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The Occurrence of δ-Tocopherylquinone in Higher Plants and Its Relation to Senescence[1]

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Abstract. δ-Tocopherylquinone (δ-TQ) content was determined in tobacco and yellow maple leaves, green ivy leaves and cactus tissues. It was found that the concentration of δ-TQ was highest in mature or senescent tissues, such as white tobacco leaves (0.02 μmole/g dry wt) while its detection was uncertain in young, green leaves from the apex of tobacco plants. Fractionation by centrifugation of senescent tobacco leaves showed that the osmiophilic globule fraction was enriched in δ-TQ. Electron microscope studies of young, mature and senescent tobacco tissues showed progressive changes in the size and number of osmiophilic globules. After chloroplast breakdown in senescent tobacco leaves, these globules became the predominant constituents of the organelle. δ-TQ which is associated with osmiophilic globules may play a role in the development of plants, particularly during senescence.

δ-Tocopherol[2] has been reported as a regular constituent of the familiar α-, γ,- and δ-tocopherol pattern in seed oils, such as soybean, linseed, arachis and others in contrast to the second predominant tocopherol pattern found in cereals and mushrooms which consists of α- and β-tocopherols and α- and β-tocotrienols (7, 8, 13). δ-Tocopherylquinone, an oxidation product of δ-tocopherol, has been reported in spinach chloroplasts in trace amounts (4).

We have found measurable amounts of δ-TQ primarily in senescent tissues—whitish basal tobacco leaves and fallen yellow maple leaves. It also occurs in green tissues of cactus and ivy leaves.

An understanding of the distribution of δ-TQ in plant tissues has gained importance since Whistance and Threlfall's (14) tracer studies in which they postulate that in the biosynthetic pathway of chloroplast lipoquinones δ-tocotrienol and δ-tocopherol are precursors to other tocotrienols and other tocopherols which differ from the δ-compounds by the degree of methylation. They found that α-tocopherol was converted to α-tocopherylquinone on the basis of specific activity data. Likewise, it is probable that oxidation of the other tocopherols gives rise to other tocopherolquinones. Evidence for this comes indirectly from the studies of Dilley *et al.* (6) who reported the occurrence of α-, β-, and γ-TQ in spinach chloroplasts and Dilley's (4) discovery of δ-TQ in spinach chloroplasts.

In this report we would like to demonstrate the occurrence of δ-TQ in 3 other higher plants—tobacco, maple leaves and cactus. Our data shows a correlation between the increase in concentration of this compound and an increase in chloroplast globule size during tobacco leaf senescence.

Materials and Methods

The plastoquinone, tocopherylquinone, and tocopherol content was determined in several species of plants by the method of Barr *et al.* (1) using whole leaves or various fractions obtained by centrifugation.

Tobacco and a barrel cactus were grown in the greenhouse. Tobacco leaves were harvested from plants approximately 3 feet tall. About 6 to 10 small, immature leaves from the apex, 5 to 10 basal senescent, whitish leaves and 6 to 10 green middle leaves from several plants were collected and pooled. Midribs were removed from leaves before grinding plant material in a Waring Blendor prior to extraction with isopropanol and heptane.

Yellow maple leaves from sugar maple, *Acer saccharum*, were gathered from the ground in early November and analyzed as above. Ivy leaves from ornamental plantings were collected while green in September.

All solvents and chemicals were treated as before (1). Standard δ-tocopherol was obtained from the Eastman Chemical Company.

Fractionations to obtain osmiophilic globules from white tobacco leaves by centrifugation were performed according to the method of Barr, Magree, and Crane (2). The gold chloride oxidation to

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[2] The following abbreviations were used: α-T, α-tocopherol; β-T, β-tocopherol; γ-T, γ-tocopherol; δ-T, δ-tocopherol; α-TQ, α-tocopherylquinone; β-TQ, β-tocopherylquinone; γ-TQ, γ-tocopherylquinone; δ-TQ, δ-tocopherylquinone; x_1 and x_2, unknown quinoidal compounds.

