respectively. These results appear to indicate that their free base and not protonated forms is preferred for the binding to the enzyme preparation. Diazepam ($pK_a = 3{\cdot}5$) did not show appreciable pH dependency in its effect upon Na^+K ATPase activity and inhibited the preparation about 22 per cent. Since pK_a value is low, this compound would have existed predominently in its base form throughout the pH range where this study was performed.

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REFERENCES

1. J. C. SKOU, *Physiol. Rev.* **45**, 596 (1965).
2. J. D. JUDAH and K. AHMED, *J. cell. comp. Physiol.* **64**, 355 (1964).
3. P. W. DAVIS and T. M. BRODY, *Biochem. Pharmac.* **15**, 703 (1966).
4. T. AKERA and T. M. BRODY, *Molec. Pharmac.* **4**, 600 (1968).
5. T. AKERA and T. M. BRODY, *Molec. Pharmac.* **5**, 605 (1969).
6. Y. ISRAEL and I. SALAZAR, *Archs. Biochem. Biophys.* **122**, 310 (1967).
7. I. UEDA and W. MIETANI, *Biochem. Pharmac.* **16**, 1370 (1967).
8. M. D. RAWSON and J. H. PINCUS, *Biochem. Pharmac.* **17**, 573 (1968).
9. R. F. Squires, *Biochem. biophys. Res. Commun.* **19**, 27 (1965).
10. A. J. TREVOR and J. T. CUMMINS, *Biochem. Pharmac.* **18**, 1157 (1969).
11. B. W. FESTOFF and S. H. APPEL, *J. clin. Invest.* **47**, 2752 (1968).
12. T. NAKAO, Y. TASHIMA, K. NAGANO and M. NAKAO, *Biochem. biophys. Res. Commun.* **19**, 755 (1965).
13. I. UEDA and T. WADA, *Analyt. Biochem.* **37**, 169 (1970).
14. T. E. WEICHSELBAUM, *Am. J. clin. Path. Tech. Sect.* **10**, 40 (1946).

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Studies on enzymatic hydrolysis of aziridines—I. The conversion of 2β,3β-imino-5α-cholestane into 2β-amino-3α-hydroxy-5α-cholestane by liver microsomes

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RECENT studies on the role of liver microsomes have shown the presence of a new type of enzyme, named epoxide hydrolase[1] or epoxide hydrase,[2] which catalyzes the hydrolysis of a variety of epoxides of olefins[3–7] and arenes[8–11] to glycols. Activity of this microsomal enzyme is so potent that except in a few cases[2,3,12] epoxides formed during the oxidation of olefins by the NADPH-dependent mixed function oxygenase (epoxidase), also located in microsomes, are immediately hydrolyzed to make recognition of their existence difficult. In view of detoxication, existence of highly active epoxide hydrolase, relative to epoxidase, appears to be important to the animal body, for most epoxides are well known to be more or less toxic. It is of interest that 2,3-epoxycholestanes are stereoselectively hydrolyzed by the hydrolase to yield a single glycol with the same absolute configuration as that of the product obtained by their acid-catalyzed hydrolysis and also that the enzyme reaction is strongly inhibited with 2β, 3β-imino-5α-cholestane **1**, suggesting that the active center of the hydrolase has a dissociating hydrogen ($Enz^- H^+$) by which the enzyme interacts with the oxiran oxygen of the

substrates.[13] The above mentioned facts encouraged us to investigate a new problem; whether aziridines are also hydrolyzed by liver microsomes or not.

Aziridine is an essential moiety for some carcinostatic agents such as mitomycins, and, together with its derivatives, has carcinogenic activity.[14] This communication presents first evidence for the hydrolysis of an aziridine to the corresponding α-aminoalcohol by liver microsomes. For obtaining information on the mode of the enzymatic hydrolysis, it is best to use a rigid-conformationed substrate whose absolute structure and reactivities with various chemical reagents are well established. The steroidal aziridine **1**,[15] used as a model substrate in the present investigation, is similar and provides reasonable information on the manner and stereoselectivity in the hydrolytic opening of the aziridine ring by the configurational assignment of the resulting aminoalcohol.

