Asymmetric Catalysis, 105[◇]

Stereoselective Hydrogenation of Folic Acid with Immobilized Optically Active Rhodium(I)/Diphosphane Catalysts

Henri Brunner*, Petra Bublak, and Martina Helget

Institut für Anorganische Chemie, Universität Regensburg,
Universitätsstraße 31, D-93053 Regensburg, Germany

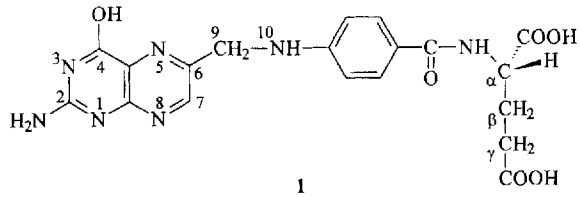
Received July 3, 1996

Keywords: Diastereoselective hydrogenation / Folic acid / Immobilized optically active rhodium(I)/diphosphane catalysts / Leucovorin / HPLC analysis of 5-formyltetrahydrofolic acid derivatives

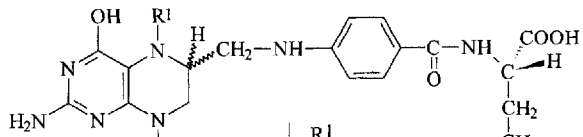
For the hydrogenation of the C=N bonds in the pyrazine ring of the vitamin folic acid (**1**) optically active rhodium(I)/diphosphane complexes immobilized on supports such as silica gel or Al_2O_3 were used. The reduction was carried out at 50 bar hydrogen pressure in an aqueous solution buffered to pH 7. Thus, 5,6,7,8-tetrahydrofolic acid (**2**) was obtained which contains a new asymmetric center at C-6 of the pterine system. Therefore, in combination with the (*S*) configuration of the natural L-glutamic acid part of the molecule two diastereomers with (6*S*,*S*) and (6*R*,*S*) configuration arise. The relatively unstable tetrahydrofolic acid (**2**) was converted into its 5-formyl derivative folinic acid (**4**) by treatment with methyl formate/formic acid in a 5:1 mixture of DMSO/pyridine. The Ca salt of folinic acid (**4**) is the widely used drug leucovorin. The diastereomers were separated by silica gel HPLC. To the column bovine serum albumine (BSA) is covalently bound. With optically active rhodium(I)/diphosphane catalysts, immobilized on silica gel supports, a diastereoselectivity of up to 90% could be achieved in the hydrogenation of folic acid (**1**).

The folates are a group of water-soluble vitamins, consisting of a pterine system, a *p*-aminobenzoic acid bridge and a L-glutamic acid moiety (folic acid **1**) or a polyglutamyl chain[2]. In the body, the biologically active species 5,6,7,8-tetrahydrofolic acid (**2**) is formed by the enzymatic reduction of folic acid in two steps. In the first step 7,8-dihydrofolic acid is obtained, which is transformed into 5,6,7,8-tetrahydrofolic acid by the enzyme dihydrofolate reductase in the second step. **2** plays an important role in the biosynthesis of precursors of DNA bases, because it acts as a carrier in the transfer of C_1 fragments, and **3** (5-methyltetrahydrofolic acid) is the methyl donor in the methylation of homocysteine to methionine[2–4].

In the chemotherapy of different kinds of cancer the carrier function of the folates is utilized. Fast growing tumor tissues need purine and pyrimidine bases for their DNA synthesis. This process can be stopped by the competitive inhibition of the enzymes thymidylate synthase and dihydrofolate reductase, which participate in the biosynthesis of nucleic acids. Pseudoirreversible inhibitors of dihydrofolate reductase are methotrexate and aminopterine. Their cytotoxicity is due to a breakdown of the DNA synthesis. The dihydrofolate reductase inhibitors cause a drop in the pool of reduced folates in the cell, preventing important metabolic processes and resulting in cell death. In particular, folate antagonists rapidly affect proliferating cells such as tumor cells[5–8].



1



	R1
2	H
3	CH_3
4	CH=O

After the application of high doses of methotrexate in the treatment of osteosarcomas, leucovorin, the Ca salt of 5-formyltetrahydrofolic acid or folinic acid (**4**) has to be administered as a rescue agent to maintain a certain level of metabolism[4,7,9]. Another important application of leucovorin is the therapy of colorectal adenocarcinomas in combination with 5-fluorouracil. Leucovorin is also used in the treatment of megaloblastic anemia, psoriasis, and rheumatic arthritis[10].

[◇] Part 104: Ref.[1].

  0009–2940/97/0101–0055 $ 15.00+.25/0

In folic acid, there is an asymmetric center of (*S*) configuration in the natural glutamic acid group. In the reduction of folic acid to tetrahydrofolic acid a new asymmetric center is generated at C-6 of the pterine system. Thus, two diastereomers with (6*S*,*S*) and (6*R*,*S*) configuration are possible for tetrahydrofolic acid. In the body, only the (6*S*,*S*) isomer is formed by enzyme catalysis[11]. In the industrial synthesis, however, a 1:1 mixture of the two diastereomers, the natural (6*S*,*S*) isomer and the unnatural (6*R*,*S*) isomer, is produced.

Early experiments indicated, that the growth of microorganisms is more effective with (6*S*,*S*)-leucovorin than with the racemic compound[12]. Further studies showed, that only the (6*S*,*S*) diastereomer acts as a carrier of C_1 groups. Moreover, the unnatural (6*R*,*S*) diastereomer is an enzyme inhibitor and medical experiments demonstrated, that it is more slowly metabolized than the natural (6*S*,*S*) isomer[8,9,13,14]. Thus, application of a 1:1 mixture of (6*S*,*S*)- and (6*R*,*S*)-leucovorin leads to an accumulation of the unnatural tetrahydrofolates in the central nervous system and to an intoxication of the patients in the long run[14-16]. Nevertheless, even today, leucovorin used in therapy is a 1:1 mixture of the (6*S*,*S*) and (6*R*,*S*) diastereomers.

