Study of biotransformation of cholesterol 3β-methyl ether by mycobacteria *Mycobacterium sp.*

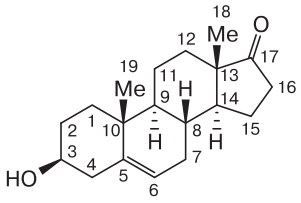
V. V. Yaderets,[a] N. V. Karpova,[a] T. S. Stytsenko,[a] V. A. Andryushina,[a] D. V. Kurilov,[b] and I. V. Zavarzin[b]★

[a]*Federal Research Center "Fundamentals of Biotechnology", Russian Academy of Sciences, korp. 1, 7 prosp. 60-letiya Oktyabrya, 117312 Moscow, Russian Federation*
[b]*N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation.*
E-mail: zavi@ioc.ac.ru

The influence of exogenous factors on the selectivity of biotransformation of cholesterol 3β-methyl ether by *Mycobacterium sp*. was studied. The main product of the microbiological transformation was Δ^5-dehydroepiandrosterone 3β-methyl ether. The presence of methyl-β-cyclodextrin in the bacterial growth medium led to a change in the ratio of the reaction products and an increase in the yield of Δ^4-androstene-3,17-dione. Aliphatic saturated and unsaturated carboxylic acids were also formed in the course of biotransformation.

Key words: mycobacteria *Mycobacterium sp*., microbiological transformation, cholesterol 3β-methyl ether, Δ^4-androstene-3,17-dione, Δ^5-dehydroepiandrosterone, Δ^5-dehydroepiandrosterone methyl ether, aliphatic saturated and unsaturated carboxylic acids, gas-liquid chromatography-mass spectrometry.

In the production of steroid medications,[1,2] sterols are used as the starting compounds, whose microbiological transformation gives[3—5] derivatives of 17-ketoandrostane **1**.



1

The interest in derivatives of ketoandrostane **1** is due to the fact that this steroid plays an important role in maintaining homeostasis in the body.[6,7] Compound **1** has been shown to be efficient in the treatment of cardiovascular, oncological, neurodegenerative, skin, autoimmune diseases and obesity, as well as in the treatment and prevention of physiological disorders associated with age.[3,8—15] In addition, compound **1** is an intermediate in the synthesis of a number of steroid medicines with high anticancer activity (abiraterone and its analogs).[13]

Earlier,[16] we studied the biotransformation of cholesterol 3β-methyl ether (**2**) by cells of *Mycobacterium sp*. (strains S-11094 and R-77). It was shown that the *Mycobacterium sp*. can transform ether **2** into derivatives of 17-ketosteroid **1**.

In the present work, we investigated the effect of exogenous factors on the selectivity of biotransformation of cholesterol ether **2** by mycobacterium *Mycobacterium sp*. We assessed the effect of the presence of compounds such as methyl-β-cyclodextrin (MCD), Tween-80, and Span-20 in the growth medium on the rate and direction of cleavage of the side chain of compound **2**. The biotransformation of cholesterol 3β-methyl ether **2** is accompanied by the formation of a large amount of fatty acids. We analyzed the content of aliphatic saturated and unsaturated carboxylic acids (fatty acid composition of the lipid fraction of the biomass from growth media).

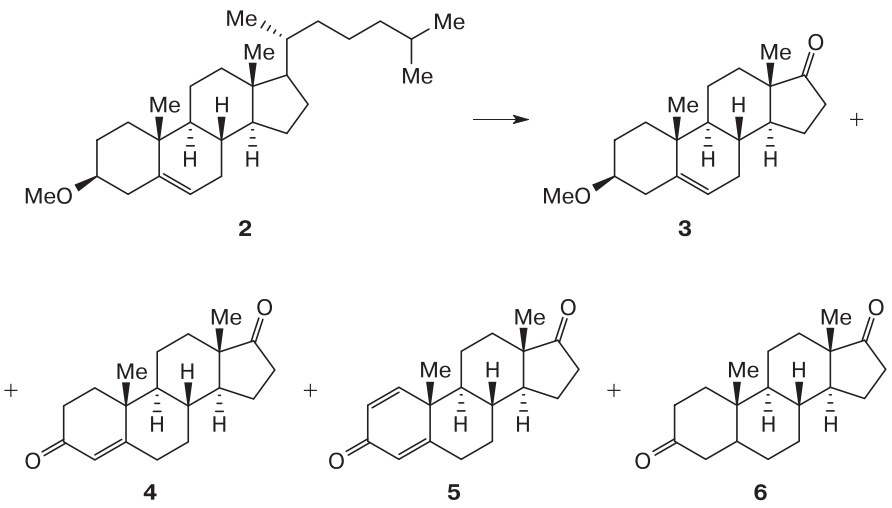
Steroids are lipophilic compounds, the absorption of which by mycobacteria depends on the direct contact of sterol particles with the cell membrane.[17] The barrier for the penetration of various exogenous compounds into the cell is the complex structure of the cell wall of mycobacteria, in which lipids and carbohydrates predominate. However, the presence of substances such as MCD, Tween-80, and Span-20 in the growth medium increases the permeability of the cell wall.

