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SYNTHESIS OF LOMAZARIN AND NORLOMAZARIN, PIGMENTS FROM *Lomandra hastilis*

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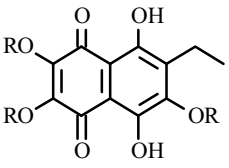
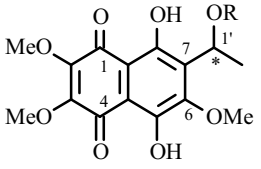
*5,8-Dihydroxy-2,3,6-trimethoxy-7-ethyl-1,4-naphthoquinone (**1**) was used to synthesize in high yield 5,8-dihydroxy-7(1′-hydroxyethyl)-2,3,6-trimethoxy-1,4-naphthoquinone (lomazarin, **3**), a pigment from* Lomandra hastilis. *Alkaline hydrolysis of lomazarin produced mainly 5,6,8-trihydroxy-2,3-dimethoxy-1,4-naphthoquinone (**9**) through a retro-aldol decomposition of the 6-keto-form of 5,6,8-trihydroxy-7(1′-hydroxyethyl)-2,3-dimethoxy-1,4-naphthoquinone (**13b**) formed during the reaction. 2,5,8-Trihydroxy-7(1′-hydroxyethyl)-3,6-dimethoxy-1,4-naphthoquinone (norlomazarin, **4a**), a pigment of* L. hastilis*, and its 3,5,8-trihydroxy-7(1′-hydroxyethyl)-2,6-dimethoxy isomer **4b** were formed as a difficultly separable mixture in addition to quinone **9**.*

Key words: echinochrome trimethyl ether, lomazarin, norlomazarin, *Lomandra hastilis*, retro-aldol reaction, spinochrome D dimethyl ether, *Echinotrix calamaris*, tricrozarin B, *Tritonia crocosmaeflora*.

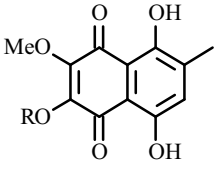
The chemistry of polymethoxylated derivatives of naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) (Structures of naphthazarin derivatives are given, unless specifically noted, as only one of the possible tautomers) is practically unstudied because of their poor availability [1]. We previously developed convenient methods for synthesizing these compounds [2], which made them convenient starting materials for the synthesis of several natural products and their analogs [3-5]. One of these promising derivatives is the trimethyl ether of echinochrome (**1**, TMEE) [2, 6]. Thus, hydrolysis of TMEE formed echinochrome (**2**) [7], a metabolite of the sea urchin *Scaphechinus mirabilis* [3], that is used as a cardioprotector and ophthalmological drug [8].



1, 2



3, 10



7, 8

1, 7, 10: R = Me; **2, 3, 8:** R = H

7 $\xrightarrow{HO^-}$ **8**

In continuation of these studies, we investigated the transformation of TMEE into lomazarin (**3**) [3, 9] and norlomazarin (**4a**) [4], metabolites (Compounds **3** and **4** contain asymmetric C-1′; however, it was not reported [4, 9] if they were optically active or a mixture of isomers.) that were previously isolated from roots of *Lomandra hastilis*. The biological activity of pigments from *L. hastilis* has not been studied because of their low content. Therefore, synthesis is the only reliable source of these compounds in amounts sufficient for studying their biological properties. A question arose during structure determinations about the mutual location of the β-hydroxyls and methoxyls (They could also be adjacent in one ring [4]) of

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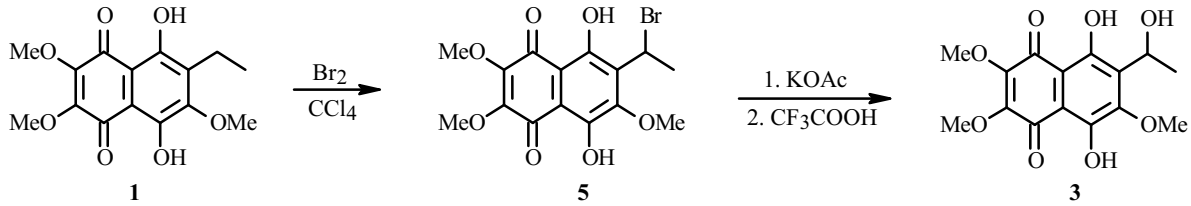
norlomazarin (**4**) relative to its backbone. Therefore, the goal of our work was to resolve the question of which actual isomer corresponds to the resonances observed in the PMR spectra [4]. Compound (**3**) was synthesized by the following scheme:



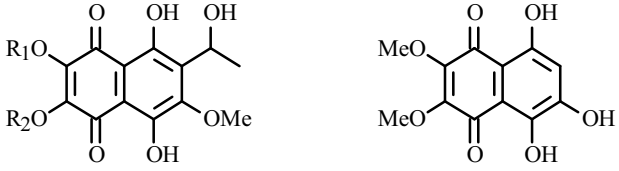
1 **5** **3**

Free-radical bromination of **1** in CCl_4 gave 1′-bromo derivative **5** in practically quantitative yield (98%). The halogen atom in **5** was readily replaced by acetoxy using KOAc in HOAc. This produced 1′-acetate **6** (72%). Hydrolysis of intermediate **6** in MeOH—CF_3COOH gave **3** also in practically quantitative yield (97%). As expected, the physicochemical properties of the synthetic product were completely identical to lomazarin (**3**) [3, 9].

2,3-Dimethoxy-1,4-naphthoquinones are by their nature analogs of vinyl ethers of carboxylic acids, which is responsible for their sensitivity to bases. This property, which was used earlier for selective conversion of the dimethyl ether of methylspinazarin (**7**) into its monomethyl ether **8** [10], was used to hydrolyze lomazarin (**3**).

Heating lomazarin in NaOH solution (1%) gave several products. Two of them were isolated as a mixture (~1:1) that consisted according to PMR of norlomazarin (**4**) [4] and its isomer. The mixture was chromatographed over a silica-gel column with elution by hexane:acetone (20:1) to isolate two fractions that were mixtures of these compounds in 2:1 and 1:2 ratios, respectively. This made it possible to assign reliably resonances of each of them in the PMR and ^{13}C NMR spectra. Resonances of protons in the PMR spectra and C atoms in the ^{13}C NMR spectra of the product mixtures were assigned based on HMBC experiments (Table 1).

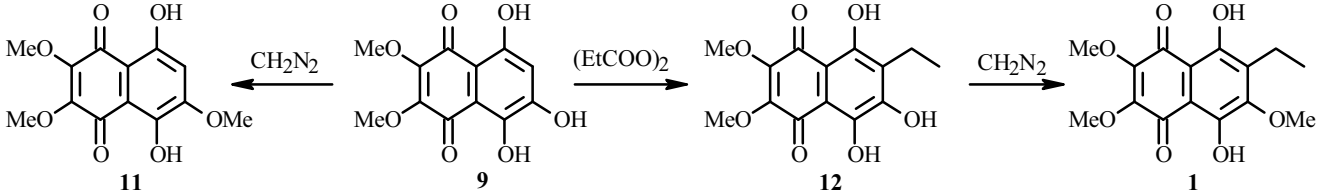
The spectral data were used to refine the structure of norlomazarin and ascribe it structure **4a**; its isomer, **4b**. Thus, in contrast with the hydrolysis of **7** [10], this reaction with lomazarin occurred nonselectively.



4a, b **9**

4a: R_1 = H, R_2 = Me; **4b:** R_1 = Me, R_2 = H

However, the main product formed by reacting **3** with dilute NaOH solution was the dimethyl ether of spinochrome D (**9**, 50%) [1], a pigment of the sea urchin *Echinotrix calamaris* [3], and also the main product obtained from base hydrolysis of **5**, **6**, and the methyl ether of lomazarin (**10**) [11]. Its structure was proved unambiguously by spectroscopic methods and a series of chemical transformations. Thus, selective methylation of **9** by CH_2N_2 gave tricrozarin B (**11**), a pigment isolated earlier from bulbs of *Tritonia crocosmaeflora* [12]. Free-radical C-ethylation of **9** by propionylperoxide gave the dimethyl ether of echinochrome (**12**), which gave **1** after treatment with CH_2N_2.



