LITERATURE CITED

Cassady, J. M.; Howie, G. A. *J. Chem. Soc., Chem. Commun.* **1974**, 512-513.
Cook, C. E.; Whichard, L. P.; Turner, B.; Wall, M. E. *Science (Washington, D.C.)* **1966**, *154*, 1189-1190.
Cook, C. E.; Whichard, L. P.; Wall, M. E.; Egley, G. H.; Coggon, P.; Luhan, P. A.; McPhail, A. T. *J. Am. Chem. Soc.* **1972**, *94*, 6198-6199.
Crossland, R. K.; Servis, K. L. *J. Org. Chem.* **1970**, *35*, 3195-3196.
Heather, J. B.; Mittal, R. S. D.; Sih, C. J. *J. Am. Chem. Soc.* **1976**, *98*, 3661-3669.
Johnson, A. W.; Rosebery, G. U.S. Patent 4 002 459, 1977.
Johnson, A. W.; Rosebery, G.; Parker, C. *Weed Res.* **1976**, *16*, 223-227.
Kendall, P. M.; Johnson, J. V.; Cook, C. E. *J. Org. Chem.* **1979**, *44*, 1421-1424.
MacAlpine, G. A.; Raphael, R. A.; Shaw, A.; Taylor, A. W.; Wild, H.-J. *J. Chem. Soc., Perkin Trans. 1* **1976**, 410-416.

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Incorporation of Maleic Hydrazide into Ribonucleic Acid of *Saccharomyces cerevisiae*

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Maleic hydrazide [MH; 6-hydroxy-3(2*H*)-pyridazinone] was incubated with *Saccharomyces cerevisiae* and was found to be incorporated into the RNA of the cells where it substituted for cytosine rather than for uracil, its structural isomer. Incorporation was found to take place in the undifferentiated RNA fraction, as well as in tRNA, and may explain the mode of action of MH with regard to its ability to interfere with protein biosynthesis and cell growth.

Maleic hydrazide [MH; 6-hydroxy-3(2*H*)-pyridazinone] has enjoyed extensive use as a commercial plant growth inhibitor since its introduction in 1949 (Schoene and Hoffmann, 1949). Dilution of as low as 1 ppm (Leopold and Klein, 1951) inhibits the growth of a vast multiplicity of plants, but a few derivatives of MH do not exhibit any inhibitory activity at all (Greulach, 1953; Baskokov and Melnikov, 1954; Parups et al., 1962; Greulach and Plylar, 1966).

Kalinin and Troyan (1973) have confirmed and clarified earlier studies by showing that the MH–triethanolamine salt inhibited the incorporation of purine and pyrimidine bases and their nucleotides into nucleic acids, thereby inhibiting RNA and DNA synthesis (Ito and Yoshinaka, 1964; Evans and Scott, 1964; Dubinina and Dubinin, 1967; Kihlman and Hartley, 1967; Lobov, 1971, 1973) and thus interfering with amino acid incorporation into polypeptide chains, causing the accumulation of free amino acids (Biswas et al., 1966) and decreasing the protein content of the cells (Ito and Yoshinaka, 1964; Kato, 1970; Lobov, 1971, 1973).

The pyrimidines thymine, uracil, and thiouracil are antagonists of MH in that they will counteract its inhibiting properties (Butenko and Baskakov, 1961; Povolotskaya, 1961; Coupland and Peel, 1972a,b), suggesting that MH interferes with pyrimidine metabolism (Greulach, 1955). Since MH and uracil are isomers, a logical hypothesis would be that the former acts by interfering with the metabolism of the latter. Such a conclusion was made (Callaghan and Grun, 1961; Callaghan et al., 1962), when MH incorporation was found to follow the pathways of RNA synthesis.

Most of the above work was carried out on intact plants or cells derived from them. The purpose of the research presented here was to incubate MH with the single-celled *Saccharomyces cerevisiae* and to determine its fate within the cell as a means of gaining possible insights into its mode of activity.

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EXPERIMENTAL SECTION

In each of the following cases, 15 untreated control samples were run concurrently with 15 samples incubated with MH.

