Chemistry of Natural Compounds, Vol. 31, No. 1, 1995

TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS XLIX. STRUCTURES OF CYCLOALPIGENIN C AND CYCLOALPIOSIDE C

M. A. Agzamova and M. I. Isaev

UDC 547.918:547.926

The structures of the new cycloartane triterpenoid cycloalpigenin C and its glycoside cycloalpioside C, isolated from the epigeal part of Astragalus alopecurus *Pall. (Leguminosae), have been determined. Cycloalpigenin C is 20R,24S-epoxycycloartane-3β,12β,16β,25-tetraol, and cycloalpioside C is the 3-O-β-D-xylopyranoside of cycloalpigenin C.*

The epigeal organs of the plant *Astragalus alopecurus* Pall. (*Leguminosae*) are distinguished by a rich content of cycloartane triterpenoids of various structures [1-3]. The glycosidic composition of the plant is represented by the 3-O-β-*D*-xylopyranosides of these methylsteroids.

The present paper is devoted to a determination of the structures of substances (**3**) and (**7**) [1] isolated from the herb *Astragalus alopecurus* and called by us cycloalpigenin C (**1**) and cycloalpioside C (**8**), respectively.

The presence in the PMR spectrum of the genin (**1**) (C_5D_5N, Table 1) of two one-proton doublets of an AB system at 0.28 and 0.66 ppm with the SSCC ^2J = 4 Hz, characteristic for a 1,1,2,2-tetrasubstituted three-membered ring, and of the signals of seven methyl groups and also the elementary composition $C_{30}H_{50}O_5$ of the compound under study enabled us to assign the new substance (**1**) to the methylsteroids of the cycloartane series [4, 5]. The IR spectrum of genin (**1**), containing an absorption band at 3040 cm^{-1}, also showed the presence of a three-membered ring in the molecule [6]. The carbon atoms forming the cyclopropane ring, C-9, C-10, and C-19 resonated in the ^{13}C NMR spectrum of cycloalpigenin C at 20.64, 27.36,and 29.56 ppm, respectively (Table 2), which confirmed the conclusion that the compound under consideration was a cycloartane.

The acetylation of cycloalpigenin C with acetic anhydride in pyridine gave the monoacetate (**2**) and the triacetate (**3**).

In the mass spectra of the genin (**1**) and the acetyl derivatives (**2**) and (**3**) the maximum peak was that of an ion with *m/z* 143, formed on cleavage of the C-17—C-20 bond. This ion, with the composition $C_8H_{15}O_2$, arises on the mass-spectrometric fragmentation of compounds having a side-chain with the 20,24-epoxy-25-ol structure. Consequently, cycloalpigenin C has a side-chain similar to the side-chain of cycloalpigenin B (**4**). This was also shown by the ^{13}C NMR spectrum of cycloalpigenin C, which contained the signals of carbon atoms linked to epoxide oxygen at 87.32 and 84.56 ppm and also of a tertiary carbon atom at 71.05 ppm. In agreement with this, in the PMR spectrum of genin (**1**) ($CDCl_3$) a triplet signal of H-24 was observed at 3.88 ppm.

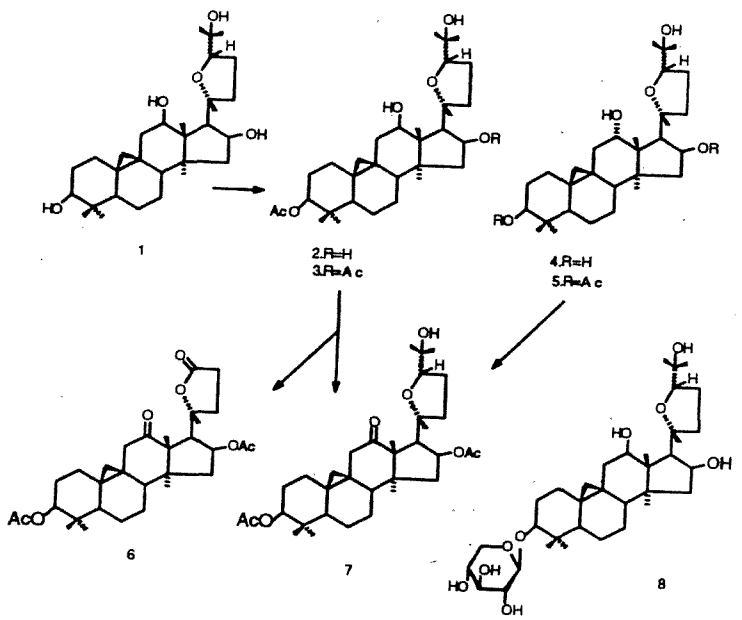
The closeness of the values of the chemical shifts of the C-20—C-25 atoms of cycloalpigenins B and C permitted the assumption that the chiral C-20 and C-24 atoms in the compounds being compared had the same stereochemistry. This means that the side-chain of cycloalpigenin C has the 20R,24S-epoxy-25-ol structure and the other three oxygen atoms are represented by hydroxy groups located in the polycyclic part of the molecule.

