Chemistry of Natural Compounds, Vol. 39, No. 2, 2003

OXIDATION OF BETULIN AND ITS MONOACETATES BY "ACTIVATED" DMSO

O. Yu. Ashavina, O. B. Flekhter, F. Z. Galin, N. N. Kabal′nova, L. A. Baltina, and G. A. Tolstikov

UDC 547.918:547.914.4.057

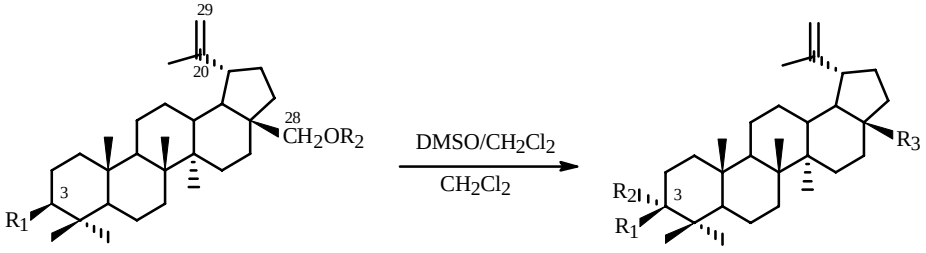
The oxidation of betulin and its 3-O- and 28-O-acetates by "activated" dimethylsulfoxide was investigated.

Key words: betulin, 3-O-acetylbetulin, 28-O-acetylbetulin, oxidation, "activated" dimethylsulfoxide.

Triterpenes of the lupane group (betulin, lupeol, betulinic acid, and their derivatives) typically have high biological activity (anti-inflammatory, cholagogic, antiviral, antitumor, immunomodulating, etc.) [1-4]. The high content of betulin (up to 35%) in white birch and the ease of its isolation [5] make it available for investigations of oxidative transformations of it and its acetates in order to find new oxidants and to synthesize new biologically active compounds.

An effective reagent for preparing carbonyl compounds by oxidation of alcohols is "activated" DMSO [DMSO in the presence of dicyclohexylcarbodiimide, $(COCl)_2$, $(CH_3CO)_2O$, Ac_2O, P_2O_5, etc.] [6]. We previously found that oxidataion of betulin and 3-O-acetylbetulin by DMSO activated by oxalylchloride produced betulonic and 3-O-acetylbetulinic aldehydes [7].

In continuation of these studies [7-9], we investigated the oxidation of betulin (**1**) and its 3-O- (**2**) and 28-O-acetates (**3**) by DMSO activated by acetic anhydride (Albright—Goldman) [10, 11]. It was found that oxidation of **1** by two and more equivalents of oxidant produces a mixture of the 3-oxo-28-O-methylthiomethyl ether of betulin (**4**) and 3-O-methylthiomethylbetulin aldehyde (**5**). Oxidation of **2** by one and more equivalents of oxidant gives in quantitative yield the 3-O-acetyl-28-O-methylthiomethyl ether of betulin (**6**). Oxidation of **3** by one and more equivalents of oxidant forms a mixture of 3-oxo-28-O-acetylbetulin (**7**) and 3-O-methylthiomethyl-28-O-acetylbetulin (**8**). It can be seen that ether formation occurs in addition to oxidative transformations owing to the facile alkylation of the hydroxyl of these triterpene alcohols. Nevertheless, this reagent can be recommended for synthesizing 3-oxo derivatives owing to the facile reaction and product separation. Furthermore, **4-6** and **8** were synthesized for the first time and are interesting as potential biologically active compounds.



1- 3 **4 - 8, 12**

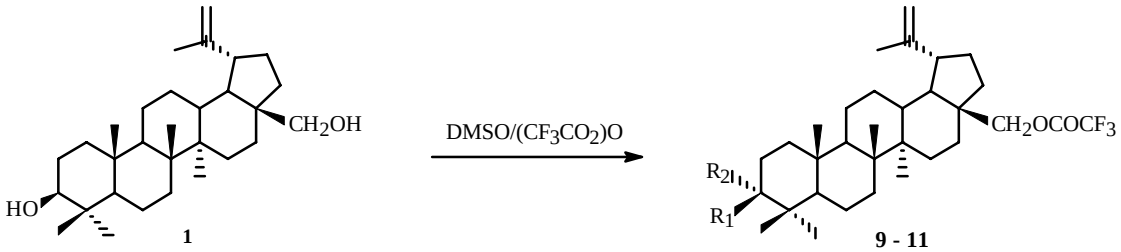
1: R_1 = OH, R_2 = H;
2: R_1 = AcO, R_2 = H;
3: R_1 = OH, R_2 = Ac

