ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2006, Vol. 32, No. 5, pp. 497–503. © Pleiades Publishing, Inc., 2006.
Original Russian Text © E.A. Piir, G.E. Morozevich, F.V. Drozdov, V.P. Timofeev, A.Yu. Misharin, 2006, published in Bioorganicheskaya Khimiya, 2006, Vol. 32, No. 5, pp. 551–558.

Δ^5-7-Ketosterols with Modified Side Chain: The Synthesis and the Effects on Viability and Cholesterol Biosynthesis in Hep G2 Cells

E. A. Piir[a], G. E. Morozevich[a], F. V. Drozdov[a], V. P. Timofeev[b], and A. Yu. Misharin[a, 1]

[a] *Orechovich Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Pogodinskaya ul. 10, Moscow, 119992 Russia*

[b] *Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, ul. Vavilova 32, Moscow, 117984 Russia*

Received August 1, 2005; in final form, October 21, 2005

Abstract—(22*E*)-3β-Hydroxysitosta-5,22-dien-7-one, (22*R*,23*R*)-3β,22,23-trihydroxysitost-5-en-7-one, and (22*R*,23*R*)-3β-hydroxy-22,23-isopropylidenedioxysitost-5-en-7-one were synthesized. The cytotoxicity and effects on cholesterol biosynthesis of the resulting 7-ketosterols, 7-ketocholesterol, and (22*S*,23*S*)-3β-hydroxy-22,23-oxidositost-5-en-7-one were studied in hepatoblastoma Hep G2 cells.

Key words: oxysterols, synthesis, Hep G2 cells, cholesterol biosynthesis, cytotoxicity

DOI: 10.1134/S1068162006050141

INTRODUCTION

7-Ketocholesterol is one of the major oxysterols of mammalian organisms.[2] In a cell culture, 7-ketocholesterol affects the biosynthesis, metabolism, and transport of lipids and oxidative processes in mitochondria, promotes inflammation, activates AHR, and is involved in the regulation of proliferation and apoptosis [1–6]. The side chain of 7-ketocholesterol is rapidly degraded by the 27-hydroxylase route in hepatocytes [7, 8]. Like 7-ketocholesterol, 7-ketositosterol, its close structural analogue, affects the growth and viability of C57BL/6 macrophages and the activity of mitochondrial dehydrogenases in the same cells [9].

We continue our studies of new analogues of biologically active oxysterols containing modified side chains [10–13], and present here (1) the synthesis of Δ^5-7-ketosterols, namely, (22*E*)-3β-hydroxysitosta-5,22-dien-7-one (**XI**), (22*R*,23*R*)-3β,22,23-trihydroxysitost-5-en-7-one (**XII**), and (22*R*,23*R*)-3β-hydroxy-22,23-isopropylidenedioxysitost-5-en-7-one (**XIII**) (scheme) and (2) a comparative examination of cytotoxicities and the effects on cholesterol biosynthesis of the synthesized 7-ketosterols (**XI**)–(**XIII**) and the earlier obtained (22*S*,23*S*)-3β-hydroxy-22,23-oxidositost-5-en-7-one (**XIV**), (22*R*,23*R*)-3β-hydroxy-22,23-oxidositost-5-en-7-one (**XV**), and 7-ketocholesterol (**XVI**) in human hepatoma Hep G2 cell line.

