carbamates and might be of the same order as that of other synergists recently described (*3*). It is also noteworthy that no significant difference in activity between compounds **3** and **4** was observed, which is in contrast with our findings in a series of derivatives of methyl farnesate, where saturation of any double bond destroyed JH activity (*5*). Molecular size does not appear to be critical, as witnessed by the identical activity of compounds **3**, **4**, and **5**.

The synthesis of compounds **7** through **11** was undertaken in an effort to find a correlation between the electron accepting ability of the terminal functional group and JH activity. However, no such relation appears to be obvious from our results. The most interesting finding was the outstanding activity of compounds **10** and **11**. Compound **9** was as active as our synthetic mixture of isomers (*9*) of *Cecropia* JH (**1**) while compound **11**, which has the abnormal terpenoid moiety of the same hormone, showed a 30-fold increase in activity over our JH mixture.

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References

1. H. Röller, K. H. Dahm, C. C. Sweeley, B. M. Trost, *Agnew. Chem. Int. Ed. Engl.* **6**, 179 (1967).
2. G. S. Meyer, H. A. Schneidermann, E. Hanzmann, J. H. Ko, *Proc. Nat. Acad. Sci. U.S.* **60**, 853 (1968).
3. W. S. Bowers, *Science* **161**, 895 (1968).
4. ———, *ibid.* **164**, 323 (1969).
5. N. Wakabayashi, P. E. Sonnet, M. W. Law, *J. Med. Chem.* **12**, 911 (1969).
6. A. C. Cope, C. M. Hofmann, C. Wyckoff, E. Hardenberg, *J. Amer. Chem. Soc.* **63**, 3452 (1941).
7. F. W. Swamer and C. R. Hauser, *ibid.* **72**, 1352 (1950).
8. W. S. Wadsworth and W. D. Emmons, *ibid.* **83**, 1733 (1961).
9. B. H. Braun, M. Jacobson, M. Schwarz, P. E. Sonnet, N. Wakabayashi, R. M. Waters, *J. Econ. Entomol.* **61**, 866 (1968).
10. W. S. Bowers and M. J. Thompson, *Science* **142**, 1469 (1963).

7 July 1969; revised 20 November 1969

Bile Pigment Formation in Plants

Abstract. *The unicellular alga* Cyanidium caldarium *evolves carbon monoxide during the syntheis of the bile pigment, phycocyanobilin. Carbon monoxide and phycocyanobilin were produced in stoichiometric amounts at comparable rates. Therefore, the mechanism of bile pigment formation in this plant parallels that in mammals.*

During the breakdown of hemoglobin in man, conversion of the porphyrin ring of heme to bile pigment is accompanied by cleavage and oxidation of the α-methyne bridge carbon to carbon monoxide (*1*). The prosthetic groups of algal biliproteins (phycocyanin and phycoerythrin) and of phytochrome in higher plants are also open-chain tetrapyrroles nearly identical to mammalian bile pigment (*2*), but little is known about the biosynthesis of these pigments. The heme precursor, δ-aminolevulinic acid (ALA), is incorporated into phycocyanobilin, the prosthetic group of phycocyanin, and the metabolic pathway for bile pigment formation in plants and animals is identical between the precursor ALA and protoporphyrin (*3*). Nevertheless, conversion of heme to phycocyanobilin by algae in vitro has not been directly demonstrated, and it is not known whether an enzymatic system is involved, similar to that recently described for the conversion of heme to bilirubin in mammalian spleen and liver (*4*). One approach to this problem would be to show that algae synthesize bile pigment and carbon monoxide concomitantly in vivo. This would suggest a similar mechanism for opening of porphyrin rings during heme degradation in plants and mammals. We now report carbon monoxide and phycocyanobilin biosynthesis in the unicellular alga *Cyanidium caldarium.*

