SUMMARY

It has been established that by using cottonseed lipase it is possible to effect the transesterification of the fatty acid radicals of mixtures of triglycerides. The transesterificates so obtained correspond completely in their structural and mechanical properties to the demands set for the fatty bases used for the production of margarine and bakers' and confectioners' articles.

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^1H AND ^{13}C NMR SPECTRA AND THE STRUCTURE OF A NEW COUMARIN, C-GLYCOSIDE DAUROSIDE D, FROM *Haplophyllum dauricum*

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On the basis of chemical transformations, ^1H and ^{13}C NMR spectra, the structure of dauroside D isolated from *Haplophyllum dauricum* has been established as 6-C-β-D-glucopyranosyl-5,7-dihydroxycoumarin. Some interesting features of the ^1H NMR spectra of its acetate have been reported and an assignment of the signals in its ^1H and ^{13}C NMR spectra has been made. Dauroside D is the first natural coumarin C-glycoside.

Previously [1] on the basis of its chemical and spectral characteristics, a structure was proposed for dauroside D isolated from *Haplophyllum dauricum* (L.) G. Don in which the position of the carbohydrate residue was not established unambiguously. For a definitive solution of this problem we have used the method of double resonance in the PMR spectrum of dauroside D (I) and its acetyl derivative, and we have also studied the ^{13}C NMR spectrum of (I).

The PMR spectrum of dauroside D taken at room temperature in DMSO-d_6 (Fig. 1) shows the following signals: at 6.00 and 7.92 ppm, one-proton doublets with J = 10 Hz relating to the H_3 and H_4 protons of the coumarin nucleus, respectively, the components of the H_4 doublet being somewhat wider than those of the H_3 doublet; at 6.25 ppm, a broadened one-proton doublet belonging to one of the aromatic protons of the coumarin skeleton. At 4.70 ppm, a doublet from the H_1' protons of the sugar moiety partially overlapping with the signal from hydroxylic protons with its center at 4.95 ppm. In the 2.95-3.85 ppm region appear signals from the H_2'-H_6' protons of the sugar residue — β-D-glucose. The cleavage of (I) with Kiliani's mixture yielded 5,7-dihydroxycoumarin [1]. Consequently, the phenolic OH groups in dauroside D are located at C_5 and C_7, and the broadened singlet at 6.25 ppm can relate only to H_6 or H_8. The determination of the position of the sugar residue in the coumarin nucleus of (I) reduced to the unambiguous assignment of the signal with δ 6.25 ppm to H_6 or H_8.

This problem was solved with the aid of double proton resonance. As can be seen from Fig. 1, and as mentioned above, the signal of the H_4 proton is somewhat broadened in comparison with the signal from the H_3 proton. It is known [2, 3] that the long-range SSCC of H_4 with H_8 in coumarin systems is $^5J_{4,8} \approx 0.6$-0.7 Hz, while $^5J_{4,6} \approx 0$.

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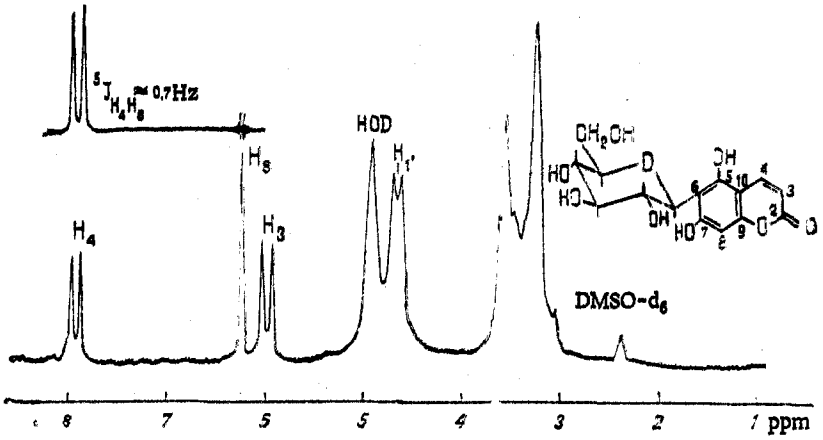

Fig. 1. PMR spectrum of dauroside D in DMSO-d_6.

