3. V. V. Chupin, E. V. Malina, I. A. Vasilenko, G. A. Serebrennikova, and R. P. Evstigneeva, Zh. Org. Khim., 16, 31 (1980).
4. É. E. Nifant'ev and D. A. Pridvoditelev, Bioorg. Khim., 7, 1285 (1981).
5. R. A. Klein, Biochem. Biophys. Acta, 210, 486 (1970).
6. H. Brockerhoff and M. Yurkowski, J. Lipid. Res., 7, 62 (1966).
7. H. J. Lucas, F. W. Mitchell, and C. N. Scully, J. Am. Chem. Soc., 72, 5491 (1950).
8. R. S. Edmundson, Chem. Ind. (London), No. 20, 1828 (1962).

# STRUCTURES OF DAUROSIDES A AND B — NEW ACYLATED COUMARIN GLYCOSIDES FROM *Haplophyllum dauricum*

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From the roots and epigeal parts of *Haplophyllum dauricum* (L.) G. Don., new acylated courmarin glycosides have been isolated: dauroside A, $C_{23}H_{28}O_{13}$, mp 145-147°C (ethanol), $[\alpha]_D^{22}$ — 72.7° (methanol), and dauroside B, $C_{30}H_{32}O_{14}$, mp 145-150°C (methanol) $[\alpha]_D^{22}$ 0° (pyridine). Their structures have been established on the basis of chemical transformations and IR, UV, mass, $^1H$ NMR and $^{13}C$ NMR spectra.

We have previously [1] reported the isolation from the epigeal part of *Haplophyllum dauricum* (L.) G. Don of umbelliferone and umbelliferone 7-O-β-D-glucopyranoside. Continuing this investigation, from the butanolic fraction of the ethanolic extract [2] we have isolated a new coumarin glycoside, dauroside A (I). Its UV spectrum is very close to that of umbelliferone 7-O-β-glucoside. The PMR spectrum of (I) (DMSO-$d_6$) contains the signals of protons due to the presence in the molecule of residues of unbelliferone, β-glucose, and α-rhamnose (see Table 1). In actual fact, the acid hydrolysis of (I) led to umbelliferone, D-glucose, and L-rhamnose. The presence of a three-proton singlet at 2.01 ppm ($CH_3CO$) and of the signal of a gem-acyl proton at 4.77 pmm in the PMR spectrum, and also of a $\nu_{C=O}$ absorption band of an ester grouping (1723 $cm^{-1}$) in the IR spectrum showed that (I) was an acylated coumarin glucoside. This was confirmed by the formation of acetic acid and of the deacetyl product (II) with mp 186-187°C on mild alkaline hydrolysis of (I) with 0.5% KOH solution at room temperature. The dauroside (I) formed a hexaacetyl derivative with the composition $C_{33}H_{38}O_{18}$ (III), $M^+$ 722. The PMR spectrum of (III) ($CDCl_3$) showed the signals of the protons of the methyls of six aliphatic acetyl groups at (ppm) 1.90 (3 H), 1.95 (6 H), and 1.98 (9 H). Consequently, (I) is an acylated bioside of umbelliferone. The mass spectrum of (III) contains, in addition to the peak of the molecular ion with m/z 722, strong peaks of ions with m/z 273 (100%), 213, 184, 153, 111, and others, corresponding to fragments of the (M — $CH_3COOH$) ion of tetraacetylrhamnopyranose [3]. The strong peak of an ion with m/z 560 is formed by the splitting out of umbelliferone from the molecular ion. Consequently, in the dauroside A molecule rhamnose is the terminal sugar.

In the PMR spectrum of (III) the ratio of the intensities of the signals of the protons in the 4.5-5.6 and 3.4-4.4 ppm intervals is 8:4, and the signal of the anomeric proton of the rhamnose residue appears at δ 4.62 ppm. These facts permit (I) to be characterized as an acylated rutinoside [4]. The position of attachment of the acetyl group and the definitive structure of dauroside A were established by a study of its $^{13}C$ NMR spectrum (Table 2). The signals in the $^{13}C$ NMR spectrum were identified on the basis of an