The commercial leucovorin synthesis is based on the hydrogenation of folic acid with PtO_2 or Pd catalysts in high-polarity solvents such as glacial acetic acid, trifluoroacetic acid or formic acid[17-19]. The reduction of folic acid is also possible with $NaBH_4$ in weakly alkaline aqueous solution[20,21]. In these reductions there is almost no optical induction from the (*S*)-configured glutamic acid moiety in the formation of the new asymmetric center at C-6 of the pterine system.

Many efforts had been undertaken to separate the two diastereomers (6*S*,*S*)- and (6*R*,*S*)-leucovorin, e.g. by preparative chromatography of folinic acid[22], fractional crystallization of its alkaline earth salts[12,23], extraction of 5-menthyloxycarbonyltetrahydrofolic acid[7,11], and fractionation of the Ca derivatives with ethylenediaminotetraacetate[24]. In addition to a couple of inefficient chemical and enzymatic approaches to synthesize diastereomerically pure (6*S*,*S*)-leucovorin[25-27], a biotechnological procedure was reported, in which dihydrofolic acid was reduced with dihydrofolate reductase to (6*S*,*S*)-tetrahydrofolic acid in 50% yield. The dihydrofolic acid must be prepared by hydrogenation of folic acid in a separate step[28,29].

Another approach to the synthesis of (6*S*,*S*)-leucovorin is the asymmetric hydrogenation of folic acid. In the literature there is only one communication, which describes a successful homogeneous hydrogenation of folic acid with a catalyst, synthesized from py_3RhCl_3, $NaBH_4$, and various chiral amides[30], which turned out to be wrong. In this study the diastereomeric excess was determined indirectly by a semiquantitative bioassay[31]. Surprisingly, there are no reports in the literature on the stereoselective hydrogenation of folic acid with rhodium(I)/phosphane complexes, which played such a dominant role in enantioselective catalysis[32,33]. Also in our hands, homogeneous rhodium(I)/phosphane catalysts proved to be ineffective in the hydrogenation of folic acid[34]. However, in a previous paper we could demonstrate, that heterogeneous hydrogenation of folic acid with immobilized rhodium(I)/diphosphane catalysts is a promising approach[34]. However, our first attempts suffered from an inferior product analysis, based on the derivatization of tetrahydrofolic acid with (−)-menthyl chloroformate, which interfered with the product composition[34]. In the present paper we describe a new efficient product analysis and much better stereoselectivities, which resulted from a screening of new procatalysts, optically active ligands, immobilization supports, and reaction conditions[35,36].

Standard Reaction

The standard procedure in the synthesis of *N*5-formyltetrahydrofolic acid (**4**) consists of two steps. In the first step, folic acid (**1**) is hydrogenated during 24 hours with an immobilized optically active rhodium(I)/diphosphane catalyst at 80°C and at a H_2 pressure of 50 atm. The solvent is a NaH_2PO_4/Na_2HPO_4 buffer of pH 7, in which folic acid dissolves on addition of NaOH. For the immobilization of the catalyst on the support, the procatalyst (usually $[Rh(cod)Cl]_2$, cod = 1,5-cyclooctadiene) and a diphosphane (ratio Rh/PP = 1:1.18) are placed in a glass vessel fitting into a 100-ml stainless steel autoclave together with 0.7−0.8 g of the support, in most cases silica. After the addition of methylene chloride a yellow-orange rhodium-phosphane complex is formed, which at first dissolves in the methylene chloride. However, it absorbs within a few minutes on the surface of the support and the supernatant solution becomes colorless. After removal of the solvent in vacuo the catalyst powder should be used immediately in the hydrogenation, because storage for some days results in decreased stereoselectivities.

The hydrogenation product 5,6,7,8-tetrahydrofolic acid (**2**) is very light- and air-sensitive and decomposes easily. As the derivatization of the 5-amino group with electron-withdrawing substituents, such as a formyl group, increases its stability appreciably, tetrahydrofolic acid (**2**) is not isolated but formylated immediately with methyl formate/formic acid in a 5:1 mixture of DMSO/pyridine at room temperature to give 5-formyltetrahydrofolic acid (**4**), which is precipitated by addition of ether. In the normal procedure, **4** is dissolved in aqueous NaOH. Addition of ethanol precipitates the Na salt of **4**, which is suitable for a subsequent HPLC determination of the (6*S*,*S*)- and (6*R*,*S*)-leucovorin ratio using a NaH_2PO_4/Na_2HPO_4 buffer. In some cases **4** was dissolved in boiling water, treated with $CaCl_2$ and precipitated with ethanol as the Ca salt, the actual drug leucovorin.

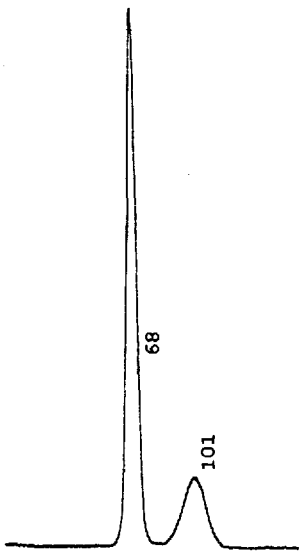
For a long time the variable water content of the folinate samples was a problem for the determination of the chemical yield by weighing. 5−6 hours of drying in high vacuo did not result in samples with a constant water content. Therefore, in Tables 1−5 for our early measurements only the diastereomeric excess of leucovorin is given and not its chemical yield. Later on we solved the problem on the basis of thermogravimetry and differential scanning calorimetry

measurements. These measurements showed that at a temperature of 130°C in vacuo the water can be completely removed from samples of the Na salt of folinic acid[36]. Thus, the chemical yield can be determined by weighing after heating of the final product at 130°C for 1 hour.

HPLC Analysis

For the analytical separation of the diastereomers of leucovorin silica gel HPLC columns were used, to which bovine serum albumine (BSA) is covalently bound[37–40]. Elution with a NaH_2PO_4/Na_2HPO_4 buffer of pH 7.5 resulted in a base line separation of the leucovorin diastereomers (Figure 1). The assignment was made on the basis of samples of pure (6*S*,*S*)-leucovorin and a 1:1 mixture of (6*R*,*S*)- and (6*S*,*S*)-leucovorin. The diastereomeric excess was calculated by using the equation % de = $(|A_S - A_R|)/(A_S + A_R) \cdot 100\%$, in which A_S and A_R are the integration areas of the (6*S*,*S*) and (6*R*,*S*) diastereomers. For repeated measurements the diastereomeric excess varied by approximately ±2.5%.

