Table 1. Mean angle of the cotyledonary branches to a horizontal axis, in erect and runner peanut plants as affected by foliar sprays with GA and seed treatments with the growth retardants CCC and Alar.

Treatment	Angle of cotyledonary branches in:	
	Erect plants	Runner plants
Control	51 a*	16 d
GA (10 ppm)	52 a	58 c
Alar (0.1%)	30 b	21 d
CCC (0.02%)	33 b	21 d

* Within each column, values followed by the same letter do not differ significantly from each other; those followed by different letters differ significantly at $P < 0.01$.

sults from heritable difference in amounts of endogenous gibberellin. This hypothesis was tested in growing tips and leaves from side branches of mature plants. Freeze-dried plant material was extracted, partitioned, and assayed with the barley endosperm bioassay (*9*).

Figure 1 presents data for runner and erect derivatives from the cross of VSM♀ × V4♂. Similar findings were obtained from runners having either plasmon and from bunch plants with either plasmon. One gibberellin detected in the growing tips and leaves of both types had an R_F of 0.5 to 0.6 corresponding to GA_3. Only slight and not significant differences were found in the amounts of gibberellin present in the two types of plants (Fig. 1).

We used a modification of the barley endosperm bioassay (*9*) to investigate possible differences in endogenous gibberellin antagonists (Fig. 1). Two significant differences were found between gibberellin inhibitors of plants having different growth habits. The same differences were found in reciprocal F_1 hybrids of V4 × Shulamit which differed in their plasmon but were all $Hb_1hb_1Hb_2hb_2$. The differences were: (i) three gibberellin inhibitors were found in both the growing tips and leaves of runner plants, whereas only two inhibitors were found in the erect ones; (ii) the amount of the inhibitor at R_F 7 to 10 was much higher in the growing tips of the runner type. The R_F of this inhibitor corresponded to that of abscisic acid in the solvent system used in the present experiment as well as in two other solvent systems—diethyl ether, methanol, acetic acid (50 : 50 : 1) and methanol, butanol, water, acetic acid (50 : 20 : 30 : 0.1).

Abscisic acid, identified in many plants (*10*), reportedly antagonizes several gibberellin-stimulated processes, including biosynthesis of amylase in barley endosperm (*11*). Endogenous inhibitors of gibberellin have been found recently in several plants (*12*). However, to the best of our knowledge the existence and endogenous level of gibberellin antagonists has not been correlated previously with a distinct morphogenetic character of a plant organ, nor have genetic differences in growth habit been correlated with levels of endogenous gibberellin antagonists, one of which may be a compound similar to abscisic acid. The effect of this acid on a physiological characteristic, bud dormancy, is well known. The possibility that native gibberellin antagonists function in geotropic growth of shoots regulated by genes and plasmatic factors is supported by the fact that the geotropic growth habit was modified by external application of synthetic gibberellin antagonists.

It is generally accepted that genetic differences between tall and dwarf varieties may be associated with ability to synthesize gibberellins (*13*). This concept is not always supported by endogenous amounts of gibberellin. For example, in tall and dwarf peas (*14*), only small and inconsistent differences in gibberellin content occur, which cannot account for the difference in growth habit. Our results suggest that in cases where there is a lack of correspondence between the responses to external application of gibberellin and the amount of endogenous gibberellin, one should examine gibberellin antagonists, including abscisic acid. These may prove to have a more important function in the regulation of plant growth than has been anticipated.

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8. Gibberellin (10 ppm) applied as a foliar spray twice weekly from 1 week after emergence. Growth retardants CCC and Alar were applied to seeds. Six weeks after emergence the angle of the side branches to a horizontal axis was measured in each plant.
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Reticulocytosis in Response to Dietary Antioxidants

Abstract. *Alpha-tocopheryl acetate, 1,2,dihydro-6-ethoxy-2,2,4-trimethylquinoline, and butylated hydroxytoluene increased the number of circulating reticulocytes when added to the diet of chickens. Hematocrit values were not reduced and erythrocyte life-spans were not shortened by the antioxidants. The reticulocytosis is attributed to delayed loss of reticular material from the maturing erythrocytes.*

Reticulocytosis has been reported to occur in response to vitamin E and to coenzyme Q_4 chromanol treatment of vitamin E–deficient monkeys, human infants, and children (*1*). The experiments described here demonstrate that antioxidants, when fed to chicks kept under normal dietary conditions, retard the maturation of reticulocytes.

