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Mechanism of Hydrogen Transfer in the Reduction of the Imine Bond by an NADH Model. Use of Cyclopropane Ring as a Chemical Probe†

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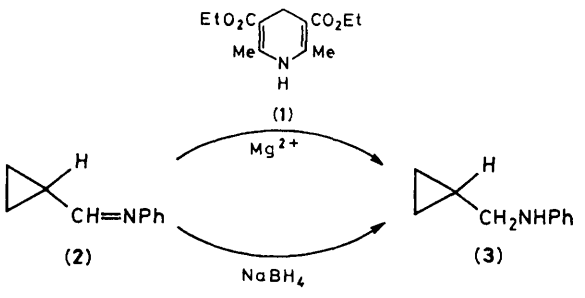
Reduction of *N*-cyclopropylmethylenephenylamine by 3,5-diethoxycarbonyl-2,6-dimethyl-1,4-dihydropyridine (Hantzsch ester) results in the exclusive formation of *N*-cyclopropylmethylaniline, whereas reduction with triphenyltin hydride gives both *N*-cyclopropylmethylaniline and n-butylaniline, a product of cyclopropane ring cleavage.

The precise nature of the hydrogen which is transferred by NADH-dependent dehydrogenases constitutes a pivotal question in regard to the mechanism of action of the co-enzyme. While in model studies both hydride[1] and initial electron transfer ($e^- + H^+ + e^-$)[2] have been considered, two recent studies[3,4] of enzyme-catalysed (HLAD) reductions, which employ chemical probes, support the 'hydride-transfer' mechanism. In one of these, the cyclopropylmethyl probe[3] was used; it being anticipated that the formation of a radical intermediate would result in opening of the 3-membered ring.[5] It has, however, been pointed out[4] that, in the enzymatic reaction, ring opening of the cyclopropylmethyl radical might be prevented owing to constraints imposed by the topology of the active site. Since such factors would be absent in an *in vitro* system, the utilization of the cyclopropyl probe in a suitable model reaction should throw light on the mechanism of the hydrogen transfer process. The results of the study of such a reaction are described in this communication.



Scheme 1

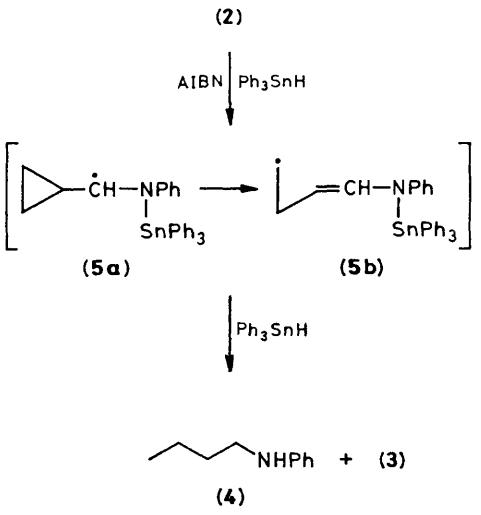
We have shown previously[6] that imines can be reduced by Hantzsch ester (**1**) in the presence of magnesium ions. Reduction of Schiff's base (**2**) by the NADH model (**1**) would be expected to proceed with or without ring-opening, depending upon whether a radical or a hydride mechanism is operative.

Imine (**2**) (Scheme 1), synthesized according to known procedures, was allowed to react with Hantzsch ester (**1**) in the presence of $Mg(ClO_4)_2$ (MeCN, room temp., 2 days). Work-up of the reaction mixture gave a single reduction product, whose structure was established as amine (**3**). No ring-opened product was observed in the g.l.c. analysis‡ of the mixture prior to work-up. The n.m.r. spectrum of the reduction product [characteristic resonance peaks at δ 2.96 (d, 2H, $>CH-CH_2-NH-$), 0.84—1.37 (m, 1H, $>CH-$), and 0.06—0.66 (m, 4H, cyclopropyl protons)] was found to be identical to the spectrum of an authentic sample of (**3**), prepared by $NaBH_4$ reduction of (**2**).

