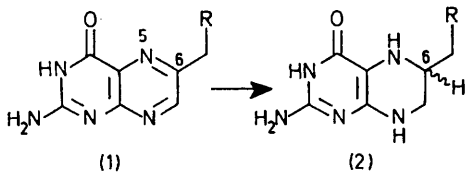
Asymmetric Hydrogenation of a Carbon–Nitrogen Double Bond in Folic Acid

By PETER H. BOYLE* and MARIE T. KEATING
(*Department of Chemistry, Trinity College, Dublin 2, Ireland*)

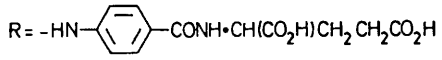
Summary Asymmetric catalytic reduction of the 5–6 carbon–nitrogen double bond in folic acid has been achieved by hydrogenation with an optically active rhodium catalyst.

TETRAHYDROFOLATES serve as essential co-factors in the formation of nucleic acids so that reduction of the pyrazine ring of folic acid (**1**) assumes a special significance. Since this reduction is stereospecific when carried out biologically,

only one form of tetrahydrofolic acid (**2**) occurs in nature, and only one form is biologically active. Reduction with borohydride, on the other hand, or hydrogenation over noble-metal catalysts, leads to a mixture of two diastereoisomeric forms,[1] epimeric at C-6. We report the successful asymmetric hydrogenation of the pyrazine ring of folic acid



(1) (2)



using an optically active catalyst. Isolated reports[2] have appeared recently on the asymmetric hydrogenation of carbon–carbon double bonds using soluble rhodium catalysts; one of the most promising of these is the rhodium(I) complex, $[(py_2)(amide)RhCl(BH_4)]^+Cl^-$ (py = pyridine), containing an optically active amide.[3] A modification of this system was used in the present work. $(py)_3RhCl_3$ was stirred under hydrogen with the desired form [(+) or (−)] of *N*-1-phenylethylformamide and $NaBH_4$. A solution of folic acid in NaOH, adjusted to pH 7 with HCl, was added to the catalyst solution. After hydrogenation, 2-mercaptoethanol was added to the reaction mixure which was then chromatographed on a DEAE cellulose column eluting with a 0·13—0·4M-NH_4OAc gradient, pH 6·9. The catalyst was eluted first, followed by tetrahydrofolic acid, identified by its u.v. spectrum and by comparison on paper chromatography with an authentic specimen. The total tetrahydrofolate content of fractions eluted from the column was estimated by monitoring the absorption at 297 nm (ϵ = 29,100). Biologically active tetrahydrofolate was estimated in the same fractions by bioassay with *L. casei* in presence of ascorbic acid as anti-oxidant. The results were expressed graphically and the percentage of the total tetrahydrofolate which was biologically active was estimated by measuring the relative areas under the two curves. This percentage gave a measure of the relative amount of the two diastereoisomers of tetrahydrofolic acid formed in the hydrogenation. With (−) catalyst, pure tetrahydrofolic acid of 46% biological activity was obtained. With (+) catalyst, on the other hand, biological activity was only 28%, the difference clearly reflecting an asymmetric reduction dependent on choice of catalyst.

The low overall level of biological activity, even when using (−) catalyst, could be due to slow decomposition of the unstable tetrahydrofolic acid during the bioassay, or alternatively could arise from the imprecision with which the u.v. extinction coefficient of tetrahydrofolic acid is known. Several widely differing ϵ values have been reported.[4] A more interesting possibility, however, is that the chiral glutamate unit in folic acid, even though far removed from the reacting centres in the pteridine nucleus, can affect the course of the reduction. This possibility will be dealt with in more detail in a later paper.

We are very grateful to the Biomedical Research Trust for support of this work, and to Mr. Sean O Broin for help with the bioassays.

(*Received, 6th March* 1974; *Com.* 261.)

[1] R. L. Blakley, 'Biochemistry of Folic Acid and Related Pteridines,' North-Holland, Amsterdam, 1969, p. 82.
[2] L. Horner, H. Siegel, and H. Büthe, *Angew. Chem. Internat. Edn.*, 1968, **7**, 942; W. S. Knowles and M. J. Sabacky, *Chem. Comm.*, 1968, 1445.
[3] P. Abley and F. J. McQuillin, *J. Chem. Soc.* (*C*), 1971, 844.
[4] Ref. 1, pp. 93—94.