18.7%, and this is submitted as the composition of the double salt.

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Isolation of the Active Principle in Claviceps Paspali—A Progress Report[1]

BY MARVIN GIEGER AND B. F. BARRENTINE

Review of Literature.—According to Brown and Ranck,[2] *Paspalum dilatum* Poir, commonly known as Paspalum, or Large Water Grass, was found to contain a fungus poisonous to livestock. These workers found the fungus to be *Claviceps paspali* (Stevens and Hall). The fungus attacks the pistils and grows as a parasite until it occupies the space between the glumes of the spikelet. Thus, the disease grows where the seed are normally produced. This was proved by feeding the sclerotia, picked from infected paspalum heads, to guinea pigs, resulting in trembling and in some cases death to the guinea pig.

Work by Dr. W. F. Hand[3] corroborates the conclusions of Brown and Ranck that the poison comes from the *Claviceps* sclerotia. Dr. Hand's ether extract of the sclerotia gave an oily residue of which 5 to 10 ml. would kill a guinea pig when given by mouth. Upon discontinuation of the isolation of the poison by Dr. Hand, the work was later taken up by this department.

Experimental

Six hundred pounds (272 kg.) of scalpings or whole paspalum seed spikes infected with the Claviceps paspali was passed through a small slow speed hammer mill containing $^5/_{16}$-inch (8-mm.) holes in the sieve. The slow speed of the mill combined with the large holes in the screen enabled the seed to be broken apart from the fungus without pulverizing either. The seed and fungus mixture was then passed over a screen containing slits just large enough to allow the paspalum seed to pass through, as they are flat, but small enough to retain the round sclerotial. The mixture retained on the screen was about 90% sclerotia.

The sclerotia were then ground and extracted with petroleum naphtha (Skelly-solve F −95°) to remove most of the oil. After most of the oil was removed and the naphtha allowed to evaporate, the oil-free sclerotia were again extracted with one of several solvents to remove the poison: namely, ethyl ether, benzene, ethyl acetate, chloroform, ethyl alcohol, or methyl alcohol. The solvent was evaporated in vacuum leaving a sticky, tarry residue. This was further purified by taking up the residue with petroleum naphtha, which dissolved some of the impurities while at the same time precipitating a cream-colored amorphous precipitate containing the active principle. The precipitate was filtered, washed three or four times with petroleum naphtha, and on standing soon dried.

One gram of this amorphous compound was dissolved in 25 ml. of ethyl ether, the solution placed in an Erlenmeyer flask with 25 ml. of a 0.5% solution of tartaric acid, and agitated by an end-over-end motion in a shaking machine for one hour. These solutions were poured into a separatory funnel and after standing long enough to separate the aqueous tartaric acid solution was drawn off, rendered just alkaline to litmus with sodium bicarbonate and extracted with ether. The ether extract was evaporated to dryness in vacuum. The very small residue obtained gave a negative test for ergot alkaloids with Smith's[4] reagent.

The foregoing procedure was repeated, using ethyl acetate, benzene and chloroform as solvents for the amorphous compound and extracting separate solutions in each case with 0.5% aqueous solutions of tartaric, malic, citric, hydrochloric, nitric, and sulfuric acids. These acid extracts were rendered just alkaline with sodium bicarbonate and extracted with ether, the ether extract evaporated in vacuum and the residue tested with Smith's reagent for ergot alkaloids, all giving negative results.

The ether, chloroform, benzene, and ethyl acetate solutions above, after having been extracted with different weak acids, were washed with water to remove any acid and evaporated to dryness in vacuum. Thirty milligrams of each residue was given to guinea pigs. Each guinea pig was badly affected in about three hours' time with intense trembling, body drawn up in knot, and the pupil of the eye presenting a glossy appearance.

Numerous and varied attempts to purify the amorphous compounds further by crystallization have been without success and, due to this fact, attempts to determine any constants other than a melting point have been postponed. The melting point was approximately 130°. The compound is not soluble in water, but is very soluble in all other organic solvents, such as ether, chloroform, acetone, benzene, ethyl acetate, and ethyl and methyl alcohols. It is not soluble in weak acids, but slowly soluble in weak alkalies. It is very easily extracted from the fungus with liquid ammonia.[5] Qualitative tests show it to contain only carbon, hydrogen, oxygen, and nitrogen. Due to not being able to purify the amorphous compound, a quantitative determination of nitrogen only was made. The nitrogen was 2.5%. A water suspension of the amorphous powder containing a solution of a mixture of emulsion and maltase for hydrolyzing agents was allowed to incubate at 37° for fifteen hours. This was then tested for hydrocyanic acid and glucose, but none was found.

A study of the therapeutic value shows that a 1 to 1000 solution of the amorphous compound administered to the

(1) Contribution from the Department of Chemistry, Mississippi Agricultural Experiment Station, State College, Mississippi. Published with the approval of the Acting Director, Mississippi Agricultural Experiment Station. Paper No. 9, New Series, December 29, 1938.

