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TRITERPENE GLYCOSIDES OF *Hedera helix*
II. DETERMINATION OF THE STRUCTURE OF GLYCOSIDE L-6d FROM COMMON IVY LEAVES

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A new hederagenin pentaoside — glycoside L-6d — has been isolated from the leaves of common ivy Hedera helix *L., fam. Araliaceae, and its structure has been determined by using various NMR-spectroscopic methods. Glycoside L-6d is hederagenin 3-O-[O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.*

The isolation and preparative purification of glycoside L-6d have been described previously [1]. The complete acid hydrolysis of L-6d enabled hederagenin to be identified as its aglycon and showed the presence of the sugars rhamnose, arabinose, and glucose. Attempted alkaline hydrolysis of the glycoside caused no changes whatever, while treatment with a solution of diazomethane led to its methyl ester. Consequently, in glycoside L-6d the carbohydrate chain was attached to the hydroxy group at the C_3 atom of the aglycon.

The structure of the carbohydrate chain of the glycoside was established with the aid of ^1H NMR spectroscopy of the methyl esters of the glycoside itself and of its full acetate. Each of the PMR spectra showed five signals (doublets) of anomeric protons and the characteristic signals of H-3, H-12, and H-23 and of the six quaternary methyl groups of the aglycon. Thus, glycoside L-6d was a hederagenin pentaoside.

The PMR spectrum of the full acetate of L-6d (Fig. 1 and the Experimental part) was interpreted with the use of one-dimensional variants of HOHAHA experiments on the observation of coherent polarization transfer in a rotating frame (RF) [2] and selective homonuclear double resonance in the difference variant [3]. HOHAHA experiments with excitation of the anomeric protons having δ 4.52, 4.62, and 4.41 ppm (Fig. 1, *a-c*), and of the proton with δ 4.05 ppm (Fig. 1, *d*), assigned to one of the rhamnose residues on the basis of its characteristic splitting, revealed the signals of the skeletal protons corresponding to each monosaccharide. It followed from the characteristic splittings of these signals that the monosaccharide residues were two β-glucopyranoses, an α-arabinopyranose, and a rhamnopyranose. Signals of the fifth monosaccharide residue (a second rhamnopyranose) were found in a series of successive homonuclear double resonance experiments, beginning with the H-6 proton, the doublet signal of which was readily identified in the strong field.

Analysis of the positions of the signals of the skeletal protons of each of the monosaccharide residues in the PMR spectrum of the full acetate of L-6d permitted the types of substitution in them to be determined. It is known that the introduction of an O-acetyl group always causes downfield shifts of the signals of the corresponding skeletal protons and that a relatively strong-field position of the signals of the protons being determined (apart from the H-5 signals, which are always present in a strong field) indicates the presence of a glycosidic bond. Thus, the signals of the skeletal protons H-2 of arabinose, H-4 of one glucose and H-6 of the other, and H-4 of one of the rhamnoses had strong-field positions. Not one of the signals of the skeletal protons of the other rhamnose had a relatively high-field position: consequently, all its hydroxy groups were acetylated and one was terminal. Thus, the carbohydrate moiety of the glycoside consisted of a linear (unbranched) pentasaccharide with a rhamnopyranose residue at the nonreducing end.

