Stereospecific Hydrogenations V:
Hydrogenation Rates Using Palladium-on-Poly-S-leucine and Palladium-on-Poly-S-valine

ROBERT L. BEAMER and WILLIAM D. BROWN

Abstract ☐ The effect of polyamino acid secondary structure (helical or nonhelical) on the rates of hydrogenations of α-methylcinnamic acid and α-acetamidocinnamic acid catalyzed by palladium-on-poly-S-leucine and by palladium-on-poly-S-valine was investigated. For each substrate, the hydrogenations proceeded at a faster rate with palladium-on-poly-S-leucine (helical catalyst) than with palladium-on-poly-S-valine (nonhelical catalyst). That α-methylcinnamic acid and α-acetamidocinnamic acid occupy entirely different sites on the catalyst surface was shown by product and cross-inhibition studies. The observation that dihydro-α-methylcinnamic acid increases the rate of hydrogenation of α-acetamidocinnamic acid on palladium-on-poly-S-valine indicates a possible allosteric mechanism analogous to that observed with enzyme catalysis.

Keyphrases ☐ Palladium-on-poly-S-leucine—α-acetamidocinnamic acid, α-methylcinnamic acid hydrogenation rates ☐ α-Acetamidocinnamic acid, α-methylcinnamic acid hydrogenation rates—palladium-on-poly-S-valine ☐ Helical conformation—polyamino acid ☐ Catalyst surface-active sites—inhibition studies

Asymmetry in polypeptides and proteins can arise both from the asymmetric carbon atoms of the individual amino acid residues (primary structure) and from helical folding of the polymeric chains (secondary structure) (1). Stereospecific hydrogenations using palladium-on-poly-S-leucine, palladium-on-poly-S-valine, palladium-on-poly-γ-benzyl-S-glutamate, and palladium-on-poly-β-benzyl-S-aspartate were described in earlier papers of this series (2–4). α-Methylcinnamic acid and α-acetamidocinnamic acid, both of which form asymmetric carbon atoms upon hydrogenation, were used as substrates in the previous investigations. The absolute configurations of the reduced substrates have been determined (5).

Hydrogenations using palladium-on-poly-S-leucine, which possesses a right-handed helical structure (6), versus those using palladium-on-poly-S-valine, which is nonhelical (7), indicated that the chirality of the helical conformation had a greater influence on the enantioselectivity of the hydrogenations than the dissymmetry arising from the amino acid residues (4).

Hydrogenation of α-methylcinnamic acid using palladium-on-poly-S-amino acids, which possess a right-handed helical structure (viz., poly-S-leucine and poly-γ-benzyl-S-glutamate), produced R(−)-dihydro-α-methylcinnamic acid; hydrogenation using these same catalysts produced S(−)-phenylalanine (after hydrolysis of the hydrogenation product) when α-acetamidocinnamic acid was the substrate (2–4). Conversely, S(+)-dihydro-α-methylcinnamic acid and R(+)-phenylalanine were formed from this substrate when either a left-handed helical polyamino acid (viz., poly-β-benzyl-S-aspartate) or a nonhelical polyamino acid (viz., poly-S-valine) was used as the carrier for the palladium (3, 4).
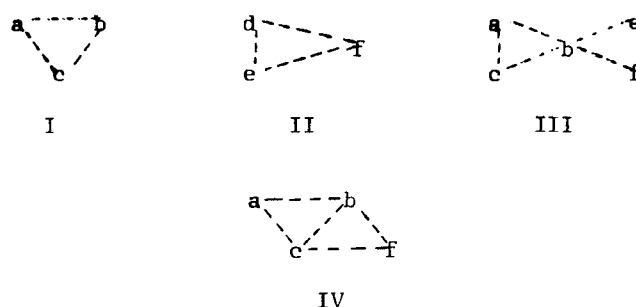

Figure 1—*Hypothetical active sites on a catalyst surface.*

The formation of products of opposite configuration when α-methylcinnamic acid and α-acetamidocinnamic acid were hydrogenated in the presence of the same catalyst led to the conclusion that these two substrates were occupying—at least in part—different sites on the catalyst surface.

The investigation described in this paper was undertaken to determine the influence of helical or nonhelical conformation (secondary structure) upon the hydrogenation rates of α-methylcinnamic acid and α-acetamidocinnamic acid. The helical or nonhelical influence on hydrogenation rates can be assessed by measuring the rate of hydrogen uptake by the respective substrate under identical conditions when catalyzed by palladium-on-poly-S-leucine (a helical catalyst) as compared with that catalyzed by palladium-on-poly-S-valine (a nonhelical catalyst).

Another objective of this investigation was to ascertain the degree to which α-methylcinnamic acid or α-acetamidocinnamic acid might occupy the same or different sites on the catalytic surface. Figure 1 illustrates substrate specific centers which could exist on a stereospecific catalytic surface.

