Chemistry of Natural Compounds, Vol. 44, No. 5, 2008

STEREOCHEMICAL ASSIGNMENTS OF 22-ISOXAZOLINYLSTEROIDS USING CIRCULAR DICHROISM

N. I. Garbuz, G. S. Yankovskaya, R. P. Litvinovskaya,*
Yu. A. Sokolov, S. V. Drach, and V. A. Khripach

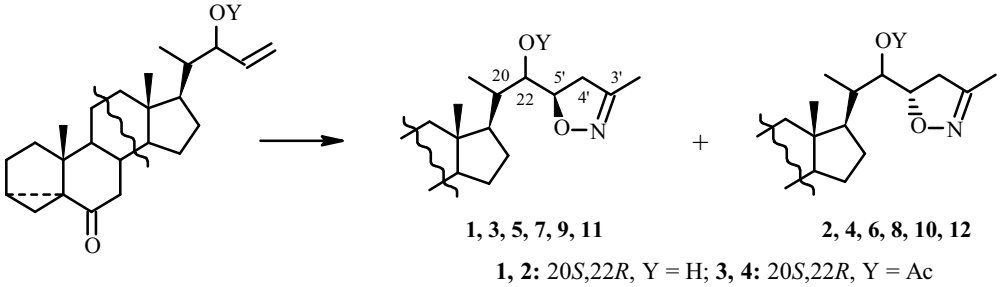
UDC 547.92:547.963

Circular dichroism (CD) spectra of 22-hydroxy- and 22-acetoxy-22-isoxazolinylsteroids were studied. The configuration of the C-5′ center of the heterocycle was established from the sign of the n-π^**-transition band of the azomethine chromophore. The band molecular ellipticity was shown to depend on the mutual placement of the isoxazoline ring and the steroid skeleton of the studied compounds.*

Key words: steroids, 22-isoxazolinylsteroids, circular dichroism method.

The ability to identify stereoisomers of 17-isoxazolinyl- and 20-hydroxy-20-isoxazolinylsteroids differing in the stereochemistry of asymmetric C-5′ in the isoxazoline ring has been demonstrated previously [1]. An empirical rule of octants that links the sign and magnitude of the molecular ellipticity of the n—π^*-transition band of the azomethine bond at 212-220 nm to the conformation of the isoxazoline ring and structural features of the compounds was proposed for determining the configuration of this center.

Herein structural studies using CD of other isoxazoline steroid derivatives with a longer side chain, 22-hydroxy- and 22-acetoxy-22-isoxazolinylsteroids **1-12**, are continued in order to establish the configuration of C-5′ and estimate the contribution of the closest environment of the isoxazoline ring to the optical activity of the azomethine chromophore. Table 1 gives the measured CD and absorption spectra of the compounds. The studied 22-isoxazolinylsteroids were synthesized according to the published method [2-4].



1, 3, 5, 7, 9, 11 **2, 4, 6, 8, 10, 12**

1, 2: 20*S*,22*R*, Y = H; **3, 4:** 20*S*,22*R*, Y = Ac
5, 6: 20*S*,22*S*, Y =H; **7, 8:** 20*R*,22*S*, Y = Ac
9, 10: 20*S*,22*S*, Y = H; **11, 12:** 20*S*,22*S*, Y = Ac

The structures of the synthesized compounds were determined from PMR and ^{13}C NMR spectra and confirmed by x-ray structure analyses (XSA) of stereoisomers **3** and **6**, which were identified as the 20*S*,22*R*,5′*R* and 20*R*,22*S*,5′*S* isomers, respectively [5, 6]. The molecular structures of these two epimers at the C-20, C-22, and C-5′ centers of the 22-isoxazolinylsteroids are very convenient for applying the rule of octants to the azomethine bond because their XSA data contain accurate information on the geometry of the isoxazoline ring and the location of side-chain and steroid-skeleton atoms closest to the ring. This differentiates advantageously the x-ray structures of the compounds from their molecular models that were used for the stereochemical assignments.

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Minsk, e-mail: litvin@iboch.bas-net.by.
Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 495-499, September-October, 2008. Original article submitted December 11, 2007.