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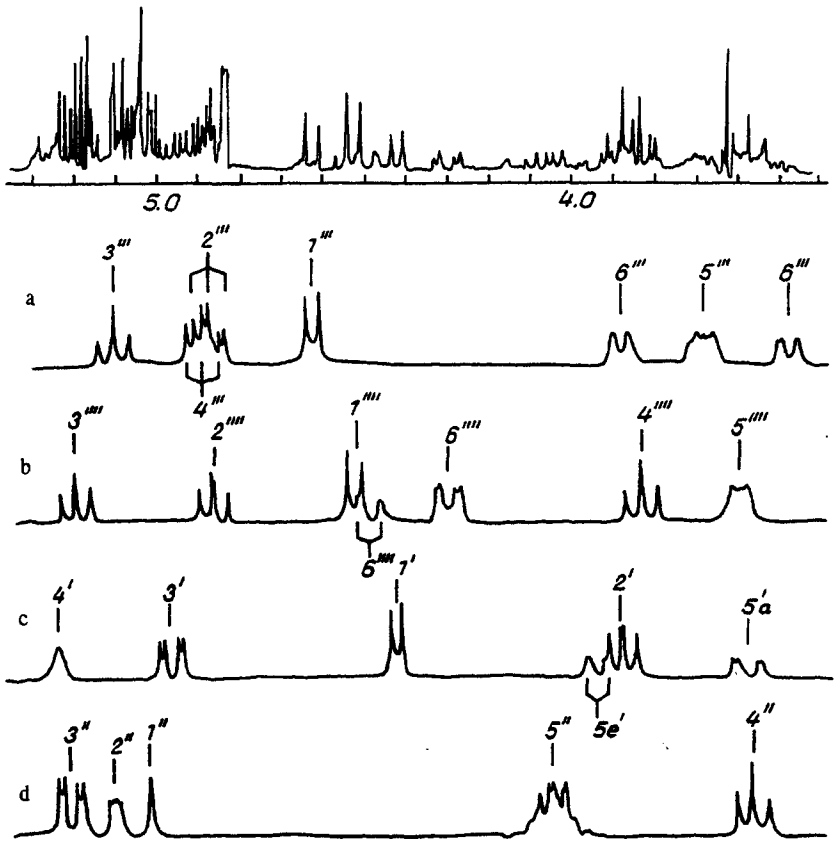


Fig. 1. Weak-field part of the ^1H NMR spectrum of the full acetate of glycoside L-6d and the HOHAHA subspectra of the monosaccharide residues.

The linkage sequence of the monosaccharides was determined in the one-dimensional variant of experiments on the observation of nuclear Overhauser effects (NOEs) in a rotating frame (CAMELSPIN) [4]. The application of the NOE procedure in a stationary frame [laboratory frame — LF] proved uninformative because of the close-to-zero NOEs resulting from the unfavorable correlation times of these molecules for an instrument with a working frequency of 250 MHz.

Figure 2 shows the PMR spectrum of deacetylated glycoside L-6d, the one-dimensional HOHAHA subspectra obtained on excitation of the anomeric protons of glucose and arabinose, the H-5''''' and H-3'' protons of the rhamnose residues, and the difference NOE spectra obtained on preirradiation of the anomeric protons. The assignment of the signals in the subspectra of the rhamnose and arabinose residues is unambiguous in view of the specific nature of their splitting (we had made an assignment of the H-5',5' signals to the axial and the equatorial atoms previously on the basis of a NOE experiment [5]). The assignment of the H2—H6 signals of the glucopyranoside residues was made on the basis of an analysis of the COSY spectrum. Figure 3 shows part of the COSY spectrum (diagonal peaks not illustrated) and the HOHAHA subspectra of one of the residues, Glc''''. The assignments for the other glucose residue, Glc''' and confirmation of the assignments for the rhamnose and arabinose residues were made similarly, and in Fig. 3 only the corresponding cross-peaks are interpreted.

The experiments on the observation of NOEs (Fig. 2) unambiguouly showed the linkage of the Rha'' and Ara' residues (Fig. 2, *b*), the Glc''' and Rha'' residues (Fig. 2, *d*), the Rha''''' and Glc'''' residues (Fig. 2, *h*), and the Ara' residue and the aglycon (Fig. 2, *e*). However, only in the last case is the type of bond obvious. In the others, because of diffusion of spin density during the period of spin-locking in the difference spectra the signals of several protons of the glycosylated residue can be seen. In this case the choice of the type of bond was made on the basis of the analysis given above of the PMR spectrum of the full acetate. This led to the conclusion that the Glc'''' residue was attached at the C_6—OH group of Glc''', since an experiment with NOEs, both in a RF and in a LF did not give unambiguous results (practically zero NOEs in the SSC because of unfavorable correlation times of the protons at the 1→6 bond and in the RSC because of the superposition of HOHAHA effects having the opposite sign to the NOEs).