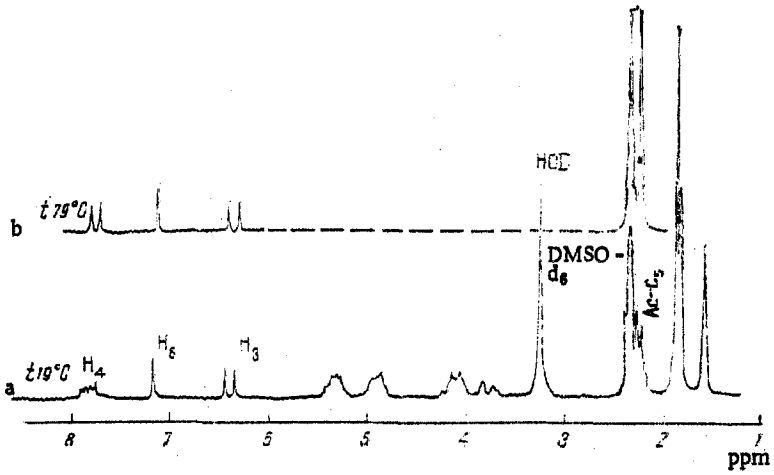

Fig. 2. PMR spectrum of the acetate of dauroside D in DMSO-d_6 at temperatures of 19°C (a) and 79°C (b).

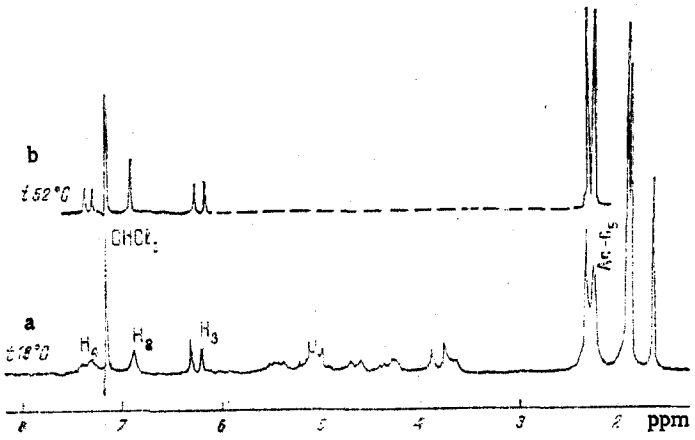

Fig. 3. PMR spectrum of the acetate of dauroside D in $CDCl_3$ at 18°C (a) and 52°C (b).

When the sample was irradiated with an addition frequency ν_2 of 792 Hz (H_4), some contraction of the signal at 6.25 ppm (long-range constant $J \approx 0.6$-0.7 Hz) was observed, while, conversely, when it was irradiated with a frequency of 625 Hz the components of the H_4 doublet contracted and the signal became identical with the signal from H_3 proton. These results of double resonance permit the unambiguous assignment of the signal at 6.25 ppm to the H_8 proton and the C_6 position of the coumarin nucleus to be established for the sugar residue.

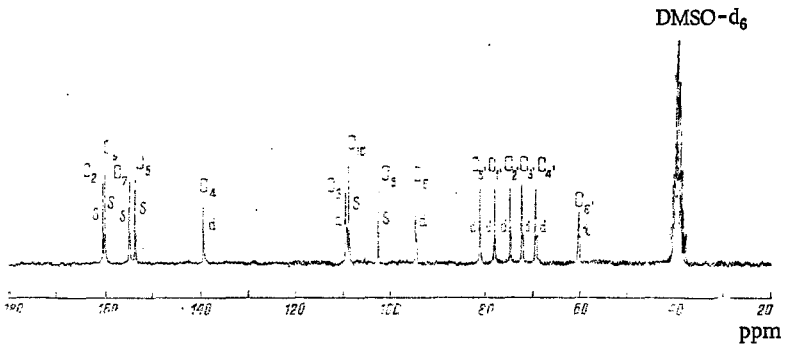

Fig. 4. ^{13}C NMR spectrum of dauroside D in DMSO-d_6.

