The precipitate that separated out was filtered off, washed with water, and crystallized from 500-530 ml of distilled water with addition of 2 g of activated charcoal. The hot filtrate was cooled to 2-5°C, held at this temperature for 10-12 h, the precipitate was filtered off, washed with distilled water, and dried at 70-80°C. Yield 45 g (63.7%, based on reacted III) of IV, mp 160-161.5°C. According to the data in [7, 10, 11], mp 158-161°C.

LITERATURE CITED

1. USSR Inventor's Certificate No. 164,289; Otkrytiya, No. 15 (1964).
2. USSR Inventor's Certificate No. 176,912; Otkrytiya, No. 24 (1965).
3. USSR Inventor's Certificate No. 180,605; Otkrytiya, No. 8 (1966).
4. USSR Inventor's Certificate No. 201,416; Otkrytiya, No. 18 (1967).
5. USSR Inventor's Certificate No. 201,417; Otkrytiya, No. 18 (1967).
6. USSR Inventor's Certificate No. 201,418; Otkrytiya, No. 18 (1967).
7. P. M. Kochergin, A. M. Tsyganova, L. S. Blinova, et al., Khim. Geterotsikl. Soedin., No. 6, 875-878 (1965).
8. M. D. Mashkovskii, Drugs, 9th edn. [in Russian], Part 2, Moscow (1984), p. 343.
9. Roumanian Patent No. 51,788 (1969); Chem. Abstr., 71, No. 124434e (1969).
10. French Patent No. 1,379,915 (1964); Chem. Abstr., 62, No. 9145 (1965).
11. G. N. Pershin, P. M. Kochergin, A. M. Tsyganova, et al., Med. Prom-st', No. 10, 12-16 (1964).
12. R. Y. Fargher and F. L. Pyman, J. Chem. Soc., 115, 217 (1919).
13. K. W. Kohlrauch and R. Seka, Chem. Ber., 71, 985 (1938).
14. A. Ladenburg, Chem. Ber., 27, 2952-2957 (1894).
15. B. Radziszewski, Chem. Ber., 15, 2706-2708 (1882).
16. B. Radziszewski, Chem. Ber., 16, 487-494 (1883).

SYNTHESIS AND MICROBIOLOGICAL HYDROXYLATION OF 16α,17α-DIHYDROXY-SUBSTITUTED 20-OXO-5α-PREGNANES

M. L. Gerasimova, T. I. Gusarova, V. M. Ryzhkova, T. M. Bykova, and L. M. Alekseeva

UDC 615.357:577.175.53].012.1.017

At the present time, production of steroids, integrated microbiological hydroxylation, hydrolysis and dehydrogenation processes are being successfully used to provide a convenient path for the synthesis of corticosteroid preparations [1, 5].

In the preceding publications [7, 8] we reported studies of the influence of substituents at C(16), C(17) of the steroid on the transformation activity of the hydroxylating cultures of the microorganisms Curvalaria lunata, Tieghemella orchidis, Cunninghamella blakesleeana, Trichothecium roseum, and others. The dependence of the direction of the hydroxylation on the structure of the substrate was studied on 16,17-substituted derivatives of the Δ^4-pregnene series.

To study the possibilities of using the 5α-pregnane derivatives for the synthesis of corticosteroids, we examined the microbiological transformation of 20-oxo-5α-pregnanes (I), containing substituents – the hydroxyl groups and the isopropylidenedioxy group – at C(16) and C(17).

Data in the literature on the microbiological transformation of 5α-pregnane derivatives are sparse, and no data are available on the selective 11-β-hydroxylation. Derivatives of 3,20-dioxo-5α-pregnane – 11β,17α,21-trihydroxy-5α-pregnane-3,20-dione and its 17-acetate are steroid metabolites; the latter has anti-inflammatory and progestagenic activity. These compounds were obtained previously by hydrogenation of hydrocortisone and its acetate with hydrogen in the presence of a palladium catalyst at room temperature [6, 9].

S. Ordzhonikidze All-Union Scientific Research Chemical Pharmaceutical Institute, Moscow.

Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 23, No. 10, pp. 1259-1263, October, 1989. Original article submitted July 15, 1988.