(1) Determination of MH in RNA. (*A*) *Cultivation of Yeast Cells.* Twenty-five cultures of *S. cerevisiae* were prepared in a medium consisting of glucose (40 g), sucrose (60 g), peptone (7 g), KH_2PO_4 (4 g), and $MgSO_4$ (2 g) in 1 L of distilled water (Lindegren, 1962). The pH was adjusted to 4.4 with diluted H_2SO_4, and the mixture subjected to continuous aeration at 36–38 °C for 2 h. Then a solution of MH (0.122 g) in 50 mL of distilled water was added to half the samples (giving solutions 0.001 M in MH) and 50 mL of distilled water was added to the rest, and the cultures were further incubated at 36–38 °C for an additional 2 h.

(*B*) *Isolation of the RNA.* The method is that of Georgiev et al. (1963) as modified by Rubin (1965). The above yeast cells were harvested by centrifugation at 1450*g* for 15 min at 0 °C. They were weighed and placed in a Mason jar with twice their weight of fine glass beads. They were then ruptured by using an Omnimixer at 5000 rpm for 40 min at 0 °C. To this cell homogenate were added 50-mL portions of 0.14 M NaCl and 90% phenol (adjusted to pH 6.0 with 1 N NaOH), as well as EDTA (0.12 g) to inhibit RNase activity. This mixture was stirred at 1000 rpm for 15 min at 0–10 °C and then centrifuged at 1450*g* for 15 min at 0 °C. The uppermost aqueous layer was removed with a pipet, and the RNA precipitated from it by adding twice its volume of 95% ethanol containing 2% potassium acetate. The two lower layers were discarded.

(*C*) *Purificaton of the RNA.* To a suspension of the above RNA in 50 mL of citrate buffer (0.05 M, pH 6.9) in

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a separatory funnel were added 35 mL of 90% phenol (adjusted to pH 6.0 with 1 N NaOH), 35 mL of chloroform, and 0.08 g of EDTA (Sibatini et al., 1960). Deproteinization was effected by shaking this system for 15 min in the cold. The lower phenol–chloroform layer was drawn off and discarded. The RNA was precipitated from the aqueous layer by adding twice its volume of 95% ethanol containing 2% potassium acetate. It was resuspended in 35 mL of 0.005 M $MgCl_2$ containing 0.35 g of DNase and allowed to stand overnight at 0 °C. The DNase was removed by extraction with 35 mL of 90% phenol (adjusted to pH 6.0), and the RNA then precipitated from the aqueous phase by adding of twice its volume of 0.005 M EDTA in 95% ethanol. Finally, the RNA was resuspended in 50 mL of citrate buffer (0.05 M, pH 6.9) and dialyzed vs. distilled water at 0.5 °C for 24 h.

(*D*) *Alkaline Hydrolysis of the RNA.* The dialyzed RNA was precipitated by adding twice its volume of 95% ethanol and collected by centrifugation at 0 °C at 1450*g* for 15 min. Then the RNA "plug", dried in a current of warm air, was dissolved in 3 mL of 0.3 M KOH in a small centrifuge tube and incubated for 18 h at 37 °C (Davidson and Smellie, 1952).

(*E*) *Separation of the Ribonucleotides by Paper Electrophoresis.* The pH of the hydrolysate was adjusted to 4.0 with 70% $HClO_4$ at 0 °C, and the precipitated $KClO_4$ removed by centrifugation at 1450*g* for 15 min. The buffer system used was a citrate buffer, pH 3.5, prepared by diluting a mixture of 1 volume of 1.0 M sodium citrate and 3 volumes of 1.0 M citric acid to 20 volumes with distilled water (Davidson and Smellie, 1952). Ten microliters of the hydrolysate was applied to the paper strip and subjected to 300 V for 3 h (Crestfield and Allen, 1955); the strips were then removed and dried, and the nucleotides observed with a short-wave UV lamp (Holiday and Johnson, 1949).

(*F*) *Isolation, Identification, and Determination of the Ribonucleotides.* The separated nucleotides were carefully cut from the electrophoresis strips, eluted with 0.01 N HCl and centrifuged for 30 min at 1450*g* to remove any suspended paper. A blank was cut from each strip and treated in the same manner. Absorbency measurements made on each band by using a Beckman Model DU spectrophotometer at 260 nm identified band 1 as uridylic acid, band 2 as guanylic acid, band 3 as adenylic acid, and band 4 as cytidylic acid (Volkin and Carter, 1951). Quantitative determinations were effected by using the known molar extinction coefficients (Beaven et al., 1960).

