduced pressure. Water (60 mL) was added, and the mixture was extracted with CH_2Cl_2 (5 × 50 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was recrystallized from methanol to give compound **4**″ as a white solid (862 mg, 2.09 mmol, 95% yield).

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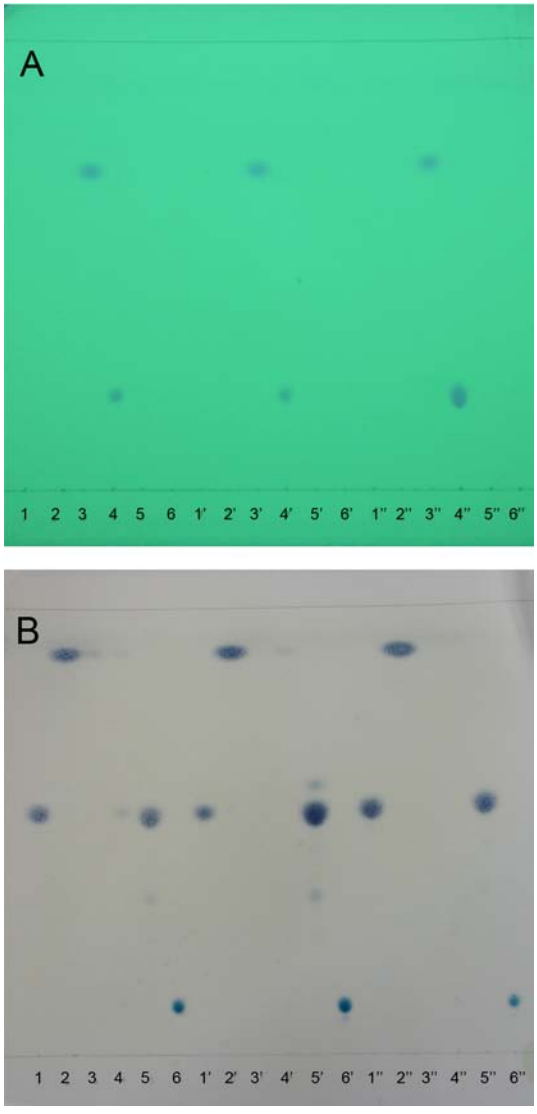

Figure 1. Scan of TLC aluminum plate (silica gel 60 F_{254}) with sterols (cholesterol **1**, stigmasterol **1′**, and phytosterols **1″**) and their synthetic intermediates and oxides developed with mobile phase *n*-hexane/ethyl acetate = 2:1 (v/v). It was observed (A) directly by UV detector at 254 nm and (B) then visualized after spraying with 20% phosphomolybdic acid reagent in ethanol followed by heating at 120 °C. The compounds are described in Table 1.

*Phytosterol Oxidation Intermediates **5″***. Compound **3″** (1.60 g, 3.52 mmol) and $CeCl_3 \cdot 7H_2O$ (3.95 g, 10.56 mmol) were dissolved in a mixture of THF/CH_3OH (5:1, 90 mL), followed by portionwise addition of $NaBH_4$ (399 mg, 10.56 mmol) at room temperature. The reaction mixture was stirred under nitrogen at room temperature for 1 h and, then, hydrolyzed with a 10% HCl solution (30 mL) and extracted with CH_2Cl_2 (4 × 50 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was recrystallized from methanol to give compound **5″** as a white solid (1.36 g, 2.99 mmol, 85% yield).

*7β-Hydroxyphytosterols **6″***. The same procedure as for compound **4″** was used: compound **5″** (1.20 g, 2.63 mmol), CH_3OH/H_2O (4:1, 100 mL), Na_2CO_3 (951 mg, 8.97 mmol). After workup, the reaction produced compound **6″** as a white solid (870 mg, 2.10 mmol, 80% yield).

