Diethylstilbestrol Di-ethylmercaptoacetate.—This ester was prepared just as was the preceding ester. The purification was carried out by evaporating the ether extract to dryness and taking the residue up in 10 cc. of ether and 40 cc. of Skellysolve A. This solution was passed through activated alumina and the alumina was then extracted with 1:4 ether–Skellysolve A. The extract was evaporated and the absorption-elution process carried out twice more. Finally the crude product (1.05 g.) was crystallized once from Skellysolve B and twice from 95% ethanol giving a yellowish product 0.30 g., m. p. 99–101°.

Anal. Calcd. for $C_{26}H_{32}O_4S_2$: C, 66.04; H, 6.83; S, 13.57. Found: C, 66.01; H, 6.73; S, 13.83.

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RECEIVED MARCH 16, 1949

COMMUNICATIONS TO THE EDITOR

THE USE OF CADMIUM IODIDE IN STARCH–IODINE COLORIMETRIC PROCEDURES

Sir:

In the course of an investigation[1] on methods of analysis for trace amounts of selenium in water, it was found that cadmium iodide and starch form a stable solution which may be used as a colorimetric reagent for a number of oxidizing substances. The reduction potential of the iodide in such a solution is a function of the *p*H. By proper adjustment of the *p*H, the iodide may be "exposed" to oxidation by oxidizing agents for controlled periods of time and in this way it was found possible to determine one oxidizing agent in the presence of others.

Cadmium iodide crystals may have a brownish discoloration which is shown by reaction with starch to be free iodine. However, after an aqueous solution of cadmium iodide is boiled for ten or fifteen minutes, a colorless solution is obtained. This solution may be added to a solution of starch to give a mixture that is apparently stable indefinitely to atmospheric oxygen and diffused sunlight.

In neutral solution, only the very strongest oxidizing agents, such as chlorine or hypochlorite, are capable of oxidizing the iodide to iodine and producing the blue starch–iodine color. At lower *p*H values, weaker oxidizing agents are able to oxidize the iodide; *e. g.*, nitrous acid is capable of oxidizing the iodide if the *p*H is below about 4.0 but the *p*H must be in the neighborhood of 1.0 or lower before selenious acid is able to react. Dissolved oxygen attacks the cadmium iodide reagent only in the most highly acid solutions, and very slowly even then.

The linear starch "A-fraction" isolated by Schoch[2] gives the best results although commercial soluble starches can be used. The color of the A-fraction starch–iodine complex produced by selenious acid in concentrations from 0.1 to 2.0 p. p. m., as selenium, follows Beer's law quite closely. The absorption band is broad with maximum absorption occurring at about 615 mμ.

Cadmium iodide in aqueous solution has been shown to form one or more auto-complexes, the nature of which has been the subject of several investigations. The complex anion may be CdI_3^-, $CdI_4^=$ or $CdI_5^{\equiv}$, with the cation Cd^{++} or CdI^+.

This reagent is undergoing further investigation in connection with the development of colorimetric methods of determining traces of substances having oxidizing properties, particularly selenium as selenious acid.

We wish to thank T. J. Schoch of the Corn Products Refining Company for providing generous samples of the linear starch A-fraction.

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(1) This investigation is supported by a research grant from the National Institutes of Health.

(2) Schoch, THIS JOURNAL, **64**, 2957–2961 (1942); "Advances in Carbohydrate Chemistry," Vol. I, ed. by Pigman and Wolfrom, Academic Press Inc., New York, N. Y., 1945, pp. 247–277.

GERMIDINE AND GERMITRINE, TWO NEW ESTER ALKALOIDS FROM *VERATRUM VIRIDE*

Sir:

Recent evidence[1,2] indicates that powdered roots and rhizomes of *Veratrum viride* may produce marked reductions of arterial pressure in patients with essential hypertension. We have isolated from this material two new, highly active ester alkaloids derived from the alkamine germine, which we have named germidine and germitrine.[3]

(1) E. D. Freis and J. R. Stanton, *Am. Heart J.*, **36**, 723 (1948).

(2) E. D. Freis, *et al.*, *J. Clin. Investigation*, **28**, 353 (1949).

(3) Drs. E. D. Freis, J. A. Stanton and F. C. Moister of the Robert Dawson Evans Memorial, Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine, have evaluated more than 100 of our individual alkaloidal fractions in patients with essential hypertension. Their results will be published elsewhere.

Preliminary fractionation of the benzene-extractable alkaloids by the excellent procedure of Jacobs and Craig[4] yielded the five known crystalline alkaloids and a large amorphous fraction; only the latter was active as a hypotensive agent at low dosage. Further fractionation of this material guided by assay[3] in hypertensive patients yielded a highly active concentrate, which on 24-plate Craig distribution, using 2 *M* acetate buffer at *p*H 5.5 and benzene as the immiscible phases, exhibited two discrete peaks. The material having a peak at tube 15 ($K = 1.67$) showed activity when administered orally in doses of 0.6–0.8 mg. per patient, while the material from the second peak, at tube 6 ($K = 0.35$), was active at about 4 mg. These fractions crystallized readily from methanol–water and ethanol–water respectively, yielding germidine (m. p. 220–223° (cor.); $[\alpha]^{25}D + 13°$ (*c*, 1.67 in chloroform)) and germitrine (m. p. 197–199° (cor.); $[\alpha]^{25}D + 11°$ (*c*, 1.54 in chloroform)).

