THE POSITIONS OF THE ACYL GROUPS IN GERMERINE AND GERMITRINE

N. V. Bondarenko

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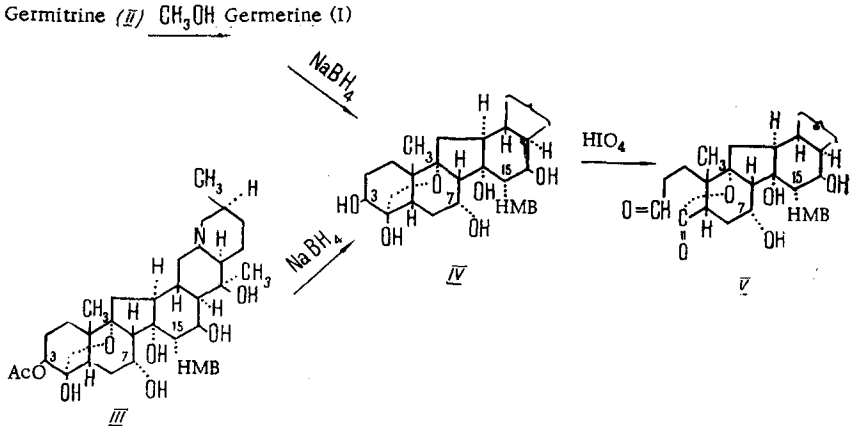
A feature of the structure of the esters of the alkaloids germine and protoverine that have been isolated from plants of the genus Veratrum is the definite arrangement of the acyl groups: all the acyl residues found in them are located in the C_3 position, only acetyl (Ac) in the C_6 and C_7 positions, and only (*l*)-2-methylbutyryl (MB) in the C_{15} position [1]. Apparent exceptions are germerine (I) and germitrine (II), which are said to have (d)-2-hydroxy-2-methylbutyryl (HMB) at C_{15} and MB at C_3. But in view of the fact that the bulk of the alkaloids contain MB in the C_{15} position, it would be logical to consider this fact a biogenetic feature of them and to check once more the correctness of literature information on the positions of the acyl groups in alkaloids (I) and (II).

An acquaintanceship with the appropriate literature showed us that the procedure for determining the positions of the acyl groups in (I) and (II) differed from that used in the investigation of the other alkaloids germine and protoverine [1]. The main difference consists in the fact that the selective splitting off of one of the acyl groups from (I) was performed by the prolonged keeping of the alkaloids in barium hydroxide solution. The reaction product formed (protoveratridine) differed from the 15-(*l*)-2'-methylbutyrylgermine (IV) obtained by treating the other ester alkaloids of germine with a solution of sodium tetrahydroborate. In view of this, we have performed the reaction of (I) with sodium tetrahydroborate in parallel with that of germidine (III). Sodium tetrahydroborate selectively splits off the acyl radicals at C_3, and therefore if germerine actually possesses the structure described, identical reaction products could not be obtained and, conversely, if the structure is as we have assumed, identical compounds should be obtained.



The results of our investigations have shown that when (I) and (III) are treated with a methanolic solution of sodium tetrahydroborate, both alkaloids give substance (IV). The latter is converted by oxidation with potassium periodate in acetic acid into an aldehydo-γ-lactone which can be obtained only if there is a free OH group in the C_3 position (α-glycol grouping). Consequently, germerine is not 15-(d)-2'-hydroxy-2'-methylbutyryl-3-(*l*)-2'-methylbutyrylgermine [1] but 3-(d)-2'-hydroxy-2'-methylbutyryl-15-(*l*)-2'-methylbutyrylgermine. This structure is not contradicated by the NMR spectrum ($CHCl_3$), either. The chemical shift of the signals of the protons of the methyl group of the HMB residue of germerine (1.40 ppm) is

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very close to that of the signals of the protons in protoveratrine A (1.37 ppm) and deacetylprotoveratrine A (1.38 ppm), which shows the similar positions of this acyl group in all the alkaloids mentioned (in the last two, HMB is located at C_3).

Since compound (II) is converted on methanolysis into germerine [2], this must also be considered to be not 7-acetyl-15-(d) 2'-hydroxy-2'-methylbutyryl-3-(*l*)-2'-methylbutyrylgermine but 7-acetyl-3-(d)-2'-hydroxy-2'-methylbutyryl-15-(*l*)-2'-methylbutyrylgermine.

EXPERIMENTAL

The NMR spectra were taken on a High R-20 A instrument with TMS as internal standard (δ scale); the IR spectra on a UR-10 spectrometer (potassium bromide); and the UV spectra on an SF-4A spectrophotometer (c 0.4 mg in 10 ml of sulfuric acid, sp. gr. 1.830). The substances were analyzed chromatographically on "M" ["slow"] paper of the Volodarskii Leningrad paper mill in the following solvent systems: 1) chloroform saturated with formamide, and 2) butan-1-ol–acetic acid–water (4 : 1 : 5). For system 1, the paper was impregnated with a solution of formamide in ethanol (1 : 2). The germerine and germidine were isolated from the combined alkaloids obtained by the treatment of the roots with rhizomes of Veratrum lobelianum Bernh. [3] with ether by chromatography on a column of cellulose [4].