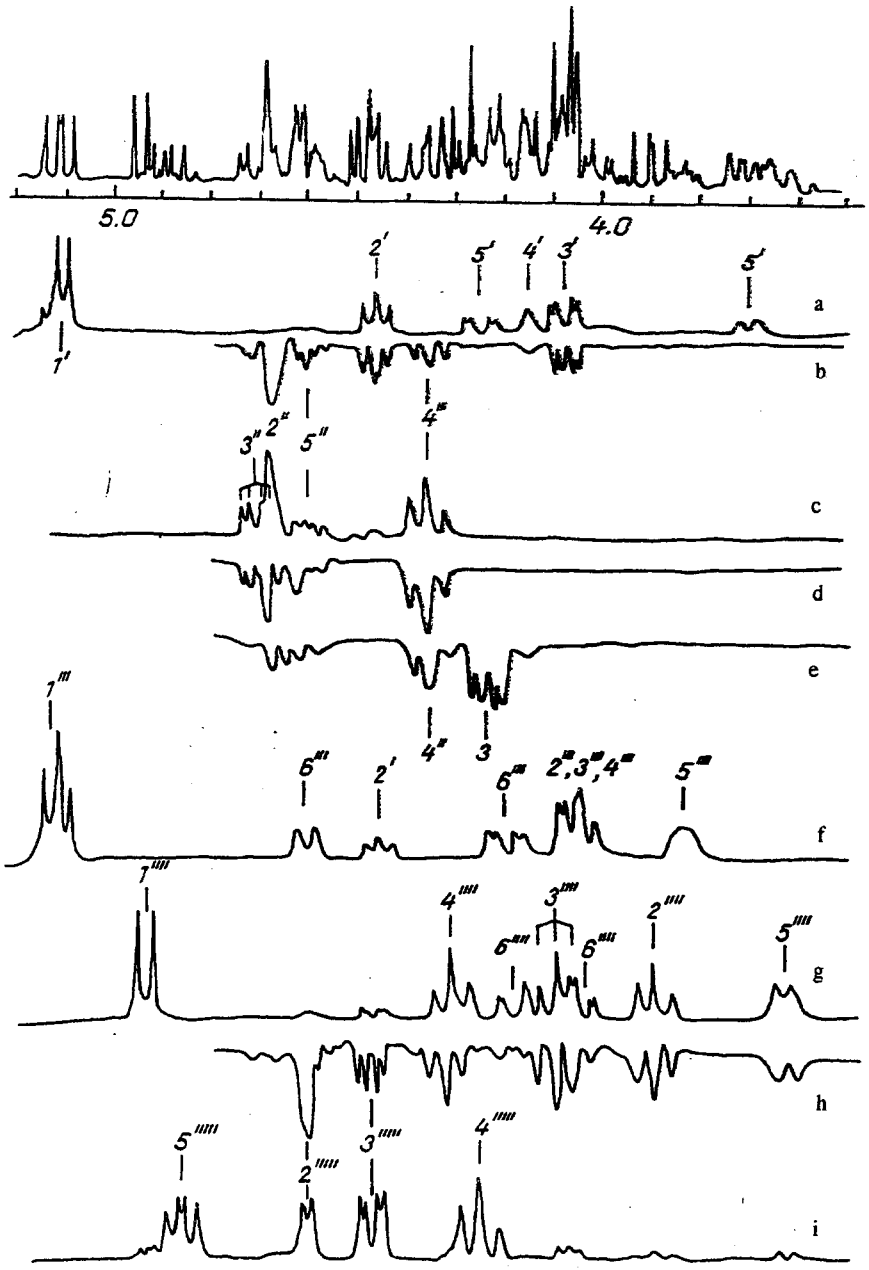


Fig. 2. Weak-field part of the ^{1}H NMR spectrum of glycoside L-6d, and the HOHAHA (*a*, *c*, *f*, *g*, and *i*) and difference CAMELSPIN-NOE (*b*, *d*, *h*, and *e*) subspectra of the monosaccharide residues.

