[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY AND THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

The Germine–Isogermine–Pseudogermine Isomerizations[1]

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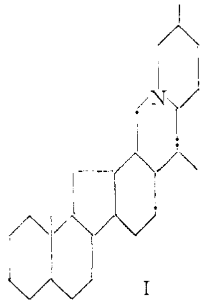
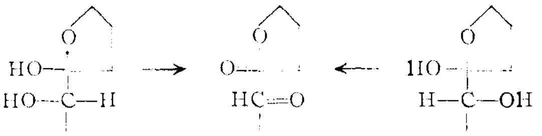
Evidence is presented for partial structures IX, X and XI for germine, isogermine and pseudogermine, respectively.

Recent studies of the hypotensive principles of several *Veratrum*[2–5] and *Zygadenus*[6] species have revealed that many of the most active compounds in this series are polyesters of germine. Germine, $C_{27}H_{43}O_8N$, was first isolated by Poethke in 1937.[7] Extensive degradative studies have been reported by Jacobs and his collaborators during the past twelve years,[8–10] and these studies recently have led to proposal of skeletal structure I for germine.[11]


I

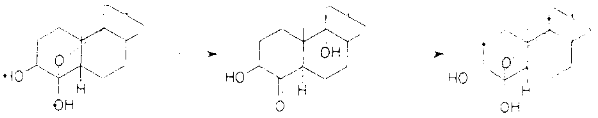
Shortly before discontinuance of research on the ester alkaloids of germine at the Squibb Institute,[2] one of us (J. F.) found that germine acetonide hydrochloride[8a] (II) consumes one mole of potassium periodate and affords a crystalline oxidation product which reduces Tollens reagent (m.p. 274–276° dec., $[\alpha]^{23}D + 2°$ (c 1.54, alc.)). Further characterization of the substance at Harvard showed it to be an aldehydo-γ-lactone (III).



II III IV

Thus infrared absorption bands at 3.65 and 5.79 μ are attributable to an aldehyde group, and a band at 5.62 μ is indicative of the γ-lactone function. Furthermore, the substance forms an oxime (m.p. 278–279° dec., $[\alpha]^{23}D + 4°$ (c 1.47, alc.)). The constitution of this aldehydo-γ-lactone offered the first experimental confirmation for the presence of an α-ketol-5-membered hemiketal system in a germine derivative. In a parallel experiment, Barton and Brooks obtained an analogous aldehydo-γ-lactone by periodic acid oxidation of cevine orthoacetate.[12]

In September, 1953, Dr. R. B. Woodward kindly informed us of his deduction concerning the veracevine–cevagenine–cevine isomerizations (V–VII).[13]



V VI VII

Experimental support for the view that veracevine and cevine contain the same α-ketol-5-membered hemiketal system and differ only in the configuration of the hydroxyl group of the α-ketol system followed soon afterward.[14] The close analogy of the germine–isogermine—pseudogermine isomerizations to the veracevine–cevagenine–cevine isomerizations suggested similar structures in the two series.[15,16]

Pseudogermine was prepared by isomerization of germine with sodium ethoxide in ethanol. (Isogermine likewise gave pseudogermine under the same conditions and this reaction completes the analogy to the cevine series.) Pseudogermine acetonide hydrochloride (IV), m.p. 283–284° dec., prepared by the standard procedure,[8a] on oxidation with potassium periodate gave an aldehydo-γ-lactone III identical with that derived from germine acetonide (II) (mixed m.p. and infrared spectral comparisons of aldehyde and oxime). The fact that the same aldehydo-γ-lactone is formed from both

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(1) Preliminary accounts of this work have been reported in Communications to THIS JOURNAL, **76**, 1200, 5259 (1954). The investigation was supported by research grants from the National Heart Institute, of the National Institutes of Health, Public Health Service (H-1563 (C3)) and Research Corporation.

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

(3) M. W. Klohs, *et al.*, *ibid.*, **74**, 1871, 4473 (1952).