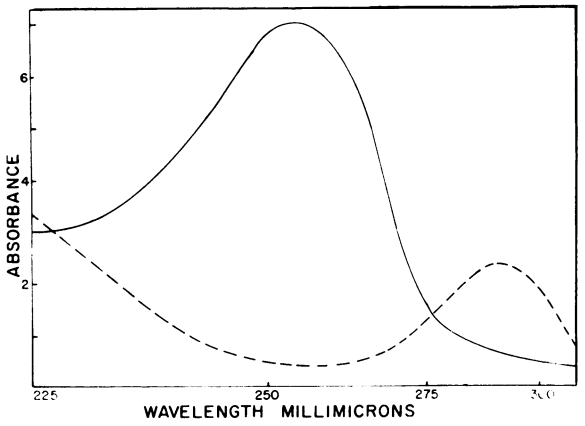


FIG. 1. The U. V. absorption spectrum of δ-tocopherylquinone in absolute ethanol. ——— line designates oxidized form with a maximum at 253 millimicrons; ------- reduced form after treatment with sodium borohydride.

convert δ-tocopherol to δ-TQ was carried out as recommended by Dilley and Crane (5). The gold chloride oxidation products of δ-tocopherol were separated on thin layer silica gel G-HR plates developed in chloroform-heptane (80:20) or benzene-methanol (98:2). The δ-TQ band was identified as a blue test spot by leucomethylene blue spray made up as previously described (1).

Unknowns x_1 and x_2 which were more polar than δ-TQ but gave blue spots with methylene blue spray like tocopherolquinones (table II) were otherwise uncharacterized by us. The amount of these unknowns was calculated from the difference in absorbancy between their oxidized and reduced forms between 250 and 270 mμ using the same extinction coefficient as for δ-TQ.

The U. V. absorption spectrum of δ-TQ purified by thin-layer chromatography with heptane-ethanol (95:5) is shown in Fig. 1. The oxidized λ_{max} is at 253 mμ. As with other tocopherolquinones, a reduced λ_{max} develops at 290 mμ upon reduction with potassium borohydride. Amounts of δ-TQ were calculated from oxidized minus reduced forms at 253 mμ arbitrarily using the same ϵ = 17.8 per ml as for α-TQ by Bucke and Hallway (3).

Tobacco leaf samples used for electron microscopy were minced into small fragments with a razor blade, fixed in 2% glutaraldehyde for 20 min, and post-fixed in 2% osmium tetroxide for 2 hr. The samples were dehydrated in a graded alcohol series and embedded in Epon as described by Luft (12). Sections were cut using a diamond knife and examined and photographed on a Phillips 200 electron microscope.

Results

Table I shows the distribution of chloroplast quinones in some higher plant tissues on the basis of dry weights or amounts of chlorophyll. It can be seen that this distribution follows 2 patterns: (1) the amount of plastoquinones found is greater than the amount of tocopherolquinones and (2) the total amount of chloroplast quinones increases in older leaves from the apex downward.

In table II the R_F values of δ-TQ are shown in 4 different solvent systems, as well as the R_F of other chloroplast lipophilic components. It can be