Sites I and II each represent separate substrate-specific sites. The two substrate-specific sites present in III have one common point, while IV illustrates two sites having two points in common. Although the configurational evidence provided in the previous investigations of this series (2–4) showed that the

α-methylcinnamic acid

dihydro-α-methylcinnamic acid
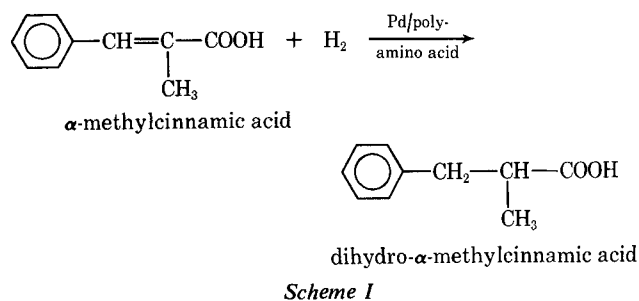
Scheme I

α-methylcinnamic acid and α-acetamidocinnamic acid must occupy different sites on the catalytic surfaces, these sites may or may not have points in common.

The hydrogenation of α-methylcinnamic acid is represented by Scheme I. Scheme II illustrates the hydrogenation of α-acetamidocinnamic acid to N-acetylphenylalanine followed by hydrolysis to phenylalanine.

Using a minimal catalyst (8, 9) and conducting the hydrogenation of α-methylcinnamic acid (Scheme I) in the presence of varying concentrations of the product, dihydro-α-methylcinnamic acid, the concentration of dihydro-α-methylcinnamic acid that produces product inhibition can be determined. In a similar manner, the concentration of N-acetylphenylalanine that inhibits the hydrogenation of α-acetamidocinnamic acid also can be determined.

By hydrogenating α-methylcinnamic acid in the presence of N-acetylphenylalanine and by hydrogenating α-acetamidocinnamic acid in the presence of dihydro-α-methylcinnamic acid (cross-inhibition studies), a determination of the degree to which the two substrates occupy the same catalytic site can be made. If dihydro-α-methylcinnamic acid inhibits the hydrogenation of α-acetamidocinnamic acid or if N-acetylphenylalanine inhibits the hydrogenation of α-methylcinnamic acid, the two substrates must be occupying the same site. On the other hand, if no cross-inhibition is seen in either hydrogenation, one must conclude that the two substrates are not occupying the same catalytic site. The concentrations of the inhibitors should correspond closely to the concentration of product that produced inhibition.

EXPERIMENTAL

Reagents—The following were used: α-methylcinnamic acid[1], α-acetamidocinnamic acid dihydrate[1], N-carbobenzyloxy-S-valine[2], leucine[3], and carbobenzyloxy chloride[2].
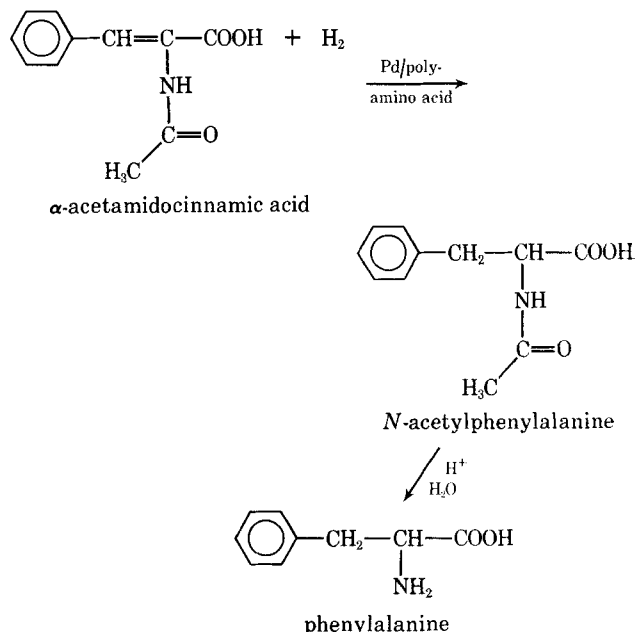
Absolute Alcohol—This reagent was prepared by taking commercial absolute alcohol and refluxing over calcium oxide for at least 24 hr. and then distilling. The product distilled at 78.5°.

Poly-S-leucine—This compound was prepared by the procedure of Beamer et al. (2), starting with S-leucine and carbobenzyloxy chloride.

Poly-S-valine—This compound was prepared by the method described by Beamer et al. (3), starting with N-carbobenzyloxy-S-valine.

Dihydro-α-methylcinnamic Acid—This compound was prepared by using 8.10 g. (0.05 mole) of α-methylcinnamic acid in 100 ml. of absolute alcohol and 1.0 g. of palladium-on-charcoal [100 mg. PdCl₂/g. charcoal prepared in the presence of sodium acetate (10)]. The hydrogenation was carried out in a Parr low-pressure hydrogenator at an initial pressure of 4.2 kg./cm.². The time required for complete hydrogenation was 1 hr. Following hydrogenation, the catalyst was removed by filtration and the ethanol was evaporated under reduced pressure. The residue was distilled under reduced pressure. The boiling point of the product was 114° at 0.25 Torr. [lit. (11) 160°/13 Torr.]. A neutralization equivalent of the product agreed with that calculated for dihydro-α-methylcinnamic acid. An IR spectrum of the product was identical with one reported for dihydro-α-methylcinnamic acid (12).

