Asymmetric Reduction of Aromatic Ketones by Sodium Borohydride in the Presence of Bovine Serum Albumin

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Summary Bovine serum albumin was used to provide a chiral template for asymmetric reduction of aromatic ketones by the achiral $NaBH_4$, and substantial (20—80%) enantiomeric excesses were obtained in the product alcohols.

THE fact that enzymes carry out a variety of highly stereoselective reactions at highly organized chiral binding sites has led to a particular interest in asymmetric syntheses by non-covalent binding of reactants in the chiral interiors of simple chemical inclusion aggregates and compounds. Unfortunately, reactions effected in cholesteric liquid crystals,[1] sodium cholate micelles,[2] and cyclodextrins[3] lead to asymmetric syntheses of only 18% enantiomeric excess at the best. This low stereoselectivity is considered to be due to the low chirality of the environment of their binding sites. We expected that some proteins capable of binding reactants into their interiors with a highly chiral environment could lead to a high stereoselectivity in asymmetric syntheses. We have found that the reductions of aromatic ketones by $NaBH_4$ in the presence of bovine serum albumin (BSA), one of carrier proteins in biological systems, can indeed result in substantial enantiomeric excesses in the product alcohols.

When acetophenone (5·0 mM) was reduced by $NaBH_4$ (0·01 M) in a 0·01 M borax buffer solution (pH 9·2) containing BSA (Fraction-V obtained from Armour) in the concentration range 0·3—2·0 mM,† asymmetric induction in the resulting alcohol was observed. The optical yield increased gradually with an increase in the amount of BSA, reaching a constant value (45%) at 1·5—1·7 mM. The stereoselectivity dropped sharply when the BSA was denatured to an unfolded random polymer by addition of

† The molecular weight of BSA, a single peptide composed of 581 amino acids, was taken as 66,000.

TABLE. Maximum optical yields of enantiomeric alcohols obtained in the reductions of aromatic ketones[a] by $NaBH_4$[b] in the presence of bovine serum albumin[c] in a 0·01 M borax buffer solution, pH 9·2, at 25 °C.

Aromatic ketone	Alcohol[d] Optical yield[e] (%)	Configuration
PhCOMe	45[f]	*R*
PhCOEt	78[g]	*R*
PhCOPr^n	27[i]	*R*
PhCOPr^i	66[k]	*R*
PhCOBu^n	14[j]	*S*
PhCOBu^t	22[l]	*R*
1-Naphthyl-COMe	67[h]	*R*
2-Naphthyl-COMe	66[m]	*R*
2-Phenanthryl-COMe	21[n]	—
3-Phenanthryl-COMe	20[o]	—

[a] 5·0 mM. [b] 0·01 M. [c] Maximum values were obtained with 1·5—1·7 mM BSA. [d] All alcohols were obtained in quantitative chemical yield and showed satisfactory spectra. [e] Optical yields were calculated from $[\alpha]_D(obs)/[\alpha]_D(max) \times 100$ (%). The optical rotations and configurations of the optically pure alcohols were referred to the following literature $[\alpha]_D$ (max) values: [f] +45·5° (MeOH; 23 °C), R. Huisgen and Ch. Ruchardt, *Annalen*, 1956, **601**, 31. [g] +32·48° (EtOH; room temp.). [h] +74·39° (EtOH; room temp.) R. H. Pickard and J. Kenyon, *J. Chem. Soc.*, 1914, 1115. [i] +35·8° (benzene; 24 °C). [j] +31·3° (benzene; 24 °C), P. A. Levene and R. E. Marker, *J. Biol. Chem.*, 1932, **97**, 379. [k] +48·3° (Et_2O; 23 °C), D. J. Cram and J. E. MaCarty, *J. Amer. Chem. Soc.*, 1957, **79**, 2866. [l] +36·2° (Et_2O; 20 °C), R. MacLeod, F. J. Welch, and H. S. Mosher, *J. Amer. Chem. Soc.*, 1960, **82**, 876. [m] +41·3° (EtOH; room temp.), T. A. Collyer and J. Kenyon, *J. Chem. Soc.*, 1940, 676. [n] 130·8° ($CHCl_3$; 25 °C). [o] 79·2° ($CHCl_3$; 25 °C), W. H. Pirkle and S. D. Beare, *J. Amer. Chem. Soc.*, 1967, **89**, 5485.