While the exclusive formation of *N*-cyclopropylmethylaniline (**3**) during the reduction of (**2**) by (**1**) strongly supports the hydride transfer mechanism, it was considered necessary to demonstrate that a cyclopropylmethyl radical, derived from (**2**), does lead to ring-opened products. In order to examine this point, the imine (**2**) was reduced by 4 equiv. of triphenyltin hydride in the presence of azoisobutyronitrile (AIBN) (cyclohexane, 18 h, reflux). A g.l.c. analysis‡ of the reaction mixture (without work-up) showed four peaks (I—IV; ratio 27 : 7 : 15 : 51). The products corresponding to these peaks were collected and examined. Peak I was found to be contaminated (n.m.r. analysis) with starting imine. Peaks III and IV were identified as n-butylaniline (**4**) (compared with authentic n-butylaniline) and *N*-cyclopropylmethylaniline (**3**), respectively (Scheme 2).

† Taken in part from the Doctorate Thesis of J. C. G. van Niel.

‡ G.l.c. analysis: glass column, 15% SE 30 on chromosorb W-AW; He gas carrier; 125 °C.



Scheme 2

The product of peak II corresponded to a dimer of (**2**) (mass spectrometry), but it has not yet been fully characterized.

The formation of both (**3**) and (**4**) in the reduction by Ph_3SnH implies that ring-opening (**5a**)→(**5b**) and reduction (**5a**)→(**3**) are competitive processes (Scheme 2), in which the ring-opening is the slower process. Although this might initially appear to be contrary to the popular notion that an intermediate of type (**5a**) undergoes a fast ring-opening reaction, recent results[7a,b] show that ring-opening is slowed down[7a] or wholly suppressed[7b] when an analogous radical centre is generated adjacent to hetero-atoms. The observation that no acyclic reduction product is formed in the reaction of (**2**) with a dihydropyridine (**1**) (NADH model), whereas significant ring-opening product (**4**) is produced when a 'radical reductant' is employed, provides persuasive evidence *against* radical intermediates and *in favour* of a hydride transfer mechanism in the Mg^{2+} ion-catalysed reduction of imine (**2**) and suggests that reduction of other imines might also proceed by a similar mechanism.

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References

1 D. S. Sigman, J. Hadju, and D. J. Creighton, 'Bioorganic Chemistry,' ed. E. E. van Tamelen, Academic Press, New York, 1978, vol. 4, p. 385; R. J. Kill and D. A. Widdowson, *ibid.*, p. 239.
2 A. Ohno, H. Yamamoto, and S. Oka, *J. Am. Chem. Soc.*, 1981, **103**, 2041; A. Ohno, T. Shio, H. Yamamoto, and S. Oka, *ibid.*, p. 2045.
3 I. MacInnes, D. C. Nonhebel, S. T. Orszulik, and C. J. Suckling, *J. Chem. Soc., Chem. Commun.*, 1982, 121.
4 S.-K. Chung and S.-U. Park, *J. Org. Chem.*, 1982, **47**, 3197.
5 B. Maillard, D. Forest, and K. U. Ingold, *J. Am. Chem. Soc.*, 1976, **98**, 7024; A. Effio, D. Griller, K. U. Ingold, A. L. J. Beckwith, and A. K. Serelis, *ibid.*, 1980, **102**, 1734.
6 U. K. Pandit, H. van Dam, and J. B. Steevens, *Tetrahedron Lett.*, 1977, 913; J. B. Steevens, 'Reducties met NADH-Modellen,' Thesis, University of Amsterdam, 1980; J. B. Steevens and U. K. Pandit, *Tetrahedron*, in the press.
7 (a) L. R. C. Barclay, D. Griller, and K. U. Ingold, *J. Am. Chem. Soc.*, 1982, **104**, 4399; (b) K. de Boer and Th. J. de Boer, unpublished results from this laboratory.