Figure 1. HPLC chromatogram of (6*S*,*S*)-leucovorin (retention time 6.8 min) and (6*R*,*S*)-leucovorin (retention time 10.1 min); diastereomeric excess: 50.9% de by integration; two Resolvosil BSA7 columns (150 × 4 mm), connected with each other; eluent: aqueous NaH_2PO_4/Na_2HPO_4 buffer of pH = 7.5; flow rate: 0.9 ml/min; pressure: 120 atm



In the case of incomplete hydrogenation, in addition to the two peaks of the leucovorin diastereomers two further peaks appear in the chromatograms, the peaks of unreacted starting material folic acid (retention time 17.5 min) and of its *N*10-formyl derivative (retention time 8.8 min). Incompletely hydrogenated samples may also give rise to other small peaks, which probably are due to partly hydrogenated folic acid and its formylation products. Completely reduced samples do show only the two diastereomer peaks in the chromatograms.

Catalysts with Different Ligands

A series of mono-, bi-, and tridentate optically active phosphanes were used in the preparation of the immobilized catalysts for the hydrogenation of folic acid[35,36]. Bidentate chelate ligands turned out to be most suitable. In Table 1 only systems are summarized for which the diastereoselectives exceeded 20% de. The standard procatalyst in this series of experiments was $[Rh(cod)Cl]_2$ and the support was silica gel 60 (125–200 μm; Merck). For the optically active ligands the corresponding acronyms were used. All the ligands were characterized by their chemical names and a leading reference in the footnotes of the tables or the references section.

Table 1. Hydrogenation of folic acid (**1**) with in situ catalysts consisting of the procatalyst $[Rh(cod)Cl]_2$ and different optically active diphosphanes[a]

No.	Ligand	% de	Config.	Yield %	Runs
1	(-)-BDPP[b]	18.4, 20.7	(*S*)	93, 94	2
2	(-)-BPPM[c]	51.3 - 54.5	(*S*)	85 - 87	6
3	(-)-Diop[d]	49.0 - 53.0	(*S*)	86 - 88	6
4	(+)-Binap[e]	50.0, 51.2	(*S*)	40, 44	2
5	(-)-Binap[f]	50.1, 50.0	(*R*)	47, 49	2
6	(-)-BDPPP[g]	25.6, 26.4	(*S*)	-	2
7	(+)-OMe-BDPPP[h]	30.2, 32.5	(*S*)	70, 72	2
8	(+)-Ph-ProNOP[i]	27.0, 31.3	(*S*)	67, 69	2
9	(+)-Ph-5-oxo-ProNOP[j]	29.7, 31.7	(*S*)	63, 67	2

[a] Standard conditions: ratio Rh/PP = 1:1.18, ratio Rh/folic acid = 1:40, H_2 pressure 50 atm, 80°C, 24 h, support: silica gel 60 (125–200 μm, Merck). – [b] (*S*,*S*)-2,4-Bis(diphenylphosphanyl)-pentane[43]. – [c] (2*S*,4*S*)-1-*tert*-Butoxycarbonyl-4-diphenylphosphanyl-2-diphenylphosphanylmethylpyrrolidine[44,45]. – [d] (4*R*,5*R*)-*trans*-4,5-Bis(diphenylphosphanylmethyl)-2,2-dimethyl-1,3-dioxolane[46]. – [e] (*R*)-2,2′-Bis(diphenylphosphanyl)-1,1′-binaphthalene[47]. – [f] (*S*)-2,2′-Bis(diphenylphosphanyl)-1,1′-binaphthalene[47]. – [g] (*S*)-1,4-Bis(diphenylphosphanyl)pentane[48]. – [h] (*R*)-1-Methoxy-2,5-bis(diphenylphosphanyl)pentane[48]. – [i] (*S*)-2-Diphenylphosphanoxymethyl-*N*-diphenylphosphanylpyrrolidine[49]. – [j] (*S*)-5-Diphenylphosphanoxymethyl-2-*N*-diphenylphosphanylpyrrolidinone[50].

The stereoselectivity of the hydrogenation of folic acid turned out to be decisively dependent on the size of the chelate ring which the ligands form with the rhodium. Chelating phosphanes forming five-membered rings, extremely efficient in the hydrogenation of dehydroamino acids, gave only poor results, including (+)-Prophos[41] (~3% de) and (+)-Norphos[42] (~15% de). (-)-BDPP, forming a six-membered chelate ring, gave a diastereomeric excess of about 20% de and good chemical yields of 93 and 94% (Table 1, no. 1).

Bidentate phosphanes, forming seven-membered chelate rings, were the most efficient ligands in folic acid hydrogenation catalysts. The cocatalyst (-)-BPPM, derived from the amino acid hydroxyproline, reproducibly gave a stereoselectivity of 50–55% de as well as high chemical yields (no. 2). In addition to (-)-BPPM the three related derivatives (-)-PPM[51], (-)-MCCPM[52], and (-)-BCPM[53] were tested in folic acid hydrogenation. In (-)-PPM the *tert*-butoxycarbonyl substituent at the N atom in the pyrrolidine ring of (-)-BPPM is replaced by a H atom. In (-)-MCCPM and (-)-BCPM instead of a diphenylphosphanyl group a bulky dicyclohexylphosphanyl group is bound to C-4 of the pyrrolidine ring. Interestingly, there was no catalysis with the (-)-MCCPM and (-)-BCPM systems and (-)-PPM gave only low stereoselectivities of 1.1 and 4.5% de.