TLC and HPLC. TLC was performed on silica gel 60 F_{254} precoated aluminum plates (Merck, Darmstadt, Germany) and observed by UV detector at 254 nm or visualized after spraying with 20% phosphomolybdic acid reagent in ethanol followed by heating at 120 °C.

Table 1. R_f Values of Sterols (Cholesterol 1, Stigmasterol 1′, and Phytosterols 1″) and Their Synthetic Intermediates and Oxides on TLC Plate[a]

compound	R_f (*n*-hexane/ethyl acetate = 2:1)
cholesterol **1**	0.66
stigmasterol **1′**	
phytosterols **1″**	
cholest-5-en-3β-ol acetate **2**	0.97
stigmast-5-en-3β-ol acetate **2′**	
phytosterol oxidation intermediates **2″**	
3β-acetoxycholest-5-en-7-one **3**	0.84
3β-acetoxystigmast-5-en-7-one **3′**	
phytosterol oxidation intermediates **3″**	
7-ketocholesterol **4**	0.30
7-ketostigmasterol **4′**	
7-ketophytosterols **4″**	
3β-acetoxycholest-5-en-7α-ol **5**	0.69
3β-acetoxystigmast-5-en-7α-ol **5′**	
phytosterol oxidation intermediates **5″**	
7β-hydroxycholesterol **6**	0.17
7β-hydroxystigmasterol **6′**	
7β-hydroxyphytosterols **6″**	

[a]TLC analyses were performed on silica gel 60 F_{254} precoated aluminum plates (Merck, Darmstadt, Germany).

HPLC analysis was performed on an Agilent 1200 series instrument (Agilent, Santa Clara, CA, USA) equipped with a manual 7725i injector, a quaternary pump system, a VWD UV−vis detector, and Agilent Chemstation software.

With a standard circulation valve and a 20 μL injection loop, in analytical system, an Agilent Eclipse XDB-C8 column (150 × 4.6 mm i.d., 5 μm) was used to analyze phytosterols, their reaction intermediates, and oxides at ambient temperature with an isocratic mobile phase (acetonitrile/isopropanol = 95:5, v/v) at a flow rate of 1.0 mL/min. The chromatograms were monitored by UV detection at 208 nm, 0.1 AUFS.

The synthesized phytosterol oxides, 7-ketophytosterols **4″** or 7β-hydroxyphytosterols **6″**, were separated by semipreparative HPLC system with a manual injector, a 2 mL injection loop, and an Agilent Eclipse XDB-C8 column (250 × 9.4 mm i.d., 5 μm). After filtration with a 0.45 μm filter membrane, 1 mL of saturated acetonitrile solution of oxides **4″** or **6″** was injected successively into the semipreparative HPLC system with acetonitrile/isopropanol = 10:1 (v/v) as mobile phase at a flow rate of 1.5 mL/min. The major fractions were manually collected into different collectors. After several injections and runs, the organic solvents in collected fractions were removed by rotary evaporation under reduced pressure at 40 °C. The pure individual compounds as white powder were obtained and stored at 4 °C under nitrogen gas. They were characterized by NMR, MS, specific rotation, etc. Until now, 350 mg of 7-ketocampesterol, 300 mg of 7-ketositosterol, 320 mg of 7β-hydroxycampesterol, and 300 mg of 7β-hydroxysitosterol were isolated by semipreparative HPLC.

Spectrometric Characterization. Melting points (mp) were measured with a Mettler Toledo FP62 (Sweden) melting apparatus. NMR spectra were taken in a Bruker 400 MHz Ultrashield spectrometer (400 and 100 MHz for ^1H and ^{13}C, respectively) with $CDCl_3$ as the solvent at room temperature. Optical rotations were measured with a Perkin-Elmer 343 polarimeter at 20 °C in a 1 dm length cell.