0009-3130/08/4405-0612

TABLE 1. Absorption and CD Bands of 22-Hydroxy and 22-Acetoxy-22-isoxazolinylsteroids

Compound	Absorption, λ, nm (ε, $mol^{-1}·cm^{-1}$)	CD, λ, nm ([θ], $deg·mol^{-1}·m^2$)	Compound	Absorption, λ, nm (ε, $mol^{-1}·cm^{-1}$)	CD, λ, nm ([θ], $deg·mol^{-1}·m^2$)
1	212 (8000) 273 (100)	212 (–16) [–30]* 285 (–8)	**8**	215 (band tail) 280 (200)	215 (+35) [+21] 285 (–8)
2	211 (9000) 273 (100)	212 (+26) [+12] 287 (–8)	**9**	213 (8000) 275 (100)	211 (+1) [-13]** 217 (+2)
3	210 (12000) 264 (3000)	208 (–22) [–36] 287 (–8)	**10**	214 (7000) 280 (200)	215 (+30) [+16] 287 (–8)
4	210 (10000) 274 (100)	210 (+40) [+26] 285 (–8)	**11**	207 (10000) 290 (300)	210 (–2) [–16] 285 (–8)
5	214 (8000) pl. 280 (400)	212 (–3) [–17] 285 (–8)	**12**	210 (9000) 289 (100)	215 (+32) [+18] 288 (–8)
6	214 (9000) pl. 280 (400)	214 (+53) [+39] 285 (–8)	**13**	214 (4000) 270 (1500)	215 (+14) 288 (–8)
7	210 (10000) 280 (800)	215 (–5) [–19] 285 (–8)	**14**	216 (360)	216 (–58)

*Molecular elliptiity after subtracting contribution of ketone π–π^*-transition band in brackets.
Weak negative CD band at 211 nm in isomer **9 in the positive range of [θ].

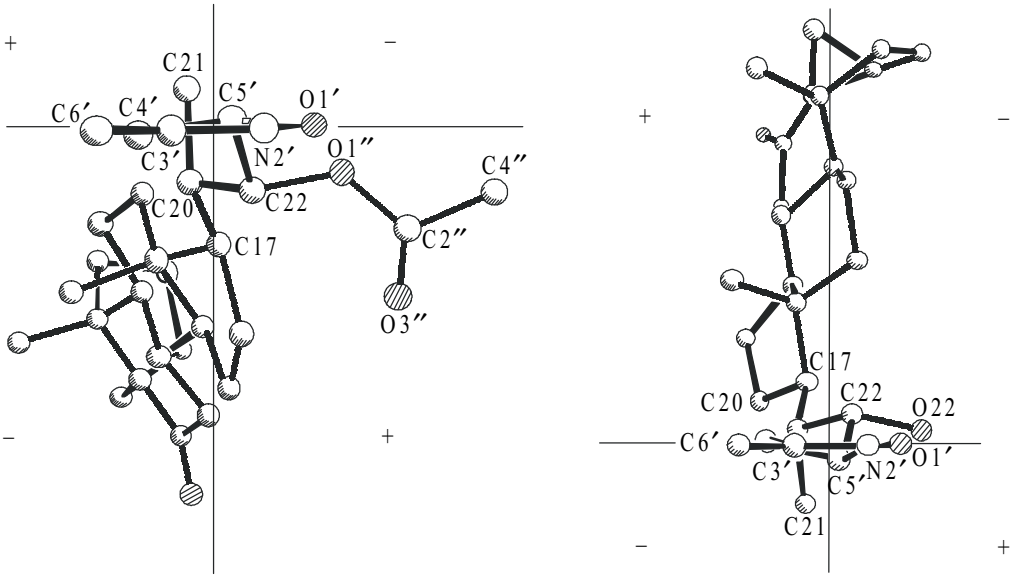


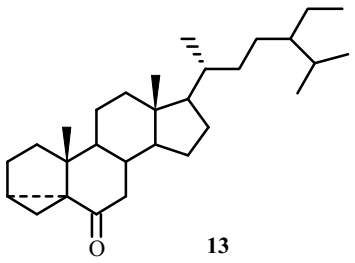
Fig. 1. Projection of stereoisomers **6** (left) and **3** (right) on rear octants of the diagram.

Figure 1 shows the octant projections of **3** and **6** that were obtained by separating their molecular structures according to the rule of octants into three mutually perpendicular planes passing through the azomethine bond. The horizontal plane of the chromophore was the plane passing through the C=N bond and the neighboring N–O bond. This plane of the chromophore can be viewed as the plane of the isoxazoline ring, from which C-4′ deviates slightly (dihedral angles O1′N2′C3′C4′ for **3** and **6** were 1.25° and –0.2° [5, 6]). Only C-5′ deviates significantly from the plane. The isoxazoline ring itself has the envelope conformation with asymmetric C-5′ on the flap.

