10 ml.)–water (1 ml.). After 45 minutes at room temperature, acetic acid was added to *p*H 6 and the methanol was evaporated *in vacuo*. The aqueous solution was made alkaline with ammonium hydroxide and extracted with chloroform (ten 10-ml. portions). The residue from chloroform crystallized from ether–petroleum ether, yield 95 mg., m.p. 210–235° dec. Recrystallization from methanol–water afforded 40 mg. of germine acetonide monoacetate (XXVI), m.p. 259–262°. The infrared spectrum of the product was identical with that of the authentic sample.

Formamido Ketone from Germine Tetraacetate (XXX).—A solution of germine tetraacetate[19] (2 g.) in pyridine (20 ml.) was added to chromic anhydride (4 g.) in pyridine (40 ml.). After 40 hours at room temperature, water (40 ml.), ammonium hydroxide (6 ml.) and chloroform (100 ml.) were added. The mixture was shaken and filtered through Supercel to clear the emulsion. After nine additional extractions with chloroform (50 ml.), the combined chloroform extracts were evaporated to dryness *in vacuo*. By cautious addition of petroleum ether to a chloroform solution of the residue, a dark amorphous solid was precipitated which was collected and rejected. The chloroform–petroleum ether solution was evaporated to dryness and the residue was taken up in acetone–petroleum ether. Again the first precipitate formed was filtered and rejected. From the filtrate, a crystalline product gradually separated (228 mg., m.p. 243–246° dec.). Recrystallization from acetone–ether gave colorless clusters of needles; yield 151 mg., m.p. 246–247° dec., $[\alpha]^{33}D$ −109° (*c* 0.55, pyr.); λ_{max} 5.78–5.85, 6.07 μ.

Anal. Calcd. for $C_{35}H_{49}O_{14}N$: C, 59.39; H, 6.98. Found: C, 59.25; H, 6.89.

In a volatile acid determination[42] 11.96[2] mg. of the formamido ketone yielded an amount of acid equivalent to 17.00 ml. of 0.004819 *N* sodium thiosulfate; calcd. for four mole equivalents of acetic acid and one mole equivalent of formic acid, as expected for structure XXX, 17.53 ml.

Formamido Ketone from Germine Pentaacetate (XXI).—Oxidation of germine pentaacetate (2 g.) by the procedure described above for the tetraacetate and crystallization of the product from ether–petroleum ether gave a microcrystalline formamido ketone (195 mg.), m.p. 205–210° dec. after sintering from 165°, $[\alpha]^{33}D$ −89° (*c* 0.72, pyr.); λ_{max} 5.78–5.85, 6.07 μ.

Anal. Calcd. for $C_{37}H_{51}O_{15}N$: C, 59.27; H, 6.86. Found: C, 59.36; H, 7.16.

In a volatile determination[42] 16.31 mg. of this formamido ketone yielded an amount of acid equivalent to 27.05 ml. of 0.004819 *N* sodium thiosulfate; calcd. for five mole equivalents of acetic acid and one mole equivalent of formic acid, as expected for structure XXXI, 27.08 ml.

(42) J. B. Niederl and V. Niederl, "Micromethods of Quantitative Organic Analysis," John Wiley and Sons, Inc., New York, N. Y., 1942, pp. 257–262. This determination and all other microanalyses reported were carried out by Dr. S. M. Nagy and associates at M.I.T. All samples were dried *in vacuo* at 110°.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

Veratrum Alkaloids. XXIX.[1] The Structures of Germitrine, Neogermitrine and Several Related Hypotensive Ester Alkaloids[2,3]

BY S. MORRIS KUPCHAN

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The structures of several naturally-occurring hypotensive esters of germine have been elucidated. The chemically-related series neogermitrine, neogermidine and germidine are formulated as IV, III and II, respectively. The chemically-related series germitrine, germerine and protoveratridine are represented by structures XIV, XIII and XII, respectively.

Alkaloid mixtures obtained from veratrum plants are employed in the treatment of hypertension. During the past decade, extensive studies of the hypotensive alkaloidal constituents of a number of veratrum[4–13] and zygadenus[14,15] species have shown that many of the most active principles are esters of the alkamine germine (I).[1] In all, eleven well-characterized germine esters have been isolated from the alkaloidal extracts of plants.[16] Some of the reported physical and chemical properties of these ester alkaloids are summarized in Table I. It is the purpose of this paper to present the structure elucidation of several of the naturally-occurring germine ester alkaloids.