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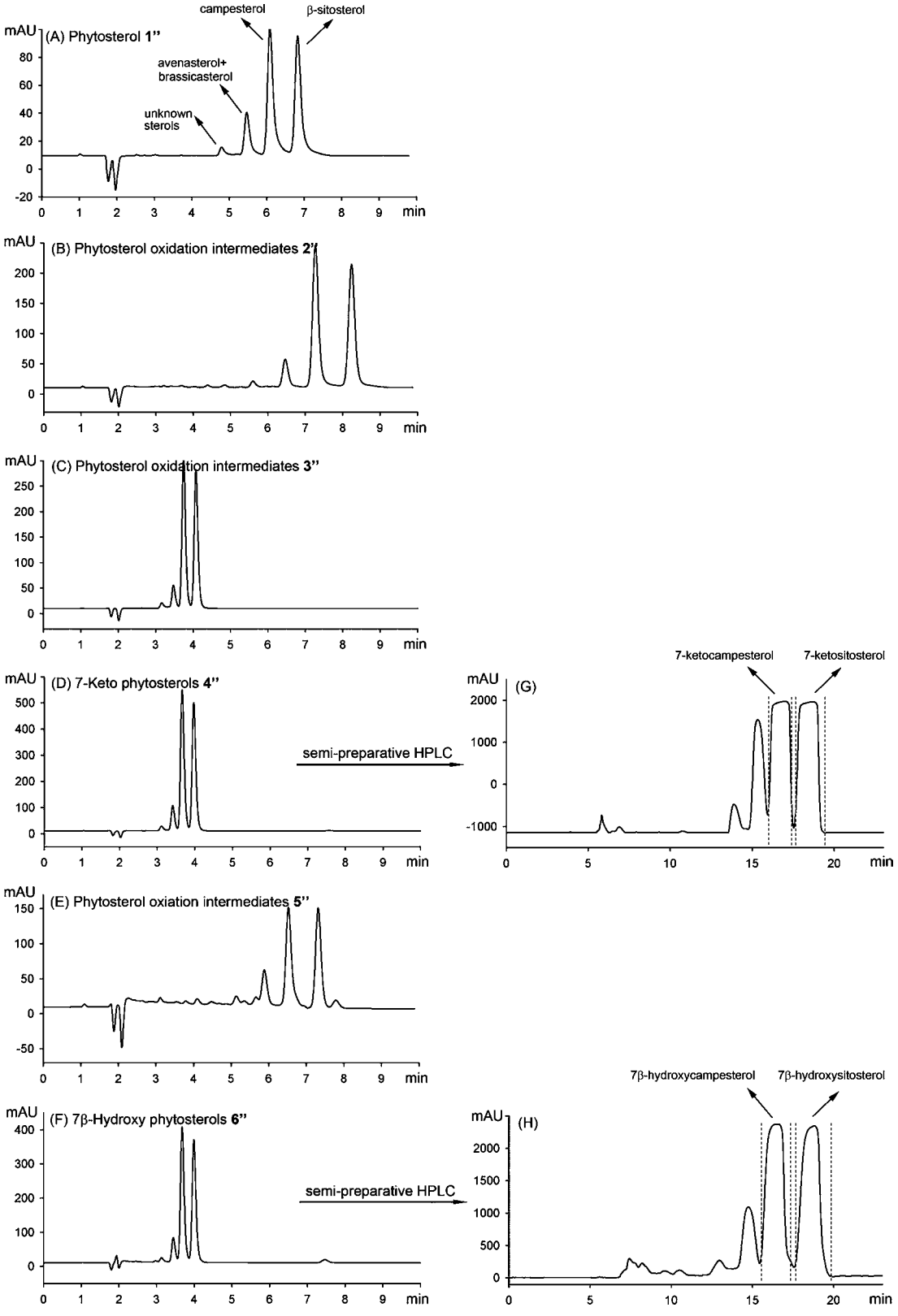