(4) G. S. Myers, W. L. Glen, *et al.*, *ibid.*, **74**, 3198 (1952); *Nature*, **170**, 932 (1952).

(5) H. A. Nash and R. M. Brooker, THIS JOURNAL, **75**, 1942 (1953).

(6) S. M. Kupchan and C. V. Deliwala, *ibid.*, **74**, 3202 (1952); **76**, 5545 (1954); S. M. Kupchan, C. V. Deliwala and R. D. Zonis, *ibid.*, **77**, 755 (1955).

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

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

(9) H. Jaffe and W. A. Jacobs, *ibid.*, **193**, 325 (1951).

(10) S. W. Pelletier and W. A. Jacobs, THIS JOURNAL, **75**, 3248 (1953).

(11) W. A. Jacobs and S. W. Pelletier, *J. Org. Chem.*, **18**, 765 (1953).

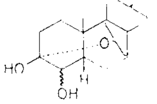
(12) D. H. R. Barton and C. J. W. Brooks, *Chemistry & Industry*, **51**, 1366 (1953); D. H. R. Barton, C. J. W. Brooks and J. S. Fawcett, *J. Chem. Soc.*, 2137 (1954).

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

(14) S. M. Kupchan and D. Lavie, THIS JOURNAL, (a) **76**, 314 (1954); (b) **77**, 683 (1955).

(15) S. W. Pelletier and W. A. Jacobs, *ibid.*, **65**, 3248 (1953); S. M. Kupchan, D. Lavie, C. V. Deliwala and B. Y. A. Andoh, *ibid.*, **75**, 5519 (1953).

(16) However, the alternative partial formulation VIII for pseudo-

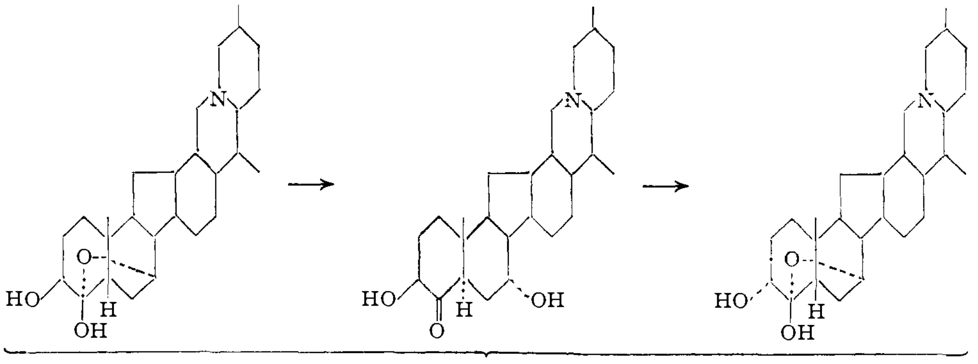


VIII

germine, with the potential ketone at C_3, could not be excluded on the basis of the evidence in hand at this point.

germine and pseudogermine acetonides suggests strongly that the parent alkamines contain the same α-ketol-5-membered hemiketal system and differ only in the orientation of the hydroxyl group of the α-ketol system, as in II and IV.

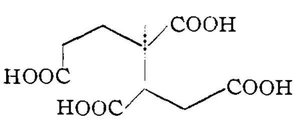
On the basis of the evidence discussed above, Barton, Jeger, Prelog and Woodward[13] have proposed that germine and pseudogermine (like veracevine and cevine) may be represented by partial structures V and VII, respectively, and that germine differs from veracevine only in the placing of one or more hydroxyl groups in the C–D–E–F system. We should like to present evidence which favors C_7 as the terminus of the ether bridge of the hemiketal system in germine and pseudogermine (as in IX–XI), rather than C_9, the terminus in veracevine and cevine.