0091-150X/89/2310-0863$12.50

TABLE 1. Dependence of the Direction of Hydroxylation on the Structure of Substituted 5α-Pregnanes and Δ^4-Pregnenes

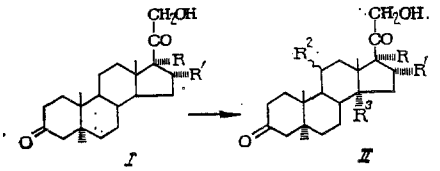
Subtrate	Hydroxylating culture							
	Curvularia lunata			Tieghemella orchidis			Cunninghamella blanesleeana	
	yield of transformation product, %							
	11β	11α	11β, 14α	11β	11α	11-keto	11β	11α
Ia	—	—	50—53	5	40—45	—	—	70—75
Id*	67	—	15 (14α)	58	26	—	31	16
Ib	38—42	—	—	5	30	—	5	60—65
Ie*	12	—	—	—	15	—	—	3
Ic	10—12	—	—	40—45	5	5—8	10—12	30—35
If*	21	—	—	73	6	—	65	6

*The results of the microbiological transformation of 16,17-substituted Δ^4-pregnenes were obtained by us previously in [8].

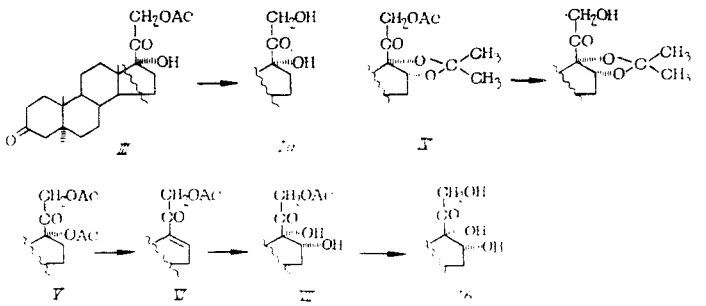
Of the common hydroxylating cultures, we selected Curvularia lunata, Tieghemella orchidis, and Cunninghamella blakesleeana.

By means of the microbiological transformation, compounds of the general formula I were converted into compounds of the general formula II.

R = OH (Ia, b, d, e; IIa-e); R^1 = H (Ia, d; IIa-c); OH (Ib, e, IId, e); $R + R^1$ = = —$OC(CH_3)_2O$— (Ic, f, IIf-h; R^2 = α-OH (IIb, d); β-OH (IIa, c, e, g); H (IIh); R^3 = α-OH (IIa); H (IIb-h); Δ^4 (Id, f); 21-acetate If).



Substrates Ia and Ic were obtained by saponification of the 21-acetoxy group of compounds III and IV, the synthesis of which was carried out previously in [2, 3]. cis-Diol Ib was obtained by splitting off the 17α-acetoxy group in diacetate V with the formation of 20-keto-pregnene VI, followed by the hydroxylation of the Δ^{16}-bond. The 17α-acetoxy group was removed by using potassium acetate with heating in DMSO. Olefin VI was oxidized by potassium permanganate in acetone in the presence of formic acid with the formation of diol VII, treatment of which with a methanolic solution of KOH in an inert gas atmosphere led to the formation of 16α,17α,21-trihydroxy-5α-prenane-3,20-dione (Ib).



The results of the transformation of the 5α-prenane derivatives (I) are listed in Table 1.

After hydroxylation of the cortexolone analog Ia saturated in ring A with the Curvularia lunata culture for 48 h, the 11β,14α-dihydroxy derivative IIa was obtained in a yield of 50-53%. The TLC analysis of a sample of the culture liquor taken after 24 h showed the presence of the initial Ia (3-5%), the 14α- and 11β-hydroxylated products (15 and 20%, respective-

ly), and the 11β,14α-dihydroxy derivative IIa (30-35%). After further 24 h, the makeup of the transformation products changed. A sample was found to contain traces of the initial compound, the 14α- and 11β-hydroxylated products (2-3 and 3-5%), and the 11β,14α-dihydroxylation product (60-65%). Thus, in the transformation of Ia, two hydroxyl groups were consecutively introduced into the substrate molecule: the 11β-hydroxy group into the 14α-hydroxy compound and 14α-hydroxy into the 11β-hydroxy compound.

In the case of fermentation of substrate Ib, the presence of a hydroxyl group at the 16α-position inhibits 14α-hydroxylation and leads to the formation of the 11β-hydroxy derivative in a 38-42% yield.

