midity probably plays a major role in this phase transformation.

The transformation of caffeine hydrate to an anhydrous state was also carried out in the same manner. The results obtained for the two purine systems are quite different. Hydrated caffeine had a fairly rapid rate of transformation at room temperature, and the conversion appeared to go through various hydrate intermediates, as evidenced through certain changes in the X-ray powder patterns with time. This latter phenomenon is in agreement with the findings of Waters and Beal (5). The general differences in the transformation processes for the two purine hydrates are consistent with structural studies on the hydrates of the two compounds (1, 6, 7). The hydrogen bonding schemes in the two hydrate crystal structures are markedly different with respect to the waters. It would be tempting to correlate the semiquantitative rate data with the crystal structures of the hydrates; however, such speculations would be premature at this time in view of the lack of structural data on the anhydrous crystals and more detailed phase transformation data.

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Stereospecific Hydrogenations Using Palladium-on-Poly-L-leucine

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Studies have been conducted concerning the stereospecific nature of catalysts prepared by depositing palladium upon a poly-L-amino acid. The poly-L-amino acid chosen for this study was poly-L-leucine which was prepared from *N*-carboxy-L-leucine anhydride. The substrates employed in this work were α-methylcinnamic acid and α-acetamidocinnamic acid. Hydrogenation of α-methylcinnamic acid catalyzed by palladium-on-poly-L-leucine produced predominately R(−)-dihydro-α-methylcinnamic acid and hydrogenation of α-acetamidocinnamic acid using the same catalyst produced, after hydrolysis, S(−)-phenylalanine.

THE STEREOSPECIFICITY of catalysts prepared from silica gels precipitated in the presence of cinchona alkaloids has been shown by Padgett and Beamer (1). Beamer and Lawson have presented evidence of substrate specificity with similarly prepared gels (2). The silica gel work was based on prior evidence indicating the existence of stereospecific and substrate-specific centers in palladium-on-charcoal catalysts (3). Also, Akabori *et al.* had demonstrated the stereospecificity of palladium-on-silk fibroin (4) and Beckett *et al.* had shown the stereospecificity of adsorption in specially prepared silica gels (5–7).

The present work concerns the observation of stereospecificity in palladium-on-poly-L-amino acids. The poly-L-amino acid chosen for this study was poly-L-leucine which was prepared by polymerization of the *N*-carboxy-α-amino acid anhydride *in vacuo*.

Stereoselection in these catalysts should occur from asymmetric induction arising from stereoselective adsorption to active sites on the catalyst surface followed by *cis*-addition of hydrogen (3).

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EXPERIMENTAL

Reagents—α-Methylcinnamic acid (Aldrich), carbobenzyloxy chloride (Nutritional Biochemicals), L-leucine (Mann Biochemicals).

Poly-L-leucine—This polypeptide was prepared from the *N*-carboxy-α-amino acid anhydride by the Bergman procedure (8) which consisted of preparation of the carbobenzyloxy amino acid (CBZ amino acid) from CBZ chloride and L-leucine. The anhydride was prepared by Leuch's procedure from the CBZ amino acid and thionyl chloride (8). By melting the anhydride under high vacuum (10^{-3} mm. Hg) the polyamino acid was obtained. The yield was 23% of theory (based on L-leucine). Infrared spectra (Nujol mull) of the resulting polyamino acid were identical with those given by Bamford *et al.* (9). Carbon, hydrogen, and nitrogen analyses indicate the product is the hemihydrate and this has been confirmed by drying in an oven. Molecular weight determinations are currently being carried out and will be reported at a later date.

Anal.—Calcd. for $C_6H_{11}NO \cdot {}^1/_2H_2O$: C, 58.99; H, 9.90; N, 11.47. Found: C, 59.66; H, 9.51; N, 11.55.

α-Acetamidocinnamic Acid—This compound was prepared by hydrolysis of the azlactone, 2-methyl-4-benzilideneoxazolin-5-one (10). The product melted from 190° to 191.5° (uncorrected). [Lit. (10) m.p. 191°–192°.]

Preparation of Catalysts—Two methods were used to prepare the catalysts.

Method A—Sixteen milliliters of a 2.5% palladous

chloride solution (representing 200 mg. of $PdCl_2$ per Gm. of polyamino acid carrier) was added to 2.0 Gm. of poly-L-leucine suspended in 200 ml. of distilled water. The mixture was shaken 1 hr. on a Parr low-pressure hydrogenator prior to the introduction of hydrogen. Following the shaking period, the system was evacuated and hydrogen was introduced. The mixture was shaken for 1 hr. in an atmosphere of hydrogen. The hydrogen pressure was 2.8 Kg./cm.^{2}. The catalyst was collected by suction filtration, washed with a total of 300 ml. of distilled water, and dried for 48 hr. in a vacuum desiccator over sulfuric acid.