11 **9** **12** **1**

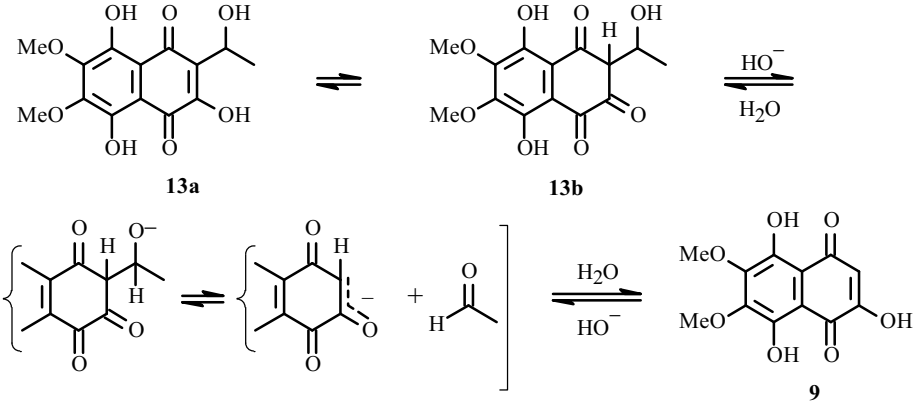
Dimethylether **9** appears as a subgroup in the structure of several natural biologically active products [3-5]. Therefore, it could become a convenient intermediate for their synthesis.

Dimethylether **9** was formed through a retro-aldol decomposition of dimethylether **13**, which was formed by hydrolysis of **3**. The keto-form **13b** was involved in the reaction. Evidently equilibrium was wholly shifted toward hydroxynaphthazarin (**9**) in this instance. Therefore, **13** was not observed among the products from base hydrolysis.

TABLE 1. Through-Space ^1H–^{13}C Correlations in 2D HMBC Experiment and ^1H Chemical Shifts (δ_H, ppm, $CDCl_3$, TMS, 30°C) of **4a** and **4b**

H atom	^{13}C correlations			
	norlomazarin (**4a**)		isomer of norlomazarin (**4b**)	
	δ_H (ppm, m, J/Hz)	δ_C	δ_H (ppm, m, J/Hz)	δ_C
2′-CH_3	1.57 (d, J = 6.5)	7	1.58 (d, J = 6.5)	7
1′-CH	5.27 (q, J = 6.5)		5.27 (q, J = 6.5)	
2-OMe	-	-	4.20 s	2
3-OMe	4.17 s	3	-	-
5-OH	13.01 s	3*, 4a, 5, 6, 7, 6-OMe*	12.04 s	3*, 4a, 5, 6, 7, 6-OMe*
6-OMe	4.14 s	6	4.10 s	6
8-OH	12.39 s	1, 2*, 7, 8, 8a	13.35 s	1, 2*, 7, 8, 8a

*Correlations observed in experiment optimized for 2 Hz.



Thus, the promise of using polymethoxynaphthazarins to synthesize natural metabolites and their analogs was demonstrated using the conversion of the trimethyl ether of echinochrome (**1**) into lomazarin (**3**) and its derivatives as an example. The retro-aldol decomposition of 5,6,8-trihydroxy-7(1′-hydroxyethyl)-2,3-dimethoxy-1,4-naphthoquinone (**13**) observed by us was of definite interest. This reaction led to dimethylether of spinochrome D (**9**), a convenient substrate for synthesizing several natural biologically active compounds.

EXPERIMENTAL

Melting points were determined on a Boetius heating stage and are uncorrected. PMR and ^{13}C NMR spectra in $CDCl_3$ were recorded on Bruker DRX-500 (500.13 and 125 MHz) and Bruker Avance-300 (300 and 75 MHz) spectrometers. Chemical shifts are given on the δ scale relative to Me_4Si. 2D heteronuclear correlation (HMBC) spectra were obtained by standard methods. HMBC experiments were optimized for $^nJ_{HC} = 2$ and $^nJ_{HC} = 10$ Hz. IR spectra in $CHCl_3$ solution were recorded on a Bruker Vector 22 spectrophotometer. Mass spectra (EI) were obtained on an LKB-9000S instrument with direct introduction with ionizing-electron energy 70 eV. Pure compounds were isolated by preparative TLC on plates (20 × 20 cm) with an unfixed silica-gel layer (H^+-form, 5-40 μm) or column chromatography over a column of silica gel (L 40/100 μm, H^+-form) [13]. The purity of compounds was monitored using TLC on Sorbfil plates (Russia) using hexane:acetone (3:1). Elemental analysis was performed on a Flash EA1112 C, H, N analyzer. Elemental analyses of all compounds agreed with those calculated.

