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The Synthesis of Monoacetylneogermitrine and Germidine from Germine

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The first synthesis of a naturally-occurring ester, germidine, from germine is described. Germine was converted to mono-D-(−)-methylethylacetylgermine by treatment with one equivalent of the acid chloride. Acetylation of this monoester with acetic anhydride led to monoacetylneogermitrine which on methanolysis yielded germidine. As a result, the positions of the acid residues in a number of natural esters may be assigned in terms of the relative ease in which the hydroxyl groups of the germine molecule are esterified.

Previous investigations[2,3] have shown that germine, one of the principal alkamines in the family of Veratrum alkaloids, undergoes stepwise and selective esterification to form mono-, di-, tri-, tetra- and occasionally pentaesters.[4-7] Certain of the synthetic di- and triesters were found to possess hypotensive activities comparable with the activities exhibited by naturally-occurring di- and triesters of germine. This observation and the fact that the molecular rotation values of the synthetic esters correspond closely to those observed for the natural esters made it seem likely that the same hydroxyl groups were esterified in both cases. No naturally-occurring ester had been prepared from germine, however, to prove this point.

Germidine[8] has been shown to be a diester of germine containing one D-(−)-methylethylacetic acid and one acetic acid residue. This ester also has been related[8] to the triester, neogermitrine, and tetraester, acetylneogermitrine, the latter compounds possessing one and two additional acetic acid residues, respectively. Since we had in hand from earlier work[2,3] a mono-D-(−)-methylethylacetylgermine (prepared from germine by acylation with one equivalent of acid chloride) an attempt was made to convert this monoester directly to germidine (or an isomer) by treatment with one equivalent of acetic anhydride in pyridine. Contrary to our previous experience whereby diesters could be prepared in this manner using larger acid residues, acetic anhydride on the monoester was less selective and gave rise to a mixture of polyesters, the majority of the monoester being recovered unchanged. The same result was obtained when the ester was treated with two equivalents of acetic anhydride in an attempt to prepare neogermitrine. Treatment of mono-D-(−)-methylethylacetylgermine with excess reagent in pyridine, however, gave an excellent yield of a triacetylmono-D-(−)-methylethylacetylgermine. A comparison of physical properties and infrared spectra of this compound in Nujol with authentic monoacetylneogermitrine[8] showed them to be identical. It can be concluded then that the methylethylacetyl radical in the synthetic esters occupies the same site in the natural esters, germidine, neogermidine (isogermidine)[9,10] and neogermitrine since they all can be converted to monoacetylneogermitrine.

Synthetic D-(−)-methylethylacetylgermine is isomeric but not identical with protoveratridine and, although it had previously been reported by Kupchan and Deliwala[9a] that protoveratridine gives rise to acetylneogermitrine on acetylation, this apparent discrepancy has now been resolved[9b] with the finding that the acetylation product of protoveratridine, triacetylprotoveratridine, is actually an isomer of acetylneogermitrine. In addition these authors have shown that neogermidine is not related to protoveratridine but does give acetylneogermitrine on acetylation.

As stated above, germidine could not be prepared by direct acetylation of methylethylacetylgermine; however, a synthetic route by way of monoacetylneogermitrine proved successful. It had been previously demonstrated that one of the acetyl groups of neogermitrine is susceptible to methanolysis particularly rapidly and that by arresting the reaction after 18 hours germidine could be isolated as the principal product.[8] By the same procedure germitrine[5] and germanitrine[11] have been converted to the corresponding diesters, germerine and germanidine.

When monoacetylneogermitrine (synthetic) was dissolved in methanol and allowed to stand at room temperature for 22 hours, germidine was isolated in 38% yield by chromatography of the product on acid-washed alumina. The diester was obtained from chloroform–ether melting at 238–239°, and the lower melting isomorphic form was obtained from alcohol–water, m.p. 202–203°. Mixed melting points of these samples with the two isomorphic forms of authentic germidine gave no depression and the infrared spectra of all four compounds taken in Nujol were identical.

It is now clear that the five hydroxyl groups of germine differ markedly in the ease in which they may be esterified and that the naturally-occurring esters possess their acid residues at the same sites as the esters prepared by direct acylation of germine. If the hydroxyl groups of germine are designated A, B, C, etc., in order of decreasing ease of esterification, then on the basis of the synthetic

(1) Squibb Institute for Medical Research, New Brunswick, N. J.

(2) F. L. Weisenborn and J. W. Bolger, *Chemistry and Industry*, 197 (1953).

(3) F. L. Weisenborn, J. W. Bolger, D. B. Rosen, L. T. Mann, Jr., L. Johnson and H. L. Holmes, THIS JOURNAL, **76**, 1792 (1954).

(4) W. Poethke, *Arch. Pharm.*, **275**, 571 (1937).

(5) J. Fried, H. L. White and O. Wintersteiner, THIS JOURNAL, **72**, 4621 (1950).

(6) H. L. White, *ibid.*, **73**, 492 (1951).

(7) In reactions in which germine mono- and diesters were treated with excess acylating agents we have observed[2,3] the formation of tetraesters only.

(8) J. Fried, P. Numerof and N. H. Coy, THIS JOURNAL, **74**, 3041 (1952).

(9) (a) S. M. Kupchan and C. V. Deliwala, *ibid.*, **74**, 3202 (1952); (b) **76**, 5545 (1954).