With the ligand (−)-Diop (no. 3) a diastereomeric excess of 49.0−53.0% and a chemical yield of 86−88% could be achieved for the (6*S*,*S*) isomer. (+)-Diop induced the undesired (6*R*,*S*) configuration in the hydrogenation product with 10.6 and 12% de[35]. In contrast to the Diop enantiomers, both Binap enantiomers (nos. 4 and 5) gave similar stereoselectivities of approximately 50% de, (+)-Binap inducing the (6*S*,*S*)- and (−)-Binap the (6*R*,*S*) configuration in the hydrogenation product. However, the chemical yields in the Binap systems were only 40−50%. Thus, the Binap ligands are inferior to (−)-BPPM and (−)-Diop in the hydrogenation of folic acid. With the ligands (−)-BDPPP (chemical yield not determined), (+)-OMe-BDPPP, (*S*)-Ph-ProNOP, and (*S*)-Ph-5-oxo-ProNOP stereoselectivities of 25−30% de and chemical yields of 60−70% were obtainend (nos. 6−9).

Different Procatalysts

In the rhodium(I) precursors, the olefin component as well as the anion can be modified. In this procatalyst screening the cocatalysts (−)-BPPM and (−)-Diop were used. The support was silica gel 60 (125−200 μm, Merck). The results are summarized in Table 2.

Table 2. Hydrogenation of folic acid (**1**) with in situ catalysts consisting of different rhodium precursors and the optically active ligands (−)-BPPM and (−)-Diop[a]

No.	Procatalyst	Ligand	% de	Config	Yield %	Runs
1	$[Rh(cod)Cl]_2$[b]	(-)-BPPM	51.3 - 54.5	(*S*)	85 - 87	6
		(-)-Diop	49.0 - 53.0	(*S*)	86 - 88	6
2	$[Rh(hxd)Cl]_2$[c]	(-)-BPPM	50.0, 52.9	(*S*)	86, 89	2
		(-)-Diop	23.1, 26.6	(*S*)	72, 75	2
3	$[Rh(nbd)Cl]_2$[d]	(-)-BPPM	32.6, 34.8	(*S*)	81, 84	2
		(-)-Diop	15.8, 22.0	(*S*)	83, 87	2
4	$[Rh(C_2H_4)_2Cl]_2$[e]	(-)-BPPM	36.7, 39.1	(*S*)	49, 52	2
		(-)-Diop	21.9, 24.4	(*S*)	64, 66	2
5	$[Rh(coe)_2Cl]_2$[f]	(-)-BPPM	21.4, 25.0	(*S*)	66, 69	2
		(-)-Diop	17.2, 18.7	(*S*)	62, 64	2
6	$[Rh(cod)OAc]_2$[g]	(-)-BPPM	41.5, 44.5	(*S*)	82, 83	2
		(-)-Diop	23.1, 25.7	(*S*)	86, 89	2
7	[Rh(cod)acac][h]	(-)-BPPM	21.4, 25.8	(*S*)	18, 20	2
		(-)-Diop	31.6, 32.6	(*S*)	28, 31	2
8	$[Rh(\eta\text{-allyl})_2Cl]_2$[i]	(-)-BPPM	40.4, 45.0	(*S*)	79, 82	2
		(-)-Diop	21.0, 23.7	(*S*)	73, 75	2

[a] Standard conditions: ratio Rh/PP = 1:1.18, ratio Rh/folic acid = 1:40, H_2 pressure 50 atm, 80°C, 24 h, support: silica gel 60 (125−200 μm, Merck). − [b] Bis{μ-chloro[1,2:5,6-η-(1,5-cyclooctadiene)]rhodium}[54]. − [c] Bis[μ-chloro{1,2:5,6-η-(1,5-hexadiene)}rhodium][55]. − [d] Bis[μ-chloro{2,3:5,6-η-(2,5-norbornadiene)}rhodium][56]. − [e] Bis[μ-chloro{di(ethylene)}rhodium][57]. − [f] Bis{μ-chloro[di(η²-cyclooctene)]rhodium}[58]. − [g] Bis[μ-acetato{1,2:5,6-η-(1,5-cyclooctadiene)}rhodium][59]. − [h] (Acetylacetonato)[1,2:5,6-η-(1,5-cyclooctadiene)]rhodium[60]. − [i] Bis[μ-chloro{di(η³-allyl)}rhodium], a rhodium(III) precursor[61].

The standard precursor $[Rh(cod)Cl]_2$ turned out to be the most efficient procatalyst for the folic acid hydrogenation. Its de values (Table 2; no. 1) are higher than those of the the corresponding 1,5-hexadiene and 2,5-norbornadiene complexes (nos. 2 and 3). On the other hand, complexes of the diolefins induce higher stereoselectivities than the complexes of the monoolefins ethylene and cyclooctene (nos. 4 and 5). Also, the exchange of the chloride anion for acetate or acetylacetonate (nos. 6 and 7) or the introduction of the η-allyl ligand (no. 8) does not increase the diastereomeric excess in the folic acid hydrogenation. Interestingly, for all the procatalysts of Table 2, (−)-BPPM proved to be a more efficient cocatalyst than (−)-Diop except for [Rh(cod)acac] (no. 7).

Catalysts Immobilized on Different Supports

In the screening of the cocatalysts and procatalysts silica gel 60 (125−200 μm, Merck) was used as the support for the immobilization of the catalyst. In this section the use of different silica gel supports, differing in particle size and particle size distribution, is described. Interestingly, the type of the silica gel has a profound influence on the chemical yield and, in particular, on the stereochemistry of the hydrogenation of folic acid (**1**). The standard procatalyst $[Rh(cod)Cl]_2$ and the ligands (−)-BPPM and (−)-Diop were used together with silica gels nos. 1− 13 (Table 3) in the heterogeneous reduction of folic acid (**1**).

Throughout, the ligand (−)-BPPM was more suitable than (−)-Diop, except for the support Florisil, a Mg silicate (MgO/SiO_2 = 15:85), for which (−)-Diop proved more effective than (−)-BPPM (no. 13). Most of the silica supports gave good chemical yields in the range between 80 and 92%. The highest stereoselectivity was achieved with the silica gel Merckosorb SI60 (no. 3). (−)-Diop gave a diastereomeric excess of 80.4 and 84.1%, and with (−)-BPPM even 87.9−92.3% de were attained. This silica gel had a medium particle size of 40 μm. For comparison, silical gel 60 (no. 1) and silica gel 60 (no. 2) have particle sizes of 63−200 μm and 125−200 μm, respectively.

