Studies on the Biosynthesis of 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one*

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The biosynthesis of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one was studied by administering to the stems of corn seedlings a number of isotopically labeled metabolites, which might serve as precursors to 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one. The results of the study show that [U-^{14}C]quinic acid, L-[*methyl*-^{14}C]methionine, D-[1-^{14}C]ribose, [2-^{14}C]glycine, and [3-^{14}C]glycerate are incorporated into the molecule. A degradation procedure was devised which permitted ^{14}C analysis of the individual carbons in positions 2 and 3, in the methoxyl carbon, and in the combined aromatic ring carbons. The pattern of labeling in 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one biosynthesized from these precursors suggests that the aromatic ring is derived from an intermediate in the shikimic acid pathway; the *O*-methyl group is formed from compounds contributing to the one-carbon pool such as methionine, glycine, and glyceric acid; and the two heterocyclic ring carbons are derived from carbons 1 and 2 of ribose.

The compound 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one (DIMBOA)[1] exists in corn seedlings and the seedlings of some other grasses as a monoglucoside. DIMBOA has two unique structural features of biosynthetic interest. It contains a benzoxazinone ring system and it is a cyclic hydroxamate.

There are few reports in the literature of the oxazine moiety occurring in natural products. Compounds containing a phenoxazinone ring system have been found in a group of pigments isolated from insects (Butenandt, 1957), in cinnabarin found in the fungus *Coriolus sanguineas* (Cavill *et al.*, 1959; Gripenberg, 1958), and in actinomycin isolated from *Streptomyces antibioticus* (Brockmann *et al.*, 1956). Biosynthetic studies of the insect pigments (Butenandt, 1957) and actinomycin (Sivak *et al.*, 1962) have shown tryptophan is incorporated into these compounds. The formation of the phenoxazine structure from a more immediate precursor is suggested by the findings of Sivak *et al.* (1962) that cell-free extracts of *S. antibioticus* catalyze the oxidative condensation of two molecules of 3-hydroxy-4-methylanthranilic acid to form actinocin.

DIMBOA and a closely related analog are the only cyclic hydroxamates isolated thus far from higher plants (Wahlroos and Virtanen, 1959) although a number of such compounds have been found as products of mold metabolism. Compounds containing the cyclic hydroxamate structure are of special interest because they all exhibit antimetabolic activity, as does DIMBOA. The biosynthesis of aspergillic acid (MacDonald, 1961) and mycelianamide (Birch and Smith, 1959) from amino acids suggests the hydroxamate moiety is formed by oxidation of an amide bond. In support of this mechanism Irving (1962) has demonstrated the presence of an enzyme in rabbit liver microsomes capable of catalyzing the hydroxylation of the nitrogen in the amide group of 2-aminofluorene. On the other hand, Emery (1963) has suggested that cyclic hydroxamate formation could result from the reaction of a carboxyl compound with the free *N*-hydroxyamino acid.

A study of the biosynthesis of DIMBOA was therefore undertaken since no previous investigation of benzoxazinone or cyclic hydroxamate biosynthesis in higher plants has been made. In addition, information concerning the biosynthesis of DIMBOA might contribute to an understanding of the mechanism of benzoxazine and cyclic hydroxamate formation in general.

The results of the present study show that [U-^{14}C]quinic acid, L-[*methyl*-^{14}C]methionine, D-[1-^{14}C]-ribose, [2-^{14}C]-glycine, and [3-^{14}C]-glycerate are specifically and extensively incorporated into DIMBOA.

EXPERIMENTAL[2]

The roots and seeds were removed from 6-day-old etiolated corn seedlings (Michigan Hybrid 350) and labeled metabolites were administered to the plants in an aqueous solution through the cut stems. The nutrient solution fed per plant contained 0.2×10^{-3} mmoles of the metabolite having 0.96 μc ^{14}C. In a typical experiment 500 seedlings were fed and this number of plants provided a sufficient amount of tissue for the isolation of about 200 mg of pure DIMBOA. Absorption of the metabolite was essentially complete within 5 hours. The nutrient solution was replaced with water at the end of this time and the plants were allowed to metabolize the administered compound for an additional 21 hours. The plants were homogenized with water in a Waring Blendor and allowed to stand for 30 minutes to insure maximum release of DIMBOA from its glucoside linkage through the action of a glucosidase liberated in the crushed plant tissue. An equal volume of ethanol was added and the plants were homogenized again. DIMBOA was isolated according to the procedure of Hamilton *et al.* (1962), mp 163–164° (decomp), reported mp 160–161°.