7(1′-Bromoethyl)-5,8-dihydroxy-2,3,6-trimethoxy-1,4-naphthoquinone (5). A solution of **1** (1 g) in commercial CCl_4 (400 mL) was treated dropwise with Br_2 (0.2 mL) and stirred in light at room temperature for 4 h (TLC monitoring). Solvent was removed in vacuo. The solid was chromatographed over a column with elution by hexane:acetone (20:1) to afford **5** (1.23 g, 98%), mp 83-89°C (acetone).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.09 (3H, d, J = 7.1, CH_3), 4.09 (3H, s, OCH_3), 4.14 (3H, s, OCH_3), 4.20 (3H, s, OCH_3), 5.75 (1H, q, J = 7.1, CH), 12.87 (1H, s, α-OH), 13.38 (1H, s, α-OH).

^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 180.0, 179.6, 162.5, 157.6, 156.2, 148.8, 147.3, 133.9, 110.2, 108.3, 61.7 (two OMe on C-6 and C-7), 61.5, 37.0, 23.7.

Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 386/388 (5) $[M]^+$, 307 (100), 291 (48), 275 (17), 263 (21).

7(1′-Acetoxyethyl)-5,8-dihydroxy-2,3,6-trimethoxy-1,4-naphthoquinone (6). A mixture of **5** (0.5 g) and AcOK (0.63 g) in AcOH (20 mL) and $CHCl_3$ (3:1) was refluxed for 2 h and evaporated at reduced pressure. The solid was dissolved in water (100 mL) and extracted with AcOEt. The extract was dried over anhydr. Na_2SO_4. Solvent was evaporated at reduced pressure. The solid was chromatographed over a silica-gel column with elution by hexane:acetone (20:1) to afford **6** (0.34 g, 72%), mp 103-105°C (acetone). IR spectrum (ν, cm^{-1}): 1603 (C=O), 1744 (CH_3C=O).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.63 (3H, d, J = 6.8, CH_3), 2.07 (3H, s, OAc), 4.08 (3H, s, OCH_3), 4.13 (6H, s, OCH_3), 6.27 (1H, q, J = 6.8, CH), 12.88 (1H, s, α-OH), 13.23 (1H, s, α-OH).

^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 180.8, 179.4, 170.4, 162.1, 157.0, 156.0, 148.8, 147.3, 131.5, 110.2, 106.5, 65.1, 61.6, 61.5, 21.1, 18.7.

Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 366 (21) $[M]^+$, 306 (100), 291 (100), 273 (22), 263 (59).

5,8-Dihydroxy-7(1′-hydroxyethyl)-2,3,6-trimethoxy-1,4-naphthoquinone (lomazarin, 3). A solution of **6** (105 mg) in MeOH—F_3COOH (2:1, 9 mL) was refluxed for 10 h (TLC monitoring) and evaporated at reduced pressure. The solid was chromatographed over a silica-gel column with elution by hexane:acetone (20:1) to afford **3** (90 mg, 97%), mp 97-100°C (acetone) (lit. [9] mp 100-101°C) (petroleum ether). IR spectrum (ν, cm^{-1}): 1602 (C=O), 3589, 3554 (OH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.57 (3H, d, J = 6.8, CH_3), 4.10 (3H, s, OCH_3), 4.13 (6H, s, OCH_3), 5.26 (1H, q, J = 6.8, CH), 12.89 (1H, s, α-OH), 13.28 (1H, s, α-OH).

^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 178.9, 177.9, 163.7, 158.6, 154.3, 148.4, 147.4, 134.7, 109.5, 106.5, 64.3, 61.7, 61.6, 23.3.

Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 324 (66) $[M]^+$, 309 (73), 306 (50), 291 (100), 263 (33).