4: $R_1 + R_2$ = O, $R_3 = {}^{28}CH_2O{}^{1'}CH_2{}^{2'}SCH_3$;
5: $R_1 = {}^{2'}CH_3S{}^{1'}CH_2O$, R_2 = H, R_3 = CHO;
6: R_1 = OAc, R_2 = H, $R_3 = CH_2OCH_2SCH_3$;
7: $R_1 + R_2$ = O, $R_3 = CH_2OAc$;
8: $R_1 = CH_3SCH_2O$, R_2 = H, $R_3 = CH_2OAc$;
12: $R_1 + R_2$ = O, R_3 = CHO

Institute of Organic Chemistry, Ufa Scientific Center, Russian Academy of Sciences, 450054, Ufa, pr. Oktyabrya, 71, fax (3472) 35 60 66, e-mail: obf@anrb.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 156-159, March-April, 2003. Original article submitted March 28, 2003.

0009-3130/03/3902-0207$25.00

We also studied oxidation of **1** by DMSO activated by $(CF_3CO)_2O$ (according to Swern) [12]. Use of two equivalents of oxidant leads exclusively to acylation of the alcohols to form a mixture of 3,28-di-O-trifluoroacetylbetulin (**9**) and 28-O-trifluoroacetylbetulin (**10**). Also, the hydroxyl of **1** could be oxidized by increasing the number of equivalents to four and lengthening the reaction time to 48 h. This produced a mixture of 28-O-trifluoroacetylbetulin (**10**), 3-oxo-28-O-trifluoroacetylbetulin (**11**), and betulonic aldehyde (**12**).



1 **9 - 11**

9: $R_1 = CF_3OCO$, $R_2 = H$;
10: $R_1 = OH$, $R_2 = H$;
11: $R_1 + R_2 = O$

The structures of **4-12** were established using IR and NMR spectroscopies. Thus, the presence in the ^{13}C NMR of signals at δ 13.7-13.9 and 73.3-75.8 ppm provided evidence that the CH_2SCH_3 group was added. The SCH_3 protons resonated at δ 2.11-2.14 ppm as sharp singlets. Oxidation was confirmed by signals for aldehyde (δ 204.9-206.1 ppm, ^{13}C) and 9.64 ppm (PMR) in **5** and **12** and the 3-oxo group (δ 217.6-218.1 ppm ^{13}C) in **4**, **7**, **11**, and **12**. Signals for the ester bonds in spectra of trifluoroacetates **9-11** appeared at δ 155-158 ppm. The signals for C-3 and C-28 also shifted to weak field (δ, 86.3 and 66.8-67.0 ppm, respectively) compared with those in the starting alcohols.

Betulin (**1**) was found to be stable to oxidation by 2-20 equivalents of DMSO activated by P_2O_5 (according to Onodera) [13] with cooling or heating to 60°C.

EXPERIMENTAL

IR spectra were recorded on a Specord M80 spectrometer in mineral oil. ^{13}C NMR and PMR spectra were recorded on a Bruker AM-300 spectrometer (75.5 and 300 MHz, respectively) in $CDCl_3$ with $SiMe_4$ internal standard. Melting points were determined on a Boetius microstage. TLC was performed on Silufol plates (Chemapol, Czech Rep.) using $CHCl_3$:CH_3OH (25:1). Compounds were developed by phosphotungstic acid in ethanol (10%) with subsequent heating at 100-120°C for 2-3 min. DMSO was distilled at reduced pressure over freshly calcined CaO. Betulin (**1**), 3-O-acetylbutlin (**2**), and 28-O-acetylbetulin (**3**) were prepared by the literature methods [14, 15]. Elemental analyses of all compounds corresponded to those calculated.

Oxidation of 1-3 According to Albright—Goldman. A solution of **1-3** (1 mmol) in CH_2Cl_2 (40 mL) was treated with DMSO (3 mL) in Ac_2O (2 mL) (1 equiv. of reagent), stirred for 24-48 h at room temperature (TLC monitoring), treated with EtOH (5 mL), stirred for 1 h, diluted with water (10 mL), and treated with NH_4OH (5 mL, 15%). The organic layer was separated and dried over $MgSO_4$. The solvent was removed in vacuum.