The observation in the PMR spectrum of genin (**1**) of the noninteracting signals of three protons geminal to hydroxy groups indicated the secondary nature of these groups. This is in complete agreement with the ^{13}C NMR spectrum of (**1**), in which the signals of three secondary carbinol carbon atoms are clearly traceable at 77.84, 72.10, and 72.27 ppm.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 89 14 75. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 88-95, January-February, 1995. Original article submitted October 25, 1994.

 0009-3130/95/3101-0070$12.50

The one-proton doublet of doublets observed in the PMR spectrum of genin (**1**) ($CDCl_3$) at 3.25 ppm ($^3J_1$ = 11, $^3J_2$ = 4.5 Hz) undergoes downfield shifts in the spectra of the acetates (**2**) and (**3**) and is found at 4.54 and 4.47 ppm, respectively. These indices, in combination with the signal of the carbonyl carbon atom in the ^{13}C NMR spectrum of cycloalpigenin C at 77.84 ppm, showed the presence of a 3β-hydroxy group in the molecule of the compound under discussion [5]. It also followed from this that the acetate (**2**) is the 3-monoacetate of cycloalpigenin C.



The triplet of doublets at 4.46 ppm with SSCCs $^3J_1$ = $^3J_2$ = 8 Hz, $^3J_3$ = 6 Hz observed in the same PMR spectrum of genin (**1**) is characteristic for 16α-H [5]. In the spectrum taken in deuteropyridine, this proton resonated at 4.77 ppm in the form of a 1:3:3:1 quartet with the SSCCs $^3J_1$ = $^3J_2$ = $^3J_3$ = 7 Hz. Thus, another secondary hydroxy group is located at C-16 and has the β-orientation. This conclusion was confirmed by the ^{13}C NMR spectrum of cycloalpigenin C, which contained the signal of the corresponding carbinol carbon atom at 72.27 ppm [1-3].

In the PMR spectrum of the acetate (**3**), in addition to the H-3 signal, the H-16 signal had also undergone a downfield shift. Consequently, product (**3**) was the 3,16-diacetyl derivative of cycloalpigenin C.

The Jones oxidation [7] of the diacetate (**3**) led to the formation of compounds (**6**) and (**7**). In its physicochemical constants and spectral parameters, the keto derivative (**7**) was identical with the analogous product obtained from cycloalpigenin B (**4**) via the diacetate (**5**). This means that cycloalpigenin C also contains a hydroxy group at C-12. Moreover, the formation of the keto derivative (**7**) from cycloalpigenins B and C confirmed the conclusion that cycloalpigenin C contains 3β- and 16β-hydroxy groups and also has the 20R,24S-stereochemistry of the side-chain. Consequently, the only difference in the structures of the compounds being compared consists in the C-12 stereochemistry, i.e., cycloalpigenins B and C are epimers with respect to this chiral center. Since the hydroxy group at C-12 in cycloalpigenin B has the α-configuration, in cycloalpigenin C this group must have the β-orientation.

Thus, cycloalpigenin C is 20R,24S-epoxycycloartane-3β,12β,16β,25-tetraol.

In the mass spectrum of product (**6**) (M^+ 528), there was no peak of an ion with *m/z* 143, and the maximum peak observed was that of an ion with *m/z* 99. This ion is formed on the fragmentation of 25-norcycloartan-20,24-olides [8]. In actual fact, the PMR spectrum of this compound lacked signals from H-12 and H-24, and in the strong field the signals of five methyl groups were observed. These facts determine compound (**6**) as 3β,16β-dihydroxy-12-oxo-20R-25-norcycloartan-20,24-olide 3,16-diacetate.

