Table III. Phytate Content of a Selected Group of Plant Sources

sample	phytate, %[a] Wade method	digestion method
sunflower protein concentrate	3.66 ± 0.07	3.78 ± 0.02
soybean	1.82 ± 0.09	1.72 ± 0.05
fababean protein concentrate	1.40 ± 0.06	1.48 ± 0.07
pea protein concentrate	1.24 ± 0.05	1.33 ± 0.06
lentil[b]	0.23 ± 0.02	0.27 ± 0.01
wheat flour[b]	0.22 ± 0.01	0.25 ± 0.01

[a] Mean and standard deviation of at least three samples. [b] Using a 5:25 dilution.

containing 1% or more phytate.

Possible interference by phenolic compounds and ascorbic acid reducing $FeCl_3$ was investigated. *p*-Hydroxybenzoic, *trans*-cinnamic, and ferulic acids, which correspond to 50% of the total phenolics in rapeseed, did not react with the Wade reagent at levels 100× that normally found. Ascorbic acid and chlorogenic acid, however, both reacted with the Wade reagent but were not eluted with the phytate fraction and thus did not interfere with the Wade procedure. Phytate levels obtained for a variety of plant materials are listed in Table III. In most cases the Wade values were slightly lower but a *t* test for paired measurements at the 5% level showed no significant differences between the two methods.

This paper demonstrates that the Wade reagent has several distinct advantages over the digestion procedure with respect to the simplicity and rapidity of the procedure. This method is ideal for screening phytate levels in soybean and rapeseed samples for breeding studies. The digestion method is time consuming and quite impractical for monitoring phytate levels in a large number of samples. This study establishes the Wade method as an effective and rapid procedure for phytate determination.

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Identification of 2-Amino-2,6-dideoxygalactose Hydrochloride in a Soil Hydrolysate

A dideoxyamino sugar, 2-amino-2,6-dideoxygalactose hydrochloride (fucosamine hydrochloride), was found for the first time in a soil hydrolysate. It was identified as its alditol acetate by gas–liquid chromatography and mass spectrometry in an eluate fraction following cation-exchange chromatography.

The presence of amino sugars in soils was first demonstrated by Bremner (1949, 1950), who showed their presence in soil hydrolysates by a colorimetric method and paper chromatography. Subsequently, a number of workers have identified glucosamine and galactosamine (Lowe, 1978). The presence of their *N*-acetyl derivatives has also been reported as well as that of another hexosamine, talosamine (2-amino-2-deoxytalose) (Wang and Cheng, 1964). More recently, muramic acid, a component of the peptidoglycan polymer of bacterial cell walls, was found in inorganic soils (Millar and Casida, 1970) and in bog soils (Casagrande and Park, 1978). In view of the relatively large number of amino sugars found in nature (Horton, 1969; Sharon, 1965) and their instability in concentrated hot acid, it has been suggested (Greenland and Oades, 1975) that others exist in the soil. If indeed this is the case, these amino sugars might be components of the "unknown" soil N which represents between 41 and 46% of the total soil N (Sowden et al., 1977). The optimal hydrolytic conditions reported for the analysis of glucosamine and galactosamine, namely, 6 M hydrochloric acid at 100 °C for periods varying between 6 and 12 h, cause significant decomposition of hexosamines, necessitating the use of correction factors ranging from 1.1 to 1.4 (Bremner, 1965). Therefore, less drastic conditions, such as those commonly used in structural determinations of bacterial amino polysaccharides (Perry et al., 1975), were chosen for the analysis of amino sugars in an orthic humic gleysol freed of plant and faunal debris. This communication presents the first report of the identification of 2-amino-2,6-dideoxygalactose hydrochloride (fucosamine hydrochloride) in a soil hydrolysate.

EXPERIMENTAL SECTION

Chemicals. 2-Amino-2,6-dideoxy-L-mannose (rhamnosamine) and 2-acetamido-1,3,4,5-tetra-*O*-acetyl-2,6-dideoxy-D-glucitol were gifts from Dr. M. B. Perry of the National Research Council, Ottawa, Canada.

General Methods. Concentrations were performed under reduced pressure at a bath temperature not exceeding 40 °C. Samples were analyzed by gas–liquid chromatography (GLC) on a Perkin-Elmer 3920 gas chromatograph, equipped with flame ionization and PN detectors. Glass columns (183 × 0.2 cm i.d.) containing

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(a) 3% ECNSS-M on Gas Chrom Q (100–120 mesh) and (b) 3% OV-17 on Gas Chrom Q (100–120 mesh) were used. The following operating conditions were employed: for column a, injection temperature 250 °C, detector temperature 260 °C, column temperature 200 and 220 °C, helium flow 50 mL/min; for column b, column temperature 200 and 220 °C, helium flow 55 mL/min; for N detection, air 150 mL/min, hydrogen 2 mL/min.