N-Acetylphenylalanine—This compound was prepared by the hydrogenation of α-acetamidocinnamic acid dihydrate, 4.8 g. (0.023 mole), using 1.0 g. palladium-on-charcoal [100 mg. PdCl₂/g.

[1] Aldrich Chemical Co.
[2] Nutritional Biochemicals.
[3] Mann Biochemical Corp.

α-acetamidocinnamic acid

N-acetylphenylalanine

phenylalanine

Scheme II

charcoal prepared in the presence of sodium acetate (10)]. Following hydrogenation, the ethanol was evaporated under reduced pressure and the white crystalline solid product recrystallized from absolute alcohol. The melting point of the product was 171–172° [lit. (13) 172°].

Preparation of Catalysts—The catalysts were prepared by depositing palladium (from a solution of palladous chloride, 2.5%) upon the respective polyamino acids according to Method II of Beamer et al. (2). The concentrations employed were 200, 100, 125, and 150 mg. of palladous chloride/g. of carrier[4]. In each study, the polyamino acid used to make the catalyst and the catalyst used came from a single batch [i.e., they were identical catalysts (8)].

Hydrogenation Studies—These studies were carried out in a Thomas microhydrogenator[5] modified by immersing the reaction flask in a 1000-ml. beaker into which water from a constant-temperature bath was circulated. All hydrogenations were conducted at a temperature of 36.0°.

The hydrogenation procedure of Ogg and Cooper (14) was used for these studies. In all studies, 30.0 mg. of catalyst was employed along with 4 ml. of absolute alcohol; the amount of substrate used was 1×10^{-4} mole (16.2 mg. of α-methylcinnamic acid and 24.1 mg. of α-acetamidocinnamic acid dihydrate). The amount of product necessary to produce product inhibition was determined to be 8 times the concentration of the substrate. An initial concentration of 8×10^{-4} mole of product or inhibitor/4 ml. of absolute alcohol was used in both product and cross-inhibition studies (131.4 mg. of dihydro-α-methylcinnamic acid and 165.8 mg. of N-acetylphenylalanine). During the hydrogenations, the volume of hydrogen consumed was recorded at set intervals. The results of the studies were processed using an IBM 7040 computer and a least-squares FORTRAN IV program[6]. This program converted all data into volumes of hydrogen at standard temperature and pressure. The program then converted the volumes into millimoles of hydrogen. It also gave values for the y-intercept and slope for each set of data. The calculated slope was an average slope, because only the first portion of the curve was linear. The various runs for each study were averaged, and the standard deviation between runs was determined.

RESULTS

The results of the hydrogenation studies are given in Tables I, II, and III and in Figs. 2–7. The catalyst concentration employed is indicated with each table.

[4] Represent minimal catalyst concentrations (8, 9).
[5] H. R. Thomas Co.
[6] The authors will make the FORTRAN IV computor program available to any interested investigator.

Table I—Hydrogenations of α-Methylcinnamic Acid and α-Acetamidocinnamic Acid Using Palladium-on-Poly-S-leucine and Palladium-on-Poly-S-valine as Catalysts

Minutes	α-Methylcinnamic Acid Poly-S-leucine[a], 0.00176 SD, mmole H₂	α-Methylcinnamic Acid Poly-S-valine[a], 0.00132 SD, mmole H₂	α-Acetamidocinnamic Acid Poly-S-leucine[a], 0.00135 SD, mmole H₂	α-Acetamidocinnamic Acid Poly-S-valine[a], 0.00187 SD, mmole H₂
0.00	0.0000 / 0.008719	0.0000 / 0.001191	0.0000 / 0.001698	0.0000
1.00	0.03309 / 0.05596	0.002252 / 0.004497	0.005434 / 0.011380	0.000682
2.00	0.07220 / 0.08152	0.006975 / 0.009448	0.017326 / 0.024799	0.001535
3.00	0.08694 / 0.08856	0.011357 / 0.013718	0.030909 / 0.037193	0.002216
4.00	0.09060 / 0.09127	0.016194 / 0.018896	0.043562 / 0.049334	0.003326
5.00	0.09511 / 0.09545	0.021497 / 0.023498	0.054689 / 0.059781	0.004519
6.00	0.09613	0.025750 / 0.028112	0.064534 / 0.069628	0.005543
7.00		0.030361 / 0.032266	0.073452 / 0.077355	0.006481
8.00		0.034064 / 0.036055	0.081007 / 0.084318	0.007504
9.00		0.038343 / 0.039905	0.087458 / 0.089835	0.008698
10.00		0.041476 / 0.043390	0.091876 / 0.093144	0.009722
11.00		0.045304 / 0.047555	0.094845 / 0.096373	0.010829
12.00		0.049132 / 0.050819	0.097216 / 0.096913	0.011682
13.00		0.052506 / 0.054083	0.098272 / 0.098986	0.012621
14.00		0.055206 / 0.056555	0.100140	0.013388
15.00		0.058132		0.014327
16.00		0.061175		
17.00		0.063764		
18.00		0.067127		
19.00		0.069378		
20.00		0.071856		0.018761
21.00		0.073881		
22.00		0.075684		
23.00		0.077488		
24.00		0.079175		
25.00		0.080641		0.023632
26.00		0.081991		
27.00		0.083717		
28.00		0.084909		
29.00		0.085759		
30.00		0.086610		0.027716
35.00		0.090178		0.032370
40.00		0.093265		0.036800
45.00		0.095468		0.041457
50.00		0.097213		0.045659
55.00		0.098199		0.049976
60.00		0.098344		0.053610
65.00				0.056336
70.00				0.058948
75.00				0.061325
80.00				0.073073
85.00				0.075969
90.00				0.078354
95.00				0.086578
100.00				0.089305
105.00				0.092032

