140°. The Ehrlich test was negative at room temperature and positive at steam-bath temperature, indicating that the acetyl group was attached to the pyrrole ring rather than phenyl.

Anal. Calcd. for $C_{16}H_{17}NO_3$: C, 70.85; H, 6.27; N, 5.17. Found: C, 71.18; H, 6.50; N, 4.99.

2-Phenyl-3-acetyl-5-methylpyrrole.—The pyrrolecarboxylic acid ester (1.5 g.) was refluxed for four hours in a solution of 1.0 g. of sodium hydroxide in 150 ml. of 50% ethanol. The product presumed to be 2-phenyl-3-acetyl-5-methyl-4-pyrrolecarboxylic acid, was precipitated by gradual addition of a mixture of 4 ml. of concentrated hydrochloric acid in 96 ml. of water. Crystallization from 50% ethanol yielded 0.6 g. of cream-colored crystals which melted at 249–251° (uncor.) with evolution of gas.

The acid was decarboxylated by heating in a test-tube immersed in an oil-bath maintained at 250–255° until gas evolution ceased. After being cooled, the dark-brown mass was crystallized from petroleum ether (b. p., 95–127°) containing a little benzene and Norit. The yield was 0.2 g. of white crystals which melted at 142–143°. The Ehrlich test at room temperature was negative at the beginning and became weakly positive after standing for several hours. At steam-bath temperature, the test was immediately positive.

Anal. Calcd. for $C_{13}H_{13}NO$: C, 78.39: H, 6.53; N, 7.04. Found: C, 78.12; H, 6.82; N, 6.85.

Ultraviolet Absorption Spectra.—The spectra were obtained with a Beckman Model DU spectrophotometer. The solvent was 95% ethanol, used without further purification. The spectrum of 2-phenyl-4-methylpyrrole is included for purposes of comparison.

Acknowledgment.—The authors are indebted to Miss Ruth Brand and Miss Jane Dixon for the carbon, hydrogen and nitrogen analyses.

Summary

Acetylation of 3,2′-nicotyrine has produced a mixture of two isomers which can be separated by fractional distillation. They have been assigned the structures of 1-methyl-2-(3-pyridyl)-5-acetyl pyrrole and 1-methyl-2-(3-pyridyl)-4-acetylpyrrole.

Structures were assigned to the two products by comparison of their ultraviolet absorption spectra with those of 2-phenyl-4-methyl-5-acetylpyrrole, 2-phenyl-3,5-dimethyl-4-acetylpyrrole and 2-phenyl-3-acetyl-5-methylpyrrole. The syntheses of these new pyrrole derivatives are described.

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[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

The Hypotensive Principles of *Veratrum Viride*

BY JOSEF FRIED, HOWARD L. WHITE AND O. WINTERSTEINER

Recent clinical investigations have shown that the peroral administration of the powdered roots and rhizomes of *Veratrum viride* is instrumental in reducing the elevated blood pressure of patients with essential hypertension.[1] This paper describes the isolation and characterization of the hypotensive principles involved.

The progress of the purification was followed by peroral assay in the hypertensive patient. In this we enjoyed the invaluable coöperation of Drs. E. D. Freis and J. R. Stanton of the Robert Dawson Evans Memorial, Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine, who have thus evaluated for us well over 100 fractions resulting from the chemical work. The details of the assay procedure and the results obtained have meanwhile been communicated by these investigators elsewhere.[2] At a later stage the clinical data were supplemented by assay for hypotensive activity in the normal anesthetized dog or cat by the intravenous route.[3] For various good reasons we relied for guidance in the fractionation work on the peroral test in the human rather than on the intravenous animal assay. Unless stated otherwise the activity figures in the text and tables refer to the average minimum effective dose (MED) per patient producing a fall in the basic mean ((systolic + diastolic)/2) blood pressure of at least 15% from the starting level on peroral administration.[2]

Of the earlier investigations of the alkaloids present in *Veratrum viride*, the most significant are those of Mitchell[4] and Wright,[5] who were able to secure in crystalline form the bases jervine, pseudojervine and rubijervine. More recently the thorough investigations of Seiferle, Johns and Richardson[6] and of Jacobs and Craig[7,8] have expanded the list of known crystalline alkaloids to nine by the isolation of germine,[6] protoveratridine,[6] veratrosine,[7] isorubijervine,[8] veratramine,[8] and the unnamed secondary base $C_{27}H_{41}O_4N$.[8] In addition there have been obtained considerable amounts of amorphous alkaloids which resisted all attempts at crystallization.

In our search for the entity or entities responsible for the therapeutic effect of *Veratrum viride* we followed in the initial steps the efficient and rational procedure devised by Jacobs and Craig,[7] which takes advantage of the fact that the free alkaloids (alkamines) are more soluble in benzene than the glycosidic alkaloids (pseudojervine and veratrosine). Thus, the former are extracted from an ammoniacal suspension of the powdered

(1) E. D. Freis and J. R. Stanton, *Am. Heart J.*, **36**, 725 (1948).

(2) E. D. Freis, J. R. Stanton and F. C. Moister, *J. Pharmacol. Exptl. Therap.*, **98**, 166 (1950).

(3) By Dr. S. Krop, private communication.

(4) C. L. Mitchell, *Am. J. Pharmacy*, **46**, 100 (1874).

(5) C. R. A. Wright, *J. Chem. Soc.*, **35**, 421 (1879).

(6) E. J. Seiferle, I. B. Johns and C. M. Richardson, *J. Econ. Entomology*, **35**, 35 (1942).

(7) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **155**, 565 (1944).

(8) W. A. Jacobs and L. C. Craig, *ibid.*, **160**, 555 (1945).

roots and rhizomes with benzene, and the latter are removed by subsequent extraction with ethanol. Only the benzene-extractable fraction showed hypotensive activity.[9] This material was further fractionated essentially according to Jacobs and Craig, except that for reasons which will become clear presently, in operations involving liberation of the free bases from their salts by alkalinization the *p*H was not allowed to rise above 10, and the temperature was kept low by ice cooling. Of the six alkamines encountered by Jacobs and Craig in this fraction, jervine, veratramine, rubijervine and isorubijervine could be isolated without difficulty. However, germine had to be secured by alkaline hydrolysis of an appropriate amorphous fraction, nor were we able to duplicate the isolation of the unnamed secondary base, $C_{27}H_{41}O_4N$, reported by these authors. In accordance with the findings in the previous investigations there remained after removal of the crystalline constituents a substantial residue which could not be crystallized. As can be seen from the activity data and percentage yields recorded in Table I, the hypotensive activity was found to reside in this amorphous fraction (MED 3.5 mg.),

TABLE I

FRACTIONATION OF CRUDE ALKALOIDS OF *Veratrum Viride*

Alkaloid or fraction	Maximum dose administered,[a] mg.	Mean effective dose[b] (MED), mg.	% of crude root
Root powder		225	100
Total benzene extractable alkaloids		10	1.1
Total alkaloids from alcohol extract	33, inactive		0.18
Jervine	9, inactive		.67
Veratramine	4, inactive		.026
Rubijervine	4, inactive		.004
Isorubijervine	4, inactive		.035
Germine	8, inactive		[c]
Amorphous fraction from benzene extr.		3.5	36
Tertiary bases		3.2	26
Combined plates 1–7 from 8-plate Craig distribution		1.8	.13
Benzene–2.5% methanol eluate from alumina chromatogram		1.2	.065
Fraction I (tube 15, K = 1.73), from 24-plate Craig distribution		0.7	.045
Fraction II (tube 6, K = 0.35), from 24-plate Craig distribution		5.5	.020

[a] This column refers to "inactive" fractions and alkaloids only. [b] The values listed in this column are those reported by Freis, *et al.* (see ref. 2). [c] Isolated by saponification of the amorphous fraction from the benzene extract.

