Table 2. The response of Walker carcinosarcoma 256 to aspartic acid, glutamic acid, and their amides.*

Concentration in final medium	Response (cells × 10^{-3}/ml)†			
	L-Aspartic acid	L-Glutamic acid	L-Asparagine	L-Glutamine
(m*M*)				
0	626	538	25	18
0.01			56	
0.02			241	
0.05	572	518	996	
0.1	696	522	1013	80
0.2	476	480	1032	212
0.5			1038	632
1.0	603	396	990	976
1.5				830
2	664	351	879	905
3				659
5	559	128	828	504
10	667	53	779	376
20	464	20	638	312

* Cells were grown 72 hours in basal medium described containing the single amino acid or amide in the concentration indicated. Medium was renewed at 48 hours. The initial inoculum was 200,000 cells per milliliter.
† Corrected to compare with initial volume.

for asparagine. The optimal concentration of L-glutamine appeared to be approximately 1.5m*M*, while the optimal concentration of L-asparagine was approximately 0.2 m*M*. These observations were confirmed using cells that had become established in the complete medium and were then exposed to a deficient medium. When the concentration of L-glutamine or L-asparagine was in excess of 10m*M*, a moderate inhibiting effect was observed.

In attempts to replace the asparagine and glutamine requirement, neither L-aspartic acid nor L-glutamic acid, with or without ammonium chloride and ATP, were able to replace the requirement for its corresponding amide. Massive quantities of glutamine were incapable of replacing the requirement for asparagine, nor could asparagine replace the requirement for glutamine. The specific requirement for both asparagine and glutamine by the Walker tumor is particularly interesting, since we have been unable to find evidence of a living system, mammalian or microbiological, which requires both of these amides and exhibits no requirement for the corresponding amino acids (*8*).

ROBERT E. NEUMAN
THOMAS A. MCCOY
Biomedical Division, Samuel Roberts Noble Foundation, Ardmore, Oklahoma

References and Notes

1. T. A. McCoy and R. E. Neuman, *J. Natl. Cancer Inst.* 16, 1221 (1956).
2. R. C. Parker, *Methods in Tissue Culture* (Hoeber, New York, ed. 2, 1950), p. 80.
3. W. H. Stein and S. Moore, *Cold Spring Harbor Symposia Quant. Biol.* 14, 179 (1949).
4. E. F. McFarren, *Anal. Chem.* 23, 168 (1951).
5. H. M. Chemical Co., Santa Monica, Calif.
6. H. Eagle, *Science* 122, 501 (1955).
7. ——— *et al., J. Biol. Chem.* 218, 607 (1956).
8. We gratefully acknowledge the technical assistance of Jane Avery, Merle D. Maxwell, and Eugene Conway.

6 April 1956

Induced Biosynthesis of Uricase in Yeast

Uric acid is assimilated by *Torulopsis utilis* (*1*), and the presence of uricase in adenine-adapted yeast has been noted (*2*). This report (*3*) deals with the uptake of uric acid by *T. utilis* cells and the subsequent increase in the uricase activity of cellular extracts.

Torulopsis utilis (ATCC 9950) was grown with aeration in the medium described previously (*2*); the cells were collected by centrifugation, washed with distilled water, and stored at 4°C until used. To increase the uricase content of the yeast, 1 g (wet wt.) of the yeast was aerated in 100 ml of medium of the same composition except that the nitrogen source was 0.1 mg/ml of uric acid. The decreasing uric acid content of the medium was followed by measurement of the optical density of a 1/10 dilution of the medium at 293 mμ after removal of the cells by centrifugation. To estimate the intracellular uric acid content, the sedimented yeast cells were suspended in 10 ml of distilled water and heated in a boiling-water bath for 10 minutes; the optical density of the extract that was obtained upon centrifugation was measured at 293 mμ. Uricase activities were measured by the method of Kalckar (*4*) on yeast extracts in *p*H 9.5 borate buffer obtained with the aid of a Hughes press (*5*) operated at dry-ice temperature. Protein was determined by the method of Lowry *et al.* (*6*) with bovine plasma albumin as the standard.

Results of typical experiments are illustrated in Fig. 1. There is a time lag of about 1 hour before the uric acid starts to disappear from the medium at a high rate (curve *A*). The disappearance of the uric acid from the medium is accompanied by the intracellular accumulation of uric acid (curve *B*); analyses of the cellular extracts with uricase show that the absorbing compound is uric acid. An estimate based on the cell count, cell volume, optical density of the medium, and optical density of the cellular extract indicated that upon completion of the removal of uric acid from the medium, the concentration of uric acid within the yeast cell is about 600 times the original concentration in the medium. The specific activity of the yeast uricase increases only after the rapid accumulation of uric acid by the yeast cell starts (curve *C*). The lag period prior to the rapid uptake of uric acid is abolished by aeration of the yeast in the medium for 2 hours prior to the addition of uric acid, the nitrogen source (curve *A'*). There is no uptake of uric acid in the absence of glucose, and anaerobic conditions do not prevent the uptake with glucose in the medium.