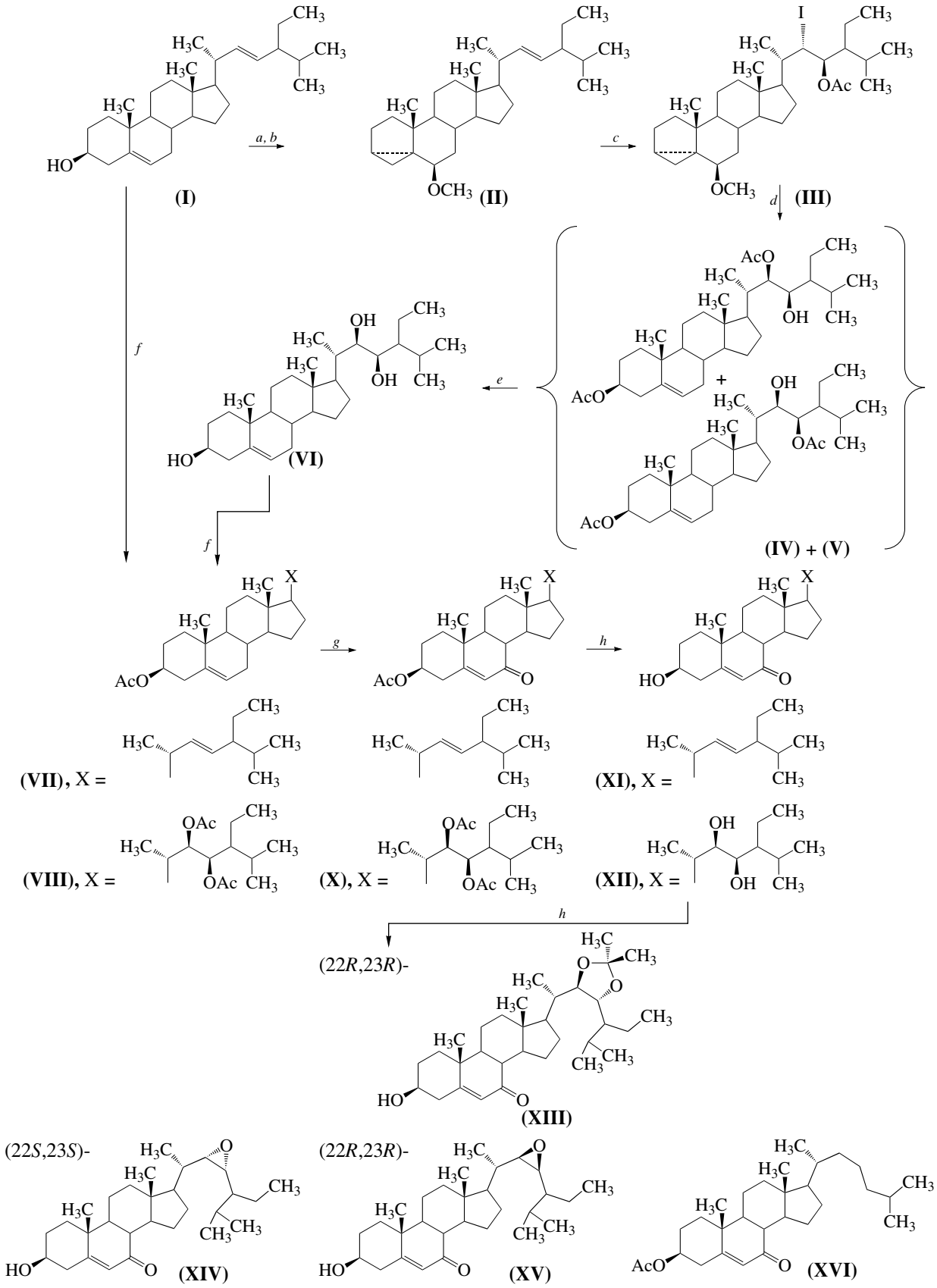
RESULTS AND DISCUSSION

The synthesis of 7-ketosterols (**XI**)–(**XIII**) from stigmasterol (**I**) is given in the scheme. Various methods of stereoselective hydroxylation of 22,23-double bond in stigmasterol derivatives have currently been developed [14–18]; however, high prices of the necessary reagents and long reaction times are shortcomings of these methods. We decided to use the classic Woodward procedure for the hydroxylation of 22,23-double bond.

Stigmasterol (**I**) was transformed into 3α,5α-cyclo-6β-methoxystigmastane (**II**) via the corresponding tosylate with the aim to protect 5,6-double bond [19]. The treatment of cyclosterol (**II**) by iodine in 95% AcOH in the presence of AcOAg at room temperature for 1 h resulted in a mixture, from which iodoacetate (**III**) was isolated as the major product in 65% yield. The presence of iodine atom in (**III**) was confirmed by a molecular ion of *m*/*z* 612 in its mass spectrum. The treatment of (**III**) with K_2CO_3 in aqueous MeOH accompanied with deacetylation led to the only product, the earlier described (22*R*,23*R*)-22,23-oxido-3α,5α-cyclo-6β-methoxysitostane [13], which unequivocally confirmed the 22*S*,23*R*-configuration of (**III**). Note that the 3α,5α-cyclo-6β-methoxy fragment of (**II**) was sufficiently stable under the conditions; it was shown in a separate experiment that the half-time of

[1] Corresponding author; phone: +7 (495) 246-3375; e-mail: alexander.misharin@ibmc.msk.ru.

[2] Abbreviations: AHR, aromatic hydrocarbon receptor; CPBA, *meta*-chloroperbenzoic acid; FCS, fetal calf serum; and MTT, [3-(4,5-dimethylthiazol-2-yl)]-2,5-diphenyltetrazolium bromide.



Reagents: *a* TosCl + Py; *b* AcONa/MeOH, heating; *c* I_2, AcOAg/95% AcOH; *d* AcOAg/AcOH, heating; *e* K_2CO_3/MeOH–H_2O; *f* Ac_2O/Py; *g* $K_2Cr_2O_7$/AcOH–Ac_2O, heating; *h* $(CH_3)_2C(OCH_3)_2$, TosOH;

cyclosterol (**II**) conversion into acetate (**VII**) in 95% AcOH at room temperature was 78 h.

Iodoacetate (**III**) was transformed into a 1 : 1 mixture of diacetylated triols (**IV**) and (**V**) in 62% yield under a reflux in glacial acetic acid in the presence of AcOAg. The presence of 5,6-double bond and 3β-acetoxy group in products (**IV**) and (**V**) was proved by the ^{1}H NMR spectrum of the mixture (see the Experimental section). Evidently, compounds (**IV**) and (**V**) are isomeric 22- and 23-monoacetates of 22,23-diol. Since the transformation of (22*S*,23*R*)-iodoacetate (**III**) into products (**IV**) and (**V**) is realized under these conditions [20] through the cyclic intermediate and requires the retention of the C23 configuration and the inversion of configuration at C22, compounds (**IV**) and (**V**) have the (22*R*,23*R*)-configuration. When the mixture of (**IV**) and (**V**) was treated with K_2CO_3 in aqueous methanol, the crystalline (22*R*,23*R*)-sitost-5-ene-3β,22,23-triol (**VI**) was obtained in 95% yield. Based on the above-described experiments, a simple preparative procedure was developed that allowed the preparation of the target triol (**VI**) from stigmasterol (**I**) in 28%yield without isolation of intermediates (see the Experimental section).

Stigmasterol (**I**) and triol (**VI**) were acetylated (Ac_2O/Py), and the corresponding acetates (**VII**) and (**VIII**) were transformed into acetylated 7-ketosterols (**IX**) and (**X**) by the treatment with $K_2Cr_2O_7$ in a mixture of AcOH and Ac_2O at 50°C using the method [21]. The yields of ketosterol triacetate (**X**) and ketosterol acetate (**IX**) were 65 and 31%, respectively. The allyl oxidation [21] in the presence of 22,23-double bond is known to lead to side reactions. More effective methods of keto group introduction in position 7 of stigmasterol derivatives have been proposed [22, 23]; however, we used the method [21] because of its simplicity and a short time. Saponification of acetates (**IX**) and (**X**) resulted in high yields of target ketosterols (**XI**) and (**XII**). The treatment of ((**XII**) with 2,2-dimethoxypropane in the presence of TosOH gave acetonide (**XIII**) in 79% yield.

