Studies were initiated in these laboratories to isolate the active ingredient from cottonseed. During the course of this investigation a compound was obtained in crystalline form which was found to be partially effective in preventing the SST-induced growth retardation in mice. This compound was identified as the purine, guanine.

TABLE I

EFFECT OF PURINES AND PURINE RIBOSIDES ON A SST-INDUCED GROWTH RETARDATION IN MICE[a]

| Diet | Av. 12-day wt. gain, g. |
|---|---|
| Control | 9.1 |
| 2% SST | 4.6 |
| 2% SST + 5% Cottonseed meal[b] | 8.6 |
| 2% SST + 15% Cottonseed meal | 10.8 |
| 2% SST + 0.02% Adenine | 8.3 |
| 2% SST + 0.08% Adenine | 8.8 |
| 2% SST + 0.02% Guanine | 7.8 |
| 2% SST + 0.08% Guanine | 7.4 |
| 2% SST + 0.02% Xanthine | 8.1 |
| 2% SST + 0.08% Xanthine | 8.9 |
| 2% SST + 0.02% Hypoxanthine | 9.5 |
| 2% SST + 0.08% Hypoxanthine | 8.2 |
| 2% SST + Adenosine equiv. to 0.02% adenine | 7.8 |
| 2% SST + Adenosine equiv. to 0.08% adenine | 9.0 |
| 2% SST + Guanosine equiv. to 0.2% guanine | 7.6 |
| 2% SST + Guanosine equiv. to 0.08% guanine | 8.1 |
| 2% SST + Inosine equiv. to 0.02% hypoxanthine | 8.2 |
| 2% SST + 1% Yeast Ribonucleic acid (GBI) | 9.0 |

[a] Eight male mice per group. [b] Proflo, Traders Oil Mill Co., Fort Worth, Texas.

The purines adenine, guanine, xanthine and hypoxanthine as well as the ribosides adenosine, guanosine and inosine were tested with growing mice using the SST containing low fat basal diet. The results obtained are shown in Table I. All of the purines and purine ribosides that were studied as well as yeast ribonucleic acid were found to be able partially to replace cottonseed in preventing the SST-induced growth retardation.

On the basis of the data presented here purines may function to modify the action of succinylsulfathiazole thus permitting the intestinal microorganisms to synthesize an as yet unknown factor which possibly plays some role in fat metabolism. On the other hand purines or nucleic acids may represent essential accessory food factors required for fat metabolism in the mouse.

In other studies, to be reported later, it has been found that other stress agents such as thyroid active materials or atabrine administered orally, or injected thyroxine cause a retardation of growth in mice fed a low fat diet. In these cases it is also possible to prevent this growth retardation by the feeding of cottonseed meal, fat, or a water extracted liver residue. It is possible that the beneficial effects of extracted liver residue in overcoming the toxicity of thyroxine or atabrine in the rat that were reported by Ershoff[2-4] may be due to the nucleic acids or their degradation products present in the extracted liver residue.

FROM THE DEPARTMENT OF BIOCHEMISTRY
RESEARCH DIVISION — JESSE W. HUFF
SHARP & DOHME, INC. — DAVID K. BOSSHARDT
WEST POINT, PENNSYLVANIA

RECEIVED JULY 28, 1952

(2) B. H. Ershoff, *Arch. Bio.*, **15**, 365 (1947).
(3) B. H. Ershoff and H. B. McWilliams, *Science*, **108**, 632 (1948).
(4) B. H. Ershoff, *J. Nut.*, **35**, 269 (1948).

## HYPOTENSIVE ALKALOIDS OF VERATRUM FIMBRIATUM GRAY

*Sir:*

Two new hypotensively active ester alkaloids, germanitrine and germinitrine, have been isolated from *Veratrum fimbriatum* Gray.

The extraction procedure employed was essentially the same as the one reported in our previous investigation of *Veratrum viride* Ait.[1] The crude amorphous fraction thus obtained was subjected to an eight plate countercurrent distribution using benzene–2*M* acetate buffer *p*H 5.5 as the solvent system with the lower phase moving. This yielded two main fractions, A (tubes 0–1) and B (tubes 2–5).

Fraction A was distributed on a 24-plate countercurrent machine with 0.5 *M* sodium acetate buffer *p*H 5.0—benzene–cyclohexane 40:60. Careful fractional crystallization of the material recovered from tubes 4–14 from acetone–water gave germanitrine, and germinitrine.