Method B—Two grams of poly-L-leucine was suspended in 200 ml. of distilled water in a 500 ml., two-necked, round-bottomed flask equipped with a dropping funnel and a motor-driven stirrer. While the mixture was stirred vigorously, 16 ml. of a 2.5% palladous chloride solution was added dropwise. After the addition of the palladous chloride solution was complete, the mixture was stirred vigorously for 1 hr. The mixture was then transferred to a Parr hydrogenation bottle and hydrogenated for 1 hr. at a hydrogen pressure of 2.8 Kg./cm.^{2}. Following hydrogenation, the catalyst was treated as has been described under *Method A*.

Elutriation Studies—The catalyst prepared by method *A* produced nonreproducible results when used to hydrogenate α-methylcinnamic acid according to the procedure of Padgett and Beamer (1).

Since the lack of reproducibility apparently stemmed from inhomogeneity within the catalyst preparation, an elutriation technique was employed to separate the catalyst into five arbitrarily assigned layers numbered 1 through 5 with 1 representing the bottom layer in the elutriation column. Each layer was then used to catalyze the hydrogenation of α-methylcinnamic acid according to the method of Padgett and Beamer (1) using 2.9 Gm. of the substrate and 0.36 Gm. of catalyst.

Hydrogenation Studies—Two substrates were used for the hydrogenation studies. These substrates were α-methylcinnamic acid and α-acetamidocinnamic acid.

α-Methylcinnamic Acid—Except for the hydrogenations using the catalysts obtained by the elutriation technique noted above, 4.05 Gm. (0.025 mole) of α-methylcinnamic acid and 0.5 Gm. of catalyst (method *B*) were employed. The hydrogenations were carried out in 50 ml. of absolute ethanol with a Parr low-pressure hydrogenation apparatus. The initial pressure was 4.2 Kg./cm.^{2} and a fall in pressure of 2.8×10^{-1} Kg./cm.^{2} was observed (theoretical: 2.9×10^{-1} Kg./cm.^{2}).

Following hydrogenation, the catalyst was removed by filtration and the solvent evaporated. The residue was vacuum distilled. The yield was 2.6 Gm. representing 63.4% of theory. The boiling point was 106°/0.35 mm. Hg (uncorrected). [Lit. (11) m.p. 160°/13 mm. Hg.] The $[\alpha]_D^{25}$ of the distilled product was −0.406 (c = 25 in benzene) was determined using a Rhudolph 200S polarimeter. The specific rotation represents 1.50% of the literature value of −27.06° (11). Infrared spectra of the product were identical with those given by Beamer and Lawson (2). The neutralization equivalent of the product agreed with that calculated for dihydro-α-methylcinnamic acid.

α-Acetamidocinnamic Acid—Hydrogenation of this substrate was carried out in 100 ml. of absolute ethanol with a Parr low-pressure hydrogenator. The weight of α-acetamidocinnamic acid was 4.8 Gm. (0.023 mole). The initial pressure of hydrogen was 4.2 Kg./cm.^{2}. One gram of catalyst was used. The observed fall in pressure was the same as that expected from theory (1.3×10^{-1} Kg./cm.^{2}).

Following hydrogenation, the catalyst was removed by suction filtration and the solvent was evaporated leaving a white, crystalline residue. The residue was treated according to the directions of Akabori (4) by boiling under reflux with 10% hydrochloric acid. Following the acid hydrolysis, the solution was concentrated under vacuum to approximately one-half its volume and treated with acetone to precipitate the amino acid hydrochloride. The phenylalanine was obtained by adjustment of a 50% v/v hydromethanolic solution to the isoelectric point of phenylalanine (pH 5.4) and collecting the resulting precipitate by suction filtration. The product weighed 3.5 Gm. representing a yield which was 92.3% of theory. The product melted with decomposition from 269° to 270° (uncorrected). [Lit. (12) m.p. 271°–273° dec.] Descending filter paper chromatography using butanol–acetic acid showed the R_f value of the product to be identical with that of known phenylalanine determined under identical conditions. The infrared spectra of the product and known phenylalanine were identical.

