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PHENOLIC CONSTITUENTS OF HEALTHY AND WOUND TISSUES IN THE GIANT CACTUS (CARNEGIEA GIGANTEA)*

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Abstract—When the saguaro cactus is wounded, bacterial infection and necrosis frequently follows, unless a protective callus layer is formed at the site of injury. This callus tissue is highly ligniferous and contains numerous phenols. The healthy cortical tissue (pulp) is mainly polysaccharide in nature, containing dopamine as the principal phenolic constituent. Dopamine concentrations increase markedly at the site of wounding.

INTRODUCTION

THE saguaro cactus (*Carnegiea gigantea*, Engelm. Britt. and Rose) is a plant unique to the Sonoran desert. Its range within the United States is essentially limited to the southwestern portion of Arizona, with the greatest concentration occurring in the general vicinity of Tucson. The saguaro may attain a maximum height of 25–35 ft[1] and may occasionally live to be 200 years old.[2] Frequently mature plants are seen with soft discolored areas that often become the site of a black exudate. Lightle *et al.*[3] first reported that this soft rotting condition was caused by the bacterium *Erwinia carnegieana* (Strandrig) and proposed the term "bacterial necrosis" for the rotting process. Recent findings[4] indicate that these bacteria may be disseminated by a number of insect vectors and that they may enter the cactus through holes in the epidermis, resulting from mechanical wounding (rocks, birds, wind, vandalism). In the vast majority of cases, the saguaro is able effectively to seal the hole against further penetration of the pathogen with a highly ligniferous wound (callus) tissue.[5]

The sequence of events which follows mechanical injury of the cortical tissue can be summarized as follows: (*a*) appearance of a red pigment, followed by blackening of the exposed incision; (*b*) rapid cell differentiation; (*c*) lignin formation concomitant with formation of polyphenols, waxes and other extractives, directly under the blackened material. The nature of the wound tissue appears to be multi-layered, with alternating sheets of lignified material and polysaccharides.

When the saguaro does not successfully form callus tissue, rotting occurs and a black exudate is excreted from the wounded site.

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[1] S. M. ALCORN and C. MAY, *Plant Disease Reptr* **46**, 156 (1962).

[2] N. L. BRITTON and J. N. ROSE, *The Cactaceae-II.* Carnegie Institute of Washington Publications, No. 248, 166 (1920).

[3] P. C. LIGHTLE, E. T. STRANDRIG and J. G. BROWN, *Phytopathology* **32**, 303 (1942).

[4] S. M. ALCORN, Personal Communication, University of Arizona, Tucson, Arizona.

[5] A. BOYLE, *Phytopathology* **39**, 1029 (1949).

The relatively large volume of easily accessible cortical tissue, and the sharp division between this tissue and the wound tissue make the saguaro an ideal model for an investigation of the mechanism of disease resistance and wound tissue formation in plants (and in particular, the role of lignification in this process) (Fig. 1).

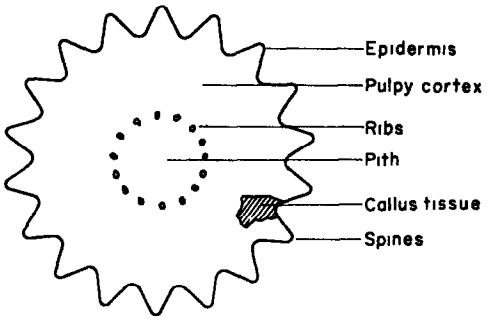


FIG. 1. CROSS SECTION OF SAGUARO STEM.

Previous studies[6,7] have indicated that the callus tissue contains 30% Klason lignin, 14% extractives in organic solvents and 53% holocellulose. This represents nearly a 50 per cent increase in the lignin content of the wound tissue over that of the normal healthy woody tissue (ribs). Nitrobenzene oxidation of the Klason lignin fraction revealed the presence of vanillin, *p*-hydroxybenzaldehyde and syringaldehyde in the ratio 6/3/2: this was identical to the ratio determined for the healthy woody tissue[7] (see Table 1), indicating that the lignin of the rib and callus tissues were of the same type. Similar ratios of these aldehydes were found in the extractives (Table 2). This is of interest, since the ribs and callus tissues are separated by the pulp, which is almost entirely polysaccharide and contains no lignin.