Room temperature hydrolysis of germidine with 0.1 *N* aqueous methanolic alkali afforded germine ($C_{27}H_{43}O_8N$),[5] acetic acid and α-methylbutyric acid. The former was identified by rotation, analysis and conversion into monoacetonylgermine hydrochloride.[6] The acids, after conversion into the *p*-phenylphenacyl esters followed by chromatography on alumina, yielded *p*-phenylphenacyl α-methylbutyrate[7] (m. p. 71–72° (cor.)) and *p*-phenylphenacyl acetate[8] (m. p. 109–110° (cor.)). Analysis of the free base and the crystalline thiocyanate (m. p. 242–244° dec. (cor.)) indicates germidine to be an ester of germine with one mole each of the above acids.

Hydrolysis of germitrine yielded germine, α-methylbutyric acid and methyl-ethylglycolic acid. The phenylphenacyl ester of the latter (m. p. 119–120° (cor.); $[\alpha]^{25}D + 5°$ (*c*, 0.64 in chloroform)) was identical with an authentic sample.

Analysis of the free base and the thiocyanate (m. p. 228–231° dec. (cor.)) indicates that germitrine is probably a mono-α-methylbutyrate dimethylethylglycolate of germine.

The specific rotations of the branched-chain acids (isolated from the total amorphous fraction because of an insufficient supply of the crystalline alkaloids) showed these materials to be *l*-α-methylbutyric and *d*-methylethylglycolic acids. Germidine and germitrine, injected intravenously in doses of 0.6–0.8 γ per kg., markedly lowered the blood pressure of anesthetized dogs and cats.[9]

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RECEIVED AUGUST 9, 1949

(4) W. A. Jacobs and L. C. Craig, *J. Biol. Chem*, **160**, 555 (1945).
(5) W. Poethke, *Arch. Pharm.*, **275**, 571 (1937).
(6) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **148**, 57 (1943).
(7) F. Kögl and H. Erxleben, *Z. physiol. Chem.*, **227**, 70 (1934).
(8) N. L. Drake and J. Bronitsky, THIS JOURNAL, **52**, 3715 (1930).
(9) Dr. S. Krop of the Division of Pharmacology will report on these findings elsewhere.

THE BIO-OXYGENATION OF 11-DESOXYCORTICOSTERONE AT C-11[1]

Sir:

A number of chemical processes[2] have been elaborated for introducing an oxygen function at C_{11} in the partial synthesis of adrenal cortical hormones. We wish to report a biochemical process, different from any hitherto described, which leads to the production of corticosterone from 11-desoxycorticosterone.

Using methods previously reported,[3,4] it was found that after perfusing 11-desoxycorticosterone (DOC) through isolated adrenal glands the perfusate contained large amounts of glycogenic activity.[5] The method of assay employed was that of Olson, *et al.*[6] The increased glycogenic activity was observed whether plasma or blood was used as the perfusion medium, and in the absence of adrenocorticotrophic hormone (ACTH). The activity could not be accounted for in terms of DOC recovered from the perfusates. Neither perfusion of the gland in the absence of DOC and ACTH,[4] nor the circulation of DOC in the absence of the gland led to significant activity; nor was it possible to demonstrate a synergistic action of DOC upon the glycogenic steroids present in adrenal extracts (Upjohn).

These observations strongly suggested that the isolated adrenal introduced an 11-oxygen function into desoxycorticosterone. Further work has resulted in the isolation from these perfusates of corticosterone, m. p. 173.3–180° (178–180.5°),[7] $[\alpha]^{30}D + 227°$ (*c*, 0.240, ethanol), as the principal crystalline transformation product. The identity was established conclusively by the melting point, 173–181°, of a mixture with an authentic sample of corticosterone (Upjohn), m. p. 173–179° (176.5–181°), and by the formation of an acetate, m. p. 150.5–152.5°, whose mixture with authentic corticosterone 21-acetate, m. p. 151.5–152.5°, melted at 150.5–152°.

Similar perfusion experiments employing 11-desoxycorticosterone 21-acetate also yielded corticosterone, the ester group apparently being hydrolyzed in the course of the perfusion.

These studies have been extended to a variety of steroids including progesterone, 4-androstene-3,17-dione, 17-hydroxyprogesterone, epi-androsterone, androsterone, 17-hydroxy-11-desoxycorticosterone and 5-pregnen-3-ol-20-one, and the

(1) The work described in this paper was supported by a grant from G. D. Searle and Company.
(2) For a comprehensive review of these processes, see L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," third edition, Reinhold Publishing Corp., New York, N. Y., p. 452, 1949.
(3) O. Hechter, *Endocrinology*, **42**, 285 (1949).
(4) O. Hechter, *Federation Proc.*, **8**, 70 (1949).
(5) O. Hechter and G. Pincus, unpublished observations.
(6) R. E. Olson, *et al.*, *Endocrinology*, **35**, 430 (1944).
(7) All comparison melting points were taken on powdered samples in open Pyrex capillaries. The melting points in parentheses were obtained for intact crystals whether determined in a capillary or on a Fisher–Johns block.