Biotransformation of ether **2** leads to a mixture of products, the ratio of which depends on the presence of exogenous additives in the reaction mixture (Scheme 1).

In this work, we used mycobacteria strains *Mycobacterium* (according to the new nomenclature *Mycolicibacterium*[18]) from the collection of the Laboratory of Biotechnology of Physiologically Active Compounds of the Federal Research Center "Fundamentals of Biotechnology" of the Russian Academy of Sciences, which are capable of transforming cholesterol and β-sitosterol to the main

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 3, pp. 533—538, March, 2021.
1066-5285/21/7003-0533

Scheme 1



steroid intermediates (*M. neoaurum* ACIM (All-Russian Collection of Industrial Microorganisms) Ac-1634,[19] *Mycobacterium sp.* R-77, *M. neoaurum* ACIM Ac-1656,[20] *M. neoaurum* 45/7-S, and *Mycobacterium sp.* S-11094). Substrate **2** in an amount of 1 g L^{-1} was introduced into the transformation medium before sterilization in a finely dispersed state (see Experimental). The selectivity of the process of microbiological transformation of compound **2** by mycobacteria was assessed by the ratio of the content of the target reaction product (**3**) in the reaction mixture to the sum of the formed side steroid identified (**4**—**6**) and unidentified components (Table 1).

As the data presented in Table 1 show, all the studied cultures of mycobacteria are capable of biotransformation of ether **2** with the formation of dehydroepiandrosterone methyl ether **3** as the main reaction product and side steroids **4**—**6**, as well as small amounts (up to 3.2% in total) of unidentified steroids. Among the tested strains, the maximum amount of the target product **3** was observed with cells of *Mycobacterium sp.* R-77 (74.6%). In this case, the content of the target product **3** in the culture liquid at the end of the reaction was 22.2% higher than in the case of cell culture *M. neoaurum* ACIM Ac-1656, for which the content of the target product **3** was minimal and equal to 52.4%. Note that in the case of biotransformation of the starting compound **2** by cells of *Mycobacterium sp.* R-77, its almost complete conversion and minimum relative content (4.0%) at the end of the process were observed. The sum of identified (**4**—**6**) and unidentified side steroids was 21.4%.

Thus, the transformation of substrate **2** by *Mycobacterium sp.* R-77 gave the maximum ratio of the target product **3** to the sum of by-products, which was equal to 3.5 : 1. The minimum ratio of 1.4 : 1 between the content of the target product **3** and the total amount of by-products was observed when cells *M. neoaurum* ACIM Ac-1656 were used for biotransformation of substrate **2**.

All the studied strains have the ability to hydrolyze the ether bond at position C(3) of the steroid molecule with the formation of products **4** and **5** in addition to the target

Table 1. Microbiological transformation of cholesterol 3β-methyl ether **2** (loading 1 g L^{-1})

Culture used	Time /h	Conversion* (%)	Relative content of steroids in reaction medium (±2.0) (%)					
			2	**3**	**4**	**5**	**6**	Other steroids**
M. neoaurum ACIM Ac-1656	76	82±2	10.2	52.4	2.6	30.3	1.3	3.2
M. neoaurum ACIM Ac-1634	76	85±2	7.5	57.6	26.0	2.4	3.5	3.0
M. neoaurum 45/7-S	76	86±1.5	5.0	58.4	3.2	29.5	1.7	2.2
Mycobacterium sp. S-11094	76	90±2.5	5.2	60.0	3.4	26.5	2.0	2.9
Mycobacterium sp. R-77	72	93±1.8	4.0	74.6	15.0	2.2	1.9	2.3