a 200 mg. PdCl₂/g. polyamino acid.

Table II—Inhibited and Uninhibited Hydrogenations of α-Methylcinnamic Acid and α-Acetamidocinnamic Acid Using Palladium-on-Poly-S-leucine as Catalyst

Minutes	Uninhibited, 0.000000 SD, mmole H₂	Inhibitor — N-Acetylphenylalanine, 0.000095 SD, mmole H₂	Inhibitor — Dihydro-α-methylcinnamic Acid, 0.00056 SD, mmole H₂
α-Methylcinnamic Acid[a]			
0.00	0.0000 / 0.0000	0.0000 / 0.0000	0.0000 / 0.0000
1.00	0.000677 / 0.000846	0.000678 / 0.001356	0.000506 / 0.000506
2.00	0.001354 / 0.002032	0.002034 / 0.002375	0.001012 / 0.001350
3.00	0.002711 / 0.003387	0.002820 / 0.003560	0.002026 / 0.002026
4.00	0.004064 / 0.004742	0.003899 / 0.004409	0.002364 / 0.002701
5.00	0.005419	0.005086	0.003376
6.00	0.006096	0.006106	0.004051
7.00	0.007452	0.006784	0.004558
8.00	0.008468	0.007802	0.005064
9.00	0.009314	0.008649	0.005739
10.00	0.009823	0.009836	0.006246
11.00	0.011347	0.010851	0.006920
12.00	0.012024	0.011866	0.007427
13.00	0.012720	0.012981	0.007933
14.00	0.013549	0.014236	0.008440
15.00	0.014226	0.015083	0.009115
20.00	0.017952	0.019319	0.012154
25.00	0.020324	0.021687	0.014348
30.00	0.024049	0.025074	0.016204
35.00	0.027635	0.028128	0.017892
40.00	0.030993	0.030155	0.019412
45.00	0.034042	0.032864	0.020930
50.00	0.034890	0.034898	0.002450
55.00	0.039429	0.037273	0.023800
60.00	0.040139	0.039307	0.025150
α-Acetamidocinnamic Acid[b]	0.000003 SD	0.000120 SD	0.000054 SD
0.00	0.0000	0.0000	0.000000
1.00	0.000681	0.000000	0.000677
2.00	0.001022	0.000678	0.001354
3.00	0.001362	0.000678	0.002032
4.00	0.002044	0.001356	0.002710
5.00	0.002725	0.001356	0.003386
6.00	0.003406	0.002034	0.004074
7.00	0.004088	0.002034	0.004741
8.00	0.004769	0.002712	0.005418
9.00	0.005450	0.002712	0.006096
10.00	0.006132	0.003390	0.006774
11.00			
12.00			
13.00			
14.00			
15.00	0.008516	0.005084	0.009482
20.00	0.011242	0.006779	0.012192
25.00	0.015329	0.008135	0.015239
30.00	0.018056	0.008982	0.017949
35.00	0.020099	0.009830	0.019980
40.00	0.023164	0.010846	0.021674
45.00	0.025548	0.011863	0.023706
50.00	0.027592	0.012710	0.026754
55.00	0.029636	0.013728	0.027770
60.00	0.031680	0.014744	0.029802

a 100 mg. PdCl₂/g. b 125 mg. PdCl₂/g.