Table I. *Distribution of Chloroplast Quinones in Some Higher Plant Tissues*

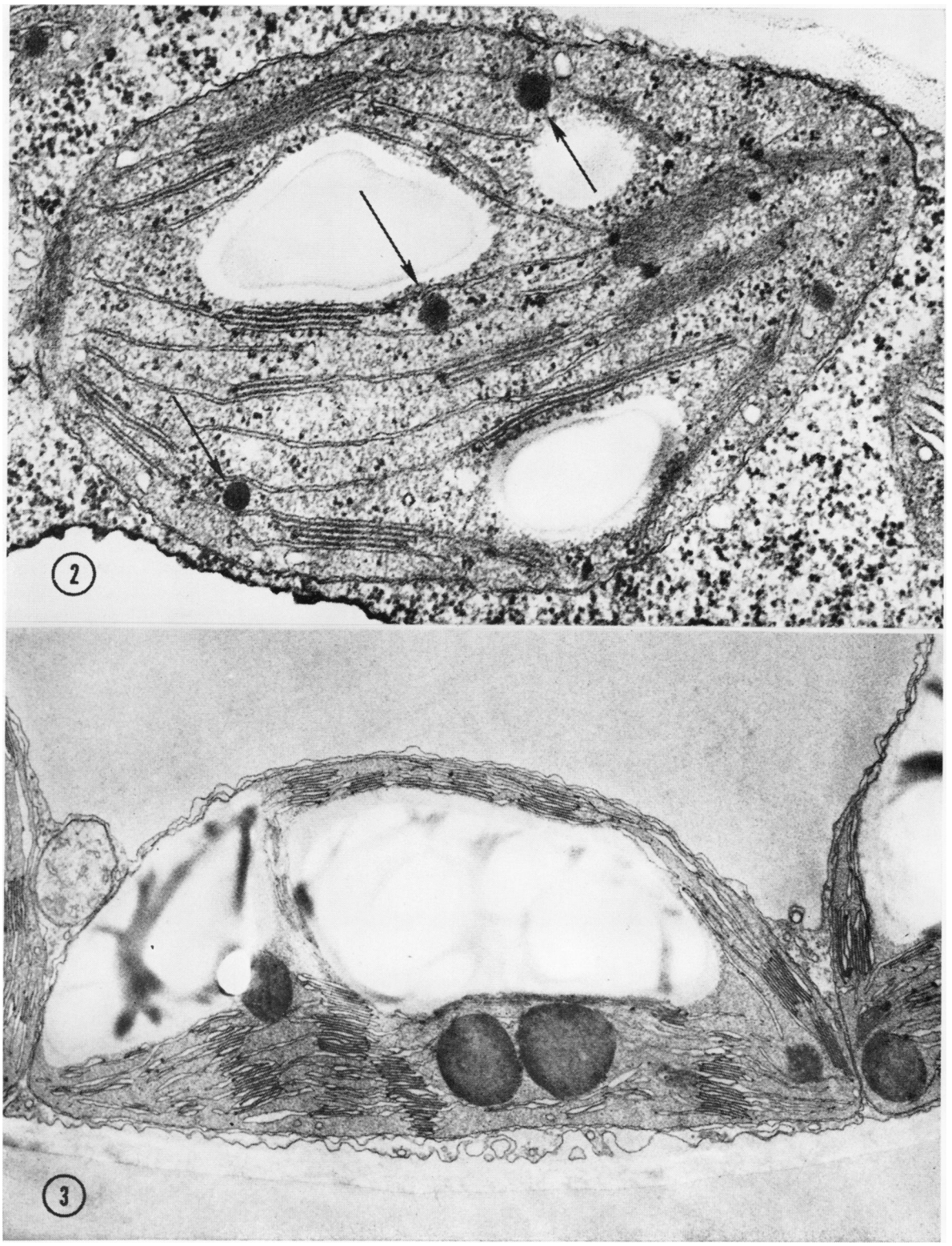
<0.01 μmole quinone indicates that it could not be detected by a spectrophotometric assay but was seen as a spot on a thin layer plate in comparison with a standard. <0.001 μmole quinone means that its detection was uncertain.

Tissue	Dry wt g	Total chlorophyll mg	Quinones[1]								
			PQA	PQB	PQC_{1-4}	PQC_{5-6}	α-TQ	γ-TQ	δ-TQ	x_1	x_2
						μmole/g dry weight					
Tobacco leaves-apex	22.5	45.8	0.43	0.04	0.13	0.01	0.04	<0.001	<0.001	0	0
Tobacco leaves-middle	74.0	128.0	0.44	0.03	0.21	0.09	0.01	0.02	0.02	0	0
Tobacco leaves-senescent	46.6	9.4	0.75	0.03	0.27	0.16	0.02	0.03	0.02	0.02	0.01
Maple leaves-yellow	128.5	...	0.08	<0.001	0.03	0.01	0.01	<0.01	<0.01	0	0
Cactus	52.0	...	0.05	0.03	0.06	0.01	0.01	0.01	0.01	0.02	0.02

[1]/ Abbreviations as indicated on the title page.

FIG. 2. (facing page) Developing chloroplast from a young, expanding apical leaf. Arrows indicate characteristic small osmophillic globules seen in these organelles. × 64,000.

FIG. 3. (facing page) A fully developed chloroplast from an expanded green leaf. Large starch granules and globules were almost always observed. × 27,000.