3-Oxo-28-O-methylthiomethyl ether of betulin (4) and 3-O-methylthiomethylbetulinic aldehyde (5) were prepared by oxidation of **1** by oxidant (2-4 equiv.) and isolation after purification by column chromatography over Al_2O_3 (benzene eluent). **Compound 4**: Yield 0.37 g (73%), R_f 0.52, mp 174-176°C. $C_{32}H_{52}O_2S$. IR spectrum (ν, cm^{-1}): 1710, 1640, 1475, 1395, 1150, 1095, 922, 810, 765, 710.

PMR spectrum (δ, ppm, J/Hz): 0.72, 0.90, 0.93, 0.99, 1.05 (s, $5CH_3$), 1.70 (s, CH_3-30), 2.14 (s, CH_3-2′), 2.45-2.55 (m, H-19), 3.50 and 3.98 (d, ^2J = 11, 2H-28), 4.55 and 4.69 (both br. signals, 2H-29), 4.63 and 4.72 (both br. signals, 2H-1′).

^{13}C NMR spectrum (δ, ppm): 13.9 (C-2′), 14.8, 15.8, 16.0, 16.7, 19.2, 19.5, 21.5, 23.7, 25.7, 26.9, 27.9, 29.9, 30.7, 34.1, 34.4, 36.8, 37.1, 37.8, 38.3, 40.7, 42.5, 46.8, 47.0, 49.3, 49.6, 55.6, 66.2 (C-28), 75.8 (C-1′), 109.7 (C-29), 150.6 (C-20), 217.9 (C-3).

Compound 5: Yield 0.07 g (14%), R_f 0.28, mp 194-196°C, $C_{32}H_{52}O_2S$. IR spectrum (ν, cm^{-1}): 1720, 1650, 1465, 1390, 1200, 1100, 930, 815, 765, 710.

PMR spectrum (δ, ppm): 0.72, 0.91, 0.95, 0.10, 1.04 (s, $5CH_3$), 1.68 (s, CH_3-30), 2.11 (s, CH_3-2′), 2.48-2.55 (m, H-19), 3.45-3.53 (m, H-3), 4.57 and 4.70 (both br. signals, 2H-29), 4.64 and 4.68 (both br. signals, 2H-1′), 9.64 (s, H-28).

^{13}C NMR spectrum (δ, ppm): 13.7 (C-2′), 14.5, 15.9, 16.0, 16.8, 19.3, 19.5, 21.0, 25.7, 26.9, 27.9, 29.8, 30.7, 33.4, 34.5, 34.4, 37.0, 37.3, 38.0, 40.7, 42.5, 46.5, 47.2, 47.6, 49.8, 49.6, 55.9, 73.3 (C-1′), 83.6 (C-3), 109.9 (C-29), 150.6 (C-20), 204.9 (C-28).

3-O-Acetyl-28-O-methylthiomethyl ether of betulin (6) was prepared by oxidation of 3-O-acetylbetulin (**2**) by oxidant (1-2 equiv.) and recrystallization from ethanol. Yield 0.48 g (88%), R_f 0.76, mp 185-187°C. $C_{34}H_{56}O_3S$. IR spectrum (ν, cm^{-1}): 1735, 1645, 1485, 1395, 1355, 1150, 1085, 1030, 890, 840, 770, 725, 710.

PMR spectrum (δ, ppm, J/Hz): 0.78, 0.88, 0.90, 0.95, 1.01 (s, $5CH_3$), 1.72 (s, CH_3-30), 2.04 (s, OAc), 2.12 (s, CH_3-2′), 2.40-2.50 (m, H-19), 3.52 and 4.00 (d, ^2J = 11, 2H-28), 4.41-4.47 (m, H-3), 4.50 and 4.60 (both br. signals, 2H-29), 4.66 and 4.70 (both br. signals, 2H-1′).

^{13}C NMR spectrum (δ, ppm): 13.9 (C-2′), 14.5, 15.8, 16.2, 16.7, 19.2, 19.5, 21.3, 21.5, 23.7, 25.7, 26.9, 27.9, 29.9, 30.7, 34.1, 34.6, 36.8, 37.1, 37.8, 38.3, 40.7, 42.5, 46.8, 47.0, 49.3, 49.6, 55.6, 66.2 (C-28), 75.7 (C-1′), 80.9 (C-3), 109.7 (C-29), 150.6 (C-20), 170.9 ($CH_3\underline{C}OO$).