When a saturated acetonide Ic is used, the fermentation by the T. orchidis culture is not clear-cut. The TLC analysis in a benzene–acetone (9:5) system showed the presence in the reaction mixture of the initial (R_f 0.77) and in addition two other compounds. The main transformation product, the 11β-alcohol (R_f 0.53) was isolated from the reaction mixture in 40% yield, the remaining products were the 11-ketone (8%; R_f 0.67) and the starting compound (35%). The ratio of the oxidation products in the culture liquor was 8:1 according to the TLC data. Thus, the most extensive transformation of the steroid was noted during the transformation of the acetonide Ic by the T. orchidis culture.

As in the case of the analog of the Δ^4-pregnene series (If), the introduction of the 16α,17α-acetonide grouping into the substrate Ic hinders the transformation when C. lunata strain is used: the fermentation proceeded to the extent of only 10-12%.

The data obtained show that the presence of substituents at C(16) and C(17), as in the case of the 16α,17α-dihydroxy-substituted Δ^4-pregnenes, substantially influences the direction of the hydroxylation. Increase in the volume of the substituent in the α-region of the substrate suppresses the α-hydroxylation process. At the same time, there are differences during the transformation of 16α,17α-substituted derivatives between the 5α-pregnane and Δ^4-pregnane series, containing the same substituents. Thus, in the case of the fermentation of compound Ia by means of the C. lunata culture, the formation of 11β,14α-dihydroxy compound was observed, in contrast to the hydroxylation of cortexolone Id by the same culture. Introduction of one hydroxyl group into the steroid does not interfere with the introduction of the second group, when the 5α-compound is used, in contrast to the Δ^4-analog, where the process proceeds with the formation of 11β- and 14α-mono-hydroxylated products. It is possible that the 5α-steroids are sterically less hindered, and the introduction of a hydroxyl into one of the positions does not eliminate the possibility of the reorientation of the compound obtained with respect to the enzyme, leading to the formation of a dihydroxy compound. This is possibly the reason why the 16α,17α-diols are more accessible in the 5α-pregnane series. Thus, during the transformation of diol (Ib), the 11α-hydroxylation product is formed in a 60-65% yield when Cunninghammella blakesleeana is used, while its analog in the Δ^4-pregnene series (Ie) practically does not undergo any changes through the action of any of the microorganism cultures studied.

The configuration of the hydroxyl group at the 11-position in compounds IIa-g was determined from the multiplicity of the proton at C(11). Examination of the molecular models showed that in the case of an equatorial proton, the signal splits as a result of equatorial-axial and di-equatorial interactions with protons at C(9) and C(12). If the proton at the 11-position is axial, the signals split with higher SSCC values with protons at the 9- and 12-positions, due to axial–axial and axial–equatorial interactions. Thus, in the spectrum of IId, the signal at δ = 3.88 ppm has the form of a sextet, which is a result of splitting with SSCC $J_1 = J_2 = 10$ Hz and $J_3 = 5$ Hz, and hence conforms with the axial position of the proton at C(11). At the same time, in the spectrum of IIg, the signal with δ = 4.5 ppm has the halfheight at a width $\Delta\nu_{1/2}$ = 10 Hz, which corresponds to the equatorial position of the proton at C(11).

To confirm the structure of compound IIa, a ^{13}C NMR spectrum was recorded under conditions of suppression of the interaction with protons. In this spectrum, signals of carbon atoms are observed at δ 90.4, 87.2, 66.6, and 66.4 ppm, which are characteristic for atoms bound to oxygen, and this confirms the presence of four hydroxyl groups in the molecule. Of the above-indicated signals, one corresponds to a secondary* carbon atom C(21), one to a tertiary C(11),

*The conclusions were based on the ^{13}C NMR spectra run under the conditions of a partial suppression of the interaction with protons.

and two to quaternary carbon atoms. One of the quaternary atoms is for C(17) and the second signal was assigned to the C(14) atom.

EXPERIMENTAL

The course of the reactions was monitored chromatographically, using Silufol plates (CSSR). The chromatograms were developed with a 1% solution of vanilin in a 10% solution of perchloric acid. The specific rotation was determined in chloroform (c = 1) on a FEP-02 apparatus (USSR). The melting points were determined on a Boetius microheating stage (GDR). The IR spectra of the compounds were run on a Perkin–Elmer spectrophotometer (Sweden) on mineral oil mulls. The ^1H and ^{13}C NMR spectra were obtained on an XL-200 spectrometer (Varian, USA). Te chemical shifts were given on a δ scale, using tetramethylsilane as internal standard. The mass spectra were recorded on a MAT-12 spectrometer (direct introduction of the sample into the source), using an ionizing voltage of 70 eV. The data of the elemental analysis of all the compounds obtained correspond to the calculated values.