(10) G. S. Meyers, W. I. Glenn, P. Morozovitch, R. Barber and G. A. Grant, *ibid.*, **74**, 3198 (1952).

(11) M. W. Klohs, M. D. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek, *ibid.*, **74**, 4473 (1952).

TABLE I

POSITION OF ACID RESIDUES IN SOME ESTERS OF GERMINE

Ester	$[\alpha]_D$(pyr.)	$(OH)_A$	$(OH)_B$	$(OH)_C$
D-(−)-Methylethylacetylgermine (synthetic)	−25°	Methylethylacetic		
Protoveratridine	− 9		Methylethylacetic	
Germerine	− 7	Methylethylglycolic	Methylethylacetic	
Neogermidine (Isogermidine)	−60	Methylethylacetic		Acetic
Germidine	−11	Methylethylacetic	Acetic	
Germitrine	−69	Methylethylglycolic	Methylethylacetic	Acetic
Neogermitrine	−78	Methylethylacetic	Acetic	Acetic

work described above and rotational contributions of the hydroxyl groups, it is possible to assign positions to acid residues of a number of natural esters. Germerine and germidine show approximately the same molecular rotation value and thus hydroxyl groups A and B are very likely involved in both esters. Since germerine is related to protoveratridine and the latter is not identical with synthetic methylethylacetylgermine, the methylethylacetic radical of protoveratridine and germerine (as well as germitrine) must occupy hydroxyl group B of the germine molecule. The diester neogermidine (isomeric with germidine) possesses a high negative rotation for a diester and since it is known[3] from synthetic work that hydroxyl group C imparts a large negative shift to the rotation on esterification, it is very likely that the acetic acid moiety of neogermidine is at this site. The position of the acid residues of six natural esters is summarized in Table I. Thus it should be possible to prepare the triester, germitrine, by successive acylation of germine with methylethylglycolic acid, methylethylacetic acid and acetic acid.

Pharmacology.—The hypotensive activity[12,13] of synthetic germidine was found to be 0.30 μg. [0.26–0.34].

Experimental[14]

Mono-D-(−)-methylethylacetylgermine.—This ester was prepared by esterification of germine with one equivalent of D-(−)-methylethylacetyl chloride as previously described[3]; m.p. 236–238°, $[\alpha]^{24}D$ −25.6° (pyridine).

Triacetylmono-D-(−)-methylethylacetylgermine (Acetylneogermitrine).—Mono-D-(−)-methylethylacetylgermine (0.610 g.) was dissolved in a mixture of acetic anhydride (15 ml.) and pyridine (15 ml.) and the solution allowed to stand overnight. The excess acetic anhydride and pyridine were removed by distillation *in vacuo*, the residue taken up in chloroform, treated with dry ammonia gas and filtered from the precipitated ammonium acetate. The chloroform solution was evaporated and the residue crystallized from acetone in colorless needles, 0.502 g., m.p. 252–253°. Recrystallization from acetone gave an analytical sample, m.p. 257–259°, $[\alpha]^{23}D$ −92° (pyridine) (reported[8] m.p. 248–249°, $[\alpha]^{24}D$ −88°).

Anal. Calcd. for $C_{33}H_{57}O_{12}N$: C, 63.40; H, 7.98. Found: C, 63.17; H, 8.02.

Mixed melting point of this product with an authentic sample of monoacetylneogermitrine (m.p. 250–252°) gave a melting point of 253–254°. Comparison of the infrared spectrum in Nujol with that of an authentic sample showed that the compounds were identical.

Monoacetylmono-D-(−)-methylethylacetylgermine (Germidine).—Synthetic monoacetylneogermitrine (0.43 g.) was dissolved in methanol (45 ml.) and the course of the methanolysis reaction was followed by the change in optical rotation of the solution. After 22 hours no further change was observed over a 2-hour period and the methanol was then removed by distillation *in vacuo*. Attempts to crystallize the residue from ether–petroleum ether gave only gummy, partially crystalline material so the product was purified by chromatography on 13 g. of Merck acid-washed alumina. Elution of the column with chloroform gave 145 mg. (38%) of germidine, m.p. 228–229°, after crystallization from ether–petroleum ether. Two recrystallizations from chloroform–ether raised the melting point to 239–240°, $[\alpha]^{24}D$ −11.6° (pyridine). Recrystallization of a sample from alcohol–water gave the isomorphic form, m.p. 202–203°.

Anal. Calcd. for $C_{34}H_{53}O_{10}N$: C, 64.22; H, 8.38; equiv. wt., 636. Found: C, 64.57; H, 8.62; equiv. wt. (perchloric acid titration), 639.

Mixed melting points of both forms taken with the corresponding form of authentic germidine showed no depression. The infrared spectra of all four compounds taken in chloroform or nujol were identical.

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(12) G. L. Maison and J. W. Stutzman, *Arch. intern. pharmacodynamie*, **85**, 357 (1951).

(13) Expressed as micrograms per kilogram of anesthetized dog per minute required for a ten-minute intravenous infusion to lower the mean arterial blood pressure 30% when administered according to the method of G. L. Maison and J. W. Stutzman.[12] The bracketed numbers express the 95% confidence limits.

(14) All melting points are corrected.