

Fig. 3. Difference spectra produced during NADPH-dependent microsomal metabolism of *N*-hydroxy-*N*-methylamphetamine (2) and *N*-methylene-1-phenyl-2-propylamine *N*-oxide (3a). The time difference between each scan is  $\sim 40$  sec. For the experimental conditions see Materials and Methods.

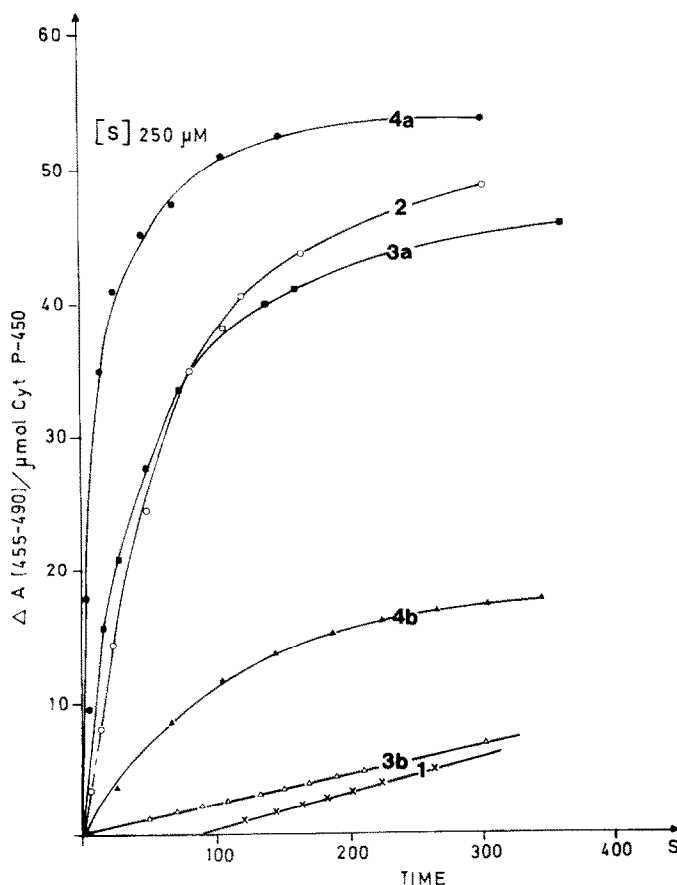


Fig. 4. Cytochrome P-450 complex formation by compounds 1-4b in microsomes isolated from phenobarbital treated rats. Microsomes ( $2 \mu\text{M}$  of cytochrome P-450) were incubated in the presence of *N*-methylamphetamine (1), *N*-hydroxy-*N*-methylamphetamine (2), *N*-methylene-1-phenyl-2-propylamine *N*-oxide (3a), *N*-(1-phenyl-2-propylidene)methylamine *N*-oxide (3b), *N*-hydroxyamphetamine (4a) and *N*-hydroxymethylamine (4b) respectively under the conditions described in Materials and Methods. All substrate concentrations were  $250 \mu\text{M}$ . One representative experiment out of 4. For standard deviations cf. Table 1.





bolic *N*-oxidation of *N*-methylamphetamine (1), undergoes further conversion to *N*-hydroxyamphetamine (4a). The latter is the ultimate precursor to the ligand forming the cytochrome P-455 complex. Our results also substantiate the notion [9–11], that there is a preference for the formation of nitrones related to 3a rather than 3b during the metabolism of amphetamines. In addition to  $\alpha$ -carbon oxidation, *N*-oxidation is indicated as a metabolic route, in this case instrumental to metabolic *N*-demethylation, but more generally instrumental to *N*-dealkylations. Thus, the results reanimate the debate on the significance of *N*-oxidation in dealkylation reactions [25, 26].

**Acknowledgements**—We thank Professor Arthur Cho, Department of Pharmacology, UCLA for encouraging and valuable discussions and Professor Sten Orrenius, Department of Forensic Medicine at the Karolinska Institute for providing the Aminco Chance. The courtesy by Dr. Mansuy, Department of Chemistry, École Normale Supérieure in performing an independent reinvestigation on the complex formation of *N*-hydroxymethylamine is also acknowledged.

Portions of this work was supported by the Swedish Natural Science Research Council, Grant No. K 3751-004, and by the IF Foundation for Pharmaceutical Research.

#### REFERENCES

1. M. R. Franklin, *Xenobiotica* **4**, 133 (1974).
2. J. Jonsson and B. Lindeke, *Acta pharm. suec.* **13**, 313 (1976).
3. D. Mansuy, P. Beaune, J.-C. Chottard, J.-F. Bartoli and P. Gans, *Biochem. Pharmac.* **25**, 609 (1976).
4. B. Lindeke, J. Jonsson, G. Hallström and U. Paulsen in *Biological Oxidation of Nitrogen in Organic Molecules*. (Ed. J. W. Gorrod), p. 47. Elsevier, Amsterdam (1978).
5. D. Mansuy, P. Gans, J.-C. Chottard and J.-F. Bartoli, *Eur. J. Biochem.* **76**, 607 (1977).
6. D. Mansuy, E. Rouer, C. Bacot, P. Gans, J.-C. Chottard and J. P. Leroux, *Biochem. Pharmac.* **27**, 1229 (1978).
7. M. R. Franklin, *Drug Metab. Disp.* **2**, 321 (1974).
8. M. Hirata, B. Lindeke and S. Orrenius, *Biochem. Pharmac.* **28**, 479 (1979).
9. R. T. Coutts, G. R. Jones, and S.-F. Liu, *Biomedical Mass Spec.* **5**, 418 (1978).
10. A. H. Beckett and K. Haya, *Xenobiotica* **8**, 85 (1978).
11. A. H. Beckett and G. G. Gibson, *Xenobiotica* **8**, 73 (1978).
12. B. Lindeke, A. K. Cho, T. L. Thomas and L. Michelson, *Acta pharm. suec.* **10**, 943 (1973).
13. O. Tangen, J. Jonsson and S. Orrenius, *Analyt. Biochem.* **54**, 597 (1973).
14. D. Mansuy, J.-C. Chottard and G. Chottard, *Eur. J. Biochem.* **76**, 617 (1977).
15. T. Nash, *J. biol. Chem.* **55**, 412 (1953).
16. P. J. Geiger and S. P. Bessman, *Analyt. Biochem.* **49**, 467 (1972).
17. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
18. J. Hamer and A. Macaluso, *Chem. Rev.* **64**, 473 (1964).
19. H. B. Huckler, B. M. Michniewicz and R. E. Rhodes, *Biochem. Pharmac.* **20**, 2123 (1971).
20. U. Paulsen, B. Lindeke and U. Jonsson, *Acta pharm. suec.* **15**, 264 (1978).
21. M. R. Franklin, *Molec. Pharmac.* **10**, 975 (1974).
22. F. F. Kadlubar, E. M. McKee and D. M. Ziegler, *Archs Biochem. Biophys.* **156**, 46 (1973).
23. L. L. Poulsen, F. F. Kadlubar and D. M. Ziegler, *Archs Biochem. Biophys.* **164**, 774 (1974).
24. R. T. Coutts and S. H. Kovach, *Biochem. Pharmac.* **26**, 1043 (1977).
25. M. H. Bickel, *Pharmac. Rev.* **21**, 325 (1969).
26. H. B. Huckler, *Drug Metab. Rev.* **2**, 33 (1973).