* Represented as $x_i \pm \Delta x$ (%), Δx (random error of direct measurements) is defined as the half value of the variation range magnitude in the aggregate of values of the feature under study, *i.e.* $\Delta x = (x_{max} - x_{min})/2$, where x_{max} and x_{min} are the maximum and minimum values from a sample of data obtained in repeated measurements.
** Unidentified steroids.

product **3**. The maximum total amount of compounds **4** and **5** was observed when *M. neoaurum* ACIM Ac-1656 was used (32.9%), while their minimum amount was observed for *Mycobacterium sp.* R-77 (17.2%).

The effect of exogenous factors on the selectivity of biotransformation of ether **2** was studied using the most efficient strain of mycobacteria *Mycobacterium sp.* R-77. The following exogenous compounds were added to the growth media: MCD (4 mmol), Tween-80 (0.8 mmol), and Span-20 (6 mmol). The resulting inoculum was then transferred into transformation flasks containing the starting sterol **2** (loading 2 g L^{-1}), which was placed into the transformation medium in a finely dispersed form prior to sterilization (see Experimental). The control for this series of experiments was the transformation of substrate **2** (loading 2 g L^{-1}) by cells of *Mycobacterium sp.* R-77 grown under standard conditions (see Experimental). The results are presented in Table 2.

The data obtained (see Table 2) correspond to the composition of the transformation medium after 96 h of reaction. The specified period of time was considered as optimal for the completion of the process of microbiological conversion. A further increase in the transformation time led to a significant decrease in the yield of the reaction products, which may indicate the use of these compounds as a source of carbon by mycobacterial cells.

The highest relative content of ether **3** (72.7%) was observed for the control variant, in which the cells of mycobacteria were grown without additional introduction of surfactants or MCD (see Table 2). In this case, the maximum ratios between the target product **3** and both the starting compound **2** and the by-product **4** were obtained, 12.1 : 1 and 4.4 : 1, respectively. The minimum relative content of compound **3** (53.5%) was observed during biotransformation with the introduction of 4 mmol of MCD into the growth medium.

Note that growing mycobacteria in a growth medium in the presence of such exogenous additives as Tween-80, Span-20, and MCD, leads to a slight slowdown in the process of biotransformation of substrate **2** by cells of *Mycobacterium sp.* R-77. This is indirectly evidenced by an increase in the residual relative content of the starting sterol in the transformation medium from 6.0% (control) to 11.8, 13.8, and 14.3% (Tween-80, Span-20, and MCD, respectively).

Carrying out the biotransformation of sterol **2** in the presence of MCD in the growth medium increases the proportion of steroid **4**, the content of which in the transformation medium at the end of the reaction (96 h) is 26.7%, which is 1.6 times higher than the corresponding value for the control (see Table 2). The presence of MCD at the stage of growing mycobacterial cells leads to a significant change in the ratio between the target product (**3**) and the starting (**2**) and side products (**4**), which are 3.8 : 1 and 2.0 : 1, respectively. These values indicate a decrease in the selectivity of the process of transformation of compound **2** into compound **3** by cells of mycobacteria *Mycobacterium sp.* R-77 in the presence of MCD in growth medium.

We also analyzed the content of aliphatic saturated and unsaturated carboxylic acids (fatty acid composition of the lipid fraction of biomass from growth media) using the strain *Mycobacterium sp.* R-77 as an example. The results are presented in Table 3.

From the analysis of the data presented (see Table 3), it follows that the addition of 0.8 mmol of MCD to the growth medium leads to a decrease in the total amount of aliphatic saturated monocarboxylic acids and unsaturated monocarboxylic acids by 1.3 and 2.4 times, respectively. In addition, there is a sharp decrease (compared to the control) in the content of such fatty acids as palmitic (1.7 times), oleic (2 times), and linoleic (4 times). This is explained by the fact that, according to the literature data,[21] the exogenous additives of cyclodextrins are capable of interaction with protein and lipid components of the outer layer of the cell wall of mycobacteria, leading to their detachment from the cell wall surface. As a result, there is an indirect effect of cyclodextrins on a decrease in the content of non-covalently bound lipids in the cell wall of mycobacteria and an increased release of certain fatty acids, which, in turn, leads to disorganization of the external lipid bilayer. This effect is indirectly confirmed by the results obtained in our study.