(9) The biologically inactive fraction extracted from the roots with ethanol yielded pseudojervine (D-glucosidojervine) but no veratrosine (D-glucosidoveratramine). Instead there was isolated a new, non-glycosidic secondary base, $C_{27}H_{41}O_3N$, which showed the same ultraviolet absorption spectrum as jervine. The experimental details will be given in a later communication.

while the crystalline alkamines were inactive in comparable doses.

During the following attempts to fractionate the amorphous bases it became evident that the hypotensive principle was not as stable as could be surmised from the good recovery of activity in the initial fractionation of the benzene extractable material. Thus extensive inactivation occurred on chromatographing the free amorphous bases on acetic acid-washed alumina, as shown by the fact that the combined eluates in a dose corresponding to more than twice the MED of the starting product failed to elicit any hypotensive response. Later it was also noted that prolonged exposure of active fractions to aqueous alkali or methanolic barium methylate at room temperature resulted in complete loss of their hypotensive power. It was this finding which impelled the introduction throughout the isolation procedure of the precautions against overalkalinization referred to above. The cause of the observed instability under the conditions mentioned will become clear later.

Table I illustrates the procedure finally adopted for the purification of the amorphous bases in terms of the potencies and yields of the active fractions obtained in each step. First the amorphous alkaloids were subjected to selective N-acetylation with acetic anhydride in methanol. The secondary bases present are thereby converted into neutral N-acetyl derivatives, while the tertiary bases remain unaffected and hence can be separated from the former by extracting the chloroform solution of the acetylated mixture with acid. Preliminary experiments had shown that the bulk of the activity is recovered in the tertiary base fraction, which accounts for about 70% of the amorphous alkaloids. While the gain in potency is thus inconsequential, and the separation not as sharp as might be expected (for instance veratramine, if present, for reasons unknown is not N-acetylated under these conditions and hence is extracted with the tertiary bases), it is our experience that the removal of the bulk of the secondary bases improves the efficiency of the subsequent operations.

Next the tertiary bases were subjected to a Craig counter-current distribution in 9 funnels, using benzene and 2 M acetate buffer at *p*H 5.5 as the solvents. The products recovered from funnels 8 and 0, representing, respectively, the most organophilic and hydrophilic fractions and accounting together for 40–50% of the starting material, were inactive with 8 mg. each. The activity was found to be scattered over the intermediate fractions 1–7 (average MED 2 mg.). The distribution curve, based on the weight of the free bases in each funnel, showed besides the two high distal peaks at plates 8 and 0, a broad middle band culminating in the region of plates 4–6. It was clear from these data that nothing was to be gained preparatively by collecting the 7 middle fractions separately. In actual practice this ma-

terial was secured by using a greatly simplified distribution scheme which required 2 funnels and involved only 14 single distributions and transfers instead of the 36 necessary in a complete 8 plate distribution series (see experimental). The product thus obtained (MED 1.8 mg.) when taken up in acetone, deposited some inactive crystalline material consisting mainly of isorubijervine, which was removed. Attempts to crystallize the remaining amorphous portion were unsuccessful. However, alkaline hydrolysis, while abolishing the activity, readily afforded crystalline germine in good yield. This finding could best be explained by assuming that the hypotensive principle was an ester of germine and hence presumably related to protoveratridine (α-methylbutyrylgermine) and the alkaloid germerine which Poethke[10] had isolated from the related species *Veratrum album* and shown to be a diester, namely, a α-methylbutyrate-methylethylglycolate, of germine. This conclusion received support by the isolation from the acidic fraction of the hydrolyzed mixture of acetic acid (as the *p*-phenylphenacyl ester), 1-α-methylbutyric acid and *d*-methylethylglycolic acid (as the free acids).

Further purification was achieved by chromatographing the active material from the 8-plate distribution in benzene solution on sulfuric acid-washed alumina, which in contrast to the acetic acid-treated preparation was found to be innocuous to the activity. After removal of some biologically inert material by continued washing with benzene the bulk of the activity was recovered in a fraction eluted with benzene containing 2.5% methanol. Comparison of the 24-plate Craig distribution diagram of this fraction with that of the starting product (plates 1–7 of 8 plate distribution) showed that the chromatographic step had removed residual amounts of the biologically inactive products, which in the 8-plate distribution had appeared in the two distal funnels. The distribution curve of the chromatographic fraction (Fig. 1) exhibited two well differentiated maxima at tubes 6 and 15, indicative of the presence of two major components only. The material in tube 15 corresponding to a distribution coefficient K = 1.73, showed very high potency in the clinical test (MED 0.7 mg.), while the peak fraction in tube 6 (K = 0.35) was considerably less potent (MED 5.5 mg.).

Redistribution in the Craig machine of the combined contents of tubes 12–20 and of tubes 5–7 showed that the two active components with K = 1.73 and 0.35, respectively, were essentially homogeneous except for the presence of a small amount of a contamination of higher K in the former. For the isolation of these two entities in larger amounts we had to resort eventually to a 24-plate distribution on a preparative scale in funnels. The results duplicated in every respect those of the analytical experiment. The fractions corresponding to the 2 maxima in the distribution curve, henceforth for convenient reference called Fraction I (tube 15, K = 1.73) and Fraction II (tube 6, K = 0.35), crystallized readily from aqueous methanol and aqueous ethanol, respectively.

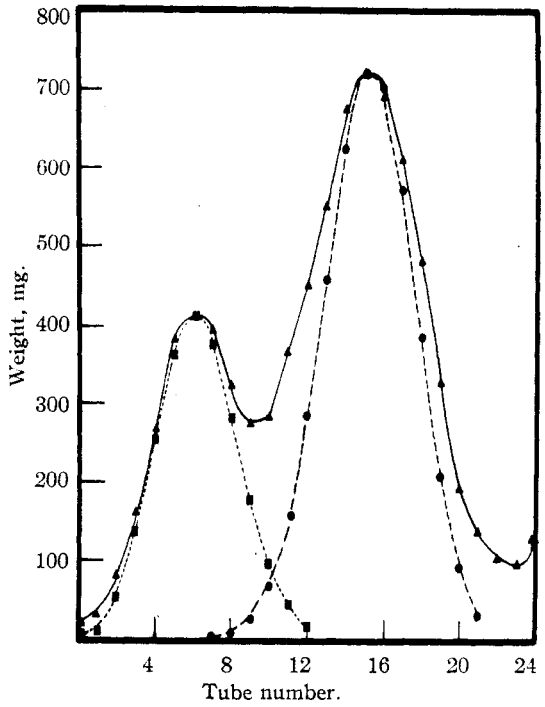

Fig. 1.—Craig counter-current distribution curve of highly purified, active concentrate of *Veratrum viride*: -▲-▲- experimental curve; --■--■-- theoretical curve, K = 0.35; -●-●- theoretical curve, K = 1.73.

The compound obtained from Fraction II after purification melted at 230–231°[11] and showed $[\alpha]^{25}D$ +13° in chloroform. Hydrolysis with 0.1 M aqueous methanolic alkali at room temperature yielded germine, acetic acid and α-methylbutyric acid. The alkamine was characterized by rotation, analysis and conversion to acetonylgermine hydrochloride,[12] while the acids were separated and identified as their *p*-phenylphenacyl esters. The analysis of the free base and of the thiocyanate (m. p. 242–244° (dec.)), and particularly the quantitative determination of the total volatile acids liberated from the former by hydrolysis with toluenesulfonic acid were in accord with the theoretical figures for $C_{34}H_{53}O_{10}N$, *i. e.*, a diester of germine containing one mole each of the above acids. This new ester alkaloid was named **germidine,** denoting a diester of germine.