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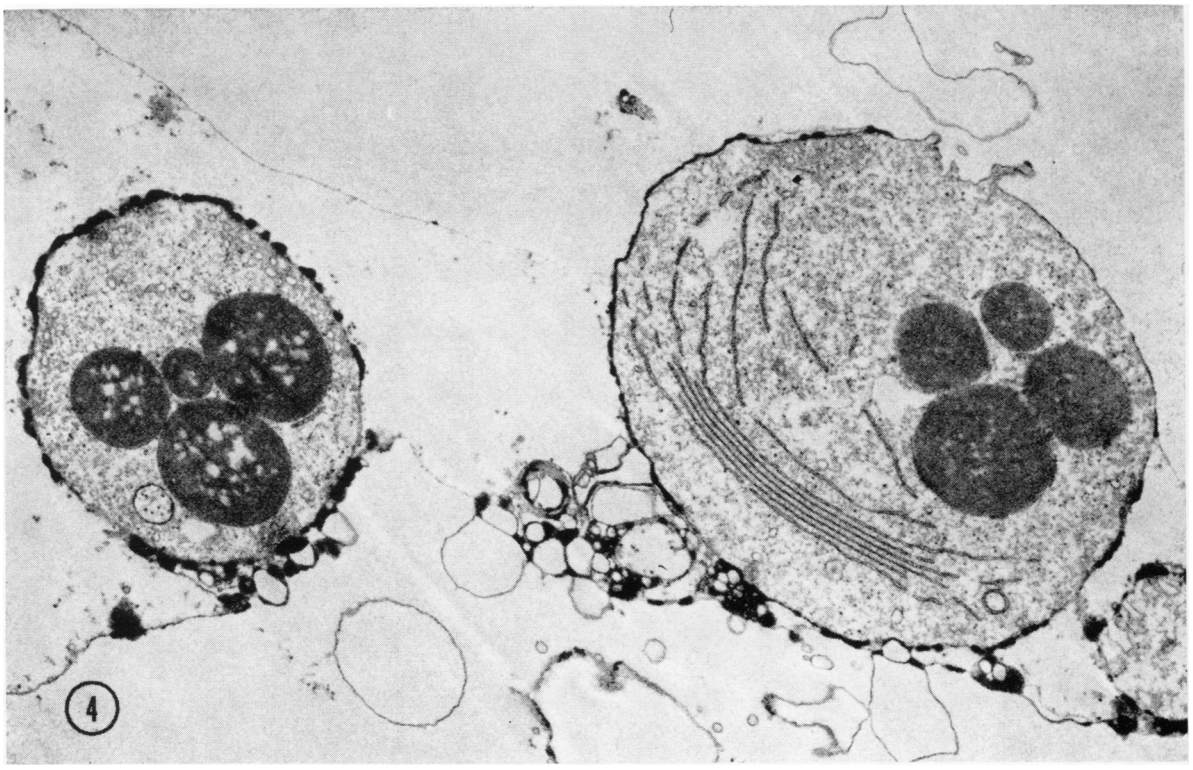

FIG. 4. Chloroplasts from senescent, yellow leaves leaves showing the lack of normal lamellar structure. Numerous, prominent glcbules were present in this tissue. × 26,000.

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Table II. *Chromatographic Properties of Chloroplast Components in 4 Different Solvent Systems*

Quinone	System I[1]	System II[2]	System III[3]	System IV[4]
	R_F	R_F	R_F	R_F
δ-Tocopherylquinone	0.21	0.45	0.45	0.50
x_1	0.17	0.37	0.34	0.49
x_2	0.14	0.28	0.24	0.54
Plastoquinone A 45	0.90	0.87	0.95	0.16
Plastoquinone A 20	0.82	0.87	0.95	0.31
Plastoquinone A 10	0.75	0.83	0.91	0.46
Plastoquinone C_2	0.52	0.63	0.79	0.40
Plastoquinone C_5	0.36	0.57	0.77	0.44
Plastoquinone C_6	0.38	0.58	0.70	0.45
Plastoquinone B_2	0.81	0.57	1.00	0.41
α-Tocopherylquinone	0.27	0.54	0.56	0.47
γ-Tocopherylquinone	0.21	0.49	0.47	0.49
Vitamin K_1	0.81	0.85	0.97	0.29
Chlorophyll *a*	0.41	0.42	0.23	0.57

[1] System I, chloroform-heptane (80:20).
[2] System II, heptane-ethanol (90:10).
[3] System III, benzene-acetone-heptane (96:4:2).
[4] System IV, plate impregnated with 4% paraffin oil and developed in acetone-water (95:5) saturated with 0.5 ml paraffin oil.

seen that in these solvent systems δ-TQ behaves as the most polar of the tocopherolquinones except compounds x_1 and x_2.

Table III presents the relative amounts of δ-tocopherol and δ-TQ found in several plant tissues. δ-Tocopherol was detected mainly as a pink spot on a thin layer plate with ferric chloride-dipyridyl spray although a spectrophotometric assay for δ-TQ was possible after gold chloride oxidation. It can be seen that the amount of δ-TQ varied from 0.007 μmole per mg chlorophyll in spinach chloroplasts to 0.98 μmole per mg chlorophyll in senescent tobacco globules.

Examination of tobacco leaf tissues used for quinone assays was carried out using electron microscopy. The chloroplasts of young, expanding, apical leaves (Fig. 2) were relatively small and had dense stroma with numerous ribosomes. The membranes were associating into grana stacks with 2 to 8 thylakoids per stack. Each chloroplast usually also contained several small densely staining globules (see arrows), and some clear starch granules.