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TABLE 1. Details of the $^1$H HMR Spectra of Coumarin Glycosides in DMSO-$d_6$ (O — TMS, δ scale)

| Protons | Dauroside A | Dauroside B |
|---|---|---|
| H-3 | 6,38, d, 10 Hz | 6,36 d, 10 Hz |
| H-4 | 8,06, d, 10 Hz | 8,03, d, 10 Hz |
| H-5 | 7,70, d, 9 Hz | 7,67, d, 8,2 Hz |
| H-6 | 7,06, d, br., 9 Hz | 6,96, q, 8,2 & 2,5 Hz |
| H-8 | 7,08, br.s. | 7,03, br.s |
| H-1′ | 5,09, d, 7 Hz | 5,44, d, 9 Hz |
| H-2′ | | 4,98, t, 9 Hz |
| H-1″ | 4,60, br.s. | 4,57, br.s |
| H-4″ | 4,77, T, 9 Hz | |
| H-α | — | 6,42, d, 16 Hz |
| H-β | — | 7,64, d, 16 Hz |
| H-2‴, 6‴ | — | 6,81, d, 8 Hz |
| H-3‴, 5‴ | — | 7,58, d, 8 Hz |
| $CH_3CO$ | 2,01, s | — |
| $CH_3$— | 0,92, d, 6 Hz | 1,08, d, 6,5 Hz |
| Other protons of the sugar moiety | 3,10—3,92 (9H, m) | 3,10—3,98 (9H, m) |
| OH groups | 4,82; 5,01 5,26; 5,28 5,50 | 4,53; 4,67 4,76; 5,53 |

TABLE 2. Details of the $^{13}$C NMR Spectra (DMSO-$d_6$, O — TMS)

| Carbon atom | Umbelliferone 7-O-β-D-glucopyranoside [5] | Linarin monoacetate (carbohydrate moiety) [7] | Dauroside A | Linarin (carbohydrate moiety) [7] | Dauroside B | Multiplicity |
|---|---|---|---|---|---|---|
| C-2 | 160,3 | | 160,0 | | 159,3(a)* | *s* |
| C-3 | 113,2 | | 113,1(a) | | 113,3(b) | *d* |
| C-4 | 144,2 | | 144,2 | | 144,1(d) | *d* |
| C-5 | 129,5 | | 129,3 | | 129,5(c) | *d* |
| C-6 | 113,8 | | 113,4(a) | | 113,8(b) | *d* |
| C-7 | 160,3 | | 160,3 | | 160,2(a) | *s* |
| C-8 | 103,4 | | 103,3 | | 103,3 | *d* |
| C-9 | 155,1 | | 154,8 | | 154,8 | *s* |
| C-10 | 113,4 | | 113,3 | | 113,4 | *s* |
| C-1′ | 100,3 | 99,7 | 99,8 | 99,9 | 97,6 | *d* |
| C-2′ | 73,3 | 73,0 | 73,0 | 73,0 | 73,9 | *d* |
| C-3′ | 77,2 | 76,2 | 76,3 | 76,3 | 73,0 | *d* |
| C-4′ | 69,9 | 69,4 | 69,5 | 69,6 | 69,8 | *d* |
| C-5′ | 76,6 | 75,3 | 75,2 | 75,6 | 75,6 | *d* |
| C-6′ | 60,9 | 65,7 | 65,7 | 66,1 | 65,8 | *t* |
| C-1″ | | 100,0 | 100,1 | 100,4 | 100,4 | *d* |
| C-2″ | | 70,2 | 70,2 | 70,2 | 70,3 | *d* |
| C-3″ | | 68,1 | 68,0 | 70,7 | 70,6 | *d* |
| C-4″ | | 73,9 | 73,7 | 72,0 | 71,8 | *d* |
| C-5″ | | 65,7 | 65,9 | 68,2 | 68,3 | *d* |
| C-6″ | | 17,2 | 17,2 | 17,7 | 17,7 | *q* |
| $CH_3CO$ | | 20,8 | 20,8 | | | *q* |
| MeCO | | 169,8 | 169,8 | | | *s* |
| C-1‴ | | | | | 124,9 | *s* |
| C-2‴, 6‴ | | | | | 130,3(c) | *d* |
| C-3‴, 5‴ | | | | | 115,7 | *d* |
| C-4‴ | | | | | 159,8(a) | *s* |
| C-7‴ | | | | | 145,2(d) | *d* |
| C-8‴ | | | | | 113,6(b) | *d* |
| C-9‴ | | | | | 165,6 | *s* |