Figure 2. Analytical HPLC chromatograms of phytosterols **1**″ (A), synthetic intermediates **2**″, **3**″, and **5**″ (B, C, and E) and oxides 7-ketophytosterols **4**″ (D), 7*β*-hydroxyphytosterols **6**″ (F), and semipreparative HPLC chromatograms of **4**″ (G) and **6**″ (H). HPLC analysis was performed on an Agilent 1200 series instrument (Santa Clara, CA, USA). Analytical HPLC conditions: Agilent Eclipse XDB-C8 column (150 × 4.6 mm i.d., 5 μm); mobile phase, acetonitrile/isopropanol = 95:5 (v/v) at a flow rate of 1.0 mL/min; UV detection at 208 nm. Semipreparative HPLC conditions: Agilent Eclipse XDB-C8 column (250 × 9.6 mm i.d., 5 μm); mobile phase, acetonitrile/isopropanol = 10:1 (v/v) at a flow rate of 1.5 mL/min; UV detection at 208 nm. Injection volume = 1 mL of saturated acetonitrile solution of **4**″ or **6**″.

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Table 2. Characterization of 7-Ketocampesterol, 7-Ketositosterol, 7β-Hydroxycampesterol, and 7β-Hydroxysitosterol

compound	property	mp (°C)	^{1}H NMR (400 MHz, $CDCl_3$), δ	^{13}C NMR (100 MHz, $CDCl_3$), δ	$[\alpha]_D^{20}$ (deg)	(ESI$^+$)-MS m/z
7-ketocampesterol	white powder, purity ≥ 95%	172.5	5.69 (1H, br s, H-6), 3.70−3.63 (1H, m, H-3), 2.42−2.37 (3H, m), 2.24 (1H, br t, J = 9.0 Hz), 2.05−1.12 (25H, m, including 1.20 (3H, s, CH_3-19)), 0.92 (3H, d, J = 6.5 Hz, H-21), 0.86−0.77 (9H, m, H-28, H-27, and H-26), 0.69 (3H, s, H-18)	202.25, 165.10, 126.11, 70.52, 54.81, 49.97, 45.43, 43.12, 41.83, 38.84, 38.28, 36.37, 36.10, 35.82, 33.73, 32.41, 31.21, 30.34, 28.53, 26.32, 21.23, 20.19, 18.85, 18.25, 17.31, 15.39, 11.97.	−88.5 (c 1, $CHCl_3$)	415.4 (32), 397.4 (100)
7-ketositosterol	white powder, purity ≥ 95%	123.8 (lit. value 121−124[18])	5.69 (1H, br s, H-6), 3.71−3.63 (1H, m, H-3), 2.53−2.37 (3H, m), 2.24 (1H, br t, J = 9.0 Hz), 2.05−1.12 (27H, m, including 1.25 (3H, s, H-19)), 0.93 (3H, d, J = 6.5 Hz, H-21), 0.87−0.81 (9H, m, H-29, H-27, and H-26), 0.69 (3H, s, H-18)	202.27, 165.11, 126.11, 70.52, 54.74, 49.97, 45.86, 45.43, 43.12, 41.83, 38.72, 38.29, 36.37, 36.08, 33.97, 31.21, 29.18, 28.54, 26.33, 26.16, 23.08, 21.23, 19.78, 19.05, 18.93, 17.31, 11.97.	−96.8 (c 1, $CHCl_3$) (lit. value −98[18])	429.2 (33), 411.3 (100)
7β-hydroxycampesterol	white powder, purity ≥ 95%	182	5.29 (1H, br s, H-6), 3.84 (1H, d, J = 7.5 Hz,, H-7), 3.57−3.51 (1H, m, H-3), 2.36−1.29 (30H, m, including 1.05 (3H, s, H-19)), 0.92 (3H, d, J = 6.5 Hz, H-21), 0.87−0.81 (9H, m, H-28, H-27, and H-26), 0.70 (3H, s, H-18)	143.48, 125.47, 73.36, 71.43, 55.99, 55.46, 48.30, 42.91, 41.74, 40.94, 39.59, 38.87, 36.96, 36.45, 36.13, 35.84, 33.76, 32.42, 31.59, 30.33, 26.38, 21.09, 20.19, 19.15, 18.95, 18.76, 18.26, 11.83.	+5.8 (c 1, $CHCl_3$)	399.4 (100), 381.3 (60), 349.0 (25), 302.2 (53)
7β-hydroxysitosterol	white powder, purity ≥ 95%	219.7 (lit. value 218−220[18])	5.29 (1H, br s, H-6), 3.84 (1H, d, J = 7.5 Hz, H-7), 3.58−3.51 (1H, m, H-3), 2.40−1.30 (32H, m, including 1.04 (3H, s, H-19)), 0.92 (3H, d, J = 6.5 Hz, H-21), 0.87−0.80 (9H, m, H-29, H-27, and H-26), 0.70 (3H, s, H-18)	143.48, 125.48, 73.36, 71.44, 55.99, 55.42, 48.30, 45.88, 42.95, 41.75, 40.94, 39.58, 36.96, 36.46, 36.10, 34.01, 31.60, 29.19, 28.54, 26.39, 26.17, 23.09, 21.09, 19.80, 19.15, 19.04, 18.84.	+6 (c 1, $CHCl_3$) (lit. value +6[18])	413.4 (100), 359.2 (20), 320.6 (78)