In conclusion, *Mycobacterium sp.* R-77 was found to be the most efficient strain for biotransformation of cholesterol 3β-methyl ether, giving a 93% conversion of the

Table 2. Content of products of biotransformation of cholesterol 3β-methyl ether **2** by the cells of *Mycobacterium sp.* R-77 (loading 2 g L^{-1})

Growth	Conversion (%)	Relative content of steroids in reaction mass (%)					
		2	**3**	**4**	**5**	**6**	Other steroids*
Control	90±2.0	6.0	72.7	16.4	1.2	0.7	3.0
MCD	86±1.5	14.2	53.5	26.7	1.3	1.2	3.1
Tween-80	88±2.1	11.8	66.6	16.2	1.0	0.8	3.6
Span-20	84±1.5	13.8	65.5	15.6	1.1	0.7	3.3

* Unidentified products.

Table 3. The effect of exogenous components on the fatty acid composition of the lipid fraction of the biomass after the completion of biotransformation on the example of the strain *Mycobacterium sp.* R-77

Component composition	Growth medium (%)				
	A_0[a]	Control[b]	MCD	Tween-80	Span-20
Aliphatic saturated monocarboxylic acids					
Hexanoic (nylon) acid, $CH_3(CH_2)_4COOH$	0.08	0.28	0.83	0.43	0.71
Heptanoic (enanthic) acid, $CH_3(CH_2)_5COOH$	0.01	0.08	0.17	0.16	0.20
Octanoic (caprylic) acid, $CH_3(CH_2)_6COOH$	0.03	0.12	0.37	0.42	0.24
Nonanoic (pelargonic) acid, $CH_3(CH_2)_7COOH$	0.05	0.10	0.30	0.21	0.25
Decanoic (capric) acid, $CH_3(CH_2)_8COOH$	0.02	0.09	0.25	0.16	0.19
Undecanoic acid, $CH_3(CH_2)_9COOH$	Traces[c]	Traces[c]	Traces[c]	Traces[c]	Traces[c]
Dodecanoic (lauric) acid, $CH_3(CH_2)_{10}COOH$	Traces[c]	0.05	0.09	0.12	0.25
Tridecanoic acid, $CH_3(CH_2)_{11}COOH$	Traces[c]	0.04	Traces[c]	Traces[c]	Traces[c]
Tetradecanoic (myristic) acid, $CH_3(CH_2)_{12}COOH$	Traces[c]	0.42	0.41	0.61	0.86
Pentadecanoic acid, $CH_3(CH_2)_{13}COOH$	Traces[c]	0.09	0.11	0.13	0.16
Hexadecanoic (palmitic) acid, $CH_3(CH_2)_{14}COOH$	0.20	11.62	6.86	9.67	11.58
Heptadecanoic (margaric) acid, $CH_3(CH_2)_{15}COOH$	0.01	0.11	0.12	0.08	0.09
Octadecanoic (stearic) acid, $CH_3(CH_2)_{16}COOH$	0.13	4.35	4.11	5.11	4.81
Total amount	0.53	17.23	13.45	17.1	19.34
Aliphatic unsaturated monocarboxylic acids					
Tetradecenoic (myristoleic) acid, $C_{13}H_{25}COOH$	—	0.22	0.17	0.21	0.29
Hexadecenoic (palmitoleic) acid, $C_{15}H_{29}COOH$	0.03	1.34	1.86	2.87	4.26
Octadecenoic (oleic) acid, $C_{17}H_{33}COOH$	0.35	28.80	14.47	13.70	15.65
Octadecadienoic (linoleic) acid, $C_{17}H_{31}COOH$	0.09	22.58	5.83	2.37	3.50
Total amount	0.47	52.95	22.33	19.15	23.7

[a] Growth medium without mycobacterial cells and exogenous additives.
[b] Growth medium with mycobacterial cells, but without the introduction of exogenous additives.
[c] <0.01% of the determined component.

substrate and a 74.6% content of the target steroid **3** in the reaction products. The presence of an exogenous additive of MCD in the growth medium shifts the direction of biotransformation toward the accumulation of product **4**, while the use of a surfactant additive leads to an indirect intensification of the processes of complete utilization of steroids to carbon dioxide and water. In addition, the content of fatty acids in biotransformation products was determined. Significant differences were revealed in the relative content of fatty acids in each of the analyzed fractions obtained from the cells of mycobacteria *Mycobacterium sp.* R-77 grown in the presence of exogenous additives.