Degradation of DIMBOA.—The isolated DIMBOA was analyzed to determine the extent of ^{14}C incorporation into the whole molecule. In those cases in which incorporation of the compound was significant, the position of the isotope was determined by degradation to isolate specific carbon atoms or groups within the molecule. The degradation procedure devised per-

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[1] Abbreviation used in this work: DIMBOA, 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one.

[2] Melting points are uncorrected. All references to petroleum ether are to that fraction boiling between 30 and 60°.

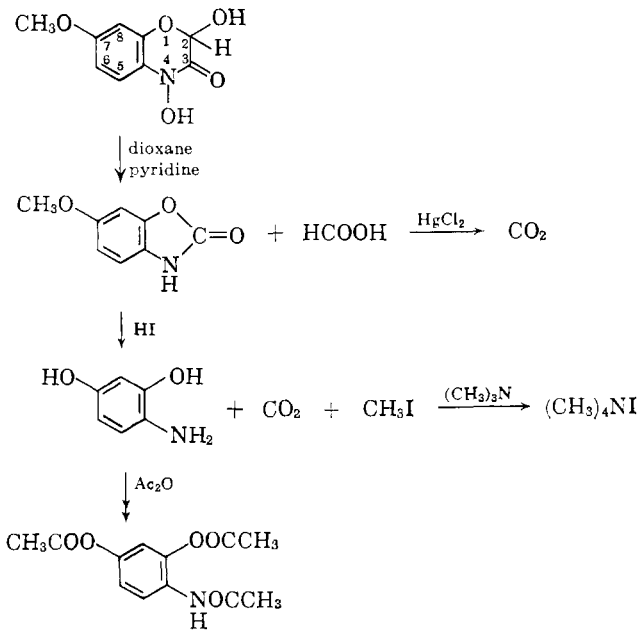

FIG. 1. The degradation procedure for DIMBOA.

mitted the isolation of carbons in positions 2 and 3, in the methoxyl carbon, and in the combined aromatic ring carbons as shown in Figure 1.

Conversion of DIMBOA to 6-Methoxybenzoxazolinone and Formic Acid.—DIMBOA (200 mg), heated for 2 hours in 20 ml of dioxane and 1 ml of pyridine, was converted to 6-methoxybenzoxazolinone with the liberation of formic acid which originated from the 2 position of the molecule (Honkanen and Virtanen, 1961). The solution was distilled under reduced pressure into a receiving flask cooled in an ice bath, formic acid was converted to the sodium salt, and the organic solvents were removed under reduced pressure. The formate was oxidized to barium carbonate according to the procedure of Sakami (1950).

The amorphous residue, obtained after the distillation of the solvents from the reaction mixture, was crystallized from hot water to yield reddish-tan needles of 6-methoxybenzoxazolinone in 60% yield, mp 153–153.5° (reported 153–153.5°, Hamilton *et al.*, 1962).

Degradation of 6-Methoxybenzoxazolinone.—Treatment of 6-methoxybenzoxazolinone with hydriodic acid produced carbon dioxide from the 3 position of DIMBOA, methyl iodide from the methoxyl group, and 2,4-dihydroxyaniline.

A suspension of 6-methoxybenzoxazolinone (80–120 mg) in 7 ml of hydriodic acid was heated under reflux conditions for 3 hours. The reaction mixture was continuously flushed with a stream of nitrogen and the volatile reaction products, carbon dioxide and methyl iodide, were swept through a series of traps. A scrubber trap containing a solution of 5% cadmium sulfate—2% stannous chloride in 0.3 N hydrochloric acid removed iodine and hydriodic acid; a saturated solution of barium hydroxide precipitated carbon dioxide as barium carbonate; and a 5% ethanolic solution of trimethylamine, cooled in a dry-ice methyl-Cellosolve bath, converted methyl iodide to tetramethylammonium iodide. The quaternary ammonium salt crystallized after several hours at room temperature. The crystals were collected, dried over calcium chloride, and recrystallized from alcohol-ether in 80% yield.

The reaction mixture, which contained 2,4-dihydroxyaniline, was diluted with five volumes of water; hydriodic acid and iodine were precipitated from solution by the addition of a sufficient amount of solid stannous chloride to decolorize the solution. The copious yellow-red precipitate was removed by filtration and the residue was washed three times with water. The combined filtrate and washes were extracted three times with equal volumes of ethyl ether and the ether, which contained unwanted reaction products and hydriodic acid, was discarded. This aqueous solution was used for the acetylation reactions.