3-Oxo-28-O-acetylbetulin (7) and 3-O-methylthiomethyl-28-O-acetylbetulin (8) were prepared by oxidation of **3** by oxidant (1-2 equiv.) and isolation after purification by column chromatography over Al_2O_3 (benzene eluent).

Compound 7: Yield 0.33 g (69%), R_f 0.88, mp 117°C. Lit. [16] mp 117-119°C. $C_{32}H_{50}O_3$. IR spectrum (ν, cm^{-1}): 1740, 1710, 1650, 1465, 1390, 1345, 1095, 930, 815, 765, 710.

PMR spectrum (δ, ppm, J/Hz): 0.84, 0.86, 0.93, 0.97, 1.02 (s, $5CH_3$), 1.70 (s, CH_3-30), 2.00 (s, OAc), 2.35-2.45 (m, H-19), 3.83 and 4.21 (d, ^2J = 11, 2H-28), 4.54 and 4.70 (both br. signals, 2H-29).

^{13}C NMR spectrum (δ, ppm): 14.0, 15.9, 16.2, 16.5, 19.3, 19.9, 21.0, 21.2, 25.7, 26.9, 27.0, 29.5, 29.6, 33.4, 34.1, 34.5, 37.0, 37.6, 39.5, 40.7, 42.5, 46.5, 47.2, 47.6, 48.6, 49.6, 54.9, 62.9 (C-28), 109.9 (C-29), 150.6 (C-20), 171.1 ($CH_3\underline{C}OO$), 218.1 (C-3).

Compound 8: Yield 0.10 g (18%), R_f 0.50, mp 180-182°C. $C_{34}H_{56}O_3S$. IR spectrum (ν, cm^{-1}): 1720, 1640, 1483, 1391, 1350, 1205, 1080, 1032, 895, 836, 725, 710.

PMR spectrum (δ, ppm, J/Hz): 0.72, 0.93, 0.95, 1.00, 1.01 (s, $5CH_3$), 1.68 (s, CH_3-30), 2.01 (s, OAc), 2.14 (s, CH_3-2′), 2.50-2.62 (m, H-19), 3.82 and 4.24 (d, ^2J = 10.9, 2H-28), 3.48-3.55 (m, H-3), 4.63 and 4.73 (both br. signals, 2H-29), 4.65 and 4.69 (both br. signals, H-1′).

^{13}C NMR spectrum (δ, ppm): 13.7 (C-2′), 14.5, 16.0, 16.5, 16.8, 19.9, 20.1, 20.5, 21.0, 23.4, 25.0, 26.1, 27.8, 29.9, 30.7, 33.8, 34.9, 37.0, 37.8, 37.9, 38.0, 40.7, 41.5, 46.4, 47.2, 49.8, 49.9, 55.9, 62.9 (C-28), 73.5 (C-1′), 83.1 (C-3), 109.9 (C-29), 150.0 (C-20), 171.9 ($CH_3\underline{C}OO$).

Oxidation of 1 According to Swern. A solution of DMSO (1.98 mL) in CH_2Cl_2 (14 mL) at -30°C under Ar was treated dropwise with $(CH_3CO)_2O$ (0.40 mL, 2 equiv.), stirred for 10 min, and treated dropwise with **1** (0.44 g, 1 mmol) in CH_2Cl_2 (50 mL). The reaction mixture was stirred at -30°C for 5 h (TLC monitoring), treated with Et_3N (0.93 mL), and stirred for 1.5 h. The temperature was adjusted to 5°C before adding HCl (21 mL, 2 M) and extracting with CH_2Cl_2 (2×15 mL). The organic layers were combined, washed with water (2×25 mL), dried over $MgSO_4$, evaporated in vacuum, and chromatographed over a column of Al_2O_3 (CH_2Cl_2 eluent).

3,28-Di-O-trifluoroacetylbetulin (9) and 28-O-trifluoroacetylbetulin (10) were prepared by oxidation of **1** by oxidant (2 equiv.).

Compound 9: Yield 0.44 g (71%), R_f 0.74, mp 210-212°C. $C_{34}H_{48}O_4F_6$. IR spectrum (ν, cm^{-1}): 1735, 1720, 1635, 1483, 1392, 1360, 1200, 1185, 1085, 1030, 1010, 890, 840, 770, 725, 715.

