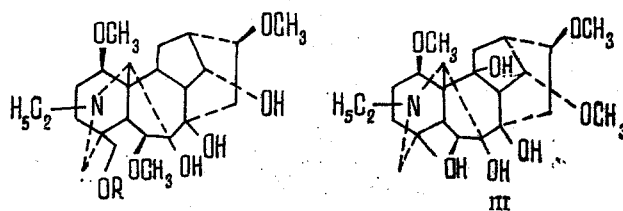


This is the first time that demethyleneeldelidine has been isolated from plants.



I. R=N-acetylanthranoyl  
 II. R=H  
 Delectine, R= anthranoyl

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#### METHOD OF DETERMINING PHYTIN IN RICE FLOUR

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UDC 615.45:615.7

Phytin — a mixture of the calcium and magnesium salts of inositol hexaphosphate — is the main phosphorus-containing reserve compound of higher plants [1]. For the sorting standardization of rice flour for its phytin content, a method for its quantitative determination [2] is used that is based on the precipitation of the phytin from an extract with lead nitrate, which does not always give satisfactory results. We have developed a simple method of quantitative determination of phytin in brans.

Phytin is obtained by extracting rice flour with 1% nitric acid and formalin is added to the acid with the aim of preventing fermentation. Under laboratory conditions, we isolated phytin from rice flour and, in parallel, from cottonseed meal with 1% nitric acid without the addition of formalin. No decrease in the amount of phytin and, consequently, no fermentation process was observed even after 4-5 days in the case of the rice flour, but in the cottonseed meal (after the extraction of the neutral lipids and phospholipids) fermentation took place (2-3 days) and the yield of phytin fell sharply.

Having shown the high solubility of phytin in 1% nitric acid containing 20% of ethanol, we extracted the phytin with this mixture, which ensured the rapid filtration of the meal. To clarify the phytin extract it was passed through a filter mass, and the filtrate was precipitated by adding concentrated ammonia solution to pH 7.0-7.5. At pH 8 and above, the phytin obtained is dark-colored. The precipitate of phytin was purified by reprecipitation and was dried in a drying chest at 80-100°C. The main index of the quality of phytin is its phosphorus content, which, calculated as phosphorus pentoxide, must not be less than 39%.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Tashkent Pharmaceutical Chemicals Factory. Translated from *Khimiya Prirodnikh Soedineni*, No. 1, pp. 129-130, January-February, 1977. Original article submitted September 14, 1976.

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The phytin that we obtained corresponded to the requirements of the State Pharmacopoeia [3] and contained more than 45% of  $P_2O_5$ .

The phytin was determined in the following way. A 25-g sample of roasted rice bran was covered with 75 ml of 1% nitric acid containing 20% of ethanol, and the mixture was stirred for 30 min. The suspension was filtered through a Schott funnel, pressed out, and washed with 25 ml of the same acid. The meal was treated twice more with 50 ml of acid each time (30 min), 25 ml of the acid being used for washing. The combined extracts were filtered through filter mass ( $\approx 2$  g) and the resulting clear yellow solution was carefully made alkaline with 25% ammonia. The phytin in the precipitate was filtered off by filtration through a Büchner funnel with a double filter paper. The filtered-off phytin paste was dissolved in 8-10 ml of 25%  $HNO_3$  and the acid solution was filtered through a paper filter and [the phytin was reprecipitated with ammonia and filtered off, and the precipitate was] washed with water and ethanol and was then dried. The yield of phytin was 1 g, which amounts to 4% of the weight of the rice flour. The dried phytin consisted of a white powder insoluble in water but soluble in dilute mineral acids and also in 1% nitric acid containing 20% of ethanol.

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#### A SIMPLE DETERMINATION OF PROTEIN

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UDC 547.96

In working with dilute solutions of highly active enzymes it is frequently necessary to know the amount of protein in the sample. At the present time there are no simple and reliable methods for its determination. Lowry's very well-known method [1] is suitable for determining fairly large amounts of protein — more than 10-15  $\mu$ g. A number of methods for the quantitative determination of protein in ultramicro amounts is known, but they are all fairly complicated and require additional equipment such as, for example, a scanning fluorimeter [2] or a special membrane [3-6].

We have attempted to find a simpler method of determining small amounts of protein in a sample. As a basis we took a method described in 1969 [7] which permitted the determination of 10-20  $\mu$ g of protein in a sample. In our modification it is possible to determine 1-10  $\mu$ g of protein. The method is as follows. A solution of protein is deposited on a sheet of filter paper and is treated with trichloroacetic acid (TCA). Then the sample is stained with a dye, the excess of dye is washed out, the stained complex is eluted, and the optical density of the eluate is measured in a spectrophotometer.

For staining the protein on the paper we tested Coomassie Blue, Amido Black 10B, and Coomassie Brilliant Blue R-250. The first and second gave no advantages whatever over the Lowry method, but the use of Coomassie Brilliant Blue R-250 gave good results. Concentrations of the dye of from 0.1 to 1% had little effect on the density of staining up to 10  $\mu$ g of protein, but with the higher concentrations of dye (0.5-1%) it was difficult to wash the dye out of the substrate, which considerably increased the adsorption of the blank. The

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Pacific Ocean Institute of Bioorganic Chemistry, Far-Eastern Center of the Academy of Sciences of the USSR, Vladivostok. Translated from *Khimiya Prirodnikh Soedinenii*, No. 1, pp. 130-131, January-February, 1977. Original article submitted July 8, 1976.

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