The series of esters related to neogermitrine has received the most chemical attention (Chart 1). Methanolysis of neogermitrine was shown to result in loss of one acetate grouping with conversion to the diester germidine.[7] Furthermore, on acetylation both alkaloids were converted to the same product, monoacetylneogermitrine. Acid hydrolysis of neogermitrine led to loss of an acetate group with conversion to the diester neogermidine. The germidine isomer neogermidine was also shown to undergo acetylation to monoacetylneogermitrine. Finally, a synthetic mono-(*l*)-2-methylbutyrate of germine has been shown to be convertible to the same acetylation product.[17] These relationships reveal that the site of attachment of the (*l*)-2-

(1) Part XXVIII, S. M. Kupchan and C. R. Narayanan, THIS JOURNAL, **81**, 1913 (1959).

(2) The investigation which forms the subject of the present paper was first outlined in part in a preliminary communication: *Chemistry & Industry*, 1092 (1956). Part of the work was performed by the author at the Department of Chemistry, Harvard University.

(3) This investigation was supported by research grants (H-1563 and H-2275) from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

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

(5) W. Poethke, *ibid.*, **275**, 357, 571 (1937).

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

(7) J. Fried, P. Numerof and N. H. Coy, *ibid.*, **74**, 3041 (1952).

(8) H. A. Nash and R. M. Brooker, *ibid.*, **75**, 1942 (1953).

(9) S. M. Kupchan and C. V. Deliwala, *ibid.*, **75**, 4671 (1953).

(10) M. W. Klohs, M. D. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek, *ibid.*, **75**, 4925 (1953).

(11) M. W. Klohs, M. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek, *ibid.*, **76**, 1152 (1954).

(12) G. S. Myers, P. Morozovitch, W. L. Glen, R. Barber, G. Papineau-Couture and G. A. Grant, *ibid.*, **77**, 3348 (1955).

(13) G. S. Myers, W. L. Glen, P. Morozovitch, R. Barber, G. Papineau-Couture and G. A. Grant, *ibid.*, **78**, 1621 (1956).

(14) S. M. Kupchan and C. V. Deliwala, *ibid.*, **76**, 5515 (1954).

(15) S. M. Kupchan, C. V. Deliwala and R. D. Zonis, *ibid.*, **77**, 755 (1955).

(16) In view of the facile deacylation of some germine ester alkaloids (*vide infra*), the possibility exists that some of the di- and monoesters may be artifacts formed during the isolation procedures.

(17) F. L. Weisenborn and J. W. Bolger, *ibid.*, **76**, 5513 (1954).

TABLE I

NATURALLY-OCCURRING GERMINE ESTERS

Ester	Formula	M.p., °C.	$[\alpha]D$	Pyr.	Acids[a]	Ref.
Germitetrine	$C_{41}H_{63}O_{14}N$	229–230	−69°		2Ac, MB, lm DMB	8, 9, 13
Germitrine	$C_{39}H_{61}O_{12}N$	216–219	−69		Ac, MB, HMB	6
Neogermitrine	$C_{36}H_{55}O_{11}N$	234–235	−78		2Ac, MB	7, 9, 10, 11, 14, 15
Germanitrine	$C_{39}H_{59}O_{11}N$	228–229	−61		Ac, MB, T	10
Germinitrine	$C_{39}H_{57}O_{11}N$	175–176	−36		Ac, T, An	10
Germerine	$C_{37}H_{59}O_{11}N$	200–203	− 7		MB, HMB	5, 6
Germidine	$C_{34}H_{53}O_{10}N$	230–231	−11		Ac, MB	6, 14
Neogermidine	$C_{34}H_{53}O_{10}N$	221–223	−60		Ac, MB	12, 14, 15
Germbudine	$C_{37}H_{59}O_{12}N$	160–164	− 8		MB, hm DMB	12
Neogermbudine	$C_{37}H_{59}O_{12}N$	149–152	−12		MB, lm DMB	12, 13
Protoveratridine	$C_{32}H_{51}O_9N$	272–273	− 9		MB	4, 5, 14