In two experiments chicks were fed balanced diets based on natural ingredients and supplemented with antioxidants as indicated in Table 1. Retic-

ulocytes were counted by the slide method as previously described (*2*) after the chicks had been on experiment for several weeks. Hematocrit values were also determined. In a third experiment the effect of dietary antioxidants on the life-span of erythrocytes was studied by labeling in vivo with selenomethionine ^{75}Se (*3*). This method estimates both production rate and survival time of erythrocytes. A different group of chicks was bled for each set of determinations of radioactivity in order to avoid the effect of blood loss on erythropoiesis which would result from repeated bleedings of the same birds. The hematocrit value of each sample was measured in order to relate radioactivity to packed cell volume. Reticulocytes were counted in the blood samples drawn 27 days after injection.

The reticulocyte counts of the chicks fed the control diets were similar to those previously reported for growing chicks (*2*). Supplementation of the diets with 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (EQ), butylated hydroxytoluene (BHT), and *dl*-alpha-tocopheryl acetate (TOC) increased reticulocyte counts significantly ($P < .01$) in all experiments. It should be noted that the levels of TOC fed were in excess of the requirement of growing chicks.

Hematocrit values were similar for the blood samples from the chicks fed the basal diets and the diets supplemented with antioxidants. It might accordingly be assumed that the observed reticulocytosis was not due to an insufficiency of red blood cells. This assumption was borne out by the results of the third experiment (Table 2) which indicate no acceleration of the rate at which red blood cells were either discharged into or removed from the circulation of the birds receiving antioxidants. Radioactivity of the fluid hemolysates from red blood cells of birds fed TOC was higher 27 days after injection of the tracer than in the case of the control birds, or the birds fed EQ. Although the markedly greater standard deviations of the values for the group treated with TOC make it difficult to calculate a valid estimate of the significance of the difference, it is apparent that erythrocyte turnover was slower in some of the birds treated with TOC.

It is emphasized that the reticulocytosis reported here occurred when antioxidants were fed to nutritionally normal animals in contrast to that reported in connection with alleviation of a vitamin E deficiency (*1*). In the present experiments the control chickens were not deficient in vitamin E and the antioxidants were administered for several weeks prior to counting of reticulocytes. The reticulocytosis is attributed to retardation of the maturation process of the cells rather than to acceleration of erythropoiesis. Reticulocytes contain more phospholipid than do mature cells (*4*), and there is other evidence that cell aging is accompanied by a decrease in total lipids, cholesterol, and phospholipids (*5*). Dietary antioxidants have been shown to moderate the adverse effects of exposure to hyperoxia (*6*), and could therefore be expected to retard autoxidation of tissue lipids under conditions of normal oxygen concentration.

If the disappearance of reticular material is related to the decline in lipids in maturing red blood cells and involves autoxidation of the lipids, delay of the maturation process could result from antioxidant protection of the lipid components.

Table 1. Effect of dietary antioxidants on reticulocyte count and hematocrit value. Results are given as means ± standard deviation. In experiment 1, eight male White Leghorn chicks per treatment were placed on test at 8 weeks of age; in experiment 2, ten male White Leghorn chicks were on each treatment from 0 to 4 weeks of age. BHT, butylated hydroxytoluene; EQ, 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline; and TOC, *dl*-alpha-tocopheryl acetate.