Compounds III, IV, V [2, 3, 10], IIc [6] were obtained previously.

17α,21-Dihydroxy-5α-pregnane-3,20-dione (Ia). A 4-g portion of III was dissolved with stirring in 300 ml of methanol. A solution of 1 g of KOH in 50 ml of methanol was added in a nitrogen atmosphere and after 15 min the suspension dissolved. The reaction solution was stirred for 1 h, and at the end of the reaction it was neutralized with glacial acetic acid. The solution was evaporated to a volume of 50 ml, 100 ml of water was added, the precipitate was filtered off, and dried in air. Yield 3.5 g (97%) of Ia, mp 198-200°C (chloroform–methanol), $[\alpha]_D$ +46.3°. IR spectrum, ν_{max}, cm^{-1}: 3480, 3460, 1680-1710. ^1H NMR spectrum in $CDCl_3$, δ, ppm: 0.68 s (18-CH_3), 1.02 s (19-CH_3), 4.48* (–CH_2–, J = 20 Hz). Mass spectrum, m/z: 348 (M^+).

16α,17α,21-Trihydroxy-5α-pregnane-3,20-dione (Ib) was obtained according to the above-described method from VII in a 56% yield, mp 169-172°C (acetone), $C_{21}H_{32}O_5$. IR spectrum, ν_{max}, cm^{-1}: 3180-3420, 1730, 1570. ^1H NMR spectrum in $CDCl_3$, δ, ppm: 0.69 s (18-CH_3), 1.01 s (19-CH_3), 4.47* (–CH_2–, J = 20.5 Hz), 4.9 qd (16-H, J = 2.5; 10 Hz).

21-Hydroxy-16α,17α-isopropylidenedioxy-5α-pregnane-3,20-dione (Ic) was obtained by the above-described method from IV in a 95% yield, mp 214-215°C (methanol). $[\alpha]_D$ +78.7°, $C_{24}H_{36}O_5$. IR spectrum, ν_{max}, cm^{-1}: 3500, 1700-1730. ^1H NMR spectrum in $CDCl_3$, δ, ppm: 0.6 s (18-CH_3) 1.16 s (19-CH_3), 1.01 s and 1.49 s [$C(CH_3)_2$], 4.44 (–CH_2–, J = 20 Hz), 5.03 d (16-H, J = 5.1 Hz). Mass spectrum, m/z: 404 (M^+),

21-Acetoxy-5α-pregn-16-ene-3,20-dione (VI). A 6.5-g portion of potassium acetate was added to a solution of 10 g of V in 110 ml of dimethyl sulfoxide. The reaction solution was stirred in an inert gas atmosphere at 120°C for 2 h, and at the end of the reaction was poured into 1000 ml of water. The precipitate was filtered, washed with water, and dried in air. Yield 9.4 g (93%) of VI, mp 149-151°C (ethyl acetate), $[\alpha]_D$ +61.6°. $C_{23}H_{32}O_4$. IR spectrum, ν_{max}, cm^{-1}: 1745, 1710, 1680, 1585, 1230. ^1H NMR spectrum in $CDCl_3$, δ, ppm: 0.94 s (18-CH_3), 1.05 s (19-CH_3), 2.19 s ($OCOCH_3$), 5.01 (–CH_2–, J = 18 Hz), 6.76 m (16-H). Mass spectrum, m/z: 372 (M^+), 357, 330, 272. UV spectrum, ν_{max} (log ε): 240.5 (2.94).

21-Acetoxy-16α,17α-dihydroxy-5α-pregnane-3,20-dione (VII). A cooled solution of 1.8 g of potassium permanganate in 14 ml of water and 81 ml of acetone was added to a solution of 3 g of VI in 260 ml of acetone, cooled to –5 ± 2°C, containing 7.5 ml of 10% formic acid. The reaction mixture was stirred for 1 min, and 10.2 ml of a 10% solution of sodium bisulfite was added. Manganese dioxide was filtered off, the filtrate was evaporated to half of its volume, and the precipitate that separated out was filtered off to yield 2.3 g (70%) of VII, mp 222-224°C. $C_{23}H_{34}O_6$. $[\alpha]_D$ +53.2°. IR spectrum, ν_{max}, cm^{-1}: 3350-3480, 1735, 1710, 1245, 1050. ^1H NMR spectrum in $CDCl_3$, δ, ppm: 0.69 s (18-CH_3), 1.0 s (19-CH_3), 2.17 s ($OCOCH_3$), 4.98 m (16-H), 4.97 (–CH_2–, J = 22 Hz).