The next stage was a comparative study of biological activities of new 7-ketosterols (**XI**)–(**XIII**) and the earlier synthesized (22*S*,23*S*)-3β-hydroxy-22,23-oxidositost-5-en-7-one (**XIV**), (22*R*,23*R*)-3β-hydroxy-22,23-oxidositost-5-en-7-one (**XV**), and 7-ketocholesterol (**XVI**) [13] in human hepatoma Hep G2 cells.

It is known that 7-ketocholesterol (**XVI**) manifests a high toxicity toward numerous cell lines; however, it is limitedly toxic for hepatocytes (particularly, for Hep G2 cells) [2, 3]. We studied cytotoxic effects of 7-ketocholesterol (**XVI**) for Hep G2 cells by the method [24] using a tetrazolium dye MMT after 24-, 48-, and 72-h incubation (Fig. 1). A considerable increase in the results of MMT assay, which indicates an increased activity of mitochondrial dehydrogenases, with the 7-ketosterol maximal concentration of 30 μM was observed after 24-h incubation. After 48-h incubation,

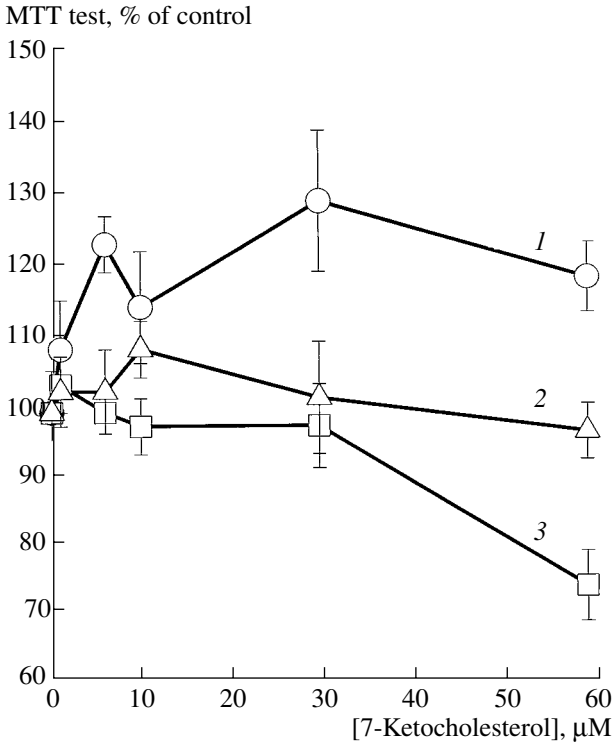


Fig. 1. The effect of 7-ketocholesterol (**XVI**) on the viability of Hep G2 cells after (*1*) 24, (2) 48, and (3) 72 h of incubation.

the results of this test were practically indistinguishable from control, whereas, after 72-h incubation, 7-ketosterol manifested a moderate toxicity (viability of ~75%) at a concentration of 60 μM. Alterations in cell morphology were also observed including a decreased spreading and the presence of rounded cells.

The effects of 7-ketosterols (**XI**)–(**XVI**) on the viability of Hep G2 cells after 48-h incubation are shown in Fig. 2. Among the compounds of this series, (22*R*,23*R*)-3β,22,23-trihydroxysitost-5-en-7-one (**XII**) was most toxic. Epimeric 22,23-epoxy-7-ketosterols differently affected the cell viability: (22*S*,23*S*)-isomer (**XIV**) displayed a rather high toxicity, whereas (22*R*,23*R*)-isomer showed no noticeable effect. Under the conditions of our experiments, we revealed no toxicity to Hep G2 cells for compounds (**XI**), (**XIII**), (**XV**), and (**XVI**).

The effects of (**XI**)–(**XVI**) on the cholesterol biosynthesis in Hep G2 cells after incubation for a short time (3 h) in the serumless medium are shown in Fig. 3. (22*S*,23*S*)-3β-Hydroxy-22,23-oxidositost-5-en-7-one (**XIV**) effectively inhibited cholesterol biosynthesis; the calculated IC_{50} value was 4 ± 0.3 μM. (22*R*,23*R*)-3β,22,23-Trihydroxysitost-5-ene-7-one (**XII**) selectively inhibited cholesterol biosynthesis at concentrations up to 20 μM without affecting fatty acid and triglyceride biosynthesis monitored in this case and thereafter by the incorporation of [^{14}C]acetate into the corresponding fractions. At a concentration of 30 μM, it inhibited biosynthesis of not only cholesterol,

