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

Veratrum Alkaloids. XXVIII.[1] The Structure and Configuration of Germine[2-4]

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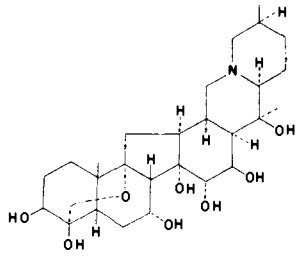
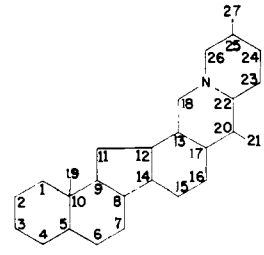
The alkaloid germine has been shown to have structure and configuration I. Alkaline isomerization of I leads to isogermine (XI) and thence to pseudogermine (XII). Acetic anhydride–pyridine acetylation of I affords a tetraacetate (XIII); acetylation under more vigorous conditions gives a pentaacetate (XIV). I forms an acetonide (XV) which is oxidized by periodic acid to an aldehydo-γ-lactone (XVI) also obtained from pseudogermine acetonide (XVII); XVI yields a diacetate (XX). The oxime XIX derived from XVI also yields a diacetate (XXII). Compound XI yields an acetonide (XVIII) which is acetylated to a diacetate (XXI). Germine acetonide (XV) affords a diacetate (XXIII) which is hydrolyzed by mineral acid to germine diacetate (XXIV). Periodic acid oxidation of XXIV yields a cyclopentenone aldehyde (XXV). Methanolysis of XXIII yields germine acetonide monoacetate (XXVI) which is hydrolyzed to germine monoacetate (XXVII) by acid. Chromic acid oxidation of XXIII affords a dehydrogermine acetonide diacetate (XXVIII) which affords a dehydrogermine diacetate (XXIX) on acid hydrolysis. Chromic anhydride–pyridine oxidation of XIII yields a formamido ketone derivative (XXX); similar oxidation of XIV affords an analogous product (XXXI).

Germine, $C_{27}H_{43}O_8N$, is the alkamine present in many polyester alkaloids which occur in *Veratrum*[5-9] and *Zygadenus*[10] species. The structure of germine is of particular interest in view of the powerful hypotensive action of its ester derivatives[11] and of the use of this antihypertensive action in clinical conditions associated with high blood pressure.[12] In this paper evidence is presented for assignment of structure and configuration I to germine.



I

Germine was first obtained by Poethke, in 1937, by alkaline hydrolysis of its esters protoveratridine and germerine.[13] Poethke described several crystalline salts and proposed the formula $C_{26}H_{41}O_8N$ for the alkamine. Craig and Jacobs further examined the question of germine's empirical formula and they advanced a $C_{27}H_{43}O_8N$ formulation on the basis of their analytical work on the alkamine and on germine acetonide hydrochloride.[14a] The latter authors also demonstrated a close structural analogy to cevine by isolating β-picoline, 2-ethyl-5-methylpyridine, cevanthrol and cevanthridine from the products of selenium dehydrogenation of germine. These results led Jacobs and Pelletier to propose skeletal structure II for germine as well as cevine.[15] Germine undergoes a series of isomerizations (germine→isogermine→pseudogermine) which parallels those of zygadenine[16] and veracevine.[17-19]



II

Our earlier work was concerned with the nature of the germine–isogermine–pseudogermine isomerizations. The oxidation of germine acetonide and pseudogermine acetonide to the same aldehydo-γ-lactone showed that these compounds contain the same α-ketol-5-membered hemiketal system. Furthermore, the close analogy of the isomerizations to the veracevine–cevagenine–cevine[20] isomerizations

(1) Part XXVII, S. M. Kupchan and C. I. Ayres, THIS JOURNAL, **81**, 1009 (1959).

(2) The investigations which form the subject of this paper were first outlined in part in two preliminary communications: *Chemistry & Industry*, 251 (1955); 1092 (1956). Parts of the investigations were performed by the authors at the Departments of Chemistry of Harvard University and the University of Wisconsin.

(3) Parts of the work were presented at the Symposium on the Chemistry of Natural Products, Technion, Haifa, Israel, June 28–29, 1955; at the XIVth International Congress of Pure and Applied Chemistry, Zurich, Switzerland, July 21–27, 1955; at the 8th Summer Seminar in the Chemistry of Natural Products, Grand Manan Island, New Brunswick, Canada, August 6–10, 1956; and at the 130th Meeting of the American Chemical Society, Atlantic City, N. J., September 16–21, 1956.

(4) 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.

(5) W. Poethke, *Arch. Pharm.*, **275**, 357 (1937).

(6) (a) J. Fried, H. L. White and O. Wintersteiner, THIS JOURNAL, **72**, 4621 (1950); J. Fried, P. Numerof and N. H. Coy, *ibid.*, **74**, 3041 (1952).

(7) M. W. Klohs, *et al.*, *ibid.*, **75**, 4925 (1953); **76**, 1152 (1954).

(8) G. S. Myers, *et al.*, *ibid.*, **77**, 3348 (1955); **78**, 1621 (1956).

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

(10) S. M. Kupchan and C. V. Deliwala, *ibid.*, **74**, 3202 (1952); **76**, 5345 (1954); S. M. Kupchan, C. V. Deliwala and R. D. Zonis, *ibid.*, **77**, 755 (1955).

(11) E. D. Fries, J. R. Stanton and F. C. Moister, *J. Pharmacol. Exptl. Therap.*, **98**, 166 (1950); G. L. Maison, E. Gotz and J. W. Stutzman, *ibid.*, **103**, 74 (1951).

(12) L. S. Goodman and A. Gilman, "The Pharmacological Basis of Therapeutics," The Macmillan Co., New York, N. Y., second edition, 1955, pp. 747–754; O. Krayer in V. A. Drill, "Pharmacology in Medicine," McGraw-Hill Book Co., New York, N. Y., second edition, 1958, pp. 515–524.

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

(14) L. C. Craig and W. A. Jacobs; (a) *J. Biol. Chem.*, **148**, 57 (1943); (b) **149**, 451 (1943).

(15) W. A. Jacobs and S. W. Pelletier, *J. Org. Chem.*, **18**, 765 (1953); THIS JOURNAL, **78**, 1914 (1956).

(16) S. M. Kupchan and C. V. Deliwala, *ibid.*, **75**, 1025 (1953).

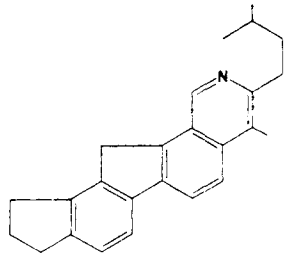
(17) S. W. Pelletier and W. A. Jacobs, *ibid.*, **75**, 3248 (1953).

(18) S. M. Kupchan, D. Lavie, C. V. Deliwala and B. Y. A. Andoh, *ibid.*, **75**, 5519 (1953).

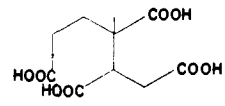
(19) S. M. Kupchan, M. Fieser, C. R. Narayanan, L. F. Fieser and J. Fried, *ibid.*, **77**, 5896 (1955).