Chloroplasts from fully expanded, mature, green leaves (Fig. 3) contained large grana stacks. One to 3 large starch granules were usually observed in these chloroplasts. There were also large globules present in almost all plastids examined. Globules of slightly larger size were observed in all chloroplasts from senescent leaf tissue (Fig. 4) and were now the dominant structure of these organelles. These globules are also distinguished from those of other tissues by the appearance of lightly staining areas within the globules. The membranes of these chloroplasts were no longer associated into grana stocks. Instead, linear or concentrically circled fragments of lamellae or dispersed small vesicles were observed.

Discussion

α-Tocopherylquinone is formed by the oxidation of α-tocopherol Similar oxidation products are derived from other members of the tocopherol family (5). α-, β-, and γ-tocopherylquinones occur in spinach chloroplasts, as shown by Dilley *et al.* (6). Dilley (4) also detected traces of δ-TQ in the same material. We have found that senescent tissues are an even better source of δ-TQ, as indicated by relatively large amounts in white, basal tobacco leaves and fallen yellow maple leaves. Some δ-TQ also occurs in cactus and green ivy leaves (table I, III). Lichtenthaler (11) found several lipophilic quinones in osmiophilic globules. Extraction of young *versus* old leaves of tobacco shows that there is little if any δ-TQ in young leaves while its concentration increases toward the base of the plant, *i.e.* in white senescent leaves reaching a maximum of 0.02 μmole per g dry weight. Two other unknown reducible quinoidal compounds designated as x_1 and x_2 were noted in senescent tobacco and cactus tissues. They appeared to be more polar than α-, β-, γ-, or δ-TQ in 4 different solvent systems (table II). These may be quinones derived from members of the tocotrienol series. Positive identification was not pos-

Table III. *Relative Amounts of δ-Tocopherol and δ-Tocopherylquinone in Several Plant Tissues*

Tissue or organelle	Total chlorophyll	δ-Tocopherol Untreated[1]	δ-Tocopherol Treated[2]	δ-Tocopherylquinone Untreated	δ-Tocopherylquinone Treated
	mg/sample	*mole/mg chlorophyll*			
Ivy	194.6	0.01	0	0.01	0.02
Spinach chloroplasts	768.7	<0.01[3]	0	0.006	0.007
Senescent tobacco globules	0.206	<0.01	0	0.87	0.89
Senescent tobacco supernatant	2.06	<0.01	0	0.05	0.05
Senescent tobacco lamellae	2.22	<0.01	0	0.13	0.16

[1] δ-T or δ-TQ content assayed before gold chloride oxidation of sample.
[2] δ-T or δ-TQ content assayed after gold chloride oxidation of sample.
[3] δ-T could not be detected by a spectrophotometric assay but was present as a spot on a thin layer plate in comparison with a standard.

sible because of small amounts present in white tobacco leaves or cactus.

Examination of tobacco leaf tissue has shown that there is a distinct pattern of development of the osmophilic globules within the chloroplasts. The small globules seen in young plastids apparently increase in size during chloroplast maturation, and develop into very large structures in the chloroplasts of senescent leaves. At this stage they are the dominant component of the organelle. With relation to these structural changes, it is of interest that δ-TQ is found to increase in concentration during leaf development and especially during senescence (table I). In addition, it was shown by fractionation studies that δ-TQ appeared to be primarily localized within the globules (table III).

Although we have shown that δ-TQ is found in low levels in the green tissue of tobacco, ivy, and cactus, Henninger *et al.* (10) have reported that it is ineffective in the restoration of ferricyanide reduction in acetone-extracted chloroplasts. From the low concentrations of δ-TQ detected in green tissues and these enzymatic studies, we feel that an active function for δ-TQ in electron transport in chloroplasts is unlikely. It is possible however, that δ-TQ is a compound which is specifically associated with senescence.

Interest in the distribution of δ-TQ is stimulated by Whistance and Threlfall's (14) studies on biosynthetic pathways of terpenoid quinones and chromanol formation using radioactive tracer techniques. These authors postulate that δ-tocotrienol and δ-tocopherol may be precursors to all other tocotrienols and tocopherols each of which can be oxidized to the corresponding tocopherolquinone by the plant. Their data show that maize and ivy leaves are able to convert α-tocopherol into α-TQ by oxidation.

Our discovery of δ-TQ in measurable amounts in senescent tobacco and yellow maple leaves thus adds evidence to the concept that tocopherols can be converted to tocopherolquinones by plants. Finding relatively significant amounts of δ-TQ, as well as other tocopherolquinones in senescent tissues may have some significance either as evidence for disruption of normal metabolic processes during aging or for oxidative degradation processes in the degenerating chloroplast.

Acknowledgments

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