Germanitrine crystallized as heavy needles; m.p. 228–229°; $[\alpha]^{24}D$ −61 ± 2° (*C* 1.0 in pyr.); 0.0 ± 2° (*C* 1.15 in $CHCl_3$). Analytical data indicate the empirical formula $C_{39}H_{59}O_{11}N$; calcd. C, 65.25; H, 8.28; N, 1.95; eq. wt., 717.87; found: C, 65.30; H, 8.26; N, 1.99; eq. wt., 721; picrate, m.p. 240–241° (dec.), $C_{39}H_{59}O_{11}N\cdot HOC_6H_6(NO_2)_3$: C, 57.07; H, 6.60; found: C, 56.68; H, 6.52. Volatile acid determination, found: 2.66 equivalents of acid. Alkaline hydrolysis of germanitrine yielded germine, acetic acid, methylethylacetic acid and tiglic acid.[2]

On methanolysis germanitrine was converted to a di-ester, germanidine, by the loss of the labile acetyl group; m.p. 221–222°; $[\alpha]^{24}D$ −4.1 ± 2° (*C* 1.0 in pyr.); + 18.1 ± 2° (*C* 0.49 in $CHCl_3$). Analytical data indicate the empirical formula $C_{37}H_{57}O_{10}N$: (calcd. C, 65.75; H, 8.50; eq. wt., 675.84; found: C, 65.66; H, 8.61; eq. wt., 672). Volatile acid determination, found: 1.97 equivalents of acid.

Germinitrine crystallized as irregular prisms; m.p. 175–176°; $[\alpha]^{24}D$ −36.0 ± 2° (*C* 1.12 in pyr.); +7.8 ± 2° (*C* 1.35 in $CHCl_3$). Analytical data indicate the empirical formula $C_{39}H_{57}O_{11}N$; calcd. C, 65.43; H, 8.03; N, 1.96; eq. wt., 715.85; found: C, 65.35; H, 8.27; N, 1.61; eq. wt., 722; picrate, m.p. 238° (dec.), $C_{39}H_{57}O_{11}N\cdot HOC_6H_2(NO_2)_3$: C, 57.19; H, 6.40; found: C, 57.17; H, 6.66. Volatile acid determination, found: 2.32 equivalents of acid. Alkaline hydrolysis of germinitrine yielded germine, acetic acid, tiglic acid and angelic acid.[2]

Fraction B was distributed on a 24-plate counter-

(1) M. W. Klohs, R. Arons, M. D. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek, THIS JOURNAL, **74**, in press (1952).
(2) The acids were identified by conversion to their *p*-phenylphenacyl esters and characterized after chromatographic separation.

current machine using 2 *M* acetate buffer *p*H 5.5 and benzene. The known triester, neogermitrine,[3] was obtained by crystallizing the material recovered from tubes 6–10 from acetone–water.

The hypotensive activity[4,5] of germanitrine, germanidine, and germanitrine was found to be 0.12 μg. [0.11–0.14], 0.77 μg. [0.46–2.3], and 0.41 μg. [0.36–0.49], respectively.

(3) J. Fried, P. Numerof and N. H. Coy, THIS JOURNAL, **74**, 3041 (1952).

(4) Edward D. Swiss and George L. Maison, Federation Proceedings, Vol. II, No. 1, March, 1952.

(5) Expressed as micrograms per kilogram of anesthetized dog per minute required for a ten-minute intravenous infusion to lower the mean arterial blood pressure 30% when administered according to the method of G. L. Maison and J. W. Stutzman. The bracketed numbers express the 95% confidence limits.

M. W. KLOHS
M. D. DRAPER
F. KELLER
S. KOSTER
W. MALESH
F. J. PETRACEK
RIKER LABORATORIES, INC.
8480 BEVERLY BOULEVARD
LOS ANGELES 48, CALIFORNIA