Acetylation of 2,4-Dihydroxyaniline.—To isolate 2,4-dihydroxyaniline it was necessary to prepare an acetylated derivative, and since the monoacetylated derivative of the degradation product, 2,4-dihydroxyacetanilide, proved difficult to crystallize in small quantities, 2,4-diacetoxyacetanilide, the triacetylated derivative, was prepared in the routine degradation procedure. Triacetylation of the 2,4-dihydroxyaniline, isolated from the hydriodic acid reaction mixture, however, could not be carried out in one reaction step; it was necessary to partially purify the monoacetylated derivative and then perform a second acetylation reaction to obtain the fully acetylated compound, 2,4-diacetoxyacetanilide.

The aqueous solution, which contained 2,4-dihydroxyaniline, was adjusted to *p*H 4 with solid sodium carbonate and the first acetylation was carried out by the addition of 0.2 ml of acetic anhydride. The reaction mixture was neutralized to *p*H 7.3 with aqueous sodium hydroxide and stirred mechanically for 20 minutes. Excess stannous ions were precipitated as stannous hydroxide at *p*H 10 and acetic anhydride was added dropwise until the *p*H reached 6.0. After separation of stannous hydroxide by filtration, the solution was continuously extracted with ethyl ether for 6 hours. The oily product, obtained after evaporation of the ether, was purified by repeated recrystallizations from ethyl acetate–petroleum ether to give white needle crystals of 2,4-dihydroxyacetanilide, mp 188–189°. Vorozhtzov and Gorkov (1932) reported the isolation of this compound as brownish crystals which melted at 164°. The monoacetylated product obtained from the degradation procedure was identical with a synthetic sample of 2,4-dihydroxyacetanilide, which analyzed as follows:

Anal. Calcd. for $C_8H_9NO_3$ (167.14): C, 57.48; H, 5.42; N, 8.41. Found: C, 57.45; H, 5.43; N, 8.31.

Preparation of 2,4-Diacetoxyacetanilide.—The second acetylation reaction was carried out at *p*H 10 by the addition of 1.0 ml acetic anhydride to the dissolved residue obtained from the continuous ether extraction described above. The reaction mixture was extracted five times with equal volumes of ethyl ether and the combined ether extracts were evaporated to dryness. The residue was dissolved in a small amount of benzene and crystallized, by the addition of petroleum ether, in 18% yield. On recrystallization from ether–petroleum ether the product, 2,4-diacetoxyacetanilide, was obtained, mp 113–114° (reported 113°, Vorozhtzov and Gorkov, 1932) and was identified by elemental analysis and paper chromatographic comparison with an authentic sample.

Anal. Calcd. for $C_{12}H_{13}NO_5$ (251.23): C, 57.40; H, 5.23; N, 5.60. Found: C, 57.30; H, 5.10; N, 5.33.

The mobilities of the degradation product and an authentic sample of 2,4-diacetoxyacetanilide on paper chromatograms were identical in two solvent systems. Mixed samples developed as a single spot when chromatographed in isobutanol–1 N formic acid (9:1) and in a solvent consisting of $CHCl_3$–methanol–1% formic

TABLE I
COMPARISON OF ^{14}C INCORPORATION INTO DIMBOA FROM PLANTS FED LABELED COMPOUNDS

Compound Fed	Specific Activity of Compound Fed (mμc/mmole)	Specific Activity of DIMBOA (mμc/mmole)	Dilution
[U-^{14}C]Quinic acid	94,000	225.5	419
L-[*methyl*-^{14}C]Methionine	480,000	367.0	1,306
D-[1-^{14}C]Ribose	480,000	193.6	2,484
[2-^{14}C]Glycine	480,000	99.6	4,820
[3-^{14}C]Calcium glycerate	480,000	35.5	13,520
DL-[7a-^{14}C]Tryptophan	94,000	2.2	43,700
[1-^{14}C]Sodium acetate	480,000	0.9	533,000
[1-^{14}C]Sodium glycollate	480,000	0	

into DIMBOA with the lowest dilution of the metabolites tested, as shown in Table I. This finding provides evidence that the aromatic ring moiety is biosynthesized through the shikimic acid pathway. Furthermore, the degradation of the molecule (cf. Table II) revealed that essentially all the ^{14}C incorporated was located in the benzenoid ring carbons. The formation of aromatic compounds from quinic and shikimic acids in higher plants is well documented. Weinstein *et al.* (1959, 1962) presented evidence for the conversion of quinic to shikimic acid which in turn gives rise to phenylalanine and tyrosine in roses and bean leaves. Neish and co-workers have shown that shikimic acid is a good precursor for lignin in wheat and maple plants (Brown and Neish, 1955), for flavonoids in buckwheat (Underhill *et al.*, 1957), and for tryptophan and gramine in barley seedlings (Wightman *et al.*, 1961).

