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Azomethine Chemistry. I. Formation of Optically Active α-Amino Acids by Asymmetric Induction[1]

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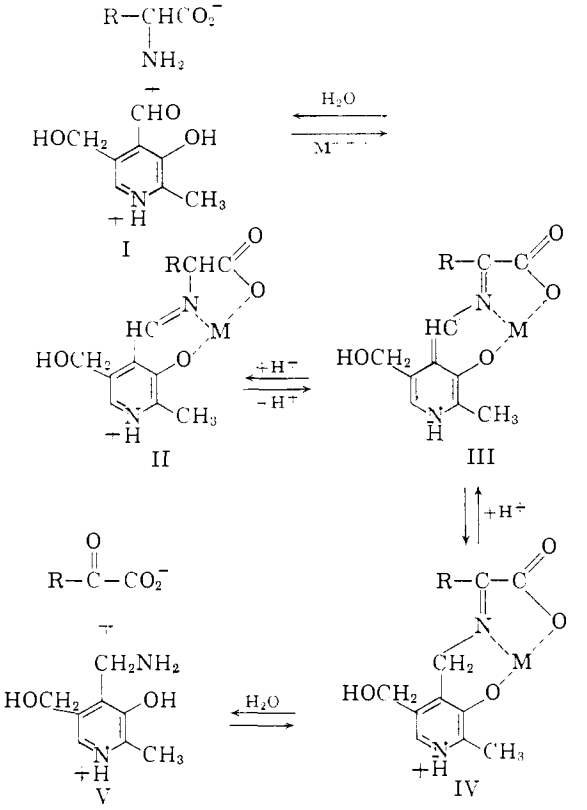
Catalytic hydrogenation of four α-keto acids in the presence of D-(+)- and L-(−)-α-methylbenzylamine afforded the corresponding optically active α-amino acid in reasonable yield. The magnitude of the induced asymmetry varied with structure of the substrate and the catalyst used.

Considerable evidence has been accumulated suggesting the essential role of the azomethine linkage in certain biochemical reactions. Although the details of the enzymic processes are obscure, several non-enzymic systems which require the presence of a carbon to nitrogen double bond are well known. For example, the elegant work of Snell, *et al.*,[3] suggests intermediates such as II, III and IV are involved in reactions requiring pyridoxal (I) and pyridoxamine (V).



Among the various enzymic reactions catalyzed by pyridoxal phosphate is transamination, the transfer of an amino group from an amino acid to a α-keto acid.[4] The conversion of pyruvic acid to L-alanine with L-glutamic acid, catalyzed by pyridoxal phosphate and an enzyme from pig heart,[4] is representative of this type of reaction. Presumably the process involves formation of II

$$CH_3COCO_2H + HO_2CCH_2CH_2\underset{\displaystyle NH_3^+}{\underset{|}{C}}HCO_2^- \rightleftharpoons HO_2CCH_2CH_2COCO_2^- + CH_3\underset{\displaystyle NH_3^+}{\underset{|}{C}}HCO_2^-$$

which is then degraded to α-ketoglutaric acid and pyridoxamine (V). Reversal of the sequence with pyruvic acid and V would generate L-alanine and I *via* IV, III and II. Of particular interest is the stereochemistry of the reverse reaction. The enzyme apparently provides an asymmetric environment for the stereospecific addition of a hydrogen atom to III, forming II and ultimately L-alanine.[5] Thus the over-all result of transamination is generation of a new asymmetric center by asymmetric induction.

A number of attempts to duplicate the conversion of a α-keto acid to the corresponding asymmetric α-amino acid are recorded. The Cu(II)–pyridoxal catalyzed non-enzymic reaction of L-alanine or L-phenylalanine with α-ketoglutaric acid has been reported[6] to yield an excess of L-glutamic acid. In both cases the ratio of L- to D-glutamic acid was about 55% to 45%. Despite this rather low result the experiment established the presence of some asymmetric intermediate, probably a chelate, which could undergo stereospecific protonation.