Attention is attracted by the fact that H-12 has practially identical SSCCs in the two genins (**1**) and (**4**) (9 and 5 Hz in (**1**); 9 and 6 Hz in (**4**)) and also the same axial orientation. Consequently, in both cases the hydroxy groups are oriented equatorially. This is possible if the conformation of ring *C* changes on passing from one genin to the other.

A consideration of Dreiding molecular models has shown that conformational transitions of ring *C* are realized: in genin (**1**) it has the C12 conformation, and in genin (**4**) the B11 conformation (Fig. 1). The closeness of the H-12—2H-11 dihedral angles in the two conpounds, determined from Newman projections, and the good agreement of the SSCCs calculated from

TABLE 1. Chemical Shifts (δ, ppm), Multiplicities, and SSCCs (J, Hz) of the Protons of Cycloalpigenins B (**4**) and C (**1**) and Their Derivatives (0 — TMS)

Compound	Positions of the protons				
	H-3	H-12/2H-11	H-16	H-17	2H-19
1	3.44 dd (11;4.5)	4.09 m*	4.77 q (7; 7; 7)	2.49 d (7)	0.28; 0.66 d (4)
	[3.25 dd (11; 4.5)]	[3.77 dd (9; 5)]	[4.46 td (8; 6)]	[2.14 d (8)]	[0.34; 0.68 d (4.5)]
2	[4.54 dd (11; 4.5)]	[3.78 dd (9; 5)]	[4.47 td (8; 6)]	[2.15 d (8)]	[0.35; 0.71 d (4)]
3	[4.47 m]	[3.76 m]	[5.27 m]		[0.31; 0.65 d (4)]
4	3.45 dd (10.5; 5.5)	4.11 dd (10; 6)	4.89 q (7; 7; 7)	3.15 d (7)	0.35; 0.47 d (4)
	[3.26 dd (11; 4.5)]	[3.86 dd (9; 6)]	[4.56 td (8; 6)]	[2.63 d (8)]	[0.41; 0.52 d (4.4)]
6	4.64 dd (10; 4.5)	–	5.51 td (7; 10)	3.27 d (10)	0.29; 0.59 d (4)
7	4.72 dd (11; 4.5)	1.91; 2.64 d (20)	5.61 q (8; 8; 8)	3.18 d (8)	0.35; 0.66 d (4)

Compound	Positions of the protons		
	H-24	CH_3 groups	OAc
1	4.09 m*	0.88; 1.00; 1.15; 1.36; 1.42; 1.73; 1.84	–
	[3.88 t (7)]	[0.78; 0.82; 0.95; 1.13; 1.22; 1.35; 1.53]	–
2	[3.88 t (7)]	[0.83; 0.83; 0.86; 1.14; 1.22; 1.35; 1.53]	[2.03]
3	[3.85 t (7)]	[0.79; 0.81; 0.81; 1.09; 1.15; 1.31; 1.46]	[1.96; 1.97]
4	3.97 t (7)	1.02; 1.14; 1.23; 1.32; 1.47; 1.50; 1.74	–
	[3.82 t (7)]	[0.79; 0.95; 0.99; 1.14; 1.24; 1.26; 1.44]	–
6	–	0.55; 0.78; 0.82; 1.35; 1.51	1.94; 2.02
7	3.83 d d (9; 6)	0.64; 0.89; 0.93; 1.28; 1.42; 1.58; 1.64	2.06; 2.16

*The spectra were taken in deuteropyridine and deuterochloroform. The indices given in square brackets were obtained in deuterochloroform. The signals marked with asterisks were superposed on one another. The spectra of compounds (**3**) and (**6**) were taken on a Tesla BS 567A instrument with HMDS as internal standard. The signals of the methyl groups had a singlet nature. Abbreviations: d) doublet; t) triplet; dd) doublet of doublets; q) 1:3:3:1 quartet; td) triplet of doublets; m) multiplet.

the Karplus equation and found from a Karplus–Conroy curve [9] with those observed experimentally confirmed the conclusion concerning the conformations of ring *C* in the cycloalpigenin B and C molecules.