The phenylalanine was dissolved in 6.4% sodium hydroxide solution, placed in a 100 mm. polarimeter tube, and read in a Rhudolph 200S spectropolarimeter giving an observed rotation of −0.26° representing an $[\alpha]_D^{25}$ of −2.6° (c = 10). The literature value for the specific rotation of phenylalanine is −48.4° (13). From these data an optical yield of 5.4% may be calculated.

TABLE I—HYDROGENATION OF α-METHYLCINNAMIC ACID USING PALLADIUM-ON-POLY-L-LEUCINE SEPARATED BY ELUTRIATION[a]

Layer	HMCA[b] Yield, Gm.	Yield,[c] %	Reduction Time, hr.	$[\alpha]_D^{25}$ [d]	Pressure Drop, Kg./cm.^{2} [e]
1	2.6	90	1	−0.146 ± .01°	7×10^{-2} [f]
2	2.48	85.5	1	−0.536 ± .01°	8.4×10^{-2}
3	2.45	84.5	1	−0.250 ± .01°	5.6×10^{-2}
4	0.00	0.00	1	...	1.4×10^{-2}
5	0.00	0.00	1	...	7×10^{-3}

[a] Weight of catalyst was 0.36 Gm. [b] HMCA = dihydro-α-methlcinnamic acid. [c] Based on 2.9 Gm. of α-methylcinnamic acid. [d] Determined in benzene using a 100-mm. tube (c = 25). The instrument used was the Rhudolph 200S spectropolarimeter. [e] Initial pressure was 4.2 Kg./cm.^{2} (gauge). [f] Hydrogen uptake for complete hydrogenation of 2.9 Gm. of substrate was calculated to be 1.04×10^{-1} Kg./cm.^{2}

RESULTS

The results of the hydrogenation experiments using the elutriated catalyst prepared by method *A* are given in Table I.

The catalyst prepared by method *B* appeared more homogeneous to the eye and in divided catalysts produced uniformly reproducible results. However, the product of the hydrogenation using the method *B* catalyst was not so optically active as that obtained from the second layer of the elu-

triated method *A* catalyst (see Table I). The hydrogenation product using method *B* catalyst was more optically active than the hydrogenation product using layers one and three of the elutriated method *A* catalyst.

The results of the other hydrogenations are given under *Experimental*. Hydrogenation of α-acetamidocinnamic acid, followed by hydrolysis, produced predominately (−)-phenylalanine.

The results of the hydrogenations using the elutriated catalyst are reproducible.

DISCUSSION

While it is too early in the studies involving these catalysts to form definite conclusions regarding the experimental evidence already gathered, one can form some preliminary ideas concerning these catalysts.

The similarities between proteins and polypeptides and the catalytic action of certain proteins (enzymes) prompted the investigation in this area. Does the stereospecificity observed from enzymes stem from the asymmetry of their individual amino acid residues or from the secondary, tertiary, or even quaternary structures of the proteins involved? Recent reports (14–16) indicate that synthetic polyamino acids, far from being random, may coil in either a righthanded or a lefthanded manner, and the direction of coiling is dependent in part on the amino acid involved. These reports are in sharp contrast to earlier data showing a more random arrangement (17).

Since these hydrogenation catalysts have been developed as models of enzymes, it might be well to critically examine our studies in the light of current concepts of enzyme specificity. The well-known theory of Koshland (18), which has been applied by Belleau (19) to include drug receptors, involves the assumption of a conformational perturbation occurring in the protein as a result of the forces of interaction between the substrate (or drug) and the enzyme (or drug-receptor). One particular interaction involved stems from the hydrophobic nature of the substrate molecule and the hydration of the protein. Other forces can also contribute to the induced perturbation (20).

It would be speculation to conceive of such a mechanism operating in the catalysts described in this paper. However, the similarities involved between synthetic polypeptides and proteins and the recent observations of Scheraga *et al.* (15) indicate something in this regard. Certain groups within the peptide chain do influence the synthetic peptide's secondary structure.

More definite conclusions may be obtained from a consideration of the absolute configurations of the products of the hydrogenations using palladium-on-polyleucine. As noted above, hydrogenations of both α-methylcinnamic acid and α-acetamidocinnamic acid yield levorotatory products. The absolute configurations of (−)-dihydro-α-methylcinnamic acid and (−)-phenylalanine have been determined (21–23). (−)-Dihydro-α-methylcinnamic acid has been shown to have the R configuration as shown in Fig. 1. (−)-Phenylalanine, however, possesses the S configuration.

