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An expeditious synthesis of spinasterol and schottenol, two phytosterols present in argan oil and in cactus pear seed oil, and evaluation of their biological activities on cells of the central nervous system

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ABSTRACT

Spinasterol and schottenol, two phytosterols present in argan oil and in cactus pear seed oil, were synthesized from commercially available stigmasterol by a four steps reactions. In addition, the effects of these phytosterols on cell growth and mitochondrial activity were evaluated on 158N murine oligodendrocytes, C6 rat glioma cells, and SK-N-BE human neuronal cells with the crystal violet test and the MTT test, respectively. The effects of spinasterol and schottenol were compared with 7-ketocholesterol (7KC) and ferulic acid, which is also present in argan and cactus pear seed oil. Whatever the cells considered, dose dependent cytotoxic effects of 7KC were observed whereas no or slight effects of ferulic acid were found. With spinasterol and schottenol, no or slight effects on cell growth were detected. With spinasterol, reduced mitochondrial activities (30–50%) were found on 158N and C6 cells; no effect was found on SK-N-BE. With schottenol, reduced mitochondrial activity were revealed on 158N (50%) and C6 (10–20%) cells; no effect was found on SK-N-BE. Altogether, these data suggest that spinasterol and schottenol can modulate mitochondrial activity and might therefore influence cell metabolism.

1. Introduction

Phytosterols are structurally related to cholesterol and are mainly C-28 and C-29 carbon steroid alcohols [1]. Phytosterols might have some benefits in preventing cardiovascular diseases, and they could also contribute to preventing cancer and inflammatory diseases [2–4]. These benefits have led to using phytosterols as nutraceuticals. As the functional food market has grown exponentially in recent years, the understanding of the potential health benefits of phytosterol–enriched foods and nutrients is continually evolving, and it is therefore necessary to have a better knowledge of these molecules. However, only few data currently exist on the biological activities of most phytosterols, mainly on spinasterol and schottenol, which are present in argan oil and in cactus pear seed oil [5]. In order to evaluate their biological activities in vitro and in vivo, high amounts of these compounds are required. Due to very low natural abundance of spinasterol and schottenol from natural sources and despite the potential of these sterols in biological studies, only three syntheses of spinasterol and schottenol are available but they require 5–10 reaction steps with low overall yield [6–8]. As part of our program directed toward the synthesis of bioactive natural products an expeditious synthesis of spinasterol and schottenol was developed. As some phytosterols have the ability to cross the blood brain barrier [9] and to modulate amyloidogenesis in mice in vivo [10], we evaluated the biological activities of these synthetic phytosterols (spinasterol and schottenol) on cells of the central nervous systems (158N murine oligodendrocytes, C6 rat glioma cells, and SK-N-BE human neuronal cells) with the crystal violet test and the MTT test allowing to evaluate cell growth and mitochondrial activity, respectively. Their effects were compared with 7-ketocholesterol (used as positive control, known to inhibit cell growth and to trigger mitochondrial dysfunction) [11], and with ferulic acid (a potent anti-oxidant) also present in argan and cactus pear seed oil [12]. Altogether, our data report

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an expeditious synthesis of spinasterol and schottenol which are able to modulate mitochondrial activity on various cell types of the central nervous system.

2. Materials and methods

2.1. Chemistry

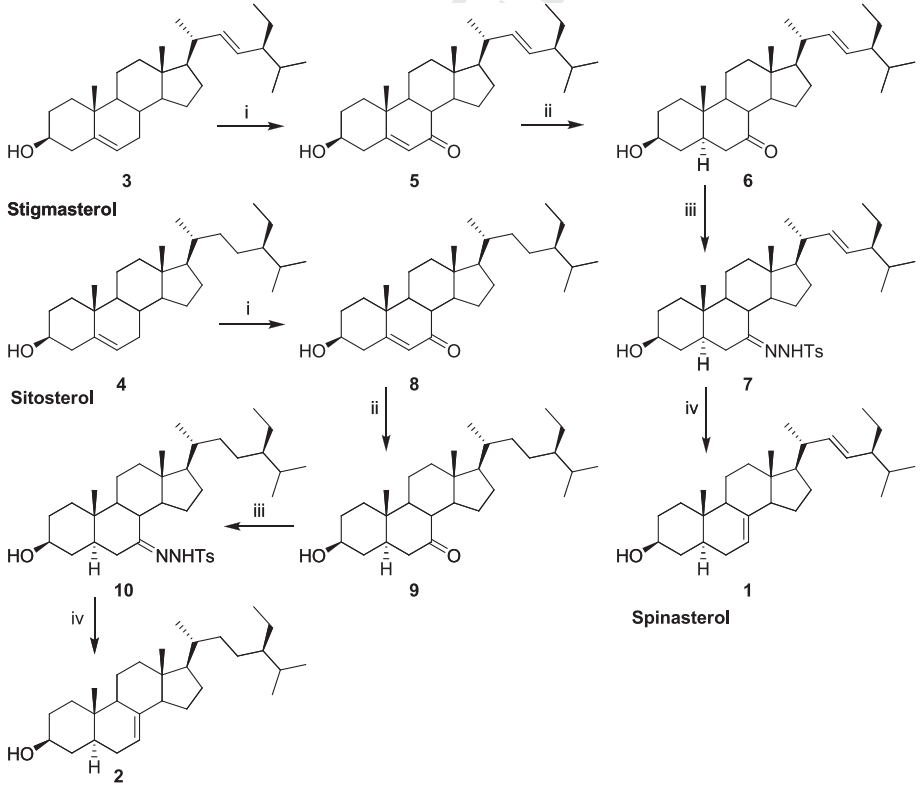
All of the reactions were carried out under an argon atmosphere. All reagents were obtained from commercial suppliers and used without further purification. Stigmasterol 90% (TCI) and Sitosterol 70% (Sigma–Aldrich) were used as such without further purification. Cu(OH)Cl·TMEDA dimmer (di-μ-hydroxo-bis[(*N,N,N′,N′*-tetramethylethylenediamine) copper(II)] chloride) was prepared according to literature [13]. Flash chromatography was carried out using silica gel (Merck Kieselgel 60, 230–400 mesh) with mixtures of ethyl acetate and petroleum ether as eluent unless specified otherwise. TLC analyses were performed on thin-layer analytical plates 60 F_{254} (Merck). The *tert*-butyl hydroperoxide (TBHP) 5–6 M solution in Decane was purchased from Sigma–Aldrich. TLC analyses were performed on thin layer analytical Plates 60 F_{254} (Merck). Melting points were measured on a Kofler Heizbank apparatus and are uncorrected. IR spectra were recorded on a PerkinElmer-Spectrum One FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Advance 400 spectrometer. High-resolution mass spectra (HRMS) were taken in electron ionization (EI) mode on Jeol GCmate.

