Table I. Recovery of Patulin as the 2,4-DNPH Derivative from Apple Juice Products

Added patulin, ppb	Percent recovery: Fresh apple cider	Pasteurized apple juice[a]: Clear		Opalescent	
0				(86 ppb)[b]	(103 ppb)[b]
50	74	71	78	76	104
120	73	64	71	73	92
340	85	75	79	76	80

[a] Samples containing substances that interfered with the official analytical method by TLC. [b] Levels of patulin found in the unspiked samples; recoveries of added patulin were corrected accordingly.

acetate alone eluted too much 2,4-DNP from the column. Benzene, the solvent employed by Schwartz and Parks, did not have sufficient solvent power to dissolve and carry the derivative. Direct evaporation of the ethyl acetate solution was found to result in a flocculent precipitate and loss of added patulin. Formation of a precipitate and loss of patulin were eliminated by passing the ethyl acetate concentrate through the micro cation-exchange column to remove extraneous materials extracted by ethyl acetate. A small amount of precipitate was usually formed upon addition of methylene chloride to the concentrated ethyl acetate solution, but this did not interfere with the subsequent determination.

Methylene chloride, although relatively nonpolar, eluted a small amount of 2,4-DNP from the column, and it was necessary to remove this excess reagent by passing the solution through a second micro cation-exchange column in order to avoid loss of the patulin derivative in subsequent manipulations.

The recovery of patulin from 50 mL of ethyl acetate containing 120 ppb patulin averaged 83% in six samples with a standard deviation of 14.2%.

Patulin in a standard solution was added to apple juice and cider samples at three concentration levels, and the samples were analyzed immediately by this method. The percent recovery is given in Table I. Blank analyses were conducted simultaneously on all samples to ascertain whether there was a detectable amount of patulin in the original sample. Patulin was found in two original samples of apple juice, and the percent recoveries in the table are the corrected values.

This study demonstrates the utility of the method described herein for analyzing samples that are refractory to the usual methods of patulin analysis. The patulin 2,4-DNP is readily isolated by TLC and develops a characteristic wine-red color when treated with NaOH. Most of the other materials on the TLC plate developed yellow-brown spots, and all were separated from the patulin derivative. The recovery of patulin, 64–104%, was within the range reported for the Scott method, 60–132%. The lower limit of detection for the new procedure is approximately 250 ng of patulin/spot, or 50 ppb in a 50-mL sample of apple juice or cider.

The ultraviolet absorbance of the 2,4-DNPH anions formed by adding alcoholic KOH to the derivative solution is often recommended for quantitative determination of carbonyl compounds (Parsons, 1966). This could not be used for quantitating the patulin 2,4-DNPH because the derivative was unstable in base, consistent with the instability of the original compound to base (Nauta et al., 1946). However, a characteristic absorption maximum at 480 nm was obtained by adding base to the patulin derivative, and this test could be used qualitatively as an additional means of confirming the identity of the derivative.

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Phytosterols in Some Tropical Tubers

Sterols were isolated from nine tropical tuber plants and the composition determined by TLC, GC, and GC–MS techniques. Cholesterol, campesterol, stigmasterol, and β-sitosterol were found to be the major sterols accumulated in these storage organs. Trace amounts of fucosterol were identified by GC–MS. β-Sitosterol was usually predominant although the pattern of distribution was irregular.

A number of herbaceous plants are cultivated in the tropics for food. These plants often have an enlarged rootstock, consisting mainly of starch, which acts as the storage organ (tuber) and is the part of the plant normally eaten. Although lipid constitutes less than 1% dry weight of tubers, its main constituents have a physiological role associated with the structure and function of membranes. The few reports (Lepage, 1968; Galliard, 1968; Walter et

