Acidic Indoles in Cold-Stored McIntosh Apples

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Indole-3-acetic acid was present in the core tissues of McIntosh apples which had been stored for 2 months at 53° F. and of apples stored at 32° F. and then removed from storage and ripened at 68° F. Only traces, as shown by bioassay, were present in the core tissues of apples stored for 2 months at 32° F. and in the tissues taken 3 weeks prior to harvest and at harvest time.

During the study of storage disorders such as core browning, scald, and rot in fruits subjected to long periods of low temperature storage, investigations were begun on the chemical composition of the fruit during storage and its possible bearing on the causes of such disorders.

Very little is known regarding the biochemistry of postharvest fruit and vegetables, especially during senescence. The production or liberation of growth substances, toxins, potential browning agents, and antifungal compounds during storage has received very little attention and requires further study.

Earlier work in this laboratory suggested a relation between the breaking of dormancy of the seeds in the fruit and the onset of "core flush" in McIntosh apples. The acidic indoles which play a role in the growth of higher plants were therefore of special interest.

The fruit auxins have received considerable attention from other workers, who have reported their presence on the basis of biological activity obtained from eluates of one-dimensional chromatograms. Most of these, with the exception of indole-3-acetic acid (IAA), remain to be characterized chemically. Luckwill (*5*) was the first to examine apple tissues for auxin activity and found three promoters and two inhibitors of stem elongation in leaves, seeds, and fruit of the developing apple. Luckwill and Powell (*6*) could find no evidence of the presence of IAA in these tissues. Reaction with the Ehrlich aldehyde reagent suggested that two of the promoters, Malus auxins 1 and 3, were indole acids. Recently von Raussendorf-Bargen (*9*) and the authors (*2*) independently identified IAA in ripe apple tissue.

This work was undertaken to follow the changes, if any, in IAA and other indole acids during cold storage of McIntosh apples. It confirms recent results of von Raussendorf-Bargen (*9*), who worked with other varieties of apples.

Materials and Methods

Material for this study of the distribution of acidic indoles in the core, cortex, and seeds of McIntosh apples was obtained from fruit picked 3 weeks prior to the regular harvest date, picked at harvest time, and after storage at 53° and 32° F. Apples removed from 32° F. storage and ripened at 68° F. under high humidity conditions provided additional material for core tissue extracts.

Chromatographically pure IAA for comparison purposes was obtained from commercial sources. Indole-3-acrylic acid was synthesized as described by Durkee and Sirois (*3*).

Extraction. Peroxide-Free Ether Extract. To obtain the diffusible acidic indoles with a minimum of oxidation, slices of core or cortex tissue (200 grams) were immersed in a solution of thiourea (0.15%). Excess thiourea solution was quickly removed by drainage and tissues were held in the cold (−1° C.) for several hours, then covered with peroxide-free diethyl ether, previously cooled to −12° C., and the whole was allowed to stand overnight at −1° C. The tissues showed no discoloration the next morning and the wet ether extract was separated from the tissues by decantation and fractionated to yield an acid fraction (*5*).

Twenty-gram quantities of seeds were extracted in the same manner but the thiourea dip was omitted. The dried extracts were stored at −12° C. for later analysis.

Boiling Ethanol Extract. To ensure that indole acids were not liberated from other compounds enzymatically during the extraction, the tissues were killed by suspending in boiling 85% ethanol. After homogenization for 3 minutes in a Waring Blendor, the filtered extract was evaporated in vacuo to the aqueous phase, extracted with peroxide-free diethyl ether, and fractionated (*5*) to yield a fraction for chromatography.

Chromatography. The entire fraction of each extract was spotted on Whatman No. 1 paper, which was developed in the solvent overnight (16 hours) using the ascending technique. Solvent fronts were allowed to move 10 inches, a distance which gave good separation of the acids. The air-dried papers were examined under ultraviolet light (SW) and then sprayed with the appropriate reagents. Substances in the extracts were identified by comparison with authentic compounds and co-chromatography in all cases.

Solvents. A. 2-Propanol–ammonia-water (8:1:1)

B. 1-Butanol–acetic acid–water (4:1:1)

C. 2-Propanol–water (4:1).

Color Reagents. Indoles were detected according to the methods outlined by Durkee and Sirois (*3*). Further confirmation was obtained using the Salkowski reagent (*1*).

Bioassay. The biological activity of eluates from paper chromatograms was measured by the pea stem elongation test based on the research of Christiansen and Thimann (*7*).

Results and Discussion

The R_f values and color reactions for the indole compounds found generally in all core and cortex tissues from McIntosh apples are summarized in Table I. Evidence of simple indole acids in seed extracts was rarely observed. Occasionally a spot at R_f 0.17 (2-propanol–ammonia-water) was found corresponding approximately to a similar spot, designated as compound A in our core and cortex tissues extracts.

Table I. Indole Acids in Ether Extract (Apple Fruit)

Compounds[a]	R_f Solvent[b] A	B	C	Color Tests: Ehrlich	DMCA[c]	Salk
IAA	0.45	0.88	0.50	Purple	Blue	Pink
Compound A	0.17	0.55	0.26	Purple	Blue	—
Compound B	0.42	—	—	Green	Blue	
Phloridzin	0.33	0.65	0.80	Pink	Purple	—
p-Aminobenzoic acid	0.25	0.85	0.70	Yellow	Purple	—

[a] IAA, phloridzin, and *p*-aminobenzoic acid identified by co-chromatography and comparison with authentic compounds. Aromatic compounds included because they react with reagents used.
[b] Solvents A, B, and C (see text).
[c] DMCA (*p*-dimethylaminocinnamaldehyde).