(20) D. H. R. Barton, O. Jeger, V. Prelog and R. B. Woodward, *Experientia*, **10**, 81 (1954).

suggested that germine possesses a 3-hydroxy-4-hemiketal system similar to that found in veracevine. At least two early experiments support this view concerning the ring A system of germine. First, the presence of the masked ketol system in ring A, with its potentiality for ring contraction, accounts for the appearance of cyclopentanofluorene derivatives such as cevanthridine (III) among the dehydrogenation products of germine.[14a,15,20] Second, the formation of the hexanetetracarboxylic acid IV by chromic acid oxidation of germine[14a] supports the location of the masked ketol system at the 3,4-position. Incidentally, the obtention of acid IV



III

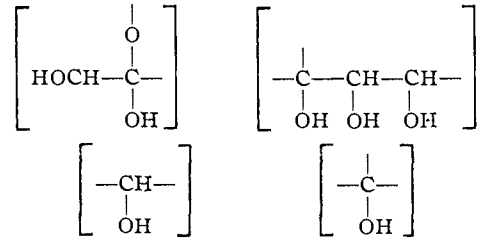


IV

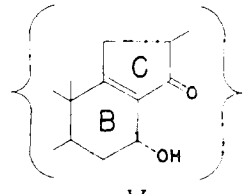
from germine excludes the attachment of oxygen functions at C_1, C_2, C_6 and C_{19} in the germine molecule.

Germine readily formed a tetraacetate which was stable to chromic acid[19]: hence, four non-tertiary hydroxyl groups are present in the molecule. Under more vigorous acetylating conditions, germine formed a pentaacetate, and, by analogy with the behavior of veracevine[21] and cevine,[22] it may be assumed that the difficultly acylable hydroxyl is the C_4-hemiketal hydroxyl. Support for this view follows from the formation of a pentaacetate from dihydrogermine (presumably formed by reduction of the masked ketone at C_4) under non-forcing acetylating conditions.[19]

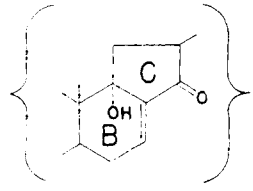
Germine consumed three moles of periodic acid; germine acetonide consumed one mole of periodic acid (in the cleavage of the 3-hydroxyl-4-hemiketal system). Hence the acetonide blocks the uptake of periodic acid by a 1,2,3-triol system or a 1,2,4,5-tetraol system. The former situation was shown to prevail by the fact that periodic acid oxidation of germine was accompanied by the production of one mole of formic acid. In addition, this result revealed that the central hydroxyl of the triol system is secondary. Germine acetonide was readily acetylated to a diacetate which consumed one mole of chromic acid and yielded a crystalline ketone (dehydrogermine acetonide diacetate) which was stable to chromic acid. Mineral acid hydrolysis of germine acetonide diacetate afforded germine diacetate, which consumed one mole of periodic acid. These facts show that: (1) the acetonide is tertiary-secondary, for two of germine's original four non-tertiary groups are still available for acetylation, and a third subsequently available for oxidation to a ketone: and (2) the triol system is tertiary-secondary-secondary, for one of the acetate groups in germine diacetate clearly occupies a terminal secondary hydroxyl of the triol system. The arguments presented thus far lead to the following tentative disposition of the functional groups of germine



The problem of locating these functional groups in the skeletal framework (II) is considerably simplified by the facile exclusion of all locations outside of ring D for the triol system. Rings A and B may be excluded on the basis of formation of the acid IV by chromic acid oxidation of germine. Rings C and E will not accommodate a tertiary-secondary-secondary triol. Ring F may be excluded on the bases: (1) that germine shows no carbinolamine properties, hence ruling out sites alpha to nitrogen for hydroxyl location and eliminating a C_{22}, C_{23}, C_{24} triol; and (2) that if the triol system were located at C_{23}, C_{24}, C_{25}, consumption of periodic acid by germine would not stop at three moles (*cf.* ref. 20). Specific location at C_{14}, C_{15}, C_{16} is made possible by the following results. When the crude product of periodic acid oxidation of germine diacetate was exposed to dilute ammonia, an unsaturated ketone was obtained, λ_{max} 238 mμ (ϵ 10,000); λ_{max} 2.90(s), 5.78–5.85(s), 5.92(s), 6.05(m)μ. The ultraviolet spectrum of the product is incompatible with the presence of a C_{15}-, C_{16}-, C_{17}-triol system in germine.[23] The ultraviolet spectrum is, however, compatible with two alternative enones derived from a C_{14}-, C_{15}-C_{16}-triol (V and VI).[23] The infrared spectral



V



VI

characteristics of the product indicate that the $\Delta^{8,9}$-14-one structure V is the correct one. The relative intensities of the carbonyl band (5.92μ) and the double bond band (6.05μ) are those characteristic of a *transoid* system. A *cisoid* system such as VI would be expected to show a C=C band of exalted intensity equal to or exceeding the intensity of the C=O band.[24] The absence of ab-

(21) Two products with rather widely different physical constants have been assigned a veracevine triacetate formulation in the literature. The first of these, m.p. 273–274°, was obtained by acetylation with acetic anhydride–pyridine at 0° (S. W. Pelletier and W. A. Jacobs, THIS JOURNAL, **75**, 3248 (1953)). This product has been re-examined and found to be veracevine diacetate (Dr. S. W. Pelletier, private communication). The second product, m.p. 239–241°, obtained by acetylation with acetic anhydride–pyridine at steam-bath temperature, is the true veracevine triacetate (reference 18).

(22) D. H. R. Barton, C. J. W. Brooks and J. S. Fawcett, *J. Chem. Soc.*, 2137 (1954).

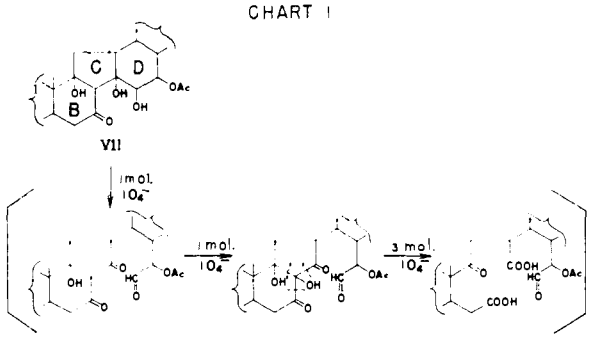
(23) R. B. Woodward, THIS JOURNAL, **63**, 1123 (1941); **64**, 76 (1942); A. E. Gillam and T. F. West, *J. Chem. Soc.*, 486 (1942).

(24) R. Hirschmann, C. S. Snoddy, Jr., C. F. Hiskey and N. L. Wendler, THIS JOURNAL, **76**, 4013 (1954); O. Wintersteiner and M. Moore, *ibid.*, **78**, 6193 (1956); D. H. R. Barton and C. R. Narayanan, *J. Chem. Soc.*, 963 (1958).