The close values of the H-12 and C-12 in the ^1H and ^{13}C NMR spectra of the compounds being compared, (**1**) and (**4**), are also due to conformational features of ring *C*. Consequently, the determination of the stereochemistry of the C-12 chiral center on the basis of the C-12 and H-12 chemical shifts and the H-12 SSCC is difficult. In answering this question, an extremely important role was played by bringing in the *γ-gauche* interaction of the 12α-OH group with H-17 [3]. Because of the descreening influence of the 12α-OH group, in the PMR spectrum of cycloalpigenin B (**4**) H-17 resonates in weaker field at 3.15 ppm (C_5D_5N) and 2.63 ppm ($CDCl_3$). In the PMR spectrum of genin (**1**), having the opposite stereochemistry of C-12, the signal of the proton under consideration was observed at 2.49 ppm (C_5D_5N) and 2.14 ppm ($CDCl_3$).

Analysis of the ^1H and ^{13}C NMR spectra of glycoside (**8**) permitted us to assign it, as well, to the cycloartane series. In confirmation of this, acid hydrolysis of (**8**) gave the genin (**1**), identical with cycloalpigenin C.

In the carbohydrate part of the hydrolysate *D*-xylose was detected by paper chromatography in comparison with authentic samples. GLC [10] showed the presence of one *D*-xylose residue in the cycloalpioside C molecule. This was also shown by the ^1H and ^{13}C NMR spectra of glycoside (**8**), which contained the signals of one *D*-xylose residue. The SSC constants of the protons and the chemical shifts of the carbon atoms of the *D*-xylose residue showed the pyranose form, $^4C_1$ conformation, and β-configuration of the pentose in the cycloalpigenin C molecule.

TABLE 2. Chemical Shifts of the Carbon Atoms of Cycloalpigenins B (**4**) and C (**1**) and of Cycloalpioside C (**8**) (δ, ppm. C_5D_5N, 0 — TMS)

Atom	Compound		
	1	4	8
1	32.14	32.60	31.81
2	31.02	31.28	29.76
3	77.84	77.93	88.22
4	40.95	41.10	41.12
5	46.87	47.74	46.90
6	20.92	21.58	20.90
7	25.68	25.98	25.41
8	45.34	48.96	45.04
9	20.64	19.93	20.23
10	27.36	26.95	26.96
11	37.24	38.80	37.05
12	72.10	72.76	72.01
13	48.68	49.90	48.53
14	52.01	50.84	51.93
15	48.25	46.49	48.16
16	72.27	72.83	72.22
17	60.17	52.29	60.11
18	13.65	21.95	13.56
19	29.56	30.35	29.16
20	87.32	87.51	87.27
21	25.96	26.18	25.90
22	38.98	38.40	38.93
23	26.10	26.92	26.06
24	84.56	83.54	84.53
25	71.05	70.76	71.05
26	26.20*	26.59*	25.68*
27	26.26*	27.45*	26.15*
28	19.98	21.20	19.87
29	27.43	27.40	27.37
30	14.66	14.89	15.19
			β-D-Xyl*p* residue
1			107.36
2			75.41
3			78.43
4			71.14
5			66.96

*Signals assigned ambiguously.

The downfield shift of the C-3 signal of cycloalpioside C in comparison with that of cycloalpigenin C unambiguously determined the position of the *D*-xylose residue at C-3.

Thus, cycloalpioside C is 20R,24S-epoxycycloartane-3β,12β,16β,25-tetraol 3-O-β-D-xylopyranoside.

EXPERIMENTAL

For general observations, see [11]. The following solvents were used: 1) benzene—ethyl acetate (3:1); 2) chloroform—methanol (15:1); 3) *n*-butyl alcohol—pyridine—water (6:4:3).

^1H and ^{13}C NMR spectra were taken on Bruker AM 400 and AC 200 instruments in deuteropyridine or deuterochloroform (δ, ppm, 0 — TMS). ^{13}C NMR spectra were also recorded under the conditions of J-modulation. The PMR spectra of compounds (**3**) and (**6**) were obtained on a Tesla BS 567A instrument with HMDS as internal standard.