Electrospray ionization tandem mass spectrometry (ESI-MS) data were measured on a Finnigan LCQ Advantage Max ion trap mass spectrometer (Thermo, USA), which was operated in positive ion mode using the following settings: spray voltage, 4.5 kV; spray current, 0.24; capillary voltage, 45.5 V; capillary temperature, 270 °C; nitrogen of pure quality as carrier gas and sheath gas flow, 24 units; scan range, m/z 50−600. One percent of acetic acid was added into the sample to aid protonation of the sample molecules before detection.

RESULTS AND DISCUSSION

Until now, the biological research of phytosterol oxides was scarce.[11] Most of these research works investigated the cytotoxicity of the mixture effects on different cell lines[10−13,22] because no commercial phytosterol oxide standards were available. It is important to obtain individual standards of oxides and, consequently, to research their concentration or possible interconversion in food, their adsorption in plasma, and their biological activities.[18] Chemical synthesis is an ideal method to obtain the products with specific structures. However, traditional chemical synthesis is generally from a pure commercially available compound or natural precursor. But so far it is a challenge to isolate or purify some major individual phytosterols with gram scale to serve the synthesis need.

Thus, in the present study, a novel synthetic strategy was employed. It provides a chemical synthesis of the oxides mixture from a sterols mixture as starting material followed by a semipreparative HPLC separation.

First, two cholesterol oxides (7-ketocholesterol **4** and 7β-hydroxycholesterol **6**) and two stigmasterol oxides (7-ketostigmasterol **4′** and 7β-hydroxystigmasterol **6′**) were laboratory-synthesized from cholesterol and stigmasterol separately as control experiments. With the same chemical

synthetic pathway as shown in Scheme 1, the phytosterol oxides with the same oxidation sites were also synthesized from phytosterol mixture **1″**. Phytosterol acetate **2″** was synthesized and underwent allylic oxidation using PCC and toluene to produce 7-ketophytosterols **4″**. From the reaction intermediate **3″**, stereoselective reduction by $NaBH_4$ and $CeCl_3$ with a deprotection reaction might produce 7β-hydroxyphytosterols **6″**.