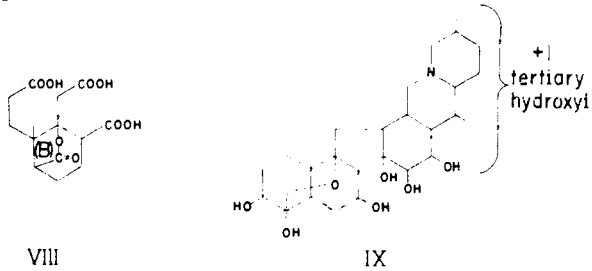
sorption characteristic of a trisubstituted double bond (the $\Delta^{7,8}$ in the case of VI) in the 11.9–12.5 μ range[25] also mitigates against the $\Delta^{7,8}$-14-ketone structure. The $\Delta^{8,9}$ location of the double bond in the unsaturated ketone indicates that a tertiary hydroxyl (or equivalent) is affixed at C_9 in germine. Incidentally, the fact that isogermine and dihydrogermine consumed three moles of periodic acid (like germine) indicates the absence of a hydroxyl group at C_{11}.

Treatment of dehydrogermine acetonide diacetate with dilute mineral acid afforded dehydrogermine diacetate. The latter compound consumed five moles of sodium periodate in the course of twenty-four hours. The result is readily compatible with the view that oxidation proceeds *via* initial cleavage of the C_{14},C_{15}-glycol system to generate a cyclic 1,3-diketone. Further oxidation of the β-diketone *via* hydroxylation at C_8 would account for the additional periodate consumption (see Chart 1).[26]

CHART 1



We believe that the five-mole periodate uptake of dehydrogermine diacetate is explicable uniquely on the basis of a structure with a keto group beta to C_{14}. Since C_{11} has been excluded as an oxygenated position (see above), dehydrogermine diacetate has a keto group at C_7 (see structure VII) and germine has a hydroxyl group at C_7. The failure to detect the lactone tricarboxylic acid VIII[20] among the products of chromic acid oxidation of germine[14a] is readily explicable on the basis of a germine formula containing a hydroxyl group at C_7. Expression IX, which incorporates seven of the eight oxygen atoms of germine, summarizes the conclusions drawn to this point.[27]

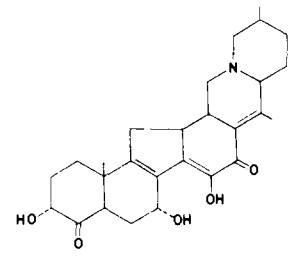


(25) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," Methuen and Co., Ltd., London, 1954.

(26) *Cf.* M. L. Wolfrom and J. M. Bobbitt, THIS JOURNAL, **78**, 2489 (1956).

(27) The sequel will show that the bulk of the evidence strongly favors a 4,9-hemiketal structure for those derivatives having a group other than hydroxyl at C_7. In those compounds which possess a free hydroxyl group at C_7 (*e.g.*, the alkamines), an unequivocal choice between the 4,9-hemiketal and the 4,7-hemiketal structure considered earlier[19] is not possible. We shall discuss our results on the basis of the preferred 4,9-hemiketal structure.

Preliminary assignment of the remaining tertiary hydroxyl group to C_{20} is made on the following basis. Neogermitrine, a naturally-occurring triester of germine, is convertible in two steps to a diosphenol for which structure X is derived.[28] The simplest explanation for the formation of the 17–20 double



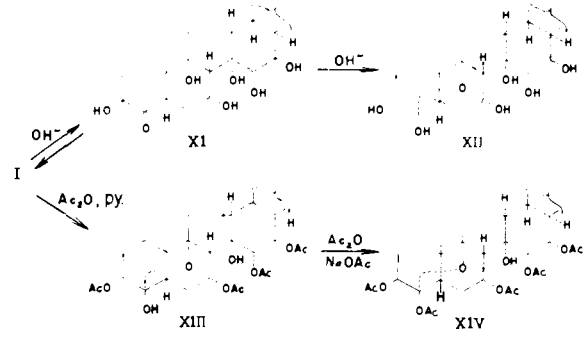
X

bond of X envisions elimination of a hydroxyl beta to the 16-keto group, *viz.*, the C_{20}-hydroxyl. Support for the location of a hydroxyl group at C_{20} follows from a study of the facile methanolysis of the acetate grouping at C_{16} in certain germine esters —an effect which has been shown to be attributable to a 1,3-diaxial interaction of the acetate at C_{16} with the hydroxyl at C_{20} (see below).

The foregoing preliminary considerations served as a basis for the development of the *structure* (apart from configurational relationships) of germine represented by formulation I. Additional corroborative evidence for this structure as well as for the configuration indicated will now be discussed in the light of the proposed formulation.

The germine–isogermine–pseudogermine isomerizations are now to be formulated as I→XI→XII.[19,27] The tetraacetates formed by acetic anhydride–pyridine acetylation of the respective isomers[19] are 3,7,15,16-tetraacetates (*e.g.*, XIII). The pentaacetate formed by more vigorous acetylation catalyzed by sodium acetate or perchloric acid is to be represented as the 3,4,7,15,16-pentaacetate XIV. Some stereochemical aspects of the isomerizations and the acylations are discussed below.

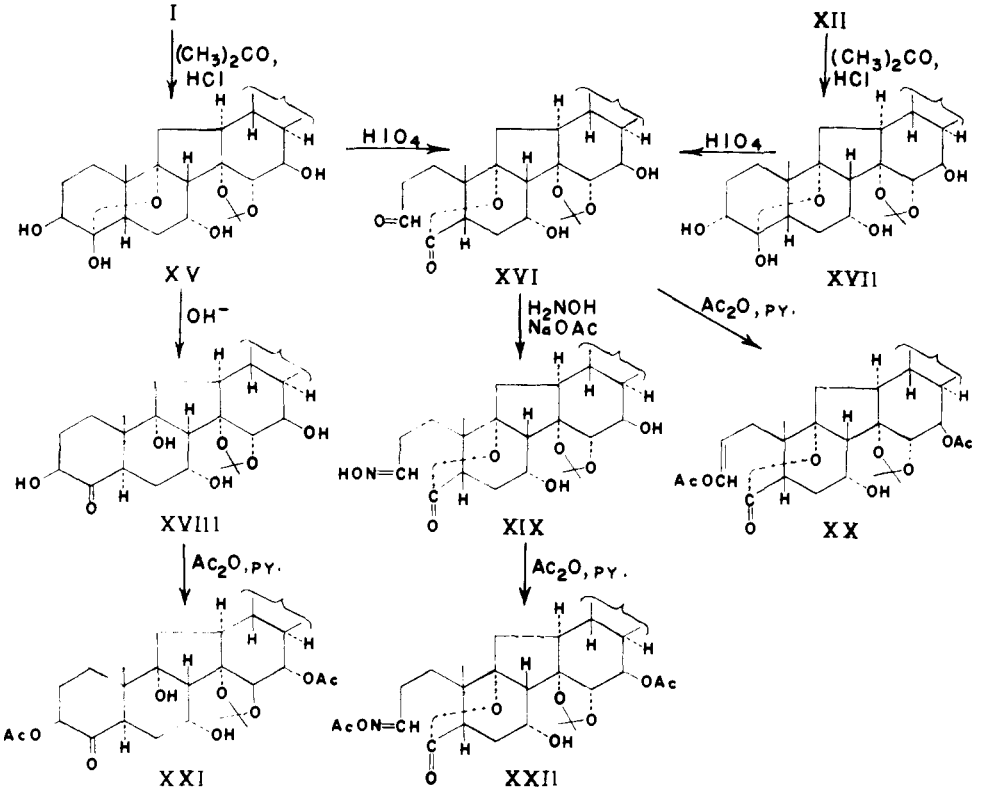
CHART 2



The acetonide derivative of germine,[14a] which has proved to be a key compound in the development of the structural argument, is formulated as the 14,15-acetonide XV. The periodic acid oxidation of germine 14,15-acetonide (XV) and pseudogermine 14,15-acetonide (XVII) to the same aldehydo-γ-lactone XVI is represented as in Chart 3. Acetyla-

(28) S. M. Kupchan, *ibid.*, **81**, 1921 (1959).