PMR spectrum (δ, ppm, J/Hz): 0.86, 0.89, 0.93, 0.97, 1.01 (s, $5CH_3$), 1.72 (s, CH_3-30), 2.40-2.50 (m, H-19), 4.90 and 5.10 (d, J = 11.0, 2H-28), 4.65-4.70 (m, H-3), 4.63 and 4.75 (both br. signals, 2H-29).

^{13}C NMR spectrum (δ, ppm): 14.0, 15.9, 16.2, 16.5, 18.3, 19.1, 21.8, 23.3, 25.1, 26.9, 27.9, 29.4, 30.7, 34.0, 34.2, 37.0, 37.8, 38.0, 38.2, 40.7, 42.5, 46.5, 47.2, 48.8, 49.9, 55.9, 66.9 (C-28), 86.3 (C-3), 109.9 (C-29), 112.8 and 116.4 (CF_3), 150.6 (C-20), 155.1 and 158.0 (CF_3COO).

Compound 10. Yield 0.13 g (24%), R_f 0.42, mp 192-194°C. $C_{32}H_{49}O_3F_3$. IR spectrum (ν, cm^{-1}): 3480, 1720, 1643, 1475, 1395, 1355, 1200, 1085, 1030, 890, 840, 770, 725, 710.

PMR spectrum (δ, ppm, J/Hz): 0.90, 0.92, 0.94, 0.97, 1.04 (s, $5CH_3$), 1.72 (s, CH_3-30), 2.50-2.60 (m, H-19), 3.15-3.20 (m, H-3), 4.95 and 5.15 (d, ^2J = 10.9, 2H-28), 4.64 and 4.72 (both br. signals, 2H-29).

^{13}C NMR spectrum (δ, ppm): 14.3, 15.4, 16.0, 16.5, 19.1, 19.8, 21.3, 23.9, 25.3, 26.3, 27.9, 30.0, 30.7, 34.3, 34.9, 37.2, 37.3, 37.5, 38.1, 40.7, 41.4, 46.3, 47.3, 49.8, 49.9, 55.9, 67.0 (C-28), 78.3 (C-3), 109.0 (C-29), 116.8 (CF_3), 150.0 (C-20), 155.5 ($CF_3\underline{C}OO$).

28-O-Trifluoroacetylbetulin (10), 3-oxo-28-O-trifluoroacetylbetulin (11), and betulinic aldehyde (12) were prepared by oxidation of **1** by oxidant (4 equiv.) for 4 h.

Compound 10. Yield 0.11 g (21%).

Compound 11. Yield 0.26 g (49%), R_f 0.56, mp 167-169°C. $C_{32}H_{47}O_3F_3$. IR spectrum (ν, cm^{-1}): 1720, 1710, 1638, 1472, 1400, 1352, 1202, 1092, 1028, 895, 832, 770, 725, 715.

PMR spectrum (δ, ppm, J/Hz): 0.92, 0.96, 0.97, 0.99, 1.02 (s, 5CH_3), 1.70 (s, CH_3-30), 2.48-2.60 (m, H-19), 4.86 and 5.10 (d, ^{2}J = 11, 2H-28), 4.65 and 4.75 (both br. signals, 2H-29).

^{13}C NMR spectrum (δ, ppm): 14.5, 15.6, 16.2, 16.5, 19.3, 19.9, 21.2, 23.9, 25.7, 26.5, 27.7, 30.0, 30.5, 34.5, 34.9, 37.0, 37.5, 37.5, 38.0, 40.4, 41.1, 46.5, 47.2, 49.6, 49.8, 55.8, 66.8 (C-28), 109.6 (C-29), 117.0 (CF_3), 151.1 (C-20), 154.8 (CF_3COO), 218.3 (C-3).

Compound 12. Yield 0.09 g (20%), mp 163°C, lit. [7] mp 163-164°C.

PMR and ^{13}C NMR spectra were analogous to those reported previously [7].

