action it may be replaced by other similar high-boiling solvents.

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[1] Chakravarti, R. N., *et al.*, *Ind. Med. Gaz.*, **88**, 422 (1953); *J. Ind. Chem. Soc.*, **31**, 173 (1954); **33**, 799 (1956).

[2] Marker, R. E., Tsukamoto, T., and Turner, D. L., *J. Amer. Chem. Soc.*, **62**, 2525 (1940).

An Unstable Modification of Aprobarbital

POLYMORPHISM in aprobarbital (5 allyl 5-*iso*propyl barbituric acid) has been reported by several workers[1-3]. Three modifications are quite stable and have been well described in the literature, and a fourth modification has been prepared by Brandstätter[2], who has reported only a melting point, and gives no preparative details. Huang[5] has attempted to prepare this modification IV, but although a new crystal habit was observed, it was found to have the same X-ray diffraction pattern as modification III.

Polymorphism was observed in this Laboratory in the course of work on the identification of toxicological barbiturate specimens by X-ray diffraction. It was noticed that aprobarbital (*inter alia*) gave different modifications on crystallization from aqueous alcohol and chloroform. Comparison of the X-ray patterns obtained on a 90° North American Phillips diffractometer with the data given by Huang[3] established that crystallization from alcohol gave modification I and that the product from chloroform was modification III.

However, under the microscope, the crystals obtained from chloroform solution were observed to be opaque, and without any clearly defined faces. This was initially assumed to be due to deposition of a microcrystalline or amorphous phase on the surface of the crystals as they dried, since, in the presence of the mother liquor, the crystals were observed to be fine transparent needles, with clearly defined faces. However, all attempts to remove any such surface layer by redissolving in chloroform, or dispersing in mineral oil, failed, and no sign of the original transparent crystal could be found.

It was desired to study the transparent crystals in the mother liquor with X-rays, but the high absorption of the X-rays by chloroform and the volatility of the solvent made this very difficult. However, it was found that the crystals could be transferred to water by adding water to the chloroform suspension of the crystals, and drawing off the chloroform layer in a sintered-glass Gooch crucible. The crystals retained their form and transparency in water, and were readily examined by X-rays while still wet. The X-ray pattern thus obtained established that these crystals were a modification of aprobarbital distinct from the three described by Huang[3], and possibly the same as that prepared by Brandstätter.

It appears that whereas this modification is reasonably stable in the presence of the mother liquor, it reverts to the more stable modification III as the crystals dry. This change was seen clearly when a suspension of the crystals in water was allowed to dry out; the needles changed their form on drying, and became rods with very well-defined end-faces, which gave the X-ray pattern of modification III.

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[1] Fischer, R., *Arch. Pharm. u. Ber. dtsch. pharm. Gesel.*, **270**, 149 (1932); *Mikrochemie*, **10**, 409 (1932). Reimers, F., *Dansk. Tids. Farm.*, **14**, 1 (1940).

[2] Brandstätter, Maria, *Z. physik. Chem.*, A, **191**, 227 (1942).

[3] Huang, T-Y., *Acta Pharm. Intern.*, **2**, 95 (1951).

Reversible Enzyme Inactivation due to N,O-Peptidyl Shift

IN the course of a study of the N,O-acyl shift at the hydroxyamino-acid residues of proteins (N,O-peptidyl shift) we observed that under certain conditions lysozyme was reversibly inactivated in a way suggestive of a direct dependence on the reversible N,O-peptidyl shift. In earlier work[1] on this rearrangement in proteins, concentrated mineral acids were employed, and concomitant, irreversible processes then make it difficult to observe any connexion between the N,O-peptidyl shift and the biological activity. However, a search for milder conditions has revealed that the reaction also takes place in formic acid at ordinary temperature, and apparently without other changes[2].

Lysozyme dissolved in anhydrous formic acid at room temperature gradually lost its enzymatic activity over a period of hours. Complete reactivation could be brought about by keeping the inactivated enzyme in aqueous solution for 4 hr. at *p*H 7·5–8·5 and room temperature. The reactivation

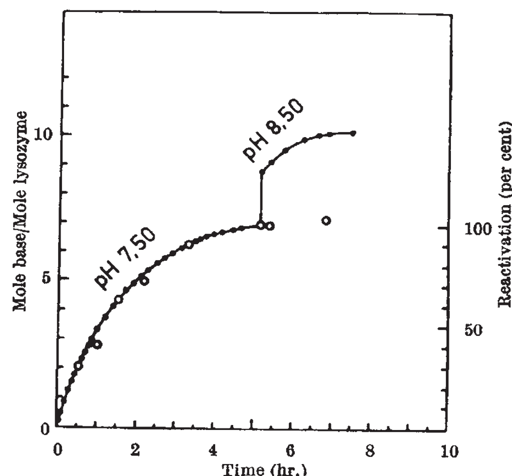

Fig. 1. Alkali consumption (—●—●—●—) during reactivation (O) of inactivated lysozyme

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