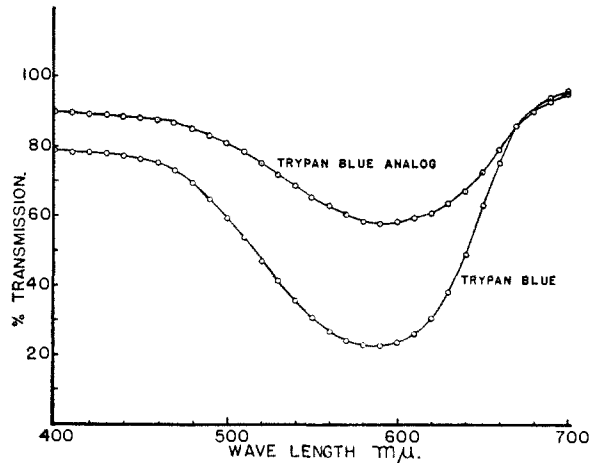

Fig. 1.

Since technical H-acid contains an impurity that forms a red dye with the tetrazonium chloride, it was purified by dissolving 27 g. of H-acid in 200 ml. of hot water and filtering. After reheating to 70°, it was salted out by the addition of 60 g. of sodium chloride. This procedure was repeated twice and the final product dried in a vacuum desiccator. This purification reduced the amount of the impurity but did not eliminate it entirely. It is therefore possible that it might be caused by mono-coupling.

Then 13.72 g. (0.04 mole) of H-acid was dissolved in 100 ml. of water containing 0.72 g. of sodium hydroxide (filtering if necessary). The solution was thus left faintly acid until just before coupling. This solution was cooled to 18°, 3.4 g. of sodium bicarbonate was added, and the tetrazonium chloride was run rather rapidly into the vigorously stirred alkaline solution of H-acid. It is important that the coupling mixture be kept alkaline, adding more sodium bicarbonate if necessary. Agitation was continued for 2 hours to ensure complete coupling. Tests were done for excess tetrazonium chloride and H-acid. The mixture was heated to 85°, 5 g. of decolorizing carbon was added, and the solution stirred for 15 minutes and filtered. The filtrate was reheated to 85° with agitation, and 27 g. of hydrated sodium acetate for each 100 ml. of solution was added slowly in four or five portions. The mixture, while still warm, was centrifuged, and the supernatant decanted. This procedure was repeated twice, and although it makes the filtration easier, it still was not possible to remove the red dye completely. For further purification, the dye was refluxed four times with 300-ml. portions of 95% ethyl alcohol which removes both the red dye and sodium acetate. To test for sodium acetate, a few drops of concentrated sulfuric acid were added to 10 ml. of filtrate until no turbidity was formed on cooling with ice. The absence of red dye was proven by the capillary test and by chromatography, using equal amounts of Hyflo-Super-cel and adsorptive magnesia. The dye was thus free of organic impurities; yield 42%.

Solubilities of the Dye.—Soluble in water, methyl alcohol, glacial acetic acid and methyl cellosolve; difficultly soluble in ethyl alcohol; insoluble in acetone, benzene, chloroform, dioxane, ether and petroleum ether.

Anal. Calcd. for $C_{33}H_{20}N_6O_{14}S_4Na_4$: N, 8.9; S, 13.6. Found: N, 8.9; S, 13.2.

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COMMUNICATIONS TO THE EDITOR

GERMBUDINE, ISOGERMIDINE AND VERATETRINE THREE NEW HYPOTENSIVE ALKALOIDS FROM VERATRUM VIRIDE

Sir:

Recent studies[1,2] have disclosed the isolation of the hypotensive ester alkaloids germitrine, neogermitrine and germidine from *Veratrum viride*. Fried, White and Wintersteiner have also shown that the triester germitrine can be partially hydrolyzed to the hypotensive diester germerine.

We wish to report that present investigations in our laboratories, on the alkaloids extractable from the ground roots and rhizomes of commercial *Veratrum viride*, have yielded germerine together with three new, highly potent, ester alkaloids for which we propose the names germbudine, isogermidine and veratetrine.