Other supports were not as useful as silica gels in the heterogeneous hydrogenation of folic acid (**1**). No catalysis took place with $[Rh(cod)Cl]_2$/(−)-BPPM or (−)-Diop catalysts immobilized from methylene choride solution onto the surface of $BaSO_4$, TiO_2, MgO, molecular sieve, sephadex, or cellulose. On Al_2O_3 (Al_2O_3 90, 63−200 μm, Merck), $[Rh(cod)Cl]_2$/(−)-BPPM induced a diastereomeric excess of 47.3−49.1% and on celite $[Rh(cod)Cl]_2$/(−)-Diop gave 23.2% de[35]. Immobilization of $[Rh(cod)Cl]_2$/(−)-BPPM and $[Rh(cod)Cl]_2$/(−)-Diop from ethanol solution onto charcoal resulted in only 4.6 and 4.9% de and 46 and 60% chemical yield in folic acid hydrogenation[36].

Repeated Use of the Immobilized Catalyst

With the procatalyst $[Rh(cod)Cl]_2$, the optically ligand (−)-BPPM and the silica gel 60 (125−200 μm, Merck) it was investigated whether the immobilized catalyst could be used repeatedly in the heterogeneous hydrogenation of folic acid (**1**). After completion of the previous run, the catalyst was separated by filtration, washed three times with water and dried for 4 h in vacuo. We tried to reuse the catalysts, loaded with $[Rh(cod)Cl]_2$/(−)-Diop or (−)-BPPM on different silica gels. However, in all successive hydrogenations the stereoselectivity decreased, e.g. in the system $[Rh(cod)Cl]_2$/(−)-BPPM with silica gel 60 (125−200 μm, Merck), from 51% de in the first run to 40% de in the second run[35].

We also tried to supply used-up catalysts with a fresh catalyst load. To this end, the catalyst obtained after com-

Table 3. Hydrogenation of folic acid (**1**) with in situ catalysts consisting of the procatalyst $[Rh(cod)Cl]_2$ and the cocatalyst (−)-BPPM and (−)-Diop immobilized on different silica gels as support[a]

No.	Silica Gel	Particle Size	Ligand	% de	Config.	Yield %	Runs
1	silica gel 60[b]	63 - 200 μm	(-)-BPPM	45.6, 46.8	(*S*)	-	2
			(-)-Diop	50.7, 52.3	(*S*)	-	2
2	silica gel 60[b]	125 - 200 μm	(-)-BPPM	51.3 - 54.5	(*S*)	85 - 87	6
			(-)-Diop	49.0 - 53.0	(*S*)	86 - 88	6
3	Merckosorb SI60[b]	medium particle size 40 μm	(-)-BPPM	87.9 - 92.3	(*S*)	86 - 89	6
			(-)-Diop	80.4, 84.1	(*S*)	86, 88	2
4	LiChrosorb SI60[b]	10 μm	(-)-BPPM	47.9, 53.9	(*S*)	85, 88	2
			(-)-Diop	29.6, 32.5	(*S*)	86, 88	2
5	LiChrosorb SI100[b]	10 μm	(-)-BPPM	45.4, 52.0	(*S*)	79, 82	2
			(-)-Diop	30.7, 32.5	(*S*)	80, 83	2
6	LiChroprep Si60[b]	40 - 63 μm	(-)-BPPM	16.2, 17.2	(*S*)	-	2
			(-)-Diop	22.6, 23.8	(*S*)	-	2
7	LiChroprep Si60[b]	15 - 25 μm	(-)-BPPM	48.4, 50.6	(*S*)	-	2
			(-)-Diop	17.2, 18.8	(*S*)	-	2
8	silica gel 60[b]	0.2 - 0.5 nm	(-)-BPPM	44.9, 48.6	(*S*)	87, 90	2
			(-)-Diop	23.2, 24.2	(*S*)	89, 92	2
9	Davisil 60[c]	60 Å	(-)-BPPM	20.7, 26.6	(*S*)	82, 84	2
			(-)-Diop	18.3, 23.4	(*S*)	85, 86	2
10	Davisil 100[c]	100 Å	(-)-BPPM	43.0, 47.5	(*S*)	84, 87	2
			(-)-Diop	19.6, 24.5	(*S*)	85, 87	2
11	SiO_2, type Aa 16/6[d]	-	(-)-BPPM	49.6, 51.0	(*S*)	75, 80	2
			(-)-Diop	22.9, 25.3	(*S*)	82, 83	2
12	SiO_2, type Aa 10/6[d]	-	(-)-BPPM	30.5, 35.1	(*S*)	77, 79	2
			(-)-Diop	19.5, 23.1	(*S*)	79, 81	2
13	Florisil[b]	75 - 150 μm	(-)-BPPM	35.3, 38.5	(*S*)	43, 45	2
			(-)-Diop	38.3, 44.9	(*S*)	39, 42	2

[a] Standard conditions: ratio Rh/PP = 1:1.18, ratio Rh/folic acid = 1:40, H_2 pressure 50 atm, 80 °C, 24 h. – [b] Merck. – [c] Aldrich. – [d] Heraeus.

pletion of the previous run was subjected to the usual immobilization procedure with $[Rh(cod)Cl]_2$ and (−)-BPPM in methylene chloride. These catalysts showed a somewhat reduced activity and an appreciably reduced stereoselectivity (Table 4)[36]. The diastereomeric excess decreased from 51.3−54.5 to 32.6−34.5 in the fourth reuse. Obviously, in the first immobilization step, surface sites on the silica gel are occupied, which are no longer available for the next catalyst loads.