An analogous study of the crystalline alkaloid from Fraction I (m. p. 197–199°, $[\alpha]^{25}D$ +11° chloroform; thiocyanate, m. p. 228–231° (dec.)) revealed that it was composed of germine, α-methylbutyric acid and methylethylglycolic acid and in this respect resembled the diester germerine

(10) W. Poethke, (a) *Arch. Pharm.*, **275**, 357 (1937); (b) **275**, 571 (1937); (c) **276**, 170 (1938).

(11) All melting points are corrected.

(12) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **148**, 57 (1943).

from *Veratrum album*. In fact, melting point and specific rotation were close to the values reported by Poethke[10b] for germerine (m. p. 195°, $[\alpha]^{20}D$ +10.8°). However, the solubility properties of our product seemed to differ from those described by that author, and, more importantly, the determination of the total volatile acids gave a figure indicating the presence of three rather than two acid radicals. Of additional observations seemingly supporting the triester nature of this alkaloid only two need to be mentioned here: First, it was difficult to see why this compound, if a diester, should be as sharply differentiable from the diester germidine by Craig distribution as was actually the case. Secondly, it had been observed that Fraction I when treated with acetic acid-washed alumina was transformed in 35% yield to a compound differing from the above product by its higher melting point (202–204°) and its considerably lower solubility in benzene and acetone. This third crystalline substance on hydrolysis likewise yielded germine, α-methylbutyric acid and methylethylglycolic acid, but the quantitative determination of the acids definitely indicated only two equivalents. As will be shown later, this product was pure germerine. Suspecting this already at the time of its isolation we assumed that it was formed by partial hydrolysis from the ester alkaloid obtained by direct crystallization of Fraction I and that the latter consequently was a triester. A mono-α-methylbutyrate-di-methylethylglycolate of germine seemed to meet best the analytical findings. We named this apparently new ester alkaloid **germitrine** to indicate its triester nature.

The results obtained up to this point, except those pertaining to the third alkaloid mentioned above, were communicated in a preliminary note.[13] Subsequent findings, however, made it necessary to revise the views presented there regarding the nature of "germitrine." The new evidence derived from the following observations.

At that time "germitrine" had been assayed only in preliminary tests by the intravenous route in the dog or cat. The MED/kg. was found to lie at about 0.6 microgram, and little difference was at first noted between the crystalline product and the amorphous Fraction I from which it was derived. When larger amounts became available for clinical assay, it developed that "germitrine" showed only about one half to one third of the potency of Fraction I on both oral and intravenous administration, and this was later confirmed by a larger number of experiments in the dog. Coincidental with the search for the more active entity which thus could be assumed to be present in Fraction I it was observed that the amorphous parent material exhibited marked levorotation in pyridine ($[\alpha]_D$ −65°), whereas the specific rotation of "germitrine" in this solvent was close to zero (in chloroform the difference is very much smaller, the respective $[\alpha]_D$ values being 0 and +11°). This was followed by the discovery that the total Fraction I after treatment with aqueous methanol (the solvent used for the crystallization of "germitrine") was no longer levorotatory in pyridine ($[\alpha]_D$ 0°). Standing in absolute methanolic or ethanolic solution produced the same effect, only at a slower rate. It was clear, therefore, that a chemical change had occurred when Fraction I was crystallized from aqueous methanol, and that the resulting crystalline product, "germitrine," was an artifact and not present as such in that fraction.

A careful reexamination of the formation and properties of this substance finally convinced us that it was not a triester of germine, but slightly impure germerine. The high value for total volatile acids which had originally misled us was traced to the presence of small amounts of acetic acid in a new sample of toluenesulfonic acid which had been used as the hydrolyzing agent in those particular determinations. Conclusive identification was achieved by comparison with a specimen of authentic germerine from *Veratrum album* kindly made available to us by Professor W. Poethke of the University of Leipzig. While there was a slight difference between the melting points of the methanolysis product (200–203°) and of the purified reference sample (198–202°), the melting point of the mixture was not depressed.

Nevertheless we were correct in our presumption that the bulk of Fraction I consists of a triester of germine. One of the three ester groups is an acetoxy group which shows the unusual property of being unstable at room temperature to methanol–water and, to a lesser degree, to absolute methanol and aqueous or absolute ethanol. The reaction involved is not hydrolysis but transesterification to methanol. This was shown by the isolation from the mother liquor of the crude germerine crystals formed from Fraction I of a volatile neutral substance which clearly was methyl acetate, since on alkaline hydrolysis and treatment with *p*-phenylphenacyl bromide it yielded acetic acid in the form of the crystalline *p*-phenylphenacyl ester.

For the isolation of the unstable precursor of germerine the effect of various solvents and solvent mixtures on the rotation of Fraction I in pyridine was studied. No or only minor changes were observed after standing for 24 hours in benzene, chloroform, acetone and 50% aqueous acetone. Employing the latter solvent mixture we succeeded in isolating the genuine triester in crystalline form and good yield (50%). This ester alkaloid differs from germerine by its higher melting point (216–219°), the more negative rotation values in pyridine (−69°) and chloroform (−4°), and its much higher solubility in acetone. The

(13) J. Fried, H. L. White and O. Wintersteiner, THIS JOURNAL, **71**, 3260 (1949). Due to an error made in preparing an abbreviated version of the note it was implied there that germidine was derived from Fraction I ($K = 1.73$) and germitrine from Fraction II ($K = 0.35$), while actually the reverse was true.

TABLE II

PROPERTIES OF GERMIDINE, GERMITRINE, GERMERINE AND PROTOVERATRINE

Substance	M. p. Base	M. p. Thiocyanate	$[\alpha]_D$ $CHCl_3$	$[\alpha]_D$ Pyridine	Hypotensive activity: Intravenous Dog, μg./kg.	Intravenous Human, mg./patient	Oral human mg./patient	Hydrolysis products
Germidine	230–231	242–244 dec.	+13°	−11°	0.8	0.1–0.4	2.5–3.5	Germine, acetic acid, α-methylbutyric acid
Germitrine	216–219	228–232 dec.	−4°	−69°	0.4–0.6	0.05–0.06	1.0–1.2	Germine, acetic acid, α-methylbutyric acid, methylethylglycolic acid
Germerine	200–203	230–232 dec.	+16°	− 7°	0.8	0.1–0.15	2.5–3.0	Germine, α-methylbutyric acid, methylethylglycolic acid
Protoveratrine (*veratrum album*)	269–272	221–223 dec.[10b]	−9°[10b]	−40°[15a]	1.0	0.1–0.15	0.9–1.5	Protoverine, acetic acid, α-methylbutyric acid, methylethylglycolic acid

volatile acid determination, carried out on the free base under carefully controlled conditions, gave a figure consistent with the presence of 3 acyl radicals. The nature of the acidic components followed from the degradation of the new alkaloid with 50% aqueous methanol to methyl acetate and further to acetic acid, and to germerine, which in this case was obtained in practically pure state. This result, taken in conjunction with the near-identity of the specific rotations in pyridine of the pure triester and Fraction I, proves conclusively that germerine is not present as such in the amorphous product (and very probably not in the root), but arises from the triester by methanolysis. As mentioned earlier, very pure germerine is also formed when Fraction I is chromatographed on acetic acid-washed alumina. Repetition of this experiment with the crystalline triester gave the same result. In this connection it was ascertained that benzene containing 2.5% methanol (the eluting agent used) does not appreciably alter the specific rotation of the triester in pyridine. This shows that the loss of the acetyl group must occur on the column, yet it does not entirely exclude participation of the methanol, since the active surface may exert a catalytic effect on the methanolysis reaction equivalent to a manyfold increase in the concentration of this reactant.