(*G*) *Acid Hydrolysis of Uridylic Acid Fraction (Band 1).* Band 1 was removed from 12 control and 12 treated strips, and the material hydrolyzed by heating with 0.5 mL of $HClO_4$ in a 4-in. test tube for 2 (Marshak and Voge, 1951). A control containing 0.2 mg of MH in a 4-in. test tube was treated identically. After being cooled, the hydrolysates were diluted with 0.5 mL of distilled water and centrifuged for 15 min at 1450*g* to separate the liquid from the blank sugar phosphate residue.

(*H*) *Identification of MH by TLC.* The nucleotide hydrolysates were neutralized with NH_4OH and placed on filter paper to dry. They were subjected to Soxhlet extraction with absolute ethanol for 2 h, the ethanol extracts evaporated to dryness, and the solid residues dissolved in 1 mL of distilled water. Five microliters of each was spotted 3 cm from the end of a thin layer composed of Mallinckrodt Silicar TLC and developed for 4 h in 60:30:10 2-propanol–1-butanol–water. After air-drying, the plates were sprayed with a freshly prepared 1:1 mixture of 1% aqueous $FeCl_3$ and 1% aqueous $K_3Fe(CN)_6$, producing blue spots in the presence of MH (Andreae, 1958).

Table I. Molar Extinction Coefficients of Ribonucleotides[a] in 0.01 M HCl

ribonucleotide	ϵ	position
uridylic acid	9 930	band 1
guanylic acid	11 800	band 2
adenylic acid	13 900	band 3
cytidylic acid	12 750	band 4

[a] Volkin and Carter (1951).

(2) Determination of MH in tRNA. (*A*) *Cultivation of Yeast Cells.* This was identical with that described under part 1A.

(*B*) *Isolation of tRNA.* The method was adapted from Holley et al. (1961). After incubation, 25 g of Celite was added to the yeast cells and the suspension suction filtered. The resultant paste was added to 50 mL of water and 112 mL of water-saturated phenol, shaken at room temperature for 1 h, and refrigerated overnight. It was then centrifuged for 15 min at 2500 rpm and 0 °C. The upper aqueous layer and middle layer were recentrifuged, and the separated aqueous layer was mixed at room temperature with 0.1 of its volume of 20% potassium acetate buffer (pH 5.0), followed by 2.5 volumes of cold 95% ethanol. After standing overnight at −20 °C, the clear supernatant was decanted and the white precipitate collected by centrifugation. This solid was dissolved in 12.5 mL of tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer, pH 7.5, and the insoluble material removed by a 30-min centrifugation. The dissolved tRNA was extracted twice at 0 °C with 25 mL of ether and insoluble material again removed by centrifugation. The solution was added at a flow rate of 8–10 drops/min to an ice-water jacketed column containing 2.5 g of DEAE-cellulose which had been previously equilibrated with cold 0.1 M Tris-HCl buffer (pH 7.5). The column was washed with an additional 65 mL of Tris-HCl buffer and then eluted with 7 mL of 1.0 M NaCl in 0.1 M Tris-HCl buffer. The first 3 mL of this eluant was discarded, and the remaining 4 mL treated with 3 volumes of 95% ethanol and the mixture kept overnight at −20 °C. The clear supernatant was decanted and the tRNA collected by centrifugation. It was washed first with 80% ethanol and then with 95% ethanol and finally dried in vacuo for 4 h and ground to a powder.

(*C*) *Alkaline Hydrolysis of tRNA.* Sixty milligrams of tRNA was hydrolyzed in 3 mL of 0.3 N KOH as described under part 1 D.

(*D*) *Separation of the Ribonucleotides by Paper Electrophoresis.* This procedure was identical with part 1E.

(*E*) *Acid Hydrolysis of Uridylic Acid Fraction (Band 1).* The method was identical with part 1F.

(*F*) *Identification of MH by TLC.* The method was identical with part 1G.

(*G*) *Isolation of MH–Nucleotide by Paper Electrophoresis.* Ten microliters of the tRNA hydrolysate (parts 2B and 2C) was applied to the paper strip and subjected to 300 V for 90 min. A Tris-HCl buffer system (pH 7.5) was used. The strips were removed and dried and the nucleotides (bands A and B) observed with a short-wave UV lamp (Holiday and Johnson, 1949).

(*H*) *Identification of MH in Band B.* Band B of the above electrophoresis was treated as described in parts 1F and 1G above and proved to be MH. Band A was negative when so treated.