Cyanidium caldarium cells grown in darkness lack pigment but produce phycocyanin (which contains the bile pigment prosthetic group, phycocyanobilin) when placed in light (*5*). Dark-grown cells were illuminated for 80 hours in 14 liters of nutrient medium (*5*) containing [5-C^{14}]-ALA [4.4×10^5 distintegrations per minute (dpm); specific activity 2.2×10^5 dpm/μmole] in an MF-114 New Brunswick fermentator. The suspension was aerated with 5 liters of 95 percent O_2 and 5 percent CO_2 recycled between the culture and the reservior with a circulating pump. At intervals during the illumination period, phycocyanobilin in cells was estimated spectrophotometrically by the opal glass method (*6*). At the same time, gas in the reservoir was passed through a train consisting of the following components connected in series: (i) Baralyme granules (National Cylinder Gas) to remove CO_2; (ii) Ascarite (Arthur Thomas) to remove CO_2; (iii) an alkali trap to ensure complete CO_2 removal; (iv) Hopcalite catalyst (*7*) to convert carbon monoxide to CO_2; and (v) a series of alkali traps to collect CO_2 derived catalytically from carbon monoxide. Derived CO_2 was measured with a *p*H meter by titration of residual alkali (v) to *p*H 8.5 with 1*N* HCl dispensed through a syringe microburet. The recovery of known amounts of standard carbon monoxide (Matheson) from the fermentator-reservoir-train assembly generally exceeded 95 percent. During experiments involving radioactivity, negligible amounts of radioactivity or titratable carbonate were detected in the alkali trap (iii) preceding Hopcalite in the recovery train, which indicated that CO_2 was quantitatively removed by the Baralyme and Ascarite and did not contaminate the terminal alkali traps.

Evolution of carbon monoxide (as measured titrimetrically and radiochemically) and synthesis of phycocyanobilin proceeded concomitantly in *C. caldarium* cells (Fig. 1). The cells evolved carbon monoxide only while making phycocyanobilin and not during growth in darkness or in light after cessation of phycocyanobilin synthesis. This is what one might anticipate if phycocyanobilin and carbon monoxide

Table 1. Synthesis for phycocyanobilin by *Cyanidium caldarium* cells incubated with [5-C^{14}]-ALA for 60 hours in light. Total label added to the 14 liter culture containing 140 g (fresh weight) of cells was 4.4×10^5 dpm. Theoretically, seven labeled ALA carbon atoms are incorporated into phycocyanobilin for each labeled carbon atom incorporated into carbon monoxide. The numbers in parentheses equal the total phycocyanobilin radioactivity and specific activity divided by 7, and therefore can be compared directly to the corresponding values for carbon monoxide.

Product	[5-C^{14}]-ALA incorporated (%)	Total incorporation (dpm)	Amount/culture (μmole)	Specific activity (dpm/μmole)
Carbon monoxide	0.75	3,290	70	47
Phycocyanobilin	5.15	22,650	74	307
		(3220)		(44)

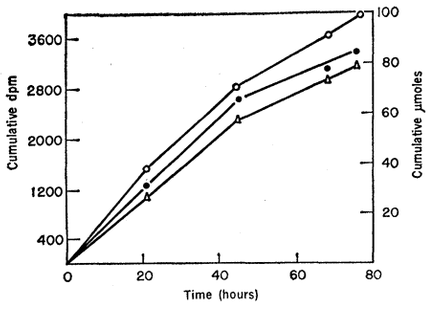

Fig. 1. Evolution of carbon monoxide (μmole, solid circles; dpm, open circles) into reservoir gas and synthesis of phycocyanobilin (μmole, triangles) by *Cyanidium caldarium* cells after administration of [5-C^{14}]-ALA (4.4×10^5 dpm; specific activity, 2.2×10^5 dpm/μmole; total ALA added, 2 μmole) in light. The disintegrations per minute were determined by driving bicarbonate from solution with acid after titration of residual alkali to *p*H 8.5. The liberated $C^{14}O_2$ (derived from $C^{14}O$) was then collected in 5 ml of ethanolamine: methoxyethanol (2 : 1), and counted (*9*) in a liquid scintillation spectrometer. Quench was determined by addition of toluene-C^{14} internal standard.

were derived from a common heme precursor derived from [5-C^{14}]-ALA.

It is known that 8 moles of ALA are necessary for the formation of 1 mole of heme, and that eight labeled carbons from [5-C^{14}])-ALA are incorporated, that is, four in the pyrrole rings and four in the methyne bridge carbons (*1*). If this pattern were followed in the formation of algal heme destined for phycocyanobilin, the specific activity of phycocyanobilin ought to be seven times that of carbon monoxide. In addition, total label incorporated into phycocyanobilin per culture should be seven times that incorporated into carbon monoxide. To explore this relationship further, phycocyanin [$E_{1cm}^{0.1\%} = 7.74$ at 618 nm in 0.1*M* phosphate buffer, *p*H 6.5 (*8*)] was isolated from cells disrupted by high-frequency sound and purified by fractionation with ammonium sulfate and by chromatography on a brushite column (*3*). The specific activity (dpm/μmole) of phycocyanobilin was determined (*8*) (Table 1).