The question of the configurations of the anomeric centers in the rhamnopyranose residues could not be solved on the basis of the spin-spin coupling constants $J_{1,2}$. However, the observation of only the H-2 signals of these residues in the difference NOE spectra on preirradiation of the anomeric protons of Rha$''''$ and Rha$''$ (Fig. 2, *b*, *h*) showed the equatorial orientation of the H-1 protons and, consequently, the α-configurations of both glycosidic bonds.

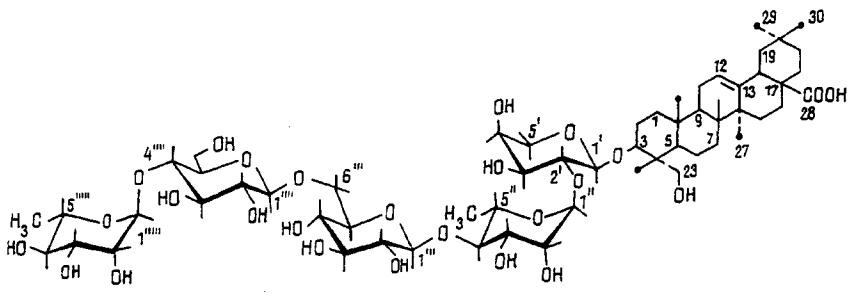
The assignment of the signals in the ^{13}C NMR spectrum of glycoside L-6d (Table 1) was made with the use of literature information for a 3-substituted hederagenin [6] and for the fragments $\text{Rha} \rightarrow^{2} \text{Ara} \rightarrow$ and $\text{Rha} \rightarrow^{4} \text{Glc} \rightarrow^{6} \text{Glc} \rightarrow$ [6],, and results on the α- and β-effects of the glycosylation of rhamnose in 1→4-bound disaccharide units $\text{Glc} \rightarrow^{4} \text{Rha} \rightarrow$ [7] and of the rhamnose residue in the fragment $\text{Xyl} \rightarrow^{4} \text{Rha} \rightarrow^{2} \text{Glc} \rightarrow$ [8] and finally confirmed the structure of glycoside L-6d. It is hederagenin 3-O-[O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

TABLE 1. Chemical Shifts of the ^{13}C Atoms of the Aglycon Moiety of the Methyl Ester of Glycoside L-6d (**1b**) (δ, ppm, 0 — TMS, C_5H_5N)

C-Atom	δ	C-Atom	δ	C-Atom	δ	C-Atom	δ	C-Atom	δ
1	39.0	7	32.8	13	144.2	19	47.0	25	16.2
2	26.3	8	39.7	14	42.0	20	30.8	26	17.4
3	80.9	9	48.1	15	28.1	21	34.0	27	26.1
4	43.5	10	36.9	16	23.7	22	32.8	28	178.1
5	47.4	11	23.3	17	46.0	23	63.8	29	33.1
6	18.1	12	125.6	18	41.8	24	14.0	30	23.8
								OCH_3	51.6

TABLE 2. Chemical Shifts of the ^{13}C Atoms of the Aglycon Moiety of the Methyl Ester of Glycoside L-6d (**1b**) (δ, ppm, 0 — TMS, C_5H_5N)

C-Atom	δ	C-Atom	δ	C-Atom	δ	C-Atom	δ	C-Atom	δ
1'	104.4	1''	101.4	1'''	106.4	1''''	105.3	1'''''	102.7
2'	76.1	2''	71.9	2'''	75.9	2''''	75.5	2'''''	72.6
3'	74.5	3''	72.6	3'''	78.6	3''''	76.4	3'''''	72.8
4'	69.3	4''	85.0	4'''	71.4	4''''	78.2	4'''''	74.0
5'	65.7	5''	68.2	5'''	77.3	5''''	77.2	5'''''	70.4
		6''	18.7	6'''	70.2	6''''	61.4	6'''''	18.6



The glycoside L-6d isolated from *Hedera helix* is a new triterpene glycoside.