As expected the triester showed about the same potency in the clinical test as Fraction I (MED 1.0–1.2 mg.). Since this fraction preponderates in weight over the less active Fraction II, there can be little doubt that this alkaloid accounts for the greater part of the hypotensive activity of the total amorphous bases and probably also of the root. In view of our previous commitment to the term germitrine as denoting a triester of germine we propose to transfer this name from the compound now identified as germerine to this genuinely new alkaloid, and shall henceforth use it with this meaning.

The properties of the two native ester alkaloids and of germerine, inclusive of the assay results obtained on intravenous injection in man and in the anesthetized dog, are listed in Table II. It can be seen that generally the ratio of the peroral to the intravenous MED in man is of the order of 20:1. For comparison the corresponding data for the triester alkaloid protoveratrine from *Veratrum album* are included. This compound, first isolated by Salzberger,[14] contains the three acids present in germitrine esterified to the alkamine protoverine, $C_{27}H_{43}O_9N$, which differs from germine by the presence of an additional oxygen atom.[10b,15b] Its pharmacological properties have been studied by a number of investigators[16,17] and lately more extensively by Krayer, Moe and Mendez,[18] so that its powerful hypotensive action in the experimental animal is already a matter of record. In the patient protoveratrine proved to be as active as germitrine by either route of administration (Table II).

Poethke[10b] had shown that the α-methylbutyric acid present in germerine is the *l*-enantiomorph but gave no optical data for the other acidic component, methylethylglycolic acid. To gain insight into the latter point we had to resort, for want of sufficient quantities of the crystalline product, to hydrolysis of the total tertiary bases. Appropriate fractionation of the volatile acidic constituents yielded *l*-α-methylbutyric acid of $[\alpha]_D$ −25° in water (Poethke,[10b] −22°), and *d*-methylethylglycolic acid, $[\alpha]_D$ +4.4° in water, and +12° in 0.2 *N* NaOH.

It was of interest to ascertain whether the instability to aqueous methanol exhibited by germitrine was also manifest in protoveratrine. That this might be the case would be surmised from the fact that protoveratrine likewise shows a much higher specific rotation in pyridine (−40°) than in chloroform (−9°), and hence appeared to contain the acetoxy group involved in this phenomenon in the identical location. Protoveratrine is only

(14) G. Salzberger, *Arch. Pharm.*, **228**, 462 (1890).

(15) W. A. Jacobs and L. C. Craig, (a) *J. Biol. Chem.*, **143**, 427 (1942); (b) **149**, 271 (1943).

(16) T. W. Eden, *Arch. exper. Path. Pharmakol.*, **29**, 440 (1892).

(17) R. Boehm, *ibid.*, **71**, 269 (1913).

(18) O. Krayer, G. V. Moe and R. Mendez, *J. Pharmacol.*, **82**, 167 (1944).

sparingly soluble in aqueous and absolute methanol, and prolonged shaking (four days, 80% methanol) was necessary to effect complete solution. From the volatile portion of the reaction products after saponification with alkali, acetic acid and a small amount of methylethylglycolic acid were isolated as the *p*-phenylphenacyl esters. The remaining alkaloidal fraction was a mixture from which only a very small fraction could be obtained in crystalline form. At any rate, the experiment showed that ester groups other than the acetoxy group are susceptible to alcoholysis in alkaloids of this type. While in germitrine the acetoxy group is obviously the most reactive one, the other groups are affected also, though more slowly. We deduce this from the observation that the melting point of the essentially pure germerine which results from the methanolysis of germitrine becomes progressively less sharp on repeated recrystallization from dilute methanol. That germerine is obtained in good yield in this reaction has to be ascribed not only to the comparatively greater speed with which the acetoxy group is methanolyzed, but also to the ease with which this alkaloid crystallizes under the conditions employed and thus escapes further attack. This conclusion also receives some support from the outcome of a similar methanolysis experiment carried out on germine pentaacetate. This polyester, which has been described as an amorphous powder by Poethke[10b] and which has now been obtained in crystalline form, is likewise much more levorotatory in pyridine than in chloroform. However, under conditions paralleling those used with germitrine (24 hours in aqueous methanol solution) a substantial part of the compound suffered methanolysis of 4 of its acetoxy groups, as evidenced by the isolation in 25% yield of a crystalline *mono*acetate. There is no precedent for such facile loss of several acetyl groups in polyacetates of ordinary hydroxylated steroids. Undoubtedly it is the nitrogenous group in the steroidal ester alkaloids which assumes the function of a basic catalyst in this reaction.

In view of the possibility that some enzymatic degradation of the active principles may occur before or during the drying of the plant material, a 150 lb. batch of fresh roots and rhizomes was put through the isolation procedure. To minimize enzymatic attack the plant parts were ground soon after collection in the presence of 95% ethanol, and then further extracted with this solvent. The residue of the alcoholic extract was then worked up in the same manner as the dried roots. No essential differences were noted in respect to the nature and amounts of the inactive alkamines isolated, and the distribution of the activity in the various fractions. At the end Fractions I and II were obtained in the usual yields. However, some additional impurities seemed to have been carried through, as the two native crystalline ester alkaloids proved to be difficult to purify.

Experimental[19]

Preparation of an Active Concentrate of *Veratrum viride* (Amorphous Bases).—Ground dried roots and rhizomes of *Veratrum viride* (60 mesh) collected in North Carolina were extracted in 75-lb. portions with benzene and dilute ammonia. The extraction was carried out in a 100-gal. ceramic crock provided with an opening of two inches in diameter close to the bottom of the crock. To this opening a pipe provided with a gate valve was attached, which led to the top of a second 100-gal. crock. The bottom of the first crock was covered with a six inch layer of crushed stone and a loose plug of glass wool held in place by a wire gauze was fitted before the outlet from the inside of the crock. To the crock were added 37.5 gal. of benzene, 1.5 liters of water, and with vigorous stirring the 75 lb. of root powder. To the stirred suspension a mixture of 3 gal. of water and 0.45 gal. of concentrated ammonia was added and stirring was continued overnight. The mixture was then allowed to settle for several hours and the solvent was percolated through the roots at a rate of 60–100 ml. per minute by opening the valve to the appropriate width. Percolation was continued with fresh quantities of benzene until the total solid content of the effluent fell to 100 mg. per liter, the total volume of benzene used being 170 gal. The benzene percolate was concentrated to 6 l. at 100–120 mm. pressure and allowed to stand in the refrigerator overnight. The resulting crystalline precipitate was filtered off and washed with several portions of fresh benzene. The combined benzene filtrate and washings were extracted with 5% tartaric acid (three 6-l. portions followed by 1-l. portions) until the extracts showed no longer a turbidity upon addition of Mayer reagent. This point was usually reached with the tenth to thirteenth extract. To the combined extracts were added with vigorous agitation at 5–10° one gallon of chloroform and sufficient 20% sodium hydroxide to raise the *p*H to 7.5–8.0. This point is easily recognized by the color change of the solution from yellow to red. Twenty per cent. sodium carbonate was then added until the *p*H reached 9.8–10.0 and this degree of alkalinity was maintained during all subsequent extractions by further additions of sodium carbonate. Sufficient crushed ice was added to maintain a temperature of between 5 and 10°. Extraction with fresh 2-l. portions of chloroform was continued until a test sample was found to be essentially free of bases (Mayer reagent). The combined chloroform extracts were evaporated to dryness *in vacuo* without previous use of a drying agent and the residue (275 g.) was dissolved in 5% acetic acid (4 l.). To this solution, which contained a small amount of undissolved tarry material, was added with vigorous stirring 550 ml. of a saturated solution of ammonium sulfate. After sixteen hours in the refrigerator the slimy precipitate was filtered through a bed of celite and washed thoroughly with 5% ammonium sulfate. The precipitate was worked up for jervine and veratramine according to Jacobs and Craig.[3] The combined filtrate and washings were alkalinized following the precautions described above and the bases removed by extraction with chloroform. The chloroform solution was dried over sodium sulfate and evaporated to dryness *in vacuo*. The residue was taken up in acetone (300 ml.) and the solution allowed to remain in the refrigerator for twenty-four hours. The copious crystalline precipitate consisting largely of rubijervine and isorubijervine was filtered off and washed with a minimum of cold acetone. The filtrate and washings were evaporated to dryness *in vacuo* leaving an amorphous residue (84 g.), the "total amorphous bases."