Table III—Hydrogenations of α-Methylcinnamic Acid and α-Acetamidocinnamic Acid using Palladium-on-Poly-S-valine as Catalyst in the Presence of Dihydro-α-methylcinnamic Acid and N-Acetylphenylalanine

| Minutes | Uninhibited, 0.000018 SD, mmole H_2 | Inhibitor | |
		Dihydro-α-methylcinnamic Acid, 0.000007 SD, mmole H_2	N-Acetyl-phenylalanine, 0.000009 SD, mmole H_2
α-Methylcinnamic Acid[a]			
0.00	0.000000	0.000000	0.000000
1.00	0.000844	0.000000	0.000849
2.00	0.001689	0.000676	0.001529
3.00	0.002365	0.000676	0.002380
4.00	0.003042	0.001014	0.003295
5.00	0.003717	0.001352	0.004080
6.00	0.004392	0.001690	0.004760
7.00	0.005068	0.002198	0.005440
8.00	0.005744	0.002536	0.006120
9.00	0.006425	0.002874	0.006800
10.00	0.007096	0.003044	0.007480
15.00	0.010474	0.004903	0.010880
20.00	0.014022	0.006763	0.014110
25.00	0.017063	0.008623	0.017340
30.00	0.019766	0.010314	0.020569
35.00	0.022132	0.011836	0.023800
40.00	0.024158	0.013357	0.026860
45.00	0.026186	0.014879	0.029921
50.00	0.028213	0.016062	0.032810
55.00	0.030240	0.017416	0.035530
60.00	0.032098	0.018768	0.038081
α-Acetamidocinnamic Acid[b]			
		0.000078 SD	0.000047 SD
0.00		0.000000	0.000000
1.00		0.001015	0.000507
2.00		0.002031	0.000845
3.00		0.003046	0.001182
4.00		0.004062	0.002196
5.00		0.005077	0.003210
6.00		0.006262	0.003886
7.00		0.007277	0.004730
8.00		0.008292	0.005068
9.00		0.009308	0.005575
10.00		0.010324	0.006082
15.00		0.015569	0.009292
20.00		0.020816	0.014192
25.00		0.026062	0.017908
30.00		0.031139	0.021118
35.00		0.036216	0.023484
40.00		0.041463	0.026862
45.00		0.046370	0.030240
50.00		0.051448	0.032606
55.00		0.055848	0.034632
60.00		0.060587	0.036998

[a] 150 mg. $PdCl_2$/g. [b] 200 mg. $PdCl_2$/g.

Table I and Figs. 2 and 3 give the results of hydrogenating α-methylcinnamic acid and α-acetamidocinnamic acid using palladium-on-poly-S-leucine and palladium-on-poly-S-valine as catalysts.

The results of the product inhibition and the cross-inhibition experiments using palladium-on-poly-S-leucine are given in Table II and Figs. 4 and 5. Table III and Figs. 6 and 7 give the results of the product inhibition studies using the palladium-on-poly-S-valine catalyst.

DISCUSSION

From the results obtained from the hydrogenation experiments involving poly-S-leucine and poly-S-valine as carriers for pal-
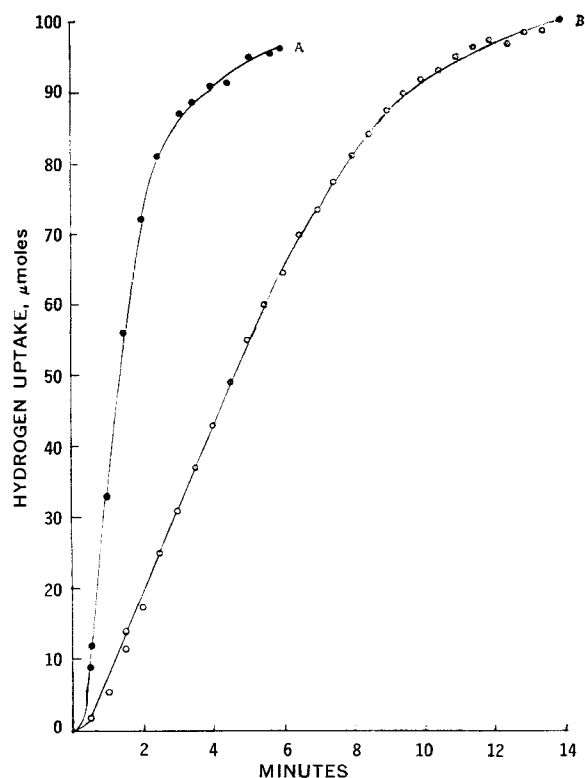

Figure 2—Hydrogenations of α-methylcinnamic acid and α-acetamidocinnamic acid by palladium-on-poly-S-leucine. Key: A, ●, α-methylcinnamic acid; and B, ○, α-acetamidocinnamic acid.