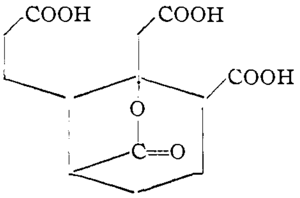

+2 *sec*-OH and 3 *tert*-OH

IX X XI

Acetylation of germine with acetic anhydride and pyridine yields germine tetraacetate,[17] stable to chromic acid, m.p. 260–261° dec., $[\alpha]^{23}\text{D}$ −98° (*c* 1.57, py.). Treatment of isogermine under the same conditions yields isogermine tetraacetate, m.p. 222–226°, $[\alpha]^{24}\text{D}$ −80° (*c* 1.63, py.). Acetylation of pseudogermine under the same conditions affords pseudogermine tetraacetate, m.p. 208–220°, $[\alpha]^{24}\text{D}$ −59° (*c* 1.70, py.). The fact that isogermine yields a tetraacetate, like germine and pseudogermine, indicates that the ether bridge of the hemiketal system terminates at a primary or secondary carbon atom. These results contrast with the acetylation experiments in the cevine series, where the ketonic isomer contains one less acylable group because a tertiary hydroxyl group is liberated by opening of the hemiketal bridge.[14b] Moreover acetylation of dihydrogermine[8b] leads to dihydrogermine pentaacetate, stable to chromic acid, m.p. 288–290° dec., $[\alpha]^{28}\text{D}$ −64° (*c* 1.41, py.). This result is compatible with the view that sodium–alcohol reduction proceeds by liberation and then reduction of a keto group with concomitant liberation of the hydroxyl group involved in the hemiketal system. Since this hydroxyl group is acylable, it is primary or secondary.

The likelihood that the hemiketal ring in germine is 5-membered indicates that the terminus of the ether bridge of the hemiketal system is at C_1, C_7 or C_{19}. The formation of the hexanetetracarboxylic acid XII[13,18] by chromic acid oxidation of germine[8a] shows the absence of oxygen from positions C_1 and C_{19} and fixes C_7 as the terminus of the ether bridge.


XII


XIII

The failure to detect the lactone tricarboxylic acid XIII[13,19] among the products of chromic acid oxidation[8a] is readily explicable on the basis of partial formulation IX for germine.

Experimental

Periodate Oxidation of Germine Acetonide Hydrochloride.—Germine acetonide hydrochloride[8a] (1.09 g.) was dissolved in 0.15 *M* potassium periodate solution (143 ml.) and the solution was allowed to remain at room temperature for four hours. After an additional 15 hours at 0° the solution was made alkaline with 20% sodium carbonate and extracted with chloroform (seven 100-ml. portions). The chloroform solution was washed with a little water, dried over sodium sulfate and evaporated to dryness *in vacuo*. The residue crystallized readily from acetone–ether to yield the aldehydo-γ-lactone (477 mg.) in the form of elongated prisms, m.p. 274–276° dec. after darkening from 230°, $[\alpha]^{23}\text{D}$ +2° (*c* 1.54, alc.). *Anal.* Calcd. for $C_{30}H_{45}O_8$: C, 65.79; H, 8.28. Found: C, 65.85; H, 8.37.

The corresponding oxime, prepared by the sodium acetate procedure, crystallized as prisms from methanol, m.p. 277–279° dec., $[\alpha]^{23}\text{D}$ +4° (*c* 1.47, alc.). *Anal.* Calcd. for $C_{30}H_{46}O_8N_2$: C, 64.03; H, 8.24; N, 4.98. Found: C, 63.93; H, 8.09; N, 5.12.

Isomerization of Germine to Pseudogermine with Alcoholic Sodium Ethoxide.—Germine (600 mg.) was added to a solution of sodium (600 mg.) in absolute alcohol (40 ml.) and the solution was heated under reflux for four hours. The solution was cooled and brought to *p*H 6.5 by addition of dilute hydrochloric acid (1:2). Sufficient water to dissolve the precipitated salt was added, and the alcohol was removed *in vacuo*. The residual solution was made alkaline with aqueous ammonia and extracted with chloroform (seven 50-ml. portions). The chloroform extract was washed with water (10 ml.), dried over sodium sulfate, and evaporated to dryness *in vacuo*. The residue was dissolved in a small volume of chloroform and induced to crystallize by seeing with germine; 55 mg. of germine was obtained by filtration. The mother liquor was evaporated to dryness *in vacuo* and

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(17) An earlier report[2a] that germine forms a pentaacetate on acetylation with acetic anhydride–pyridine has been found to be in error. Recent acetyl determinations both at Harvard and at the Squibb Institute agree with a tetraacetate formula.