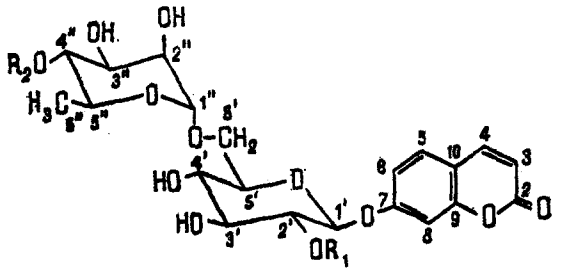
*The assignments in the vertical columns with the same pairs of letters may be interchanged.

analysis of the spectra obtained under the conditions of complete and partial (off-resonance) decoupling from protons, and also by comparing them with the chemical shifts of the carbon atoms of unbelliferone 7-O-β-glucopyranoside [5], of acacetin 7-O-rutinoside (linarin), and of linarin monoacetate [6, 7]. As can be seen from Table 2, the values of the chemical shifts of the carbons of the sugar moiety of linarin monoacetate and of (I) have very close values, which indicates the complete identity of the sequence of attachment of the sugars and of the position of the acetyl group. Thus, dauroside A had the structure of 7-[6′-(4″-O-acetyl-α-L-rhamnopyranosyloxy)-β-D-glucopyranosyloxy]coumarin (I).

It must be mentioned that the carbohydrate moeity of dauroside A proved to be identical with that of the flavonoid acetylpectolinarin isolated from certain species of *Linaria* [8].

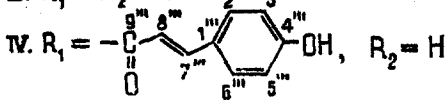
The chromatographic separation of an ethyl acetate fraction of the ethanolic extract of the roots yielded dauroside B (IV), with mp 148-150°C (methanol). The acid hydrolysis of (IV) formed umbelliferone, D-glucose, L-rhamnose, and p-coumaric (p-hydroxycinnamic) acid. Its IR and PMR spectra (Table 1) showed that (IV) was also a cylated coumarin glycoside. The mild alkaline hydrolysis of (IV) formed p-coumaric acid and a glycoside (umbelliferone 7-O-rutinoside), which proved to be identical with the compound (II). The formation of the latter shows that dauroside B is also an acylated derivative of umbelliferone 7-O-rutinoside (II). On acetylation with acetic anhydride in pyridine, (IV) gave a hexaacetyl derivative with the composition $C_{40}H_{42}O_{19}$ (V), the PMR spectrum of which showed the signals of the protons of the methyls of one aromatic acetyl group (2.22 ppm, 3 H, s) and of five aliphatic acetyl groups (1.95, 6 H, s; 1.99, 6 H, s; and 2.02 ppm, 3 H, s). The presence in the mass spectrum of (V) of intense peaks of ions with m/z 273 (100%), 213, 184, 153, and 111 excludes the attachment of the p-coumaric acid to the rhamnose residue. The site of attachment of the p-coumaric acid was established by a comparative study of the $^{13}C$ NMR spectra of dauroside B, umbelliferone 7-O-β-glucopyranoside [5], and linarin [6, 7].

A comparison of the chemical shifts in the $^{13}C$ spectra of (IV) and of linarin showed that they differed mainly by the chemical shifts of the carbon atoms of the glucose residue. It has been established [9-12] that the acylation of a hydroxy group of a sugar leads to a downfield shift of the signal of the carbon atom to which it is attached, while the neighboring carbon atoms experience upfield shifts. It can be seen from the figures in the table that the C-1' and C-3' signals in the spectrum of (IV) are shifted upfield by 2.3 and 3.3 ppm, respectively, while the C-2' signal is shifted downfield by 2.9 ppm. This shows that the hydroxyl at C-2' in the molecule of (IV) is acylated by p-coumaric acid. The signals of the carbon atoms of the p-coumaric acid were identified by comparison of the spectrum (IV) with that of tiliroside [13]. Thus, dauroside B has the structure of 7-[6'-O-(α-L-rhamnopyranosyl)-2'-O-(p-coumaroyl)-β-D-glucopyranosyloxy]coumarin (IV).



