1930. Preliminary investigation showed no alkaloids nor glucosides to be present. A saponin was indicated by all the well-known tests. Previous to attempting its isolation the fatty oil was investigated. Eight hundred twenty-nine grams of ground seeds dried at 105° C. yielded 3.39 per cent of fatty oil by petroleum ether extraction. The chemical and physical properties checked well with those found by Weedon. A sample of the oil stored in a well filled bottle in diffused light has become very viscid, indicating polymerization. This, of course, is to be expected from the high iodine value of the oil.

Saponin.—After the above petroleum ether extraction, the seeds were extracted with 80 per cent alcohol. When this was distilled off 8.5 Gm. of a syrupy residue remained. It gave a negative test for saponin. It had an odor of caramel and gave the osazone of dextrose. After standing for several months over concentrated sulfuric acid it partially crystallized. Apparently the saponin had hydrolyzed during extraction and is but another instance illustrating the well known difficulty of isolating these bodies in a pure condition Repeated efforts, using also methyl alcohol extracts from three Kg. of seeds, failed to produce a pure saponin; therefore, the following efforts were made to get the sapogenin or product of hydrolysis other than the sugar.

Sapogenin.—Using the procedure of Liang, and Noller (6), the dried alcohol-water extract was dissolved in just sufficient methyl alcohol containing 5 per cent HCl and refluxed continuously for forty-eight hours. The dark brown precipitate which formed was washed with water, dried in air and extracted two hours in a Soxhlet extractor using carbon tetrachloride. The solvent was allowed to evaporate at room temperature, leaving a cream-colored material. Hot methyl alcohol was used to dissolve all the material that it would and normal isopropyl alcohol was used to dissolve the remainder.

The methyl alcohol solution was chilled in an ice-bath and some material separated which on drying in air was a light tan color and glistened. The mother liquor was concentrated and again chilled producing a second crop of material. The quantities were so small that further purification was not attempted, but the material appeared crystalline. Melting points of these two portions were not very sharp, ranging from 48° C. to 54° C.

The normal isopropyl alcohol solution was also chilled and a white crystalline material separated. This was washed with water, dried and the melting point determined as 47° C., uncorrected. Further crystallization from the mother liquor gave crystals whose melting points checked closely. The crystals gave negative tests for sulfur, nitrogen and halogens. Since the quantity of material was so small no further data could be obtained.

It is indicated that an alcoholic-aqueous extract of these seeds yields, upon hydrolysis with hydrochloric acid, one or more sapogenins and it is hoped that subsequent work with larger quantities will enable their identification.

SUMMARY

The available data on the toxicity of *Glottidium vesicarium* (Jacq.) Harper have been pointed out and the previous work on the seeds, consisting principally of an investigation of the fatty oil, has been reviewed. The data on the fatty oil have been checked and negative tests for alkaloids and glucosides have been obtained. Positive tests for saponin have been obtained but the saponin appears to be hydrolyzed during extraction, and thus far attempts at isolation have failed. One or more hydrolysis products have been obtained by refluxing an alcoholic-aqueous extract with 5 per cent HCl.

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Crystalline Xanthophyll from Wheat Germ

*By O. Gisvold**

While attempting to isolate the tocopherols by new methods, a crystalline xanthophyll was obtained from the nonsaponifiable portion of wheat germ. A small amount of an allophanate of one of the tocopherols was also isolated.

EXPERIMENTAL

Twenty-five pounds of wheat germ were extracted with hot Skelly-solve B in a Barnsdahl continuous extraction apparatus. There was obtained 1200 Gm. of oil which was saponified in the usual manner. The nonsaponifiables were separated from the soap by continuous extraction with

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Skelly-solve B. The nonsaponifiable material thus obtained was dissolved in alcohol and most of the sterols were removed from this solution by fractional crystallization.