Table 4. Hydrogenation of folic acid (**1**) with in situ catalysts consisting of $[Rh(cod)Cl]_2$ and (−)-BPPM, loaded on catalysts used in previous hydrogenation reactions[a]

No.	Ligand	Use	% de	Config.	Yield %	Runs
1	(-)-BPPM	1	51.3 - 54.5	(*S*)	85 - 87	6
2	(-)-BPPM	2	43.2, 44.8	(*S*)	84, 86	2
3	(-)-BPPM	3	40.3, 41.6	(*S*)	83, 84	2
4	(-)-BPPM	4	35.2, 37.2	(*S*)	79, 80	2
5	(-)-BPPM	5	32.6, 34.5	(*S*)	75, 76	2

[a] Standard conditions: ratio Rh/PP = 1:1.18, ratio Rh/folic acid = 1:40, H_2 pressure 50 atm, 80 °C, 24 h, support: silica gel 60 (125−200 μm, Merck).

Variation of the Catalyst Substrate Ratio

In all the experiments described, the catalyst-to-folic acid ratio was 1:40. We tried to improve this ratio in the two systems procatalyst $[Rh(cod)Cl]_2$, cocatalysts (−)-BPPM and (−)-Diop on silica gel 60 (125−200 μm, Merck) (Table 5).

In both series the same trends were observed, a decrease of the diastereomeric excess with rising amounts of substrate and, more seriously, a drop of the chemical yields (Table 5). After work-up and derivatization, there were peaks of folic acid and its derivatization products in the HPLC chromatograms. Thus, by increasing the amount of substrate with respect to the catalyst the folic acid hydrogenation under standard conditions was no longer quantitative.

Table 5. Hydrogenation of folic acid (**1**) with in situ catalysts consisting of $[Rh(cod)Cl]_2$ and (−)-BPPM or (−)-Diop[a]; variation of the catalyst/substrate ratio

No.	Ligand	Cat./Substr.	% de	Config.	Yield %	Runs
1	(-)-BPPM	1:40	51.3 - 54.5	(*S*)	85 - 87	6
2	(-)-BPPM	1:80	49.1, 51.7	(*S*)	49, 51	2
3	(-)-BPPM	1:120	46.3, 48.7	(*S*)	46, 48	2
4	(-)-BPPM	1:160	38.9, 42.9	(*S*)	36, 38	2
5	(-)-Diop	1:40	49.0, 53.0	(*S*)	86, 88	2
6	(-)-Diop	1:80	39.9, 42.4	(*S*)	52, 55	2
7	(-)-Diop	1:120	35.6, 37.0	(*S*)	40, 42	2
8	(-)-Diop	1:160	27.6, 30.7	(*S*)	26, 29	2

[a] Standard conditions: ratio Rh/PP = 1:1.18, H_2 pressure 50 atm, 80 °C, 24 h, support: silica gel 60 (125−200 μm, Merck).

In conclusion, it is possible to reduce folic acid (**1**) successfully to tetrahydrofolic acid (**2**) by using immobilized rhodium(**1**)/diphosphane catalysts with a high stereoselectivity, but, unfortunately, with a low substrate-to-catalyst ratio (40:1). However, compared to the separation of the (6*R*,*S*)- and (6*S*,*S*)-leucovorin diastereomers, in which half of the material cannot be used, in the stereoselective hydrogenation of folic acid (**1**) there is almost no waste, which has to be thrown away.

We thank the *Deutsche Forschungsgemeinschaft*, the *Fonds der Chemischen Industrie*, the *BMBF* and the *BASF, Ludwigshafen*, for support of this work.

Experimental Section

All reactions were carried out under nitrogen by using standard Schlenk techniques. Solvents were dried under N_2 by standard procedures. Water was bidistilled and saturated with nitrogen. The phosphate buffers were obtained by dissolution of sodium monophosphate and sodium biphosphate (Merck) in water and were saturated with nitrogen. The transition-metal complexes $[Rh(cod)Cl]_2$[54], $[Rh(hxd)Cl]_2$[55], $[Rh(nbd)Cl]_2$[56], $[Rh(C_2H_4)_2Cl]_2$[57], $[Rh(coe)_2Cl]_2$[58], $[Rh(cod)OAc]_2$[59], [Rh(cod)acac][60], and $[Rh(\eta\text{-allyl})_2Cl]_2$[61] were prepared by reported methods. The bidentate phosphanes were commercially avaible or obtained as described in the literature. The supporting materials were degassed in vacuo and flushed with nitrogen. Folic acid was commercially avaible from Fluka. – ^1H NMR: Bruker WM 250 and Bruker ARX 400. – Analytical HPLC: Liquid chromatograph Spectra Physics SP8000, detector Spectra Physics SP8210 with wavelength 254 nm. – pH determination: pH meter CG 820 (Schott).

Preparation of the Immobilized Catalysts: Under nitrogen, 7.0 mg (0.014 mmol) of $[Rh(cod)Cl]_2$ (0.028 mmol of Rh) and 0.033 mmol of the bidentate phosphane (ratio rhodium/phosphane = 1:1.18) were dissolved in 10 ml of methylene chloride in a glass vessel fitting into a 100-ml stainless steel autoclave, which was connected with a vacuum line via a joint, and 0.7–0.8 g of the catalyst support was added. The intensely orange suspension was stirred for 30 min at room temperature. During this time the rhodium-phosphane complex that formed was adsorbed on the support, which was apparent from the decolorization of the solution. The methylene chloride was removed in vacuo and the yellow-orange catalyst powder was dried for 30 min.

Catalytic Hydrogenation of Folic Acid (**1**): Into the glass vessel, containing the heterogeneous catalyst, a solution of folic acid in an aqueous phosphate buffer was given. The solution was obtained by suspending folic acid (500 mg, 1.13 mmol) in 25 ml of a NaH_2PO_4/Na_2HPO_4 buffer (pH 7) and dissolving it with about 45.0 mg (1.13 mmol) of sodium hydroxide. The buffer was obtained by appropriate mixing of 67.0 mmol/l solutions of NaH_2PO_4 and Na_2HPO_4. The glass vessel was inserted into a nitrogen-purged 100-ml stainless steel autoclave, which was flushed 3 times with 20 atm of H_2. Then the H_2 pressure in the reactor was adjusted to 50 atm. The reaction mixture was stirred for 24 h at 80–82 °C in an oil bath. After cooling to room temperature, the yellow solution was separated from the heterogeneous catalyst, which usually had become brown, by filtration. The solid residue was washed twice with 3.0 ml of the phosphate buffer. From the combined aqueous solutions the water was removed in vacuo (water bath, 45 °C), leaving a viscous residue, which contained the light- and air-sensitive 5,6,7,8-tetrahydrofolic acid (**2**).