TABLE 1. ANALYSIS OF THE LIGNIN DEGRADATION PRODUCTS OF THE SAGUARO[7]

		Aldehydes in lignin % of lignin			
Tissue	Lignin*	Vanillin (I)	Syring-aldehyde (II)	*p*-Hydroxybenz-aldehyde (III)	Ratio (I):(II):(III)
Rib	21·90	0·66	0·30	0·20	1·0:0·44:0·30
Callus	30·40	1·20	0·50	0·40	1·0:0·43:0·33

* Results in per cent of oven-dry (105°) unextracted wood samples.

From these data, it was concluded that either injury or infection of the cortical tissue (pulp) causes metabolic changes, which lead to the rapid formation of lignin, phenols, melanin and other non-polysaccharide materials. With a view to elucidating the chemical nature of this metabolic process, we undertook an examination of the phenolic fraction of the healthy pulp, wounded pulp and callus tissue.

[6] J. W. BERRY, A. Ho and C. STEELINK, *J. Org. Chem.* **25**, 1267 (1960).
[7] C. STEELINK and J. W. BERRY, *Arid Lands Colloquia*, p. 39. The University of Arizona (1959–60).

TABLE 2. ANALYSIS OF THE HYDROLYSATE OF EXTRACTIVES FROM THE SAGUARO

Tissue	Extractives*	Aldehydes in the extractives % of extractives: Vanillin (I)	Syringaldehyde (II)	*p*-Hydroxybenzaldehyde (III)	Ratio (I):(II):(III)
Rib	2·80	0·13	0·05	0·03	1·0:0·38:0·23
Callus	10·50	0·07	0·02	0·01	1·0:0·29:0·14

* Results in per cent of oven-dry (105°) unextracted wood samples.

RESULTS

Samples of callus tissue were successively extracted with petroleum ether, ethyl ether and ethanol/benzene (1/2). The ethanol/benzene extract revealed the presence of 3,4-dihydroxybenzoic acid, vanillic acid and *p*-hydroxybenzoic acid (see Table 3). Trace amounts of *p*-coumaric acid and ferulic acid were also observed. The original ether fraction of the callus tissue was found to contain quercetin which was also found in the ethanol/benzene fraction. Its concentration was 0·1 per cent of the total callus, and is probably responsible for the yellow color of the callus. No quercetin was found in the light-yellow ribs (secondary xylem). In addition, two isoquinoline alkaloids have been reported to occur in the cactus: carnegine (I)[8] and 4-hydroxycarnegine (II).[9]

TABLE 3. R_f VALUES (×100) FOR PHENOLIC ACIDS EXTRACTED FROM CALLUS TISSUE OF SAGUARO CACTUS

Compound	Solvent* A	B	C	D	Color with DASA†
Compound (extracted from callus tissue)					
1	85	03	57	26	Purple
2	92	12	60	—	Orange
3	92	14	64	—	Yellow
Authentic compounds					
3,4-Dihydroxybenzoic acid	84	07	58	30	Purple
Vanillic acid	95	14	60	63	Orange
p-Hydroxybenzoic acid	94	18	65	49	Yellow

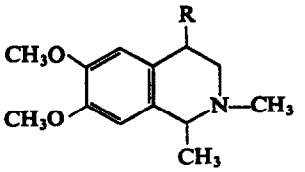
* A: Butanol/acetic acid/water (4/1/5) organic phase; B: butanol/2% ammonia, organic phase; C: 5% acetic acid; D: benzene/dioxane/acetic acid (90/25/4).
† DASA: diazotized sulfanilic acid.

In contrast to the callus extracts, the cortical tissue (pulp) contained very few phenolic components. Dopamine (3,4-dihydroxyphenylethylamine) was found to be the major phenol in the pulp* in concentrations around 1 per cent of the total pulp. A glycoside of

* This compound was observed chromatographically by Booth;[10] however, he was not able to characterize it.

[8] G. HEYL, *Arch. Pharm.* **266**, 668 (1928).
[9] J. E. HODGKINS, Personal communication, Texas Christian University, Ft. Worth, Texas.
[10] J. A. BOOTH, Ph.D. Thesis, University of Arizona, Tucson, Arizona (1964).