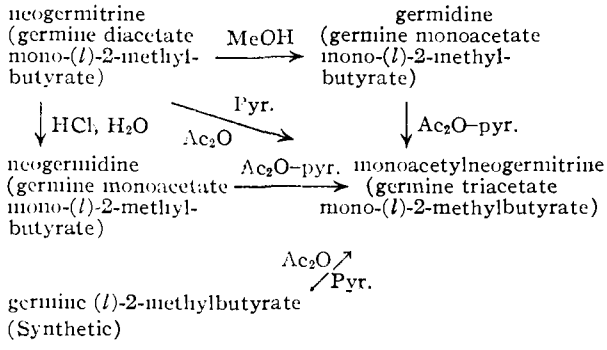
[a] Ac = acetic acid; MB = (*l*)-2-methylbutyric acid; HMB = (*d*)-2-hydroxy-2-methylbutyric acid; lm DMB = low melting 2,3-dihydroxy-2-methylbutyric acid; hm DMB = high melting 2,3-dihydroxy-2-methylbutyric acid; T = tiglic acid; An = angelic acid.

methylbutyryl residue is the same in each of the four precursors of monoacetylneogermitrine.[18]

Periodic acid oxidation studies have revealed the site of attachment of the 2-methylbutyryl residue. Germidine and neogermitrine were stable to periodic acid; neogermidine consumed one mole of periodic acid and yielded an amorphous aldehydo-γ-lactone (λ_{max} 3.65, 5.62, 5.80–5.85). Hence, neogermidine

CHART 1

CORRELATION OF DERIVATIVES OF GERMINE (*l*)-2-METHYLBUTYRATE



possesses a free α-ketol-hemiketal system in rings A and B. Furthermore, germidine contains an ester group on a hydroxyl group of the ring A glycol and a second ester group at C_{15}. These facts and the aforementioned conclusion that the location of the 2-methylbutyryl residue is the same in these alkaloids fix the site of attachment of the residue at C_{15}.

It now becomes possible to assign structures to the polyesters of the neogermitrine series. Since monoacetylneogermitrine can be obtained by stepwise acylation of germine,[17] it is reasonable to assume that the same hydroxyl groups are acylated as in the analogous germine 3,7,15,16-tetraacetate.[1] The acetate group of germidine therefore is located at C_3 and germidine is assigned structure II. Assignment of the acetate group of germidine to C_3 is in accord with the molecular rotatory data for other germine 3-acetate derivatives (see Table II).

The second acetate of neogermitrine is assigned to C_7 (leading to expression IV for neogermitrine

(18) This conclusion is based on the assumption that no transesterification takes place during the acetylation reactions. The assumption is supported by the fact that no transesterification during acylation of polyoxygenated veratrum alkaloids has ever been observed in our laboratory or reported by others.