Oxidation of 1 According to Onodera. A solution of **1** (0.44 g, 1 mmol) in CH_2Cl_2 (50 mL) under Ar at 0°C was treated with DMSO (0.15 mL) and P_2O_5 (0.29 g, 2 equiv.), stirred for 2 h (TLC monitoring) at room temperature or with heating with a reflux condenser at 60°C for 3 h, treated dropwise with Et_3N (0.50 mL), stirred for 30 min, treated with HCl (20 mL, 10%), and extracted with CH_2Cl_2 (2×15 mL). The organic layers were combined, washed with saturated NaCl solution (25 mL) and water (2×25 mL), dried over $MgSO_4$, and evaporated in vacuum. The reaction was also carried out with 5, 10, and 20 equiv. of reagent. The physicochemical properties and spectral data of the isolated product corresponded to those of starting betulin [5, 17].

ACKNOWLEDGMENT

The work was supported by the Russian Foundation for Basic Research (project Nos. 01-03-33131 and 02-03-81007).

REFERENCES

1. T. Fujioka, Y. Kashiwada, R. E. Kilkuskie, L. M. Cosentino, L. M. Ballas, J. B. Jiang, W. P. Janzen, I. S. Chen, and K. H. Lee, *J. Nat. Prod.*, **57**, 243 (1994).
2. E. Pisha, H. Chai, I. S. Lee, T. E. Chagwedera, N. R. Farnsworth, G. A. Cordell, C. W. W. Beecher, H. H. S. Fong, A. D. Kinghorn, D. M. Brown, M. C. Wani, M. E. Wall, T. J. Hieken, T. K. Das Gupta, and J. M. Pezzuto, *Nat. Med.*, **1**, 1046 (1985).
3. M. C. Recio, R. M. Giner, S. Manez, and J. L. Rios, *Planta Med.*, **61**, 9 (1995).
4. O. B. Flekhter, L. T. Karachurina, L. R. Nigmatullina, T. A. Sapozhnikova, L. A. Baltina, F. S. Zarudii, F. Z. Galin, L. V. Spirikhin, G. A. Tolstikov, O. A. Plyasunova, and A. G. Pokrovskii, *Bioorg. Khim.*, **28**, 6, 543 (2002).
5. P. Jaaskelainen, *Pap. Puu*, **63**, No. 10, 599 (1981).
6. T. T. Tidwell, *Synthesis*, 857 (1990).
7. O. B. Flekhter, O. Yu. Ashavina, E. I. Boreko, L. T. Karachurina, N. I. Pavlova, N. N. Kabal′nova, O. V. Savinova, F. Z. Galin, S. N. Nikolaeva, F. S. Zarudii, L. A. Baltina, and G. A. Tolstikov, *Khim-Farm. Zh.*, **36**, 6, 21 (2002).
8. O. Yu. Ashavina, O. B. Flekhter, N. N. Kabal′nova, E. V. Ivanova, F. Z. Galin, and G. A. Tolstikov, in: Materials of the Young Scientists Scientific Educational Conference "Current Problems in Organic Chemistry," Novosibirsk, April 2-6, 2001, 52.
9. O. Yu. Ashavina, N. N. Kabal′nova, S. A. Grabovskii, O. B. Flekhter, F. Z. Galin, and G. A. Tolstikov, in: Materials of the VIth International Conference "Bioantioxidant," Moscow, April 16-19, 2002, 47.
10. J. D. Albright and L. Goldman, *J. Am. Chem. Soc.*, **87**, 4214 (1963).

11. J. D. Albright and L. Goldman, *J. Am. Chem. Soc.*, **89**, 2416 (1967).
12. C. M. Amon, M. G. Banwell, and G. L. Gravatt, *J. Org. Chem.*, **52**, 4851 (1987).
13. D. F. Taber, J. C. Amedio, and K.-Y. Jung, *J. Org. Chem.*, **52**, 24, 5621 (1987).
14. O. B. Flekhter, L. T. Karachurina, V. V. Poroikov, L. R. Nigmatullina, L. A. Baltina, F. S. Zarudii, V. A. Davydova, L. V. Spirikhin, I. P. Baikova, F. Z. Galin, and G. A. Tolstikov, Bioorg. Khim., **26**, 3, 215 (2000).
15. L. F. Tietze, H. Heinzen, P. Moyna, M. Rischer, and H. Neunaber, *Leibigs Ann. Chem.* 1245 (1991).
16. A. V. Korovin and A. V. Tkachev, *Izv. Akad. Nauk, Ser. Khim.*, 2, 292 (2001).
17. J. S. Alves, J. C. M. de Castro, M. O. Friere, E. V. Leitao da-Cunha, J. M. Baebosa-Filho, and M. S. de Silva, *Magn. Reson. Chem.*, **38**, 201 (2000).