11β→14α,17α,21-Tetrahydroxy-5α-pregnane-3,20-dione (IIa). A 4-g portion of Ia was introduced into the fermenter in a methanol solution and subjected to transformation by means of C. lunata culture. At the end of the process, and after the separation of the mycelium, the culture liquor was extracted with 4.5 liters of ethyl acetate (3 × 1.5 liters). The extract was evaporated to a volume of 500 ml, washed with water (50 ml), and 0.5 g of anhydrous sodium sulfate and 0.5 g of charcoal were added, and the mixture was filtered. The solvent

*A type AB center of signals.

was evaporated to dryness to yield 2.93 g (50%) of IIa, mp 235-237°C (methylene chloride–methanol). $C_{21}H_{32}O_6$. IR spectrum, ν_{max}, cm^{-1}: 3560, 3420, 3330, 3180, 1720, 1695. Mass spectrum, m/z: 380 (M^+, 100%), 362, 344, 329, 326.

11α,17α,21-Trihydroxy-5α-pregnane-3,20-dione (IIb). From 1.2 g of Ia, after the transformation by the action of the C. blakesleeana culture, 0.87 g (69.6%) of IIb was obtained, mp 228-230°C (acetone). Literature data [4], mp 228°C. $C_{21}H_{32}O_5$. ^1H NMR spectrum in $CDCl_3$, δ, ppm: 0.65 s (18-CH_3), 1.14 s (19-CH_3), 3.90 sextet (11β-H, J = 10, 10, 5 Hz), 4.46 (–CH_2–, J = 19.6 Hz). Mass spectrum, m/z: 364 (M^+, 100%), 346, 328, 287.

11α-16α,17α,21-Tetrahydroxy-5α-pregnane-3,20-dione (IId). At the end of the fermentation process of Ib by means of C. blakesleeana, extraction, and evaporation of the solvent, the precipitate was ground with ether, and 0.32 g (61.5%) of IId was obtained, mp 214-216°C (acetone). $C_{21}H_{32}O_6$. ^1H NMR spectrum in $CDCl_3$, δ, ppm: 0.69 s (18-CH_3), 1.12 s (19-CH_3), 3.88 sextet (11β-H, J = 10, 10, 5 Hz), 4.86 (16-H), 4.5 (–CH_2–, J = 19.6 Hz). Mass spectrum, m/z: 380 (M^+, 100%), 362, 344, 332.

11β,16α,17α,21-Tetrahydroxy-5α-pregnane-3,20-dione (IIe). A 2.5-g portion of Ib was introduced into the fermenter in dimethylformamide. At the end of the fermentation process, by means of the C. lunata culture, extraction and evaporation of the extractant, the residue was ground with ether, and the precipitate was filtered off. Yield 1 g (38.3%) of IIe, mp 223-225°C (methanol). $C_{21}H_{32}O_6$. IR spectrum, ν_{max}, cm^{-1}: 3430-3495, 1705, 1700. Mass spectrum, m/z: 380 (M^+, 100%), 362, 344, 332.

21-Acetoxy-11α-hydroxy-16α,17α-isopropylidenedioxy-5α-pregnane-3,20-dione (IIf, 21-Acetate). From 0.65 g of Ic after the transformation by means of the C. blakesleeana culture, 0.24 g of IIf was obtained, which was dissolved in 6 ml of acetic acid containing 0.5 ml of acetic anhydride. A 0.5-g portion of barium acetate was added, and the mixture was allowed to stand for 24 h. The reaction solution was poured into 50 ml of water, the precipitate was filtered off, washed with water to pH 7 to yield 0.11 g of IIf 21-acetate, mp 204-206°C (methanol). Mass spectrum, m/z: 463 (M^+), 361, 344, 326. $C_{24}H_{36}O_6$.