I. $R_1 = H$, $R_2 = COCH_3$

II. $R_1 = R_2 = H$

IV. $R_1 =$  , $R_2 = H$

It must be mentioned that in the spectra of (I) and (IV) the protons of the hydroxy groups appear as individual signals in the 4.53-5.53 ppm interval (see Table 1). This is due to the fact that, in DMSO, proton exchange between the individual OH groups is stabilized by hydrogen bonds with the solvent and therefore it is possible to detect nonequivalent OH groups [14]. When the spectra were recorded in Py-$d_5$ solutions, the signals of the OH groups fused.

## EXPERIMENTAL

General Observations. Thin-layer chromatography (TLC) was performed on Silufol plates in the solvent system 1) chloroform–methanol (71:16) and 2) chloroform–methanol (9:1), and paper chromatography (PC) on FN-11 paper in the butan-1-ol–pyridine–water (6:4:3) system. For column chromatography we used silica gel L 100/160 μ (Chemapol). UV spectra were taken on a Hitachi spectrophotometer, and IR spectra on a UR-20 instrument in tablets with KBr. The PMR and $^{13}C$ NMR spectra were obtained on a Varian XL-200 spectrometer in DMSO-$d_6$

(substances (I) and (IV)), O — TMS, and a JNM-4H-100 instrument in $CDCl_3$ ((II) and (IV)) and in Py-$d_5$ (I) with HMDS as internal standard. Mass spectra were recorded on a MKh-1310 instrument at an ionizing voltage of 50 V.

Isolation of Dauroside A. The butanolic fraction of the ethanolic extract of the epigeal part [2] in an amount of 124 g was chromatographed on a column of silica gel (1900 g). The substances were eluted with chloroform and with mixtures of chloroform and methanol. The chloroform eluates yielded umbelliferone [1]. At a composition of the mixture of 97:3, 0.589 g of dauroside A was isolated. The mixture with a ratio of 93:7 eluted 0.66 g of umbelliferone 7-O-β-glucopyranoside [1]. Both these substances were recrystallized from ethanol.

Dauroside A (I), $C_{23}H_{28}O_{13}$, mp 145-147°C (ethanol), $R_f$ 0.58 (system 1), $[\alpha]_D^{22}$ — 72.7 ± 2° (c 1.1; methanol), $\lambda_{max}$, $cm^{-1}$ (ethanol), nm: 215, 241 sh, 251, 297 sh, 321 (log ε 4.18, 3.65, 3.53, 3.94, 4.08); $\nu_{max}^{KBr}$ ($cm^{-1}$): 3300-3540 (OH), 1723 (ester C=O), 1710 (α-pyrone C=O), 1626, 1615, 1560 (C=C). PMR spectrum in Py-$d_5$ (ppm, δ scale): 1.13 (d, 6 Hz, —$CH_3$); 1.90 (s, —$COCH_3$); 3.80-4.58 (m, 9 H, carbohydrate moiety); 4.78-4.98, (m, OH groups); 5.21 (br.s, H-1"); 5.47 (d, 7 Hz, H-1'); 5.56 (t, 10 Hz, h-4"); 6.05 (d, 9.5 Hz, H-3); 6.97 (dd, 8.5 and 2 Hz, H-6); 7.14 (br.s, H-8); 7.23 (d, 8.5 Hz, H-5); 7.43 (d, 9.5 Hz, H-4).

Isolation of Dauroside B. The dried and comminuted roots (8 kg) of the plant *Haplophyllum dauricum* gathered in August, 1980 (Mongolian Peoples' Republic, Uvur-Khangaiskii aimak) were extracted eight times with methanol and twice with chloroform at room temperature. The concentrated ethanolic extract was diluted with water (1:1) and was shaken out successively with chloroform and ethyl acetate. The solvents were distilled off to give 450 g of chloroform fraction and 32 g of ethyl acetate fraction. The ethyl acetate fraction (30 g) was separated by column chromatography. The substances were eluted by hexane—chloroform, chloroform, and chloroform—methanol in various proportions. This gave justicidin B and daurinol [2] and, at a composition of the mixed solvent chloroform—methanol of 9:1, 0.503 g of dauroside B.