CD spectra of analogous twisted asymmetric derivatives of cyclopentanone or bicyclic ketones with a strong effect on the chromophore of asymmetric C atoms of the ring itself that was viewed as a first-order effect and dominated over the effect of the ring substituents (second-order effect) have been described [7, 8]. For our compounds, C-5′ deviates from the plane of the isoxazoline ring and will have a strong asymmetric perturbation on electrons of the C=N bond that are involved in the

n—π^*-transition. Asymmetric centers of the side chain, their substituents, and more distant steroid skeleton atoms should play a secondary role. Figure 1 shows that the positive short-wavelength Cotton effect (CE) of 5′*S*-isomer **6** is due primarily to the location of asymmetric center C-5′ in the positive right lower octant whereas the negative CE of 5′*R*-isomer **3** is due to its location in the negative right upper octant (here and henceforth, the rear octants).

Assignments were also made for the remaining stereoisomers with the *S*- or *R*-configuration of asymmetric C-5′ (Table 1), thereby confirming the ability to establish its configuration by CD for 22-isoxazolinylsteroids. The positive CE for the *S*-isomers was always more intense than for the negative CE of the corresponding *R*-isomers. This was due to the contribution to the short-wavelength band of other chromophores, primarily ketones, the π—π^*-transition band of which fell in the range 210-215 nm [9]. We used model compound **13**, which does not contain an isoxazoline ring in the side chain and has a rather large positive CE at 215 nm, to eliminate the contribution of the ketone to the short-wavelength CE (Table 1).

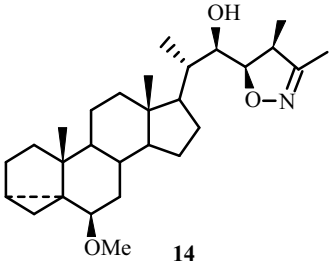


13

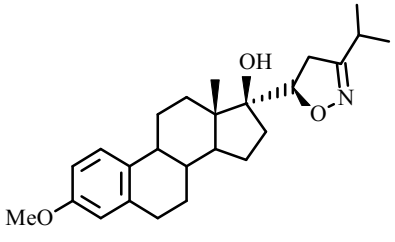
The significant distance between the ketone and the isoxazoline ring and the rigidity of the steroid skeleton suggest that this group makes an equal contribution (+14 deg·mol^{-1}·m^2) to the [θ] value of the short-wavelength CD band of stereoisomers **1**-**12**. However, these do not consider the optical activity of the n—π^*-transition of the acetoxy carboxyl of the corresponding stereoisomers. The band of this electronic transition is usually located near 210 nm [10] and also overlaps the band of the C=N n—π^*-transition. This is consistent with the hypsochromic shift and the increase of ε for the short-wavelength band in UV spectra of the acetoxy derivatives compared with spectra of the starting 22-hydroxy-22-isoxazolinylsteroids (Table 1).

The XSA of epimeric **3** and **6** in combination with the rule of octants revealed their individual stereochemical details, which facilitated the correct construction of molecular models of the other studied compounds. Cyclic C-5′ and side-chain C-22 that was closest to it were situated on different sides of the plane of the isoxazoline ring, which corresponded to the sterically more favorable pseudo-equatorial orientation of the exocyclic C-22—C-5′ bond, which is formed during addition of nitroxide to the terminal Δ^{23}-steroid. Sequential placement in octants of different sign was characteristic for **3** and **6** in the crystalline state for side-chain atoms C-22, C-20, and C-21. Most atoms of the steroidal skeleton were located in the same octant as asymmetric C-20. Octants into which these atoms of **3** and **6** fell had positive signs, which was the rule for stereomers with different configurations of all three asymmetric centers of the side chain. It was also obvious that C-5′ in stereoisomer **6** deviated from the horizontal plane of the chromophore more than in isomer **3**.

The last stereochemical feature deserved special attention because the molecular ellipticity of the n—π^*-transition band of the azomethine bond should depend substantially on the degree of deviation of this asymmetric center from the chromophore plane. In fact, dihedral angle C3′N2′O1′C5′ (φ_1) in stereomers **3** and **6**, which determined the deviation of C5′ from the chromophore plane, was 6.3 and −13.9°, respectively [5, 6]. One of the reasons for such different angles might have been structural features of the stereoisomers, in particular, the presence of an intramolecular H-bond (IaHB) between the hydroxyl and the O atom of the isoxazoline ring in stereoisomer **6** [6].



