plates) solution of 103 g. of sodium bicarbonate in 500 ml. of water cooled to 12–15°. In portions, 177 g. of powdered iodine was now added during the course of an hour. Stirring was continued another hour and by this time the dark oil first formed had changed to a black, lumpy solid heavier than water.

A mixture of this crude solid and 20 g. of sodium hydrosulfite was steam distilled. After 8 hours and 7 l. of distillate had collected, no more product came over. The cooled product, originally an oil, crystallized to a mass of radiating white needles. Recrystallization from a small amount of hot ethanol gave 85 g. (74%) of long white needles of product. Several recrystallizations from ethanol served to purify it, m.p. 59–60°.

Anal. Calcd. for C_7H_7ClIN: C, 31.43; H, 2.64; N, 5.31. Found: C, 31.58; H, 2.87; N, 5.40.

3-Chloro-5-iodotoluene (VIII).[3]—A mixture of 75 g. of 2-chloro-6-iodo-*p*-toluidine and 1 l. of 30% aqueous hypophosphorous acid was cooled to 0–5° and a solution of 19.5 g. of sodium nitrite in 100 ml. of water was added dropwise during one hour. Stirring was continued at 0–5° for 2 hours, then at room temperature for 2 hours. Upon addition of 1 l. of water a heavy black oil separated. It was washed with 6 100-ml. portions of warm 6 *N* hydrochloric acid. These extracts on dilution with water gave 31 g. of unreacted amine.

The black oil insoluble in 6 *N* hydrochloric acid was washed with aqueous hydrosulfite and then distilled. A tarry residue remained in the flask. The product weighed 14 g. and boiled at 238–245° (747 mm.). 3-Chloro-5-iodotoluene as prepared by treatment of diazotized 3-chloro-5-aminotoluene with potassium iodide[3] is reported to boil at 240–243° at atmospheric pressure.

3,3'-Dimethyl-5,5'-dichlorobiphenyl (VII): By the Ullman Reaction on 3-Chloro-5-iodotoluene.—The Ullman reaction on 3-chloro-5-iodotoluene as reported by McAlister and Kenner,[3] proved difficult to repeat until an active copper bronze was used.

In portions, 8 g. of active copper bronze was added to 6 g. of vigorously boiling (metal-bath at 265°) 3-chloro-5-iodotoluene. The bath temperature was raised to 300° during one-half hour, after which the reaction was complete. The cooled mixture was extracted with 50 ml. of hot benzene. By evaporation 2 g. (64%) of brownish needles resulted. Recrystallization from ethanol gave white needles, m.p. 95–97° (cor.). For further purification, they were dissolved in 20 ml. of cyclohexane and the solution was poured through a column (2 × 20 cm.) of alumina (ALCOA F-20). Elution with 500 ml. of cyclohexane and evaporation of the solvent gave white needles. These were recrystallized from 20 ml. of hot ethanol and then the chromatographic procedure was repeated. A final crystallization gave long white needles of 3,3'-dimethyl-5,5'-dichlorobiphenyl, m.p. 100–101° (cor.) (lit.[3] 101–102°).

A mixture of this material and VII (from the deamination of VI) also melted at 100–101° (cor.). The infrared spectra of the two substances were identical.

3,3'-Dimethyl-5,5'-dichlorobenzidinedibenzenesulfonamide (V): By Benzenesulfonylation of VI.—A solution of 1 g. of 3,3'-dimethyl-5,5'-dichlorobenzidine (VI) in 5 ml. of benzenesulfonyl chloride was boiled for 20 minutes, then poured into 50 ml. of pyridine. This solution was boiled for a few minutes then poured into 200 ml. of water. The product was crystallized from glacial acetic acid (Darco) to give 0.3 g. of small white prisms which after two more crystallizations from glacial acetic acid had an m.p. 247–248° (cor.). This was identical with the product obtained by addition of hydrogen chloride to the 3,3'-dimethyl-5-chlorodiphenoquinonedibenzenesulfonimide (IV) as shown by a melting point of the mixture and infrared absorption spectra.

URBANA, ILLINOIS

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Neogermitrine, a New Ester Alkaloid from Veratrum Viride[1]

BY JOSEF FRIED, PAUL NUMEROF AND NETTIE H. COY

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A new triester alkaloid, neogermitrine, possessing hypotensive activity of the order of that of germitrine has been isolated from *Veratrum viride*. This alkaloid, while present as the main active constituent in two batches of root collected during the summer seasons of 1948 and 1949, had not been encountered in a previous batch collected during 1947. Neogermitrine, $C_{36}H_{55}O_{11}N$, is a diacetate-mono-(levo)-α-methylbutyrate of the alkamine germine. On stepwise degradation with dilute methanol it yielded first the known diester alkaloid germidine and finally germine. The infrared spectra of germine and of the ester alkaloids derived from it have been recorded and found to be useful for identification purposes.