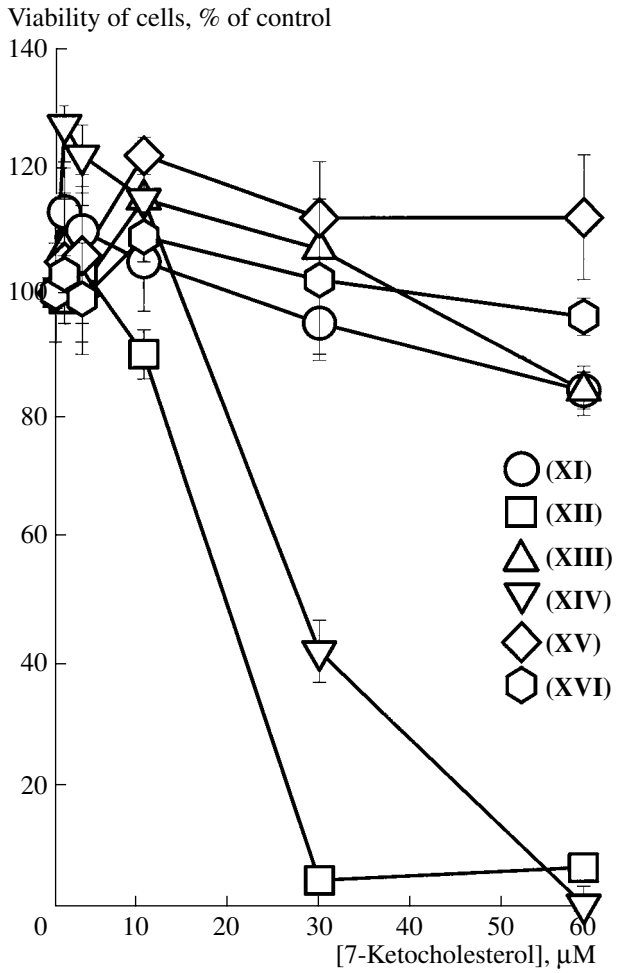


Fig. 2. The effects of (**XI**)–(**XVI**) on the viability of Hep G2 cells after 48-h incubation (the curve numbers correspond to the compound numbers).

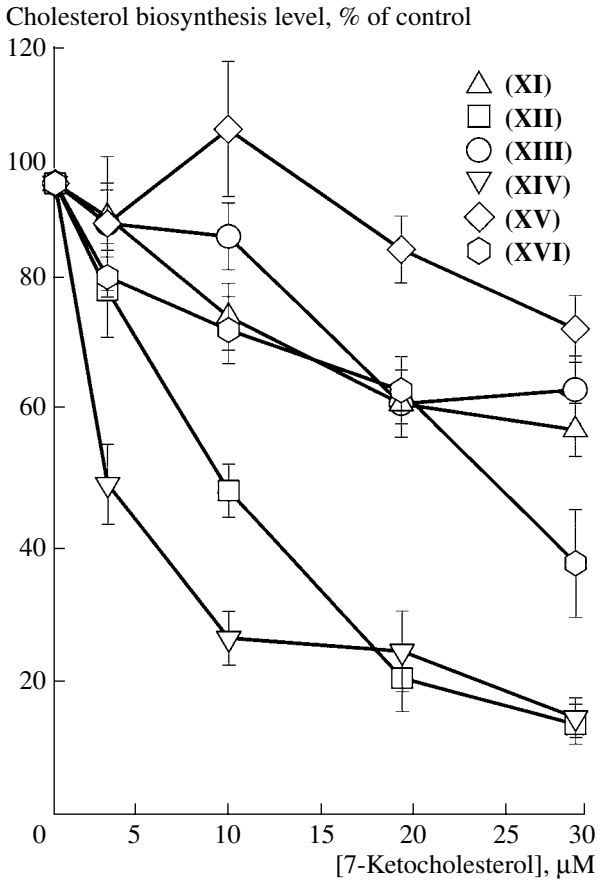


Fig. 3. The effects of (**XI**)–(**XVI**) on the cholesterol biosynthesis level in Hep G2 cells after 3-h incubation in the serumless medium (the curve numbers correspond to the compound numbers).

but also of fatty acids and triglycerides. The inhibitory effects of (**XI**), (**XIII**), and (**XV**) were small. (22*R*,23*R*)-3β-Hydroxy-22,23-isopropylidenedioxysitost-5-en-7-one (**XIII**) stimulated fatty acid and triglyceride biosynthesis by 40% at a concentration of 30 μM. Our results demonstrate that side chain modifications essentially affect 7-cholesterol cytotoxicity and its ability to regulate cholesterol biosynthesis in Hep G2 cells. (22*R*,23*R*)-3β,22,23-Trihydroxysitost-5-en-7-one (**XII**) efficiently decreased the cell viability and markedly inhibited cholesterol biosynthesis. In comparison with ketosterol (**XII**) (22*S*,23*S*)-3β-hydroxy-22,23-oxidositost-5-en-7-one (**XIV**) was a more potent inhibitor of cholesterol biosynthesis, but was less toxic.

One may presume that further studies of the aforementioned compounds in various cell cultures can be of interest for both the study of the role of oxysterols in regulatory processes and the evaluation of pharmacological potential of these compounds.

EXPERIMENTAL

Stigmasterol was obtained from ICN; reagents and solvents were from Aldrich, Merck, and MedKhimLab (Russia); media, sera, and plastic utensil for cell cultures were from Gibco BRL and Costar; PBS and MTT, from Sigma; and [1-^{14}C]AcONa, from Amersham. Stigmasteryl acetate (**VII**) was obtained by treatment of stigmasterol (**I**) with excess Ac_2O in pyridine; 6β-methoxy-3α,5α-cyclostigmastane (**II**) was synthesized by the method [19]; (22*S*,23*S*)-3β-hydroxy-22,23-oxidositost-5-ene-7-one (**XIV**) and (22*R*,23*R*)-3β-hydroxy-22,23-oxidositost-5-ene-7-one (**XV**) were prepared as in [13], and 7-ketocholesterol was obtained according to [21]. Mps of crystalline compounds were determined in a glass capillaries.

Column chromatography was carried out on silica gel G (70–200 μm (Merck), andTLC, on precoated HPTLC Kieselgel plates (Merck). Substance spots on plates were detected with 3% ammonium molybdate in 5% H_2SO_4 and/or by 5% $SbCl_3$ in dry $CHCl_3$ followed by heating.

^{1}H- and ^{13}C NMR spectra were registered on an AMX-III-400 (Bruker) in $CDCl_3$; chemical shifts are given in ppm; coupling constants, in Hz; chemical shifts of $CHCl_3$ in ^{1}H- and ^{13}C NMR spectra were 7.25 and 77.16 ppm, respectively. Mass spectrum of compound (**III**) was registered on a Kratos MS-890 mass spectrometer at electron impact ionization with energy of 70 eV.