RESULTS AND DISCUSSION

The mole fractions of ribonucleotides separated by paper electrophoresis were determined spectrophotometrically by using the extinction coefficients listed in Table I. The

Table II. Mole Fractions of Ribonucleotides in Treated and Untreated RNA ($N = 15$)

band	source of value	mole fraction[a]	significance[b]
uridylic acid (no. 1)	literature[c]	0.240 ± 0.003	
	control	0.239 ± 0.005	
	MH treated	0.379 ± 0.005	$P < 0.001$
guanylic acid (no. 2)	literature[c]	0.276 ± 0.003	
	control	0.276 ± 0.005	
	MH treated	0.275 ± 0.005	NS
adenylic acid (no. 3)	literature[c]	0.268 ± 0.003	
	control	0.268 ± 0.005	
	MH treated	0.267 ± 0.004	NS
cytidylic acid (no. 4)	literature[c]	0.216 ± 0.005	
	control	0.216 ± 0.005	
	MH treated	0.079 ± 0.005	$P < 0.001$

[a] Arithmetic mean ± standard deviation. [b] Probability of no significant difference from the t test. [c] Guschlbauer et al. (1965).

Table III. Mole Fractions of Ribonucleotides in Treated and Untreated tRNA ($N = 15$)

band	source of value	mole fraction[a]	significance[b]
uridylic acid (no. 1)	literature[c]	0.246	
	control	0.225 ± 0.004	
	MH treated	0.248 ± 0.004	$P < 0.001$
guanylic acid (no. 2)	literature[c]	0.300	
	control	0.303 ± 0.004	
	MH treated	0.299 ± 0.004	NS
adenylic acid (no. 3)	literature[c]	0.192	
	control	0.194 ± 0.004	
	MH treated	0.197 ± 0.004	NS
cytidylic acid (no. 4)	literature[c]	0.284	
	control	0.277 ± 0.004	
	MH treated	0.255 ± 0.004	$P < 0.001$

[a] Arithmetic mean ± standard deviation. [b] Probability of no significant difference from the t test. [c] Cantoni et al. (1963).

results obtained for 15 samples of MH-treated RNA and 15 untreated control samples of RNA are catalogued in Table II.

Since MH is an isomer of uracil, it was anticipated that substitution of it for uracil in the RNA would occur. The data of Table II show, however, that 63% of the cytosine in the RNA had been replaced by MH, with a corresponding increase in the content of band 1 (uridylic acid plus MH–nucleotide).

So that the presence of MH in the uridylic acid fraction could be confirmed, band 1 was separated and hydrolyzed and the constituent bases were subjected to TLC. Duplicate samples of control RNA, treated RNA, and pure MH were run; the treated RNA exhibited spots identical with those of the pure MH (R_f 0.86), whereas the control did not. The nature of the MH was confirmed by the $FeCl_3$–$K_3(CN)_6$ color test.

Proof that the MH was incorporated also into tRNA was obtained by isolating the tRNA, hydrolyzing it, and analyzing its ribonucleotide content spectrophotometrically. The results are contained in Table III and indicate once again that MH has substituted for cytosine in the tRNA.

Band 1 was separated as before and hydrolyzed and the bases were subjected to TLC. Again the treated sample gave a spot corresponding to that given by pure MH (R_f 0.86) and confirmed by the color test, while the control did not.

The original tRNA hydrolysate was again subjected to paper electrophoresis, this time in a Tris-HCl buffer at pH 7.5. Under these conditions, the four usual ribonucleotides are identically charged and thus travel at about the same rate, giving a single band (band A). But the MH–ribonucleotide, bearing a higher charge, moves further to form a second band (band B). The contents of this band were confirmed by TLC and the spot color test to be MH.

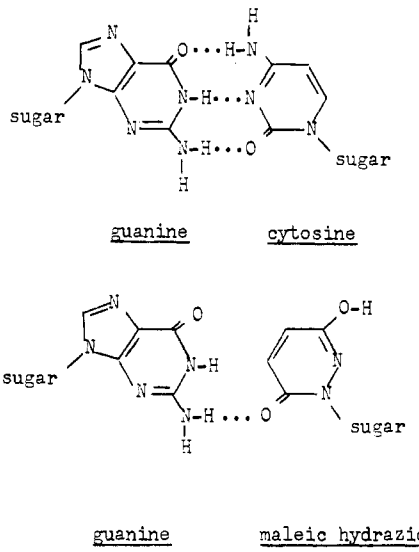

Figure 1. Hydrogen bonding within nucleic acids between guanine–cytosine and guanine–maleic hydrazide.