A recent investigation[2,3] having as its purpose the isolation and characterization of the hypotensive principles of *Veratrum viride* revealed that the hypotensive activity resided exclusively in an amorphous fraction,[4] from which all the previously known crystalline alkaloids had been removed. Further fractionation of this amorphous residue by chromatography and countercurrent distribution led to the isolation of the alkaloids germitrine and germidine, which were shown to be esters of the previously known alkamine germine,[5] the former with one mole each of acetic, (levo)-α-methylbutyric and (dextro)-methylethylglycolic acids,[6] and the latter with acetic and (levo)-α-methylbutyric acids. Germitrine, the more abundant and the more active of the two alkaloids, accounted for the bulk of the hypotensive activity present in the root.

More recently, with the objective of preparing larger amounts of the two active alkaloids, a 50-lb. batch of root was processed essentially as outlined previously.[2] A highly active concentrate was obtained, which could be separated by 24-plate countercurrent distribution between benzene and 2 *M* acetate buffer at *p*H 5.5 into two components with peaks at tubes 5 and 15 as shown in Fig. 1. This curve agrees in its essentials with the one reported

(1) Presented before the Division of Medicinal Chemistry of the American Chemical Society, Cleveland, Ohio, April 8 to 12, 1951.

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

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

(4) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **160**, 555 (1944).

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

(6) These acids, which in our previous publication had been referred to as *l*-α-methylbutyric and *d*-methylethylglycolic acid, should properly carry the designations levo- and dextro-, respectively. In a paper by Stenhagen, *et al.*, (*Arkiv Kemi*, **24B**, 1 (1947)), which came to our attention only recently, the former acid has been configurationally related to D-glyceraldehyde.

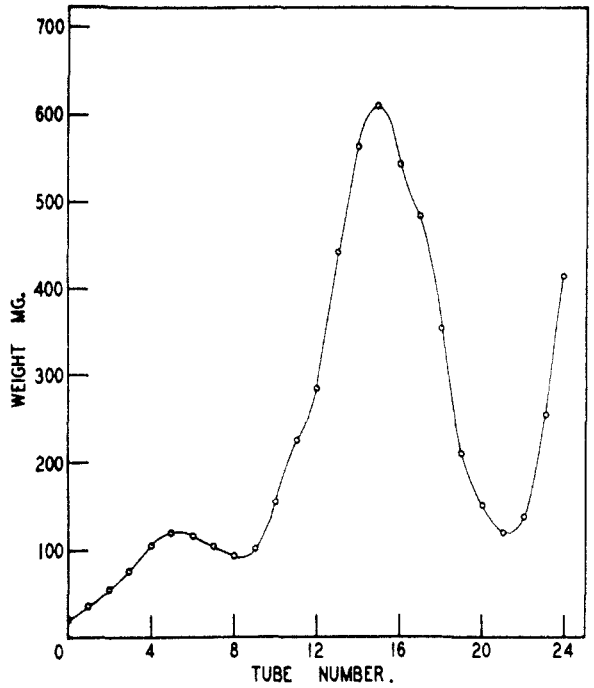


Fig. 1.—Craig countercurrent distribution curve for active concentrate of *Veratrum viride* showing peaks due to germidine (tube 5) and neogermitrine (tube 15).

in our earlier paper except for a slight shift of one of the maxima from tube 6 to tube 5, and for the presence of material in tubes 23 and 24, which in the previous case had been removed in the chromatographic step preceding the 24-plate distribution. Crystallization of the material present in tube 5 and neighboring tubes from dilute ethanol yielded, as in the past, germidine, identified by its characteristic double melting point (202–203°, and 231–233° when seeded with the higher melting form), its specific rotation ($[\alpha]^{28}D$ −11° in pyridine) and its infrared spectrum. Extensive fractional crystallization of the contents of tube 15 and adjoining tubes afforded, contrary to previous experience, a new alkaloid, which, as will be shown below, is likewise a triester of germine and was therefore named neogermitrine. In addition, there were obtained from this fraction a small amount of germitrine and a new alkaloid (m.p. 227–230°, $[\alpha]D$ −7° in pyridine), the latter in amounts too small for further characterization.