11β,21-Dihydroxy-16α,17α-isopropylidenedioxy-5α-pregnane-3,20-dione (IIg). A 2-g portion of Ic was subjected to inoculation with the T. orchidis culture. At the end of the process, after the extraction and evaporation of the solvent, the residue was ground with ether. Yield 1.33 g of a transformation product containing two compounds, with R_f 0.67 and R_f 0.53. After crystallization from a 1:1 chloroform–methanol mixture, 0.83 g of IIg was obtained, R_f 0.53, yield 40%, mp 231-233°C. $C_{24}H_{36}O_6$. IR spectrum, ν_{max}, cm^{-1}: 3470, 3520, 1600-1730. ^1H NMR spectrum in $CDCl_3$, δ, ppm: 0.83 s (18-CH_3), 1.26 s (19-CH_3), 1.15 and 1.47 s [$C(CH_3)_2$], 4.44 s (–CH_2–, J = 20 Hz), 4.5 m (11α-H), 5.05 d (16-H, J = 4.6 Hz). Mass spectrum, m/z: 420 (M^+), 361, 344, 326.

21-Hydroxy-16α,17α-isopropylidenedioxy-5α-pregnane-3,11,20-trione (IIh) was obtained by crystallization from methanol of the mother liquor of IIg, mp 215-217°C. $C_{24}H_{36}O_6$. IR spectrum, ν_{max}, cm^{-1}: 3470, 1715, 1705, 1700. ^1H NMR spectrum in $CDCl_3$, δ, ppm: 0.54 s (18-CH_3), 1.2 s (19-CH_3), 1.19 and 1.55 s [$C(CH_3)_2$], 4.42 s (–CH_2–, J = 20 Hz), 5.1 d (16-H, J = 5.1 Hz). Mass spectrum, m/z: 418 (M^+), 359, 342, 301.

The medium for cultivating T. orchidis and C. blakesleeana contained 30 g of glucose, 5 g of corn extract, 3 g of peptone, 3 g of a fermentolysate of the biomass of microorganisms, 5 g of KH_2PO_4, and 100 ml of water, pH 6.8-7.2. The inoculates were cultivated in 0.75-liter flasks on a rotatory shaking device, rotating at 220 rpm at 26-28°C. The transformation was carried out on a growing culture. To cultivate C. lunata, a medium was used consisting of 30 g of saccharose, 2.5 g of a fermentolysate of a biomass of the microorganisms, 2 g of $NaNO_3$, 3.2 g of $(NH_4)_2HPO_4$, 1 g of K_2HPO_4, 0.5 g of KCl, and 0.5 g of $MgSO_4$, dissolved in 1000 ml of water, at pH 6.1-6.2. For the transformation, a mycelium washed out from the medium was used. The process was carried out in a phosphate buffer at pH 6.0-6.3. The concentration of the initial steroid was 0.5 g/liter, steroids Ia and Ic were introduced in the form of a 2-3% solution in methanol, Ib in a reprecipitated state (a solution of 50 mg of Ib in 0.5 ml of dimethylformamide was added to 5 ml of ice water; the precipitate was filtered and placed in a flask). The transformation was carried out in flasks under the same conditions as the cultivation of inoculates, or (on using larger batches) in glass fermenters with feeding of the air at a rate of 0.5 liter/min and a working stirrer rotating at 400 rpm; time of transformation 24-48 h. The process was monitored by TLC of the steroids, extracted from the sample with chloroform on Silufol-254 plates, in methylene

chloride—methanol—water (19:1:0.1) and chloroform—acetone (7:3) systems. The chromatograms were treated with a 1% solution of vanilin in 10% perchloric acid, followed by heating, with identification of the transformation products by comparison with the corresponding standards.

LITERATURE CITED

1. USSR Inventor's Certificate No. 517,489; Otkrytiya, No. 33 (1977).
2. M. L. Gerasimova, T. I. Gusarova, V. B. Nikitin, et al., Khim.-farm. Zh., No. 9, 1100 (1989).
3. K. K. Koshoev and A. A. Shchelochkova, Ref. Zh. Khim., No. 17(1), No. 17zh529DP (1974).
4. US Patent No. 265,744; Chem. Abstr., 48, No. 21, 12817e (1954).
5. US Patent No. 3,030,278 (1962).
6. French Patent No. 1,552,761; Chem. Abstr., 72, No. 7, 32142a (1970).
7. V. M. Ryzhkova, T. I. Gusarova, G. A. Klubnichkina, et al., Khim.-farm. Zh., No. 12, 69-71 (1980).
8. V. M. Ryzhkova, T. M. Bykova, G. A. Klubnichkina, et al., Khim.-farm. Zh., No. 7, 871-876 (1986).
9. M. Harnik, J. Org. Chem., 28, No. 12, 3386-3391 (1963).
10. H. J. Ringold, G. Rosenkranz, and F. Sondheimer, J. Am. Chem. Soc., 78, No. 4, 820-825 (1956).