The effect of various inhibitors on the uptake of uric acid by the yeast cell was investigated. Potassium cyanide (0.038*M*), an inhibitor of uricase (*7*), completely stopped the uptake under all conditions. Sodium arsenate ($10^{-3}M$) inhibited the uptake if it was present during a 2-hour aeration period prior to the addition of uric acid but not if it was added with the uric acid after preaeration; if the yeast cells that have been exposed to arsenate for 2 hours are removed from the medium by centrifugation, washed, and resuspended in fresh medium without the inhibitor, uptake occurs upon the addition of uric acid. The inhibition of uptake by $10^{-3}M$ arsenate is prevented if 0.02*M* phosphate is present with the arsenate during the experiment.

A consideration of these results has led to the following tentative conclusions and working hypotheses. (i) In the system described, when there was a lag period in the induced synthesis of uricase, the lag is dependent on the penetration of the cell wall by the substrate. (ii) The accumulation of uric acid by the cell involves active transport (*8*). (iii) Glucose metabolism, accompanied by the

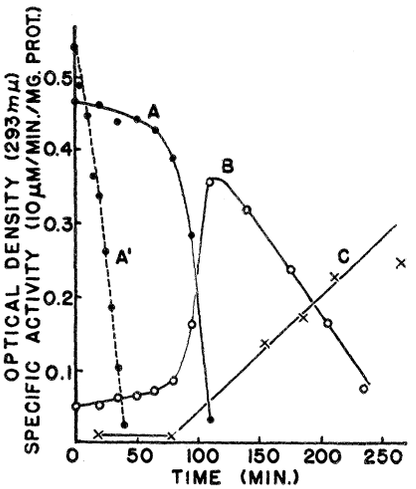


Fig. 1. The intracellular accumulation of uric acid by *T. utilis* and the resulting formation of uricase. (*A*) Optical density of the uric acid medium, yeast added at zero time; (*B*) optical density of the corresponding extracts of boiled yeast; (*C*) specific activity (uricase) of the corresponding extracts of frozen yeast; (*A'*) optical density of the uric acid medium, yeast aerated for 2 hours prior to the addition of uric acid to the medium at zero time.

formation of energy-rich phosphate bonds, probably at the glyceraldehyde-3-phosphate dehydrogenase step only, is required for the uptake of uric acid. This is consistent with the known action of arsenate (*9*) on glucose metabolism. (iv) After the maximal accumulation of uric acid by the yeast cell, the uricase activity of the cell increases at a constant rate until all of the uric acid is metabolized. (v) After the maximal accumulation of uric acid by the yeast cell, the uric acid is metabolized at a constant rate.

The properties of the yeast uricase are being investigated and compared with animal uricase. A detailed study of the uptake of uric acid by the yeast cell is in progress in these laboratories.

ALLAN H. ROUSH
A. J. DOMNAS
Department of Biology, Illinois Institute of Technology, Chicago

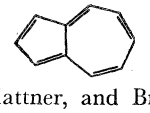
References and Notes

1. F. J. Di Carlo, A. S. Schultz, D. K. McManus, *J. Biol. Chem.* **189**, 151 (1951).
2. A. H. Roush, *Arch. Biochem. and Biophys.* 50, 510 (1954).
3. This investigation was supported by research grant C-2510 from the National Cancer Institute, U.S. Public Health Service.
4. H. M. Kalckar, *J. Biol. Chem.* **167**, 461 (1947).
5. D. E. Hughes, *Brit. J. Exptl. Pathol.* **32**, 97 (1951).
6. O. H. Lowry *et al.*, *J. Biol. Chem.* **193**, 265 (1951).
7. H. R. Mahler, G. Hübscher, H. Baum, *ibid.* **216**, 625 (1955).
8. H. T. Ussing, in *Ion Transport across Membranes*, H. T. Clarke, Ed. (Academic, New York, 1954).
9. O. Warburg and W. Christian, *Biochem Z.* **303**, 40 (1939).

26 March 1956

On the Space Group and Molecular Orientation of Azulene

In their investigation of the structure of the nonbenzenoid hydrocarbon azulene,



Günthard, Plattner, and Brandenberger (*1*) reported that the x-ray space group extinctions of the crystal correspond to $P2_1/a$, and that the unit cell contains 2 molecules. This requires positioning of the molecules on centers of symmetry, which is possible only if disorder is present. Günthard (*2*) was unable to solve the structure in this space group.

We have reexamined the structure (*3*). Lattice constants of the monoclinic crystal are $a = 7.89 \pm 0.03$ A, $b = 6.06 \pm 0.03$ A, $c = 7.94 \pm 0.03$ A, $\beta = 103° \pm 0.5°$, $a:b:c = 1.301:1:1.130$. We also find x-ray extinctions which would lead one to assign $P2_1/a$ as space group. The calculated specific gravity of 1.165 compares well with the measured value of 1.17. There are indeed 2 molecules per cell. A test for piezoelectricity was negative.