Dauroside B (IV), $C_{30}H_{32}O_{14}$, mp 148-150°C (from methanol), $R_f$ 0.50 (system 1), $[\alpha]_D^{22}$ 0 ± 3° (c 1.1; pyridine); $\lambda_{max}$ (ethanol), nm: 229 sh, 294 sh, 318 (log ε 4.19, 4.34, 4.45); $\nu_{max}^{KBr}$ ($cm^{-1}$): 3280-3540 (OH), 1706 (α-pyrone C=O), 1693 (ester C=O), 1608, 1590, 1518 (C=C), 1000-1100 (C—O of a glycoside).

Acid Hydrolysis of (I). A solution of 20 mg of (I) in 5 ml of 5% $H_2SO_4$ solution was heated on the water bath for 3 h. The precipitate of aglycone that deposited after cooling was filtered off and recrystallized from ethanol, and it was identified as umbelliferone (TLC, mixed melting point). D-glucose and L-rhamnose were identified by PC and GLC in the neutralized and evaporated filtrate.

Alkaline Hydrolysis of (I). A solution of 25 mg of (I) in 6 ml of 0.5% KOH was kept at room temperature for 30 min. Then it was neutralized with 5% HCl and evaporated at room temperature to 0.5 ml. Acetic acid was detected from characteristic odor and by PC with a marker.

The solution was then evaporated to dryness, and the residue was dissolved in methanol and chromatographed on a column of Sephadex LH-20. This gave a deacetyl product with mp 186-189°C (from methanol), $\nu_{max}^{KBr}$ ($cm^{-1}$); 3217-3510 (OH), 1712 (α-pyrone C=O), 1616, 1568, 1517 (C=C), 1040-1113 (C—O of glycosides), 841.

Acetylation of (I). A solution of 35 mg of (I) in 0.5 ml of pyridine was treated with 1.5 ml of acetic anhydride and the mixture was left at room temperature. After 24 h the acetyl derivative was isolated in the usual way: $C_{33}H_{38}O_{18}$, mp 115-116°C. Mass spectrum, m/z (%): $M^+$ 722 (0.1), 561 (3), 560 (11), 479 (11), 331 (3), 317 (11), 274 (37), 273 (100), 271 (3), 257 (4), 229 (7), 215 (5), 213 (13), 197 (4), 185 (4), 184 (4), 171 (13), 169 (13), 163 (12), 162 (9), 155 (13), 145 (6), 129 (8), 128 (13), 112 (6), 111 (13), 109 (12).

PMR spectrum ($CDCl_3$), ppm: 1.10 (3 H, d, 6 Hz, —$CH_3$); 1.90 (3 H, s, —$COCH_3$); 1.95 (6 H, s, 2 $COCH_3$); 1.98 (9 H, s, 3 $COCH_3$); 3.47-3.95 (4 H, m, H-5', 6', 5"); 4.62 (1 H, br.s,

H-1"); 4.80-5.30 (7 H, m, H-1', 2', 3', 4', 2", 3", 4"); 6.16 (1 H, d, 9.5 Hz, H-3); 6.78 (2 H, m, H-6,8); 7.35 (1 H, d, 9 Hz, H-5); 7.51 (1 H, d, 9.5 Hz, H-4).

Acid Hydrolysis of (IV). A mixture of 23 mg (IV) and 5 ml of 5% $H_2SO_4$ solution was heated on the water bath for 3.5 h. After cooling, the deposit of aglycone was filtered off, and this was identified by TLC and a mixed melting point was umbelliferone. The filtrate was evaporated on dryness and glucose and rhamnose were identified in the residue by PC and GLC, while p-coumaric acid was identified by TLC in system 2 and by mass spectrometry ($M^+$ 164).

Alkaline Hydrolysis of (IV). A solution 28 mg of (IV) in 6 ml of 0.5% KOH was kept at room temperature for 30 min. Then it was neutralized with 3% HCl and evaporated to dryness at room temperature. The residue was dissolved in methanol and chromatographed on a column of Sephadex LH-20. p-Coumaric acid with mp 206-209°C and a glycoside identical with (III) (TLC, mixed melting point, and IR spectrum) were isolated.

Acetylation of (IV). Compound (IV) (40 mg) was acetylated with acetic anhydride (2 ml) in the presence of pyridine (0.6 ml). An acetyl derivative $C_{40}H_{42}O_{19}$, mp 86-87°C, was obtained. Mass spectrum, m/z (%): 709 (26), 708 (M − 162, 68), 667 (11), 666 (30), 664 (13), 626 (9), 477 (7), 464 (4), 463 (83), 435 (7), 421 (22), 331 (21), 273 (100), 259 (75), 213 (87), 190 (11), 189 (82), 184 (8), 171 (75), 169 (10), 163 (7), 153 (7), 148 (7), 147 (58), 146 (36), 139 (55), 128 (7), 127 (9), 111 (13), 109 (11).

