Phytochemistry, 1966, Vol. 4, pp. 851 to 856. Pergamon Press Ltd. Printed in England

PHYSICO-CHEMICAL STUDIES OF THE METABOLIC END-PRODUCT OF 3-AMINO-1,2,4-TRIAZOLE IN YEAST

JEROME F. FREDRICK and ARTHUR C. GENTILE

New York Research Laboratories of the Dodge Chemical Company (Boston), Bronx, N.Y.; and Department of Botany, the University of Massachusetts, Amherst, Mass.

(*Received* 17 *May* 1965)

Abstract—The first step in the metabolism of the phytotoxic compound, 3-amino-1,2,4-triazole in yeast is its linking with glucose to form an amine glucoside. This compound may then be phosphorylated by hexokinase and split by yeast aldolase. Isolation of the end-product of this metabolism has made possible the delineation of its physico-chemical properties and an interpretation of its molecular structure via infrared studies.

INTRODUCTION

3-AMINO-1,2,4-TRIAZOLE (3AT) has been involved in a wide spectrum of phytotoxic reactions in many different species.[1–6] Its metabolic paths in different yeasts have recently been studied.[7–9] The intermediates which were isolated as a result of those studies show that the metabolism of 3AT while subject to individual minor variations in different species, is, nontheless generally similar.

The addition of 3AT metabolic intermediates, such as the amine glucoside, N-(1H-1,2,4-triazol-3-yl)-glucopyranosylamine, GAT, and its phosphorylated derivative, PGAT, to purified yeast extracts, has resulted in the isolation of a relatively inert metabolic end-product of 3AT.[10] Similar end-products have been reported by Massini[11] and Carter and Naylor.[12] There is general agreement that these end-products of phytometabolism contain the intact triazole nucleus with a three carbon side chain attached to the nucleus via the 3-amino group.[10–12]

The action of yeast aldolase on PGAT has been shown to liberate a phosphotriose which appears to be dihydroxyacetone phosphate, DHAP (Fig. 1). This finding indicates that the phosphorylated amine glucoside is split by the yeast enzyme into a phosphotriose and an aminotriazole fragment with a three carbon side chain.[10] The individual metabolic steps of 3AT in yeast are shown in Fig. 1.

The isolation and purification of this fragment containing the triazole nucleus, 3ATX,

[1] K. A. SUND, *Agric. Food Chem.* **4**, 57 (1956).
[2] K. A. SUND, *Physiol. Plantarum* **14**, 260 (1961).
[3] B. J. ROGERS, *Weeds* **5**, 5 (1957).
[4] D. RACUSEN, *Arch. Biochem. Biophys.* **74**, 106 (1958).
[5] J. F. FREDRICK and A. C. GENTILE, *Arch. Biochem. Biophys.* **86**, 761 (1960).
[6] C. G. MCWHORTER and W. K. PORTER, *Physiol. Plantarum* **13**, 444 (1960).
[7] J. F. FREDRICK, *Physiol. Plantarum* **14**, 734 (1961).
[8] A. C. GENTILE and J. F. FREDRICK, *Physiol. Plantarum* **12**, 862 (1959).
[9] J. F. FREDRICK, *Physiol. Plantarum* **15**, 186 (1962).
[10] J. F. FREDRICK, *Int. J. Exp. Botany* **22**, 67 (1965).
[11] P. MASSINI, *Biochim. et Biophys. Acta* **36**, 548 (1959).
[12] M. C. CARTER and A. W. NAYLOR, *Physiol. Plantarum* **14**, 62 (1961).