Cycloalpigenin C (1) — substance (**3**) in [1], $C_{30}H_{50}O_5$, mp, 242-244° (from MeOH), $[\alpha]_D^{21} -34.5 \pm 2°$ (c 0.58; MeOH). IR spectrum (KBr, ν, cm^{-1}): 3550-3300 (OH), 3040 (CH_2 of a cyclopropane ring). Mass spectrum, m/z (%): M^+ 490(1.3), 475(1.7), 472(5.9), 457(4.2), 454(3.4), 439(7.1), 429(3.8), 421(3.8), 413(4.6), 396(10.5), 395(10.1), 377(5.5), 353 (3.8), 313(4.2), 245(11.8), 143(100), 125(18.9). For the ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

Cycloalpioside C (8) — substance (**7**) in [1], $C_{35}H_{58}O_9$, mp. 223-225° (from MeOH), $[\alpha]_D^{26} + 25.7 \pm 2°$ (c 0.71; C_5H_5N). IR spectrum (KBr, ν, cm^{-1}): 3600-3280 (OH), 3040 (CH_2 of a cyclopropane ring). PMR spectrum (C_5D_5N, 0-TMC): 0.24 and 0.60 (2H-19, d, ^2J = 4 Hz), 0.87; 0.97; 1.27; 1.38; 1.44; 1.73; 1.85 (7 × CH_3, s), 2.50 (H-17, d, ^3J = 8 Hz), 3.41

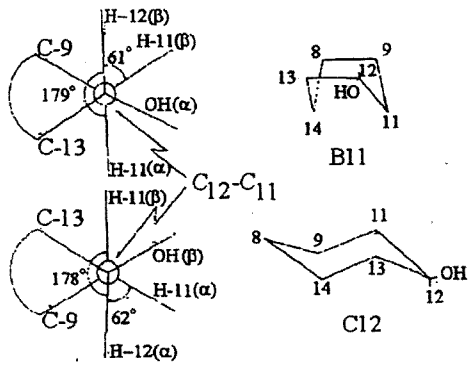


Fig. 1. Conformations of ring *C* in the molecules of cycloalpigenins B (**4**) and C (**1**) and the corresponding Newman projections along the C_{12}—C_{11} bond.

(H-3, dd, $^3J_1$ = 11.5, $^3J_2$ = 4.5 Hz), 3.70 (H-5a of D-xylose, dd, $^3J_1$ = 11, $^3J_2$ = 9 Hz), 3.98 (H-2 of D-xylose, t, ^3J = 7.5 Hz), 4.06 (H-12, dd, $^3J_1$ = 9, $^3J_2$ = 4.5 Hz), 4.11 (H-3 of D-xylose and H-24, t), 4.18 (H-4 of D-xylose, td, $^3J_1$ = $^3J_2$ = 9, $^3J_3$ = 5, Hz), 4.32 (H-5e D of D-xylose, dd, $^3J_1$ = 11, $^3J_2$ = 5 Hz), 4.79 (H-16, m), 4.80 (H-1, ^3J = 7.5 Hz). For the ^{13}C NMR spectrum, see Table 2.

The 3-Monoacetate (2) and 3,16-Diacetate of Cycloalpigenin C (3) from (1). Cycloalpigenin C (75 mg) was acetylated with 0.3 ml of acetic anhydride in 0.7 ml of pyridine at room temperature for 24 h. Then the solvent was evaporated off and the reaction products were chromatographed on a column, with elution by system 1. This led to the isolation of 55 mg of the amorphous diacetate (**3**), $C_{34}H_{54}O_7$, $[\alpha]_D^{26}$ + 43.8 ± 2° (c 1.05; MeOH). IR spectrum (KBr, ν, cm^{-1}): 3470(OH), 3060 (CH_2 of a cyclopropane ring), 1745, 1250 (ester groups). Mass spectrum, m/z (%): M^+ 574(2.7), 559(0.8), 556(0.7), 538(0.2), 514(22.5), 496(2.5), 481(3.0), 471(5.2), 455(2.3), 439(6.3), 438(8.2), 437(6.8), 421(3.0), 395(3.8), 378(7.9), 374(7.0), 367(2.9), 314(6.1), 287(6.6), 161(7.0), 143(100), 125(15.0). For the PMR spectrum, see Table 1.