(18) O. Jeger, R. Mirza, V. Prelog, Ch. Vogel and R. B. Woodward, *Helv. Chim. Acta*, **37**, 2295 (1954).

(19) F. Gautschi, O. Jeger, V. Prelog and R. B. Woodward, *ibid.*, **37**, 2280 (1954).

the residue was crystallized from methanol–water. Colorless prisms (328 mg.) were obtained, which softened to a bubbly mass at 165–175° and liquefied gradually until a meniscus formed at 205–208°, $[\alpha]^{24}\text{D}$ +12° (*c* 1.47, alc.). The infrared spectrum of the product in chloroform was identical with the spectrum of a sample of pseudogermine prepared by the procedure of Pelletier and Jacobs.[10]

Isomerization of Isogermine to Pseudogermine with Alcoholic Sodium Ethoxide.—Isogermine[8a] (200 mg.) was treated with alcoholic sodium ethoxide by the procedure described above. Crystallization of the crude reaction product from methanol-water gave pseudogermine (54 mg.), characterized by mixed melting point, rotation and infrared spectral comparison with an authentic sample.

Pseudogermine Acetonide.—Pseudogermine (700 mg.) was dissolved in a small volume of absolute alcohol by the addition of sufficient hydrochloric acid (1:1) to turn congo red. After addition of acetone (3 ml.), rubbing induced crystallization, and 615 mg., m.p. 280–283° dec., was obtained. Recrystallization from absolute alcohol gave pseudogermine acetonide hydrochloride in the form of colorless plates, m.p. 283–284° dec.

Anal. Calcd. for $C_{30}H_{47}O_8N \cdot HCl \cdot H_2O$: C, 59.63; H, 8.34. Found: C, 59.86; H, 8.44.

The hydrochloride (214 mg.) was dissolved in water (6 ml.) and the solution was made alkaline with 20% sodium carbonate solution and extracted with chloroform (six 10-ml. portions). The chloroform extract was washed with water (10 ml.), dried over sodium sulfate, and evaporated to dryness *in vacuo*. Crystallization from the same solvents gave translucent plates (71 mg.), m.p. 237–239° dec., $[\alpha]^{23}\text{D}$ +27° (*c* 1.51, alc.).

Anal. Calcd. for $C_{30}H_{47}O_8N$: C, 65.53; H, 8.62. Found: C, 65.46; H, 8.65.

Periodate Oxidation of Pseudogermine Acetonide Hydrochloride.—Pseudogermine acetonide hydrochloride (587 mg.) was oxidized with potassium periodate solution by the procedure described for the oxidation of germine acetonide hydrochloride. Crystallization of the crude product from acetone–ether yielded the aldehydo-γ-lactone (182 mg.) obtained earlier from germine acetonide hydrochloride. The identity of the two products was demonstrated by mixed m.p. and infrared spectral comparisons of the respective samples of aldehydo-γ-lactone and oxime.

Germine Tetraacetate.—A mixture of anhydrous germine (1 g.), acetic anhydride (20 ml.) and pyridine (20 ml.) was heated on the steam-bath for two hours. Evaporation of the excess reagents *in vacuo* at room temperature left an amorphous solid, which was dissolved in water (7 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 acetone; yield 938 mg., m.p. 248–251° dec. Two further recrystallizations from the same solvent afforded colorless prisms, m.p. 260–261° dec., $[\alpha]^{23}\text{D}$ −98° (*c* 1.57, py.).