14



15

TABLE 2. Dihedral Angles of Isoxazoline Ring in Isoxazolinylsteroids Studied by XSA

Dihedral angle, deg	Isoxazolinylsteroid			
	3 [5]	**6** [6]	**14** [11]	**15** [12]
$C^{5'}O^{1'}N^{2'}C^{3'}$ (φ_1)	6.3	− 13.9	14.3	−3.3
$O^{1'}N^{2'}C^{3'}C^{4'}$ (φ_2)	1.2	− 0.2	3.5	0.2
$C^{5'}C^{4'}C^{3'}N^{2'}$ (φ_3)	−7.8	13.2	−18.7	2.8
$O^{1'}N^{2'}C^{3'}C^{6'}$ (φ_4)	−179.6	−177.7	177.1	176.2

TABLE 3. Calculated Dihedral Angles Characterizing Mutual Placement of Isoxazoline Ring and Steroid Skeleton in 22-Isoxazolinylsteroids

Stereoisomer	Dihedral angle, deg		Stereoisomer	Dihedral angle, deg	
	$C^{4'}C^{5'}C^{22}C^{20}$ (φ_5)	$C^{16}C^{17}C^{20}C^{22}$ (φ_6)		$C^{4'}C^{5'}C^{22}C^{20}$ (φ_5)	$C^{16}C^{17}C^{20}C^{22}$ (φ_6)
1	71.0	58.3	**4**	174.8	62.7
3	161.6	55.0	**6**	179.8	175.4
2	56.3	65.2	**8**	−178.4	61.5

Therefore, it seemed interesting to analyze the dihedral angles characterizing the geometry of the isoxazoline ring in crystals of stereoisomers **3** and **6** and in two other isoxazolines **14** and **15** that we studied using XSA [11, 12] and to compare the absolute values of [θ] of the n—π^*-transition band of the C=N bond and their CD spectra.

The comparison of dihedral angles (Table 2) showed that the geometry of this strained five-membered heterocycle was consistent with our previous hypothesis [1] that it was a highly flattened ring with C-5′ deviating from its plane. Small differences in the geometry of the heterocycle for these compounds that were due to the degree of C-5′ deviation from the plane of the chromophore and to a smaller extent that of C-4′ were obviously the result of their intra- and intermolecular interactions.

The most planar heterocycle of isoxazolinylsteroid **15** that had small angles φ_1—φ_3 (Table 2) may have been due to formation of a dimer through an intermolecular H-bond (IeHB) of the 17β-OH and the N atoms of isoxazoline rings [12]. On the other hand, involvement of the heterocyclic O atom in formation of an IaHB OH...O in steromers **6** and **14** [6, 11] increased the folding of the ring that was accompanied by a larger deviation of C-5′ from its plane in these isomers than in **3** (Table 2). The different effect of an IeHB and IaHB on the geometry of the isoxazoline ring can be explained logically because an IeHB tends to stretch the heterocycle whereas an IaHB contracts it. Rupture of the relatively weak IaHB [13] in **6** and **14** and the IeHB in **15** under CD measurement conditions in ethanol will restore the "normal" geometry of the isoxazoline ring. This was confirmed by the similar absolute values of [θ] for the n—π^*-transition band of the C=N bond for **6** and **3** (Table 1). However, the absolute value of [θ] for the band of this transition for **14** was 1.5 times greater than for its 5′-epimeric homolog **6**. This was due primarily to the strong effect on the chromophore of the second asymmetric center in the isoxazoline ring. In fact, as follows from the rule of octants, asymmetric atoms C-4′ and C-5′ are situated in octants of the same (negative) sign.

Thus, the comparison of the geometric and optical parameters of the four isoxazolinylsteroids studied by XSA suggested that the observed differences in the geometry of the heterocycle were due to features of the crystal structures of these compounds. Molecules of the various isoxazolinylsteroids in the solvated state during CD measurements obviously had the same ring geometry if their structural modifications did not impose, like for **14**, changes in the ring itself. The intensity of the CE of the n—π^*-transition of the azomethine bond depended apparently only on its steric surroundings.