Attempted Isolation of the Tocopherols by Means of Their Lead Salts.—An alcoholic solution of normal lead acetate was added to the alcoholic solution of the nonsaponifiable material. Precipitation did not occur, even after concentration or cooling in the ice chest. The alcohol was completely removed on the steam bath and the residue treated with Skelly-solve B. The material insoluble in this solvent was suspended in alcohol and decomposed by hydrogen sulfide. A very small amount of a semi-solid was obtained.

When basic lead acetate was substituted for normal lead acetate the same results were obtained.

Attempted Isolation of the Tocopherols by Means of Their Barium Salts.—Essentially the same procedure was followed as outlined above with the exception that methanol was used as the solvent and a methanol solution of barium hydroxide as the precipitant. The results were negative.

Attempted Isolation of the Tocopherols by Extraction of a Skelly-solve B Solution of the Nonsaponifiables by Means of Aqueous and Alcoholic Sodium Hydroxide.—The nonsaponifiable material was dissolved in Skelly-solve B. It was extracted with 5, 10 and 20 per cent solutions of aqueous sodium hydroxide. When these solutions were neutralized with phosphoric acid and extracted with ether, a very small amount of a semisolid material was obtained. The nonsaponifiable portion was then extracted with an approximately 25 per cent solution of sodium hydroxide in 25 to 33 per cent alcohol. The alkaline extractive was colored and, upon decomposition with phosphoric acid and extraction with ether, yielded a small amount of an oil. From this oil an exceedingly small amount of an allophanate, m. p. 145° C., was obtained. This allophanate failed to give the color reactions characteristic of sterols and by virtue of its melting point was thought to be one of the tocopherols.

Crystalline Xanthophyll.—The petroleum ether solution that had been extracted with an alcoholic solution of alkali was allowed to stand in the ice chest for some time. Rosettes of garnet red leaflets separated from this solution. They were collected upon a fritted glass filter, washed with Skelly-solve B and dried. They melted at 175° C. and exhibited an absorption max. at 475, 450 in alcohol. A portion of the material when dissolved in Skelly-solve B would partition itself into 85 per cent methanol. A solution of the pigment in chloroform gave a bluish violet color when carefully stratified with strong sulfuric acid.

SUMMARY

Crystalline xanthophyll was obtained from the nonsaponifiable portion of wheat germ oil while attempting to isolate the tocopherols by new methods.

Colormetric Determination of Thiamin Chloride in Certain Pharmaceutical Preparations

*By M. E. Auerbach**

Recently, Prebluda and McCollum (1) have described in detail a chemical reagent for thiamin chloride. Using the same reagent Melnick and Field (2) have developed procedures which make possible a quantitative determination in a variety of substances. The principle elaborated by these investigators is that thiamin will couple with diazotized *p*-amino acetophenone to produce a purplish red compound. This compound is insoluble in water, but is readily soluble in various organic solvents, thus providing a colored solution suitable for comparison with standards. Melnick and Field prefer to use xylol as the color solvent. We use toluol, which is equally serviceable. For the analysis of thiamin dissolved in a simple, colorless menstruum, it is unnecessary to extract the colored compound from its mother liquor. In such cases, we simply add enough isopropanol to dissolve the color, and proceed directly to the quantitative comparison with a reference solution.

A certain drawback appears in the work quoted, in that at least 12 hours are stated to be necessary for the development of the maximum color intensity. During the past two years, we have employed a modification of the Prebluda-McCollum test which permits a complete analysis of thiamin in certain pharmaceutical products within 15 to 30 minutes. The same method, with minor alterations, has served for the determination of the stability and incompatibilities of thiamin chloride under various conditions and in various media.

EXPERIMENTAL

Conditions for the Analysis.—A quantity of test sample is taken which will yield as nearly as possible 100 micrograms of thiamin. A standard solution is made by dissolving exactly 50 mg. of pure dry thiamin chloride (or an equivalent calculated from actual analysis) in 500 cc. of 50% (volume) ethanol containing one drop of 4*N* HCl. This standard is stable at room temperature for several months, but

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