TABLE II

MOLECULAR ROTATIONS OF THE GERMINE ESTERS IN PYRIDINE

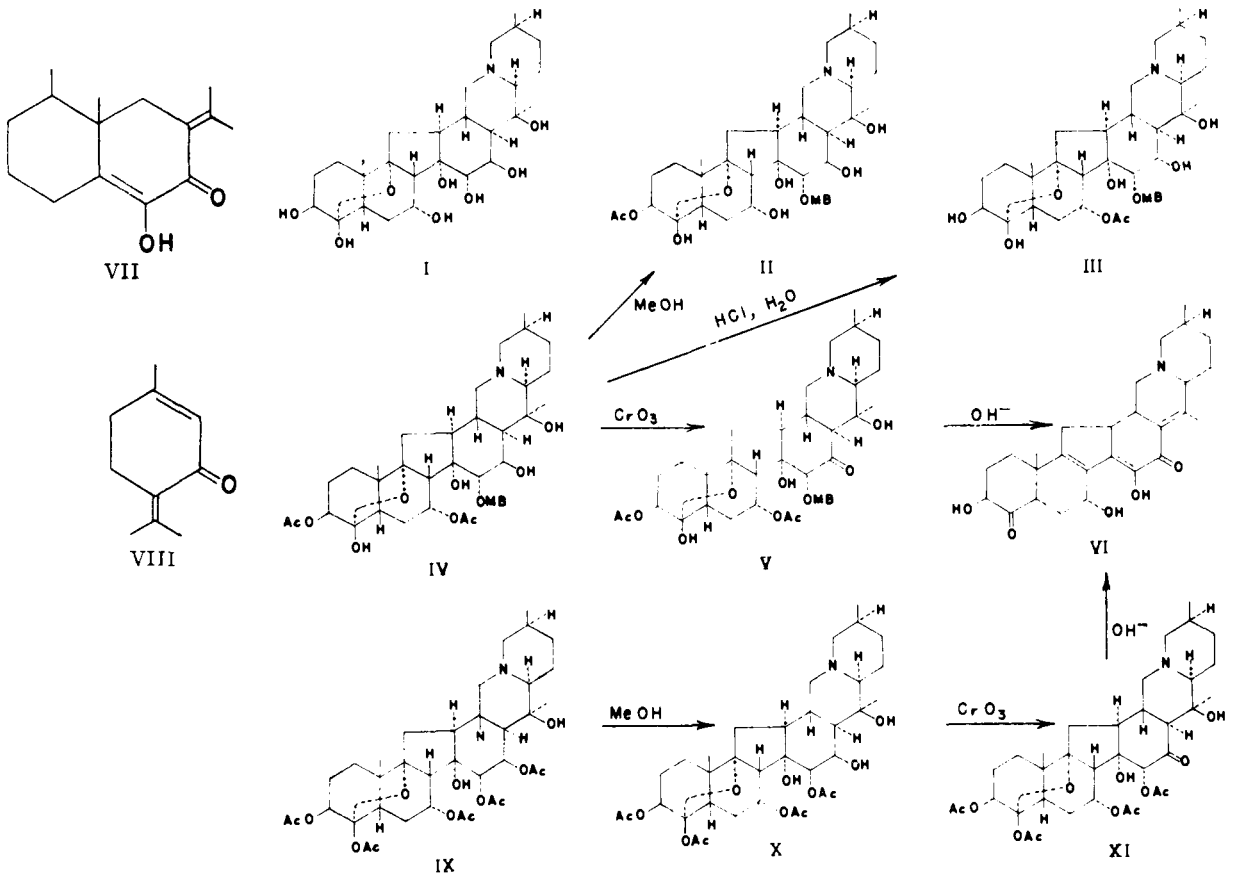
	Alkaloid	[M]o	Basis of calculation	Contribution of acyl groups 3	7	15	16
1.	Germine	− 15°					
2.	Germine 3-acetate[1]	+ 55	2–1	+70°			
3.	Germine 16-acetate[1]	−105	3–1				− 90°
4.	Germine 3,16-diacetate	− 22	4–3	+83			
5.	Germine 15-(*l*)-2-methylbutyrate[17]	−154	5–1		−139°		
6.	Germidine[6]	− 70	6–5	+84			
7.	Neogermidine[14]	−382	7–5		−228		
8.	Neogermitrine[7]	−529	8–6		−459		
9.	Monoacetylneogermitrine[7]	−705	9–8				−176
10.	Germerine[5]	− 49					
11.	Germitrine[5]	−508	11–10		−459		
12.	Germanidine	− 27					
13.	Germanitrine	−438	13–12		−411		
14.	Desacetylgermitetrine[18]	− 60					
15.	Germitetrine[5,18]	−547	15–14		−487		
16.	Germine 14,15-acetonide 3-acetate[1]	+237					
17.	Germine 14,15-acetonide 3,16 diacetate[1]	+158	17–16				− 79
18.	Germine isotetraacetate	−339					
19.	Germine 3,4,7,15,16-pentaacetate	−468	19–18				−129

and, consequently, expression III for neogermidine) on the basis of the following sequence. Oxidation of neogermitrine with chromic anhydride–acetic acid yielded neogermitrone, formulated as the 16-dehydro derivative V. Alkaline treatment of neogermitrone yielded a product to which the cross-conjugated diosphenol structure VI is assigned. The structural assignment is made on the basis of analysis, infrared spectrum and ultraviolet spectrum. The ultraviolet spectrum showed λ_{max} 319 (ε 12,700), 285 mμ (ε 7000); λ_{max} 0.01 *N* KOH 373 (ε 9,500), 343 mμ (ε 7,800). The shift of the principal peak in alkali, and, in particular, the change in relative intensity of the peaks are characteristic of the diosphenol chromophore.[19] A chromophore related to that of VI is present in hydroxyeremophilone (VII); λ_{max} 312.5 (ε 9,500), 277.5 mμ (ε, 6,000).[20] The absorption of VII in

(19) D. H. R. Barton and J. F. Eastham, *J. Chem. Soc.*, 424 (1953).