Although the physical constants for neogermitrine and germitrine differ sufficiently to distinguish these two alkaloids from each other, a definite need for additional means of identification was felt. With this in mind we have determined the infrared spectra of the ester alkaloids present in *Veratrum viride*, as well as those of germine, isogermine and protoveratrine with the results shown in Table I. A critical evaluation of the data presented in this table has convinced us that although the spectra show certain similarities they differ sufficiently from each other to permit the identification not only of neogermitrine and germitrine but of all the ester alkaloids encountered in this work, especially when obtained in amounts too small for full analytical characterization. As expected, the spectra of the ester alkaloids examined exhibited one or two strong bands in the region characteristic for ester carbonyl (5.7–5.9 μ). In contrast, the alkamine germine showed only a weak band in the double bond region at 6.05 μ, possibly due to the carbon–carbon double bond postulated by Craig and Jacobs[7] to be present in germine. If this interpretation of the low intensity band is correct the double bond in germine cannot be located in the 8,14-position of a steroid nucleus as proposed by Jacobs and Sato,[8] since, according to Jones, *et al.*,[9] $\Delta^{8,14}$-stenols do not show any absorption in this region. It is, moreover, premature to discuss the position of this double bond as long as the exact nature of the ring skeleton present in germine is not established. The assumption of a steroid nucleus in germine analogous to the well documented steroidal structures of rubijervine[10] and isorubijervine[11] is purely speculative and open to question, especially in view of recent findings that jervine[12] and veratramine,[13] two of the most abundant alkaloids in *Veratrum viride* appear to possess modified steroidal structures. We have also had occasion to examine the infrared spectrum of isogermine, a substance first obtained by Craig and Jacobs[14] by treatment of germine with dilute alkali. This isomerization reaction has been pictured[8] as involving a shift of the double bond from the 8,14- to the 14,15-position, an interpretation which is not supported by our spectral data. The infrared spectrum of isogermine exhibits an intense band at 5.78 μ indicative of a carbonyl group, which from the mode of formation of isogermine can only be an aldehyde or a keto group. The presence of such a group is also evident from the ultraviolet absorption spectrum of isogermine, which shows a low intensity band (ϵ 35) at 284 mμ characteristic for the isolated keto or aldehyde group.[14a]

Neogermitrine on saponification with dilute alkali in 50% methanol at room temperature afforded the alkamine germine and a mixture of acids, which after chromatographic separation of the *p*-phenylphenacyl esters on alumina according to the general procedure described in our previous publication[2] were identified as acetic acid and (levo)-α-methylbutyric acid. No other acidic components were detected in spite of careful examination of all the chromatographic fractions obtained. Titration of the total volatile acids after hydrolysis of the alkaloid with *p*-toluenesulfonic acid indicated the presence of three equivalents of acid. Neoger-

(7) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **149**, 451 (1943).
(8) W. A. Jacobs and Y. Sato, *ibid.*, **175**, 57 (1947).
(9) L. N. Jones, P. Humphries, E. Packard and K. Dobriner, THIS JOURNAL, **72**, 86 (1950).
(10) Y. Sato and W. A. Jacobs, *J. Biol. Chem.*, **179**, 623 (1949).
(11) Y. Sato and W. A. Jacobs, *ibid.*, **191**, 63 (1951).
(12) J. Fried, O. Wintersteiner, M. Moore, B. M. Iselin and A. Klingsberg, THIS JOURNAL, **73**, 2970 (1951).
(13) W. A. Jacobs and Y. Sato, *J. Biol. Chem.*, **191**, 71 (1951).
(14) L. C. Craig and W. A. Jacobs, *ibid.*, **148**, 57 (1943).
(14a) After this manuscript had been prepared for publication a paper by H. Jaffe and W. A. Jacobs (*ibid.*, **193**, 325 (1951)) appeared in which these authors, essentially on the basis of ultraviolet and infrared absorption spectra, revise the views previously expressed by Jacobs and Sato (ref. 8) concerning the nature and position of the double bonds in germine and in isogermine. Our spectral data and the conclusions based thereon are in agreement with those presented in the above publication. We wish to acknowledge Dr. Jacobs' priority but are presenting our own data in unaltered form since they were obtained independently.

TABLE I

INFRARED SPECTRA[a]