The value of the SSCC for the H_1' and H_2' protons (J = 9.5 Hz) in the spectrum taken in Py-d_5 corresponds to their diaxial orientation, which shows the β-glucopyranose form of the sugar residue in dauroside D [4]. We obtained an extremely interesting result on the analysis of the PMR spectrum of the acetate of dauroside D (II) taken in DMSO-d_6.

As can be seen from the spectrum obtained at room temperature (19°C, Fig. 2a), the signal of the H_4 proton appears in the form of two doublets with an intensity of 1 H and its center at 7.84 ppm, and the signal of the methyl of one of the acetyl groups appears in the form of a three-proton doublet at 2.33 ppm. This fact is apparently due to the existence of two preferred (hindered) conformations of the acetyl grouping and to different mutual influences of this group on the chemical shift of the H_4 proton. With a rise in the temperature to 79°C (Fig. 2b), as the result of the effective rotation of the acetyl group this influence is averaged out and the signal of the methyl protons of the acetyl group at C_5 becomes a singlet and that of the H_4 proton a broadened doublet. When the temperature is lowered at room temperature, the original pattern of the spectrum is restored. An analogous but less pronounced mutual influence of H_4 and Ac—C_5 appears in the spectrum of (II) taken in $CDCl_3$ (Fig. 3a). At room temperature the signal of the H_4 proton has highly broadened components and the signal of the acetyl group at C_5 is likewise highly broadened. This broadening is also eliminated by raising the temperature to 52°C (Fig. 3b), as the result of the effective rotation of the acetyl group.

The experiments that we performed on the dilution of solutions of dauroside D acetate showed that both in DMSO-d_6 solution and in $CDCl_3$ solution the effect that we have described of the mutual influence of the acetyl group at C_5 and the H_4 protons is practically independent of the concentration. This gives grounds for stating that the observed effect has an intramolecular nature. In addition to PMR spectra, we studied the ^{13}C NMR spectra of dauroside D in DMSO (Fig. 4). Its ^{13}C NMR spectrum shows nine signals from sp^2-hybridized carbon atoms of the coumarin skeleton in the 94-161 ppm region and from six sp^3-hybridized carbon atoms of the sugar moiety of the molecule. On the basis of the chemical shifts and multiplicities of the signals in an off-resonance experiment, and also by comparing them with literature figures [5-8], we made the assignment of the signals of the carbon atoms in the ^{13}C spectrum of dauroside D the results of which are given in Fig. 4 and in the Experimental section. The chemical shift of the C_1' carbon of dauroside D (δ 77.7 ppm) is not characteristic for coumarin O-glycosides in which the chemical shifts of C_1' amount to 100-101 ppm [5]. At the same time, it is close to the chemical shift of the C_1' carbon atoms of flavonoid C-glycosides (~79 ppm) [7, 8]. The chemical shifts of the C_1' and C_6 carbons show that the sugar residue of dauroside D is attached to the coumarin skeleton by a carbon—carbon bond and not through oxygen. Thus, on the basis of the PMR and ^{13}C NMR spectra we have established the structure of dauroside D as 6-C-β-D-glucopyranosyl-5,7-dihydroxycoumarin (I) and have made an assignment of the signals in the PMR and ^{13}C NMR spectra of (I) and its acetate and have also observed an interesting feature of the PMR spectra of the latter. Dauroside D is the first natural coumarin C-glycoside.

EXPERIMENTAL

General Observations. The purity of the substances was checked by chromatography on Silufol in the chloroform—methanol (71:16) system. IR spectra were recorded on a UR-20 instrument in tablets with KBr, UV spectra on a EPS-3T photometer in C_2H_5OH, and mass spectra on a MKh-1303 instrument fitted with a system for the direct introduction of the sample into

the ion source. PMR spectra were obtained on a JNM-4H-100/100 MHz spectrometer (δ scale, 0 — HMDS), and ^{13}C NMR spectra on a Varian XL-200 instrument in DMSO-d_6, 0 — TMS.