(20) A. E. Gillam, J. I. Lynas-Gray, A. R. Penfold and J. L. Simonsen, *ibid.*, 60 (1941).

CHART 2



alkali has unfortunately not been reported. The failure of the ring D system of VI to aromatize, perplexing at first glance, finds ample precedent in the reluctance of piperitenone (VIII) to change to its phenolic isomer.[21]

Professor D. H. R. Barton has kindly informed me of unpublished experiments which demonstrated the sequence: germine pentaacetate→germine isotetraacetate→dehydrogermine isotetraacetate→ a diosphenol. I have shown that the diosphenol obtained by this sequence is the same as the diosphenol derived from neogermitrine and propose that the sequence be formulated IX→X→XI→ VI.

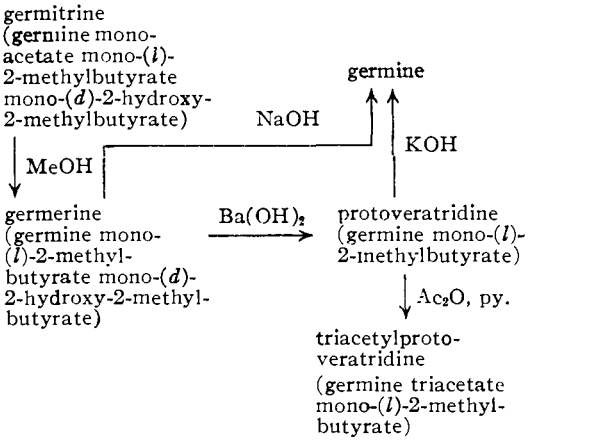
A second chemically related series of ester alkaloids consists of germitrine, germerine and protoveratridine (Chart 4). It has been shown that methanolysis of germitrine results in the loss of one acetate grouping with conversion to the diester germerine.[6] Partial hydrolysis of germerine with barium hydroxide afforded the monoester protoveratridine.[5] Hydrolysis of protoveratridine with methanolic potassium hydroxide yielded germine.[5]

Germerine consumes periodic acid at a negligible rate. Hence, like germidine, germerine contains an ester group on a hydroxyl group of the ring A glycol and a second ester group at C_{15}.

(21) W. Kuhn and H. Schinz, *Helv. Chim. Acta*, **36**, 161 (1953); *cf.* the discussion in D. H. R. Barton, O. Jeger, V. Prelog and R. B. Woodward, *Experientia*, **10**, 81 (1954).

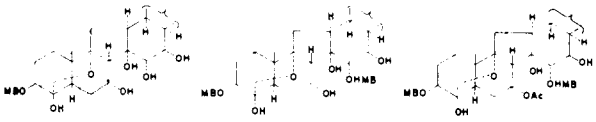
Since protoveratridine is an isomer of synthetic germine-15-(*l*)-2-methylbutyrate,[14,17] it is the (*l*)-2-methylbutyrate residue which is located in ring A, *i.e.*, at C_3 or C_4. A germine 4-monoester on non-forcing acetylation would form a pentaester.[1] In fact, protoveratridine is known to form a tetraester (presumably the 3,7,15,16) on acetylation[14] and a germine 4-(*l*)-2-methylbutyrate structure

CHART 3



is therefore precluded. Hence protoveratridine is germine 3-(*l*)-2-methylbutyrate (XII) and germerine consequently has structure XIII. The structural parallel of the diesters germidine (II) and

CHART 4



XII XIII XIV

germerine (XIII) is in accord with the similarity in their molecular rotations.[17]

The acetate group of germitrine shows the same reactivity as the C_7-acetate of neogermitrine and a strikingly similar contribution to the molecular rotation. On this basis, the acetate of germitrine is assigned to C_7, leading to structure XIV for germitrine. Incidentally, the same considerations suggest that the acetate groupings lost in the transformations of germitetrine to desacetylgermitetrine[13] and of germanitrine to germanidine[10] are likewise affixed at C_7.