8 M urea[4] and also when the reduction was conducted in the presence of naphthalene (5·0 mM), a good guest compound for BSA;[5] for example, the 12% enantiomeric excess obtained with 0·6 mM BSA decreased to 2 and 1%, respectively. These findings suggest that one BSA protein molecule binds about 3 acetophenone molecules in its chiral interior‡ and subsequent asymmetric reduction by $NaBH_4$ takes place there. Asymmetric reductions of other aromatic ketones (5·0 mM) can also produce enantiomeric alcohols with maximum optical yields ranging from 14 to 78% at 1·5—1·7 mM BSA, depending upon the substituent (Table).§ All the alcohols produced have the *R* configuration except in the reduction of PhCOBu^n, although at present we cannot comment on the reasons for this stereochemical outcome.

The present method for asymmetric reduction has another attractive feature in addition to the high stereoselectivity. The aqueous protein solution remaining after extraction of the reaction mixture with ether can be reused repeatedly for syntheses of enantiomeric alcohols without change of stereoselectivity if the contaminated ether solvent is thoroughly removed *in vacuo*. For example, in the case of propiophenone the first repeated reduction showed an asymmetric induction of 77% and the optical yield in the second repeat remained almost unchanged.

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‡ One BSA molecule forms different aggregates with several molecules of hydrophobic guest compound, depending on the ratio between their concentrations (see ref. 5).

§ The optical yields of alcohols, purified by preparative t.l.c. or g.l.c., were calculated using the values of optical rotations for the optically pure alcohols. The n.m.r. spectra of the crude alcohols in the presence of the chiral europium shift reagent, tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorato]europium(III) (H. L. Goering, J. E. Eikenberry, and G. S. Koermer, *J. Amer. Chem. Soc.*, 1971, **93**, 5913), also provided an estimate of the optical yields. Values from optical rotation and n.m.r. spectroscopy were in good agreement.

[1] F. D. Saeva, P. E. Sharpe, and G. R. Olin, *J. Amer. Chem. Soc.*, 1975, **97**, 204; W. H. Pirkle and P. L. Rinaldi, *ibid.*, 1977, **99**, 3510; L. Verbit, T. R. Halbert, and R. B. Patterson, *J. Org. Chem.*, 1975, **40**, 1649.
[2] T. Sugimoto, Y. Matsumura, T. Imanishi, S. Tanimoto, and M. Okano, *Tetrahedron Letters*, in the press.
[3] F. Cramer and W. Dietsche, *Chem. and Ind.*, 1958, 892; *Chem. Ber.*, 1959, **92**, 1739; C. van Hooidonk and J. C. A. E. Breebaart-Hansen, *Rec. trav. Chim.*, 1970, **70**, 289; 1972, **91**, 958; C. van Hooidonk and C. C. Groos, *ibid.*, 1970, **70**, 845; C. van Hooidonk, *ibid.*, 1972, **91**, 1103; K. Flohr, R. M. Paton, and E. T. Kaiser, *Chem. Comm.*, 1971, 1621; Y. Iwakura, K. Uno, F. Toda, S. Onozuka, K. Hattori, and M. L. Bender, *J. Amer. Chem. Soc.*, 1975, **97**, 4432; Y. Kitaura and M. L. Bender, *Bioorg. Chem.*, 1975, **4**, 237.
[4] H. A. McKenzie, M. B. Smith, and R. G. Wake, *Biochim. Biophys. Acta*, 1963, **69**, 222.
[5] F. Helmer, K. Kiehs, and C. Hansch, *Biochemistry*, 1968, **7**, 2858.