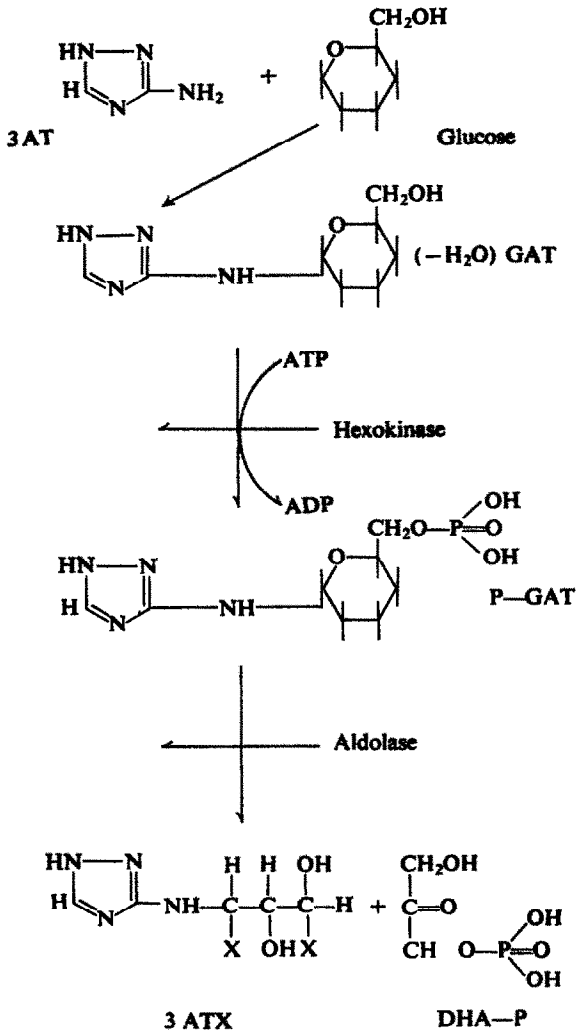


FIG. 1. THE METABOLIC PATHWAY OF 3-AMINO-1,2,4-TRIAZOLE IN YEASTS.

The diagram shows a summary of the various intermediates which have been isolated using *Cryptococcus neoformans* and *Saccharomyces cerevisiae*. The metabolic intermediates appear to be much the same in all plant life treated with the defoliant.

has made possible the delineation of its molecular structure via i.r. and other physicochemical studies, the results of which are reported here.

RESULTS

The i.r. spectrum of the isolated compound, 3ATX, is shown in Fig. 2. Although the two positions shown in Fig. 1 for this compound are somewhat in doubt, the absence of carboxyl groups in the compound appears certain. The absorption maxima associated with the carboxyl group or its charged anion are missing from the 5·4 μ–6·5 μ region of its spectrum.[13]

[13] R. J. KOEGEL, R. A. MCCALLUM, J. P. GREENSTEIN, M. WINITZ and S. M. BIRNBAUM, *Ann. N.Y. Acad. Sci.* 69, 94 (1957).

The relatively strong absorption peak at 2·8 μ–2·9 μ appears to be due to stretching vibrations of the hydroxyl groups.[14,15] The presence of both primary (CH_2OH) and secondary (CHOH) hydroxyl groups is indicated by the many well-defined peaks in the 6·5 μ–7·5 μ region of the i.r. spectrum.[14,15] In fact, a comparison of the spectrum of 3ATX with that of (±)-glyceraldehyde in this region shows that the peaks for each are identical (Fig. 3). This area of the spectrum is assigned to bending vibrations of the —O—H groups in alcohols.[16,17]

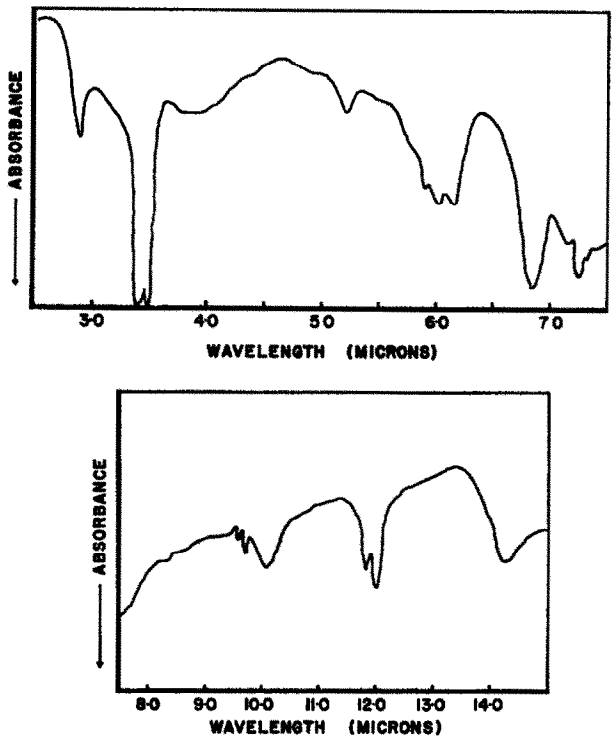


FIG. 2. THE I.R. SPECTRUM OF THE END-PRODUCT OF 3AT METABOLISM IN YEAST.
Top, the i.r. spectrum for the region 2·5 μ to 8·0 μ. Bottom, the spectrum from 8·0 μ to 15·0 μ. See text for frequency assignments of groups in molecule.