Experimental[22]

Periodic Acid Oxidations.[23]—The titrations were performed as in Part XXV.[1] Germidine and neogermitrine consumed no periodic acid in 20 hours. Germerine consumed 0.1 mole equivalent in 2 hours, 0.3 in 6 hours and 0.5 in 23 hours. Periodic acid oxidation of ring glycols in germine has been shown to proceed rapidly; in each case studied oxidation has been substantially complete within an hour. We feel that the observed consumption of periodic acid by germerine is attributable to slow cleavage of the 2-hydroxy-2-methylbutyryl residue at C_{15}, with liberation of the ring D periodate-sensitive triol system.

Neogermidine (30 mg.) was dissolved in 5% acetic acid (5 ml.) and water (15 ml.) and 0.05 *M* periodic acid (5 ml.) was added. Titration of a 5-ml. aliquot after 1 hour showed an uptake of 0.9 mole equivalent. Titration of a second 5-ml. aliquot after 5 hours showed that the consumption was unchanged. The remainder of the solution (15 ml.) was made alkaline with ammonia and extracted with chloroform (six 15-ml. portions). Evaporation of the chloroform left an amorphous residue (7.5 mg.) which showed infrared absorption at 3.65, 5.62, 5.80–5.85 μ. These bands have been shown to characterize the ring A aldehydo-γ-lactone structure.[24]

Acid Hydrolysis of Neogermitrine to Neogermidine.[25]—Neogermitrine (324 mg.) was dissolved in a mixture of concentrated hydrochloric acid (1 ml.) and water (9 ml.) and the solution was allowed to stand at room temperature for six hours. The solution was made alkaline with ammonia and extracted with chloroform. The chloroform solution was dried over sodium sulfate and evaporated to dryness *in vacuo*. The residue was dissolved in chloroform and chromatographed on Merck acid washed alumina (9 g.) using chloroform and chloroform–1% methanol as eluents. The first 100 ml. chloroform fraction yielded 109 mg. of amorphous residue: crystallization from ether gave recovered neogermitrine (99 mg.). The next 200 ml. of chloroform yielded 24 mg. of residue apparently consisting of neogermitrine and germidine, as indicated by paper chromatographic comparison with authentic samples by the ethylene chloride–cellosolve acetate–pyridine method of Levine and Fischbach.[26] The following fractions eluted with chloroform–1% methanol (300 ml. total) afforded a residue (50 mg.) with an R_f identical with that of authentic neogermidine.[14] Crystallization from ether gave needles (31 mg.), m.p. 224–227° (dec.). Recrystallization from benzene afforded prisms, m.p. 224–226° (dec.). The melting point was not depressed by admixture with the authentic specimen of neogermidine. The infrared spectra were identical.

Oxidation of Neogermitrine to Neogermitrone (V).—Neogermitrine (800 mg.) was dissolved in 0.16 *N* chromic anhydride in 98.5% acetic acid (40 ml.) and the solution was allowed to stand at room temperature for 24 hours. The solution was cooled in an ice-bath and treated first with dilute sodium bisulfite to a green color and then with 20% sodium hydroxide until alkaline. The solution was extracted with chloroform (nine 100-ml. portions) and the combined chloroform extracts were washed with water (20 ml.), dried over sodium sulfate, and evaporated to dryness *in vacuo*. The residue crystallized from alcohol, yield 550 mg., m.p. 222–224° dec. Recrystallization from alcohol afforded colorless needles (440 mg.), m.p. 215–216° dec., $[\alpha]^{23}D$ −192° (*c* 1.43, pyr.).

Anal. Calcd. for $C_{36}H_{53}O_{11}N$: C, 63.98; H, 7.90. Found: C, 63.86; H, 8.09.