Table I. Relative Percentages of Sterols in Some Tropical Tubers

Tuber plant	Quantity of sterol (mg) from 500 g (wet wt) of tuber	Relative percentage of sterols[a]			
		Cholesterol	Campesterol	Stigmasterol	β-Sitosterol
Alocasia macrorrhiza	82.8	0.1	24.0	11.9	64.0
Colocasia esculenta	117.8	2.7	8.6	28.1	60.6
Xanthosoma sagittifolium	56.6	0.5	17.5	9.0	73.0
Ipomoea batatas	75.2		28.5	11.0	60.5
Dioscorea alata	42.4	0.5	20.0	6.0	73.5
Dioscorea bulbifera	94.6	2.2	22.6	46.7	28.5
Dioscorea rotundata	79.3	4.1	0.1	32.0	63.8
Dioscorea cayenensis	83.8	3.5	0.1	30.0	66.4
Dioscorea dumetorum	119.4	9.7	24.9	49.7	15.7

[a] Percentages were determined by measuring GLC peak areas. Standard error ca. 5% (two experiments).

al., 1971; Hudson and Ogunsua, 1974) on lipid composition in tuber plants have been on the potato and these merely mentioned the presence of free sterols, sterol esters, steryl glucosides, and acylated steryl glucosides. In most cases, the sterols were not isolated and characterized. Studies on the physiological significance of sterol metabolism in the development and maintenance of plant tubers have recently been initiated in our laboratories. The metabolic aspects of this work demanded a knowledge of the identity, composition, and amounts of the different sterols present in the tubers. The purpose of this communication is to report the results obtained for nine tropical tubers.

MATERIALS AND METHODS

All the tubers were grown by local farmers in the Bendel State of Nigeria and were collected immediately after harvest in May 1976. Healthy tubers were selected and only varieties of unequivocal identity were utilized. The tubers were cleaned, peeled, and weighed. Each (500 g) was homogenized with water in a Waring blender at room temperature. This was immediately hydrolyzed by heating for 2 h under reflux with 2 N hydrochloric acid, using 10 mL/g of plant material. After cooling and filtering the mixture, the acid-insoluble residue was washed with water, before neutralization with 10 mL/g of 5% (w/v) ammonia solution. When it had drained, the insoluble residue was dried in an oven at 60 °C for 16 h before it was extracted in a Soxhlet with light petroleum 40/60 for 24 h. The solvent was removed on a rotatory vacuum evaporator and the oily residue was chromatographed on a column of silica gel M.F.C. (Hopkin and Williams, 40 g/g of extract) packed in hexane. Elution with hexane–ethyl acetate (9:1) removed nonpolar hydrocarbon material. Further elution with hexane–ethyl acetate (9:2) afforded the sterols which were estimated gravimetrically and spectrophotometrically (Williams and Goodwin, 1965). The sterols were chromatographed on thin layers of silica gel G (Merck F_{254}) with chloroform–acetone (4:1) as solvent. The sterols were acetylated and separated on silver nitrate impregnated plates by the method of Nordby and Nagy (1973). Authentic sterol standards were included in the TLC plates. The sterols were further analyzed by GLC on glass columns packed with 1% Dexsil-300 and 3% SE-30 on Gas-Chrom Q 80–100 mesh and operated at 265 °C. GC–MS on the sterol mixtures was carried out on AE[1] MS 30 fitted with a Pye series 104 gas chromatograph using the 1% Dexsil column at 265 °C.

RESULTS AND DISCUSSION

The sterols identified in these tubers (Table I) are similar to those reported for a large variety of higher plants (Goad and Goodwin, 1972). GC–MS on eight of the sterol mixtures showed traces of fucosterol and an unidentified sterol whose molecular ion was at 396 but exhibited a fragmentation pattern not comparable with common phytosterols (Knights, 1967; Knights and Brooks, 1969). Cholesterol, fucosterol, and the unidentified sterol were absent in *Ipomoea batatas*. Cycloartenol and other 4,4-dimethyl sterols identified in potato tubers (Bae and Mercer, 1970; Kusano et al., 1973; Hartmann and Benveniste, 1974) were undetected in all tubers investigated. Since these 4,4-dimethyl sterols are biosynthetic intermediates, their absence may be due to the physiological state of the tubers analyzed.

Grunwald (1971) studied the effect of sterols on the permeability of red beet and barley root slices. He found that cholesterol, stigmasterol, and sitosterol greatly reduced membrane permeability whereas ergosterol increased cell permeability, indicating that membrane stability effects are very specific with respect to the configuration of the sterols present. It is therefore possible to understand the choice of sterols accumulated in these tubers which require reinforced membranes in view of their natural environment.

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