Extraction of Fresh Roots and Rhizomes of *Veratrum viride*.—Fresh roots and rhizomes of *Veratrum viride* (150 lb.) from which rotted and other spoiled parts had been removed, were ground in a meat grinder and the resulting mash transferred immediately into 95% alcohol (20 gal.) to stop enzymatic action. The suspension was poured into the extraction crock described above and 95% alcohol was percolated through the root. The percolate totaling 115 gal. was concentrated *in vacuo* to 5 gal. and the

(19) All melting points are corrected.

large amount of tarry material which had separated out was removed by filtration through cheesecloth. The aqueous filtrate was extracted with six 1-liter portions of chloroform to remove tarry impurities and the ice cold aqueous solution was alkalinized to *p*H 9 with 25% sodium hydroxide and eventually to *p*H 9.8 with 20% sodium carbonate. The alkaline mixture was extracted with thirty 1-liter portions of chloroform and the combined chloroform extracts concentrated to small volume (300 ml.). Benzene was added and the chloroform removed by vacuum distillation. The benzene solution was placed in the refrigerator and the resulting crystalline precipitate was removed by centrifugation and washed with fresh portions of benzene. The benzene solution and washings upon freeze drying yielded a tan powder (127 g.), which was worked up in the same manner as the amorphous bases obtained from the dried root powder. The counter-current distribution curve of the material thus obtained exhibited the two maxima characteristic of germidine and germitrine.

Removal of Secondary Bases by N-Acetylation.—The total amorphous bases (30 g.) were dissolved in absolute methanol (300 ml.) containing acetic anhydride (12 ml.). After standing at room temperature for four hours the solution was evaporated to dryness *in vacuo*. The residue was dissolved in chloroform (215 ml.) and extracted with 5% tartaric acid (one 300-ml. and ten 100-ml. portions) until the extract gave a faint turbidity with Mayer reagent. The combined extracts were carefully alkalinized with 20% sodium hydroxide at 5° to *p*H 7.5–8.0 and then with 20% sodium carbonate to *p*H 9.8–10.0 and extracted with chloroform. The combined chloroform extracts were dried over sodium sulfate and the solvent removed *in vacuo*. The dried residue (21 g.) represents the tertiary bases.

Fractionation of Tertiary Bases by Simplified 8-Funnel Counter-current Distribution.—The tertiary bases were further fractionated by distribution between benzene and 2 molar acetate buffer of *p*H 5.5 (prepared by mixing 2 l. of a sodium acetate solution containing 544 g. of the trihydrate with 340 ml. of an acetic acid solution containing 40.8 g. of glacial acetic acid). Two separatory funnels were set up each containing 50 ml. of benzene per gram of tertiary bases. The material was added to the first funnel and an equal volume of buffer was added. Insoluble material separated here, which adhered to the walls of the separatory funnel and was removed by decanting from it the liquid phases. After shaking for two minutes the layers were separated and the benzene phase was extracted with seven more 50-ml. portions of buffer, these last seven buffer extracts being combined. The first buffer extract was extracted with seven 50-ml. portions of benzene. These seven benzene extracts were combined and extracted once with 35 ml. of 2% sodium bicarbonate. The seven combined buffers were alkalinized to *p*H 8.5 with 10% sodium hydroxide at 5°, then to *p*H 9.8–10.0 with 20% sodium carbonate and extracted with chloroform. The resulting chloroform extracts were combined with the seven benzene extracts, the solution dried over sodium sulfate and the solvents removed *in vacuo*. The material obtained (*ca.* 45% of the weight of the tertiary bases) represents plates 1–7 of an 8-plate distribution and contains the bulk of the hypotensive activity.

Chromatography of Plates 1–7.—A solution of the material from tubes 1–7 (15 g.) in benzene (150 ml.) was passed through a column of 35 mm. diameter containing alumina (300 g.), which had previously been washed with sulfuric acid until the *p*H of an aqueous suspension of the alumina was 4.5, and reactivated at 150° for 36 hours. Elution of the column with benzene (25 l.) removed inactive partially crystalline material (3.5 g.), which consisted mostly of rubijervine and isorubijervine. The active fraction was obtained by subsequent elution with benzene containing 2.5% of methanol. Elution with this solvent mixture proceeded rapidly after the first 500 ml. had passed through the column. Fractions of 150 ml. each were collected and evaporated to dryness *in vacuo* until a total of 5 g. of material (lyophilized weight) was obtained. This required between 500 and 600 ml. of eluant. The material was dissolved in 20 ml. of benzene and allowed to stand in the refrigerator overnight. The resulting crystalline precipitate (rubijervine) was removed by filtration and the filtrate was lyophilized. The product obtained in this manner was active in doses of 1–1.2 mg. in the hypertensive individual and consisted essentially of a mixture of germitrine and germidine. Continued elution with the above solvent mixture (3 l.) yielded material of lower activity (2.5–2.8 mg.) which contained a higher proportion of germidine.

Separation of Germitrine and Germidine by 24-Plate Counter-current Distribution.—Twenty-five 1-l. separatory funnels numbered from 24 to 0[20] were filled with 400 ml. of benzene each. To the funnel numbered 24 was added the above chromatographed material (8 g.) and 400 ml. of 2 *M* acetate buffer of *p*H 5.5. The funnel was shaken for two minutes and a small amount of oily material adhering to the wall of the funnel was separated off by decantation. The buffer layer was transferred to the funnel bearing the next lower number (23), fresh buffer added to funnel 24 and the contents of the two funnels equilibrated by shaking for two minutes. This procedure of transfer, addition of fresh buffer to funnel 24 and equilibration of the layers was continued until the first buffer layer had reached funnel 0. The contents of each funnel was then cooled to 5 to 10° by the addition of crushed ice and alkalinized with 20% sodium carbonate (80 ml.) to a *p*H of about 9.5. The layers were separated and the aqueous phases were extracted each with three portions of chloroform (100 ml.). The benzene and chloroform solutions from each funnel were combined and washed with 40 ml. of water. The organic layers were dried over sodium sulfate and evaporated to dryness *in vacuo*. The residual materials were lyophilized from benzene. Figure 1 shows the weight of the material derived from each funnel as a function of the funnel number. The specific rotations of some of the fractions in pyridine and chloroform are as follows

Funnel	4	6	7	12	15	16	19	21
$[\alpha]_D$, deg., in								
Pyridine	−8	−20	−23	−64	−64	−59	−49	−22
Chloroform		− 4			− 7	0		

Germidine.—This alkaloid was best obtained by crystallization of funnels (tubes) 3–7 from dilute alcohol. The material from tube 4 (227 mg.) after three crystallizations yielded thin rectangular plates (112 mg. and additional pure material from the mother liquors) which melted at 230–231° (dec.). In one instance involving the preparation of germidine from tubes 6 and 7 repeated crystallization did not raise the melting point above 198–200°. Seeding of this material with the higher melting form raised the melting point to 230°; $[\alpha]^{25}D$ +13° (*c*, 1.67 in chloroform); −11° (*c*, 1.84 in pyridine). For analysis the product was dried to constant weight at 110°. The two sets of figures refer to samples derived from tubes 4 and 5, respectively.

Anal. Calcd. for $C_{34}H_{53}O_{10}N$: C, 64.22; H, 8.38. Found: C, 64.56, 64.17; H, 8.37, 8.17.