ladium, one must conclude that the helical character of the poly-S-leucine favorably influences the hydrogenation rate. Table I and Figs. 2 and 3 show that both α-methylcinnamic acid and α-acetamidocinnamic acid were reduced faster with palladium-on-poly-S-leucine than with palladium-on-poly-S-valine. Previous studies (4)
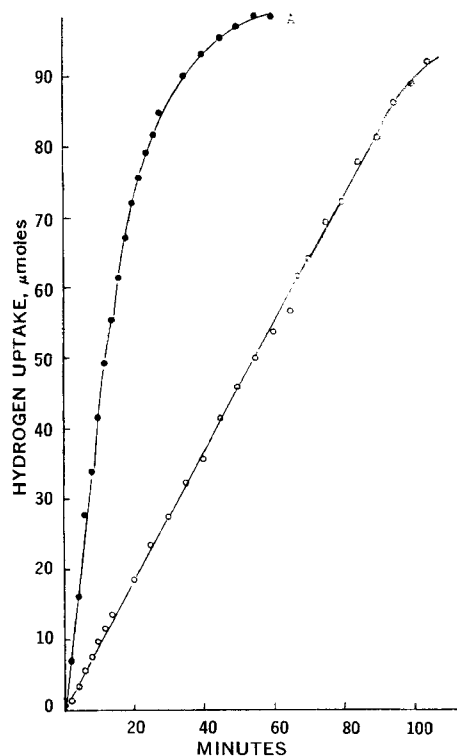

Figure 3—Hydrogenations of α-methylcinnamic acid and α-acetamidocinnamic acid by palladium-on-poly-S-valine. Key: A, ●, α-methylcinnamic acid; and B, ○, α-acetamidocinnamic acid.
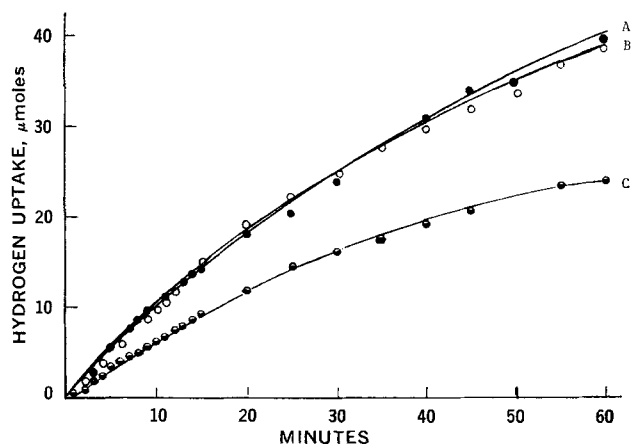
Figure 4—*Hydrogenations of α-methylcinnamic acid by palladium-on-poly-S-leucine in the presence of* N-acetylphenylalanine *and dihydro-α-methylcinnamic acid. Key: A, ●, α-methylcinnamic acid; B, O, α-methylcinnamic acid with* N-acetylphenylalanine; *and C, ◒, α-methylcinnamic acid with dihydro-α-methylcinnamic acid.*

using these same catalysts and substrates showed a consistently higher optical yield with the poly-S-leucine catalyst than with the poly-S-valine catalyst. Apparently, not only does the α-helix provide increased stereoselection, but it also provides a closer fit between the catalyst and substrate. With both catalysts, α-methylcinnamic acid was hydrogenated at a faster rate than α-acetamidocinnamic acid. The faster rate could result from a greater affinity of α-methylcinnamic acid for the catalyst surface, from the greater steric hindrance provided by the more bulky acetamido group as compared to the smaller methyl group, or from a combination of both of these effects.

The hydrogenation of 1×10^{-4} mole of α-methylcinnamic acid using palladium-on-poly-S-leucine was inhibited by the addition of 8×10^{-4} mole of the product of the hydrogenation, dihydro-α-methylcinnamic acid, to the reaction mixture. A similar inhibition was seen with the hydrogenations of α-methylcinnamic acid using palladium-on-poly-S-valine. However, these same reactions were not inhibited by the presence of 8×10^{-4} mole of N-acetylphenylalanine, the product of the reduction of α-acetamidocinnamic acid.

The hydrogen uptake by 1×10^{-4} mole of α-acetamidocinnamic acid catalyzed by 30 mg. of palladium-on-poly-S-leucine was product inhibited by the presence of 8×10^{-4} mole of N-acetylphenylalanine, but 8×10^{-4} mole of dihydro-α-methylcinnamic acid in the reaction mixture did not inhibit the reaction. The same results were obtained when α-methylcinnamic acid was hydrogenated in the presence of the palladium-on-poly-S-valine catalyst. Dihydro-α-methylcinnamic acid (8×10^{-4} mole/4 ml. of solvent) increased the rate of the palladium-on-poly-S-valine-catalyzed hydrogenation of α-acetamidocinnamic acid, while N-acetylphenylalanine in the same concentration slowed the hydrogenation rate.
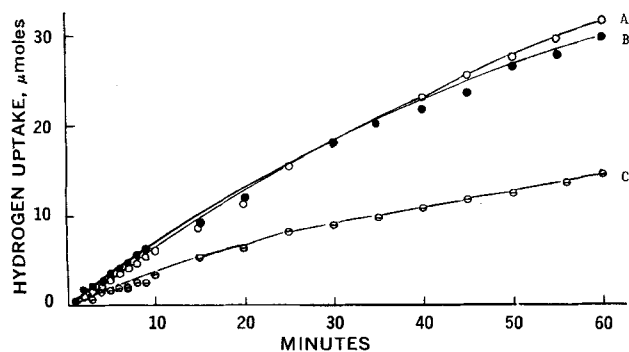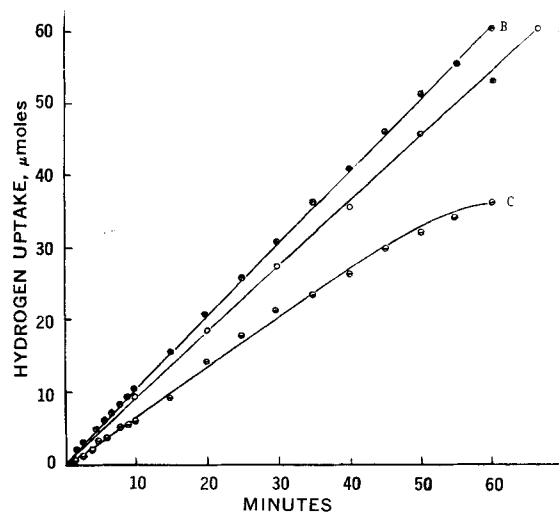