2.1.1. -Ethylcholest-5, 22-dien-3-hydroxy-7-one (compound **5**; Fig. 1)

To a solution of stigmasterol 3 (1 mmol) and CuCl(OH)·TMEDA (23.2 mg, 0.05 mmol) in CH_2Cl_2 (8 mL) and MeOH (2 mL) was added t-BuOOH (2 mL, 10 mmol) [13]. After, the reaction was stirred at room temperature for 40 h, solvent was removed under reduced pressure. The residue was purified by flash column chromatography with EtOAc–petroleum ether (40–60) as eluent to yield 5 (247 mg, 58%) as a white solid. mp 150–152 °C; IR (neat): *v*: 3349, 2958, 2938, 2865, 1677, 1633, 1042 cm^{-1}. ^{1}H NMR ($CDCl_3$) 5.71 (s, 1H, 6-CH), 5.19 (dd, 1H, *J* = 15.16, 8.56 Hz, 23-CH), 5.043 (dd, 1H, *J* = 15.16, 8.64 Hz, 22-CH), 3.731–3.677 (m, 1H, 3-CH), 1.21 (s, 3H, 19-CH_3), 1.04 (d, 3H, *J* = 6.5 Hz, 21-CH_3), 0.86 (d, 3H, *J* = 6.5 Hz, 26-CH_3 or 27-CH_3), 0.82 (t, 3H, *J* = 7.0 Hz, 29-CH_3), 0.81 (d, 3H, *J* = 6.5 Hz, 26-CH_3 or 27-CH_3), 0.71 (s, 3H, 18-CH_3), ^{13}C NMR ($CDCl_3$) δ = 202.29, 165.24, 138.07, 129.5, 126.06, 70.49, 54.7, 51.21, 50.04, 49.95, 45.39, 42.99, 41.83, 40.23, 38.58, 38.3, 36.36, 31.87, 31.17, 29.03, 26.4, 25.36, 21.41, 21.2, 21.04, 19.00, 17.31, 12.24, 12.19. HRMS: *m/z* calcd for $C_{29}H_{46}O_2$ [M] 426.3498, found 402.3489. ^{1}H, ^{13}C NMR, and IR data were in accordance with literature values [14] (Suppl. Fig. 1).

2.1.2. -Ethylcholest-5-en-3-hydroxy-7-one (compound **8**; Fig. 1)

Sitosterol (414 mg, 1 mmol) was oxidized by same procedure described for **3** and purified over silica gel using EtOAc–petroleum ether (40–60) as eluent to give compound **8** (223 mg, 52%) as a white solid. mp 122–124 °C; IR (neat): *v*: 3524, 2938, 2861, 1660, 1627, 1062 cm^{-1}. ^{1}H NMR ($CDCl_3$) 5.7 (d, *J* = 0.84 Hz, 1H, 6-CH),



i: CuCl(OH).TMEDA(5% mol, 0.05 equiv), 10 equiv of tBuOOH(5 M in decane), CH_2Cl_2-MeOH (4:1), rt, 40 h, (58% for **5** and 52% for **8**); ii: 10% Pd/C, 4 equiv of ammonium formate, EtOAc-MeOH (1:1), 70°C, 1 h, (91% for **6** and 94% for **9**); iv: 1.2 equiv of $TsNHNH_2$, MeOH, 75°C, 3 h; v: 10 eq LiH, Toluene-THF (1:1), 110°C, 5h, (73%(**1**) and 75%(**2**) for 2 two steps).

Fig. 1. Synthesis scheme of spinasterol and schottenol.

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