Base Hydrolysis of 3 and Its Derivatives 5, 6, and 10. Compounds **3**, **5**, **6**, and **10** (0.27 mmol) in aqueous NaOH (1%, 10 mL) were refluxed for 3 h, cooled, neutralized with conc. HCl, and extracted with AcOEt. The extract was dried over anhydr. Na_2SO_4. Solvent was evaporated at reduced pressure. The solid was chromatographed over a silica-gel column with elution by hexane:acetone (10:1) to afford **9** (50-55%) and a mixture (1:1, PMR) of **4a** and **4b** (20-25%). The mixture of **4a** and **4b** (50 mg) was chromatographed over a silica-gel column (d = 15 mm, h = 60 cm) with elution by hexane:acetone (20:1). Two bands were isolated and contained mixtures of **4a** and **4b** in 2:1 and 1:2 ratios, respectively.

5,6,8-Trihydroxy-2,3-dimethoxy-1,4-naphthoquinone (9), mp 195-197°C (acetone) (lit. [1] mp 194-196°C). IR spectrum (ν, cm^{-1}): 1601 (C=O), 3408 (OH), 3520 (OH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.06 (3H, s, OCH_3), 4.16 (3H, s, OCH_3), 6.46 (1H, s, H-3), 7.08 (1H, br.s, OH-2), 12.27 (1H, s, α-OH), 13.12 (1H, s, α-OH).

^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 177.5, 167.8, 167.0, 165.1, 156.4, 150.5, 146.1, 110.4, 108.0, 106.0, 61.7, 61.6

Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 266 (100) $[M]^+$, 251 (79), 237 (25), 223 (26), 205 (26).

2,5,8-Trihydroxy-7(1′-hydroxyethyl)-3,6-dimethoxy-1,4-naphthoquinone (norlomazarin, 4a). PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.57 (3H, d, J = 6.8, CH_3), 4.14 (s, 3H, OCH_3), 4.18 (s, 6H, OCH_3), 5.27 (1H, q, J = 6.8, CH), 7.00 (1H, br.s, OH-2), 12.39 (1H, s, α-OH), 13.01 (1H, s, α-OH).

^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 183.7 (C-4), 180.9 (C-1), 158.1 (C-8), 155.2 (C-6), 153.8 (C-5), 143.8 (C-2), 140.6 (C-3), 133.8 (C-7), 108.9 (C-4a), 105.3 (C-8a), 64.2 (C-1′), 61.9 (6-OMe), 60.8 (3-OMe), 23.3 (C-2′).

3,5,8-Trihydroxy-7(1′-hydroxyethyl)-2,6-dimethoxy-1,4-naphthoquinone (4b). PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.58 (3H, d, J = 6.8, CH_3), 4.10 (3H, s, OCH_3), 4.20 (6H, s, OCH_3), 5.27 (1H, q, J = 6.8, CH), 7.00 (1H, br.s, OH-2), 12.04 (1H, s, α-OH), 13.35 (1H, s, α-OH).

^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 182.8 (C-1), 182.1 (C-4), 158.7 (C-8), 153.0 (C-5), 153.0 (C-6), 143.0 (C-3), 141.1 (C-2), 136.0 (C-7), 108.4 (C-4a), 105.7 (C-8a), 64.4 (C-1′), 61.7 (6-OMe), 60.9 (2-OMe), 23.3 (C-2′).

5,6,8-Trihydroxy-2,3-dimethoxy-7-ethyl-1,4-naphthoquinone (12). A boiling solution of **9** (100 mg) in *t*-BuOH (15 mL) was treated dropwise with an ether solution of propionyl peroxide [14] (TLC monitoring). The reaction was stopped at about 50% conversion. Solvent was evaporated. Chromatography (PTLC) using hexane:acetone (3:1) isolated **9** (50 mg,

50%) and **12** (28 mg, 24%), mp 150-152°C (acetone) (lit. [1] mp 153-154°C). IR spectrum (ν, cm^{-1}): 1597 (C=O), 3412 (OH), 3522 (OH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.15 (3H, t, J = 7.4, CH_3), 2.62 (2H, q, J = 7.4, CH_2), 4.05 (3H, s, OCH_3), 4.13 (3H, s, OCH_3), 7.21 (1H, br.s, OH-2), 12.14 (1H, s, α-OH), 13.49 (1H, s, α-OH).