CHART 3



tion of XVI with acetic anhydride–pyridine afforded a diacetate, stable to Fehling solution. This diacetate showed infrared absorption at 5.62 (γ-lactone), 5.80 (acetate) and 5.97 μ (double bond) and is represented by formula XX.[29] Acetylation of the oxime of the aldehydo-γ-lactone XIX yielded the acetonide-aldehyde-oxime-γ-lactone diacetate XXII. Germine acetonide (XV) isomerized readily on alkaline treatment to give isogermine 14,15-acetonide (XVIII). Attempted preparation of XVIII from isogermine (XI) by reaction with acetone and concentrated hydrochloric acid in the usual manner[14a] was unsuccessful. However, reaction in acetone in the presence of hydriodic acid did yield XVIII. Acetylation of XVIII with acetic anhydride pyridine gave isogermine 14,15-acetonide 3,16-diacetate (XXI).

The diacetate formed by acetylation of germine 14,15-acetonide[30] is now formulated as XXIII. Hydrolysis of XXIII with 2,4-dinitrophenylhydrazine in strong alcoholic sulfuric acid[30] afforded in small yield a germine monoacetate. The monoacetate is formulated as germine 16-acetate on the basis of its periodic acid consumption (Table I) and rotation in pyridine.[28,31] Hydrolysis of XXIII with dilute hydrochloric acid gave germine 3,16-diacetate (XXIV). As noted earlier, periodic acid oxidation of XXIV followed by exposure of the initial product to dilute base gave a product with partial structure V. The formation of the cyclopentenone is clearly explicable in terms of initial glycol cleavage at C_{14}, C_{15} to a β-hydroxycyclopentanone, followed by β-elimination to XXV. Methanolysis of XXIII gave germine 14,15-acetonide 3-acetate (XXVI). Dilute acid hydrolysis of XXVI afforded germine 3-acetate (XXVII).

TABLE I

PERIODIC ACID OXIDATIONS

Substrate	Mole equivalents of HIO_4 consumed[a] (hr.)
Germine (I)	2.7 (1), 2.9 (4)
Isogermine (XI)	2.8 (1), 2.9 (4)
Pseudogermine (XII)	2.7 (1), 2.9 (4)
Dihydrogermine[14b]	2.7 (1)
Germine 3,7,15,16-tetraacetate (XIII)	0 (24)
Germine 14,15-acetonide (XV)	0.8 (1), 1.0 (4)
Pseudogermine 14,15-acetonide (XVII)	1.0 (1), 1.2 (4)
Isogermine 14,15-acetonide (XVIII)	0.9 (1), 1.1 (4)
Isogermine 14,15-acetonide 3,16-diacetate (XXI)	0 (18)
Germine 14,15-acetonide 3,16-diacetate (XXIII)	0 (18)
Germine 3,16-diacetate (XXIV)	1.0 (1)
Germine 16-acetate	1.7 (1), 2.2 (6)
Germine 3-acetate (XXVII)	1.8 (1), 2.0 (6)
7-Dehydrogermine 3,16-diacetate (XXIX)[b]	1.4 (2), 1.9 (4); 3.4 (24), 4.6 (48)

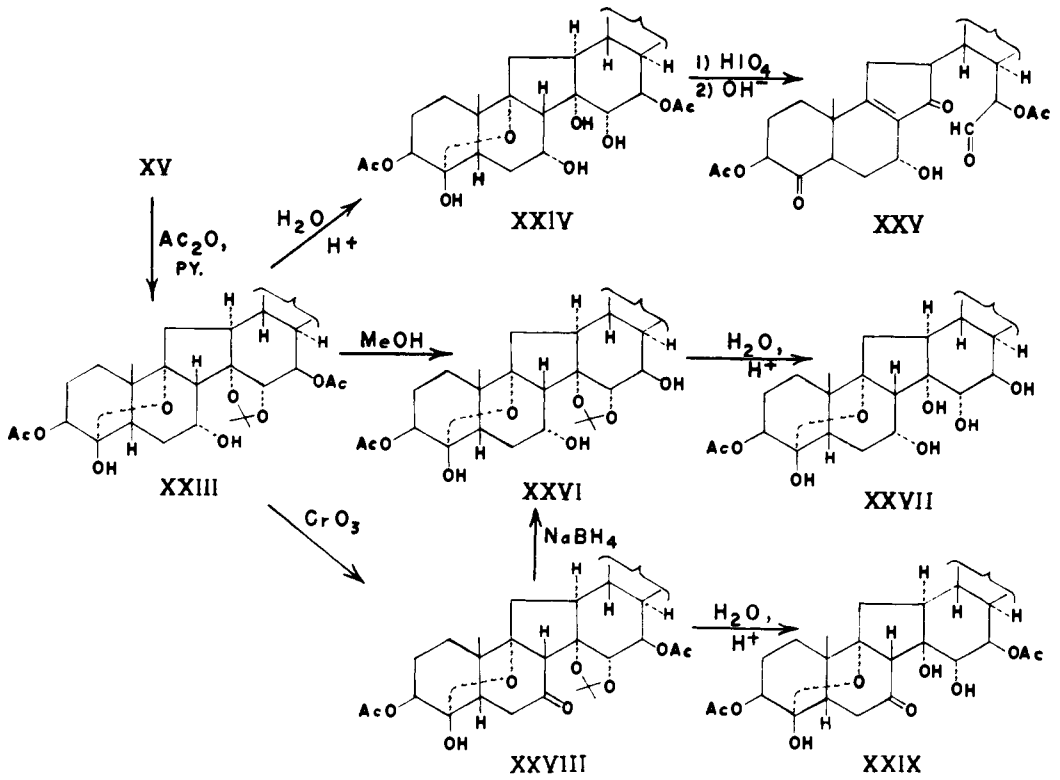
[a] The last uptake recorded in each case is the one beyond which no significant change occurred on further standing. [b] To test the effect of pH on the rate of oxidation[26] of XXIX, the compound was oxidized also with sodium periodate–dilute acetic acid; results: 1.9 (2 hr.), 2.7 (5 hr.), 4.7 (24 hr.), 5.3 (48 hr.).

(29) *Cf.* A. M. Sladkov and G. S. Petrov, *J. Gen. Chem. U.S.S.R.*, **24**, 459 (1955).

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

(31) *Cf.* the hydrolysis of cevine 3,16-diacetate to cevine 16-acetate in the presence of perchloric acid: D. H. R. Barton, C. J. W. Brooks and P. De Mayo, *J. Chem. Soc.*, 3950 (1954).