Radioactivity was measured in a toluene scintillator on an LKB counter. The cell viability based on the MTT assay [24] was determined on a Microtiter plate reader (LKB).

The human cell line Hep G2 obtained from the European collection of cell cultures (ECACC) was cultured at 37°C in the atmosphere containing 5% CO_2 in the 1 : 1 OptiMEM : F12 medium containing 10% FCS in 24- or 96-well plates. The cells were kept for 24 h in the serumless medium before the experiments.

(22*S*,23*R*)-3α,5α-Cyclo-6β-methoxy-22-iodo-23-acetoxysitostane (III). Water (0.5 ml) and AcOAg (220 mg, 1.3 mmol) were added to a solution of cyclosterol (**II**) (240 mg, 0.5 mmol) in AcOH (10 ml). Finely ground iodine (130 mg, 1.1 mmol) was then added in portions for 20 min under vigorous stirring. The mixture was stirred for another 40 min, and the precipitate was separated and washed with toluene (3 × 15 ml). The filtrate was combined with the toluene extract, water (10 ml) was added, and the toluene layer was successively washed with saturated $NaHCO_3$ and water, dried with Na_2SO_4, and evaporated. The residue was chromatographed on a column eluted with 3 : 1 hexane–EtOAc. Compound (**III**) (260 mg, 0.35 mmol, 65%) was isolated as a colorless glass-like film; ^{1}H NMR: 0.43 (1 H, m), 0.64 (1 H, m), 0.75 (3 H, s), 0.76 (3 H, d, *J* 6.8), 0.88 (3 H, d, *J* 6.8), 0.89 (3 H, t, 7.5), 1.01 (3 H, s), 2.05 (3 H, s), 2.78 (1 H, s), 3.32 (3 H, s), 4.36 (1 H, d, *J* 7.5), and 5.43 (1 H, d, *J* 10.5); MS, *m/z* (*I*, %): 612 (4) [M^+]; 597 (15), 580 (18), 557 (34), 443 (15), 393 (100).

(22*R*,23*R*)-3β,22-Diacetoxysitost-5-en-23-ol (IV) and (22*R*,23*R*)-3β,23-diacetoxysitost-5-en-23-ol (V). A mixture of (**III**) (185 mg, 0.3 mmol), AcOAg (86 mg, 0.5 mmol) and glacial AcOH (10 ml) was refluxed for 1 h, and the precipitate was separated and washed with toluene (3 × 10 ml). The filtrate was combined with the toluene extract, combined with water (10 ml); the toluene layer was washed with saturated $NaHCO_3$ and water, dried with Na_2SO_4 and evaporated. The residue was chromatographed on a silica gel column in 3 : 1 hexane–ethyl acetate to give a mixture of (**IV**) and (**V**) as a clear film; yield 105 mg (0.2 mmol, 66%); ^{1}H NMR: 0.68 (3 H, s), 0.81 (d, *J* 6.6) and 0.85 (3 H total, d, *J* 6.6), 0.87 (3 H, t, *J* 7.5), 0.92 (3 H, d, *J* 6.8), 1.00 (3 H, s), 1.08 (3 H, d, *J* 6.8), 2.02 (3 H, s), 2.05 (s) and 2.10 (3 H total, s), 3.66 (m) and 4.91 (1 H total, m), 3.72 (m) and 5.06 (1 H total, m), 4.59 (1 H, m), 5.36 (1 H, m).

(22*R*,23*R*)-3β,22,23-Trihydroxysitost-5-ene (VI). A mixture of (**IV**) and (**V**) (210 mg, 0.4 mmol), K_2CO_3 (1 g), methanol (5 ml), and water (3 ml) was refluxed for 30 min, and chloroform (20 ml) and water (10 ml) were added. The chloroform layer was separated, the aqueous layer was extracted with a 2 : 1 $CHCl_3$ –MeOH (10 ml); the combined extract was dried with Na_2SO_4; and the residue was recrystallized from acetonitrile to give (**VI**); yield 167 mg (0.38 mmol, 95%); mp 182–184°C; ^{1}H NMR: 0.71 (3 H, s), 0.86 (3 H, d, *J* 6.8), 0.93 (3 H, d, *J* 6.8), 0.95 (3 H, t, *J* 7.5), 1.00 (3 H, s), 1.02 (3 H, d, *J* 6.8), 3.51 (1 H, m), 3.55–3.68 (2 H, m), 5.34 (1 H, m); ^{13}C NMR: 11.70, 13.96, 14.10, 14.45, 17.72, 18.54, and 19.30 (C18, C19, C21, C26, C27, C28, and C29), 21.03, 21.67, 24.48, 26.87, 27.96 and 29.61 (C2, C7, C11, C15, C16, and C25), 31.62, 31.79, 31.88, 37.21, and 39.71 (C1, C8, C10, C12, and C20), 42.25, 42.35, 49.60, 50.06, 52.64, and 56.36 (C4, C9, C13, C14, C17, and C24); 70.65, 71.72, 72.30 (C3, C22, and C23), 121.48, and 140.76 (C5 and C6).