TABLE II
DISTRIBUTION OF ^{14}C IN THE DIMBOA MOLECULE FROM LABELED PRECURSORS

Compound Isolated in Degradation	[U-^{14}C]Quinic Acid		L-[*methyl*-^{14}C]-Methionine		[1-^{14}C]-Ribose		[2-^{14}C]-Glycine		[3-^{14}C]Calcium Glycerate	
	Sp. Act. (mμc/mmole)	(%)	Sp. Act. (mμc/mmole)	(%)	Sp. Act. (mμc/mmole)	(%)	Sp. Act. (mμc/mmole)	(%)	Sp. Act. (mμc/mmole)	(%)
DIMBOA	224.3	100.0	367.5	100.0	193.2	100.0	99.6	100.0	35.5	100.0
HCOOH (position 2)	0	0	0	0	7.5	3.9	0	0	0	0
CO_2 (position 3)	0.4	0.1	0	0	120.8	62.5	0.1	0.1	1.9	5.3
Tetramethylammonium iodide (methoxyl carbon)	7.1	3.1	377.5	102.7	11.0	5.7	97.8	98.2	13.2	37.2
2,4-Diacetoxyacetanilide (benzenoid ring carbons)	223.5	99.5	2.0	0.5	60.9	31.5	0	0	22.2	62.5

acid (10:1:1) (Reio, 1960) with R_F values of 0.81 and 0.94, respectively. The compounds were located on chromatograms with hydroxylamine following the procedure of Carles *et al.* (1961) for the detection of esters.

Determination of Radioactivity.—All compounds analyzed for ^{14}C were converted to barium carbonate. Recovery studies, carried out on the compounds subjected to the combustion procedure—DIMBOA, tetramethylammonium iodide, and 2,4-diacetoxyacetanide—indicated that oxidation was essentially complete. ^{14}C determinations were made with Nuclear-Chicago Model D47 proportional gas-flow counter and a 192A scaler. All measurements were corrected for self-absorption. In all instances the counts were at least two times background.

RESULTS AND DISCUSSION

The extent of ^{14}C incorporation of a number of labeled compounds into DIMBOA, is shown in Table I. The compounds are listed in decreasing order of incorporation as judged by the dilution, the ratio of the specific activity of the compound fed to the specific activity of DIMBOA isolated.

The position of isotopic incorporation in DIMBOA was determined by ^{14}C analysis of the compounds isolated from the degradation procedure. Table II shows the distribution of ^{14}C in positions 2 and 3, in the methoxyl carbon, and in the combined aromatic ring carbons of the DIMBOA molecule isolated from plants fed metabolites which were most readily incorporated.

[U-^{14}C]*Quinic Acid Incorporation into DIMBOA.*—Uniformly labeled quinic acid was incorporated

An alternate pathway for the biosynthesis of aromatic compounds in plants is the head-to-tail condensation of acetate units described by Birch *et al.* (1955). The ring biosynthesis of DIMBOA, however, does not involve this pathway since [1-^{14}C]acetate administered to corn plants was not incorporated.

The chemical structure of DIMBOA suggests that a nitrogen-containing compound in the shikimic acid pathway could be a more immediate precursor and the source of the nitrogen in the hydroxamate group. A common intermediate might give rise to both DIMBOA and anthranilic acid in this pathway, or DIMBOA might be more directly derived from tryptophan or its degradation product, 3-hydroxyanthranilic acid. The latter possibility was tested but the participation of tryptophan in the biosynthesis of DIMBOA appears unlikely since the incorporation of ring-labeled DL-[7a-^{14}C]tryptophan occurred with a very large dilution in comparison with other precursors, as shown in Table I.

