XVIa mixture. The product contained ca. 80% of tetraene which was "contaminated" with residual brominated products, or starting material. Analysis showed 4-5% Br, which fitted well with NMR data: 80% tetraene, based on ratio of olefinic hydrogens to others ($C\underline{H}_2OCO$, allylic H). UV absorption showed the typical high intensity of conjugated diene ($\epsilon_{230} \sim 20,000$). IR showed absorption at 980 cm^{-1} (conjugated E,E-diene) which replaced the 730 cm^{-1} and 970 cm^{-1} absorption of Z- and E-double bonds, respectively, in **XV-XVa**: NMR spectrum of **XVI-XVIa** showed overlapping of two spectra of E,E-isomer and Z,E-isomer, as compared with known spectra of pheromones (11, and Shani and Klug, unpublished data). By measuring the contribution of each isomer (Fig. 1) one can calculate as much as 30% of Z,E-isomer in the mixture, which fits well with the residual Z-izomer of jojoba oil, after its isomerization with HNO_2, or during the NBS reaction (see above). Qualitative support for this was found in the IR spectrum, where we noticed weak absorptions at 985 and 950 cm^{-1}, typical of Z,E-izomer (Shani and Klug, unpublished data).

Basic hydrolysis of the tetraene **XVI-XVIa** afforded almost pure dien-ol **XVIII-XVIIIa**, which exhibited typical NMR spectrum of E,E-isomer, overlapping Z,E-isomer. A still lower percentage of bromine (2.9%) contaminated the product. The acidic fraction contained, besides the diene-acid **XVII-XVIIa**, hydroxylic products, which always were formed from the dibromide **XV-XVa**.

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Calcium-Phytate Complex Formation Studies

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ABSTRACT

A calcium-phytate complex has been prepared. The chemical composition indicates that five calciums combine per phytate molecule. The solubility product of this calcium-phytate is about 10^{-22} in 0.2 M KCl.

INTRODUCTION

Phytic acid, myo-inositol hexaphosphate, is a strong chelating agent that can bind metal ions to form insoluble phytates. Oilseeds have high levels of phytic acid. Much of the extensive literature on the subject stemmed from McCance and Widdowson's (1) demonstration that phytate decreased the absorption and urinary excretion of calcium. Erdman (2) has recently summarized the nutritional implications of phytates. We report here the preparation of a calcium-phytate complex and its solubility properties under certain conditions.

MATERIALS AND METHODS

Phytic acid was prepared from sodium phytate, Lot No. 107C-0066, Sigma Chemical Co., by passage through cation-exchange columns of Rexyn 101 (H), research grade. The sodium content of the acid was ca. 0.2 ppm as judged by atomic absorption spectrophotometry. All other solutions were prepared from reagent-grade chemicals with deionized, distilled water.

An Radiometer PHM64 research pH meter in conjunction with a GK2401C electrode was used for all pH measurements at 25.0 ± 0.05 C. All solubility experiments were carried out at 25 ± 1 C in 250-mL round-bottom flasks rotated at 75 rpm.

For the preparation of the calcium-phytate complexes, 20 to 30 mL of ca. 0.05 M phytic acid was mixed with 100 mL of 0.2 M KCl in a 250-mL beaker. To this solution was added a quantity of ca. 1 M calcium chloride so that the phosphorus/calcium ratio in the reaction mixture varied from 1/1 to 1/4. This solution was then adjusted to between pH 5 and 6 by slowly adding 1 M KOH with stirring. Stirring was continued for an additional 1/2 to 1 hr. The solution was filtered and the precipitate washed with three 20-mL portions of boiling distilled water. The precipitate was allowed to air-dry overnight, and then it was dried at 120 C under vacuum for 1 hr prior to analysis.

