NMR SPECTRUM OF GERMERINE

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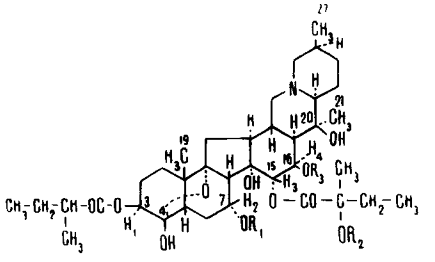
We have previously [1] described the isolation from the Central-Asian plant Veratrum lobelianum of a base with mp 203-205°C, $[\alpha]_D + 9.98°$ and its identification as germerine $C_{37}H_{59}O_{11}N$ [2, 3]. However, the NMR spectrum of germerine has not been investigated [4].

In the present communication we give the results of a study of the NMR spectra of germerine and of its triacetyl (II) and tribenzoyl derivatives.

In order to isolate the signals of the free hydroxy groups of germerine, we performed deuterium exchange by adding a few drops of CD_3OD to a solution of the substance in $CDCl_3$. The table gives the features of the spectra and the assignment of the signals of the methyl and methine protons. The identification of the signals of the C_{19}, C_{21}, and C_{27} protons was complicated by the fact that the signals of the methyl protons of the side chain fell in the same region. The spectrum of the acetyl derivative of germerine showed the presence in it of three singlets at δ 1.97, 2.00, and 2.04 ppm (three proton units each), showing that a triacetylgermerine had been formed. Fermerine contains only two secondary hydroxy groups.

It is known that tertiary hydroxyls at C_4 and C_{20} of a molecule with a steroid skeleton are not acetylated under ordinary conditions. Since germerine gives a triacetate, it may apparently be assumed that in addition to two secondary hydroxy groups the tertiary hydroxyl of the side chain is also acetylated. This is confirmed by the fact that in the spectra of the acetyl and benzoyl derivatives (II) and (III) the signal from $\underset{\substack{| \\ OR}}{C}-CH_3$ (R = Ac and Ph) undergoes a considerable paramagnetic shift by 0.15-0.24 ppm as compared with germerine (I). At the same time, when (I) was benzoylated a weak diamagnetic shift of the signals of the other methyls took place.

On considering the chemical shifts and the spin–spin coupling constants of the signals of the H_1, H_2, H_3 and H_4 protons at C_3, C_7, C_{15}, and C_{16} we obtained useful information on the configuration of these centers. A proton attached to a carbon atom bearing a hydroxy group undergoes a considerable paramagnetic shift when the hydroxy group is acetylated [5]. This fact enabled us to assign the weak-field signals at 4.96 and 5.29 ppm to H_1 and H_3 respectively; in the spectra of the acetyl and benzoyl derivatives (II and III), the chemical shifts of these signals change insignificantly, and they are found in the weakest fields because of the influence of the ester group of the side chain.



I $R_1=R_2=R_3=H$ II $R_1=R_2=R_3=CO-CH_3$ III $R_1=R_2=R_3=COC_6H_5$

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TABLE 1. Chemical Shifts (δ, ppm) and Spin–Spin Coupling Constants (J, Hz) of the Protons of Germerine and Its Derivatives

Substance	$19-CH_3$ s	$21-CH_3$ s	$27-CH_3$ d	Side chain					H_1	H_2	H_3	H_4
				$C(OH)-CH_3$ s	$CH-CH_3$ d	CH_2-CH_3 t	$OCOCH_3$ s	$OCOC_6H_5$ m				
Germerine (I) $R_1=R_2=R_3=H$	0,94	1,14	1,03 $J=7,0$	1,35	1,11 $J=7,0$	0,85 1,07 $J=7,0$	—	—	4,96 m $\Delta W=8,0$	4,48 m $\Delta W=12-13$	5,29 d $J=3,0$	4,22 m $\Delta W=7,0$
Acetate (II) $R_1=R_2=R_3=COCH_3$	0,97	1,11	1,01 $J=7,0$	1,50	1,07 $J=7,0$	0,85 1,04 $J=7,0$	1,97 2,00 2,04	—	4,95 q $J_1=4,0$ $J_2=2,0$	5,47 m $\Delta W=13,0$	5,44 d $J=3,0$	4,94 m $\Delta W=8,0$
Benzoate (III) $R_1=R_2=R_3=COC_6H_5$	0,91	1,09	0,83 $J=7,0$	1,59	1,05 $J=7,0$	0,67 0,91 $J=7,0$	—	7,39 7,89	4,99 m $\Delta W=8,0$	5,45 m $\Delta W=13-14$	5,18 d $J=3,0$	5,62 m $\Delta W=8,0$

Note. s–singlet; d–doublet; t–triplet; q–quartet; m–multiplet; ΔW–half-width of the signal, Hz.

The H_2 and H_4 signals in the spectra of (II) and (III) are shifted downfield by 0.99 and 0.72 ppm, respectively, in (II) and by 0.97 and 1.40 ppm, respectively, in (III). The half-widths, ΔW of the signals of the H_1, H_2, H_3 and H_4 protons, which characterize the sums of the spin–spin coupling constants of these protons with the neighboring ones show that they have equatorial positions and the substituents at C_3, C_7, C_{15}, and C_{16} have the axial orientations [5]. The values of ΔWH_2 are approximately twice as great as ΔWH_1 and ΔWH_4. This is due to the fact that there are three protons adjacent to H_2 and only two each adjacent to H_1 and H_4.

EXPERIMENTAL

The NMR spectra were obtained on a JNM-4H-100 MHz instrument (deuterochloroform solution). HMDS was used as internal standard. The chemical shifts are given in the δ scale.

The Acetate (II). Germerine (I) (0.2 g) was acetylated with 4 ml of acetic anhydride in 6 ml of pyridine at room temperature for 72 h. After the appropriate working up, the reaction product was purified by passage through a column filled with silica gel and was eluted with a mixture of benzene and acetone (10 : 1). The first five fractions yielded the triacetate (II) with mp 192-194°C (acetone–petroleum ether, 40-70°C), R_f 0.64 on TLC in silica gel using system 1 [benzene–acetone (1 : 1)].

The Benzoate (III). A cooled solution of 0.3 g of germerine (I) in 2 ml of pyridine was treated dropwise with 0.6 ml of benzoyl chloride, and the mixture was left at room temperature for three days. After the elimination of the pyridine in vacuum, the residue was dissolved in water, and the solution was made alkaline with ammonia and extracted with ether. The residue after the distillation of the ether was dissolved in benzene and chromatographed on a column of silica gel. The benzene–acetone (100 : 25) eluate yielded the amorphous tribenzoyl derivative of germerine (III) with R_f 0.43 on TLC in system 1, while germerine in the same system has R_f 0.33.

SUMMARY

In a comparative study of the NMR spectra of germerine and its acetyl and benzoyl derivatives, an assignment has been made of the signals of the protons of the methyl groups, and the equatorial orientation of the protons at C_3, C_7, C_{15}, and C_{16} in it has been confirmed.

LITERATURE CITED

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