Anal. Calcd. for $C_{27}H_{39}O_8N\,(COCH_3)_4$: C, 62.02; H, 7.59; acetyl, 25.40. Found: C, 61.77; H, 7.50; acetyl, 25.22.

The same product was obtained by room-temperature acetylation with acetic anhydride–pyridine[2a] and by heating with acetic anhydride alone for two hours.

Isogermine Tetraacetate.—Isogermine (400 mg.) was acetylated with acetic anhydride and pyridine as described above for germine. The crude product crystallized from ether–petroleum ether. Recrystallization from the same solvents afforded small needles (136 mg.), m.p. 222–226° dec., $[\alpha]^{24}\text{D}$ −80° (*c* 1.63, py.).

Anal. Calcd. for $C_{27}H_{39}O_8N(COCH_3)_4$: C, 62.02; H, 7.59; acetyl, 25.40. Found: C, 61.69; H, 7.49; acetyl, 25.85.

Pseudogermine Tetraacetate.—Pseudogermine (400 mg.) was acetylated with acetic anhydride and pyridine by the procedure described above for germine. The crude product crystallized from ether (U.S.P.)–petroleum ether. Recrystallization from the same solvents gave a micro-crystalline solid (140 mg.), m.p. 190–210°, $[\alpha]^{24}\text{D}$ −59° (*c* 1.70, py.).

Anal. Calcd. for $C_{27}H_{39}O_8N(COCH_3)_4 \cdot H_2O$: C, 60.41; H, 7.68; acetyl, 24.75. Found: C, 60.34; H, 7.29; acetyl, 24.44.

Dihydrogermine Pentaacetate.—Dihydrogermine[8b] (300 mg.) was acetylated with acetic anhydride and pyridine by the procedure described above for germine. The crude product crystallized from chloroform–ether. Two recrystallizations from acetone afforded clusters of prisms (191 mg.), m.p. 284–285° dec., $[\alpha]^{28}\text{D}$ −64° (*c* 1.41, py.).

Anal. Calcd. for $C_{27}H_{40}O_8N(COCH_3)_5$: C, 61.56; H, 7.68; acetyl, 29.82. Found: C, 61.51; H, 7.68; acetyl, 29.82.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE POLYTECHNIC INSTITUTE OF BROOKLYN]

The Decarboxylation of Pyrazinium Betaine Salts[1]

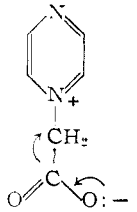
BY EUGENE V. HORT AND PAUL E. SPOERRI

RECEIVED MAY 11, 1955

Betaine salts containing the pyrazinium nucleus decompose extremely readily with loss of carbon dioxide. The products of this decomposition with 2,5-dimethylpyrazine are 1-alkyl-2,5-dimethylpyrazinium salts.

In the course of a study on the quaternization of 2,5-dimethylpyrazine (DMP), the reaction with α-haloacids was found to proceed in an unusual manner. The expected betaine salts could not be isolated. Even at room temperature, carbon dioxide was evolved and only decarboxylated quaternary salts obtained. This ease of decarboxylation contrasts sharply with the stability normally exhibited by betaine salts. For example, 1-carboxymethylpyridinium bromide[2] and carboxymethyltrimethylammonium bromide[3] are reported to melt with decomposition at 200 and 233°, respectively.

It is possible to explain the unusual ease of decarboxylation of the pyrazinium compounds on the basis of resonance stabilization of the decarboxylation intermediate. The loss of carbon dioxide from a betaine molecule I leaves a negatively charged methylene group on the quaternary nitrogen II. This can be stabilized by a form III in which the negative charge is borne by the comparatively electronegative nitrogen atom.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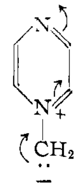
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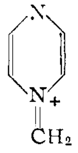
III

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[1] From the Ph.D. Thesis of E. V. Hort, June, 1950.

[2] M. Krüger, *J. prakt. Chem.*, [2] **43**, 280 (1891).

[3] H. Stoltzenberg, *Z. physiol. Chem.*, **92**, 471 (1914).