Der Nachweis und die Identifikation der einzelnen Verbindungen erfolgte auf papierchromatographischem Wege. Die Nachweisreaktionen und die R_f-Werte für das Lösungsmittel Ac/H_2O (15:85) sind in Tabelle 1 wiedergegeben. Als weitere Lösungsmittel wurden benutzt: $Bu/Ac/H_2O$ (3:2:95), Aceton/Ameisensre./H_2O (5:15:80), Bu/ges. 1,5 n NH_3, 20%iges KCl.

Tabelle 1. *Nachweisreaktionen von Phlorizin und seinen Abbauprodukten*

Verbindungen	R_f-Werte Ac/H_2O 15:85	Diazot. Sulfanilsäure	Diazot. Nitranilin	Fluoreszenz mit Ammoniak
Phlorizin	0,56	gelb	gelb-braun	—
Phloretin	0,28	gelb	gelb-braun	—
p-Oxyhydrozimtsäure	0,81	rot	dunkel-violett	—
Phloroglucin	0,65	gelb	gelb-braun	blau
p-Oxybenzoesäure	0,70	gelb	rot	—

Auch die Zwischenprodukte werden je nach den Versuchsbedingungen mehr oder weniger schnell weiter abgebaut und verschwinden schließlich ganz. Unter natürlichen Bedingungen wird Phlorizin jedoch dauernd aus absterbenden und zerfallenden Wurzelrückständen nachgeliefert, so daß wir über einen langen Zeitraum auch mit einer Anwesenheit der Abbauprodukte im Boden rechnen müssen.

Weitere Untersuchungen über den Einfluß der identifizierten Verbindungen auf das Wachstum von Apfelsämlingen sind eingeleitet, hierüber wird an anderer Stelle ausführlich berichtet.

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Changes in Sugars and Nitrogenous Compounds of Tree Barks from Summer to Winter

The causes of the increases in cold hardiness of plants with the onset of colder weather in autumn has long been sought in changes in extractable chemical compounds. Two of the most promising compounds have been sugars and proteins. However, it seems important to analyze for individual nitrogenous substances rather than haphazard mixed groups of them[1]). This is probably also true of sugars, since oligosaccharides may increase while monosaccharides remain about the same from summer to winter[2]). This report briefly summarizes determinations of nitrogenous and sugar constituents in a number of barks of mature and exotic trees growing wild in Westchester county, New York.

Sugars were extracted as previously described[2]), except that the bark material (only inner bark from the cambium out to the first unhomogeneous layer, 4 ft. above ground) was first Waring blended with 180 ml. of 80% ethanol, filtered, and the solid material Soxhlet-extracted with a fresh quantity of 80% ethanol for 18 hrs. The two extracts were then combined and evaporated at 20 to 30° C. by an air stream to 15 ml. A comparison of some typical summer (August) results with those for winter (December) is shown in table 1. The most remarkable thing is the increase in tri- and tetrasaccharides, apparently raffinose and stachyose in most cases.

Nitrogenous compounds were extracted from the same sort of inner bark material. This was chopped into small pieces about 1 cm. on a side and extracted for 18 hours with 180 ml. of 80% ethanol in a Soxhlet apparatus. The water-alcoholic extract was then evaporated to 5 ml. and chromatogrammed for amino acids and amides using n-butanol, water, acetic acid, 4:5:1 (v:v:v) in the descending direction, then drying the paper, spraying with the electrophoretic solvent (M/4 CH_3COOH) and placing in the electrophoresis apparatus at 2° C. for 3 hours using 400 V and 6 milliamps. The paper was dried and sprayed with 0·1% ninhydrin in water-saturated butanol. The solid material in the thimble of the Soxhlet extractor was dried 3 hours at 60° C. and total "protein" nitrogen determined by the standard macrochemical Kjeldahl technique.

Table 1. *Sugar in tree barks of species shown as per cent X 10 of fresh weight.*

tr = trace; 1 = 0·1 to 0·2; 5 = 0·3 to 0·6; 10 = 0·7 to 1·2; 20 = 1·3 to 2·7; 30 = 2·8 to 3·6 *). I Stachyose, II Raffinose, III Melezitose, IV Sucrose, V Glucose, VI Fructose.

	I	II	III	IV	V	VI
August 1957						
Pinus strobus		tr		5		
Acer platanoides				5		
Betula lenta				5		5
Robinia pseudoacacia				5		
Ailanthus altissima		1		10		
Cornus florida		tr	tr	10		5
Fraxinus americana	5	1		5		
Quercus coccinea				5	1	1
Carya ovata		1		5		1
December 1957						
Pinus strobus	tr	5		5	5	5
Acer platanoides	5	5		20	1	5
Betula lenta	5	1		5	10	10
Robinia pseudoacacia	5	5		30	1	5
Ailanthus altissima	5	5		30	5	5
Cornus florida	5	20	10	20	5	10
Fraxinus americana	10	10		20	5	5
Quercus coccinea		5		20	5	5
Carya ovata		1		10	10	20

*) More detailed data on these and other species will be published elsewhere.

In *Robinia* the following free compounds declined from summer to winter: serine, threonine, tyrosine, leucine, phenylalanine, valine, alanine, cystine (?), and glutamic acid. Proline increased markedly while aspartic acid remained about the same. These results are somewhat similar to those of ZIEGLER[3]). A compound appearing in the position for glutamine but giving a sky blue color with ninhydrin became stronger in winter.

Water soluble protein from *Robinia* was hydrolyzed and then chromatogrammed by the above 2-dimensional procedure. The inner bark was homogenized in water, filtered, centrifuged, and the supernatant decanted and precipitated with trichloroacetic acid and recentrifuged. The latter centrifugate was hydrolyzed according to LINSKENS[4]). No outstanding changes in the amino acid or amide spots were observed from summer to winter. Two sugars: desoxyribose, and sucrose (?) also appeared but did not change in intensity with the season.

Table 2. *Per cent protein nitrogen in inner barks of species shown based on dry weight*

	August	October	December
Pinus strobus	0·49	0·50	0·82
Acer platanoides		0·50	0·87
Betula lenta		0·29	0·53
Betula populifolia	0·43	0·49	0·62
Robinia pseudoacacia	2·38	2·33	2·49
Ailanthus altissima		0·84	0·86
Cornus florida		0·50	0·92
Quercus montana	0·68	0·88	1·01
Quercus alba	0·38	0·44	0·63
Prunus serotina	0·52	0·75	0·81
Liriodendron tulipifera	0·53	0·50	0·72
Fagus grandifolia	0·43	0·54	0·54

Total "protein" nitrogen showed definite increases in all species tested from summer into winter (table 2). It thus appears that both proteins and sugars increase in autumn in all these tree barks and many free amino acids and amides go possibly into the production of new protein. The increase in sugars is probably accounted for by the hydrolysis of starch and this appears to be temperature controlled.

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