disadvantages of oven drying (*7*). However, cost per sample is higher than when conventional drying methods are used, and conditions of sample storage are critical, since enzyme inactivation is minimal. Storage conditions must be carefully controlled to avoid chemical changes if freeze-dried samples are to be stored for more than short periods of time (*7, 11*).

The results of this study supported the conclusions of Link (*9*) that no universal method for drying plant tissue can be relied on for accurate results, because plant tissues vary so widely in chemical and physical nature and in enzyme content and that individual experimentation is required to determine an appropriate drying temperature. It seems clear, then, that an investigator must consider carefully sampling and drying procedures in relation to the objectives of a study, especially if small amounts of easily altered constituents are to be determined.

Literature Cited

(1) Bath, I. H., *J. Sci. Food Agr.* **11,** 560 (1960).
(2) Bathurst, N. O., Allison, R. M., *New Zealand J. Sci. Tech.* **31(B),** 1, (1949).
(3) Collins, F. D., Shorland, F. B., *Ibid.*, **26(A),** 372 (1945).
(4) Davies, A. W., Evans, R. A., Evans, W. C., *J. Brit. Grassland Soc.* **3,** 153 (1948).
(5) Heinze, P. H., Murneek, A. E., Missouri Agr. Expt. Sta., Res. Bull. **314** (October 1940).
(6) Jones, D. I. H., *J. Sci. Food Agr.* **13,** 83 (1962).
(7) Laidlaw, R. A., Wylam, Clare B., *Ibid.*, **3,** 494 (1952).
(8) Lindahl, I., Davis, R. E., Sheperd, W. O., *Plant Physiol.* **24,** 285 (1949).
(9) Link, K. P., *J. Am. Chem. Soc.* **47,** 470 (1925).
(10) Melvin, J. F., Simpson, Beulah, *J. Sci. Food Agr.* **14,** 228 (1963).
(11) Perkins, H. J., *Can. J. Plant Sci.* **41,** 689 (1961).
(12) Pirie, N. W., in "Modern Methods of Plant Analysis," K. Paech, M. V. Tracey, eds., p. 50, Springer-Verlag, Berlin, 1956.
(13) Smith, Dale, *Crop Sci.* **2,** 75 (1962).
(14) Weinmann, H., *Plant Physiol.* **22,** 279 (1947).
(15) Wylam, C. B., *J. Sci. Food Agr.* **4,** 527 (1953).

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PEANUT FLOUR CONSTITUENTS

Cyclic Imino Acid Derivative from Peanut Flour

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An imino acid derivative, melting at 236–8° C., which has not been reported previously as a constituent of an edible product was isolated from peanut flour (0.02% yield) in the course of a chromatographic investigation of the nonprotein fraction. Its identity as *N*-methylhydroxyproline was proved by elemental analysis, molecular weight determination, and comparison of the infrared spectrum of the compound with that of *N*-methylhydroxyproline synthesized in the laboratory. The structure of the synthetic product was confirmed by elemental and functional group analysis as well as by nuclear magnetic resonance.

THE alcohol-soluble components of peanut flour are being investigated at this laboratory to assess their contribution not only to the biological value of peanut flour, but also to the formation of flavor and aroma in roasted peanut products. This paper describes the procedure used in the isolation of an imino acid derivative not previously reported as a constituent of an edible product and the methods used to establish its identity.

Experimental Procedure

Approximately 100 pounds of lightly roasted blanched peanuts served as the original material for this investigation. The fractionation procedure used to obtain the water-soluble fraction is identical to that used for the isolation of pinitol (*7*).

The column chromatographic separations were made using a 9-cm. i.d. × 40-cm. column, packed with Whatman cellulose powder. The powder was prewashed by extraction with methanol in a Soxhlet apparatus for 3 hours. It was applied to the column in a slurry and packed thoroughly by running the solvent, butanol–acetone–water (2:2:1), through the column. This solvent was used for all the column fractionations of material from peanuts. The synthetic product, *N*-methylhydroxypyroline, was prepared by the method of Leuchs and Felser (*8*) and separated from the reaction mixture by chromatography on the same column, using butanol–acetone–water (4:1:1) as the developing solvent. This fractionation was followed by paper chromatography on Whatman 3MM paper, using the same developing solvent. Only the fractions containing one component with an R_f identical to that of the natural crystals were combined.