The stoichiometry of carbon monoxide and phycocyanobilin produced per culture were in good agreement. The specific activity (dpm/μmole) of phycocyanobilin was seven times that of carbon monoxide, as was total incorporation of [5-C^{14}]-ALA per culture. In three additional experiments, in which labeled ALA was omitted, *C. caldarium* cells produced 35, 73, and 54 μmole of carbon monoxide, respectively, which corresponds to synthesis of 34, 70, and 53 μmole of phycocyanobilin, respectively.

Thus, carbon monoxide is a normal byproduct of phycocyanobilin synthesis. Evolution of carbon monoxide and synthesis of phycocyanobilin proceed at equimolar rates in *C. caldarium* cells exposed to light. The results, therefore, support the concept that phycocyanobilin arises from precursor heme, and suggest that the mechanism of algal heme catabolism is similar to that described in mammalian systems.

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References and Notes

1. R. Lester and R. F. Troxler, *Gastroenterology* **56**, 143 (1969).
2. H. W. Siegelman, D. J. Chapman, W. J. Cole, in *Porphyrins and Related Compounds*, T. W. Goodwin, Ed. (Academic Press, London, 1968), pp. 107–120.
3. R. F. Troxler and R. Lester, *Biochemistry* **6**, 3840 (1967).
4. R. Tenhunen, H. S. Marver, R. Schmid. *Proc. Nat. Acad. Sci. U.S.* **61**, 748 (1968).
5. R. F. Troxler and L. Bogorad, *Plant Physiol.* **41**, 491 (1966).
6. K. Shibata, in *Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience, New York, 1959), vol. 7, pp. 77–109.
7. Mine Safety Appliances Co., Pittsburgh, Pa.
8. R. F. Troxler and R. Lester, *Plant Physiol.* **43**, 1737 (1968).
9. Toluene : methoxyethanol (2 : 1) containing 4 g of 2,5-bis-2(5-*t*-butyl benzoxazolyl)β thiophene (Packard Instrument Co., Downers Grove, Illinois).
10. Supported in part by PHS grants AM-09881 and AM-07301, and Career Development Award 12,127. We thank W. J. Williams for [5-C^{14}]-ALA.

15 September 1969; revised 24 November 1969

White Pine Blister Rust: Simply Inherited Resistance in Sugar Pine

Abstract. *Segregation ratios of offspring from disease-free sugar pines suggest that resistance to the white pine blister rust fungus is under major gene control and simply inherited.*

Resistance to disease in cultivated plants is often conditioned by single genes with major effects. Heritable variation in resistance to diseases in forest trees also has been demonstrated, but modes of inheritance in most cases are poorly understood and probably more complex. We report one, if not the first, instance of an effective and simply inherited factor for resistance to a major forest disease in a commercially important species.

Table 1. Blister rust infection on sugar pine progenies from rust-free parent trees.

Parents		No. of seedlings		Healthy (%)
Seed	Pollen	Infected	Healthy	
K36	K17	18	21	54
K16	K17	23	16	41
K60	K17	26	21	45
K60	K10	23	19	45
K36	K73	7	12	63
K70	Wind	2	36	95
Other progenies*		1359	28	2
Controls*		402	0	0

* Combined data from 1962–1964 outplantings, involving 29 hand-pollinated crosses (with K60, K36, and K16 as seed parents in most crosses), 6 wind-pollinated progenies, and controls.

White pine blister rust, caused by *Cronartium ribicola* Fischer, has become chronically epidemic on white pines in north temperate forests the world over, with devastating effects. Ever since this disease was introduced to North America on infected planting stock around 1900, its continued spread and intensification on indigenous white pines has seriously jeopardized the future management and, in some areas, even existence of these economically and ecologically important trees. Attempts to control the spread of the rust by eradication of its alternate hosts (*Ribes* spp.) have been costly and relatively ineffective in areas of high disease hazard. Use of antibiotics to effect therapy of diseased stands has not justified the early enthusiasm it inspired. Presently, production of trees with genetic resistance offers the most feasible and promising approach to curtailing wholesale loss of regeneration over vast areas where climatic conditions favor disease development. In spite of the high susceptibility of all North American species, occasional rust-free individuals do exist in severely infected stands, prompting the hope that some of their apparent resistance might be genetically transmissible. These rust-free phenotypes have provided the initial germ plasm for several breeding programs in eastern (*Pinus strobus* L.) and western (*P. monticola* Dougl.) white pines in the United States and Canada over the past three decades (*1*). Progress in some of these has been encouraging, if slow and difficult. Progeny tests have shown generally low but usable levels of resistance from a relatively small proportion of the parent selections (*1, 2*). Apparently several