We constructed molecular models of stereoisomers taking into account the details noted aobve for the molecular structures of stereomers **3** and **6** and using dihedral angles from their x-ray structures and from structures of stereoisomeric isoxazolinylsteroids calculated separately by us here and previously [13] in order to make stereochemical assignments for the 22-isoxazolinylsteroids **1-12**. Application of the rule of octants to the azomethine bond in the molecular models of the stereoisomers enabled the sign and value of the CE in the range 208-215 nm to be linked to the placement in the octant diagram of asymmetric C-5′ and other asymmetric centers and their substituents. The asymmetric center of the isoxazoline ring was situated in the negative right upper octant for all 5′R-stereomers with a negative CE in this range; for 5′S-stereomers with a

positive CE, in the positive right lower octant. The placement of the other two asymmetric centers of the side chain and their substituents in the octant diagram of the 5′*R*- and 5′*S*-stereoisomers in the lower and upper octants, respectively, was determined by the configuration of these centers. Thus, the CE intensity of one of the stereoisomers in the series (5′*R*)-22-hydroxy-22-isoxazolinylsteroids **1**, **5**, and **9**, and in the series of their 5′*S*-isomers **2**, **6**, and **10**, **1** and **6**, respectively, was more than two times greater than the CE intensity of the other two stereoisomers (Table 1). The high intensity of the CE for **1** and **3** was due to the primary placement of the steroid skeleton and C-5′ in octants of the same sign. On the other hand, placement of C-5′ and most atoms of the steroid skeleton in octants of different signs (lower right for 5′*R*-steromers **5** and **9** and upper right for 5′*S*-stereomers **2** and **10**) greatly decreased the CE intensity.

These trends in the intensity change of the short-wavelength CE were observed for 22-oxygenated 22-isoxazolinylsteroids only in the 5′*R* series and not in the 5′*S* series (Table 1). Thus, stereoisomer **4** had the strongest CE among (5′*S*)-22-acetoxy derivatives whereas the (5′*S*)-isomer corresponding to it **2** had the weakest CE. Compound **6** had the strongest CE; its acetoxy derivative **8**, a comparatively weak CE.

The observed difference in the CE intensity ratio of (5′*S*)-22-hydroxy- and (5′*S*)-22-acetoxy-22-isoxazolinylsteroids cannot be explained by the specifics of optical activity generation by the acetoxy carboxyl chromophore, which should be comparatively weak. The molecular ellipticity of the $n—\pi^*$-transition band of the analogous chromophore located directly in the steroid skeleton in 7-oxa-6-keto- and 6-oxa-7-ketosteroids [14] was an order of magnitude less than [θ] of the $n—\pi^*$-transition band of the azomethine bond in the isoxazolinylsteroids examined by us. It can be assumed that an acetoxy group in the steriod side chain had a lower [θ] for the $n—\pi^*$-transition band. On the other hand, the acetoxy group is a bulky substituent located next to the isoxazoline ring and can affect the optical activity of the $n—\pi^*$-transition of the azomethine bond.

Application of the rule of octants to the azomethine chromophore of 22-acetoxy-22-isoxazolinylsteroids and the corresponding 22-hydroxy-22-isoxazolinylsteroids and comparison of their [θ] for the $n—\pi^*$-transition band (Table 1) showed that the acetoxy group in all stereomers except stereomer **3** made the expected contribution based on its placement in the octant diagram. Whereas this contribution was comparatively small in stereomers **7**, **11**, and **12** (2-3 deg·mol^{-1}·m^2), it was significant in **4** and **8** (+14 and −18 deg·mol^{-1}·m^2, respectively). A small negative contribution was observed for stereoisomer **3** instead of the expected positive contribution of the acetoxy group (−3 deg·mol^{-1}·m^2).

Conformational analysis of 22-acetoxy-22-isoxazolinylsteroids **3**, **4**, and **8** and the corresponding 22-hydroxy-22-isoxazolinylsteroids **1**, **2**, and **6** was performed in order to explain the anomalous dichroic behavior of the first group. A comparison of the calculated dihedral angles C4′C5′C22C20 (φ_5) and C16C17C20C22 (φ_6) in stereoisomers **3** and **1** (Table 3) showed the different mutual placement in them of the isoxazoline ring and the steroid skeleton. This resulted in more atoms of the steroid skeleton being placed in the negative lower left octant for **3** than for **1** and increased the strength of the negative CE. Comparison of dihedral angles φ_5 and φ_6 in two other pairs of isoxazolinylsteroids gave an analogous result. Fundamental changes of φ_5 in acetoxy derivatives **4** and **8** compared with 22-hydroxysteroids **2** and **6** resulted in placement of more atoms of the steroid skeleton in the positive right upper and negative left upper octants, respectively. This increased significantly the [θ] values of their short-wavelength band. Differences in dihedral angles φ_5 and φ_6 of 22-hydroxy-22-isoxazolines **7**, **11**, and **12** and the corresponding 22-hydroxy derivatives **5**, **9**, and **10** will be smaller.