Treatment	After 2 weeks	After 3 weeks	
	Reticulocytes (%)	Reticulocytes (%)	Hematocrit (%)
	Experiment 1		
Control	11.8 ± 2.0	13.8 ± 2.3	30.8 ± 2.1
BHT (0.5 g/kg)	11.9 ± 2.5	19.2 ± 5.5	30.9 ± 2.5
BHT (5.0 g/kg)	16.7 ± 3.8	19.3 ± 7.1	30.9 ± 2.3
EQ (0.5 g/kg)	18.5 ± 4.6	23.0 ± 5.3	30.1 ± 2.2
EQ (5.0 g/kg)	29.6 ± 6.9	46.7 ± 12.0	28.3 ± 3.3
TOC (120 unit/kg)	20.7 ± 3.3	31.6 ± 6.8	30.5 ± 3.1
	Experiment 2		
Control	12.5 ± 2.1		28.0 ± 1.9
EQ (0.5 g/kg)	16.3 ± 2.4		27.8 ± 4.7
EQ (5.0 g/kg)	18.6 ± 4.6		27.3 ± 5.7
TOC (220 unit/kg)	18.7 ± 2.0		29.8 ± 2.1

Table 2. Erythrocyte turnover in chicks fed antioxidants. Male White Leghorn chicks were fed a balanced diet supplemented as indicated from 0 to 7 weeks of age. They were then injected intravenously with 1 μc of ^{75}Se-labeled L-selenomethionine. Their red cells were tested 2, 9, and 27 days later for ^{75}Se (count/min), and the percentage of reticulocytes was determined on day 27. Results are average counts per minute ± standard deviation of fluid lysates from 1 ml of packed cells. EQ, 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline; TOC, *dl*-alpha-tocopheryl acetate.

Treatment	Turnover after injection			
	Day 2* (count/min)	Day 9† (count/min)	Day 27†	
			(count/min)	Reticulocytes (%)
Control	302 ± 63	926 ± 194	579 ± 61	11.8 ± 2.5
EQ (2.5 g/kg)	252 ± 119	949 ± 269	554 ± 96	16.9 ± 3.0
TOC (220 unit/kg)	308 ± 95	887 ± 316	784 ± 243	18.6 ± 5.1

* Twenty chicks per treatment. † Ten chicks per treatment.

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3,4-Dihydroxyproline: A New Amino Acid in Diatom Cell Walls

Abstract. *An analog of proline, 2,3-*cis*-3,4-*trans*-3,4-dihydroxy-*L*-proline, was found in the cell walls of the eight species of diatoms studied and was isolated from the proteinaceous material of the wall of* Navicula pelliculosa. *The properties of this substance are described; its structure was confirmed by nuclear magnetic resonance spectroscopy.*

The mature wall of the diatom is composed of a siliceous shell tightly enclosed by, and interlocked with, an organic casing (*1*) of an unusual and complex chemical structure. In addition to the known amino acids and a number of sugars, the cell wall contains a great many as yet unidentified compounds positive to the ninhydrin test, sugars and uronic acids (*2*) which constitute proteinaceous components, and a number of distinctive polysaccharides, several of which are sulfated (*2*). We describe here the isolation and characterization of one of the ninhydrin-positive compounds.

For the isolation of the compound, dried cell wall material (50 g) was hydrolyzed with 6*N* HCl in an atmosphere of nitrogen for 24 hours at 100°C. The hydrolyzate was evaporated at reduced pressure; the residue, dissolved in water, was passed through Dowex AGW 50-X4 ion-exchange column (hydrogen form, 20 to 50 mesh) and eluted with 2*N* aqueous ammonia. The eluate was concentrated at reduced pressure and passed through Dowex AG 2-X8 ion-exchange column (acetate form, 200 to 400 mesh) at 45°C; the column was washed with water and then with 0.1*N* acetic acid. Acidic amino acids were retained, and at this stage the eluate contained basic and neutral amino acids. These amino acids were separated by ion-exchange chromatography on Dowex AGW 50-X4 column (pyridinium form, 200 to 400 mesh) at 45°C, buffered at *p*H 3.1 with a solution (0.1*M*) of pyridine and formic acid (*p*H 3.1) (*3*). The new amino acid was eluted between methionine sulfoxide and threonine.

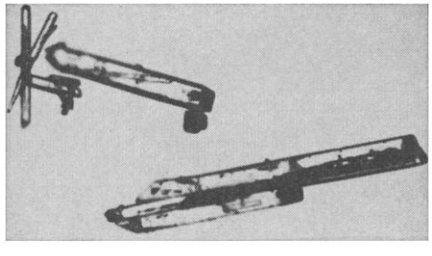

Fig. 1. Photomicrograph of crystals of 2,3-*cis*-3,4-*trans*-3,4-dihydroxy-L-proline (from aqueous ethanol) (× 146).