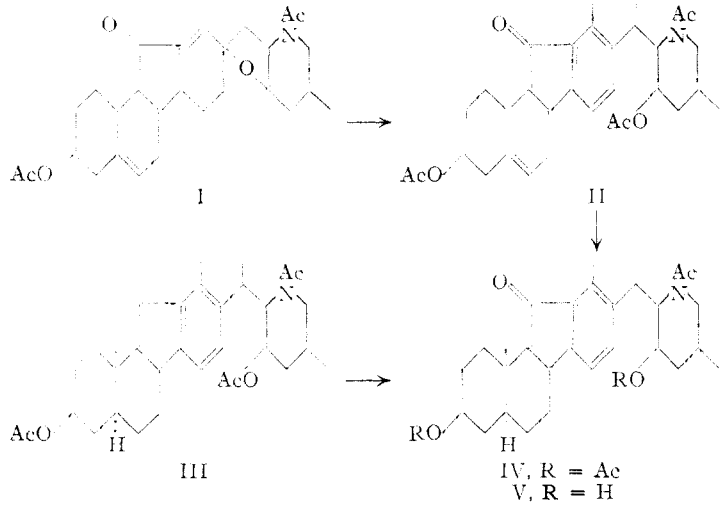
RECEIVED JULY 28, 1952

## THE STRUCTURAL CORRELATION OF JERVINE AND VERATRAMINE

*Sir:*

As reported in preliminary communications from this Laboratory,[1] O,N-diacetyljervine (I) on acetolysis with acetic anhydride–acetic acid containing a catalytic amount of sulfuric acid gave rise to a triacetate, $C_{33}H_{43}O_6N$, (m.p. 239–240°, $[\alpha]^{24}D$ −29°[2]), which on the basis of its ultraviolet and infrared characteristics ($\lambda_{max}^{alc}$ 251 mμ, log ε 4.08; 300 mμ, log ε 3.30; IR: intense bands at 5.75, 5.88 and 6.09 μ, indicative, respectively, of O-acetyl, ketonic carbonyl and N-acetyl) and other evidence was assigned the indanone structure II. On the other hand, there has been obtained by chromic acid oxidation of triacetyldihydroveratramine ($C_{33}H_{47}O_6N$, now assigned structure III[3]) a compound $C_{33}H_{45}O_6N$ (m.p. 241–245°, $[\alpha]^{21}D$ +59°) which likewise exhibited the above spectral properties, and the new keto group of which, like that of II, was unreactive to ketone reagents.[3] We have now reduced II catalytically with palladium–calcium carbonate in ethanol to its 5,6-dihydro derivative IV,[4] and found the latter identical in all respects, inclusive of the infrared characteristics over the whole measurable range, with the oxidation product from triacetyldihydroveratramine (m.p. 242–245°, $[\alpha]^{23}D$ +57.5°; *Anal.* Calcd. for $C_{33}H_{45}O_6N$: C, 71.83; H, 8.22. Found: C, 71.94; H, 8.26). The respective N-acetates (V), prepared by hydrolysis with methanolic potassium hydroxide, were likewise identical (m.p. 264–266.5°, $[\alpha]^{23.5}D$ +71.7°, +68.8°; *Anal.* Calcd. for $C_{29}H_{41}O_4N$: C, 74.46; H, 8.84. Found: C, 74.57; H, 8.64). The vicinal effect of the indanone grouping on the contribution to molecular rotation of $C_5$ is evident in the abnormally high $\Delta[M]_D$ for the saturation of the double bond (+484° for II → IV, +495° for O-desacetylated II → V) as compared with the values +399° for triacetylveratramine → triacetyldihydroveratramine, +404° for N-acetylveratramine → N-acetyldihydroveratramine, and +243° for normal $\Delta^5$-stenyl acetates.[5]

The significance of this result lies in the fact that it renders extremely remote the possibility of a rearrangement of the carbon skeleton in the formation of the acetolysis product II from diacetyljervine, since (1) the presence in veratramine of a preformed benzenoid ring has been assured not only by ultraviolet spectrophotometry[6] but also by chemical means,[3] (2) the conversion of triacetyldihydroveratramine to IV obviously cannot involve a change in the skeleton, and (3) it is reasonable on biogenetic grounds to accord also to jervine the abnormal ring structure which has now been shown to pre-exist in veratramine.




THE SQUIBB INSTITUTE
FOR MEDICAL RESEARCH
NEW BRUNSWICK, NEW JERSEY
O. WINTERSTEINER
NORMAN HOSANSKY[7]

RECEIVED JULY 21, 1952

(1) J. Fried, O. Wiutersteiner, A. Klingsberg, M. Moore and B. M. Iselin, THIS JOURNAL, **73**, 2970 (1951); O. Wintersteiner and M. Moore, Abstracts, XIIth Internat. Congress of Chemistry, New York, Sept. 10-13, 1951, p. 292.

(2) All melting points corrected; all rotations in chloroform.

(3) Ch. Tamm and O. Wintersteiner, THIS JOURNAL, **74**, 3842 (1952).

(4) The catalytic hydrogenation of II presented unexpected difficulties in that it did not proceed smoothly under any of the conditions tried, and invariably gave rise to mixtures. Thus, with $PtO_2$ in acetic acid the indanone carbonyl was partly reduced, and the product obtained by reoxidation with chromic acid, (m.p. 214–217°, $[\alpha]D$ +57.5°) was obviously not pure IV. On the other haud, hydrogen uptake in the reaction catalyzed with palladium was very sluggish, and the crude product was contaminated with less dextrorotatory impurities (apparently for the most part unchanged starting material), which could not always be completely removed by recrystallization or eliminated by rehydrogenation, so that the yield of pure dihydro product was always low.

(5) D. H. R. Barton, *J. Chem. Soc.*, 512 (1946).

(6) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **160**, 555 (1945).

(7) This paper is part of the dissertation to be presented by Norman Hosansky in partial fulfillment of the requirements for the Ph.D. degree in the Graduate School of Rutgers University.