Figure 6—*Hydrogenations of α-acetamidocinnamic acid by palladium-on-poly-S-valine in the presence of* N-acetylphenylalanine *and dihydro-α-methylcinnamic acid. Key: A, O, α-acetamidocinnamic acid with no inhibitor; B, ●, α-acetamidocinnamic acid with dihydro-α-methylcinnamic acid; and C, ⊖, α-acetamidocinnamic acid with* N-acetylphenylalanine.

The increase in hydrogenation rate observed with α-acetamidocinnamic acid when catalyzed by palladium-on-poly-S-valine in the presence of dihydro-α-methylcinnamic acid is reminiscent of allosteric facilitation of some enzyme-catalyzed reactions (15).

The results of the inhibition experiments lead to the conclusion that the two substrates occupy two entirely different reaction sites (*i.e.*, I and II of Fig. 1). This not only confirms the conclusion arising from the configurational studies (2–4), but extends the conclusions of these studies by eliminating partial interaction between sites (*i.e.*, as seen in III and IV of Fig. 1).

SUMMARY AND CONCLUSIONS

The polyamino acid secondary structures of α-methylcinnamic acid and α-acetamidocinnamic acid were found to affect hydrogenation rates in reactions catalyzed by palladium-on-poly-S-
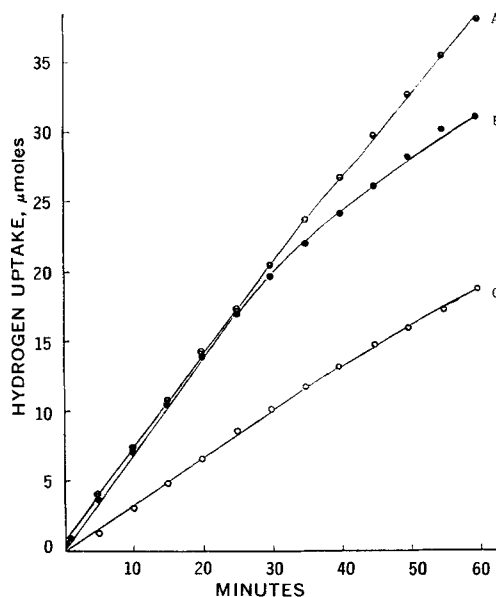

Figure 5—*Hydrogenations of α-acetamidocinnamic acid by palladium-on-poly-S-leucine in the presence of* N-acetylphenylalanine *and dihydro-α-methylcinnamic acid. Key: A, O, α-acetamidocinnamic acid; B, ●, α-acetamidocinnamic acid with dihydro-α-methylcinnamic acid; and C, ◒, α-acetamidocinnamic acid with* N-acetylphenylalanine.

Figure 7—*Hydrogenations of α-methylcinnamic acid by palladium-on-poly-S-valine in the presence of* N-acetylphenylalanine *and dihydro-α-methylcinnamic acid. Key: A, ⊖, α-methylcinnamic acid with* N-acetylphenylalanine; *B, ●, α-methylcinnamic acid with no inhibitor; and C, O, α-methylcinnamic acid with dihydro-α-methylcinnamic acid.*

leucine *versus* those catalyzed by palladium-on-poly-*S*-valine. The catalyst possessing the α-helical conformation (palladium-on-poly-*S*-leucine) gave a faster rate of hydrogenation than the nonhelical catalyst (palladium-on-poly-*S*-valine).

The hydrogenation rates for α-methylcinnamic acid were faster than those for α-acetamidocinnamic acid with both of the catalysts.

Inhibition studies show that the two substrates, α-methylcinnamic acid and α-acetamidocinnamic acid, occupy entirely different sites on the catalyst surface in both the palladium-on-poly-*S*-leucine- and the palladium-on-poly-*S*-valine-catalyzed reactions.