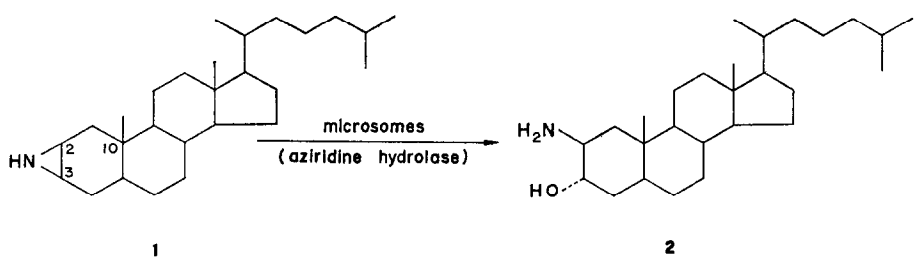
To a homogeneous suspension of 10 μmoles of **1** in 0·1 M phosphate buffer, pH 7·4, was added twice washed microsomes, isolated from 2 g of male rabbit liver by the previously reported method[16] and suspended in 0·1 M phosphate buffer, pH 7·4. This mixture was incubated at 37° under nitrogen. The suspension of the substrate was prepared before use by shaking a solution of **1** in minimum ether with 1·5 ml of the buffer and methanol (4:1), containing 0·2% of Triton X-100, evaporating the organic solvent under reduced pressure, and adjusting the volume with the buffer to 2 ml. The total volume of the incubation mixture was 6 ml. After 3 hr the reaction was stopped by addition of 1 ml of 5N NaOH, and the mixture extracted with ether following saturation with sodium chloride.

T.L.C. of the extract on silica gel in benzene saturated with 28% ammonia and acetone (1:1) suggested the formation of only one metabolite which was visualized as a single spot by a ninhydrin reagent at R_f 0·24. The aziridine **1**, which also showed a ninhydrin-positive spot at R_f 0·71, was found to be completely stable in 0·1 M phosphate buffer, pH 7·4, in the presence of boiled microsomes under the above mentioned conditions. The polar metabolite was identified as a *trans* diaxially opened product, 2β-amino-3α-hydroxy-5α-cholestane **2**,[15] by co-T.L.C. with the authentic specimen. Trifluoroacetylation of the metabolite, isolated by preparative T.L.C., and subsequent analyses by T.L.C. in benzene and acetone (4:1) and by V.P.C. on a 0·75% SE-30 and a QF-1 columns at the column temperatures of 270° and 250°, respectively, also indicated it to be identical with a trifluoroacetate of **2** and, furthermore, homogeneous; the derivative of the metabolite showed a single spot at R_f 0·46 and was eluted as a single peak at 9·6 and 7·8 min from the former and the latter columns, respectively.

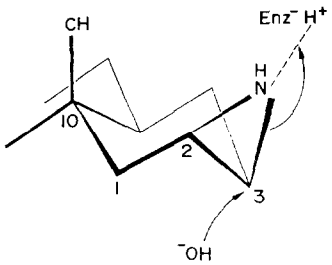
For further identification of the metabolite, a large scale of the mixture, including 80 μmoles of **1**, was incubated until it was completely hydrolyzed (20 hr), and the metabolite formed was separated on a silica gel column, packed using benzene saturated with 28% ammonia, to yield 18 mg of crystals. After recrystallization from ethanol and ether, identification was carried out by mass spectrometry (*m/e* 403 (M^+)), mixed melting point test on an admixture with authentic **2**, and superimposability of their i.r. spectra.