A variety of substrates, including azomethines, have been hydrogenated in the presence of a catalyst deposited on an asymmetric surface. In general however, the yields and optical purity of the amino acids obtained by this method have been rather low. Akabori, *et al.*,[7] have employed a palladium-on-silk catalyst for this purpose. Hartung, *et al.*,[8] have discussed the theoretical basis for asymmetric induction by a hydrogenation catalyst and have clarified the role of the asymmetric surface in this process.

Another approach to the conversion of an α-keto acid to an optically active α-amino acid would involve reduction of an asymmetric azomethine or related intermediate. Akabori and

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(2) Petroleum Research Fund Fellow, 1961.

(3) D. E. Metzler, M. Ikawa and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 648 (1954).

(4) P. P. Cohen, in "The Enzymes," J. B. Sumner and K. Myrback, eds., Vol. 1, Academic Press, Inc., New York, N. Y., 1951, p. 1040.

(5) Under certain conditions the hydrogen transfer step (II → III) is non-stereospecific; *e.g.*, the racemization of L-alanine by an enzyme from *streptococcus faecalis*, W. A. Wood and I. C. Gunsalus, *J. Biol. Chem.*, **190**, 403 (1951).

(6) J. B. Longenecker and E. E. Snell, *Proc. Natl. Acad. Sci., U. S.*, **42**, 221 (1956).

(7) S. Akabori, Y. Izumi and S. Sakurai, *Nippon Kagaku Zasshi*, **77**, 1374 (1956); S. Akabori, S. Sakurai, Y. Izumi and Y. Fujii, *Nature*, **178**, 323 (1956).

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TABLE I

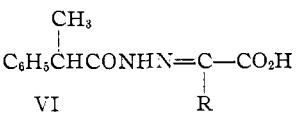
α-AMINO ACIDS PREPARED BY ASYMMETRIC INDUCTION

$$RCOCO_2R + C_6H_5\overset{*}{C}H(NH_2)CH_3 \xrightarrow[2,\ Pd(OH)_2/H_2]{1,\ cat./H_2} R\overset{*}{C}H(NH_2)CO_2H + C_6H_5CH_2CH_3$$

Substrate	Cat.	Config. of amine	% yield of amino acid	$[\alpha]^{26}D$ obsd.	$[\alpha]^{25}D$ (c 2, 5 N HCl) lit.[a]	Total % of excess enantiomorph
CH_3COCO_2H	Pd[b]	D	47.9	−10.3[c]	+14.6[d]	85.2, D-(−)
CH_3COCO_2H	Pd	L	43.1	+11.9[e]		90.7, L-(+)
$CH_3COCO_2CH_2C_6H_5$	Pd	L	22.0	+10.8[e]		87.0, L-(+)
$CH_3CH_2COCO_2H$	Pd	L	84.9	+13.0[f]	−20.7[g]	81.4, L-(+)
$CH_3CH_2COCO_2H$	$Pd(OH)_2$	D	71.7	− 9.00		71.7, D-(−)
$CH_3CH_2COCO_2H$	PtO_2	D	59.2	− 2.60		56.3, D-(−)
$(CH_3)_2CHCOCO_2H$	Pd	D	15.7	− 7.82[h]	−27.5[g]	64.2, D-(−)
$(CH_3)_3CCOCO_2H$	$Pd(OH)_2$	D	0.0			
$C_6H_5CH_2COCO_2H$	Pd	D	53.8	+ 4.5[i]	+34.8[g]	56.4, D-(+)
$C_6H_5CH_2COCO_2H$	Pd	L	65.0	− 4.2		56.0, L-(−)

[a] The largest value reported, regardless of sign, has been used. [b] 10% palladium-on-charcoal. [c] c 2.31 in 6 N HCl. [d] J. R. Parikh, J. P. Greenstein, M. Winitz and S. M. Birnbaum, *J. Am. Chem. Soc.*, **80**, 953 (1958). [e] c 2.03 in 5 N HCl. [f] c 2.02 in 5 N HCl. [g] S. M. Birnbaum, L. Levintow, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **194**, 455 (1952). [h] c 2.06 in 5 N HCl. [i] c 2.00 in H_2O.

