

*intermedia* alkaloids were found localized in the germ or seedcoat rather than in the gum portion of the seed. Preliminary feeding tests of the gum indicate no short-term toxicity for rats other than retarded growth (Table V).

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## PEANUT FLOUR CONSTITUENTS

### Isolation and Identification of Pinitol from Peanut Flour

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Pinitol, a monomethyl ether of D-inositol has been isolated from peanut flour. Its identity was proved by elemental analysis, molecular weight determination, and comparison of the infrared spectra of the compound and of its derivative, diisopropylidene pinitol, with the infrared spectra of authentic samples of these substances.

A SYSTEMATIC INVESTIGATION of the minor constituents in peanuts is being made at this laboratory (2), the primary interest being in those that contribute to flavor and aroma. Many of the precursors to flavor factors are concentrated in the alcohol-soluble portion of defatted peanut flour; this extract represents about 3.5% of the blanched peanut. The present emphasis is on the isolation of constituents found in the water-soluble fraction of the alcohol extract, and this report deals with the isolation and identification of pinitol, a monomethyl ether of D-inositol, the structure of which has been established by Anderson (7).

#### Experimental

The fractionation procedure used in obtaining the alcohol-soluble fraction (D) in Figure 1 was the same as that used by Morris and Lee (2). This lyophilized material was extracted thoroughly with absolute ethyl alcohol; after removal of the solvent and lyophilization, a product (F) was obtained which represented 1.3% of the blanched peanut. Fifty-gram batches of the extract (F) were then refluxed with acetone on a steam bath. The acetone solution was decanted and

filtered. More acetone was added and the procedure repeated until no residue remained on evaporation of the decanted solvent. After drying by lyophilization, this acetone-soluble portion (H) was shaken with chloroform and water. Three layers formed in the separatory funnel: a clear water-soluble layer, a clear chloroform-soluble layer, and an emulsion of the two at the interface. Heating on a steam bath followed by centrifuging broke this emulsion so that all of the material was water-soluble or chloroform-soluble. The water-soluble fraction (J) was washed with 1-butanol, and the resulting 1-butanol-insoluble fraction (J-w) represented 0.2% of the peanut. This fraction was the starting material for chromatographic separation.

Four grams of J-w (Figure 1) were placed on a column (9 cm. diam.  $\times$  40 cm.) of cellulose powder, and the column was developed with a mixture of 1-butanol-acetone-water (2:2:1). One hundred fractions, 70 ml. each, were collected, and J-w was resolved into four peaks by this procedure, as indicated by the curve obtained by plotting the weight of material eluted against the volume of eluant. These are Peaks I, II, III, and IV (Figure 1).

Crystals formed as the solvent was evaporated from fractions comprising Peak II. These fractions (Peak II) were combined and rechromatographed on the same column with the same developing solvent. Fractions in which crystals again formed represented 0.021% of the blanched peanut (II-B, Figure 1). The noncrystalline material in these fractions was dissolved in methanol and decanted. The crystals (Figure 2) remaining amounted to approximately 5.4% of II-B or 0.001% of the peanut.

The crystals (m.p. 187-188° C.)  $[\alpha]_D^{25} + 65.7^\circ$  ( $C=2$  in  $H_2O$ ) had a sweet taste yet gave a negative Molisch test. Tests for acidic and ester groups were also negative. Qualitative elemental tests showed the absence of nitrogen, sulfur, phosphorus, magnesium, calcium, or halogen. Analysis: C, 43.36; H, 7.33;  $CH_3O$ , 16.15; mol. wt. 204 (differential vapor pressure osmometer method). Calculated for  $C_7H_{14}O_6$ : C, 43.29; H, 7.27;  $CH_3O$ , 15.98; mol. wt. 194.19.

With this information, tentative identification of the compound was established by reference to the Sadtler Index (4) which indicated only one compound with such a formula and melting point—i.e., pinitol, a monomethyl ether of