The eluate was concentrated under reduced pressure and the residue (45 mg), dissolved in aqueous ethanol, yielded 36 mg of crystals (fine prisms) (Fig. 1), melting at 262°C with decomposition. The specific rotation $[\alpha]_D^{20}$ was −61.2° at a concentration of 0.5 percent in H_2O (*4*). Analysis showed the values for $C_5H_9NO_4$ were: C, 40.81; H, 6.04; and N, 9.53 percent. The calculated values were: C, 40.82; H, 6.17; and N, 9.52 percent. As judged by conformity to the rule of Lutz-Jirgensons (*5*), the compound has L configuration.

Reaction with ninhydrin on paper gave a yellow color which turned brownish-yellow when heated over 100°C. When the paper was sprayed with isatin solution (*6*) a white spot appeared against the yellowish background, producing a white fluorescence under ultraviolet light. This reaction is similar to that of 3-substituted proline (*7*).

Thin-layer chromatography on cellulose sheets (*8*) in a number of solvents gave the following R_F values: solvent A, 0.27; solvent B, 0.25; solvent C, 0.24. In the amino acid analyzer (*9*) the peak due to the compound appeared 116 ml ahead of methionine sulfoxide; this position is similar to that of *trans*-3-hydroxyproline (*10*). The new amino acid showed a ninhydrin color at a maximum absorption of 440 nm, similar to that of proline-analog compounds.

Because a nitroso derivative, obtained by treatment with nitrous oxide, was converted to the original compound by hydrolysis (*11*), the new compound appears to be a cyclic imino acid.

The structure and stereochemistry of the new amino acid was elucidated by nuclear magnetic resonance (NMR) spectroscopy (*12*). The compound did not show a chemical shift contributed by the 3- or 4-methylene group in the pyrrolidine ring (Fig. 2) and hence appears to be a 3,4-dihydroxyproline. Comparison of the NMR spectrum with that of 3-hydroxyprolines (*13*) and 4-hydroxyprolines (*14*) allows no alternative assignment.

We concluded that, of the four isomers of 3,4-dihydroxyproline, the structure of the new amino acid is 2,3-*cis*-3,4-*trans*-3,4-dihydroxyproline (Fig. 3); two of the other isomers have been synthesized (*15*). This was confirmed by mass spectroscopy and x-ray diffraction analysis (*16*).

Analysis with paper chromatography (*17*) and the amino acid analyzer showed that the new compound is also present in acid hydrolyzates of the walls of seven marine diatom species of differing physiological characteristics and

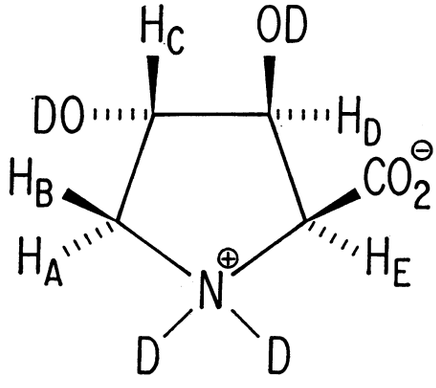

Fig. 2. Assignment of NMR data for 2,3-*cis*-3,4-*trans*-3,4-dihydroxyproline. H_A = 3.36 parts per million (1 proton, doublet) $J_{AB} = 13$ hz; $H_B = 3.72$ ppm (1 proton, double doublet) $J_{BC} = 3.5$; $H_C = 4.42$ ppm (1 proton, doublet) $J_{AC} \sim 0.7$; $H_D = 4.48$ ppm (1 proton, doublet) $J_{CD} \sim 1$; $H_E = 4.39$ ppm (1 proton, doublet) $J_{DE} = 4$.

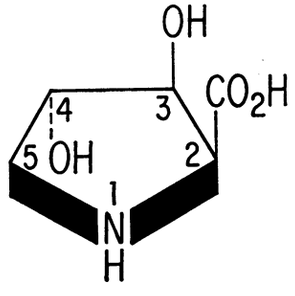

Fig. 3. Structure of 2,3-*cis*-3-4-*trans*-3,4-dihydroxyproline.