The benzene-extractable alkaloids, obtained by the procedure of Jacobs and Craig,[3] were separated into a crystalline non-ester alkaloid fraction and an amorphous fraction which contained the bulk of the hypotensive activity. This amorphous material was subjected to a 24-plate Craig counter-current distribution between benzene and 2 *M* acetate buffer at *p*H 5.5. The known triester neogermitrine was obtained by crystallizing the material in tubes 12–20 from acetone. Crystallization of the material in tubes 4–11 from benzene gave germerine (m.p. 203–205°; $[\alpha]^{26}D$ − 14.2° (*c*, 1 in pyridine), +6° (*c*, 1 in chloroform); the sample was identified further by comparison of its infrared spectrum, and by mixed melting point with authentic germerine kindly provided by Dr. J. Fried). In a personal communication, the latter disclosed that Dr. D. R. Walters of Squibb and Sons has also isolated germerine from *Veratrum viride*.

The alkaloids in tubes 0–3 were given a 72-plate Craig distribution between benzene and 2 *M* acetate at *p*H 6.5 and three fractions from this distribution, A, B and C, were crystallized from benzene to give three new alkaloids.

Fraction A (tubes 1–3), yielded germbudine (m.p. 158–160°, $[\alpha]^{27}D$ − 8.4° (*c*, 1 in pyridine), + 10.7° (*c*, 1 in chloroform)). Analytical data indicate the empirical formula $C_{37}H_{59}O_{18}N$ (calcd. C, 61.2; H, 8.20; eq. wt., 726; found: C, 61.0; H, 8.21; eq. wt., 732). Volatile acid determination, found: 0.91 equivalent of acid. Alkaline hydrolysis of germbudine afforded the alkamine germine and an acid fraction. The acids were converted to their *p*-phenylphenacyl esters which were separated chromatographically into the ester of α-meth-

(1) J. Fried, H. L. White and O. Wintersteiner, THIS JOURNAL, **71**, 3260 (1949); **72**, 4621-4630 (1950).

(2) J. Fried and P. Numerof, Abst. 119th Meeting A.C.S., Cleveland, Ohio, April, 1951.

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

ylbutyric acid and what appeared to be the ester of an unidentified acid (D). The presence of the latter was suggested by an examination of its infrared spectrum. It is being studied further.

The diester isogermidine crystallized from fraction B (tubes 12–18) (m.p. either 221–222° or 229–230°, depending on which form separated; $[\alpha]^{28}D$ − 63.2° (*c*, 1 in pyridine), −26.0° (*c*, 1 in chloroform)). Calcd. for $C_{34}H_{53}O_{10}N$: C, 64.2; H, 8.40; eq. wt., 635. Found: C, 64.0; H, 8.33; eq. wt., 646, 655. Hydrolysis of isogermidine yielded germine, α-methylbutyric acid and acetic acid. The acids were identified by conversion to their *p*-phenylphenacyl esters.

Fraction C (tubes 19–36) gave crystalline veratetrine (m.p. 269–270° (dec.); $[\alpha]^{26}D$ − 31° (*c*, 1 in pyridine), $[\alpha]^{28}D$ − 2° (*c*, 1 in chloroform)). The analytical data indicate the empirical formula $C_{43}H_{64}O_{16}N$ (calcd. C, 60.7; H, 7.58; N, 1.65; eq. wt., 850.5; found: C, 60.8; H, 7.56; N, 1.4; eq. wt., 840). We have also isolated the same alkaloidal ester from *Veratrum album*. Volatile acid determination, found 3.2 equivalents of acid.

Alkaline hydrolysis of veratetrine yielded the alkamine isoprotoverine (identified by melting point and by comparison of its infrared and ultraviolet absorption spectra with those of authentic isoprotoverine) and an acid fraction. The acids were converted to their *p*-phenylphenacyl esters which were separated chromatographically into the esters of α-methylbutyric acid, acetic acid and the unidentified acid "D," obtained above from germbudine. The weights of the phenylphenacyl esters suggest veratetrine gives on hydrolysis one equivalent each of α-methylbutyric acid and acid D, together with two equivalents of acetic acid.

The hypotensive activity of germbudine, isogermidine and veratetrine have been determined in the anesthetized dog by a modification[4] of the method of Maison and Stutzman.[5] In comparison with a mixed alkaloidal ester preparation from *Veratrum viride* ("Deravine"), which produced a 30% fall in the mean arterial blood pressure of the anesthetized dog at a dose level of 0.2 γ per kg. per min., their relative activities are 0.97, 0.12 and 0.87, respectively.