PMR spectrum in $CDCl_3$, ppm: 1.12 (3 H, d, 6 Hz, $CH_3$); 1.95 (6 H, s, 2 $COCH_3$); 1.99 (6 H, s, 2 $COCH_3$); 2.02 (3 H, s, $-COCH_3$), 2.22 (3 H, s, Ar—$OCOCH_3$); 3.45-4.00 (4 H, m, H-5', 6, 5"); 4.64 (1 H, br.s, H-1"); 4.85-5.48 (7 H, H-1', 2', 3', 4', 2", 3", 4"); 6.14 (1 H, d, 10 Hz, H-3); 6.22 (1 H, d, 16 Hz, $H_\alpha$); 6.80 (1 H, q, 8, and 2.5 Hz, H-6); 6.83 (1 H, d, 2.5 Hz, H-8); 7.01 (2 H, d, 8 Hz, H-2"', 6"'); 7.34 (1 H, d, 8 Hz, H-5); 7.42 (2 H, d, 8 Hz, H-3"', 5"'); 7.50 (1 H, d, 10 Hz, H-4); 7.59 (1 H, d, 16 Hz, $H_\beta$).

## SUMMARY

Two new coumarin glycosides have been isolated from the epigeal part and roots of *Haplophyllum dauricum* (L.) G. Don. — daurosides A and B. By the performance of certain chemical transformations and on the basis of the results of a study of IR, UV, mass, $^1H$ HMR, and $^{13}C$ NMR spectra it has been established that dauroside A has the structure of 7-[6'-O-(4"-O-acetyl-α-L-rhamnopyranosyl)-β-D-glucopyranosyloxy]coumarin and dauroside B that of 7-[6'-O-(α-L-rhamnopyranosyl)-2'-O-(p-coumaroyl)-β-D-glucopyranosyloxy]coumarin.

## LITERATURE CITED

1. D. Batsurén, É. Kh. Batirov, and V. M. Malikov, Khim. Prir. Soedin., 659 (1981).
2. D. Batsurén, É. Kh. Batirov, V. M. Malikov, V. N. Zemlyanskii, and M. R. Yagudaev, Khim. Prir. Soedin., 295 (1981).
3. H. Budzikiewicz, C. Djerassi, and D. H. Williams, Structure Elucidation of Natural Products by Mass Spectrometry, Holden-Day, San Francisco, Vol. II (1964), p. 204.
4. H. Rosler, T. J. Mabry, M. F. Cranmer, and K. Kagan, J. Org. Chem., 30, 4346 (1965).
5. N. J. Cussans and T. N. Huckerby, Tetrahedron, 31, 2719 (1975).
6. V. M. Chari, M. Jordan, H. Wagner, and P. W. Thies, Phytochemistry, 16, 1110 (1977).
7. H. Ina and H. Iida, Phytochemistry, 20, 1176 (1981).
8. G. G. Zapesochnaya, V. I. Sheichenko, and A. I. Ban'kovskii, Khim. Prir. Soedin., 313 (1974).
9. M. R. Vignon and Ph. J. A. Vottero, Tetrahedron Lett., 2445 (1976).
10. Y. Terui, K. Tori, and N. Tsuji, Tetrahedron Lett., 621 (1976).
11. K. Yamasaki, R. Kasai, Y. Masaki, M. Okihara, O. Tanaka, H. Oshio, S. Takagi, M. Yamaki, K. Masuda, G. Nonaka, M. Tsuboi, and I. Nishioka, Tetrahedron Lett., 1231 (1977).
12. K. R. Markham, B. Ternai, R. Stanley, H. Geiger, and T. J. Mabry, Tetrahedron, 34, 1389 (1978).
13. M. Kuroyanagi, M. Fukuoka, K. Yoshohira, S. Natori, and K. Yamasaki, Chem. Pharm. Bull., 26, 3594 (1978).
14. N. M. Sergeev, NMR Spectroscopy [in Russian], Moscow (1981), p. 190.