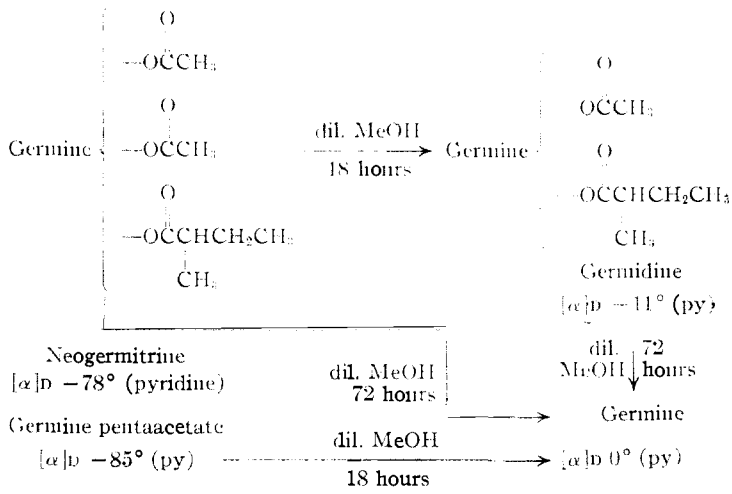
Germine	Isogermine	Germidine	Germerine	Neogermitrine	Germitrine	Protoveratrine
3.04–3.26 (4)	2.99 (3)	2.92–3.15 (5)	3.10 (4)	2.97 (4)	2.99 (5)	2.87 (4)
3.50 (10)	3.20 (8)	3.50 (10)	3.50 (10)	3.50 (10)	3.24 (1)	2.99 (8)
6.05 (1)	3.50 (10)	5.76 (10)	5.74 (10)	5.71 (10)	3.50 (10)	3.50 (10)
6.80 (7)	5.78 (10)	6.80 (7)	5.81 (10)	5.82 (10)	5.90 (5)	5.74 (10)
7.21 (7)	5.90 (1)	7.21 (5)	6.80 (10)	6.80 (10)	6.80 (10)	6.78 (4)
7.62 (1)	6.80 (10)	7.41 (1)	7.21 (7)	7.21 (10)	7.21 (6)	7.21 (5)
7.82 (1)	7.21 (10)	7.52 (1)	7.41 (2)	7.41 (1)	7.36 (1)	7.41 (1)
7.98 (1)	7.41 (5)	7.82 (1)	7.62 (1)	7.82 (1)	7.52–7.93 (6)	7.46 (2)
8.24 (4)	7.57 (6)	7.98 (10)	7.77 (1)	7.98 (8)	8.34 (2)	7.62 (2)
8.34 (1)	7.72 (5)	8.60 (7)	7.98 (6)	8.49 (1)	8.65 (6)	7.98–8.82 (10)
8.60 (6)	7.91 (7)	8.95 (5)	8.24 (1)	8.65 (4)	8.95 (4)	9.25 (2)
8.82 (2)	8.13 (4)	9.13 (1)	8.39 (1)	8.82 (1)	9.30 (7)	9.46 (7)
9.04 (4)	8.24 (1)	9.25 (10)	8.60 (1)	9.00 (5)	9.50 (1)	9.63 (1)
9.34 (8)	8.34 (4)	9.34 (1)	8.73 (10)	9.30 (8)	9.66 (10)	9.78 (10)
9.62 (8)	8.49 (1)	9.54 (10)	8.91 (6)	9.50 (2)	9.90 (2)	10.20 (3)
9.97 (4)	8.63 (1)	9.74 (1)	9.20 (2)	9.74 (1)	10.23 (4)	10.46 (8)
10.20 (4)	8.82 (10)	10.16 (6)	9.46 (10)	9.82 (6)	10.58 (10)	10.95 (10)
10.42 (1)	9.30 (1)	10.42 (10)	9.63 (10)	10.09 (2)	11.10 (8)	11.10 (1)
10.54 (10)	9.38 (8)	10.84 (3)	9.82 (10)	10.23 (2)	11.41 (3)	11.59 (1)
10.84 (1)	9.50 (10)	11.10 (10)	10.37 (10)	10.42 (7)	11.62 (1)	11.92 (1)
11.10 (8)	9.67 (8)	11.55 (3)	10.58 (10)	10.58 (1)	11.79 (1)	12.07 (2)
11.62 (3)	9.90 (7)	11.75 (2)	11.10 (6)	10.76 (1)	12.36 (2)	12.31 (1)
11.82 (1)	10.20 (3)	12.01 (4)	11.38 (1)	11.10 (7)	12.52 (4)	12.55 (4)
12.04–12.16 (1)	10.34 (8)	12.36 (4)	11.59 (1)	11.52 (4)	12.81 (1)	12.74 (3)
12.36 (3)	10.73 (5)	12.68 (2)	12.13 (2)	11.79 (1)	13.02 (2)	13.20 (3)
12.81 (1)	10.95 (10)	13.30 (1)	12.34 (2)	12.01 (1)	13.30 (1)	13.53 (1)
13.11 (1)	11.06 (1)		12.55 (1)	12.10 (3)	13.56 (1)	
	11.38 (6)		12.81 (2)	12.31 (2)		
	11.79 (7)		13.30 (1)	12.55 (2)		
	12.04 (2)		13.53 (1)	12.81 (2)		
	12.38 (4)			13.10 (1)		
	12.56 (2)			13.30 (1)		
	12.78 (5)			13.53 (1)		
	13.23 (4)					
	13.65 (2)					

[a] The spectra were run as nujol mulls using a Perkin–Elmer Model 12B instrument. The wave lengths are in microns. Intensities were estimated visually on a scale 0 to 10.

mitrine is therefore a triester of germine with either two moles of acetic acid and one mole of (levo)-α-methylbutyric acid or with two moles of the latter and one mole of the former. In order to decide between these two alternatives the amount of α-methylbutyric acid present in the above distillate was determined taking as a criterion its levo-rotation in water ($[\alpha]^{25}D$ −25°). The found value of 0.95 mole equivalent of α-methylbutyric acid established the composition of neogermitrine as that of a mono-α-methylbutyrate diacetate of germine. The carbon and hydrogen figures obtained with the intact alkaloid are in agreement with the formula $C_{36}H_{55}O_{11}N$, which is the composition required by such a triester. Neogermitrine thus contains the same acids as germidine (monoacetyl-mono-α-methylbutyrylgermine), but differs from the latter in that it possesses two acetyl groups instead of a single one. The presence of this additional acetyl group in neogermitrine causes a striking increase in the levo-rotation in pyridine from a value of −11° for germidine to that of −78° for neogermitrine, which corresponds to a change in the molecular rotation of $\Delta[M]^{25}D$ −465°. A similar change ($\Delta[M]^{25}D$ −470°) had previously been observed[2] with the pair germitrine–germerine, which likewise differs by one acetyl group. Such close correspondence in the molecular rotation changes accompanying the introduction of an acetyl group into germidine and germerine to form neogermitrine and germitrine, respectively, obviously suggests identical sites of attachment of this group on the alkamine germine in both neogermitrine and germitrine. The same conclusion has been arrived at as a result of a comparison of the reactivities of the acetoxy groups in the two alkaloids toward aqueous methanol. As has already been reported,[2] germitrine, when dissolved in this medium, readily loses its acetyl group to methanol by transesterification. Analogously, when neogermitrine was treated with dilute methanol under the conditions described in the experimental part one of the acetyl groups was split off as evidenced by the isolation of the diester germidine (Chart 1). This finding not only gave support to the above conclusions concerning the location of the (labile) acetoxy group, but established at the same time that, save for the presence in neogermitrine of the additional acetyl in this position, germidine and neogermitrine possess identical structures. When the methanolysis re-