Alkaline Treatment of Neogermitrone.—Neogermitrone (150 mg.) was treated with methanol (15 ml.) and 50% sodium hydroxide solution (0.3 ml.) and the solution was heated under reflux for 12 minutes. The resulting red solution (with green fluorescence) was treated with acetic acid (2 ml.) and evaporated *in vacuo*. The oily residue was treated with water (5 ml.) and dilute ammonium hydroxide to *p*H 8.5 and extracted with chloroform (ten 10-ml. portions). The residue obtained by evaporation of the combined chloroform extract was taken up in ether (20 ml.) and filtered from the highly colored insoluble precipitate. The clear ethereal solution was concentrated to dryness *in vacuo*. The light-tan solid residue (70 mg.) showed m.p. 170–180° dec., λ_{max} 319 (ϵ 12,700), 285 mμ (ϵ 7,000); $\lambda_{max}^{0.1\ N\ KOH}$ 373 (ϵ 9,500), 343 mμ (ϵ 7,800); λ_{max} 5.85, 6.00–6.20 μ.

Anal. Calcd. for $C_{27}H_{35}O_5N$: C, 71.49; H, 7.78. Found: C, 71.27; H, 8.22.

Germine 3,4,7,15-Tetraacetate ("Isotetraacetate") (X).—Germine pentaacetate (5 g.) was dissolved in methanol (100 ml.) by heating under reflux, water (50 ml.) was added and the solution was allowed to stand at room temperature for 19 hours. The methanol was evaporated *in vacuo* until crystallization began; chloroform (80 ml.) was then added to extract the alkaloid. The aqueous layer was extracted with nine 20-ml. portions of chloroform. The combined chloroform extracts were evaporated to dryness and the residue crystallized from benzene. Colorless needle clusters (2.43 g.) were obtained, m.p. 302–304° dec., $[\alpha]^{24}D$ −50° (*c* 0.89, pyr.).

Anal. Calcd. for $C_{27}H_{39}O_8N(COCH_3)_4$: C, 62.02; H, 7.59; acetyl, 25.40. Found: C, 61.86; H, 7.58; acetyl, 25.02.

Concentration of the benzene mother liquors yielded a second crop of 400 mg., m.p. 297–300° dec.

16-Dehydrogermine 3,4,7,15-Tetraacetate (XI).—Germine 3,4,7,15-tetraacetate (1.5 g.) was dissolved in 0.16 *N* chromic anhydride in 98.5% acetic acid (60 ml.) and the solution was allowed to stand at room temperature for 24 hours. The reaction mixture was worked up as described above for neogermitrone. The residue from chloroform crystallized from ether; yield 1.1 g. of microcrystalline product, m.p. 223–225° dec. This material was recrystallized by solution in chloroform, evaporation to an amorphous residue, and solution in ether. Clusters of colorless rods (950 mg.) were obtained, m.p. 226–228° dec., $[\alpha]^{23}D$ −137° (*c* 1.98, pyr.).

Anal. Calcd. for $C_{27}H_{37}O_8N(COCH_3)_4$: C, 62.20; H, 7.31. Found: C, 62.22; H, 7.34.

Alkaline Treatment of 16-Dehydrogermine 3,4,7,15-Tetraacetate.—16-Dehydrogermine 3,4,7,15-tetraacetate (150 mg.) was treated with methanol and 50% sodium hydroxide solution in the manner described above for neogermitrone. As in the case of neogermitrone, a red solution with green fluorescence was soon formed. Work-up as above yielded a tan solid residue (60 mg.), m.p. 170–180°, with ultraviolet and infrared spectral characteristics identical with those of the diosphenol from neogermitrone.

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(22) Melting points are corrected for stem exposure. Values of $[\alpha]D$ have been approximated to the nearest degree. Ultraviolet absorption spectra were determined in 95% ethanol on a Cary recording spectrophotometer (model 11 MS). Infrared spectra were determined on a Baird double beam infrared recording spectrophotometer (model B) in chloroform. Microanalyses were carried out by Dr. S. M. Nagy and his associates at M.I.T. on samples dried *in vacuo* at 110°.

(23) Experiment by Drs. C. R. Narayanan and C. I. Ayres. The germerine was generously supplied by Dr. Josef Fried of The Squibb Institute for Medical Research.

(24) S. M. Kupchan, M. Fieser, C. R. Narayanan, L. F. Fieser and J. Fried, THIS JOURNAL, **77**, 5896 (1955).

(25) Experiment by Dr. C. I. Ayres.

(26) J. Levine and H. Fischbach, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 191 (1957).