CHART 4



The oxidation of germine 14,15-acetonide 3,16-diacetate to 7-dehydrogermine 14,15-acetonide 3,16-diacetate is represented by XXIII→XVIII. Sodium borohydride reduction of XXVIII afforded germine 14,15-acetonide 3-acetate. The hydrolysis product of XXVIII, 7-dehydrogermine 3,16-diacetate (XXIX), readily formed a crystalline oxime.

Germine has sixteen asymmetric carbon atoms: C_3, C_4, C_5, C_7, C_8, C_9, C_{10}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{20}, C_{22} and C_{25}. The absolute configuration has been related with the configuration at C_{10} in steroids *via* the hexanetetracarboxylic acid IV.[32] The configurations at C_3, C_4, C_5 and C_9 are related to C_{10} as shown in formula I by the steric requirements of the isomerization sequence I→XI→XII.[20]

Alkaline equilibration studies of the germine isomers show that the order of stability is isogermine (3-β-hydroxy-4-keto-9-α-hydroxy-A/B-*trans*, XI) <germine (3-β-hydroxy-4,9-hemiketal, I)<pseudogermine (3-α-hydroxy-4,9-hemiketal, XII). This order contrasts with those in the zygadenine and veracevine series, where the order is 3-β-hydroxy-4,9-hemiketal isomer <3-β-hydroxy-4-keto-9-α-hydroxy-A/B *trans* isomer<3-α-hydroxy-4,9-hemiketal isomer.[16-18,20,33] The greater relative stability of the hemiketal forms in the germine series may be attributable to hydrogen bonding interaction of the α-oriented hydroxy group at C_7 with the α-oriented basic ethereal oxygen of the 4,9-hemiketal bridge. The infrared spectra of some relevant germine derivatives support this view. Thus, the infrared spectrum of germine 14,15-acetonide 3,16-diacetate (XV) shows (in addition to the usual hydroxyl bands at 2.90–2.95μ) a band at 3.01μ which is attributable to a strong intramolecular hydrogen bond.[25,34] The band is absent from the spectra[34] of isogermine 14,15-acetonide 3,16-diacetate (XXI), 7-dehydrogermine 14,15-acetonide 3,16-diacetate (XXVIII) and zygadenine 14,15-acetonide 3,16-diacetate.[33] The latter compounds lack either the basic ethereal oxygen of the 4,9-hemiketal bridge or the 7-α-hydroxyl group necessary for the postulated strong intramolecular hydrogen bond.

Additional support for assignment of α-orientation to the hydroxyl group at C_7 follows from another experiment. Acetylation of germine with acetic anhydride–pyridine (conditions which lead to acetylation of the C_4-hemiketal hydroxyl in veracevine[18,20]) afforded germine 3,7,15,16-tetraacetate (XIII). The sluggishness of acetylation of the C_4-hydroxyl is consistent with the view that acetylation of the 7-α-hydroxyl group proceeds rapidly, and that the resulting 7-α-acetoxy group hinders reaction of the C_4-hydroxyl group. More vigorous acetylation apparently overcomes this hindrance and yields germine 3,4,7,15,16-pentaacetate (XIV).

Preliminary assignment of α-orientation to the hydroxyl groups at C_{14} and C_{15} was made on the basis of the inertness of the C_7-hydroxyl group in

(32) F. Gaütschi, O. Jeger, V. Prelog and R. B. Woodward, *Helv. Chim. Acta*, **38**, 296 (1955).

(33) S. M. Kupchan, THIS JOURNAL, **78**, 3546 (1956); **81**, 1925 (1959).

(34) These spectra were run on 1% solutions in carbon tetrachloride on a Perkin–Elmer model 112 single beam spectrophotometer with a calcium fluoride prism. We thank Professor Robert C. West for the measurements and for helpful discussions concerning their interpretation.

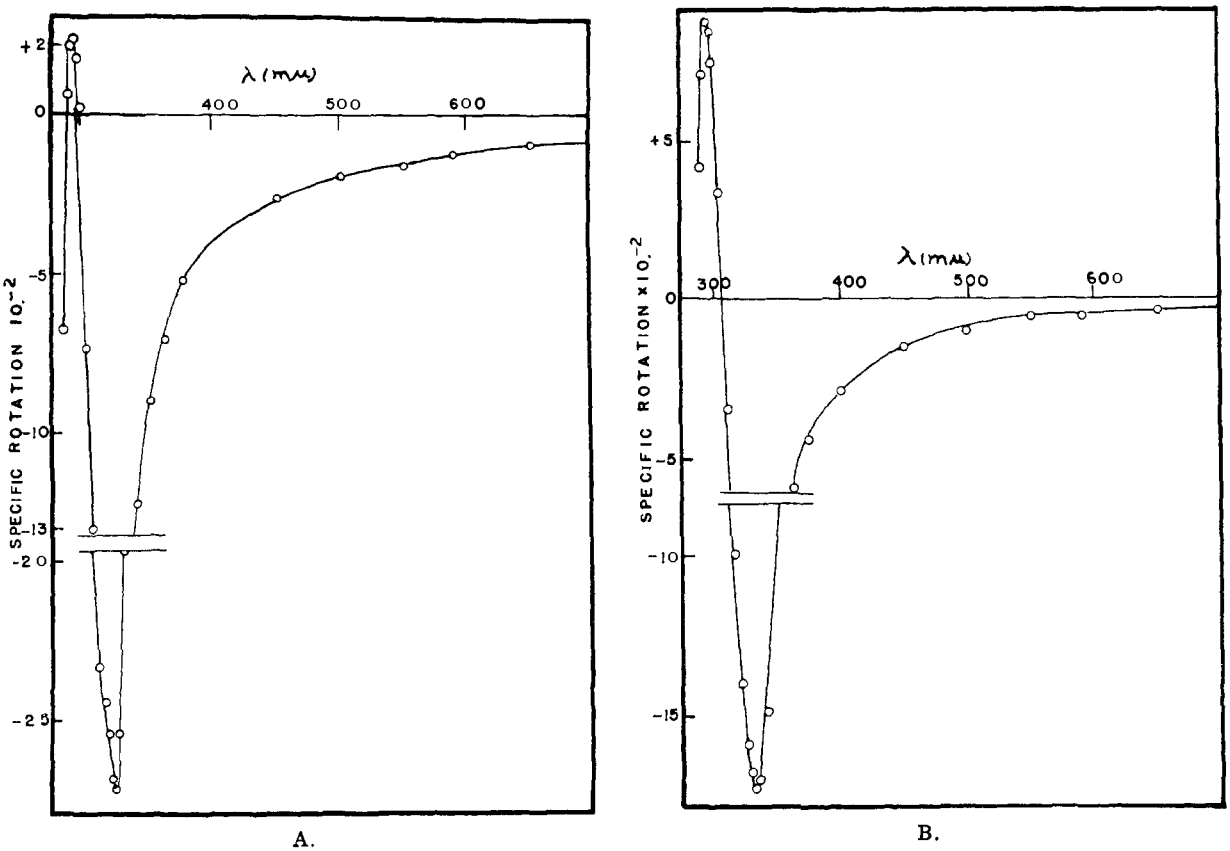


Fig. 1.—Optical rotatory dispersion curves in dioxane of: A, 16-dehydrogermine 3,4,7,15-tetraacetate (*c* 0.083 from 700 to 330 mμ, *c* 0.016 from 325 to 282.5 mμ); B, 16-dehydrocevine 3,4-diacetate (*c* 0.082 from 700 to 325 mμ, *c*, 0.016 from 320 to 290 mμ).