Obviously the two substrates must be interacting—at least, in part—at two different sites on the catalyst surface. Analysis of the structure of α-methylcinnamic acid indicates four points which could bind with the catalytic surface: the olefinic group (C=C) at which reduction takes place, the carboxyl group which could form a salt bond with a cationic group or could form a hydrogen bond with a suitable group on the catalyst, a phenyl group which could bind by van der Waal's forces, and a methyl group which also could bind by van der Waal's forces. The methyl group is replaced by an acetamido group in α-acetamidocinnamic acid, but all the other groups are common to the two substrates. The methyl group is nonpolar but can bind to the catalyst by van der Waal's forces. The acetamido group can bind to the catalyst by hydrogen bonds or by induced dipole interaction. If the respective points of attachment are suitably situated and if two active centers or sites of reaction are envisioned, the production of the configurations described above can be explained. Schemes I and II illustrate possible mechanisms explaining the observed results. The hydrogen in these figures is shown enter-

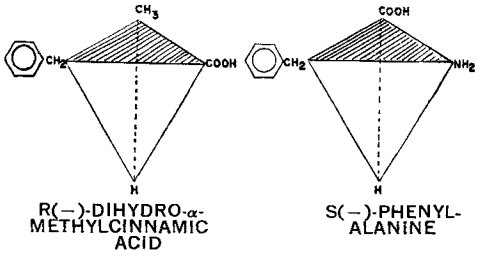
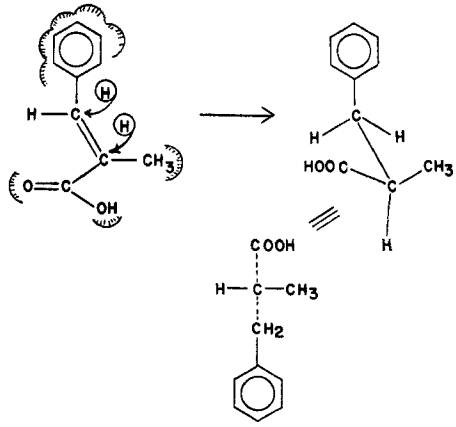
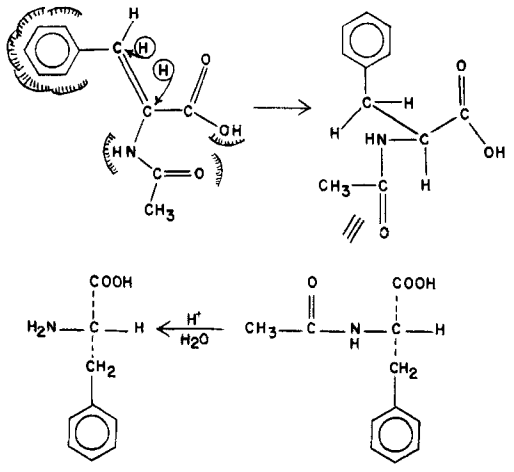


Fig. 1—Absolute configurations of (—)-dihydro-α-methylcinnamic acid as reported by Schrecker (21) and (—)-phenylalanine as reported by Karrer et al. *(22, 23).*



Proposed Scheme for the Stereospecific Hydrogenation of α-Methylcinnamic Acid Catalyzed by Palladium-on-Poly-L-leucine. The Hydrogen Should Be Viewed as Entering the Molecule Cis *and from the Catalytic Surface,* i.e., *the Hydrogen Attack is in a Direction from Out of the Page and Toward the Side Away from the Viewer*

Scheme I



Proposed Scheme for the Stereospecific Hydrogenation of α-Acetamidocinnamic Acid Catalyzed by Palladium-on-Poly-L-leucine. The Hydrogen Should be Viewed as Entering the Molecule Cis *and from the Catalytic Surface,* i.e., *the Hydrogen Attack is in a Direction from Out of the Page and Toward the Side Away from the Viewer*

Scheme II

ing the α-methylcinnamic and acid *cis* and from the catalytic side, *i.e.*, beneath the molecule or toward the side of the molecule away from the viewer. Stereoselective adsorption apparently occurs prior to addition of hydrogen and stereospecific hydrogenation is the result.

Later work with palladium-on-synthetic polypeptides may provide answers to the questions raised in this paper and indicate the fidelity of this model in explaining the complex phenomena of enzymatic catalysis.

SUMMARY

Hydrogenations using palladium-on-poly-L-leucine with α-methylcinnamic acid and α-acetamidocinnamic acid as substrates gave optically active products. The absolute configurations of the hydrogenation products indicate the two substrates fit—at least, in part—different active sites.

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