^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 183.1, 175.2, 159.6, 158.8, 153.3, 150.2, 146.1, 126.6, 106.6, 106.5, 61.63, 61.58, 16.3, 12.6.

5,8-Dihydroxy-2,3,6-trimethoxy-1,4-naphthoquinone (tricrozarin B, 11). A solution of **9** (50 mg) in diethylether (10 mL) was stirred, treated dropwise with diazomethane in diethylether [15] (TLC monitoring), and evaporated to afford **11** (52 mg, 100%), mp 169-173°C (acetone) (lit. [11] mp 176-177°C) (methanol).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.96 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 4.16 (3H, s, OCH_3), 6.41 (1H, s, H-3), 12.93 (1H, s, α-OH), 13.04 (1H, s, α-OH).

^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 174.6, 172.2, 170.1, 161.4, 159.2, 150.2, 149.5, 146.9, 109.4, 107.5, 105.0, 61.62, 61.56, 56.7.

5,8-Dihydroxy-2,3,6-trimethoxy-7-ethyl-1,4-naphthoquinone (TMEE, 1). A solution of **12** (25 mg) in diethylether (10 mL) was stirred, treated dropwise with diazomethane in diethylether [15] (TLC monitoring), and evaporated to afford **1** (26 mg, 100%).

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REFERENCES

1. R. E. Moore, H. Singh, C. W. J. Chang, and P. J. Scheuer, *Tetrahedron*, **23**, 3271 (1967).
2. V. P. Anufriev and V. L. Novikov, *Tetrahedron Lett.*, **36**, 2515 (1995).
3. R. H. Thomson, *Naturally Occurring Quinones*, 2nd Ed., Academic Press, London-New York (1971), p. 734.
4. R. H. Thomson, *Naturally Occurring Quinones*, 3rd Ed., Chapman & Hall, London-New York (1987).
5. R. H. Thomson, *Naturally Occurring Quinones*, 4th Ed., Blackie Academic and Professional, London-New York (1997), p. 746.
6. V. A. Anufriev, G. B. Elyakov, S. G. Polonik, N. D. Pokhilo, O. P. Shestak, A. Y. Yakubovskaya, S. A. Osadchii, G. A. Tolstikov, and E. E. Shul′ts, RF Pat. No. 2,277,083 (2006); *Byull. Izobret.*, No. 15 (2006).
7. S. G. Polonik, N. D. Pokhilo, V. V. Makhan′kov, and V. F. Anufriev, *Khim. Prir. Soedin.*, 73 (2008).
8. G. B. Elyakov, O. B. Maksimov, N. P. Mishchenko, E. A. Kol′tsova, S. A. Fedoreev, L. I. Glebko, N. P. Krasovskaya, and A. A. Artyukov, RF Pat. No. 2,137,472 (1999); *Byull. Isobret.*, No. 26 (1999); RF Pat. No. 2,134,107 (1999); *Byull. Izobret.*, No. 22 (1999).
9. R. G. Cooke and J. B. Robinson, *Aust. J. Chem.*, **23**, 1695 (1970).
10. J. R. Cannon, Y. Matsuki, V. A. Patrick, and A. H. White, *Aust. J. Chem.*, **40**, 1191 (1987).
11. N. D. Pokhilo, M. I. Kiseleva, V. V. Makhan′kov, and V. F. Anufriev, *Khim. Prir. Soedin.*, 228 (2008).
12. K. Masuda, S. Funayama, K. Komiyama, and I. Umezava, *J. Nat. Prod.*, **50**, 958 (1987).
13. G. V. Malinovskaya, A. Ya. Chizhova, and V. F. Anufriev, *Izv. Akad. Nauk Ser. Khim.*, 1019 (1999).
14. V. Karnojitzki, *Les Peroxides Organiques*, Hermann, 6 Rue de la Sorbonne, Paris V (1958).
15. W. Bachmann and W. Struve, in: *Organic Reactions*, R. Adams, ed., J. Wiley and Sons, New York (1942).