CHART 1

METHANOLYSIS OF NEOGERMITRINE



action was extended over a period of about two to three days the yield of germidine became negligible and the only product isolated in crystalline form was a substance which had previously been encountered as the end-product of the methanolytic degradation of pentaacetylgermine and formulated as a monoacetyl derivative of germine[2] mainly on the basis of its volatile acid content. It has now been found that the volatile acid content of this product corresponds to only a small fraction and not to a full mole of acetic acid, as had been reported previously. From this new finding as well as from the reasonably close agreement (but not identity) of the melting points and infrared spectra of this methanolysis product and of germine, we conclude that the former represents not as originally assumed a monoacetyl derivative of germine, but germine itself contaminated by small amounts of partially degraded germine ester. Monoacetylgermine should therefore be stricken from the literature.

Acetylation of neogermitrine and of germidine with pyridine–acetic anhydride yielded monoacetyl neogermitrine (triacetyl-mono-α-methylbutyrylgermine). One of the five acylable hydroxyl groups of germine thus resists esterification. The esters germidine, neogermitrine and monoacetyl-neogermitrine represent a series of progressively acylated derivatives of germine, in which each higher member is derived from the one below it by the addition of one acyl group. A comparison of the hypotensive activities of the members of this series strikingly illustrates the influence of the number of ester groups on the physiological activity. Table II records the relative hypotensive potencies in the anesthetized dog of the above three esters, and of the alkamine germine. It can be seen that maximum potency is reached with the triester neogermitrine, and that the lowest and the highest members of the group show only insignificant, if any, activity. It is furthermore of interest that the triesters neogermitrine and germitrine are about equally potent in spite of the fact that they differ in one of the ester groups. This may perhaps indicate that maximum activity depends less on the nature of the ester groups than on their number and site. Such a view receives support from the finding that of a number of tri-, tetra- and pentaesters prepared by White[15] the triester triisobutyrylgermine, although considerably less potent than germitrine, was the most active.

The occurrence in the triester fraction of the present batch of *Veratrum viride* of both neogermitrine and germitrine, as contrasted to the presence in a previous batch[2] of germitrine only, prompted an inquiry into the derivation of all the source materials used in this work, a question which had not received much attention before. We were informed by our supplier that all of the batches of root employed by us were collected during the summer months in the Eastern United States in an area extending from North Carolina to Maine. The batch of root which had yielded only germitrine in the triester fraction was collected during 1947, whereas the present batch containing largely neogermitrine was part of the 1948 collection. Most recently, a batch of root collected in the same broadly defined area during the 1949 season has been found to yield almost exclusively neogermitrine. It may be concluded that the observed differences in the composition of the ester alkaloid fraction were referable either to the year-to-year variability of climate, to local conditions of growth at the sites chosen for collection or possibly to the occurrence of different strains within the general area mentioned, or to a combination of these factors.[15a]

TABLE II

DEGREE OF ESTERIFICATION AND HYPOTENSIVE ACTIVITY

Substance	Number of ester groups	Rel. potency (Dog) germitrine = 1000
Germine	0	0.2[a]
Germidine	2	240[b]
Neogermitrine	3	870[b]
Monoacetyl-neogermitrine	4	<80

[a] This value is of little significance since it may be due to contamination with minute amounts of the highly active ester alkaloids, from which germine is prepared by saponification. [b] Private communication from Dr. G. L. Maison, Dept. of Pharmacology, Boston University School of Medicine.

Experimental

Isolation of Neogermitrine.—The fractionation of the benzene-soluble alkaloids derived from a 50-lb. batch of *Veratrum viride* collected in the Summer of 1948 in the Eastern United States were carried out as described in a previous paper from this Laboratory.[2] In the final step, which consists of a 24-plate Craig distribution in separatory funnels, 5.2 g. of chromatographed material was used and the distribution curve shown in Fig. 1 was obtained. The isolation of germidine and neogermitrine from the peak tubes 5 and 15, respectively, and from neighboring tubes proceeded as follows:

A portion (160 mg.) of the combined contents of tubes 5, 6 and 7 was dissolved in a little ethanol. Careful addition of water to incipient turbidity caused crystallization of ger-

(15) H. L. White, THIS JOURNAL, **73**, 492 (1951).