Experimental

Sorbfil plates (IMID Ltd., Russia) were used for preliminary TLC analysis of biotransformation products.

Qualitative and quantitative component compositions of steroids involved in the biotransformation process, as well as the fatty acid composition of the lipid fraction of the biomass, were determined by GLC-MS. The analytical study was carried out on an Agilent Technologies instrument composed of a 7890 gas chromatograph (HP-5 column, 50 m×320 μm×1.05 μm) and a 5975C mass-selective detector with a quadrupole mass analyzer.

The starting substrate **2** was obtained according to our procedure described earlier.[16]

Microbiological transformation. The work was performed with bacterial strains of the genus *Mycobacterium* (according to the new nomenclature *Mycolicibacterium*[18]): *M. neoaurum* ACIM Ac-1656, *M. neoaurum* ACIM Ac-1634, *M. neoaurum* 45/7-S, *Mycobacterium sp.* S-11094, and *Mycobacterium sp.* R-77. These bacterial cultures were stored at 28±0.5 °C on slant agar containing the following components (g L^{-1}): glucose 10.0, soy flour 10.0, lemon acid 2.2, urea 0.5, NH_4Cl 1.0, KH_2PO_4 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.05, $CaCO_3$ 1.5 (medium pH 6.8—7.2).

The biomass of mycobacteria aged 10—14 days obtained on a slant agar medium was transferred into 750-mL conical flasks with 100 mL of the medium of similar composition. In accordance with the objective of the experiment, exogenous compounds, MCD (4 mmol), Tween-80 (0.8 mmol), or Span-20 (6 mmol), were introduced into the growth medium before sterilization. The mycobacteria were grown on a shaker for 94—98 h at 28—30 °C and stirring at 220 rpm. Next, the inoculum of mycobacteria in an amount of 20 vol.% (of the volume of the transformation liquid) was transferred into baffled flasks with a transformation medium of the following composition (g L^{-1}): glucose 20.0, soy flour 15.0, lemon acid 2.2, urea 0.5, NH_4Cl 1.0, KH_2PO_4 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.05, $CaCO_3$ 1.5 (medium pH 6.8—7.2). The loading of the starting compound (substrate), cholesterol 3β-methyl ether (**2**), was 1—2 g L^{-1}. The

substrate was introduced into the transformation medium in a finely dispersed form with the addition of Tween-80 (0.5 mmol) before sterilization.

The biotransformation products were analyzed using TLC (sorbent silica gel) and GLC-MS.

For preliminary TLC analysis, aliquots of the culture liquid (1 mL) were collected at intervals determined by the objectives of the experiment. Steroids were extracted by washing the aliquot of the culture liquid with a four-fold volume of ethyl acetate or chloroform. The steroid compounds were separated by TLC, using a mixture of hexane—acetone (2 : 1) as an eluent. Chromatograms were visualized by treating chromatographic plates with 1% solution of vanillin in 10% $HClO_4$ with subsequent heating until colored spots appeared.

To isolate the biotransformation products, an aliquot of the culture liquid (100 mL) was extracted twice with an equal volume of ethyl acetate. Then the ethyl acetate solution was concentrated under reduced pressure. The oily residue obtained after evaporation of the solvent was dried in a drying oven at 60 °C (to constant weight). The component composition of steroids involved in the biotransformation process was analyzed by GLC-MS without additional derivatization of the analyzed components.

The composition of fatty acids of the lipid fraction in the biomass from growth media (both in the absence and in the presence of mycobacterial cells and exogenous additives) was determined by GLC-MS after additional derivatization of the analyzed components (as the corresponding silyl esters).

Statistical processing of the data obtained was carried out based on the results of three transformations using the Excel program.

Gas-liquid chromatography-mass spectrometry. The component composition of steroids involved in the biotransformation process was analyzed without additional derivatization of the components (direct injection). The substance collected from the corresponding reaction (transformation) mixture was dissolved in ethyl acetate with stirring and shaking. The resulting ethyl acetate suspension was centrifuged; the supernatant was separated from the precipitate by decantation. The resulting solution was analyzed.