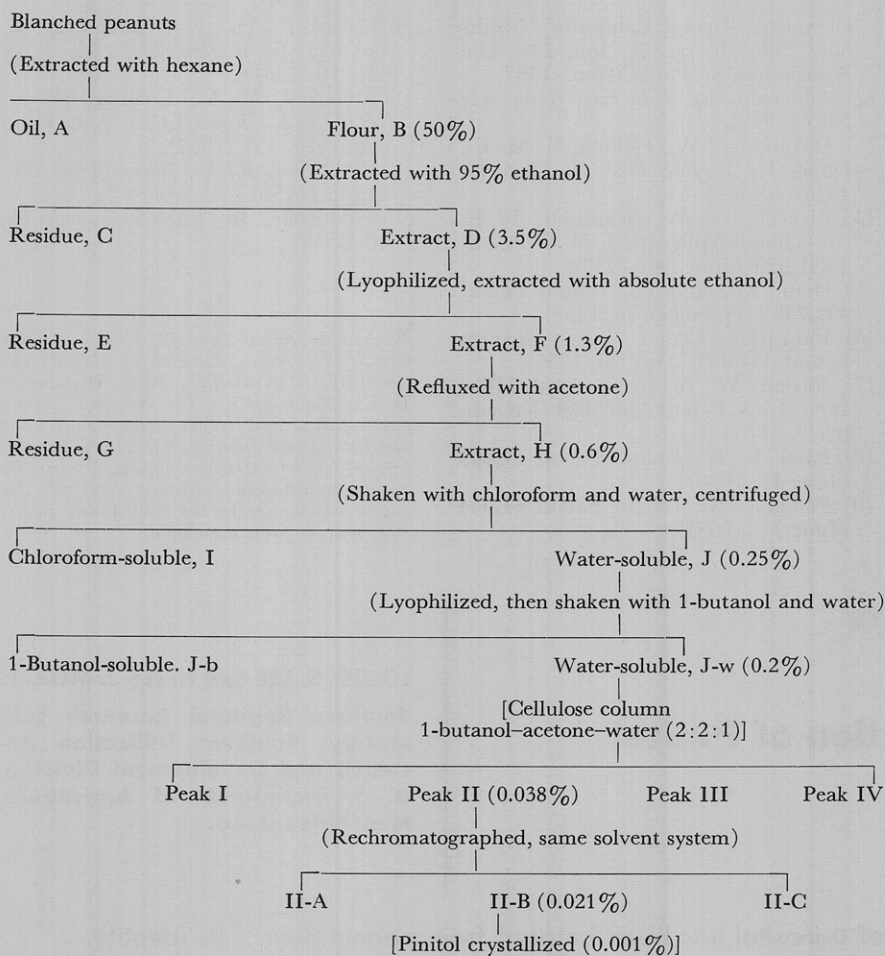


Figure 1. Schematic diagram of isolation of pinitol

D-inositol. Its identity was confirmed by infrared absorption. The spectra (potassium bromide disk method) of the crystals isolated from peanut flour and of an authentic sample of pinitol showed the two compounds to be identical with characteristic absorption maxima at 10.35, 11.04, 11.58, and 13.28 microns. The melting point was not depressed on admixture with this authentic sample.

The acetone derivative, diisopropylidene pinitol, was prepared according to Anderson (7) with the following modification. After shaking about 40 mg. of the pinitol with 25 ml. of acetone containing 1.2% hydrogen chloride for 3 hours, the solution was quickly neutralized with 2*N* NaOH. Diethyl ether was added and the mixture shaken in a separatory funnel. On evaporation of

the ether layer, needle crystals collected around the lip of the beaker and sublimed onto the watch glass covering it. They melted at 105–106.5° C. which agreed well with the melting point (104.5–106° C.) reported in the literature (7) for diisopropylidene pinitol. Analysis: C, 56.91; H, 8.20. Calculated for C<sub>13</sub>H<sub>22</sub>O<sub>6</sub>: C, 56.92; H, 8.09. The infrared spectra of the derivative and of the diisopropylidene pinitol prepared by Anderson (7) and reported in the Sadtler Index (4) showed these two compounds to be identical.

The occurrence of pinitol in rather large amounts (1 to 2% of the dry material) in the flowers and leaves of a number of *Leguminosae* has been reported by Plouvier (3). Since very little pinitol was obtained from peanut flour,

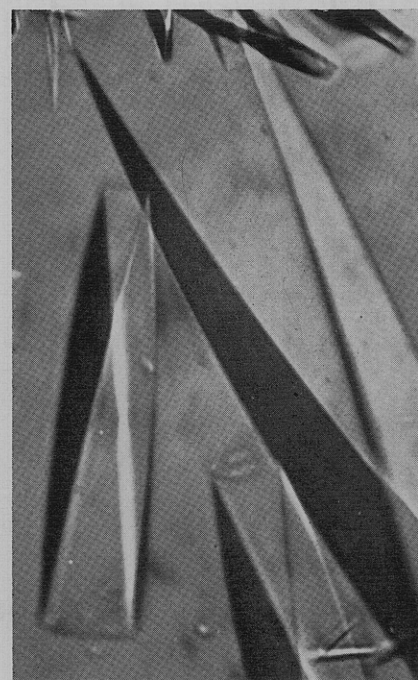


Figure 2. Photograph of pinitol isolated from peanuts

Magnification 150

the conclusion is that it occurs in much smaller amounts in the kernel of this legume (*Arachis hypogaea*).

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