Material containing the crystalline product from peanuts was obtained from a composite of 16 fractionations of the water-soluble fraction (*7*). This was divided into lots of about 4 grams each, which were rechromatographed separately using butanol–acetone–water (2:2:1) as the developing solvent. As the solvent evaporated, crystals formed in fractions which represented 0.02% of the peanut flour. The crystals were washed several times with methanol and the supernatant liquid was decanted.

When the melting behavior of this material was observed, it was noted that at 200–04° C. a sublimate separated as needle crystals, leaving plates in the residue. Consequently, the impure material was heated in a microsublimator to 180° C. under vacuum to separate this sublimate, which constituted ap-

proximately 1% of the crystalline mixture. The discolored residue was dissolved in a few milliliters of water and filtered through charcoal. Crystals formed as the water evaporated. About 50 mg. of pure material were accumulated by repeating this procedure several times. The entire sample was submitted for nuclear magnetic resonance analysis, and the material recovered after the analysis was used for the other qualitative and quantitative tests.

Melting points were determined in a sealed tube on a Büchi apparatus, and the microsublimator was made according to a model illustrated by Cheronis and Entrikin (*3*). Optical rotation was measured on an ETL-NPL automatic polarimeter using the mercury 5461-A. line. The nuclear magnetic resonance spectra were obtained in deuterium oxide, and the infrared by the potassium bromide disk method. Two tests for amines were carried out according to Feigl (*4*)—the fusion with fluorescein chloride, and the thiocyanate test—and the Hinsberg test for tertiary amines was performed by the method of Walsh and Merritt (*12*). The molecular weight was determined by the differential vapor pressure osmometer method. C, H, O, N, methoxyl, and *N*-methyl determinations were made by a commercial firm. In all cases standard reagent grade solvents were used with no further purification.

Table I. R_f Values

Solvent	Proline	OH-Proline	Peanut Acid Crystals	N-methyl-hydroxy-proline	4-Methyl-hydroxy-proline
1-Butanol–acetone–water, 4:1:1	0.15	0.08	0.13	0.13	0.11
1-Butanol–water–acetic acid, 6:3:1 (organic layer)	0.21	0.11	0.16	0.16	0.14

Table II. Confirmation of Identification

Physical and Chemical Properties	Peanut Acid Crystals	Synthesized N-methylhydroxyproline
Melting point, °C.	236–238	236–238
Solubility	Sol. water	Sol. water
	Sl. sol methanol	Sl. sol methanol
	V. sl. sol ethanol	V. sl. sol ethanol
	Insol. ether	Insol. ether
Color with ninhydrin	None	None
Color with isatin	None	None
Tests for amines	Positive	Positive
Elemental analyses, %		
C	49.29	49.61
H	7.65	7.49
N	9.44	9.45
O	33.30	...
Group analyses, %		
	...[a]	Methoxyl, 0.0
	...[a]	*N*-methyl, 30.30
NMR	Band at 3.09 p.p.m.	Band at 3.09 p.p.m.
IR	Showed compounds identical	

[a] Insufficient sample for group analysis.

Results and Discussion

Records of the quantitative fractionation established that the unknown compound was isolated from a fraction representing 0.02% of the original 100 pounds of peanuts. On rapid heating the crystals melted at 236–38° C. (decomposition), with softening at 234° C. These were soluble in water, $[\alpha]_{5461\ A.}^{20°} -106.5$ (*c.*, 0.28 in H_2O) slightly soluble in methanol, very slightly soluble in ethanol, and insoluble in ether. Infrared analysis indicated the presence of an ionized carboxyl group as a band appeared at 6.1 microns. This band was lost and a new one appeared at 5.75 microns on the infrared spectrum prepared after treatment of the material with concentrated hydrochloric acid.

Although the crystalline material gave no indication of purple color with ninhydrin or blue with isatin, it was strongly positive to other tests for amines: fusion with fluorescein chloride, the thiocyanate test, and the Hinsberg test for tertiary amines. Therefore, although the material was neutral to litmus, the possibility of its being an amino- or imino-type acid was explored. A standard neutralization titration showed a typical titration curve, which indicated a pK_a value of 9.4. The equivalent weight calculated from this titration was 143.2. The molecular weight by the differential vapor pressure osmometer was 152.