EXPERIMENTAL

For general observations, see [1]. We have described the isolation and purification of glycoside L-6d (**1**) previously [1].

Full Acetate of the Methyl Ester of Glycoside L-6d (1a). Compound (**1**) (100 mg) was acetylated with acetic anhydride in pyridine (1:1, 30°C, 10 h) and, after evaporation to dryness, the residue was chromatographed on silica gel with elution by chloroform—methanol (100:1). The purified acetate (150 mg) was esterified with an ethereal solution of diazomethane, and the (**1a**) obtained was chromatographed on silica gel in the chloroform—methanol (200:1) system. This gave 100 mg of (**1a**). PMR spectrum of (**1a**) (δ, ppm, $CDCl_3$): 4.41 d ($J_{1,2}$ = 6.5 Hz, H-1'), 3.88 (dd, $J_{2,3}$ = 9.1 Hz, H-2'), 4.96 (dd, $J_{3,4}$ = 3.5 Hz, H-3'), 5.24 (H-4'), 3.94 (dd, $J_{4,5}$ = 3.3 Hz, $J_{5,5}$ = 13.0 Hz, H-5'e), 3.50 (H-5'a); 4.83 (d, $J_{1,2}$ = 2.2

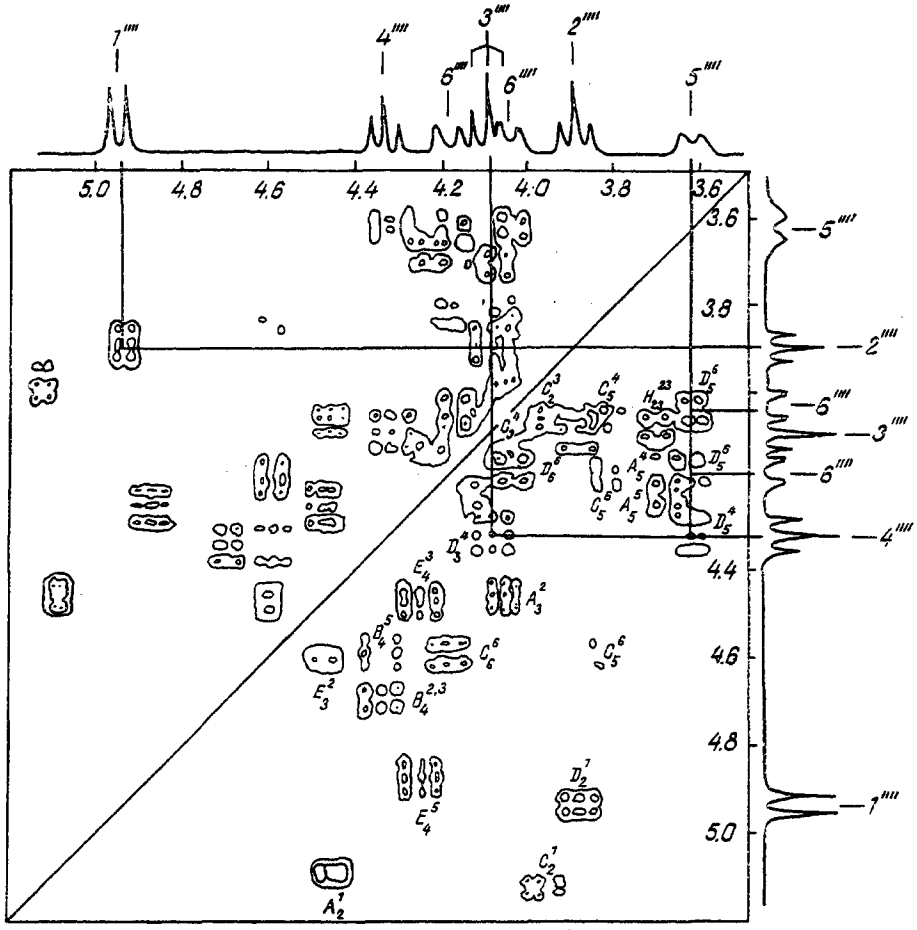


Fig. 3. Weak-field part of the COSY spectrum of glycoside L-6d and one of the HOHAHA subspectra (Glc′′′′) The residues are denoted arbitrarily by letters: *A* — Ara′; *B* — Rha′′; C — Glc′′′; *D* — Glc′′′′; *E* — Rha′′′′′.