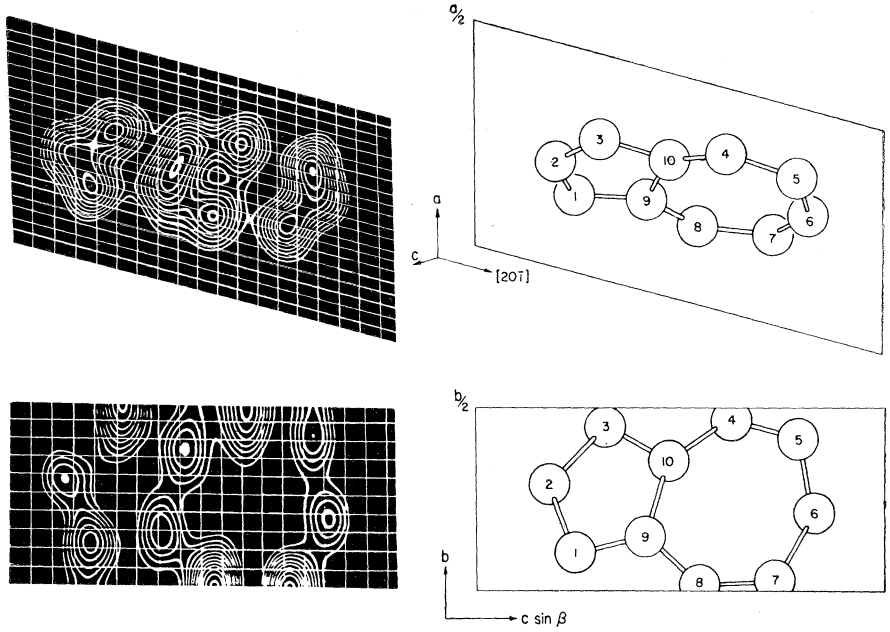


Fig. 1. Fourier projections of azulene on (010) and (100) planes and corresponding projections of molecule.

There is no evidence of disorder in any layer lines of the Weissenberg patterns. If random orientation of the molecules is present, it is thus on a molecular level; that is, there are no small ordered regions, of the nature of twin domains. Since the dipole strength of azulene is high, such a random arrangement is unlikely; that is, one would at least expect to find small ordered domains. We have examined the possibility of the random arrangement in $P2_1/a$ crystallographically; and we have compared this possibility with consequences of the assumption of a space group of lower symmetry—either $P2_1$ or Pa, with the extinctions which suggest a screw-axis along b owing to a pseudo translational symmetry in that direction. In the case of the lower symmetries, we have assumed that piezoelectricity is unobservable owing to weak electromechanical coupling.

$P2_1$ can be excluded because the glide a cannot be included as a pseudo-symmetry. Pa is a distinct possibility. If the projection of molecules on the b-axis is symmetrical about a point lying halfway between the glide planes, $(0k0)$ reflections will appear only if k is even; this would then explain the extinctions that led one to expect a twofold screw-axis. Taking the glide planes at $(x,0,z)$ and $(x,1/2,z)$, the polar axis of azulene will be on or very close to $(x,1/4,z)$ when adjoining molecules are at most reasonable distances.

Intensity data were obtained by the multiple-film technique using $CoK\alpha$ radiation ($\lambda = 1.79$A). The approximate orientation of the molecule on the (010) plane was derived from a weighted reciprocal lattice $(h0l)$, in which strong reflections were observed in a distorted hexagonal arrangement. The vector transform of the projected molecule thus derived was in good agreement with the peaks of a sharpened Patterson, computed using $F^2(h0l)/\Sigma f_i^2$ as coefficients. Since glide a is the only symmetry element, the molecule could be located at any place in the asymmetric unit of the (010) projection. For the phase calculation, the center of the polar axis of the molecule was located at (0,1/2). The real part of the structure factor for Pa then corresponds to the *entire* factor for the case of a disordered structure in $P2_1/a$; and it thus is a simple matter to compare possible structures in Pa and $P2_1/a$.

Symmetry Pa was fully supported by successive S-FAC and X-RAC calculations, which resulted in a well-defined Fourier projection $\rho(x,z)$. The R-factor was reduced to less than 20 percent for $F(h0l)$ in Pa. The R-factor for the already unlikely case of a random structure in $P2_1/a$ could not be reduced below 24 percent. Fourier projections $\rho(x,z)$ and $\rho(y,z)$ are shown in Fig. 1. Atomic coordinates derived from these are given in Table 1.

The y parameters result in no observable intensities for odd-order $(0k0)$ spectra. The latter parameters are not yet determined with sufficient accuracy, owing to overlapping in the (100) projection, to justify reporting of interatomic distances. At this stage of the analysis the molecule appears to be planar, with the plane inclined about 63°