4-hydroxybenzoic acid was a minor constituent. This glycoside was also found in the black exudate of a diseased saguaro, which had not formed the callus tissue. Minute amounts of ferulic acid were also detected in the pulp.



(I) R=H; (II) R=OH

Cores taken from pulp areas adjacent to callus tissue were found to have significantly more dopamine than cores taken from healthy pulp portions (Table 4). The concentration of dopamine decreased when the core was exposed to air. However, when cores were placed in ascorbic acid solutions immediately after removal from the plant, the dopamine content (as measured by its absorption at 283 nm) was increased. This indicates that reduction of the oxidized dopamine (λ_{max}=253 nm) had occurred. To insure a uniform procedure for measuring maximum dopamine concentration, all subsequent cactus sections were immediately immersed in ascorbic acid solutions subsequent to their removal from the plant.

TABLE 4. CHANGES IN DOPAMINE CONCENTRATION OF SAGUARO CACTUS AS INFLUENCED BY WOUNDING

Sample	Treatment *	Sample number	% Dopamine (fresh wt.)	Absorption (nm) λ_{max}	Absorption (nm) λ_{min}	Color
Fresh	a	1	0·30	283	251	Yellow
Wounded	a	2	0·58	283 475	251 380	Red-Brown
Fresh	b	4	0·40			Yellow
Wounded	b	5	0·64			Maroon

* Treatment: a, fresh tissue extracted with 95% ethanol; b, fresh tissue extracted with 95% ethanol containing 0·5 g ascorbic acid/l.

A study of the changes in dopamine content after wounding was carried out in the following manner: A core was removed from the healthy pulp tissue of a 45 cm plant. This core contained 1·4% dopamine. After one hour's standing, the wounded area had turned black. A second core was taken adjacent to the first core; its dopamine content was 2·1 per cent. Subsequent cores were taken at 1 cm intervals away from the original core, at the same time as that for the second core. The results of the spectrophotometric analysis of these cores showed a gradual decrease in dopamine concentration to a value of 1·6 per cent. It was interesting that samples taken near the base of the plant (where a heavy callus layer always appears) revealed a high dopamine content.

When a saguaro is wounded or sliced, the exposed area first turns red, and then turns black. This sequence of color changes is reminiscent of the reaction of DOPA (3,4-dihydroxyphenylalanine) upon oxidation to melanin. In order to establish the nature of this reaction sequence, we oxidized dopamine in aqueous solution with tyrosinase and air, following the

course of oxidation by u.v. absorption spectroscopy (Fig. 2). The spectral data indicated that dopamine appeared to follow the same sequence as DOPA upon oxidation. Ascorbic acid was found to inhibit the oxidation completely. However, if ascorbic acid was added to the solution after it had turned red, the spectrum of the resulting decolorized solution was not identical to the spectrum of the original solution. This indicated a species which was different from the original dopamine. When the enzymatic oxidation was attempted in acid solutions at lower pH values than 3, no reaction occurred. If the acid oxidation was carried out with MnO_2, instead of tyrosinase, a yellow solution developed that had an u.v. absorption spectrum very similar to that of an orthobenzoquinone. A sample of pure dopamine in water exposed to air blackened very slowly; however, when a small amount of aqueous extract of the cactus pulp is added to this solution, blackening proceeds very rapidly.* No dopamine was found in the callus tissue.

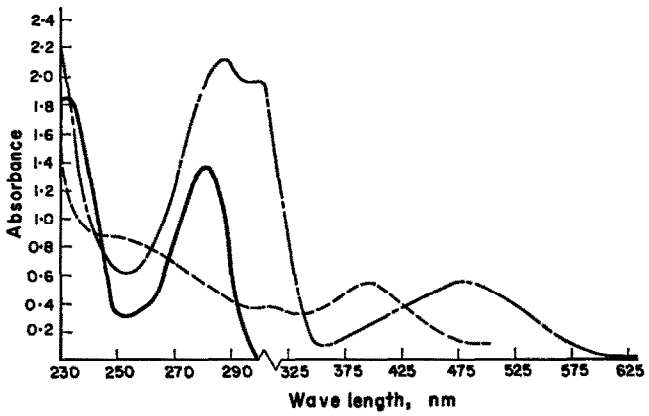


FIG. 2. ABSORPTION SPECTRA OF DOPAMINE DERIVATIVES.
Dopamine in water (————); dopamine in 10^{-3} M HCl, after oxidation with MnO_2 (– – – – –); dopamine in water, after oxidation with air and tyrosinase (— — — —).