Hz, H-1″), 5.05 (dd, $J_{2,3}$ = 3.9 Hz, H-2″), 5.20 (dd, $J_{3,4}$ = 9.8 Hz, H-3″), 3.57 (t, $J_{4,5}$ = 9.9 Hz, H-4″), 4.05 (dq, H-5″), 1.28 (d, $J_{5,6}$ = 6.3 Hz, H-6″); 4.62 (d, $J_{1,2}$ = 8.2 Hz, H-1‴), 4.87 (dd, $J_{2,3}$ = 10.0 Hz, H-2‴), 5.10 (t, $J_{3,4}$ = 9.6 Hz, H-3‴), 4.88 (t, $J_{4,5}$ = 9.7 Hz, H-4‴), 3.66-3.73 (H-5‴), 3.88 (dd, $J_{5,6}$ = 2.3 Hz; $J_{6A,6B}$ = 11.0 Hz, H-6A‴), 3.49 (dd, $J_{5,6}$ = 6.8 Hz, H-6B‴); 4.52 (d, $J_{1,2}$ = 7.8 Hz, H-1⁗), 4.86 (dd, $J_{2,3}$ = 9.3 Hz, H-2⁗), 5.19 (t, $J_{3,4}$ = 9.3 Hz, H-3⁗), 3.83 (t, $J_{4,5}$ = 9.5 Hz, H-4⁗), 3.59 (H-5⁗), 4.49 (dd, $J_{5,6}$ = 1.6 Hz, $J_{6A,6B}$ = 12.6 Hz, H-6A⁗), 4.30 (dd, $J_{5,6}$ = 3.8 Hz, H-6B⁗), 5.01 (d, $J_{1,2}$ = 1.7 Hz, H-1⁗), 5.09 (dd, $J_{2,3}$ = 3.2 Hz, H-2⁗), 5.19 (dd, $J_{3,4}$ = 10.1 Hz, H-3⁗), 5.04 (t, $J_{4,5}$ = 10.1 Hz, H-4⁗), 3.84 (dq, H-5⁗), 1.15 (d, $J_{5,6}$ = 6.3 Hz, H-6⁗); 4.18 (d, $J_{23A,23B}$ = 11.8 Hz, H-23A), 3.76 (d, H-23B), 0.72, 0.73, 0.90, 0.92, 0.95, 1.09 (eV s, 6 CH_3).