germine 14,15-acetonide to acetylation (see Chart 4, XV→XXIII). Examination of molecular models indicates that only an α-oriented 14,15-isopropylidene grouping would cause steric hindrance to acetylation of the C_7-hydroxyl. Subsequent support for assignment of α-orientation to the C_{14}-hydroxyl (and therefore to the C_{15}-hydroxyl in a *cis* relationship to the C_{14}-hydroxyl) came from a study of the facile methanolysis of C_7-acetates (*e.g.*, the ready methanolysis of neogermitrine and germitrine[6,28]). The ready methanolysis was shown to be explicable in terms of a facilitation by a hydroxyl group bearing a *cis*-1,3-diaxial relationship to the ester group.[35] In the case of the C_7-acetate of germine, the facile methanolysis lends support to assignment of α-orientation to the C_{14}-hydroxyl group. Furthermore a *cis*-1,3-diaxial relationship of the substituents at C_7 and C_{14} requires that the hydrogen at C_8 be β-oriented; *i.e.*, that rings B and C be *trans*-fused, as in all other naturally occurring steroids. The failure of the di-secondary glycol system at C_{15}, C_{16} to form an acetonide indicates that these groups are *trans*, *i.e.*, that the hydroxyl at C_{16} is β-oriented.

The C_{16}-acetate in the germine series is readily methanolyzed (*e.g.*, XXIII-XXVI) and this requires, on the basis of a 1,3-diaxial facilitation, a β-(axial-) orientation of the C_{20} hydroxyl.[35] The *cis*-1,3-diaxial disposition of the C_{16}- and C_{20}-hydroxyl groups requires, in turn, that rings D and E be *trans*-fused, *i.e.*, that C_{13} be β-oriented and C_{17} α-oriented[36] (see models).

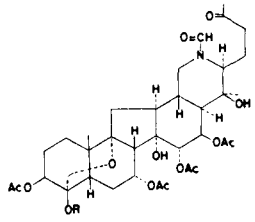
Of the thirteen asymmetric carbon atoms of germine discussed up to this point, eleven (C_3, C_4, C_5, C_8, C_9, C_{10}, C_{13}, C_{14}, C_{16}, C_{17}, C_{20}) are also present in the veracevine molecule. *In every case*, the configurations at the common asymmetric carbon atoms are similar.[1] It is likely indeed that the configurations at C_{12}, C_{22} and C_{25}, the remaining common asymmetric carbon atoms, are also alike. Support for assignment of α-configuration to the hydrogen at C_{12} was obtained from a comparison of molecular rotatory dispersion curves of 16-dehydrogermine 3,4,7,15-tetraacetate[28] and 16-dehydrocevine 3,4-diacetate[36] (Fig. 1).[37] The striking similarity of the two curves indicates strongly that the configurations of rings C, D and E in germine and cevine are identical. The presumption that the configurations at C_{22} and C_{25} in germine are the same as those in cevine is supported by the following experiment. Twenty-four-hour oxidation of germine 3,7,15,16-tetraacetate (XIII) with chromic anhydride–pyridine afforded a neutral product. This product is assigned the formamido-ketone

(35) S. M. Kupchan and W. S. Johnson, THIS JOURNAL, **78**, 3864 (1956); *cf.* H. B. Henbest and B. J. Lovell, *Chemistry & Industry*, 278 (1956); *J. Chem. Soc.*, 1965 (1957).

(36) D. H. R. Barton, C. J. W. Brooks and P. de Mayo, *ibid.*, 3950 (1954).

(37) We thank Professor Carl Djerassi and Dr. E. J. Eisenbraun (Wayne State University) for the optical rotatory dispersion measurements and their interpretation.

structure XXX on the basis of analyses and infrared spectral characteristics which parallel those of analogous oxidation products in the cevine series.[38] Similar oxidation of germine 3,4,7,15,16-pentaacetate (XIV) gave an analogous formamido-ketone (XXXI).



XXX : R=H
XXXI : R=Ac

Acknowledgments.—We thank Professor William S. Johnson cordially for stimulating discussions and suggestions. We gratefully acknowledge the generous assistance of Dr. Harold A. Nash, Pitman-Moore Co., Indianapolis 6, Ind., in supplying veratrum alkaloid extracts for the preparation of the germine used in this work.

Experimental[39]

Germine Pentaacetate (XIV). A. By Sodium Acetate-catalyzed Acetylation.—A mixture of anhydrous germine (1 g.), sodium acetate (1 g.) and acetic anhydride (10 ml.) was heated under reflux for 30 minutes. The solution was cooled in ice and the excess acetic anhydride decomposed by cautious addition of methanol (10 ml.). Evaporation of the solvents *in vacuo* left an amorphous residue, which was dissolved in water (8 ml.). The solution was made alkaline with ammonia and extracted with chloroform (seven 25-ml. portions). The chloroform extract was washed with water (10 ml.), dried over sodium sulfate and evaporated to dryness *in vacuo*. The residue crystallized from ether; yield 235 mg., m.p. 279–281° dec. Two recrystallizations from acetone–petroleum ether afforded clusters of prisms, m.p. 285–287° dec., $[\alpha]^{23}D$ −65° (*c* 0.65, pyr.).

Anal. Calcd. for $C_{27}H_{38}O_8N(COCH_3)_5$: C, 61.74; H, 7.42; acetyl, 29.90. Found: C, 62.10; H, 7.71; acetyl, 29.08.

B. By Perchloric Acid-catalyzed Acetylation.—To a solution of germine (1 g.) in acetic anhydride (10 ml.) cooled to −10°, cold 70% perchloric acid (0.3 ml.) was added dropwise with swirling. The solution was kept at −10° for 1.5 hours. The excess acetic anhydride was then decomposed with methanol (10 ml.) and the mixture was worked up as above. Crystallization from ether afforded 550 mg. of germine pentaacetate, m.p. 279–282° dec. Recrystallization from acetone–petroleum ether gave 400 mg. of product, m.p. 284–286° dec.

Acetylation of germine 3,7,15,16-tetraacetate[19] under the same conditions gave approximately the same yield of pentaacetate.

Diacetate from the Aldehydo-γ-lactone Derived from Germine Acetonide (XX).—Germine acetonide hydrochloride[14a] (2 g.) was dissolved in absolute alcohol (4 ml.) and 0.06 *M* sodium periodate solution (66 ml.) and the solution was allowed to remain at room temperature for four hours. Ammonia was added to bring the *p*H to 7.5 and the solution was extracted with chloroform (eight 100-ml. portions). The chloroform solution was washed with a little water, dried over sodium sulfate and evaporated to dryness *in vacuo*. The amorphous residue was treated with pyridine (20 ml.) and acetic anhydride (20 ml.) and the solution was heated on the steam-bath for two hours. The highly colored solution was cooled and treated cautiously with methanol (25 ml.). The mixture was worked up as above and the residue from chloroform was dissolved in ether–petroleum ether. Filtration of the highly colored insoluble solid gave a yellow filtrate, from which clusters of needles (680 mg.) separated. Recrystallization from ether-petroleum ether gave the diacetate in the form of colorless needles (224 mg.), m.p. 265–268° dec., $[\alpha]^{23}D$ −13° (*c* 1.02, pyr.).

