THE POSITIONS OF THE ACYL GROUPS IN GERMERINE AND GERMITRINE

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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 C₃ position, only acetyl (Ac) in the C₅ and C₇ positions, and only (l)-2-methylbutyryl (MB) in the C₁₅ position [1]. Apparent exceptions are germine (I) and germitrine (II), which are said to have (d)-2-hydroxy-2-methylbutyryl (HMB) at C₁₅ and MB at C₃. But in view of the fact that the bulk of the alkaloids contain MB in the C₁₅ 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 acquaintance 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 C₃, 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.

Germitrine (II) \text{CH₃OH} \text{Germerine (I)}

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 peroxidate in acetic acid into an aldehydo-γ-lactone which can be obtained only if there is a free OH group in the C₃ position (α-glycol grouping). Consequently, germerine is not 15-(d)-2'-hydroxy-2'-methylbutyryl-3-(l)-2'-methylbutylgermine [1] but 3-(d)-2'-hydroxy-2'-methylbutyl-15-(l)-2'-methylbutyrylgermine. This structure is not contradicted by the NMR spectrum (CHCl₃), either. The chemical shift of the signals of the protons of the methyl group of the HMB residue of germerine (1.40 ppm) is...
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 C3).

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 a SF-4A spectrophotometer (ε 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, C₃₇H₅₉O₁₁N, mp 202-204°C (benzene), [α]₂°₁ = -7° (c 0.91; pyridine), Rf 0.49 (1). IR spectrum, cm⁻¹: 3360 (OH), 2940, 1465, 1385 (−CH₃ and −CH₂), 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 OCOCH₃ group. The UV spectrum of a sulfuric acid solution of the alkaloid taken 24 h after dissolution (λmax 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 germaine in them. The ether treatment of the acidified hydrolysate yielded two organic acids which were identified by their Rf 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, C₃₈H₅₃O₁₀N, mp 200-202°C (ethanol), [α]₂°₁ = -11° (c 0.8; pyridine), Rf 0.6 (1) [3]. The NMR spectrum of the alkaloid has the three-proton singlet of an OCOCH₃ 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 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.

The fractions including a substance with Rf 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 Rf values of both samples were 0.31.

By the same treatment, the fractions containing a substance with Rf 0.0 (1) yielded a crystalline precipitate with mp 219-221°C (methanol), Rf 0.51 (2), which coincides with the Rf 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.