Precursors to Shikimic Acid.—The contribution of ^{14}C from ribose and glycerate to the aromatic ring of DIMBOA can readily be interpreted in terms of known reactions by which these compounds are converted to shikimic acid. The studies of Srinivasan *et al.* (1955), employing *Escherichia coli* mutants, have shown that 5-dehydroquinic acid results from the cyclization of a 7-carbon sugar which in turn is formed by the condensation of phosphoenolpyruvate and erythrose-4-phosphate. The shikimic acid pathway in plants and the more thoroughly investigated pathway in microorganisms appears to be essentially the same since the same intermediates have been found to occur in both systems. Nandy and Ganguli (1961) have reported phosphoenolpyruvate and eryth-

rose-4-P are also optimal substrates for 5-dehydroshikimate biosynthesis in mung-bean seedlings. If shikimate arises in a similar manner in corn plants, [3-^{14}C]glycerate would presumably enter the pathway as phosphoenolpyruvate and the tracer carbon would then become incorporated in the aromatic ring of DIMBOA. The findings of this study are consistent with the described pathway since a significant amount of the radioactivity incorporated into DIMBOA from [3-^{14}C]glycerate was associated with the aromatic ring.

Table II shows that [1-^{14}C]ribose contributed to the aromatic ring carbons of DIMBOA to an even greater extent than glycerate, probably through generation of both the 3- and 4-carbon compounds utilized in formation of shikimic acid. This interpretation is supported by the isotope studies of Sprinson and coworkers (Sprinson, 1955) on shikimic acid formation from the products of pentose metabolism. These investigators reported that ^{14}C analysis of the shikimate carbon atoms was consistent with the view that [1-^{14}C]pentose gives rise to phosphoenolpyruvate by glycolysis and to erythrose in the pentose pathway.

Synthesis of the Methoxyl Carbon from [methyl-^{14}C]-Methionine, [2-^{14}C]Glycine, and [3-^{14}C]Glycerate.—The 7-methoxyl carbon of DIMBOA appears to be formed by a transmethylation process from methionine. The data show that essentially all the label incorporated from [*methyl*-^{14}C]methionine was recovered from the methoxyl position. The α-carbon of [2-^{14}C]glycine, a known methyl precursor, was also specifically utilized for 1-carbon formation as 98.2% of the isotope incorporated was present in the methoxyl carbon. The contribution of the α-carbon of glycine however was almost 4-fold less than that from methionine as shown by the dilution figures in Table I. The evidence for biosynthesis of *O*-methyl groups in higher plants from methionine is in accord with the findings of others (Byerrum *et al.*, 1954; Dubeck and Kirkwood, 1952).

[3-^{14}C]Glycerate also provided carbon for the formation of the methoxyl group of DIMBOA as 37.2% of the incorporated radioactivity was associated with the methoxyl carbon. Presumably the glycerate was first converted to serine which in turn participated in 1-carbon metabolism. This finding is in agreement with evidence presented by Hanford and Davies (1958) that crude extracts of pea epicotyls are capable of converting 3-phosphoglycerate to 3-phosphoserine, presumably by way of the intermediate 3-phosphohydroxypyruvic acid.

Incorporation of [1-^{14}C]Ribose into the Heterocyclic Ring of DIMBOA.—The data obtained on the degradation of DIMBOA from plants fed [1-^{14}C]ribose indicate that the two carbon atoms completing the oxazine ring are derived from carbons 1 and 2 of ribose. Table II shows that carbon 3 of DIMBOA contained 62.5% of the incorporated isotope and presumably corresponds to the C-1 of ribose.

Ribose is known to give rise to a number of 2-carbon compounds some of which were considered as possible precursors in the biosynthesis of DIMBOA. Experimentally, however, the 2-carbon compounds [1-^{14}C]acetate, [2-^{14}C]glycine, and [1-^{14}C]glycollate were not incorporated into the 2 and 3 positions of DIMBOA. The lack of ^{14}C incorporation into these two carbons following glycolate feeding further implies that glyoxylate does not serve as the 2-carbon precursor since the latter compound is an immediate metabolic product of glycolate in plants. The incorporation of glycolaldehyde into the oxazine ring of DIMBOA was not ruled out by isotope studies. However, no well-defined system in which free glycolaldehyde results from pentose metabolism has been demonstrated. The enzyme transketolase catalyzes the formation of an "active glycolaldehyde" intermediate in the pentose pathway of plant metabolism. The 2-carbon compound, which is bound to the cofactor thiamine pyrophosphate, originates from the 1 and 2 carbons of ribose. A transfer of this glycolaldehyde by the accepted mechanism of nucleophilic attack on an amine or a hydroxyl group of a DIMBOA precursor does not seem mechanistically feasible. Although a 2-carbon precursor derived from ribose has not been definitively eliminated, a pathway consistent with the known mechanisms of ribose metabolism and the experimentally determined labeling pattern is not readily apparent.