In two volatile acid determinations[21] (*p*-toluenesulfonic acid) 8.99 mg. and 9.96 mg. of germidine consumed 2.91 ml. and 2.96 ml. of 0.01 *N* sodium hydroxide, calcd. for germine monoacetate-mono-α-methylbutyrate: 2.83 ml. and 3.13 ml., respectively.

Germidine Thiocyanate.—Material from tube 5 of the above distribution experiment (47 mg.) was dissolved in a few drops of 5% acetic acid and a concentrated solution of ammonium thiocyanate was added until no further precipitation occurred. The oily precipitate crystallized on rubbing and was recrystallized twice from dilute methanol.

(20) The reverse numbering was used in order to conform to the numbering of the tubes conventionally used when the distribution is run in the Craig machine (L. C. Craig, *J. Biol. Chem.*, **155**, 519 (1944)).

(21) Pregl-Roth, "Die quantitative organische Mikroanalyse," Julius Springer, Berlin, 1935, p. 235.

The substance melted at 242–244° (dec.). The analytical sample was dried at 110° for two hours.

Anal. Calcd. for $C_{34}H_{53}O_{10}N\cdot HNCS$: C, 60.50; H, 7.82; N, 4.03; S, 4.61. Found: C, 60.70; H, 7.73; N, 4.11; S, 4.75.

Hydrolytic Cleavage of Germidine to Germine, Acetic Acid and α-Methylbutyric Acid.—To a solution of germidine (108 mg.) in methanol (3.3 ml.) was added 1.07 *N* sodium hydroxide (0.66 ml.) and water (1.65 ml.). The mixture was allowed to stand at room temperature for sixteen hours and the methanol was removed *in vacuo*. The residual aqueous solution and precipitate was extracted with chloroform (two 10-ml. and two 5-ml. portions), the chloroform layer was washed with water (5 ml.) and evaporated to dryness *in vacuo*. The crystalline residue (82 mg.) after two recrystallizations from methanol yielded heavy prisms (53 mg.), which began to sinter at 160° and melted at 220–225°; $[\alpha]^{26}D$ +4.0° (*c*, 2.0 in 95% alcohol). The melting point of germine has been reported[10b] at about 220° after sintering at 160–170°; $[\alpha]^{20}D$ +4.7° (*c*, 2.0 in abs. alcohol).

Anal. Calcd. for $C_{27}H_{43}O_8N$: C, 63.63; H, 8.50. Found (after drying at 110°): C, 63.69; H, 8.72.

The hydrochloride of the acetone compound prepared from the above sample decomposed at 275° after previous sintering at 265°. Craig and Jacobs have reported[22] monoacetonyl germine hydrochloride to melt at 275° (dec. uncor.).

The alkaline solution and water washings from which the germine had been removed by extraction were adjusted to *p*H 8.0 with 0.1 *N* hydrochloric acid and the solution was lyophilized. The residue was dissolved in water (1.5 ml.) and the solution adjusted to *p*H 6.5 by the addition of a few drops of 0.1 *N* hydrochloric acid. Alcohol (4 ml.) and *p*-phenylphenacyl bromide (94 mg.) were added and the mixture refluxed for one hour. After dilution with water (3 ml.) the alcohol was removed *in vacuo* and the residual solution and precipitate extracted with benzene (three 5-ml. portions). The benzene solution was dried over sodium sulfate and the solvent removed *in vacuo*. The residue (82.4 mg.) was dissolved in a mixture (12 ml.) of 3 parts of hexane and 1 part of benzene and chromatographed on sulfuric acid–washed alumina (5.5 g.). Elution of the column with the same solvent mixture yielded in the first 20 ml. excess *p*-phenylphenacyl bromide (m. p. 125–126°) immediately followed in the next 15 ml. by a second substance which after purification from hexane melted at 71.5–72°. The *p*-phenylphenacyl ester of *d,l*-α-methylbutyric acid has been reported[23] to melt at 70.6°; that of the *d*-enantiomorph at 71°.[24]

Anal. Calcd. for $C_{19}H_{20}O_3$: C, 77.01; H, 6.80. Found: C, 76.90; H, 6.61.

Continued elution with benzene–hexane (1:3, 70 ml.) yielded a third crystalline fraction, which after three crystallizations from ether–hexane melted at 109–110° and gave no melting point depression when mixed with an authentic sample of *p*-phenylphenacyl acetate.

Anal. Calcd. for $C_{18}H_{16}O_3$: C, 75.58; H, 5.55. Found: C, 75.22; H, 5.72.

Germitrine.—Germitrine was isolated from the highly levorotatory material present in funnels 12–17. Three crystallizations of such material (412 mg.) from dilute acetone yielded heavy prisms (231 mg.), the properties of which were not changed on further crystallization (m. p. 216–219° (dec.); $[\alpha]^{25}D$ (after drying at 110°) −69° (*c*, 0.85 in pyridine); −4° (*c*, 1.5 in chloroform)).

Anal. Calcd. for $C_{39}H_{61}O_{12}N$: C, 63.65; H, 8.36. Found (after drying at 110°): C, 63.45; H, 8.47.

In a volatile acid determination 6.92 mg. of substance yielded an amount of acid equivalent to 2.50 ml. of 0.01 *N* sodium hydroxide; calcd. for an ester of germine with one mole each of acetic, α-methylbutyric and methylethylglycolic acid, 2.70 ml.[25]

Germitrine Thiocyanate.—Germitrine (15 mg.) was dissolved in 5% acetic acid (12 drops) and a concentrated solution of ammonium thiocyanate (3 drops) was added. An amorphous precipitate appeared which solidified rapidly on rubbing. Two recrystallizations from dilute acetone yielded a product (13.7 mg.) melting at 231–232° (dec.). When mixed with the thiocyanate of germerine of m. p. 230–232° the melting point was depressed to 220–221.5°. The thiocyanates of germitrine and germerine differ markedly in their solubilities in acetone. The former is readily and the latter sparingly soluble in that solvent, $[\alpha]^{25}D$ −55° (*c*, 0.7 in pyridine).

Anal. Calcd. for $C_{39}H_{61}O_{12}N\cdot HNCS$: C, 60.43; H, 7.87; S, 4.02. Found (after drying at 110°): C, 60.56; H, 8.08; S, 4.19.

Methanolytic Degradation of Germitrine to Germerine and Methyl Acetate.—A solution of germitrine (50 mg.) in methanol (2 ml.) and water (1 ml.) was allowed to stand at room temperature. At the end of about one hour crystals began to appear, which increased in amount during the second hour. The crystals were redissolved by gentle warming and the mixture allowed to crystallize for a period of fifteen hours. The crystals (26 mg.) were centrifuged and washed with 50% methanol (1 ml.). The air dried material melted at 203–204° (slight browning), $[\alpha]^{25}D$ −8.7° (*c*, 0.69 in pyridine); +11° (*c*, 1.54 in chloroform). There was no depression in melting point when the sample was mixed with an authentic specimen of germerine isolated from *Veratrum album*.[26]

Anal. Calcd. for $C_{37}H_{59}O_{11}N$: C, 64.05; H, 8.57. Found (after drying at 110°): C, 64.03; H, 8.21.