Sakurai[9] reported that catalytic reduction of the D-acylhydrazones (VI) of pyruvic acid (R = CH_3) and phenylpyruvic acid (R = $C_6H_5CH_2$) afforded a 11.2% yield of L-alanine, $[\alpha]^{19}D$ + 1.21°, and a 10.9% yield of L-phenylalanine,



VI

$[\alpha]^{16}D$ − 1.55°. Maeda[10] has reported a similar sequence which gave essentially the same results. Since the azomethine intermediates II-IV are doubtless involved in the enzymic process, the scheme represented in Fig. 1 appeared to represent a more reasonable approximation of biological transamination. While this method would not be

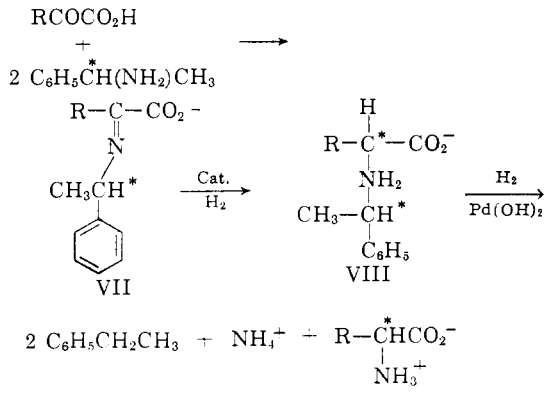


Fig. 1.

comparable to the pyridoxal-catalyzed transaminations (a metal catalyst rather than an enzyme surface is present) the results should provide information on the stereochemistry of hydrogen addition to azomethines of α-keto acids. A sequence of this type was recently employed[11] for the synthesis of R,R-α,α′-dimethyldibenzylamine (IX). Since IX was easily hydrogenolyzed to R-(+)-α-methylbenzylamine with 10% palladium-on-charcoal catalyst,[11] a similar result was envisioned with VIII. However, when VIII (R = CH_3), $[\alpha]^{25}D$ + 87.5°, obtained by hydrogenation of a mixture of pyruvic acid and D-(+)-α-methylbenzylamine, was further hydrogenated no debenzylation occurred. Likewise the use of various acidic solvents with 10% palladium-on-charcoal, platinum oxide or Raney nickel catalysts were not successful. However, hydrogenation of VIII (R = CH_3) in the presence of palladium hydroxide-on-charcoal catalyst[12] afforded a 78% yield of D-(−)-alanine, $[\alpha]^{25}D$ −13.3° (optical purity 91.1%). This result indicated that asymmetric induction did in fact occur during the reduction of VII (R = CH_3) and that the N-α-methylbenzylamino acids could be debenzylated with little or no loss of configurational integrity. The scheme was, therefore, applied to several other α-keto acids; the results are shown in Table I.

The intermediate azomethines were not isolated but rather directly hydrogenated. When benzyl pyruvate was treated with L-(−)-α-methylbenzylamine bimolecular condensation products of pyruvate were observed. These probably account for the low yield of L-(+)-alanine obtained. The free α-keto acids were subsequently used to minimize this side reaction. As the size of the alkyl group of the α-keto acid was increased, the yield of amino acid was reduced. The steric effect of the alkyl portion on the yield of α-amino acid was particularly apparent when 3-methyl-2-oxobutyric acid and 3,3-dimethyl-2-oxobutyric acid were subjected to the reaction conditions. The former afforded a 15.7% yield of D-(−)-valine, but in the latter case no *t*-leucine was obtained.