Anal. Calcd. for $C_{30}H_{43}O_8N(COCH_3)_2$: C, 64.64; H, 7.82; acetyl, 13.63. Found: C, 65.09; H, 7.90; acetyl, 13.22.

Diacetate from the Oxime of the Aldehydo-γ-lactone (XXII).—The oxime of the aldehydo-γ-lactone[19] (900 mg.) was treated with pyridine (10 ml.) and acetic anhydride (10 ml.) and the solution was heated on the steam-bath for 2 hours. The reaction mixture was worked up as described for the aldehydo-γ-lactone diacetate above, and the product crystallized slowly from ether–petroleum ether; yield 258 mg., m.p. 206–210° after sintering from 140°, $[\alpha]^{23}D$ −26° (*c* 0.98, pyr.).

Anal. Calcd. for $C_{30}H_{44}O_8N_2(COCH_3)$: C, 63.14; H, 7.79; N, 4.33; acetyl, 13.31. Found: C, 63.54; H, 7.64; N, 4.53; acetyl, 13.60.

Isogermine Acetonide (XVIII). A. From Germine Acetonide.—Germine acetonide[14a] (2 g.) was dissolved in methanol (140 ml.) and 1 *N* NaOH (4 ml.) was added. The solution was heated under reflux for 30 minutes. It was brought to *p*H 6 with dilute acetic acid, diluted with water (10 ml.), and the methanol was removed *in vacuo* on the steam-bath. The aqueous solution was brought to *p*H 8 with ammonia and the suspension was extracted with chloroform (ten 15-ml. portions). The residue crystallized readily from benzene; yield 960 mg. of colorless needles, m.p. 293–295° dec., $[\alpha]^{23}D$ −34° (*c* 1.16, pyr.).

Anal. Calcd. for $C_{30}H_{47}O_8N$: C, 65.53; H, 8.62. Found: C, 65.68; H, 8.59.

B. From Isogermine.—The isogermine used for this conversion was prepared by a procedure which was found superior to those described earlier[14] and the method is therefore described in detail. The sodium salt of the oxime prepared from germine[40] (1 g.) was treated with pyruvic acid (0.85 g.), acetic acid (3 ml.), water (4 ml.) and 50% NaOH (1 ml.) and the mixture was heated on the steam-bath for 90 minutes. The *p*H was brought to 9 with dilute ammonia and the solution was extracted continuously with chloroform for 3 hours. The residue obtained by evaporation of the chloroform was crystallized from methanol; yield 358 mg. of isogermine, m.p. 259–262° dec.

To a solution of isogermine (1 g.) in methanol (12 ml.) and constant-boiling hydriodic acid (0.75 ml.) was added acetone (150 ml.) and the solution was allowed to stand at room temperature overnight. The solution was evaporated to dryness *in vacuo* and the residue was treated with dilute ammonia and extracted with chloroform (ten 15-ml. portions). The residue from chloroform crystallized from benzene; yield 755 mg. of isogermine acetonide, m.p. 289–293° dec.

Isogermine Acetonide Diacetate (XXI).—Isogermine acetonide (1.45 g.) was dissolved in pyridine (20 ml.) and acetic anhydride (20 ml.) and the solution was heated on the steam-bath for two hours. After decomposition of the excess acetic anhydride with methanol (25 ml.), the reaction mixture was worked up in the usual manner and the residue from chloroform was crystallized from ether–petroleum ether. Clusters of needles (911 mg.), m.p. 170–185° dec., were obtained. Recrystallization from the same solvents afforded 705 mg., m.p. 225–230° dec. after shrinking from 170° to a bubbly mass at 190–195° and resolidifying at about 210°, $[\alpha]^{23}D$ −56° (*c* 1.43, pyr.).

Anal. Calcd. for $C_{30}H_{45}O_8N(COCH_3)_2$: C, 64.43; H, 8.11; acetyl, 13.58. Found: C, 64.28; H, 8.13; acetyl, 13.49.

Acid Hydrolysis of Germine Acetonide Diacetate. A. Germine Diacetate (XXIV).—Germine acetonide diacetate[30] (500 mg.) was dissolved in 1:4 dilute hydrochloric acid (12 ml.) and the solution was allowed to stand at room tempera-

(38) S. M. Kupchan, W. S. Johnson and S. Rajagopalan, THIS JOURNAL, **80**, 1769 (1958); *Tetrahedron*, in press.

(39) 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) unless otherwise specified. Unless specified to the contrary, reagent chloroform was used as the solvent.

(40) H. Jaffé and W. A. Jacobs, *J. Biol. Chem.*, **193**, 325 (1951).

ture for 15 minutes. Dilute ammonium hydroxide was added to *p*H 8 and the solution was extracted with chloroform (ten 15-ml. portions). The chloroform was dried over sodium sulfate and brought to dryness *in vacuo*, leaving a residue which crystallized readily from methanol. Filtration afforded colorless needle clusters (370 mg.) which melted at 205–210° after softening from 170°, $[\alpha]^{23}D$ −4° (*c* 1.19, pyr.).

Anal. Calcd. for $C_{27}H_{41}O_8N(COCH_3)_2 \cdot CH_3OH$: C, 61.42; H, 8.22; acetyl, 13.76. Found: C, 61.00; H, 7.89; acetyl, 14.21.

B. Germine 16-Acetate.—Acid hydrolysis of germine acetonide diacetate (600 mg.) with 2,4-dinitrophenylhydrazine and strong sulfuric acid[30] afforded only a small yield of crystalline product (36 mg.) upon crystallization of the crude product from chloroform–petroleum ether. Recrystallization from chloroform afforded a microcrystalline product (21 mg.), m.p. 225–227°, $[\alpha]^{23}D$ −19° (*c* 0.93, pyr.).

Anal. Calcd. for $C_{27}H_{42}O_8N(COCH_3)$: C, 63.13; H, 8.22; acetyl, 7.80. Found: C, 62.73; H, 8.17; acetyl, 7.40.

Evaporation of the chloroform–petroleum ether mother liquors above afforded a residue which crystallized from methanol; 230 mg. of germine diacetate was obtained.

Periodic Acid Titrations.—Between 25 and 30 mg. of substrate was dissolved in 5% acetic acid (5 ml.) and water (15 ml.) and 0.05 *M* periodic acid (5 ml.) were added. (Concentrations were approximately 0.01 *M* of periodic acid and 0.002 *M* with respect to substrate.) Five-milliliter aliquots were withdrawn at appropriate time intervals and titrated by the procedure described by Jackson ("Organic Reactions," Vol. II, p. 361). Several compounds were also titrated with sodium periodate; in every case tested the results were not significantly different from the periodic acid value in Table I.

Formic acid formed during the periodic acid oxidation of germine was determined by the method of Dyer.[41] In a typical determination, the volatile acid generated during oxidation and titrated with standard alkali was found 0.94 mole equivalent; the formic acid by reduction of mercuric chloride and gravimetric determination of the calomel formed was found 0.87 mole equivalent.