In the course of this investigation, it was noted that the blackening of a wounded area was significantly greater if the injury was inflicted near the site of a previous wound (callus). A similar phenomenon has been recently observed for other plant species by Stahmann.[11]

DISCUSSION

The sequence of events which appear to follow injury of the cortical tissue can be summarized as follows: (*a*) increase of dopamine concentration, (*b*) enzymatic oxidation to melanin, (*c*) rapid cell differentiation and (*d*) lignin formation with concomitant formation of polyphenols, as well as waxes and other unexamined extractives. Spectral data indicate the dopamine is oxidized to the quinone (reversible), then to dopaminochrome (irreversible) and finally melanin (irreversible).

The evidence indicates that wound tissue formation in the saguaro is a result of a major stimulation of the shikimic acid pathway (the conversion of saccharides to aromatic compounds) as well as a result of the stimulation of the acetate pathway (leading to quercetin,

* Booth[10] noted a rapid increase in the respiration and an increase in polyphenoloxidase activity when saguaro seedlings were injured.

[11] B. CLARE, D. J. WEBER and M. A. STAHMANN, *Science* **153**, 62 (1966).

which has been reported as an abnormal metabolite of deseased plants[12]). In addition, the presence of previous wound tissue appears to stimulate increased production of dopamine in the surrounding healthy tissue.

EXPERIMENTAL

Equipment

Ultraviolet absorption spectra were obtained on a Cary 11 Recording Spectrophotometer. Chromatographic separations were carried out by (*a*) ascending techniques on thin-layer cellulose or silica plates or (*b*) descending techniques with Whatman No. 1 filter paper.

Materials

Mature saguaro plants were obtained through the courtesy of the Saguaro National Monument, Department of Interior, Tucson, Arizona. Seedlings were donated by Professor Stanley Alcorn of the Department of Plant Pathology, University of Arizona.

Methods

The identities of all phenolic compounds in the cactus extracts were established by co-chromatography with authentic samples. Further, the bands were eluted from the chromatograms with ethanol; the u.v. absorption spectra of these eluates were compared to the spectra of authentic samples. Characteristic shifts of the major peaks in the spectra with NaOH were used to confirm the identities of the compounds.

Hydrolysis of the ethanol extracts of the cactus was carried out by refluxing in 2 N HCl for 24 hr. After the ethanol was removed from the hydrolysate, the aqueous solution was neutralized to pH 7 and extracted with ether to remove phenols. The pH of the aqueous layer was then reduced to pH 4; the aqueous layer was then extracted with ether to remove aromatic acids. The ether extracts were chromatographed, as described above.

Quercetin was isolated from the ether extract of the callus tissue, converted to its penta-acetate and identified by mixed melting point with an authentic sample.

Dopamine was quantitatively determined by measuring the absorptivity of its solution at 283 nm. Chromatography of the dopamine solution extracted from the pulp revealed no compounds which interfered at this wavelength.

To determine the variation in concentration of dopamine with distance from the site of injury, cores were taken from the pulp, immediately extracted with cold ethanol containing ascorbic acid and powdered sugar (to remove traces of chlorophyll). After filtration, the absorptivity of aliquots of the solution were measured spectrophotometrically against suitable blanks.

The oxidation of dopamine was carried out in aqueous solution, and the resulting changes were followed spectrophotometrically. Enzymatic oxidation was effected by the addition of tyrosinase (or aqueous extracts of the cactus pulp) to the dopamine solution in the quartz spectrometer cell. Chemical oxidation at pH 3 was carried out with MnO_2; the mixture was centrifuged and the supernatant examined spectrophotometrically.

Acknowledgements—We wish to acknowledge the generous support of this work by the American Cancer Society Institutional Grant and the National Institutes of Health, Grant GM-12288.

[12] O. T. DIENER, *Ann. Rev. Phytopath.* **1**, 197 (1964).