A Finnigan Model 3100 mass spectrometer connected to a Finnigan Model 9500 gas chromatograph by means of a jet separator was used for gas chromatography–mass spectrometry (GC–MS) analyses. The mass spectrometer was interfaced with a Model 6100 computer-controlled data acquisition system. A 150 × 0.37 cm i.d. glass column packed with 3% OV-17 on Gas Chrom Q (100–120 mesh) was used. The column was either run isothermally at 200 °C or programmed from 190 to 240 °C at a rate of 8 °C/min. The helium flow rate was at 30 mL/min. The mass spectra were recorded at 70 eV.

Reference Compounds. 2-Acetamido-1,3,4,5-tetra-*O*-acetyl-2,6-dideoxygalactitol, 2-acetamido-1,3,4,5-tetra-*O*-acetyl-2,6-dideoxyglucitol, and 2-acetamido-1,3,4,5-tetra-*O*-acetyl-2,6-dideoxymannitol were run under the same conditions on both columns and for GC–MS as the soil samples. Identities of the soil amino sugars were confirmed by comparing their retention times and mass spectra with those of the reference compounds that have been similarly treated. These techniques do not differentiate D and L enantiomorphs of the component sugar.

Soil. Soil was taken from the surface horizon (0–15 cm) of the Bainesville clay loam (orthic humic gleysol) on the Experimental Farm, Ottawa, Ontario. The soil which was stored below 0 °C had a pH of 6.3 and contained 3.7% organic C and 0.32% N.

The soil was freed of plant and faunal remains by using the wet sieving process of Roulet et al. (1963). Soil (162 g) was suspended in 400 mL of distilled water and kept at 0 °C overnight to soften any aggregates and then was shaken for 1 h and immediately passed over sieves of different mesh sizes ranging from 420 to 105 μm. All operations were done at 4 °C. The water suspension containing soil of particle sizes smaller than 105 μm was centrifuged at 3000 rpm. The supernatant was decanted, and the soil residue was ground in a mortar and freeze-dried, weight 78 g. Analysis showed 4.82% organic C and 0.41% N. Analysis of a thin section of the soil after centrifugation, using a polarizing light microscope, showed no plant or faunal debris.

Hydrolysis. Soil (20 g) in 3 M hydrochloric acid (500 mL) was stirred and heated in an evacuated flask for 18 h at 105 °C. After being cooled, the mixture was centrifuged at 3000 rpm. The soil residue was washed once with 3 M hydrochloric acid. The washing and supernatant were combined and evaporated under reduced pressure. Water was added several times during evaporation to remove volatile acid and prevent development of a high concentration of acid. The residue was finally dried in a desiccator over potassium hydroxide, weight 12.8 g.

Ion-Exchange Chromatography. Cation-exchange chromatography was used for the partial purification of carbohydrates following hydrolysis. The residue was taken up in 90 mL of distilled water. The nonsoluble material was removed by centrifugation. The supernatant was neutralized with sodium bicarbonate, centrifuged, and applied to a column (45 × 2 cm) of Ag 50W-X8 (H^+) resin. Neutral sugars were eluted with water (200 mL), and the amino sugar components were eluted with 2 M hydrochloric acid in two successive fractions, 1 and 2 of 150 mL each. Finally, the resin was washed with 6 M hydrochloric acid (150 mL) to give fraction 3. Under the above conditions, amino acids as well are eluted from the column. As the characterization of amino sugars was based on gas liquid chromatography and mass spectrometry of their fully acetylated hexitol derivatives, amino acids did not interfere.

All fractions were evaporated under reduced pressure, dried, and stored at −20 °C. Weights of the fractions were (1) 0.025, (2) 3.3, and (3) 0.44 g.

Gas–Liquid Chromatography (GLC). All three fractions eluted with hydrochloric acid were analyzed by GLC for amino sugars after reduction and acetylation using both flame ionization and N detectors. Sodium borohydride (5–10 mg) was added to a solution of fraction 1 (0.6 mg) in water (1 mL) and left for 2 h at room temperature. After acidification with acetic acid, the solution was evaporated to dryness in vacuo. Methanol containing a few drops of acetic acid was added 5 times and evaporated from the residue to remove boric acid. Acetic anhydride (2 mL) was added to the residue, and the mixture was heated at 120 °C for 2 h and then evaporated in a desiccator over potassium hydroxide. A fraction of a chloroform extract of the product (1 μL) was used for GLC analysis.

Fraction 2 (250 mg) was dissolved in 2 mL of distilled water, and sodium borohydride (250 mg) was added. The exothermic reaction was controlled by putting the reaction flask in ice water. After 2 h at room temperature, the black precipitate was removed by centrifugation. The supernatant was acidified with acetic acid and evaporated to dryness. The resulting alditols were isolated, acetylated, and analyzed by GLC as described above.

Fraction 3 (222 mg) was reduced, acetylated, and analyzed by GLC as described for fraction 2.

For quantitative determination, myoinositol (50 μL of a 2 mg/mL solution) was added as an internal standard prior to reduction with sodium borohydride. Each GLC analysis was repeated at least 3 times, and no difference was observed.