In a volatile acid determination 8.49 mg. of substance yielded an amount of acid equivalent to 2.27 ml. of 0.01 *N* sodium hydroxide; calcd. for an ester of germine with one mole each of α-methylbutyric acid and methylethylglycolic acid, 2.29 ml.[25]

The methanol–water supernate and washing were distilled *in vacuo* at a bath temperature not exceeding 30° into a receiver immersed into a Dry Ice-bath. When all the liquid had distilled over, water (1 ml.) was added to the distillation residue and distilled into the Dry Ice-cooled receiver. The dry distillation residue after recrystallization from dilute methanol yielded an additional amount (5.4 mg.) of germerine (m. p. 198–200°). To the distillate 0.01 *N* sodium hydroxide (10 ml., was added (the second drop rendered the solution alkaline toward phenolphthalein) and the solution refluxed for two hours with the exclusion of carbon dioxide. The saponification mixture was then titrated (phenolphthalein) with 0.01 *N* sulfuric acid (5.35 ml.), the methanol distilled off *in vacuo* and the remaining aqueous solution lyophilized. In a blank experiment methanol (2.6 ml.) and water (1.5 ml.) were refluxed with 0.01 *N* sodium hydroxide (10 ml.) and then titrated with 0.01 *N* sulfuric acid (9.3 ml.). The difference between the amounts of alkali consumed in the actual and in the blank experiments is equivalent to the amount of acid liberated in the saponification (0.0395 millimole). The lyophilized residue containing the sodium salt of the acid was dissolved in water (0.5 ml.) acidified to *p*H 6 with a few drops of hydrochloric acid and after addition of alcohol (1.5 ml.) and *p*-phenylphenacyl bromide (14 mg.) the mixture was refluxed for two hours. The resulting esters (10 mg.) were worked up and chromatographed on sulfuric acid-washed alumina (3 g.) as described above. Elution with benzene–hexane, 1:3, afforded at first some unchanged reagent (m. p. 123–125°)

(22) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **148**, 57 (1943).

(23) N. L. Drake and E. P. Veitch, Jr., THIS JOURNAL, **57**, 2624 (1935).

(24) F. Kögl and H. Erxleben, *Z. physiol. Chem.*, **227**, 70 (1934).

(25) This value is corrected for the finding that only 87% of the methylethylglycolic acid (or its dehydration product, tiglic acid) are found in the distillate under the conditions of the volatile acid determination.

(26) The authors wish to thank Prof. W. Poethke of the University of Leipzig, Germany, for generously supplying this sample. It was recrystallized three times from dilute methanol and then melted at 198–202°.

followed by *p*-phenylphenacyl acetate (2.8 mg.) m. p. 110–111°; no depression when mixed with an authentic sample.

Conversion of Germitrine into Germerine by Chromatography on Acetic Acid-Washed Alumina.—A solution of germitrine (50 mg.) in benzene (5 ml.) was passed through a column of acetic acid-washed alumina (*p*H 5.6). The column was washed with benzene (50 ml.) and allowed to stand topped by a layer of benzene for 48 hours at room temperature. It was then eluted with benzene containing 2.5% methanol (50 ml.). The eluate was evaporated to dryness *in vacuo*, and the residue (41 mg.) crystallized twice from acetone. The crystals (22.5 mg.) melted at 202–205° (dec.) and showed no depression in melting point when mixed with either germerine obtained by methanolysis of germitrine or the sample of germerine derived from *Veratrum album;* $[\alpha]^{25}D$ −5.4° (*c*, 0.83 in pyridine), +15.7° (*c*, 1.02 in chloroform).

Anal. Calcd. for $C_{37}H_{59}O_{11}N$: C, 64.05; H, 8.57. Found (after drying at 110°): C, 63.93; H, 8.87.

Germerine Thiocyanate.—To a solution of germerine (30 mg.) in 5% acetic acid (1 ml.) was added dropwise a concentrated solution of ammonium thiocyanate until precipitation was complete. The resulting product after 3 crystallizations from dilute methanol melted at 230–232° (dec.). Germerine thiocyanate has been reported to melt at 221–223°.[10b] In contrast to the thiocyanate of germitrine this salt is very slightly soluble in acetone.

Anal. Calcd. for $C_{37}H_{59}O_{11}N \cdot HNCS$: C, 60.62; H, 8.05; S, 4.25. Found (after drying at 110°): C, 60.56; H, 7.98; S, 4.25.

Hydrolytic Cleavage of Germerine to Germine, α-Methylbutyric Acid and Methylethylglycolic Acid.—Germerine (100 mg.) was hydrolyzed with dilute methanolic sodium hydroxide and the hydrolysis mixture worked up as described above for germidine. The crystalline residue from the chloroform extract (65 mg.) after recrystallization from methanol melted at 220–225° with previous sintering at 155–165°, $[\alpha]^{27}D$ −4.3° (*c*, 1.9 in 95% alcohol).[10b]

Anal. Calcd. for $C_{27}H_{43}O_8N$: C, 63.63; H, 8.50. Found: C, 63.84; H, 8.74.

The hydrochloride of the acetone compound was prepared from the above sample of germine and analyzed after drying at 110°.

Anal. Calcd. for $C_{30}H_{47}O_8N \cdot HCl$: C, 61.45; H, 8.26. Found: C, 61.45; H, 8.88.

The sodium salts of the acids obtained by neutralization of the aqueous solution were allowed to react with *p*-phenylphenacyl bromide and the resulting *p*-phenylphenacyl esters chromatographed on sulfuric acid-washed alumina as described for germidine. Elution with benzene–hexane yielded at first unreacted bromide (m. p. 123–125°) followed immediately by *p*-phenylphenacyl-α-methylbutyrate (33 mg. crude), which after several recrystallizations from hexane melted at 72°. Elution with benzene (150 ml.) gave crystals (23 mg. crude), which after several crystallizations from ether melted at 119°.

Anal. Calcd. for $C_{19}H_{20}O_5$: C, 73.06; H, 6.45. Found: C, 73.13; H, 6.40.

The product resulting from the reaction of methylethylglycolic acid (m. p. 72–73°) with *p*-phenylphenacyl bromide melted at 119° and gave no depression when mixed with the above sample.

Isolation of *l*-α-Methylbutyric Acid and *d*-Methylethylglycolic Acid from an Active Amorphous Concentrate.—A portion of the tertiary base fraction (6.0 g.) was saponified with 0.25 *N* sodium hydroxide (120 ml.) and methanol (180 ml.) for 18 hours at room temperature. The methanol was removed *in vacuo* and the precipitated bases extracted with chloroform. The alkaline solution was acidified with hydrochloric acid (congo) and 90 ml. of water was distilled from it *in vacuo* (50 mm.). The aqueous distillate was extracted ten times with chloroform and the chloroform extracts fractionated carefully to remove the solvent. The residual liquid was distilled in a micro sublimation apparatus at a bath temperature of 40° and 25 mm. pressure. The distillate had the characteristic odor of a valeric acid, $[\alpha]^{25}D$ −25° (*c*, 1.35 in water). The rotation of *l*-α-methylbutyric acid has been reported as −22°.[10b]

Anal. Calcd. for $C_5H_{10}O_2$: C, 58.75; H, 9.86; neutr. equiv., 102. Found: C, 57.55; H, 9.75; neutr. equiv., 101.

The aqueous solution from which the α-methylbutyric acid had been removed by extraction with chloroform was extracted with ether in a continuous extraction apparatus. The ether was distilled off and the oily residue was sublimed at ordinary pressure at a bath temperature of 80–120°. After an oily forerun crystals appeared, which after resublimation melted at 72.5–73.5°. Poethke[10b] has reported a melting point of 72–73° for methylethylglycolic acid derived from germerine; $[\alpha]^{24}D$ +4.4° (*c*, 1.6 in water); +12° (*c*, 1.28 in 0.2 *N* sodium hydroxide).

Anal. Calcd. for $C_5H_{10}O_3$: C, 50.90; H, 8.54. Found: C, 50.83; H, 8.95.