Derivatization of Tetrahydrofolic Acid (**2**) *to 5-Formyltetrahydrofolic Acid* (**4**): The residue, containing 5,6,7,8-tetrahydrofolic acid (**2**), was suspended in 10 ml of a 5:1 mixture of DMSO/pyridine and 6.5 ml of methyl formate and dissolved with 2.5 ml of formic acid. After 24 h and 48 h methyl formate (6.5 ml) was added again. After 72 h, 10 ml of ethanol and 10 mg of charcoal were added and the mixture was filtered. To the filtrate, ether (150 ml) was added. The voluminous precipitate was filtered off, washed 3 times with ether and dried in vacuo.

Sodium Salt of 5-Formyltetrahydrofolic Acid: 5-Formyltetrahydrofolic acid (**4**) (200 mg) was dissolved in 5.0 ml of a 0.1 N NaOH solution. After the addition of 50 ml of ethanol (99%) the solution was cooled to −20 °C for 3 d. Na folinate precipitated as a fine yellow powder, which was filtered off, washed with ether and dried in vacuo at 130 °C for 1 h. Then the yield was determined by weighing. ^1H NMR (D_2O, NaOD (40%), Na salt of 3-(trimethylsilyl)propionic acid-d_4): δ = 1.93–2.25 (m, 2H, C(β)H_2, glutamic acid), 2.31–2.34 (m, 2H, C(γ)H_2, glutamic acid), 3.15–3.50 (m, 5H, C(7)H_2 – C(6)H – C(9)H_2), 4.24–4.32 (m, 1H, C(α)H, glutamic acid), 4.81 (s, H_2O), 6.78/6.84 and 7.69/7.75 (AA'BB' system, 4H, ^3J = 8.8, C_6H_4), 8.72 (s, 1H, N5-formyl).

Analytical HPLC: The diastereomers of leucovorin were separated on two Resolvosil BSA7 columns (150 × 4 mm) connected with each other. The eluent was a buffer of pH 7.5, obtained by appropriate mixing of 50.0 mmol/l solutions of NaH_2PO_4 and Na_2HPO_4. The eluent and the HPLC system were degassed with helium. The leucovorin isomers were separated at a flow-rate of 0.9 ml/min and detected at a wavelength of 254 nm. The retention times were 6.8 min for (6*S*,*S*)-leucovorin, 10.1 min for (6*R*,*S*)-leucovorin and 17.5 min for unreacted folic acid (**1**).

[1] H. Brunner, L. Wagenhuber, *J. Organomet. Chem.*, in press.
[2] W. Friedrich, *Vitamins*, De Gruyter, Berlin, **1988**.
[3] R. L. Blakley in *The Biochemistry of Folic Acid and Related Pteridines* (Eds.: A. Neuberger, E. L. Tatum), vol. 13, North-Holland Publishing Company, Amsterdam, **1969**.
[4] G. H. Hitchings, *Angew. Chem.* **1989**, *101*, 903; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 879.
[5] B. A. Chabner, M. Slavik, *Chemother. Rep.* **1975**, *6*, 1.
[6] N. Jaffe, S. Howell, *Adv. Cancer Chemother.* **1979**, *1*, 111.
[7] U. Lönn, S. Lönn, *Biochem. Biophys. Res. Commun.* **1986**, *138*, 54.
[8] F. M. Sirotnak, P. L. Chello, D. M. Moccio, R. L. Kisliuk, G. Combepine, Y. Gaumont, J. A. Montgomery, *Biochem. Pharm.* **1979**, *28*, 2993.
[9] C. Temple, J. D. Rose, W. R. Laster, J. A. Montgomery, *Cancer Treat. Rep.* **1981**, *65*, 1117.
[10] *Physician's Desk Reference*, 38th Ed., Medical Economics Co., Oradell, NJ, **1984**, p. 821 and 1078.
[11] J. C. Fontecille-Camps, C. E. Brugg, C. Temple, J. D. Rose, J. A. Montgomery, R. L. Kisliuk, *J. Am. Chem. Soc.* **1979**, *101*, 6114.
[12] D. B. Cosulich, J. M. Smith, H. P. Broquist, *J. Am. Chem. Soc.* **1952**, *74*, 4215.
[13] R. P. Leary, Y. Gaumont, R. L. Kisliuk, *Biochem. Biophys. Res. Commun.* **1973**, *56*, 484.
[14] G. K. Smith, P. A. Benkovic, S. J. Benkovic, *Biochemistry* **1981**, *20*, 4034.
[15] J. A. Straw, J. M. Covey, D. Szapary, *Cancer Res.* **1981**, *41*, 3936.
[16] J. A. Straw, W. T. Wynn, D. Szapary, *Cancer Res.* **1984**, *44*, 3114.
[17] M. May, T. J. Barger, M. Lansford, J. M. Ravel, G. L. Sutherland, W. Shive, T. J. Bardos, *J. Am. Chem. Soc.* **1951**, *73*, 3067.
[18] B. Roth, M. E. Hultquist, M. J. Fahrenbach, E. L. R. Stokstad, D. B. Cosulich, H. P. Broquist, R. P. Parker, J. M. Smith, *J. Am. Chem. Soc.* **1952**, *74*, 3247.
[19] A. Bobst, M. Viscontini, *Helv. Chim. Acta* **1966**, *49*, 875.
[20] C. Temple, R. D. Elliot, J. D. Rose, J. A. Montgomery, *J. Med. Chem.* **1979**, *22*, 731.
[21] L. Rees, C. J. Suckling, H. C. S. Wood, *J. Chem. Soc., Chem. Commun.* **1987**, 470.
[22] C. K. Mathews, F. M. Huennekens, *J. Biol. Chem.* **1969**, *235*, 3304.
[23] H. R. Mueller, M. Ulman, J. Conti, G. Muerdel, *PCT Int. Appl. WO 88 08,844* **1988**; *Chem. Abstr.* **1989**, *111*, P 102722q.
[24] G. Vecchi, *PCT Int. Appl. WO 93 15,076* **1993**; *Chem. Abstr.* **1993**, *119*, P 226424d.
[25] J. Feeney, B. Birdsall, J. P. Albrand, G. C. K. Roberts, A. S. Burgen, P. A. Charlton, D. W. Young, *Biochemistry* **1981**, *20*, 1837.
[26] P. A. Charlton, D. W. Young, *J. Chem. Soc., Chem. Commun.* **1979**, 734.
[27] L. Rees, E. Valente, C. J. Suckling, H. C. S. Wood, *Tetrahedron* **1986**, *42*, 117.
[28] T. Uwajima, T. Oshiro, T. Eguchi, Y. Kuge, A. Horiguchi, A. Igarashi, K. Mochida, M. Iwakura, *Biochem. Biophys. Res. Commun.* **1990**, *171*, 684.
[29] T. Eguchi, T. Oshiro, Y. Kuge, K. Mochida, T. Uwajima, *Eur. Pat. Appl. EP 350934* **1990**; *Chem. Abstr.* **1990**, *112*, P 234008s.
[30] P. H. Boyle, M. T. Keating, *J. Chem. Soc., Chem. Commun.* **1974**, 375.