(4) F. R. Skelton, Marjorie Beck and G. A. Grant, *Fed. Proc.*, Vol. 11, No. 1, Part I. p. 390, March, 1952.

(5) C. L. Maison and J. W. Stutzman, *Arch. Int. Pharmacodyn.*, **85**, 357 (1951).

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RECEIVED MAY 14, 1952

THE ANALYSIS AND SEPARATION OF GLUCURONIC AND GALACTURONIC ACIDS BY ION EXCHANGE[1]

Sir:

Methods for the analysis of uronic acids in biological material have always been rather limited due to the interference caused by sugars. Recently Dische[2] has described a series of color tests, which depend upon the rate and difference in color formation to distinguish between glucuronic and galacturonic acids, polyuronides, and sugars. Roboz[3] has previously shown that galacturonic acid could be separated from other material by adsorption on an anion-exchange resin (IRA-400); then recovered by elution with 25–50% acetic acid. However, no methods reported so far have effected both a qualitative and quantitative determination of the uronic acids, with a complete recovery of the pure individual uronic acids as crystalline materials; such a method will be described here.

In these experiments, a dilute alkaline solution of galacturonic, glucuronic, and mannuronic acids was absorbed quantitatively on the acetate form of the strong base anion exchanger Dowex-1. The uronic acids were eluted with 0.15 *M* acetic acid and the fractions analyzed by a slight modification of the orcinol method as described by Brown[4] for pentose determination (samples were heated at 100° for at least one-half hour). This assay method, as applied here, obeys Beer's law through the concentration range of 2 to 60 μg./ml. of uronic acid.

The separation of galacturonic and glucuronic acids in the presence of arabinose and galactose is shown in Fig. 1. The same separation can be achieved with a formate system using 0.01 *M* formic acid as the eluting agent. Mannuronic acid was eluted from the column in the same fraction as the glucuronic acid and a separation of these two could not be effected in the systems reported here. The free sugars, which were not absorbed, were collected in the first fraction and determined colorimetrica ly (galactose was determined by the anthrone method of Dreywood[5] as developed by Morris[6];

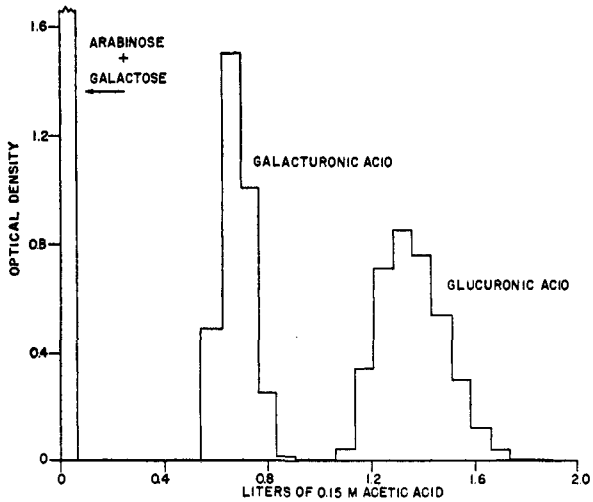

Fig. 1. The separation of galacturonic and glucuronic acids in the presence of sugar: exchanger, 0.85 sq. cm. × 12 cm. Dowex-1, *ca.* 300 mesh, acetate form; eluting agent, 0.15 *M* acetic acid at ~2.5 ml./min.; test material, 5.0 mg. each of arabinose and galactose, 10 mg. each of galacturonic and glucuronic acids in 10 ml. of 0.02 *M* sodium hydroxide [galactose was determined by anthrone method at 620 mμ; the other materials by orcinol method at 660 mμ].

(1) Work performed under Contract W-7405-eng-26 for the Atomic Energy Commission.

(2) Z. Dische, *J. Biol. Chem.*, **167**, 189 (1947); **171**, 725 (1947); **183**, 489 (1950); also *Arch. Biochem.*, **16**, 409 (1948).

(3) E. Roboz, Internat. Congr. of Pure and Applied Chem., Abstracts XII, 156 (1951).

(4) A. H. Brown, *Arch. Biochem.*, **11**, 269 (1946).

(5) R. Dreywood, *Ind. Eng. Chem., Anal. Ed.*, **18**, 499 (1946).

(6) D. L. Morris, *Science*, **107**, 254 (1948).