Periodic Acid Oxidation of Germine Diacetate.—Germine diacetate (1 g.) was dissolved in alcohol (5 ml.) and 0.05 *M* periodic acid (40 ml.) and left at room temperature for 15 hours. The solution was brought to *p*H 8 with aqueous ammonia and extracted with chloroform (seven 50-ml. portions). The residue from chloroform showed no absorption in the ultraviolet other than the high end absorption characteristic of these alkaloids. The amorphous solid was dissolved in alcohol (5 ml.) and water (5 ml.) and ammonium hydroxide (3 ml.) was added. The solution was allowed to stand at room temperature for three hours and was then diluted with water (10 ml.) and extracted with chloroform. The chloroform was evaporated *in vacuo* and the residue was triturated with ether and filtered; yield 574 mg., m.p. 180–190°, $[\alpha]^{23}D$ −56° (*c* 1.18, pyr.); λ_{max} 238 mμ (ε 10,000); λ_{max} 2.90(s), 5.78–5.85(s), 5.92(s) and 6.05(m) μ.

Anal. Calcd. for $C_{27}H_{37}O_7N(COCH_3)_2 \cdot H_2O$: C, 62.92; H, 7.67; acetyl, 14.55. Found: C, 63.16; H, 7.65; acetyl, 13.87.

Alkaline Treatment of Isogermine.—Isogermine (1 g.) was dissolved in methanol (7 ml.), water (15 ml.) and 1 *N* sodium hydroxide (5 ml.) by warming on the steam-bath. The solution was kept at 50° for 6 hours and was then extracted continuously with chloroform for 20 hours. The chloroform extract was concentrated to a small volume (approx. 6 ml.) and seeded with germine. Filtration the next day afforded a crystalline product (554 mg.), m.p. 250–255° after sintering from 165°, $[\alpha]^{28}D$ −6° (*c* 1.00, alc.). The infrared spectrum of this material (Nujol mull) suggested that the material was a mixture of germine and isogermine, with germine predominating. The specific rotation indicated a mixture of approximately 80% germine ($[\alpha]D$ +5°) and 20% isogermine ($[\alpha]D$ −47°). Recrystallization of the product from methanol afforded prisms (390 mg.), m.p. 219–226° after sintering from 155°. The infrared spectrum of this material (Nujol mull) was identical with that of germine.

The chloroform mother liquors above yielded a second crop of crystalline product (40 mg.) upon concentration. This material showed a rotation ($[\alpha]^{28}D$ −33°, *c* 1.00, alc.) and infrared spectrum (Nujol) indicative of impure isogermine. Evaporation of the chloroform mother liquor to dryness gave an amorphous residue (301 mg.), $[\alpha]^{28}D$ 0° (*c* 1.00, alc.). The infrared spectrum of this residue suggested that it consisted of pseudogermine (predominantly) and isogermine.

Alkaline treatment of germine under the same conditions yielded a mixture practically indistinguishable in composition from that above.

Germine Acetonide Monoacetate (XXVI).—Germine acetonide diacetate (500 mg.) was dissolved in methanol (15 ml.) and water (7 ml.) and the solution was allowed to stand at room temperature for 18 hours. Concentration of the solution on the steam-bath led to crystallization of needles (370 mg., m.p. 259–262° dec.). Recrystallization from methanol–water afforded colorless needle clusters (250 mg.), m.p. 263–265° dec., $[\alpha]^{23}D$ +40° (*c* 1.36, pyr.).

Anal. Calcd. for $C_{30}H_{46}O_8N(COCH_3)$: C, 64.95; H, 8.35; acetyl, 7.26. Found: C, 64.33; H, 8.11; acetyl, 7.15.

Germine 3-Acetate (XXVII).—Germine acetonide monoacetate (480 mg.) was dissolved in 1:4 dilute hydrochloric acid (10 ml.) and the solution was allowed to stand at room temperature for 15 minutes. Dilute ammonia was added to *p*H 8.5 and the solution was extracted with chloroform (ten 15-ml. portions). The chloroform was dried over sodium sulfate and brought to dryness *in vacuo*, leaving a residue which crystallized from ether. Filtration afforded colorless clusters of needles (288 mg.), m.p. 219–221°, $[\alpha]^{23}D$ +10° (*c* 1.05, pyr.).

Anal. Calcd. for $C_{27}H_{42}O_8N(COCH_3)$: C, 63.13; H, 8.22; acetyl, 7.80. Found: C, 63.49; H, 8.25; acetyl, 7.49.

7-Dehydrogermine Acetonide Diacetate (XXVIII).—Germine acetonide diacetate (2 g.) in pyridine (20 ml.) was added to chromic anhydride (4 g.) in pyridine (40 ml.) and the solution was allowed to stand at room temperature for 3 hours. Water (40 ml.) and ammonium hydroxide (4 ml.) were added and the solution was shaken with chloroform (100 ml.) and filtered through Supercel to clarify the emulsion. After eight additional extractions with chloroform (50 ml.) the combined chloroform extracts were brought to dryness *in vacuo*. The residue crystallized from alcohol; yield 690 mg., m.p. 261–263° dec. Recrystallization from acetone–petroleum ether gave colorless elongated prisms (530 mg.), m.p. 267–269° dec., $[\alpha]^{23}D$ −42° (*c* 0.86, pyr.).

Anal. Calcd. for $C_{30}H_{43}O_8N(COCH_3)_2$: C, 64.64; H, 7.82; acetyl, 13.63. Found: C, 64.81; H, 8.03; acetyl, 13.19.

Similar yields of product were obtained by oxidation of germine acetonide diacetate (2 g.) in acetic acid (5 ml.) and carbon tetrachloride (60 ml.) with 0.66 *N* chromic anhydride in 98.5% acetic acid (70 ml.).[36]

7-Dehydrogermine Diacetate (XXIX).—7-Dehydrogermine acetonide diacetate (1.25 g.) was dissolved in acetic acid (2 ml.) and 1:4 dilute hydrochloric acid (10 ml.) and the solution was allowed to stand at room temperature for 4 hours. Work-up with dilute ammonia and chloroform in the usual manner gave a residue which crystallized from acetone–ether. Recrystallization from the same solvents gave clusters of plates (550 mg.), m.p. 235–237° dec., $[\alpha]D$ −68° (*c* 2.23, pyr.).

Anal. Calcd. for $C_{27}H_{39}O_8N(COCH_3)_2$: C, 62.92; H, 7.67; acetyl, 14.55. Found: C, 62.56; H, 7.65; acetyl, 13.55.

The corresponding oxime, prepared by the pyridine procedure, crystallized from alcohol, m.p. chars at 250°, does not melt to 300°.

Anal. Calcd. for $C_{31}H_{46}O_{10}N_2$: C, 61.37; H, 7.64; N, 4.62; acetyl, 14.19. Found: C, 61.58; H, 7.52; N, 4.92; acetyl, 13.05.

Sodium Borohydride Reduction of 7-Dehydrogermine Acetonide Diacetate.—7-Dehydrogermine acetonide diacetate (200 mg.) in methanol (30 ml.)–water (3 ml.) was treated with a solution of sodium borohydride in methanol

(41) J. R. Dyer in David Glick, "Methods of Biochemical Analysis," Vol. III, Interscience Publishers, Inc., New York, N. Y., 1956, pp. 130–131.

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°.

MADISON 6, WISC.

[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.

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(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).