The analysis of the fatty acid composition in the lipid fraction of the biomass was carried out after additional derivatization of the analyzed components (as the corresponding silyl esters). For this, the silylating agent, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), was added to the dried substance extracted from the appropriate growth medium. The resulting suspension of the substance in the silylating reagent was kept for 30 min at 70 °C with periodic stirring and shaking, then cooled and diluted with hexane. The resulting solution was analyzed.

The conditions for analysis by GLC-MS (temperature program of chromatography and parameters of mass spectrometric detection) of the component composition of steroids involved in the biotransformation process (I) and the composition of fatty acids in the lipid fraction of biomass (II) were similar.

The conditions for analysis by GLC-MS are shown below. Temperature program of chromatography: at 40 °C the isotherm for 2 min; then programmed heating up to 250 °C at a rate of 5 °C min^{-1}; at 250 °C the isotherm for 15 min; then programmed heating up to 320 °C at a rate of 25 °C min^{-1}; at 320 °C the isotherm for 45 min; a splitless injector; the injector temperature 250 °C; the interface temperature 280 °C; carrier gas helium; the flow rate 1 mL min^{-1}; achieving chromatogram of samples by total ionic current.

Conditions for mass spectrometric detection: energy of ionizing electrons 70 eV; positive ion mode for registration of mass spectra in the m/z range from 20 to 450 at a rate of 2.5 scan s^{-1}; ChemStation E 02.00 software. The component composition (qualitative analysis) was identified in accordance with the database of full mass spectra (NIST), as well as by comparing the values of the corresponding chromatographic retention times and chromatographic linear indices of retention of compounds-tester. The relative content (%) of the components in the analyzed mixture (quantitative analysis) was calculated from the ratio of the chromatographic peak areas by a simple normalization method.

Cholesterol 3β-methyl ether (2). Chromatographic retention time 73.65 min. $C_{28}H_{48}O$. MS, m/z (I_{rel} (%)): 353 (12), 341 (1), 329 (8), 301 (8), 275 (9), 255 (8), 247 (7), 213 (8), 199 (5), 185 (5), 173 (6), 159 (11), 145 (18), 133 (12), 119 (18), 105 (27), 95 (25), 81 (26), 71 (55), 57 (41), 43 (100).

Δ^5-Dehydroepiandrosterone 3β-methyl ether (3). Chromatographic retention time 63.02 min. $C_{20}H_{30}O_2$. MS, m/z (I_{rel} (%)): 302 [$M^{+\bullet}$] (21), 270 (65), 255 (62), 231 (42), 213 (32), 199 (13), 185 (12), 171 (17), 159 (31), 145 (37), 131 (35), 119 (49), 105 (76), 91 (97), 79 (71), 71 (100), 55 (38), 41 (62).

Δ^4-Androstene-3,17-dione (4). Chromatographic retention time 65.98 min. $C_{19}H_{26}O_2$. MS, m/z (I_{rel} (%)): 286 [$M^{+\bullet}$] (67), 271 (7), 258 (6), 244 (44), 229 (9), 215 (7), 201 (21), 187 (13), 173 (13), 163 (12), 148 (40), 131 (20), 124 (61), 107 (47), 91 (100), 79 (91), 67 (53), 55 (64), 41 (53).

$\Delta^{1,4}$-Androstadiene-3,17-dione (5). Chromatographic retention time 64.48 min. $C_{19}H_{24}O_2$. MS, m/z (I_{rel} (%)): 284 [$M^{+\bullet}$] (9), 159 (18), 122 (100), 105 (16), 91 (81), 79 (30), 67 (20), 55 (25), 41 (25).

Androstane-3,17-dione (6). Chromatographic retention time 64.77 min. $C_{19}H_{28}O_2$. MS, m/z (I_{rel} (%)): 288 [$M^{+\bullet}$] (100), 255 (40), 244 (43), 229 (39), 217 (71), 147 (39), 135 (18), 124 (35), 109 (32), 93 (33), 79 (61), 67 (68), 55 (69), 41 (68).

The present publication does not contain a description of the research performed by the authors involving humans or animals as objects.

The authors declare that they have no conflicts of interest in financial or any other area.