Stereoselective formation of the same *trans* diaxial aminoalcohol **2** has also been reported earlier in the acid-catalyzed hydrolysis of **1**,[15] suggesting that the enzymatic hydrolysis is possibly initiated by association of a dissociating hydrogen from the active center of the enzyme protein with nitrogen of the aziridine ring, followed by attack of a hydroxyl anion from water, in a concerted manner, on the aziridine carbon at 3-position from the α-side opposite to the enzyme. The suggested mode of the enzymatic hydrolysis could permit the least sterically hindered transition state of the reaction. This would be the most reasonable interpretation for the formation of the single aminoalcohol, for if ring opening occurs *cis* between nitrogen and C_3 or either *trans* or *cis* at the other bond, the steroidal amino group associated with the active center of the enzyme is oriented equatorial and, consequently, the hydroxyl anion will have to approach to the resulting carbonium ion from the side almost same as the enzyme surface since these steps, if possible, are reasonably thought to proceed concertedly.

Although similarity in the mode of the enzymatic hydrolysis of the aziridine ring of **1** to that of the oxiran ring of 2β, 3β-epoxycholestane[13] and the fact that hydrolysis of the latter by liver microsomes



1 **2**



is inhibited with the former[13] might suggest that a single enzyme catalyzes both types of hydrolysis, we propose "aziridine hydrolase" tentatively for the name of the present enzyme.

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REFERENCES

1. T. WATABE and E. W. MAYNERT, *Fedn Proc.* **27**, 302 (1968).
2. D. M. JERINA, J. W. DALY, B. WITKOP, P. ZALTZMAN-NIRENBERG and S. UDENFRIEND, *J. Am. chem. Soc.* **90**, 6525 (1968).
3. T. WATABE and E. W. MAYNERT, *Pharmacologist* **10**, 203 (1968).
4. G. T. BROOKS, S. E. LEWIS and A. HARRISON, *Nature, Lond.* **220**, 1034 (1968).
5. K. C. LEIBMAN and E. ORTIZ, *Fedn Proc.* **27**, 302 (1968).
6. G. T. BROOKS, A. HARRISON and S. E. LEWIS, *Biochem. Pharmac.* **19**, 255 (1970).
7. T. WATABE, Y. UENO and J. IMAZUMI, *Biochem. Pharmac.* **20**, 912 (1971).
8. D. M. JERINA, J. W. DALY, B. WITKOP, P. ZALTZMAN-NIRENBERG and S. UDENFRIEND, *Archs Biochem. Biophys.* **128**, 176 (1968).
9. D. M. JERINA, H. ZIFFER and J. W. DALY, *J. Am. chem. Soc.* **92**, 1056 (1970).
10. D. M. JERINA, J. W. DALY, B. WITKOP, P. ZALTZMAN-NIRENBERG and S. UDENFRIEND, *Biochemistry* **9**, 147 (1970).
11. H. PANDOV and P. SIMS, *Biochem. Pharmac.* **19**, 299 (1970).
12. G. T. BROOKS, *Residue Reviews* **27**, 81 (1969).
13. T. WATABE, S. KANEHIRA, K. KIYONAGA and S. HARA, *2nd Symposium on Drug Metabolism and Action, Kyoto* (1970), p. 59
14. S. ODASHIMA, *Taishya* **4**, 316 (1967).
15. A. HASSNER and C. HEATHCOCK, *J. org. Chem.* **30**, 1748 (1965).
16. T. WATABE, H. YOSHIMURA and H. TSUKAMOTO, *Chem. pharm. Bull. (Tokyo)* **12**, 1151 (1964).

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Effects of various hydrazines upon the metabolism of gamma aminobutyric acid (GABA)-1-^{14}C by rats

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STUDIES on the effects of simple hydrazines on GABA metabolism have been concerned mainly with the central nervous system. Administration of hydrazine to mice has been shown to cause a several fold increase of brain GABA levels, presumably because GABA transamination was more inhibited than was formation of GABA through decarboxylation of glutamic acid.[1–3] Monomethylhydrazine