Preparative synthesis of (22*R*,23*R*)-3β,22,23-trihydroxysitost-5-ene (VI) from stigmasterol (I). Tosyl chloride (8.64 g, 40 mmol) was added to a solution of stigmasterol (8.24 g, 20 mmol) in anhydrous pyridine (50 ml). The mixture was stirred for 14 h, poured into a mixture of saturated $NaHCO_3$ (500 ml) and ice (100 g), stirred for 2 h, and the precipitate was separated. The aqueous solution was extracted with toluene (2 × 100 ml). The combined toluene extract was washed with saturated Na_2SO_4, dried with Na_2SO_4, and evaporated to a volume of 40 ml. The resulting solution was slowly poured into a solution of AcONa (20 g) in methanol (250 ml) under reflux, and the mixture was refluxed for another 40 min and evaporated. Toluene (200 ml) and water (50 ml) were added to the residue; the toluene solution was washed with water (2 × 50 ml), dried with Na_2SO_4, and evaporated to dryness. The residue was dissolved in AcOH (40 ml); water (2 ml) and AcOAg (6.72 g, 40 mmol) were added followed by the addition of finely ground I_2 (5.04 g, 40 mmol) in portions for 20 min. The mixture was stirred at room temperature for 40 min, Ac_2O (15 ml) and AcOAg (3.36 g, 20 mmol) were added, and the mixture was refluxed under stirring for 1 h and filtered. The filtrate was evaporated, the residue was dissolved in 1 : 1 toluene–ethyl acetate (50 ml), and the resulting solution was washed with saturated $NaHCO_3$ and evaporated. Potassium carbonate (20 g), methanol (100 ml), and water (40 ml) were added to the residue, and the mixture was refluxed under stirring for 40 min. After cooling, chloroform (200 ml) and water (20 ml) were added; the organic layer was separated; and the aqueous layer was extracted with $CHCl_3$ containing 10% MeOH (3 × 50ml). The combined chloroform extract was washed with Na_2SO_4 and evaporated. The residue was treated with boiling light petroleum (40 ml) and kept for 14 h at room temperature. The resulting precipitate was twice recrystallized from a minimal volume of acetone to give 2.45 g (5.6 mmol, 28%) of (**VI**). Mp and ^{1}H- and ^{13}C NMR spectra coincided with those given above.

(22*R*,23*R*)-3β,22,23-Triacetoxysitost-5-ene (VIII). A mixture of 3β,22,23-trihydroxystigmast-5-ene (**VI**) (446 mg, 1 mmol), Ac_2O (2 ml), and anhydrous pyridine (5 ml) was refluxed for 1 h, cooled, diluted with pyridine (10 ml) and methanol (5 ml), kept for 10 min, twice reevaporated with water, and the resulting glasslike film was dried in a vacuum to give (**VIII**); yield 620 mg (1 mmol, quantitative); ^{1}H NMR: 0.66 (3 H, s), 0.82 (3 H, d, *J* 6.8), 0.91 (6 H, d, *J* 6.8), 0.92 (3 H, t, *J* 7.5), 0.99 (3 H, s); 2.02 (3 H, s), 2.03 (3 H, s), 2.08 (3 H, s), 4.58 (1 H, m), 5.03 (1 H, m), 5.24 (1 H, m), and 5.36 (1 H, m).

(22*E*)-3β-Acetoxystigmasta-5,22-dien-7-one (IX) and (22*R*,23*R*)-3β,22,23-triacetoxystigmast-5-en-7-one (X). Acetic anhydride (10 ml) and finely ground $K_2Cr_2O_7$ (0.36 g, 1.20 mmol) were added to a solution of (**VII**) or (**VIII**) (1 mmol of each) in AcOH (10 ml), and the mixture was stirred at 50°C for 50 min with TLC monitoring in 5 : 1 hexane–EtOAc. The resulting mixtures were poured into toluene (150 ml), shaken for 5 min, and, after 15 min, the toluene solution was filtered through a paper filter into stirred saturated $NaHCO_3$ solution (100 ml). The toluene solution was separated, aqueous layer was extracted with toluene (100 ml), and combined toluene extracts were washed with water, dried with Na_2SO_4, and evaporated. (22*E*)-3β-Acetoxystigmasta-5,22-dien-7-one (**IX**) was isolated by chromatography on a silica gel column in 5 : 1 hexane–EtOAc; yield 146 mg (0.31 mmol, 31%); colorless clear film; ^{1}H NMR: 0.69 (3 H, s), 0.79 (3 H, t, *J* 7.5), 0.83 (3 H, d, *J* 6.8), 0.85 (3 H, d, *J* 6.8), 1.01 (3 H, d, *J* 6.8), 1.20 (3 H, s), 2.04 (3 H, s), 4.70 (1 H, m), 5.04 (1 H, m), 5.24 (1 H, m), 5.69 (1 H, d, *J* 1.2). (22*R*,23*R*)-3β,22,23-Triacetoxystigmast-5-en-7-one (**X**) was isolated by crystallization from light petroleum; yield 303 mg (0.65 mmol, 65%); ^{1}H NMR: 0.69 (3 H, s), 0.83 (3 H, d, *J* 6.8), 0.92 (3 H, t, *J* 7.5), 0.92 (3 H, d, *J* 6.8), 0.94 (3 H, d, *J* 6.8), 1.19 (3 H, s); 2.02 (3 H, s), 2.03 (3 H, s); 2.07 (3 H, s), 4.70 (1 H, m); 5.01 (1 H, m), 5.16 (1 H, m), and 5.69 (1 H, d, *J* 1.2).