Methyl Ester of Glycoside L-6d (1b). Compound (**1a**) (80 mg) was deacetylated with a catalytic amount of sodium methanolate in an excess of dry methanol (40°C, 24 h); the solution was deionized with KU-2-8 cation-exchange resin in the H^+ form and was evaporated to dryness. After chromatography on silica gel with elution by chloroform—ethanol—water (10:5:1), 30 mg of pure (**1b**) was obtained. PMR spectrum of (**1b**) (δ, ppm, C_5D_5N): 5.10 d, $J_{1,2}$ = 6.2 Hz, H-1′), 4.47 (dd, $J_{2,3}$ = 8.0 Hz, H-2′), 4.07 (dd, $J_{3,4}$ = 3.8 Hz, H-3′), 4.15 (H-4′), 3.69 (dd, $J_{4,5a}$ = 2.2 Hz, $J_{5a,5c}$ = 12.0 Hz, H-5′a), 4.24 (dd, $J_{4,5e}$ = 4.2 Hz, H-5′e); 6.06 (d, $J_{1,2}$ = 1.5 Hz, H-1″), 4.67 (dd, $J_{2,3}$ = 3.3 Hz, H-2″), 4.71 (dd, $J_{3,4}$ = 9.0 Hz, H-3″), 4.36 (t, $J_{4,5}$ = 9.2 Hz, H-4″), 4.60 (dq, H-5″), 1.76 (d, $J_{5,6}$ = 6.5 Hz, H-6″); 5.13 (d, $J_{1,2}$ = 7.5 Hz, H-1‴), 3.98 (dd, $J_{2,3}$ = 9.7 Hz, H-2‴), 4.01-4.09 (m, H-3‴, H-4‴), 3.84 (H-5‴), 4.61 (dd, $J_{5,6A}$ = 2.5 Hz, $J_{6A,6B}$ = 12.0 Hz, H-6A‴), 4.20 (dd, $J_{5,6B}$ = 6.8 Hz, H-6B‴); 4.94 (d, $J_{1,2}$ = 7.7 Hz, H-1⁗), 3.90 (dd, $J_{2,3}$ = 9.3 Hz, H-2⁗), 4.10 (t, $J_{3,4}$ = 9.5 Hz, H-3⁗), 4.33 (t, $J_{4,5}$ = 9.5 Hz, H-4⁗), 3.63 (H-5⁗), 4.19 (dd, $J_{5,6A}$ = 3.0 Hz, $J_{6A,6B}$ = 12.5 Hz, H-6A⁗), 4.04 (dd, $J_{5,6B}$ = 5.5 Hz, H-6B⁗); 5.78 (d, $J_{1,2}$ = 1.5 Hz, H-1⁗), 4.61 (dd, $J_{2,3}$ = 3.5 Hz, H-2⁗), 4.48 (dd, $J_{3,4}$ = 9.5 Hz, H-3⁗), 4.27

(t, $J_{4,5}$ = 9.5 Hz, H-4''''), 4.88 (dq, H-5''''), 1.67 (d, $J_{5,6}$ = 6.4 Hz, H-6''''); 4.24 (dd, $J_{2e,3}$ = 4.0 Hz, $J_{2a,3}$ = 11.5 Hz, H-3), 3.71 (d, $J_{23A,23B}$ = 11.5 Hz, H-23A), 4.08 (d, H-23B).

REFERENCES

1. V. I. Grishkovets, A. E. Kondratenko, N. V. Tolkacheva, A. S. Shashkov, and V. Ya. Chirva, Khim. Prir. Soedin., 742 (1994) [in this issue].
2. S. Subramanian and A. Bax, J. Magn. Reson., **71**, 325 (1987).
3. M. M. Benidze, O. D. Dzhikiya, T. A. Pkheidze, É. P. Kemertelidze, and A. S. Shashkov, Khim. Prir. Soedin., 537 (1987).
4. A. A. Bothner-By, R. L. Stephens, J. Lee, C. D. Warren, and R. W. Jeanloz, J. Am. Chem. Soc., **106**, No. 3, 811 (1984).
5. A. S. Shashkov, V. I. Grishkovets, A. A. Loloiko, and V. Ya. Chirva, Khim. Prir. Soedin., 363 (1987).
6. V. I. Grishkovets, N. V. Tolkacheva, A. S. Shashkov, and V. Ya. Chirva, Khim. Prir. Soedin., 522 (1992).
7. A. S. Shashkov, A. I. Usov, Yu. A. Knirel', and B. A. Dmitriev, Bioorg. Khim., **7**, No. 9, 1364 (1981).
8. T. Konoshima and T. Sawada, Chem. Pharm. Bull., **30**, No. 8, 2747 (1982).