TLC or column chromatography (CC) was usually used for the preliminary separation of phytosterol oxides on the basis of their different oxidation sites.[23,24] In the present study, as shown in Figure 1 and Table 1, TLC R_f values of synthetic phytosterol oxidation intermediates (**2″**, **3″**, and **5″**) and oxides (**4″** and **6″**) were similar to the data of the corresponding structural derivatives of cholesterol (**2**−**6**) or stigmasterol (**2′**−**6′**). The mobilities of these compounds were $R_{f(2'')} > R_{f(3'')} > R_{f(5'')} > R_{f(1'')} > R_{f(4'')} > R_{f(6'')}$ using *n*-hexane/ethyl acetate = 2:1 (v/v) as eluant. No separation could be achieved between individual compounds in each kind of phytosterol derivate by TLC. This result declared that R_f values strongly depended on their chemical structures in sterol compounds, but little on their side-chain structures.

Indeed, because of its higher resolution, gas chromatography (GC) has some obvious advantages over TLC and HPLC. It was used in most previous research to separate complex phytosterols and their oxides and analyze their content in foods and biological samples.[20,21] However, a time-consuming silylation or derivatization step is necessary before the injections,[23,25] so the reference oxides could not be finally obtained after the structural modification and the destructive testing. For the preparative and collection purpose, HPLC should be an ideal technique because it could be operated under milder column temperature and nondestructive detection of sterols. In the present study, with an Agilent Eclipse XDB-C8 column and acetonitrile/isopropanol = 95:5 (v/v) as mobile phase, this analytical HPLC system could separate not only individual phytosterol peaks in the starting mixture, except avenasterol with brassicasterol coelution (Figure 2A), but also the peaks in the synthetic intermediates (Figure 2B,C,E) and the peaks in target oxides (Figure 2D,F). All of these compounds could be eluted out of the column in <10 min. No sample pretreatment step was necessary before HPLC separation.

When we regarded the four phytosterol peaks in Figure 2A as one group, this "four-in-one" phenomenon could also be clearly observed in Figure 2B−F with the similar peak shape and area ratio. The retention time of these groups was $R_{t(6'')} \approx R_{t(4'')} < R_{t(3'')} < R_{t(1'')} < R_{t(5'')} < R_{t(2'')}$, which was consistent with the order of corresponding analogues of cholesterol or stigmasterol derivates (the chromatograms are not shown). This revealed that the individual sterols in the starting mixture participated in the chemical reactions at the same time with the same reaction rate. As long as the separation of starting sterols was achieved, the separation of sterol derivatives should be also realized.

On the basis of the above results, scale-up to semipreparative HPLC was carried out to isolate four major individual compounds in synthesized **4″** and **6″** (Figure 2G,H). Isocratic condition was conducive to the stability of separation and allowed the minimal consumption of eluents. About 5 mg of each target compound was collected manually per run when 1 mL of saturated acetonitrile solution of **4″** or **6″** was separately injected into the HPLC system. About 300 mg of each individual phytosterol oxide, including 7-ketocampesterol, 7-ketositosterol, 7β-hydroxycampesterol, and 7β-hydroxysitosterol, was collected after several runs. Their purity was found to be ≥95% with GC detection. They were also characterized by their ^1H and ^{13}C NMR profiles, optical rotations, and electrospray ionization mass spectra (ESI-MS) (Table 2). The data of 7-ketositosterol and 7β-hydroxysitosterol were consistent with the information in the literature.[18] 7-Ketocampesterol and 7β-hydroxycampesterol were first isolated and characterized as pure compounds.

To the best of our knowledge, this is the first report to synthesize the mixed oxides directly from a phytosterols mixture. The facile RP-HPLC system used here not only monitored and analyzed the reaction process but also isolated high-purity individual oxides in sufficient amounts for the further systematic biological investigations in vitro or in vivo. On the basis of the above results, we might predict that other individual phytosterol oxides (19-hydroxy-, 25-hydroxy-, 7α-hydroxy-, 5α,6α-epxy-, 5β,6β-epoxy-, etc.) could be produced according to this method. This strategy thus provided a simple and easy-to-handle method to obtain the individual target sterol oxides or other different structural sterol derivatives.

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Notes

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