Germerine, $C_{37}H_{59}O_{11}N$, mp 202-204°C (benzene), $[\alpha]_D^{20}$ −7° (c 0.91; pyridine), R_f 0.49 (1). IR spectrum, cm^{-1}: 3360 (OH), 2940, 1465, 1385 (−CH_3 and −CH_2), 1738, 1250 (ester C=O). In the NMR spectrum there is a three-proton signal of the α-methyl group of HMB (1.39 ppm) and no signal from an $OCOCH_3$ group. The UV spectrum of a sulfuric acid solution of the alkaloid taken 24 h after dissolution (λ_{max} 246, 315, 406, 528 nm) did not coincide with the spectrum taken after 1.5 h, which shows the presence of the amino alcohol germine in the substance [5]. The chromatographic analysis of the products of alkaline hydrolysis in system 2 confirmed the presence of germine in them. The ether treatment of the acidified hydrolyzate yielded two organic acids which were identified by their R_f values in the butan-1-ol–1.5 N ammonia (1 : 1) system as (*l*)-α-methylbutyric and (d)-α-hydroxy-α-methylbutyric acids, which have been obtained in the hydrolysis of protoveratrine A and deacetylprotoveratrine A.

Germidine, $C_{34}H_{53}O_{10}N$, mp 200-202°C (ethanol), $[\alpha]_D^{20}$ −11° (c 0.8; pyridine), R_f 0.6 (1) [3]. The NMR spectrum of the alkaloid has the three-proton singlet of an $OCOCH_3$ group (2.06 ppm) and lacks the signal of the α-methyl group of HMB. Chromatographic analysis of the products of alkaline hydrolysis of the alkaloid showed that it contained acetic and α-methylbutyric acids, and also the amino alcohol germine. The presence of the latter is also confirmed by the nature of the spectra of sulfuric acid solutions of the alkaloids [5]. Thus, the analytical results correspond to those for (I) and (III).

Conversion of Germerine and Germidine into 15-(*l*)-2-Methylbutyrylgermine. A solution of sodium tetrahydroborate (72 mg in 5.6 ml of methanol) was added to a solution of 72 mg of (I) in 2 ml of methanol [6]. After 15 h, the solution was acidified with glacial acetic acid and evaporated under reduced pressure at 20°C. The residue was dissolved in water and the solution was cooled to 0°C, treated with cold 5% ammonia solution, and extracted with chloroform. The chloroform extract was washed with water, dried with anhydrous sodium sulfate, and evaporated under reduced pressure. The residue, which consisted of a mixture of two substances (by paper chromatography in system 1) was separated on a column of cellulose [4]. Eight hours after the charging of the chromatograph, the column of cellulose was divided into sections with a size of 1-1.5 cm. Those sections of the column that contained alkaloids were eluted with 5% acetic acid. The acetic acid solutions were cooled to 0°C, brought to the pH 9.0 with 10% aqueous ammonia, and extracted with chloroform.

The fractions including a substance with R_f 0.07 (1) were combined, washed with water, dried with anhydrous sodium sulfate, evaporated to small volume, and mixed with an equal amount of diethyl ether. The crystalline precipitate that formed (14 mg) had mp 222-225°C (the melting point of protoveratridine obtained by treating germerine with a solution of barium hydroxide is 266-267°C) [7]; IR spectrum, cm^{-1}: 1740, 1250 (ester C=O). The melting point of a mixture with compound (IV) obtained similarly from germidine gave no depression of the melting point. On comparative chromatography in system 1 (the chromatogram being run for 18 h at 20°C), the R_f values of both samples were 0.31.

By the same treatment, the fractions containing a substance with R_f 0.0 (1) yielded a crystalline precipitate with mp 219-221°C (methanol), R_f 0.51 (2), which coincides with the R_f value of a sample of germine obtained by the methanolysis of germidine by the method of Fried et al. [8]. A mixture showed no depression of the melting point.

Oxidation of 15-(*l*)-2'-Methylbutyrylgermine with Potassium Periodate [6]. A mixture of a solution of 10 mg of (IV) in 1 ml of 5% acetic acid and 3 ml of a 0.25% solution of potassium periodate was left at 21°C for 24 h. Then its pH was brought to 9.0 by the addition of 10% ammonia solution and it was treated with chloroform. The chloroform solution was evaporated to dryness and the residue was dried over anhydrous calcium chloride. Its IR spectrum showed absorption bands at 2712, 1779, and 1724 cm^{-1} showing the aldehydo-γ-lactone structure of ring A.

SUMMARY

The positions of the acyl residues at C_3 and C_{15} in germerine and germitrine have been corrected, showing that germerine is 3-(d)-2'-hydroxy-2'-methylbutyryl-15-(*l*)-2'-methylbutyrylgermine and germitrine is 7-acetyl-3-(d)-2'-hydroxy-2'-methylbutyryl-15-(*l*)-2'-methylbutyrylgermine.

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