(22*E*)-3β-Hydroxystigmasta-5,22-dien-7-one (XI) and (22*R*,23*R*)-3β,22,23-trihydroxystigmast-5-en-7-one (XII). Compounds (**IX**) and (**X**) were refluxed with a tenfold excess of K_2CO_3 in 2 : 1 MeOH–H_2O for 30 min. Products (**VIII**) and (**XI**) were extracted with $CHCl_3$, and chloroform extracts were dried with Na_2SO_4 and evaporated. (22E)-3β-Hydroxystigmasta-5,22-diene-7-one (**XI**) was recrystallized from 7 : 3 MeOH–H_2O mixture; mp 124–126°C; Found, %: C 81.35; H 10.70. $C_{29}H_{46}O_2$. Calc., %: C 81.63; H 10.87. quantitative yield; ^{1}H NMR: 0.69 (3 H, s), 0.78 (3 H, d, *J* 6.8); 0.79 (3 H, t, *J* 7.5); 0.84 (3 H, d, *J* 6.8); 1.01 (3 H, d, *J* 6.8); 1.20 (3 H, s); 3.67 (1 H, m); 5.01 (1 H, m), 5.16 (1 H, m), and 5.68 (1 H, d, *J* 1.2). ^{13}C NMR: 12.36, 14.37, 17.48, 19.16, 21.17, 21.39, and 21.57 (C18, C19, C21, C26, C27, C28, and C29), 25.50, 26.56, 29.16, and 29.85 (C11, C15, C16, and C25), 31.40, 32.03, 36.55, and 38.78 (C1, C2, C12, and C20), 40.35, 42.00, 43.15, 45.58, 50.02, 50.17, 51.39, and 54.93 (C4, C8, C9, C10, C13, C14, C17, and C24), 70.71 (C3), 126.30, 129.71, and 138.21 (C5, C22, and C23), 165.09 (C6), and 202.22 (C7).

(22*R*,23*R*)-3β,22,23-Trihydroxystigmast-5-ene-7-one (XII) was recrystallized from 1 : 2 EtOAc–hexane; mp136°C; quantitative yield; ^{1}H NMR: 0.72 (3 H, s), 0.93 (3 H, d, *J* 6.8), 0.95 (3 H, t, *J* 7.5), 0.95 (3 H, d, *J* 6.8), 1.03 (3 H, d, *J* 6.8), 1.20 (3 H, s), 3.55–3.72 (3 H, m), 5.68 (1 H, d, *J* 1.2); ^{13}C NMR: 12.05, 14.25, 14.37, 14.60, 17.44, 17.93, and 18.73 (C18, C19, C21, C26, C27, C28, and C29), 21.37, 21.92, 26.66, 27.09, and 28.50 (C11, C15, C16, C24, and C25), 31.50, 36.54, 37.44, and 38.83 (C1, C2, C12, and C20), 42.00, 42.59, 43.75, 45.57, 49.78, 50.70, and 51.56 (C4, C8, C9, C10, C13, C14, and C17); 70.66, 70.76, and 72.29 (C3, C22, and C23); 126.15 (C5), 165.39 (C6), and 202.28 (C7). Found, %: C 76.05; H 10.39. $C_{29}H_{48}O_4$. Calc., %: C 75.61; H 10.50.

(22*R*,23*R*)-3β-Hydroxy-22,23-isopropylidenedioxystigmast-5-en-7-one (XIII). A mixture of ketosterol (**XII**) (92 mg, 0.2 mmol), 2,2-dimethoxypropane (3 ml), and TosOH (5 mg) was stirred for 10 min, diluted with $CHCl_3$ (20 ml), washed with saturated $NaHCO_3$ (2 × 5 ml), dried with Na_2SO_4, and evaporated. Acetonide (**XIII**) was isolated by chromatography on a silica gel column eluted with 2 : 1 hexane–acetone; colorless gel-like film; Found, %: C 76.30; H 10.32. $C_{32}H_{52}O_4$. Calc., %: C 76.75; H 10.47. yield 80 mg (0.16 mmol, 79%); ^{1}H NMR: 0.70 (3 H, s), 0.93 (3 H, d, *J* 6.8), 0.93 (3 H, t, *J* 7.5), 0.94 (3 H, d, *J* 6.8), 1.03 (3 H, d, *J* 6.8), 1.19 (3 H, s), 1.36 (6 H, s), 3.56 (1 H, m), 3.86–4.00 (2 H, m), and 5.68 (1 H, s); ^{13}C NMR: 11.54, 13.27, 14.12, 17.28, 18.64, and 19.67 (C18, C19, C21, C26, C27, and C29), 21.22, 23.31, 26.50, 26.82, 27.31, 27.56, 28.61, and 29.68 (C11, C15, C16, C24, C25, C28, and $(\underline{CH_3})_2C(O)_2$); 31.21, 36.39, 38.39, and 38.71 (C1, C2, C10, and C12), 41.82, 43.80, 45.44, 46.50, 49.70, 49.91, and 51.46 (C4, C8, C9, C13, NC14, C17, and C20), 70.52, 72.29. and 79.72 (C3, C22, and C23), 106.66 106.66 $(CH_3)_2\underline{C}(O)_2$), 126.06 (C5), 165.10 (C6), and 202.00 (C7).

Cytotoxicity of compounds (XI)–(XVI) to Hep G2 cells [24]. Hep G2 cells were incubated in 96-well plates in a serumless medium containing the tested compounds at varied concentrations for 48 h [for 7-ketocholesterol (**XVI**), the incubation was also carried out for 24 and 72 h], the cultural medium was removed, PBS (125 μl) containing MTT (1 mg/ml) was added into each well, and the cells were incubated for 4 h at 37°C. The reaction was terminated by addition into each well of a solution of 0.1 M HCl in isopropanol containing 10% Triton X-100 (125 μl). Optical density was measured every 2 h at 540 and 690 nm in each well. The results of the MMT assay (the difference in absorption at 540 and 690 nm) were averaged for six independent determinations. The result of the MMT assay in the absence of the tested compounds was taken as 100%.