REFERENCES

(1) B. Jirgensons, "Optical Rotatory Dispersion of Proteins and Other Macromolecules," Springer-Verlag, New York, N. Y., 1969, pp. 40, 48–49.

(2) R. L. Beamer, C. S. Fickling, and J. H. Ewing, *J. Pharm. Sci.*, **56**, 1029(1967).

(3) R. L. Beamer, R. H. Belding, and C. S. Fickling, *ibid.*, **58**, 1142(1969).

(4) *Ibid.*, **58**, 1419(1969).

(5) A. W. Schrecker, *J. Org. Chem.*, **22**, 33(1957); P. Karrer and K. Ehrhardt, *Helv. Chim. Acta*, **34**, 2202(1951); P. Karrer, P. Portmann, and M. Suter, *ibid.*, **31**, 1917(1948).

(6) J. A. Ferretti, *Chem. Commun.*, **10**, 30(1967); E. Shechter and E. R. Blout, *Proc. Nat. Acad. Sci. USA*, **51**, 794(1964); E. R. Blout, in "Optical Rotatory Dispersion," C. Djerassi, Ed., McGraw-Hill, New York, N. Y., 1960.

(7) E. R. Blout, C. DeLoze, S. M. Bloom, and G. D. Fasman, *J. Amer. Chem. Soc.*, **82**, 3787(1960); S. M. Bloom, G. D. Fasman, C. DeLoze, and E. R. Blout, *ibid.*, **84**, 458(1962).

(8) J. G. Young, W. H. Hartung, and H. H. Daniels, *J. Org. Chem.*, **18**, 229(1953).

(9) J. G. Young and W. H. Hartung, *ibid.*, **18**, 1659(1953).

(10) W. D. Cash, F. T. Semeniuk, and W. H. Hartung, *ibid.*, **21**, 999(1956).

(11) I. M. Heilbron, "Dictionary of Organic Compounds," vol. II, Oxford University Press, New York, N. Y., 1936, p. 738.

(12) R. L. Beamer and W. W. Lawson, *J. Pharm. Sci.*, **55**, 53 (1966).

(13) R. C. Weast, S. M. Selby, and C. D. Hodgman, "Handbook of Chemistry and Physics," Chemical Rubber Co., Cleveland, Ohio, 1967, p. C501.

(14) C. L. Ogg and F. J. Cooper, *Anal. Chem.*, **21**(11), 1400 (1949).

(15) D. E. Koshland, Jr., and K. E. Neet, *Ann. Rev. Biochem.*, **37**, 359(1968).

DRUG STANDARDS

Use of Ceric Sulfate and Cupric Perchlorate for Titrimetric Analyses of Phenothiazine Derivatives

L. G. CHATTEN, R. A. LOCOCK, and R. D. KRAUSE

Abstract ☐ A method was developed in which phenothiazine derivatives were titrated visually to a colorless end-point with ceric sulfate. Quantitative recoveries were obtained only for chlorpromazine, acetylpromazine, trifluoperazine, and triflupromazine. The method was also applied to pharmaceutical dosage forms of these drugs. UV photometric detection of the end-point was found to be applicable only to thiethylperazine and thioridazine. Attempts to develop a quantitative procedure for phenothiazines by photometric titration with cupric perchlorate in acetonitrile were unsuccessful.

Keyphrases ☐ Phenothiazine derivatives and dosage forms—analysis ☐ Ceric sulfate—phenothiazine titration ☐ Titration, visual—phenothiazine derivatives, analysis ☐ UV photometric titration—phenothiazine derivatives, analysis

In 1940, Michaelis *et al.* (1) showed, by potentiometric titration, that the oxidation of phenothiazine (I) and several of its derivatives proceeds through two successive and distinct one-electron steps. Scheme I illustrates the sequence in the oxidation of an *N*-substituted phenothiazine. A highly colored free radical or semiquinone (II) results from the first oxidation step. The loss of another electron from this intermediate gives a phenazathionium ion (III). The phenazathionium ion from the second step can then react with water to produce a sulfonium base (IV), which will lose a proton to yield the phenothiazine sulfoxide (V).

The formation of colored semiquinone products by the action of a variety of oxidizing agents has been used for the detection and assay of phenothiazines (2–15). The 1963 edition of the British Pharmacopoeia (16) included an oxidimetric assay procedure for chlorpromazine tablets, using ceric sulfate as the titrant and dilute sulfuric acid as the solvent. The end-point was determined visually using the phenanthroline–ferrous complex as indicator. This method is not in the 1968 British Pharmacopoeia (17). During this investigation, Agarwal and Blake (18) reported the titration of phenothiazines and some dosage forms with ceric sulfate. The end-point was determined photometrically by following the reaction at 420 nm., the wavelength of maximum absorbance of ceric sulfate.

The purposes of the present investigation were: (*a*) to develop a simple assay for phenothiazine drugs