(15a) ADDED IN PROOF.—The work-up of yet another batch of root by Dr. D. R. Walters of our laboratories yielded germerine and no germidine in the diester fraction. Germerine had not previously been isolated from *Veratrum viride*.

midine in characteristic thin plates. After two recrystallizations from the same solvents the crystals (32 mg. and additional small amounts of pure material from the mother liquors) melted at 202–203°.[16] When seeded with the higher melting form of germidine the melt resolidified and melted again at 231–233°, $[\alpha]^{23}D$ −11° (*c* 1.0 in pyridine); reported[2]: m.p. 198–200° and 230–231°, $[\alpha]^{25}D$ −11° (pyridine). The infrared spectrum of this sample was identical in every respect with that of an authentic specimen of germidine.

The contents of tubes 13–17 (2.30 g.) were combined and carefully fractionated by crystallization from dilute acetone. In all the crystallizations the material was dissolved in the minimum amount of acetone at room temperature, whereupon water was added until crystallization just began, and the mixture was allowed to come to equilibrium at room temperature. During our first attempt to purify neogermitrine the crystallizing solutions were allowed to cool in the refrigerator, and this resulted in impure preparations resembling germitrine in their physical characteristics. Two recrystallizations at room temperature produced material (1.18 g.) melting at 230–231° which represented essentially pure neogermitrine. It was recrystallized once more without change in melting point and dried at 110° *in vacuo* for analysis and rotation, $[\alpha]^{23}D$ −78° (*c* 0.38 in anhydrous pyridine); $[\alpha]^{23}D$ 0° (*c* 0.42 in $CHCl_3$).

Anal. Calcd. for $C_{36}H_{55}O_{11}N$: C, 63.80; H, 8.18; N, 2.04. Found[17]: C, 64.31, 64.34; H, 8.29, 8.54; N, 2.53.

In a volatile acids determination[18] 30.92 mg. of neogermitrine consumed 13.90 ml. of 0.01 *N* sodium hydroxide; calcd. for germine diacetate mono-α-methylbutyrate: 13.68 ml.

Additional amounts (*ca.* 400 mg.) of neogermitrine were obtained by systematic fractionation of the mother liquors according to the triangular scheme. Some of the neogermitrine fractions obtained in this manner melted somewhat higher (up to 235–237°) but appeared less pure on inspection of the crystals under the microscope. In the center fractions were found small amounts of a substance crystallizing in fine long needles, which was readily distinguishable from the heavier prisms of neogermitrine. This substance melted at 227–230° and had $[\alpha]^{24}D$ −6.5 (*c* 0.31 in $CHCl_3$). The amounts available were too small for rigorous purification and analysis. From the lowest mother liquors on the right-hand side of the crystallization diagram there was isolated after a large number of crystallizations a small amount (34 mg.) of heavy prisms which melted at 214–218°. This melting point is in accord with that reported for germitrine.[2] Identity with the latter alkaloid was established by comparison of the infrared spectra and by methanolytic degradation to germerine. For this purpose 10 mg. of the material was "recrystallized" twice from dilute methanol. The resulting material melted at 197–200° and its infrared spectrum was identical with that of an authentic sample of germerine; reported for germerine,[2] m.p. 203–204°.

Hydrolytic Cleavage of Neogermitrine to Germine, Acetic Acid and (*levo*)-α-Methylbutyric Acid.—To a solution of neogermitrine (152 mg.) in methanol (4.5 ml.) was added 1.07 *N* sodium hydroxide (1.2 ml.) and water (2.3 ml.). The mixture was allowed to stand at room temperature for 18 hours and the methanol was removed *in vacuo*. The residual aqueous solution was extracted with eight 10-ml. portions of chloroform, the chloroform layer washed with a small volume of water (5 ml.) and evaporated to dryness *in vacuo*. The crystalline residue (122 mg.) after two recrystallizations from methanol yielded small prisms which melted at 216–221° after prolonged sintering beginning at 160–165°, $[\alpha]^{24}D$ +5° (*c* 0.82 in 95% alcohol). The above data agree with those reported for germine.[5] The infrared spectrum was identical with that of an authentic specimen of germine.

Anal. Calcd. for $C_{27}H_{43}O_5N$: C, 63.63; H, 8.50. Found (after drying *in vacuo* at 110°): C, 63.84; H, 8.41.

The hydrochloride of the acetone compound prepared from the above sample of germine decomposed at 250–260°. Craig and Jacobs[7] have reported a melting point of 275° (dec., uncor.) for monoacetonylgermine hydrochloride.

(16) All melting points are corrected.

(17) The two sets of carbon and hydrogen values refer to two different fractions.

(18) Pregl-Roth, "Die Quantitative Organische Mikroanalyse," Julius Springer, Berlin, 1935, p. 235.