If the spectrum of 3AT is subtracted from that of 3ATX, using the method suggested by Jones,[18] the differential spectrum resulting therefrom is identical with that of glycerol with the exception of some very strong absorption maxima which appear in the 3ATX spectrum in the 3·4 μ–3·5 μ region; these peaks are indicative of C—H stretching vibrations.[19]

The isolated and purified compound does not react with ninhydrin indicating the absence of an alpha-amino group. This conclusion is supported by the i.r. spectrum since

[14] H. H. ZEISS and M. TSUTSUI, *J. Am. Chem. Soc.* **75**, 897 (1953).
[15] A. D. CROSS, *Practical Infra-Red Spectroscopy*, p. 61. Butterworths, London (1960).
[16] E. K. PLYLER, *J. Research Nat. Bur. Standards* **48**, 281 (1952).
[17] L. P. KUHN, *J. Am. Chem. Soc.* **74**, 2492 (1952).
[18] R. N. JONES, E. AUGDAHL, A. NICKON, G. ROBERTS and D. J. WHITTINGHAM, *Ann. N.Y. Acad. Sci.* **69**, 38 (1957).
[19] R. N. JONES and C. SANDORFY, *Chemical Applications of Spectroscopy*, p. 247, Interscience, New York (1956).

maxima associated with the amino group are missing.[13] The other absorption maxima in the 3ATX spectrum are all associated with the triazole nucleus.[20,21]

Impure 3ATX when chromatographed on Whatman No. 3MM paper in a solvent system of *n*-butanol–acetic acid–water, does indicate traces of an amino acid which appears to be alanine. However, the purified compound is chromatographically homogeneous and shows no traces of ninhydrin-positive material.

Electrophoresis of 3ATX on Whatman No. 1 paper indicated that the compound always moved towards the cathode, regardless of the pH of the buffer. This cathodic migration was fairly constant at pH values of 5·0 to 9·5, and was on the average 2·5 mm. These electro-

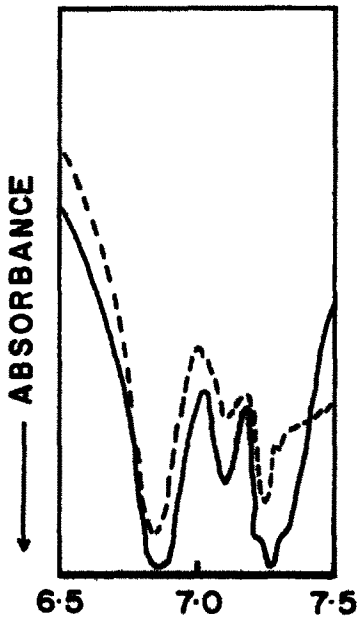


FIG. 3. COMPARISON OF 6·5 μ–7·5 μ REGIONS OF THE I.R. SPECTRA OF 3ATX AND DL-GLYCERALDEHYDE. Note that this region, indicative of stretching and bending vibrations of alcoholic O—H groups is identical. (----) is 3ATX; (——) is DL-glyceraldehyde.

phoretic studies substantiate the i.r. data as to the lack of carboxyl groups in the molecule. The cathodic migration is probably due to the ring nitrogens and the secondary amino group in position 3 (Fig. 1).

DISCUSSION

The end-product of 3AT metabolism in plants has been the subject of intensive investigations. Massini postulated that this metabolic end-product was 3-amino-1,2,4-triazolyl-alanine,[11] and indeed, Carter and Naylor have found that their "Compound 1" isolated from beans, gave a violet–blue color with ninhydrin. This finding would be in line with the observation that the 3-amino side-chain contains an alanine residue, possessing an alpha

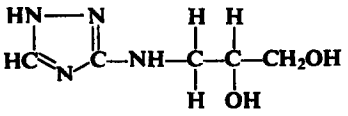
[20] J. F. FREDRICK and A. C. GENTILE, *Arch. Biochem. Biophys.* **92**, 356 (1961).
[21] R. L. M. SYNGE, *Biochem. J.* **48**, 429 (1951).

amino group.[12] However, they were unable to account for certain other reactions of the compound which left doubt as to its identity with Massini's compound.