References

1. P. Fernandes, A. Cruz, B. Angelova, H. M. Pinheiro, J. M. S. Cabral, *Enz. Microb. Technol.*, 2003, **32**, 688; DOI: 10.1016/S0141-0229(03)00029-2.
2. M. V. Donova, O. V. Egorova, *Appl. Microbiol. Biotechnol.*, 2012, **94**, 1423; DOI: 10.1007/s00253-012-4078-0.
3. P. Sahu, B. Gidwani, H. J. Dhongade, *Steroids*, 2020, **153**, 108507; DOI: 10.1016/j.steroids.2019.108507.
4. M. J. Cupp, T. S. Tracy, *Dietary Supplements. Toxicology and Clinical Pharmacology*, Humana Press, New York, 2003, 410 pp.
5. L. Stárka, M. Dušková, M. Hill, *J. Steroid Biochem. Mol. Biol.*, 2015, **145**, 254; DOI: 10.1016/j.jsbmb.2014.03.008.
6. V. B. Rozen, *Osnovy endokrinologii* [*Fundamentals of Endocrinology*], Izd-vo MGU, Moscow, 1994, 384 pp. (in Russian).

7. N. P. Goncharov, G. V. Katsiya, *Andrologiya i genital′naya khirurgiya* [*Andrology and Genital Surgery*], 2015, **16**, 13; DOI: 10.17650/2070-9781-2015-1-13-22 (in Russian).
8. K. Rutkowski, P. Sowa, J. Rutkowska-Talipska, A. Kuryliszyn-Moskal, R. Rutkowski, *Drugs*, 2014, **74**, 1195; DOI: 10.1007/s40265-014-0259-8.
9. C. J. Teixeira, K. Veras, C. R. DeOliveira Carvalho, *J. Mol. Med*., 2020, **98**, 39; DOI: 10.1007/s00109-019-01842-5.
10. C. M. Klinge, B. J. Clark, R. A. Prough, *Vitamins and Hormones*, 2018, **108**, 1; DOI: 10.1016/bs.vh.2018.02.003.
11. M. S. Ritsner, *CNS Neurosci. Ther.*, 2010, **16**, 32; DOI: 10.1111/j.1755-5949.2009.00118.x.
12. Pat. RF 2592370; *Byul. Izobret*. [*Invention Bull.*], 2016, No. 20 (in Russian).
13. V. A. Kostin, A. S. Latysheva, V. A. Zolottsev, Ya. V. Tkachev, V. P. Timofeev, A. V. Kuzikov, V. V. Shumyantseva, G. E. Morozevich, A. Yu. Misharina, *Russ. Chem. Bull.*, 2018, **67**, 682; DOI: 10.1007/s11172-018-2122-7.
14. M. Garrido, M. Cabeza, F. Cortés, J. Gutiérrez, E. Bratoeff, *Eur. J. Med. Chem.*, 2013, **68**, 301; DOI: 10.1016/j.ejmech.2013.02.031.
15. N. Dhingra, T. R. Bhardwaj, N. Mehta, T. Mukhopadhyay, A. Kumar, M. Kumar, *Arch. Pharm. Res.*, 2011, **34**, 1055; DOI: 10.1007/s12272-011-0702-2.
16. V. A. Andryushina, T. S. Stytsenko, N. V. Karpova, V. V. Yaderets, I. V. Zavarzin, D. V. Kurilov, *Russ. Chem. Bull.*, 2019, **68**, 2355; DOI: 10.1007/s11172-019-2711-0.
17. L. B. Xiong, H. H. Liu, L. Q. Xu, W. J. Sun, F. Q. Wang, D. Z. Wei, *Microb. Cell. Fact.*, 2017, **16**, 89; DOI: 10.1186/s12934-017-0705-x.
18. A. Oren, G. Garrity, *Int. J. Syst. Evol. Microbiol.*, 2018, **68**, 1411; DOI: 10.1099/ijsem.0.002711.
19. Pat. RF 2231553; *Byul. Izobret*. [*Invention Bull.*], 2007, No. 18 (in Russian).
20. Pat. RF 2297455; *Byul. Izobret*. [*Invention Bull.*], No. 11 (in Russian).
21. P. Awadhiya, T. Banerjee, *Int. J. Pharm. Sci. Res.*, 2018, **9**, 1935; DOI: 10.13040/IjPSR.0975-8232.

Received May 18, 2020;
in revised form January 19, 2021;
accepted January 22, 2021