MH, then, is absorbed by *S. cerevisiae* and converted into a nucleotide which is incorporated into the RNA of the cell, displacing cytidylic acid.

The cloverleaf pattern of typical specific tRNA is well-known. The anticodon, by which the tRNA is attached to the proper codon on the mRNA to give the required sequence, is distinctive, but the sequence on the 3′ end holding the amino acid is common to every tRNA.

Two possibilities exist to explain the effects of the MH. First, they may stem from the substitution of MH for cytosine (opposite guanine along the DNA template) during the original formation of the tRNA strand. A similar theory has been advanced regarding 5-fluorouracil, which also replaces cytosine in RNA (Bujard and Heidelberger, 1966). This means that MH must have some ability to hydrogen bond with the guanine in the DNA, and its presence in the cell in concentrations much higher than that of cytosine would explain its considerable substitution in terms of mass action. Once the strand has formed, it must fold up into the proper arrangement and be held in it by hydrogen bonding. Again, these would involve many guanine–MH interactions.

Figure 1 compares the hydrogen bonding potentials of the guanine–cytosine and the guanine–MH pairs. As can be seen, the strong triple hydrogen bonding in the former is reduced to a single interaction in the latter. The pairing of MH with other bases in RNA has been considered (Cradwick, 1975). The possibility of the coordination of it with adenine, as illustrated there, has been ruled out by the present work. Cradwick does mention the alternative of a pairing with guanine, but only if the maleic hydrazide exists in a diketo form. Our proposal leaves it in its usual hydroxypyridazinone form. Such an incorporation might have two effects. First, it might reduce the rate of formation of the tRNA, thus lowering its concentration in the cell and the rate at which protein synthesis can occur. Second, and more important, it might seriously impair the stability of the cloverleaf structure of the tRNA, which would tend to denature and become inactive.

On the other hand, incorporation of MH into mRNA would disrupt protein biosynthesis on both the transcription and translation levels and would interfere with the

binding of tRNA and proper sequence of amino acids in the polypeptide chain.

Further work will be needed to choose between these possibilities. There is, of course, no present proof that MH acts in an identical fashion in higher plants.