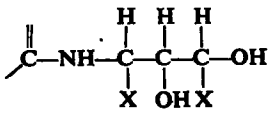
It is of interest that the 3ATX isolated from the present studies, when chromatographed *before* purification, gave a ninhydrin-positive additional spot which had the R_f values associated with alanine.[21] When the compound was purified, this spot vanished. It is possible that a coprecipitation of alanine or other amino acids present in the extract with 3ATX occurred.

The i.r. spectrum indicates that the 3ATX is devoid of carboxyl groups. This evidence is further substantiated by the behaviour of the compound upon electrophoresis. Contrary to Carter and Naylor's observations on their "Compound 1", no anodic migration of 3ATX was observed.[22] Likewise, the absence of a free amino group leaves little doubt that 3ATX is not an amino acid derivative of 3AT.

The fact that 3ATX and dihydroxyacetone phosphate result from the action of purified yeast aldolase on the phosphorylated derivative of the amine glucoside of 3AT, would add to the i.r. data in support of structure (I) for this metabolic end-product.[10] Since the fragment



(I)



(II)

which logically would result from the splitting of PGAT by aldolase with the liberation of DHAP must have the minimum structure (II) in the side-chain (Fig. 1) it is obvious that two hydrogens must attach at the "X" positions. The strong absorption in the infra-red at 3·4 μ–3·5 μ, indicates that N-carbon of the side-chain must be a methylene group (Fig. 2). Ample evidence is available in the i.r. spectrum of 3ATX to substantiate the primary and secondary alcoholic hydroxyl groups. If the O—H bending regions (6·5 μ–7·5 μ) are compared for (±)-glyceraldehyde and 3ATX, the identity seems obvious (Fig. 3). Therefore, it would seem that the structure shown above for 3ATX is the only logical one. Further studies are necessary to explain the hydrogenation of the two carbons in the propanediol side-chain. These studies are now underway.

EXPERIMENTAL

3AT was added to purified yeast extracts.[10] After 4 hr incubation at 30°, the extracts were added to equal volumes of absolute ethanol and boiled for 2–3 min. The hot extracts were filtered through Whatman No. 54 paper, and the paper was washed with 3 ml of hot deionized water. The combined filtrates were concentrated to ¼ vol. over a steam bath.

The concentrate was passed through Amberlite IR-120 C.P. resin (H^+ form) in a prepared column (Mallinckrodt, St. Louis, Missouri). The column was subsequently washed with 50 ml of deionized water followed by 50 ml of 80% ethanol and again by 50 ml of water. The column was eluted with 25 ml of 1·0 N-HCl. Each fraction was tested with Sund's alkaline nitroprusside reagent.[1] Those fractions of the eluate giving a positive reaction were combined and concentrated to near-dryness over a steam bath. The residue was taken up with 10 ml of boiling 80% ethanol. An amorphous white precipitate separated after 4 hr at

[22] M. C. CARTER and A. W. NAYLOR, *Physiol. Plantarum* 14, 20 (1961).

20°. This precipitate was washed with cold 80% ethanol and chromatographed on Whatman No. 3 MM paper using *n*-butanol: acetic acid: water (4:1:5 v/v) as the developing solvent.[10] The area of the chromatograph at R_f 0·08–0·10 was cut out and eluted with hot ethanol. The eluate was concentrated as described above and the white crystalline precipitate was dried *in vacuo* at room temperature.

The crystalline material thus obtained was used for all of the tests described. The i.r. studies were done with a mull of the material in Nujol; the scanning was with a Perkin–Elmer Infracord Spectrophotometer.

The electrophoretic studies were performed using the technique of Carter and Naylor.[22]

Acknowledgements—This study was supported in part by a grant from the Professor J. Parker Memorial Fund of the Dodge Institute for Advanced Studies, Miami, Fla. (JFF) and by grant CA 04052-07 from the Public Health Service, National Cancer Institute (ACG).

The authors are indebted to Dr. Louis A. Carpino for the i.r. spectroscopy.