Elemental Analyses. Found: C, 49.29; H, 7.65; N, 9.44; and O, 33.30. Calculated for $C_6H_{11}NO_3$: C, 49.64; H, 7.64; N, 9.66; and O, 33.09. Molecular weight, 145.2.

In 1954, Hulme (*6*) isolated from apples a $C_6H_{11}NO_3$ amino acid which was later proved to be 4-methylhydroxyproline (*7*). This same acid was later isolated from pears by Burroughs (*2*), who kindly supplied a sample. The infrared spectrum of this acid was similar to that determined for the peanut acid, but it showed that the two compounds were not identical; the difference appeared in the 6- to 7-micron range. The 6.4-micron band, often attributed to N-H grouping, is not present in the spectrum of the peanut acid. The possibility was considered that the acid from apples and pears was identical with that from peanuts, except that the amino acid from the pear has both the methyl and hydroxyl groups attached to the same carbon, and that from the peanut has an *N*-methyl group. This was corroborated by the positive test for tertiary amines and by the NMR spectrum. Comparison of this spectrum with standards indicated that the strong band at 3.09-p.p.m. chemical shift from TMS may be assigned to an *N*-methyl group.

R_f values obtained on paper chromatograms of proline, hydroxyproline, the peanut acid, synthetic *N*-methylhydroxyproline, and 4-methylhydroxyproline are given in Table I. All of these compounds were detected as yellow spots on a purple background after dipping the chromatograms in a cold saturated solution of potassium permanganate in a mixture of acetone and pyridine (*9*). Another set of chromatograms was exposed to iodine vapors, the spots marked, then after all the iodine had sublimed the chromatograms were dipped in either isatin or ninhydrin. However, only proline, hydroxyproline, and 4-methylhydroxyproline were detected with these reagents, since *N*-methylhydroxyproline, like the peanut acid, did not produce color with isatin or ninhydrin but was detected after exposure to iodine vapors alone. This lack of color development with isatin or ninhydrin may be explained on the basis of the *N*-methyl group. McCaldin (*9*) reports that the CH_2–CH_2–NH linkage is necessary for the formation of the indigo blue color with isatin. The purple color obtained with ninhydrin is given by all α-amino acids, β-amino acids, and primary and secondary aliphatic amines. Tertiary aliphatic amines, as well as all groups of aromatic amines, do not react with ninhydrin under the usual conditions (*4*). In the work reported in this paper, an extremely weak ninhydrin test was obtained when the chromatograms were treated using a modified method of Russell (*11*), who detected *N*-methylleucine in the presence of valine.

Identification of the compound as *N*-methylhydroxyproline was confirmed by the series of comparisons shown in Table II. Group analysis of the synthesized compound gave methoxyl, 0.0%; and *N*-methyl, 30.30%. Although there was evidence of decomposition during the determination of *N*-methyl, the fact that a value was obtained indicates the presence of this group.

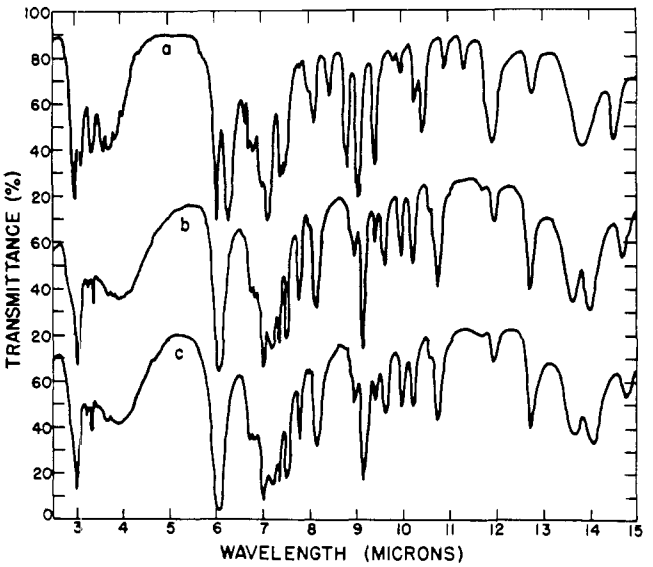

Figure 1. Infrared curves

a. 4-Hydroxymethylproline
b. Peanut acid crystals
c. *N*-methylhydroxyproline

Nuclear magnetic resonance data confirmed this. The melting point of the peanut acid crystals was not depressed on admixture of the synthetic compound and infrared analysis showed the two compounds to be identical (Figure 1).