RESULTS AND DISCUSSION

The conditions for the analysis of amino sugars were similar to those used by Perry and Daoust (1973). These conditions separate all eight dideoxyamino sugars. The chromatograms obtained with both detectors compared very well. As expected, myoinositol added as an internal standard was not detected by the N detector. The first fraction contained all the glucosamine and almost all the galactosamine. The second fraction, after reduction and acetylation, showed several peaks on the GLC chromatogram. One peak corresponded to galactosaminol acetate: the retention time and mass spectrum were identical with those of an authentic sample treated in the same way. The regular chromatogram on GC–MS was scanned for m/e 316, 315, and 302. The peak having all three m/e values was further analyzed. The mass spectrum showed the same principal mass spectral fragmentations as those observed for 2-acetamido-1,3,4,5-tetra-*O*-acetyl-2,6-dideoxyhexitols (Horton et al., 1977): m/e 316 (0.3, M − $AcNH_2$), 315 (0.8, M − AcOH), 302 (1, M − CH_2OAc), 260 (0.8, M − CH_2OAc − $CH_2{=}C{=}O$), 242 (2, M − CH_2OAc − HOAc), 201 (3), 195 (4), 182 (4), 144 (13, $AcOCH_2{-}{}^{+}CH{-}NHAc$), 140 (15), 102 (23), 98 (22), 84 (47, $CH_2{=}{}^{+}C{-}NHAc$), 43 (100). The identity of the deoxyamino sugar was based on its retention times on two columns (a and b), t_R (relative to myoinositol hexaacetate) = 1.21 and 0.87, respectively, and the mass spectrum by comparison with an authentic sample of 2-acetamido-1,3,4,5-tetra-*O*-acetyl-2,6-dideoxy-

galactose, run under the same conditions.

Analysis of fraction 3 showed only traces of nitrogenous substances. Thus, the identity of the dideoxy sugar present in soil is definitely 2-amino-2,6-dideoxygalactose (fucosamine). The fact that this compound had not been identified before is not surprising because of the hydrolytic conditions used previously. As reported by Wheat (1966), fucosamine is converted to oxonorleucine in 6 M hydrochloric acid in the overnight hydrolysis of a fucosamine-containing polysaccharide. A recent study of a pneumococcal polysaccharide (Benzing-Purdie and Perry, 1980) showed that the conditions used here are appropriate for the determination of dideoxyamino sugars in polysaccharides.

The internal standard myoinositol was used in order to obtain a quantitative estimation of the dideoxyamino sugar in the soil studied. The value obtained was 8 $\mu g/g$ of soil. However, the actual amount in soil is probably higher because, as in the case of glucosamine and galactosamine, there is considerable loss during cation-exchange chromatography and also all the fucosamine may not have been released upon acid hydrolysis (Bondietti et al., 1972; Nelson et al., 1979). On comparison of the amount of fucosamine with that of another amino sugar recently identified in the soil, muramic acid (Millar and Casida, 1970), the value is approximately 1 order of magnitude lower. It should be noted, however, that while the latter is a component of nearly all bacterial cell walls, fucosamine is not so widespread. It has been found in the lipopolysaccharides of seven strains of *Pseudomonas aeruginosa*, a potentially dangerous bacterial pathogen (Horton et al., 1977) and plant pathogen (Cother et al., 1966), and in a specific lipopolysaccharide of a *Chromobacterium violaceum* strain (Hepper, 1975). In one instance, it has been reported that *Achromobacter georgiopolitanum*, another soil bacterium, synthesized a fucosamine-rich polysaccharide (Hepper, 1975). The contribution of fucosamine to the total N in the soil is very small, but its identification is of considerable importance. The origin of soil polysaccharides cannot yet be assigned with confidence (Hayes and Swift, 1978). The presence of fucosamine in soil supports the hypothesis that they are of microbial origin. As stated by Finch et al. in 1971, "The evidence for cell wall origin would be far greater if some soil polysaccharides were known to contain muramic acid, fucosamine, viosamine, and possibly neuraminic acid". Two of these compounds have now been identified. Although it is difficult to assess whether all fucosamine originates from the living biomass or whether some is associated with the nonliving humus fraction, it seems more likely that the latter is the case. As more work is done on the structural determination of bacterial polysaccharides, the amounts of fucosamine in soil may be correlated with the presence in soil of certain bacterial genera.

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Differential Identification of Toxic Mushrooms by Pyrolysis–GLC Coupling

The authors propose the use of alcoholic extracts analyzed by pyrolysis–GLC for the identification of mushrooms. The "fingerprint" pyrogram should be compared with a preestablished catalog of pyrograms. The method is rapid, easy, and may be used with as little as 15 mg (dry weight) of material. It is thought that the method can be used to detect mushroom poisoning. Sixteen mushrooms of various species have been analyzed to illustrate the possibilities of the method.

In cases of intoxication by toxic mushrooms, symptomatology is not enough for identification. Gastroenteritis, even appearing after eating a dish of mushrooms, is not always related to the mushrooms. Identification needs to be ascertained by other means. Sometimes it is possible to find mushrooms which have not been cooked and identification might then proceed from determination of botanical characteristics. In other cases the only material

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