(2) H. B. Brown and E. M. Ranck, "Forage Poisoning Due to Claviceps on Paspalum," Tech. Bull. No. 6, Miss. Expt. Sta., 1915.

(3) W. F. Hand (unpublished notes).

(4) M. I. Smith, *Pub. Health Repts.*, **45**, 1466–1481 (1930).

(5) E. H. Stuart, U. S. Patent 2,067,866, 1937, to Eli Lilly & Co.,

isolated uterus of a rabbit and also a guinea pig failed to give a single contraction. Fifty milligrams was dissolved in Wesson oil and fed to a pregnant guinea pig. After a few hours the pig developed the characteristic tremble. The following day she was much worse and died the following night, but without aborting the foetus.

A post mortem showed the lungs filled with blood, congested. The heart was flabby or soft and blood coagulated. The kidneys were congested and filled with coagulated blood. The large intestine and stomach were filled with gas; the liver was soft, brownish in color and very tender. The adrenal was enlarged and the outside walls of the uterus were very congested and inflamed. Five embryos measured approximately 3 inches (7 cm.) in length from crown to rump. The union between placenta discs was lost and embryos fell out as soon as the uterus was opened. If administered to anesthetized cats, this amorphous compound caused a fall in blood pressure. It was estimated that one milligram of this material has a depressor action equivalent to 0.002 mg. of histamine hydrochloride. By intravenous injection in mice, the lethal dose of the amorphous compound was found to be 22.5 mg. per kg.

Discussion

It may be definitely concluded that the active constituent in this compound is not an ergot alkaloid, as the experimental data show the base could not be combined with a weak acid, nor did it respond to the alkaloidal test characteristic of the ergot alkaloids. Methods for the isolation of alkaloids of ergot of rye by Thompson,[6] Stuart,[5] Arthur Stoll and Ernst Burckhardt,[7] Smith, Sidney and Timmis,[8] Tswett perfected by Kuhn, Winterstein, and Karrer,[9] have been used in this work, but all were of no value thus far.

Hydrolysis with enzymes leads one to believe it is not a glucoside.

It was thought at one time the fungus contained the same alkaloids as ergot of wheat or rye, but the pharmacological data prove this to be an erroneous idea.

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(6) Marvin R. Thompson, *J. Am. Pharm. Assoc.*, **24**, Nos. 1-3 (1935).

(7) Arthur Stoll and Ernst Burckhardt, U. S. Patent 2,080,954.

(8) Sidney, Smith and G. M. Timmis, English Patent 460,387.

(9) M. Tswett perfected by R. Kuhn, A. Winterstein and P. Karrer, French patent, démandé le 9 janvier 1935, à 16h 25m, à Paris.

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On the Hydration of Dihydropimaric Acid

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In recent communications we described the formation of a lactone on hydration of dihydroabietic acid present in heat-treated rosin [Hasselstrom, U. S. Patents 2,121,032, 2,121,033 (1938); Hasselstrom, Brennan and McPherson, THIS JOURNAL, **60**, 1267 (1938); Hasselstrom and McPherson, *ibid.*, **60**, 2340 (1938)].

We have now been able to prepare a similar lactone by hydration of dihydropimaric acid, m. p. 241–243° (corr.), $(\alpha)_D$ +19.2°, obtained on recrystallization of hydrogenated rosin (Staybellite A-2, by courtesy of the Hercules Powder Company) with methanol according to the procedure described in a previous paper [Hasselstrom and Bogert, THIS JOURNAL, **57**, 2118 (1935)]. The methyl ester of dihydropimaric acid was obtained by means of diazomethane, m. p. 78.5–79.5 (corr.). *Anal.* Calcd. for $C_{21}H_{34}O_2$: C, 79.2; H, 10.8. Found: C, 78.8; H, 10.8. Ruzicka and Frank (*Helv. Chim. Acta*, **15**, 1297 (1932)] have recorded the melting point 79–80° for the methyl ester of the dihydropimaric acid.

A mixture of 3.5 g. of dihydropimaric acid and 40 cc. of sulfuric acid, sp. gr. 1.84, was stirred intermittently for about twenty minutes at 5°, then poured onto cracked ice. The mixture was extracted with ether, the ether solution washed with water, with dilute potassium hydroxide solution and with water. After drying with anhydrous sodium sulfate, the ether was evaporated and the residue, a yellow oil, was dissolved in hexane. On standing, 1.6 g. of white crystals separated which, after recrystallization from acetone, melted constantly at 143–144° (corr.). *Anal.* Calcd. for $C_{20}H_{32}O_2$: C, 78.9; H, 10.5. Found: C, 78.9; H, 10.6; $(\alpha)_D$ −40° (in ethanol). In a mixed melting point test with an authentic sample of the lactone of hydroxytetrahydroabietic acid a depression was observed, the melting point of the mixture being 102–112° (corr.).