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LITERATURE CITED

Andreae, W. A. *Can. J. Biochem. Physiol.* **1958**, *36*, 71.
Baskokov, Y. A.; Melnikov, N. N. *Zh. Obshch. Khim.* **1954**, *24*, 1216.
Beaven, G. H.; Holiday, E. R.; Johnson, E. A. In "The Nucleic Acids"; Chargaff, E.; Davidson, J. N., Eds.; Academic Press: New York, 1960; Vol. I, p 493.
Biswas, P. K.; Hall, O.; Mayberry, B. D. *Weeds* **1966**, *14*, 329.
Bujard, H.; Heidelberger, C. *Biochemistry* **1966**, *5*, 3339.
Butenko, R. G.; Baskokov, Y. A. *Plant Physiol.* **1961**, *7*, 323.
Callaghan, J. J.; Appleton, M. D.; Haab, W.; Portanova, R. *Proc. Penna. Acad. Sci.* **1962**, *36*, 91.
Callaghan, J. J.; Grun, P. *Biophys. Biochem. Cytol.* **1961**, *10*, 567.
Cantoni, G. L.; Ishikura, H.; Richards, H. H.; Tanaka, K. *Cold Spring Harbor Symp. Quant. Biol.* **1963**, *28*, 123.
Cradwick, P. D. *Nature (London)* **1975**, *258*, 774.
Coupland, D.; Peel, A. J. *Planta* **1972a**, *103*, 249.
Coupland, D.; Peel, A. J. *Planta* **1972b**, *105*, 66.
Crestfield, A. M.; Allen, F. W. *Anal. Chem.* **1955**, *27*, 424.
Davidson, J. N.; Smellie, R. M. S. *Biochem. J.* **1952**, *52*, 594.
Dubinina, L. G.; Dubinin, N. P. *Dokl. Akad. Nauk. SSSR* **1967**, *175*, 213.
Evans, H. J.; Scott, D. *Genetics* **1964**, *49*, 17.
Georgiev, G. P.; Lamarina, O. P.; Smirnov, M. N.; Lerman, M. I.; Severtzov, A. N. *Nature (London)* **1963**, *201*, 1291.
Greulach, V. A. *Science (Washington, D.C.)* **1953**, *117*, 601.
Greulach, V. A. *J. Elisha Mitchell Sci. Soc.* **1955**, *71*, 2.
Greulach, V. A.; Plylar, D. B. *J. Elisha Mitchell Sci. Soc.* **1966**, *82*, 18.
Guschlbauer, W.; Richards, E. G.; Beurling, K.; Adams, A.; Fresco, J. R. *Biochemistry* **1965**, *4*, 964.
Holiday, E. R.; Johnson, E. A. *Nature (London)* **1949**, *163*, 216.
Holley, R. W.; Apgar, J.; Doctor, B. P.; Farrow, J.; Marini, M. A.; Merrill, S. H. *J. Biol. Chem.* **1961**, *236*, 200.
Ito, H.; Yoshinaka, Y. *Kobe Diagaku Kyoikugakubu Kenkyu Shuroku, Shizen Kagaku-hen* **1964**, *32*, 69.
Kalinin, F. L.; Troyan, V. M. *Gidgazid Maleinovoi Kisloty. Regul. Rosta Rast.* **1973**, 246.
Kato, T. *Engei Gakkai Zasshi* **1970**, *39*, 178.
Kihlman, B. A.; Hartley, B. *Exp. Cell Res.* **1967**, *48*, 629.
Leopold, A. C.; Klein, W. H. *Science (Washington, D.C.)* **1951**, *114*, 9.
Lindegren, C. C. "Yeast Genetics"; Biological Research Laboratory, Southern Illinois University: Carbondale, IL, 1962.
Lobov, V. P. *Fiziol. Rast. (Moscow)* **1971**, *18*, 116.
Lobov, V. P. *Gidrazid Maleinovoi Kisloty. Regul. Rosta Rast.* **1973**, 234.
Marshak, A.; Vogel, H. J. *J. Biol. Chem.* **1951**, *189*, 597.
Parups, E.; Hoffman, I.; Morley, N. V. *Can. J. Biochem. Physiol.* **1962**, *40*, 1159.
Povolotskaya, K. L. *Izv. Akad. Nauk. SSSR, Ser. Biol.* **2**, 250.
Rubin, C. S. Undergraduate Thesis, University of Scranton, 1965.
Schoene, D. L.; Hoffman, O. L. *Science (Washington, D.C.)* **1949**, *109*, 588.
Sibatini, A.; Yamana, K.; Kimura, K.; Takahashi, T. *Nature (London)* **1960**, *198*, 215.
Volkin, E.; Carter, C. E. *J. Am. Chem. Soc.* **1951**, *73*, 1516.

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Crystal and Molecular Structure of Herbicides. 4. Bromoxynil

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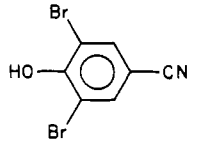
The crystal and molecular structure of bromoxynil [3,5-dibromo-4-hydroxybenzonitrile, $C_7H_3NBr_2O$, triclinic, $P\bar{1}$, a = 9.154 (3) Å, b = 11.752 (3) Å, c = 8.345 (4) Å, α = 109.03 (3)°, β = 105.62 (4)°, γ = 95.87 (5)°, Z = 4, Mo Kα radiation, ρ_{calcd} = 2.301 g cm^{-3}, μ = 106.8 cm^{-1} (correction made)] has been determined by three-dimensional X-ray analysis. The structure was solved by direct methods and refined to a final R = 0.065 for 1606 observed reflections [$|F_o| > 3\sigma(F_o)$]. Bromoxynil crystallizes with two nearly identical molecules in the asymmetric unit.

Bromoxynil (I) is a known inhibitor of the Hill reaction



(I)

(photosynthetic electron transport) as it will cause 50% inhibition at a concentration of 18 μM. As a result, the plant's energy supply is disrupted, eventually leading to death (Corbett, 1974).

One would like to have a good understanding of the exact mechanism(s) being followed in the course of a herbicide's interaction so that the compound will selectively eliminate unnecessary plants yet will not harm crops or man. It is usually assumed that Hill reaction inhibitors operate by binding to some site or sites in the chloroplast. If this is correct, and it is difficult to see how some other mechanism could operate, one would ideally require information about the nature of the site. We have embarked on a series of molecular structure determinations of herbicides (Baughman et al., 1978, 1980a,b) to better infer important structural features of this site. The distance and angle information afforded by X-ray crystallography can supply this information.

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