A Postulated Mechanism for the Biosynthesis of the Oxazine Ring of DIMBOA.—An alternate explanation for the incorporation of ribose carbons into the oxazine ring of DIMBOA would entail a condensation of ribose with an appropriate aromatic intermediate followed by cleavage of the 3, 4, and 5 carbons. The participation of ribose in the formation of heterocyclic rings in which only the 1 and 2 carbons are retained in the final product has been demonstrated for the biosynthesis of the imidazole of histidine (Moyed and Magasanik, 1960), the pyrrole ring of tryptophan (Yanofsky, 1955), and the azine moiety of pteridines (Weygand *et al.*, 1961). The evidence obtained from this study suggests that the heterocyclic ring of DIMBOA is biosynthesized from ribose in a similar manner.

The incorporation of the 1 and 2 carbons of ribose into the oxazine ring of DIMBOA could result from a reaction sequence analogous to the pathway established for the pyrrole ring of tryptophan (Smith and Yanofsky, 1960; Yanofsky, 1956; Doy *et al.*, 1961) and the postulated route for the biosynthesis of the azine ring of pteridines (Weygand *et al.*, 1961). The reaction sequence would be initiated by a condensation of ribose, or a phosphorylated derivative, with an amino-substituted aromatic compound derived from the shikimic acid pathway to form a ribosyl intermediate; an Amadori type rearrangement to yield the 1-deoxy-2-keto derivative followed by ring closure to include carbons 1 and 2 of ribose in the oxazine ring; elimination of the triose moiety; and oxidation of the molecule to the levels found in DIMBOA.

The biosynthesis of DIMBOA by a condensation reaction of ribose with an amine further suggests that the carbon-nitrogen bond is formed prior to the *N*-hydroxylation reaction to yield the cyclic hydroxamate in the molecule.

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Denaturation of Phytochrome

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The absorption spectra of phytochrome were found to be sensitive to the association between the chromophoric group and the protein moiety. The effects of protein denaturation were studied by measuring the absorption spectra of both forms of the photochromic pigment system. One form of phytochrome, P_{FR}, was much more susceptible to denaturation by urea, to attack by pronase and trypsin, and to sulfhydryl-reacting reagents than the other form, P_R. The results suggest that light-induced interconversions between P_R and P_{FR} involve changes of protein conformation as well as changes of the chromophoric group.

Action spectra of the effects of light on various aspects of plant growth and development (Borthwick and Hendricks, 1960) revealed the presence and action of a red-far-red photochromic pigment. The pigment, called phytochrome, was detected spectrophotometrically in intact plant tissue and was extracted as a soluble protein (Butler *et al.*, 1959). Procedures for purifying the chromoprotein have been described (Siegelman and Firer, 1964). The reversible photoconversion between the two forms of phytochrome[1] is retained in the purified solutions.

The best source of phytochrome has been dark-grown seedling tissue. The phytochrome extracted from different species of seedlings, however, showed differences in ease of purification and in resistance to denaturation. In particular, the phytochrome extracted from barley seedlings was less stable than that from maize or oats, and differences in the absorption spectra of barley phytochrome suggested that the chromoprotein might be altered by the purification procedures. These observations led to the studies of denaturation reported below. The term "denaturation" is used in a very general sense to indicate an unknown alteration of the protein.

The absorption spectra of both forms of phytochrome were found to depend upon the state of the protein. The absorption bands in the long-wavelength region of the spectrum, where other proteins do not absorb, provide a specific tag for phytochrome molecules and permit the phytochrome protein to be studied in the presence of large amounts of other proteins. In the present paper the absorption spectra have been used to indicate the progress and degree of denaturation. Complete denaturation was assumed when the red and far-red absorption bands of the chromophores disappeared. This operational assay for denaturation is thus limited to those parts of the protein which influence the chromophoric group.

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[1] Abbreviations used in this work: P_R, red-absorbing form of phytochrome; P_{FR}, far-red-absorbing form of phytochrome; EDTA, ethylenediaminetetraacetic acid.

$$P_R \underset{\text{far-red light}}{\overset{\text{red light}}{\rightleftharpoons}} P_{FR}$$

Materials and Methods

Phytochrome was purified through the Sephadex G-200 step according to the procedures described (Siegelman and Firer, 1964). Unless otherwise noted, the phytochrome was purified from dark-grown oat