The solubility experiments on the calcium-phytate complexes were done in the following manner. From 0.2 to 0.8 g of calcium-phytate was placed into round-bottomed flasks along with 100 mL of a solution 0.2 M in KCl containing from 1.5 to 3.5 mL of 0.1 M HCl. The flask was rotated for

TABLE I

Precipitation of Calcium-Phytate at Varying pH and P/Ca Ratios

pH	P/Ca[a]	%P	%Ca	P/Ca[b]
5.0	1/1	18.85	20.63	1.18
5.3	1/2	18.98	20.64	1.19
5.5	1/3	18.89	20.58	1.19
6.0	1/4	18.56	19.83	1.21

[a] In reaction mixture.
[b] In precipitate.

TABLE II

Calculations of the Solubility Product of $C_6H_8O_{24}P_6 \cdot 8H_2O$ in 0.2 M KCl at 25 C

pH	-log Ca	-log $(P_{10})/(P_T)$	-log (P_T)	-log (P_{10})	-log K_S
5.22	2.244	8,070	2.943	11.013	21.9
5.34	2.284	7.710	2.975	10.684	21.8
5.66	2.456	6.650	3.155	9.804	21.8
5.80	2.533	6.230	3.252	9.482	22.0

about 24 hr and then left to stand for 1 hr in order to let the precipitate settle. Approximately 20 mL of the clear supernatants were removed through filter sticks for pH measurements and analysis of calcium content by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

A typical analysis for calcium-phytate precipitated at pH 5 was: C, 7.20; H, 2.16; P, 18.97; Ca, 20.37. Calculated for $C_6H_8O_{24}P_6Ca_5 \cdot 8H_2O$: C, 7.24; H, 2.43; P, 18.69; Ca, 20.15.

Table I gives the results of several analyses of the calcium salt precipitated at varying pH and P/Ca ratios. Although the results of the experiments listed in Table I indicate that the composition of the salt is independent of both the P/Ca ratio and the indicated pH range, in some instances, precipitation of salts with a simple P/Ca ratio was unsuccessful. In these instances, the precipitates may represent mixtures of variously substituted calcium phytate salts. Although a hexa calcium phytate salt has been reported (3) it is notable that, despite rather widely varying P/Ca ratios in the reaction mixture, the only simple P/Ca obtained in the precipitates was 1.2 which is indicative of the penta-substituted phytate. Moreover, in a similar study, Hoff-Jǿrgensen (4) reported only the penta-substituted phytate.

In order to calculate the solubility product of the calcium-phytate complex, it is necessary to know the concentration of the phytate ion with 10 negative charges. Although the total phytate concentration may be determined analytically, the ratio (phytate ion with 10 negative charges)/(total phytate) can be determined only by use of the ionization constants of phytic acid. Thus, from mass balance and the ionization constants, Hoff-Jǿrgensen (4) derived the following relationship which is applicable over the pH range studied in this investigation where P_{10} is the phytate ion with 10 negative charges, P_T is the total phytate concentration, and the K's are the various ionization constants of phytic acid (Equation I). These constants have been determined by potentiometric titration (4).

$$\frac{(P_{10})}{(P_T)} = \frac{K_5K_6 \cdot K_7 \ldots K_{10} \cdot \frac{1}{(H^+)^{10}}}{\frac{1}{(H^+)^4} + \frac{K_5}{(H^+)^5} + \frac{K_5 \circ K_6}{(H^+)^6} + \ldots \frac{K_5 \cdot K_6 \ldots K_{12}}{(H^+)^{12}}} \qquad [I]$$

For the calculation of the solubility product, $(Ca)^5 (P_{10}) = K_S$, calcium was determined analytically, $(P_{10})/(P_T)$ was calculated from Equation I, and (P_T) was calculated from 5 (P_T) = (Ca). These calculations are summarized in Table II.

The solubility product is almost constant in the pH range of ca. 5-6 and has value of ca. log K_S = -22. In a similar investigation, Hoff-Jǿrgensen prepared phytic acid by H_2SO_4 precipitation of the barium salt. According to Barre et al. (5), phytic acid prepared in such a manner can never be entirely free of H_2SO_4. It is apparent that in the pH range such as that existing in the intestine, a considerable portion of any dissolved calcium in the presence of phytic acid will be removed in the form of a complex.

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