Isolation of Dauroside D. On further elution of the coumarins [9] (see the isolation of dauroside A) with chloroform—methanol (89:11 by volume), fractions 101-105 yielded 0.125 g of dauroside C, and fractions 110-120 3.0 g of dauroside D. The latter was twice recrystallized from methanol.

Dauroside D, $C_{15}H_{16}O_9$, mp 214-215°C, $[\alpha]_D$ +108.6° (c 0.35; pyridine), R_f 0.33, λ_{max} (ethanol; nm): 225, 253 sh., 262, 333 (log ε 4.28, 3.99, 3.99, 4.22); ν_{max}^{KBr}, cm^{-1}: 3460, 3400, 3175 (OH groups), 1723 (C=O of an α-pyrone), 1637, 1620, 1586 (C=C bonds), 1463, 1380, 1360, 1321, 1263, 1198, 1151, 1118, 1090, 1080, 1028, 911, 875, 831, 740.

^{13}C NMR (DMSO-d_6): 60.0 (t, $C_{6'}$), 69.1 (d, $C_{4'}$), 72.0 (d, $C_{3'}$), 74.6 (d, $C_{2'}$), 77.7 (d, $C_{1'}$), 80.8 (d, $C_{5'}$), 94.6 (d, C_8), 102.2 (s, C_6), 108.6 (s, C_{10}), 109.2 (d, C_3), 139.4 (d, C_4), 153.7 (s, C_5), 154.9 (s, C_7), 159.9 (s, C_9), 160.4 (s, C_2).

Mass spectrum, m/z (%): 322 (M − H_2O, 100), 304 (M − $2H_2O$, 22), 286 (M − $3H_2O$, 28), 274 (10), 273 (17), 270 (14), 268 (15), 231 (26), 230 (26), 206 (35), 202 (9), 191 (13), 190 (9), 178 (21), 162 (9), 150 (9), 145 (9), 132 (12), 125 (8), 123 (11), 121 (13), 120 (17), 111 (15), 109 (15), 105 (21), 98 (10), 97 (25), 95 (24), 85 (14), 83 (21), 81 (20), 77 (12), 71 (22), 69 (30).

Acetylation of (I). A solution of 0.15 g of (I) in 1.5 ml of pyridine was treated with 3 ml of acetic anhydride and the mixture was left at room temperature. After 24 h, it was diluted with ice water, and the resulting precipitate was filtered off with suction, washed several times with distilled water, dried, and recrystallized from methanol. This gave a substance $C_{27}H_{28}O_{15}$, M^+ 592, mp 202-204°C.

Hydrolysis of (I) with Kiliani's Mixture. The hydrolysis of 0.11g of (I) was carried out with 8 ml of Kiliani's mixture on the water bath for 6 h. Then the reaction mixture was diluted with water and extracted with ethyl acetate. The ethyl acetate was distilled off and the residue was separated by preparative TLC in a fixed layer of silica gel in the chloroform—methanol (9:1) system. This led to the isolation of 13 mg of a substance which, by comparison with an authentic sample, was identified as 5,7-dihydroxycoumarin [1]. In the aqueous fraction of the hydrolysate after neutralization on AV-10G anion-exchange resin (OH form), D-glucose and a small spot of D-arabinose were detected by the methods of GLC and PC with markers in the butan-1-ol—pyridine—water (6:4:3) system.

SUMMARY

On the basis of chemical transformations and ^1H and ^{13}C NMR spectra, the structure of the first coumarin C-glycoside dauroside D has been established as 6-C-β-D-glucopyranosyl-5,7-dihydroxycoumarin. Some features of the PMR spectra of its acetate have been reported and an assignment has been made of the signals in the ^1H and ^{13}C NMR spectra.

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