The alkaline solution and water washings, from which the germine had been removed by chloroform extraction, was neutralized with 0.1 *N* hydrochloric acid, made just alkaline toward phenolphthalein and 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 (6 ml.) and *p*-phenylphenacyl bromide (212 mg.) were added and the mixture was refluxed for two hours. After dilution with water (3 ml.) the alcohol was removed *in vacuo* and the resulting aqueous solution and precipitate extracted with chloroform. The residue from the chloroform solution (207 mg.) was dissolved in benzene (6 ml.) and hexane (3 ml.) and chromatographed on sulfuric acid-washed alumina (13 g.). Elution of the column with benzene–hexane (2:1) yielded at first *p*-phenylphenacyl bromide (m.p. 126–127°), which was followed immediately by the *p*-phenylphenacyl ester of α-methylbutyric acid (37 mg. crude). After a number of crystallizations from hexane this substance melted at 70–71° and showed no depression when mixed with an authentic sample melting at 71–72° derived from germerine. Continued elution of the column with the same solvent mixture yielded as a separate band the characteristic leaflets of *p*-phenylphenacyl acetate (68 mg. crude), which after crystallization from ether–hexane melted at 109.5–111°. The melting point of this sample was not depressed on admixture of an authentic sample of *p*-phenylphenacyl acetate. Subsequent elution of the column with the more polar solvents and solvent mixtures benzene, benzene–ether, benzene–acetone and benzene–methanol yielded only insignificant amounts of material.

Determination of (*levo*)-α-Methylbutyric Acid in the Mixture of Volatile Acids from Neogermitrine.—Neogermitrine (107.0 mg.) was saponified at room temperature with 0.15 *N* sodium hydroxide in 50% methanol (6 ml.) for a period of 18 hours. The mixture was placed in a micro distillation apparatus and the methanol distilled off at ordinary pressure. Since Poethke[5] had shown that saponification of germerine with (non-aqueous) methanolic potassium hydroxide led to the formation of methyl esters which distilled with the methanol, the above methanolic distillate was treated with excess dilute aqueous alkali at 100° for four hours. Back titration with dilute acid, however, showed only a negligible consumption of alkali.

To the above methanol-free solution was added 25% aqueous *p*-toluenesulfonic acid (2 ml.) and the mixture was distilled at ordinary pressure. Water was added at intervals to the distilling flask to maintain a constant volume and the distillate was collected in an ice-cooled receiver. Three fractions were collected and the optical rotation of these fractions was determined in a 2-dm. tube. The first fraction (3 ml.) showed α = −0.23°, which corresponds to 13.8 mg. of (levo)-α-methylbutyric acid ($[\alpha]^{25}D$ −25°). The second fraction (2 ml.) contained 1.6 mg. (α = 0.04°) and the third fraction (2 ml.) was devoid of optical activity. 107 mg. of neogermitrine thus yielded a total of 15.4 mg. of (levo)-α-methylbutyric acid; calcd. for a mono-α-methylbutyrate diacetate of germine: 16.2 mg. of (levo)-α-methylbutyric acid.

Methanolytic Degradation of Neogermitrine to Germidine.—A solution of neogermitrine (41.5 mg.) in methanol (0.3 ml.) and water (0.1 ml.) was allowed to stand at room temperature. A small amount of neogermitrine which had crystallized out initially went into solution as the reaction proceeded. After 18 hours, water was added dropwise to incipient turbidity and a seed of germidine was added. Crystallization ensued which was allowed to proceed to completion in the refrigerator. The resulting material (20.4 mg.) after two recrystallizations from dilute alcohol melted at 198–200°. Seeding of the melt with the higher melting form of germidine caused resolidification, whereupon the product melted at 226–228°, $[\alpha]^{24}D$ −11° (*c* 0.40 in pyridine). The above constants are characteristic for germidine.[2] The infrared spectrum of this sample was identical with that of an authentic specimen of germidine.

Methanolytic Degradation of Neogermitrine to Germine.—A solution of neogermitrine (41 mg.) in methanol (0.8 ml.) and water (0.4 ml.) was allowed to stand at room temperature for three days. The solution was then concentrated to dryness *in vacuo* and taken up in a small volume of dry methanol. On standing in the refrigerator crystallization took place and the resulting precipitate was recrystallized twice from methanol. The purified product crystallized in well defined, small prisms, which sintered at 160°, liqui-

fied at 170° and formed a meniscus at 190–195°. The infrared spectra of the above sample and of a sample prepared by methanolysis of germine pentaacetate were identical. This material represents germine contaminated with small amounts of germine esters.

Anal. Calcd. for $C_{27}H_{43}O_5N$: C, 63.63; H, 8.50. Found: C, 63.23; H, 8.64.

In a volatile acid determination[18] 6.795 mg. consumed 0.40 ml. of 0.01 *N* NaOH; calcd. for germine monoacetate, 1.23 ml.