[31] The hydrogenation procedure of ref.[30] was repeated several times. Using the HPLC analysis described in the present paper, the diastereomeric excess turned out to be 2.6–5.6% de.
[32] K. E. Koenig in *Asymmetric Synthesis* (Ed.: J. D. Morrison), vol. 5, Academic Press, Orlando, **1985**, p. 71.
[33] H. Brunner, W. Zettlmeier, *Handbook of enantioselective catalysis with transition metal compounds*, VCH, Weinheim, **1993**.
[34] H. Brunner, C. Huber, *Chem. Ber.* **1992**, *125*, 2085.
[35] P. Bublak, Dissertation, University of Regensburg, **1994**.
[36] M. Helget, Dissertation, University of Regensburg, **1996**.
[37] R. J. Mullin, D. S. Dutch, *Chemistry and Biology of Pteridines*, De Gruyter, Berlin, **1989**, p. 167.
[38] K. E. Choi, R. L. Shilsky, *Anal. Biochem.* **1988**, *168*, 398.
[39] I. W. Wainer, R. M. Stiffin, *J. Chromatogr.* **1988**, *424*, 158.
[40] L. Silan, P. Jadaud, L. R. Whitfield, I. W. Wainer, *J. Chromatogr.* **1990**, *532*, 227.
[41] (*R*)-(+)-1,2-Bis(diphenylphosphanyl)propane, M. D. Fryzuk, B. Bosnich, *J. Am. Chem. Soc.* **1978**, *100*, 5491.
[42] (*S*,*S*)-2,3-Bis(diphenylphosphanyl)bicyclo[2.2.1]hept-5-ene, H. Brunner, W. Pieronczyk, *Angew. Chem.* **1979**, *91*, 655; *Angew. Chem. Int. Ed. Engl.* **1979**, *18*, 620.
[43] J. Bakos, I. Toth, B. Heil, L. Marko, *J. Organomet. Chem.* **1985**, *279*, 23.
[44] K. Achiwa, *J. Am. Chem. Soc.* **1976**, *98*, 8265.
[45] H. B. Kagan, T.-P. Dang, *J. Am. Chem. Soc.* **1972**, *94*, 6429.
[46] B. A. Murrer, J. M. Brown, P. A. Chaloner, P. D. Nicholson, D. Parker, *Synthesis* **1979**, 350.
[47] H. Takaya, K. Mashima, K. Koyano, M. Yagi, H. Kumobayashi, T. Taketomi, S. Akutagawa, R. Noyori, *J. Org. Chem.* **1986**, *51*, 629.
[48] H. Brunner, H. J. Lautenschlager, W. A. König, R. Krebber, *Chem. Ber.* **1990**, *123*, 847.
[49] C. Hatat, N. Kokel, A. Mortreux, F. Petit, *Tetrahedron Lett.* **1990**, *31*, 4139.
[50] A. Roucoux, F. Agbosson, A. Mortreux, F. Petit, *Tetrahedron: Asymmetry* **1993**, *4*, 2279.
[51] (2*S*,4*S*)-4-Diphenylphosphanyl-2-diphenylphosphanylmethylpyrrolidine, ref.[44].
[52] (2*S*,4*S*)-4-Dicyclohexylphosphanyl-2-diphenylphosphanylmethyl-*N*-methyl-1-pyrrolidinecarboxamide, H. Takeda, S. Hosokawa, M. Aburatani, K. Achiwa, *Synlett*, **1991**, 193.
[53] (2*S*,4*S*)-1-*tert*-Butoxycarbonyl-4-dicyclohexylphosphanyl-2-diphenylphosphanylmethylpyrrolidine, H. Takahashi, M. Hattori, M. Chiba, T. Morimoto, K. Achiwa, *Tetrahedron Lett.* **1986**, *27*, 4477.
[54] W. P. Fehlhammer, W. A. Herrmann, K. Öfele, *Metallorganische Komplexverbindungen* (Ed.: G. Brauer), *Handbuch der präparativen anorganischen Chemie*, Enke Verlag, Stuttgart, **1981**, vol. III, p. 1799.
[55] G. Giordano, R. H. Crabtree, *Inorg. Synth.* **1990**, *19*, 218.
[56] E. W. Abel, M. A. Bennett, G. Wilkinson, *J. Chem. Soc.* **1959**, 3178.
[57] R. Cramer, *J. Am. Chem. Soc.* **1964**, *86*, 217.
[58] W. P. Fehlhammer, W. A. Herrmann, K. Öfele, *Metallorganische Komplexverbindungen* in G. Brauer (Ed.), *Handbuch der präparativen anorganischen Chemie*, Enke Verlag, Stuttgart, **1981**, vol. III, 1896.
[59] J. Chatt, L. M. Venanzi, *J. Chem. Soc.* **1957**, 4735.
[60] J. Powell, B. L. Shaw, *J. Chem. Soc. A* **1968**, 583.

[96132]