Indole-3-acetic acid was identified in ripe apples after 2 months' storage at 53° F. and in apples taken from 32° F. storage and ripened for several days at 68° F. under high humidity conditions. However, bioassay suggests that trace quantities of IAA (below 0.5 μg. per 200 grams of fresh tissue) are present in McIntosh apples prior to harvest, at harvest, and during low temperature storage (32° F.) but these levels remain chemically nondetectable, the limits of detection under our conditions being 0.5 μg. on paper chromatograms (Tables II and III).

IAA was detected by color reactions produced by spraying with three chromogenic sprays (Table I) and chromatography in three solvents. Further confirmation was obtained through cochromatography and bioassay, using the pea stem test after elution from papers developed in two solvents (*2*).

IAA was found on chromatograms of both ethanol and ether extracts and was therefore not produced through enzymatic action during the ether extraction.

Two other substances found on chromatograms of the acid extracts reacted with both Ehrlich and DMCA reagents (Table I). Compound A occurred in most extracts but appeared to be in higher concentrations in the core tissue of 53° F. apples (Table II) than in the cortex. The reverse was true for 32° F. storage. The low solubility of this substance in ethanol and ether but high solubility in water suggested a hydrophilic complex, perhaps with an amino acid. von Raussendorf-Bargen (*9*) reported that malonyltryptophan, found in other plants by Good and Andreae (*4*), occurred in unhydrolyzed extracts of ripened apples. Compound A may be this substance, since it did not react with Salkowski reagent.

Compound B was found with IAA in 53° F. stored apples. It gave a green color with Ehrlich reagent, turning to turquoise after 3 hours. The R_f value in 2-propanol–ammonia-water and color reactions are identical with those of synthetic indole-3-acrylic acid. A substance having the same R_f value as indole-3-acrylic acid and occurring with IAA in ripe apples was reported by von Raussendorf-Bargen as 2-hydroxyindole-3-acetic acid (*9*). This may be identical with our compound B. Chromatograms developed in other acidic or neutral solvents failed to reveal this substance, and it may be considered an artifact of ammoniacal chromatography which occurs only in tissue from apples after storage at 53° F., the conditions under which IAA is also detected. von Raussendorf-Bargen (*9*) considered it to be derived from IAA during extraction.

Two other substances, not indole compounds, were detected in the core tissues of McIntosh apples by their reaction with Ehrlich and DMCA reagents (Table I) and were tentatively identified as *p*-aminobenzoic acid and phloridzin.

Since IAA was not detected and other indoles were infrequently detected in apple seeds, there is some doubt as to the importance of this hormone in the dormancy-breaking mechanism. Nitsch (*8*) found two substances in apple seed endosperm which exhibited the properties of a gibberellin and von Raussendorf-Bargen and others (*9*) have suggested that the Malus auxin II of Luckwill (*5*) may be a gibberellin. It is possible that gibberellins are the important factors in seed germination processes.

Our results agree with those of von Raussendorf-Bargen (*9*), in that there is an increase in free IAA in ripened apples but the concentration of this auxin at harvest time or before is at a very low level and remains so when ripening is retarded by storage at 32° F. Because of these very low levels, Luckwill (*5*) was probably unable to detect IAA in developing fruit through use of the Ehrlich reagent.

From the evidence so far obtained, it would appear that core flush occurs in the tissues containing low levels of IAA and other acidic indoles. To establish any real biochemical relation between seed germination and core flush, further research on other apple auxins—e.g., gibberellins—and inhibitors (phenols, etc.) is necessary.

Table II. Chemically Detectable Indole Acids of Core Tissues of McIntosh Apples under Different Storage Conditions[a]

Compounds	3 to 4 Weeks before Harvest	Harvest	53° F. Storage, 2 Months	32° F. Storage, 2 Months	Ripened at 68° F.
IAA	−	−	++	−	++
Compound A	+	+	++(+)	+(++)	+
Compound B	−	−	+	−	+

[a] + Relative amounts of compounds as estimated by spot size.
( ) Relative differences for cortex extracts.

Table III. Chromogenic and Biological Tests for IAA in Core Tissue of McIntosh Apples

Development and Storage	Ehrlich Test (Chromatogram)	Bioassay, Pea Stem Elongation, Mm. Control	0.5 μg. IAA	Extract 200 g. eluate[a] (R_f 0.45)
3 Wk. prior to harvest	−	1.213 ± 0.185[b] (100)[c]	2.450 ± 0.350 (202)	1.750 ± 0.186 (144)
Harvest	−	1.525 ± 0.146 (100)	3.475 ± 0.156 (228)	1.950 ± 0.143 (128)
32° F. storage				
Nov. 1961	−	2.125 ± 0.136 (100)	4.250 ± 0.271 (200)	2.200 ± 0.153 (103)
Jan. 1962	−	1.875 ± 0.124 (100)	4.275 ± 0.225 (228)	2.025 ± 0.115 (140)
53° F. storage				
Nov. 1961	+	1.175 ± 0.154 (10)	2.625 ± 0.119 (224)	2.850 ± 0.230 (242)
Jan. 1962	+	1.300 ± 0.073 (100)	3.050 ± 0.223 (235)	2.900 ± 0.269 (223)

[a] 2-Propanol–ammonia-water (8:1:1).
[b] Standard error based on 9 degrees of freedom.
[c] ( ) Per cent elongation over controls.

Acknowledgment

The authors thank Peter Thivierge and Alice Savage for technical assistance in chromatography and bioassay procedures used throughout this investigation and also the Genetics and Plant Breeding Institute which kindly supplied the plant material for this study.

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Received for review May 21, 1964. Accepted November 27, 1964. Contribution from Food Research Institute, Canada Department of Agriculture.