No record of the isolation of *N*-methylhydroxyproline from an edible source has been found. However, it was isolated from the bark of *Croton gubougia* (*5*) in 1919, and more recently from the heartwood of *Afrormosia elata* (*10*). *N*-methylhydroxyproline was synthesized by Leuchs and Felser (*8*) by methylation of hydroxyproline. Since the synthetic material from hydroxy-L-proline prepared by their method was proved identical with the material isolated from peanuts, the identity of the peanut product as *N*-methylhydroxyproline is established. Morgan (*10*) reports that the 4-hydroxy-*N*-methyl-L-proline isolated from *Afrormosia elata* is levorotatory. As the hydroxyproline derivative from peanut flour is also strongly levorotatory, it may be concluded that this compound is also 4-hydroxy-*N*-methyl-L-proline.

Since this material has never been reported as a constituent of an edible product, its role in nutrition as well as in plant metabolism should be determined. The new compound could possibly serve as a methyl donor.

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Literature Cited

(1) Biemann, K., Deffner, G. G. J., *Nature* **191,** 380 (1961).
(2) Burroughs, L. F., Dalby, S., Kenner, G. W., Sheppard, R. C., *Ibid.*, **189,** 394 (1961).
(3) Cheronis, N. D., Entrikin, J. B., "Semimicro Qualitative Organic Analysis," 2nd ed., p. 74, Interscience, New York, 1960.
(4) Feigl, F., "Spot Tests," Vol. **II,** 4th ed., pp. 191, 208, 211, Elsevier, Amsterdam, 1954.
(5) Goodson, J. A., Clewer, H. W. B., *J. Chem. Soc.* **1919,** p. 923.
(6) Hulme, A. C., *Nature* **174,** 1055 (1954).
(7) Lee, L. S., Morris, N. J., J. AGR. FOOD CHEM. **11,** 321 (1963).
(8) Leuchs, H., Felser, H., *Ber. Chem. Ges.* **41,** 1726 (1908).
(9) McCaldin, D. J., *Can. J. Chem.* **38,** 1229 (1960).
(10) Morgan, J. W. W., *Chem. Ind. (London)* **1964,** p. 542.
(11) Russell, D. W., *J. Chromatog.* **4,** 251 (1960).
(12) Walsh, J. T., Merritt, C., Jr., *Anal. Chem.* **32,** 1378 (1960).

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FERTILIZER STABILITY

Decomposition of Mixed Fertilizers during Drying

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LARGE scale continuous processes and the use of high proportions of ammoniating solutions containing heat-sensitive salts have led to considerable difficulty in maintaining nitrogen guarantees during the manufacture of high analysis mixed fertilizers. A survey conducted by TVA (*6*) showed that the nitrogen content of 10–10–10 (10–4.37–8.30 NPK) and 12–12–12 (12–5.24–9.96) grade fertilizers was consistently low. About half of the samples were deficient in nitrogen to an extent that exceeded the tolerances set by state laws. In these cases the manufacturers gave assurances that the nitrogen input was at least equal to and usually more than that guaranteed.

As part of the same investigation, a pilot plant study on the manufacture of 12–12–12 (12–5.24–9.96) grade fertilizers disclosed that the loss of nitrogen, in a form that would explain why the losses could not be detected by scrubbing samples of exhaust gas, resulted from the decomposition of ammonium nitrate to form N_2O or N_2. Near the distributors of the pilot plant ammoniator there were localized areas of high acidity and observed temperatures as high as 260° F. Such conditions favor serious loss of nitrogen by decomposition of ammonium nitrate.

Stability of pure ammonium nitrate has been studied extensively (*3–5, 7*), and in the temperature range 210° to

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