On continuing to wash the column with the same system, we obtained 10 mg of the monoacetate (**2**), $C_{32}H_{52}O_6$, mp 304-306° (from MeOH), $[\alpha]_D^{26}$ + 31.4 ± 2° (c 0.47; $CHCl_3$–MeOH, 1:1). IR spectrum (KBr, ν, cm^{-1}): 3500 (OH), 3040 (CH_2 of a cyclopropane ring), 1740, 1250 (ester group). Mass spectrum, m/z (%): M^+ 532(0.9), 517(1.2), 514(1.3), 499(1.9), 496(1.4), 481(3.4), 472(8.6), 455(3.9), 437(6.4), 429(4.3), 421(5.7), 411(3.6), 395(9.3), 377(9.3), 314(6.4), 295(5.7), 287(11.4), 269(6.4), 251(7.1), 143(100), 125(40.0). For the PMR spectrum, see Table 1.

3β, 16β-dihydroxy-12-oxo-20R-25-norcycloartan-20, 24-olide 3, 16-diacetate (6) and 3β,16β,25-Trihydroxy-20R,24S-epoxycycloartan-12-one 3,16-diacetate (7) from (3). A solution of 38 mg of the diacetate (**3**) in 20 ml acetone cooled to −13°C was treated with 0.05 ml of the Jones reagent [7], and the mixture was stirred at the same temperature for 1 h. The reaction was stopped by the addition of 2 ml of methanol. After the usual working up, the reaction products were chromatographed on a column, with elution by system 1. This gave 12 mg of product (**7**), $C_{34}H_{52}O_7$ mp 94-95°C (from MeOH), $[\alpha]_D^{26}$ +45 ± 2° (*c* 0.6; MeOH). This product coincided with the analogous keto derivative of cycloalpigenin C obtained via the diacetate (**5**) [3], the indices of their ^1H and ^{13}C NMR spectra also agreeing.

Further elution of the column with the same system yielded 10 mg of the nor- product (**6**), $C_{31}H_{46}O_7$, mp. 237-240° (from MeOH), $[\alpha]_D^{18}$ + 50 ± 2° (c 0.24; MeOH). IR spectrum (KBr, ν, cm^{-1}) : 3040 (CH_2 of a cyclopropane ring), 1773 (γ-lactone C=O), 1734, 1246 (ester groups), 1707 (C=O at C-12). Mass spectrum, *m/z* (%): M^+ 528 (8), 468 (24), 425 (48), 408 (20), 394 (28), 205 (32), 99 (100). For the PMR spectrum, see Table 1.

Cycloalpigenin C from (8). Cycloalpioside C (70 mg) was hydrolyzed with 10 ml of a 0.5% methanolic solution of sulfuric acid at 50°C for 4 h. After the usual working up and column chromatography in system 2, the genin fraction of the products yielded 32 mg of cycloalpigenin C, identified by traditional methods.

In the carbohydrate fraction of the hydrolyzate, after appropriate working up, *D*-xylose was detected by PC in system 3 in the presence of authentic samples.

GLC analysis [10] of glycoside (**8**) showed the presence of one *D*-xylose residue.

REFERENCES

1. M. A. Agzamova and M. I. Isaev, Khim. Prir. Soedin., 377 (1991).
2. M. A. Agzamova and M. I. Isaev, Khim. Prir. Soedin., 379 (1994).
3. M. A. Agzamova and M. I. Isaev, Khim. Prir. Soedin., 515 (1994).
4. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 431 (1985).
5. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 156 (1989).
6. K. Nakanishi, Infrared Spectra. Practical, Holden-Day, San Francisco (1962) [Russian translation, Mir, Moscow (1965), p. 25].
7. C. Djerassi, R. R. Engle, and A. Bowers, J. Org. Chem., **21**, 1547 (1956).
8. M. I. Isaev, M. B. Gorovits, N. D. Abdullaev, M. R. Yagudaev, and N. K. Abubakirov, Khim. Prir. Soedin., 572 (1981).
9. H. Günther, NMR Spectroscopy: An Introduction, Wiley, New York (1980) [Russian translation from the German, Mir, Moscow (1984), p. 122].
10. M. A. Agzamova, M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 719 (1986).
11. M. A. Agzamova, M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 455 (1986).