Methanolysis of Pentaacetylgermine.—A solution of pentaacetylgermine (109.4 mg.) in methanol (16 ml.) and water (8 ml.) was allowed to stand at room temperature for 18 hours. After removal of the solvents *in vacuo* the residue was taken up in a small amount of dry methanol and allowed to crystallize in the refrigerator. Three recrystallizations from methanol yielded material (9 mg.) which sintered at 165–170° but did not completely liquefy until at about 195°. The infrared spectrum of this material was identical with that of the material described in our earlier publication.[2]

Volatile acid determinations[18] on two independently prepared samples weighing 6.173 and 5.559 mg., respectively, yielded amounts of acid equivalent to 0.34 and 0.14 ml. of 0.01 *N* NaOH, respectively; calcd. for germine monoacetate: 1.21 and 1.09 ml., respectively.

Monoacetylneogermitrine.—Neogermitrine (32 mg.) was acetylated with acetic anhydride (1 ml.) and pyridine (1 ml.) for 15 hours at room temperature. Evaporation of the reagents *in vacuo* left a crystalline residue, which was recrystallized twice from acetone in which it is rather sparingly soluble. The thus purified product (22.9 mg.) melted at 248–249° (dec.) and had $[\alpha]^{24}D$ −88° (*c* 0.45 in pyridine).

Anal. Calcd. for $C_{35}H_{57}O_{12}N$: C, 63.41; H, 7.98. Found: C, 63.28; H, 7.95.

In a volatile acid determination[18] 14.57 mg. consumed 7.79 ml. of 0.01 *N* NaOH; calcd. for germine triacetate mono-α-methylbutyrate, 8.08 ml.

Acetylation of germidine under the above conditions yielded a product having the same properties.

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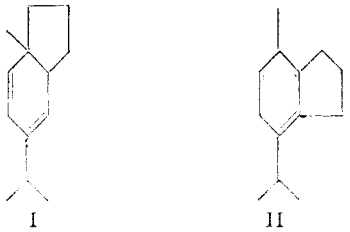
[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY OF HARVARD UNIVERSITY AND COLUMBIA UNIVERSITY]

Picrotoxin. II. The Skeleton of Picrotoxinin. The Total Synthesis of *dl*-Picrotoxadiene

BY HAROLD CONROY[1]

Picrotoxadiene, a degradation product of picrotoxinin, has been synthesized by an unambiguous route, and shown to be *cis*-5-isopropyl-8-methylhydrin-4,6-diene. The skeleton of picrotoxinin has been shown to be that of 1,2,6,7,9-pentoxy-1,4-dicarboxy-5-isopropenyl-8-methylhydrindane. The unique relationship of picrotoxinin to the steroid family is pointed out.

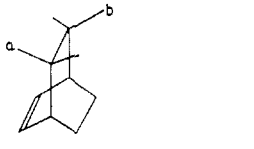
Picrotoxadiene,[2] a hydrocarbon retaining the relevant features of the picrotoxin skeleton, has been obtained by a systematic, stepwise degradation of the unsaturated hydroxyketolactone, picrotoxinide,[2] in turn prepared by pyrolysis of dihydro-α-picrotoxininic acid.[3] It was shown that picrotoxadiene must have either of the structures (I) or (II), although I was favored



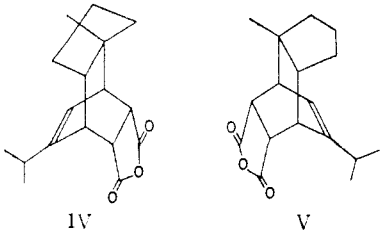
I II

because of the ultraviolet spectrum (λ_{max} 258 mμ, log ε 3.6; expected for I: 260 mμ, for II: 272 mμ) and because the substance offered resistance to dehydrogenation under mild conditions. Accordingly, a synthesis of 5-isopropyl-8-methylhydrin-4,6-diene was undertaken and is reported in detail herein.[4]

The structure (I) implies two forms differing in the configuration at the ring junction, *i.e.*, *cis* and *trans*. To decide which isomer should be the aim of synthesis, consider the following argument: *trans* groups, (a) and (b), protruding from the bicyclo-[2.2.2]octene system (III) are held rigidly at opposed angles, but in the (hypothetical) adduct (IV)[5] from maleic anhydride and the *trans* form of I, *these must be joined to the same atom*. The *cis*-adduct (V)[5] is essentially strain-free. The strain



III



IV V

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(2) For the first article of this series, see H. Conroy, THIS JOURNAL, **74**, 491 (1952).

(3) P. Horrmann, *Ber.*, **46**, 2793 (1913).

(4) First reported in a Communication to the Editor, H. Conroy, THIS JOURNAL, **73**, 1889 (1951).

(5) Two points of stereochemistry implied in the expressions (IV) and (V) have been assumed for purposes of the discussion; although these points may be incorrectly represented, the argument is not thereby invalidated. Thus the configuration of the angular methyl group in each case has been almost arbitrarily assigned, while the anhydride grouping is shown *endo* only on the basis of the rule of Alder [K. Alder and G. Stein, *Angew. Chem.*, **50**, 510 (1937); K. Alder, *et al.*, *Ann.*, **514**, 1 (1934); K. Alder and E. Windemuth, *Ber.*, **71**, 1939 (1938)].