Thus, the investigation established that CD can be used to assign the configuration of asymmetric C5′ in several 22-isoxazolinylsteroids regardless of the possible location of other chromophores in the range of the $n—\pi^*$-transition band of the azomethine bond. The rule of octants proposed by us previously [1] is applicable to the $n—\pi^*$-transition of the isoxazoline C=N bond and to isoxazolinylsteroids with a side chain of different length.

EXPERIMENTAL

CD spectra of studied compounds in ethanol solution at concentrations of $3\cdot10^{-4}$-$7\cdot10^{-4}$ M in quartz cuvettes (0.2 and 0.5 cm) were recorded on a Jasco-20 spectropolarimeter. The sensitivity of the instrument was 0.005°/cm; time constant 4; scan rate 1 nm/min in the range 200-240 nm and 10 nm/min in the range 240-350 nm. The relative uncertainty of the measurement of molecular ellipticity of the short-wavelength band was less than 20%. The intensity of the short-wavelength CE was adjusted to that of the long-wavelength CE as an internal standard in order to avoid concentration effects. The value of the latter varied little. Absorption spectra were measured on a Specord M-400 spectrophotometer at the same concentrations and cuvette thicknesses as the CD spectra. Conformational analysis of the compounds was carried out by the AM-1 quantum-chemical method [15].

ACKNOWLEDGMENT

We thank A. S. Lyakhov for providing the x-ray projections of stereomers **3** and **6**.

REFERENCES

1. N. I. Garbuz, G. P. Yankovskaya, A. V. Baranovskii, R. P. Litvinovskaya, and V. A. Khripach, *Khim. Prir. Soedin.*, 391 (1994).
2. R. Litvinovskaya, S. Drach, and V. Khripach, *Mendeleev Commun.*, 215 (1995).
3. R. P. Litvinovskaya, S. V. Drach, and V. A. Khripach, *Zh. Org. Khim.*, **35**, 1653 (1999).
4. R. P. Litvinovskaya, S. V. Drach, and V. A. Khripach, *Zh. Org. Khim.*, **37**, 243 (2001).
5. R. P. Litvinovskaya, S. V. Drach, V. A. Tereshko, and V. A. Khripach, *Zh. Obshch. Khim.*, **66**, 859 (1996).
6. R. P. Litvinovskaya, A. S. Lyakhov, A. A. Govorova, S. V. Drach, and V. A. Khripach, *Bioorg. Khim.*, **23**, 147 (1997).
7. P. Crabbe, *Introduction to the Chiroptical Methods of Chemistry*, Impresos Offsali, G.S. A., Mexico (1971).
8. G. Snatzke, ed., *Optical Rotary Dispersion and Circular Dichroism in Organic Chemistry; Including Applications from Inorganic Chemistry and Biochemistry, Proceedings of NATO Summer School Held at Bonn*, 24 September-1 October 1965, Heyden, London (1967).
9. B. A. Kazanskii, ed., *Reactions and Research Methods of Organic Compounds* [in Russian], Khimiya, Moscow (1967), p. 12.
10. O. V. Sverdlova, *Electronic Spectra in Organic Chemistry* [in Russian], Khimiya, Leningrad (1973), pp. 112-116, 139.
11. R. P. Litvinovskaya, M. A. Aver′kova, A. S. Lyakhov, N. V. Koval′, A. V. Baranovskii, and V. A. Khripach, *Zh. Obshch. Khim.*, **75**, 1345 (2005).
12. R. P. Litvinovskaya, A. S. Lyakhov, S. V. Drach, V. A. Khripach, and A. A. Govorova, *Zh. Obshch. Khim.*, **70**, 1571 (2000).
13. N. I. Garbuz, Yu. A. Sokolov, L. P. Solovei, R. P. Litvinovskaya, S. V. Drach, A. V. Baranovskii, and V. A. Khripach, *Zh. Prikl. Spektrosk.*, **73**, 145 (2006).
14. N. I. Garbuz, G. P. Yankovskaya, Zh. N. Kashkan, O. P. Kananovich, and N. V. Kovganko, *Khim. Prir. Soedin.*, 76 (1992).
15. M. J. S. Dewar, E. G. Zoebisch, and E. F. Haaly, *J. Am. Chem. Soc.*, **107**, 3902 (1985).