Methanolysis of Protoveratrine.—Finely powdered protoveratrine (150 mg.) was suspended in methanol (5 ml.) and water (1 ml.). The suspension was shaken on the machine for four days after which time all the crystals had dissolved. The solution was distilled to dryness at reduced pressure at a bath temperature not exceeding 25° and the distillate collected in a Dry Ice-cooled vessel. Water (1 ml.) was then added to the dry residue and distilled over into the receiver. The methyl esters present in the distillate were saponified with dilute sodium hydroxide (the amount of free acid present was negligible) and the sodium salts of the acids allowed to react with *p*-phenylphenacyl bromide. The phenacyl esters upon chromatography on alumina afforded *p*-phenylphenacyl acetate (47 mg. crude) which after recrystallization melted at 109–110.5°, and *p*-phenylphenacyl methylethylglycolate (6.6 mg. crude) which after recrystallization melted at 117–118°. Only a very small amount of the basic fraction containing the partially deacylated alkaloids could be crystallized.

Germine Pentaacetate.[10b]—Anhydrous germine (250 mg.) was acetylated for eighteen hours at room temperature with pyridine (6 ml.)–acetic anhydride (6 ml.). The mixture was evaporated to dryness and taken up in a minimum of chloroform. Crystallization took place rapidly upon addition of dry ether, and the resulting material was recrystallized from the same solvent mixture. The pure pentaacetate (199 mg.) formed small prisms and melted at 256–257° (dec.), $[\alpha]^{25}D$ −85° (*c*, 0.6 in pyridine).

Anal. Calcd. for $C_{27}H_{38}O_8N(COCH_3)_5$: C, 61.74; H, 7.42; acetyl, 29.9. Found: C, 61.51; H, 7.46; acetyl, 29.6.

Methanolysis of Germine Pentaacetate.—A solution of germine pentaacetate (100 mg.) in methanol (16 ml.)–water (8 ml.) was allowed to stand at room temperature for eighteen hours. After removal of the solvents *in vacuo* the residue was dissolved in a small amount of chloroform and dry ether was added to incipient turbidity. After standing in the refrigerator for eighteen hours a white powder (20 mg.) was obtained which after two recrystallizations from dry methanol melted to droplets of a clear resin at 166–168°, and coalesced to form a meniscus at 183–186°; $[\alpha]^{25}D$ 0°(*c*, 0.94 in pyridine).

Anal. Calcd. for $C_{27}H_{42}O_8N(COCH_3)$: C, 63.14; H, 8.22; acetyl, 7.80. Found: C, 62.86; H, 8.56; acetyl, 7.43.

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Summary

It has been shown that the hypotensive properties of the roots and rhizomes of *Veratrum viride*, the American or Green Hellebore, are principally due to two hitherto undescribed crystalline alkaloids which have been named germidine and germitrine. Germidine has been identified as a mixed diester of the known alkamine germine, $C_{27}H_{43}O_8N$, with acetic acid and *l*-α-methylbutyric acid. Germitrine is a triester of germine containing in addition to the above acids one mole of *d*-methylethylglycolic acid.

Germitrine undergoes rapid methanolysis in 50% aqueous methanol at room temperature with the loss of the acetyl group and the formation of the diester alkaloid germerine (germine *l*-α-methylbutyrate *d*-methylethylglycolate), already known as a constituent of *Veratrum album*. The compound originally obtained from the triester fraction by crystallization from aqueous methanol and designated germitrine in our preliminary communication[13] was actually slightly impure germerine. Germitrine is also degraded to germerine by chromatography on acetic acid-washed alumina.

Germitrine is a powerful hypotensive agent. As little as half a microgram per kg. given intravenously elicits a marked fall in blood pressure in the anesthetized dog. The two diester alkaloids are somewhat less active.

Protoveratrine (*Veratrum album*), the corresponding triester of the alkamine protoverine, is likewise unstable in aqueous methanolic solution; in this case the volatile methanolysis products yielded on hydrolysis acetic acid as well as methylethylglycolic acid. Germine pentaacetate on similar treatment is partially degraded to a monoacetate.

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[CONTRIBUTION FROM THE LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY]

2-Amino-4-hydroxy-6-pteridinecarboxaldehyde

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A pterincarboxaldehyde was first prepared from the natural fermentation *L. casei* factor by sulfurous acid cleavage by Hutchings, *et al.*[1] The fermentation *L. casei* factor has been synthesized and shown to be pteroyl-γ-glutamyl-γ-glutamylglutamic acid.[2] Therefore, the sulfurous acid method of cleavage should be applicable to the general class of pteroyl- compounds. Reported herein are two convenient methods of synthesizing 2-amino-4-hydroxy-6-pteridinecarboxaldehyde (I).

The first method of synthesis of I is the cleavage of pteroylglutamic acid with sodium sulfite solution acidified with excess acetic acid. This resulting solution reacted with a slight excess of iodine to precipitate I.

Petering, *et al.*,[3a] in a preliminary report indicated a pterincarboxaldehyde formation by oxidation of a polyhydroxyalkylpterin with lead tetraacetate. Forrest and Walker[4] by a similar oxidation of 6-tetrahydroxybutylpterin obtained a pterincarboxaldehyde characterized as I by the formation of a 2,4-dinitrophenylhydrazone and oxidation to 2-amino-4-hydroxy-6-pteridinecarboxylic acid (IV). Karrer, *et al.*,[5] have also reported a number of hydroxyalkylpterins.

Angier, *et al.*[6] have shown that the synthesis of substituted alkylpterins through a dihydropterin often results in methylpterins rather than the desired substituted methylpterins.

Weygand, *et al.*,[7] have confirmed the findings of Angier, *et al.*,[6] by the preparation of 2-amino-4-hydroxy-7-[D-*erythro*-2′,3′,4′-trihydroxybutyl]-pteridine from *p*-tolyl-D-isoglucosamine and 2,4,5-triamino-6-hydroxypyrimidine and subsequently periodic oxidation to 2-amino-4-hydroxypteridine-7-acetaldehyde.

Weygand, *et al.*,[7] also prepared I by periodic oxidation of 2-amino-4-hydroxy-6-[D-*arabo*-tetrahydroxybutyl]-pteridine. His product was characterized by analysis, oxidation to IV, preparation of a Schiff base with *p*-toluidine and methyl *p*-aminobenzoate and the preparation of pteroylglutamic acid.

The biological interest in the pteridine carboxaldehyde (I), such as the inhibition of xanthine oxidase by I as reported by Kalckar, *et al.*,[8] and Van Meter,[9] makes it desirable to have a preparative synthesis which will eliminate the possibility of isomer and pteridine acetaldehyde contaminants.

(1) Hutchings, *et al.*, THIS JOURNAL, **70**, 10 (1948); *Annals N. Y. Acad. Sci.*, XLVIII, 273 (1946).

(2) Boothe, *et al.*, *ibid.*, **70**, 1099 (1948); Mowat, *et al.*, *ibid.*, **70**, 1096 (1948); Boothe, *et al.*, *ibid.*, **71**, 2304 (1949); Mowat, *et al.*, *ibid.*, **71**, 2308 (1949); Semb, *et al.*, *ibid.*, **71**, 2310 (1949); Angier, *et al.*, *ibid.*, in press.

(3) (a) Petering and Weisblat, THIS JOURNAL, **69**, 2566 (1947); (b) Petering and Schmidt, *ibid.*, **71**, 3977 (1949).

(4) Forrest and Walker, *J. Chem. Soc.*, 83 (1949).

(5) (a) Karrer, *et al.*, *Helv. Chim. Acta*, **30**, 1031 (1947); (b) **31**, 777 (1948); (c) **32**, 423 (1949).

(6) Angier, *et al.*, THIS JOURNAL, **70**, 3029 (1948).

(7) Weygand, *et al.*, *Ber.*, **82**, 25 (1949).

(8) Kalckar, *et al.*, *J. Biol. Chem.*, **174**, 771 (1948).

(9) Van Meter and Oleson, *ibid.*, in press.