The effect of compounds (XI)–(XVI) on cholesterol biosynthesis in Hep G2 cells [25]. Hep G2 cells were incubated in 24-well plates in a serumless medium at varied concentrations for 3 h, the medium was replaced by a fresh one containing 0.1 mM [1-^{14}C]AcONa (1 μCi per ml of water) and the tested compounds, and the incubation was carried out for 3 h. The cells were washed thrice with PBS, the lipids were extracted with a 3 : 2 mixture of hexane–isopropanol, the extracts were separated by TLC in 70 : 29 : 1 hexane–Et_2O–AcOH in the presence of internal standards (cholesterol oleate, trioleine, oleic acid, and cholesterol). The chromatograms were treated with iodine vapors, the areas corresponding to the standards were collected in vials for radioactivity counting. The level of cholesterol biosynthesis (cpm/mg of cell protein for 3 h) was calculated by incorporation of the radioactive label into cholesterol. The incorporation of the radioactive label in the absence of tested compounds was taken as 100%. Each determination was repeated thrice in three independent experiments.

ACKNOWLEDGMENTS

We are grateful to A.P. Pleshkova (Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences) for registration of mass-spectra.

The work was supported by the Russian Foundation for Basic Research, project no. 03-04-48700, and the program Molecular and Cell Biology of the Presidium of Russian Academy of Sciences.

REFERENCES

1. Smith, L.L., *Biological Effects of Cholesterol Oxides*, Peng, S.-K. and Morin, R.J., Eds., Boca Raton, FL: CRC, 1992, pp. 7–31.
2. Smith, L.L., *Lipids*, 1996, vol. 31, pp. 453–487.
3. Schroepfer, G.J., *Physiol. Rev.*, 2000, vol. 80, pp. 361–554.
4. Guardiola, F., Codony, R., Addis, P.B., Rafecas, M., and Boatella, J., *Food. Chem. Toxicol.*, 1996, vol. 34, pp. 193–211.
5. Lemaire, S., Lizard, G., Monier, S., Miguet, C., Gueldry, S., Volot, F., Gambert, P., and Neel, D., *FEBS Lett.*, 1998, vol. 440, pp. 434–439.
6. Savouret, J.-F., Antenos, M., Quesne, M., Xu, J., Milgrom, E., and Casper, R.F., *J. Biol. Chem.*, 2001, vol. 276, pp. 3054–3059.
7. Norlin, M., von Bahr, S., Bjorkhem, I., and Wikvall, K., *J. Lipid Res.*, 2003, vol. 44, pp. 1515–1522.
8. Lyons, M.A. and Brown, A.J., *Lipids*, 2001, vol. 36, pp. 701–711.
9. Adcox, C., Boyd, L., Oehrl, L., Allen, J., and Fenner, G., *J. Agric. Food Chem.*, 2001, vol. 49, pp. 2090–2095.
10. Misharin, A.Yu. and Timofeev, V.P., *Bioorg. Khim.*, 2004, vol. 30, pp. 84–88; *Rus. J. Bioorg. Chem.*, 2004, vol. 30, pp. 75–79.
11. Piir, E.A., Medvedeva, N.V., Kashirina, N.M., Shevelev, A.Ya., and Misharin, A.Yu., *Bioorg. Khim.*, 2004, vol. 30, pp. 547–551; *Rus. J. Bioorg. Chem.*, 2004, vol. 30, pp. 492–496.
12. Flegentov, G.Yu., Piir, E.A., Medvedeva, N.V., Tkachev, Ya.V., Timofeev, V.P., and Misharin, A.Yu., *Bioorg. Khim.*, 2005, vol. 31 pp. 312–319; *Rus. J. Bioorg. Chem.*, 2005, vol. 31, pp. 279–285.
13. Flegentov, G.Yu., Tkachev, Ya.V., Piir, E.A., Pleshkova, A.P., Timofeev, V.P., and Misharin, A.Yu., *Bioorg. Khim.*, 2005, vol. 31, pp. 528–534; *Rus. J. Bioorg. Chem.*, 2005, vol. 31, pp. 475–481.
14. Kolb, H., Van Nieuwenhze, M.S., and Sharpless, K.B., *Chem. Rev.*, 1994, vol. 94, pp. 2483–2547.
15. McMorris, T.C., Chavez, R.C., and Patil, P.A., *J. Chem. Soc., Perkin Trans. 1*, 1996, pp. 295–302.
16. Brosa, C., Soca, L., Terricabras, E., and Ferrer, J.C., and Alsina, A., *Tetrahedron*, 1998, vol. 54, pp. 12337–12346.
17. Ramyrez, J.A., Teme Centurion, O.M., Gros, E.G., and Galagovsky, L.R. *Steroids*, 2000, vol. 65, pp. 329–337.
18. Michelini, F.M., Ramyrez, J.A., Berra, A., Galagovsky, L.R., and Alche, L., *Steroids, 2004*, vol. 69, pp. 713–720.
19. Fieser, L.F. and Fieser, M., *Natural Products Related To Phenantrhrene, 3rd Ed*, New York: Reinhold Publishing Corp., 1949.
20. Wilkinson, S.J., *Advanced Organic Chemistry*, vol. 2, New York: Wiley, 1980. Translated under the title *Obshchaya organicheskaya khimiya*, vol. 2, Moscow: Khimiya, 1982.
21. Marshall, C.W., Ray, R.E., Laos, J., and Riegel, B., *J. Am. Chem. Soc.*, 1957, vol. 79, pp. 6308–6313.
22. Jones, S.R. and Selinsky, B.S., *J. Org. Chem.*, 1998, vol. 63, pp. 3786–3789.
23. Uekawa, T., Ishigami, K., and Kitahara, T., *Biosci. Biotechnol. Biochem.*, 2004, vol. 68, pp. 1332–1337.
24. Mosmann, T., *J. Immunol. Methods*, 1983, vol. 65, pp. 55–63.
25. Goldstein, J.L., Anderson, R.G.W., and Brown, M.S., *Methods Enzymol.*, 1979, vol. 98, pp. 241–261.