In the examples studied the configuration of the α-amino acid produced was the same as that of the α-methylbenzylamine from which it was derived. A similar result was observed in the single case previously reported.[11] The magnitude of the

(9) S. Akabori and S. Sakurai, *Nippon Kagaku Zasshi*, **78**, 1629 (1957).

(10) G. Maeda, *ibid.*, **77**, 1011 (1956).

(11) C. G. Overberger, N. P. Marullo and R. G. Hiskey, *J. Am. Chem. Soc.*, **83**, 1374 (1961).

(12) This catalyst was called to our attention by Dr. M. E. Munk. The catalyst was developed by Mr. W. M. Pearlman, Parke, Davis and Company, Ann Arbor, Michigan, who generously provided directions for the preparation.

induced asymmetry apparently depends on the nature of the alkyl portion of the keto acid as well as the catalyst used. As the size of the alkyl group was increased the optical purity of the enantiomer produced decreased. Since an equivalent of ammonia was produced in the debenzylation, an attempt was made to determine whether the amino acid was partially racemized during hydrogenation. When the rotation of the phenylalanine obtained from phenylpyruvic acid was followed as a function of time no appreciable change was noted. Further, the presence of equivalent amounts of acid did not affect the optical purity of the products. Of the three catalysts used for the reduction of the azomethine in the production of butyrine, 10% palladium-on-charcoal gave a considerably greater excess of one enantiomer. The enhanced ability of palladium catalysts to promote asymmetric induction has been previously noted.[8]

Since the effect of the amine portion of the azomethine has not been investigated, the present results cannot be fully evaluated. However, it is clear that substrates of this type afford a high degree of stereospecificity when reduced with palladium catalysts and in a broad sense resemble the enzyme–pyridoxal phosphate transamination. The application of other reducing agents and a detailed study of this unique system is currently in progress.

Experimental[13]

Pyruvic acid and 2-oxobutyric acid were obtained commercially and purified prior to use. The D-(+)- and L-(−)-α-methylbenzylamine were obtained from commercial racemic amine,[14] $[\alpha]^{26}D$ +40.0° (neat) and −40.0° (neat).

Preparation of Benzyl Pyruvate.—The ester was obtained in 45% yield by slow distillation of equimolar quantities of benzyl alcohol and pyruvic acid; b.p. 137–137.5° at 16 mm., reported[15] b.p. 138° at 16 mm. The semicarbazone derivative melted at 176–177°, reported[15] 176°.

Preparation of Phenylpyruvic Acid.—To 4.0 g. (0.02 mole) of 5-benzylidene-2-thioxo-4-oxazolidone, obtained in 60.2% yield from 2-thioxo-4-oxazolidone,[16] was added 160 ml. of 10% barium hydroxide solution. The mixture was refluxed 20 minutes, acidified and the crude phenylpyruvic acid (0.61 g., 19%) filtered. Recrystallization from chloroform gave a white solid, m.p. 151–156° (cor.); reported[17a] m.p. 156–157°,[17b] 150–154°.

Preparation of 3-Methyl-2-oxobutyric Acid.—Treatment of 42.3 g. (0.28 mole) of isobutyryl bromide with 29.0 g. (0.32 mole) of cuprous cyanide gave 8.1 g. (30%) of 3-methyl-2-oxobutyryl nitrile, b.p. 115–116°, reported[18] b.p. 116–118°.

The nitrile obtained above was hydrolyzed with 25 ml. of concd. hydrochloric acid (75 minutes at 25°) and afforded 6.1 g. (62%) of the crude α-keto acid, $n^{25}D$ 1.4020. The substance is reported[18] to decompose on distillation and thus the crude sample was used in the hydrogenation.

Preparation of Palladium Catalysts.—The 10% palladium-on-charcoal was prepared by the standard method.[19]

The palladium hydroxide-on-charcoal catalyst was prepared as follows.[12] A rapidly stirred mixture of 68.6 g. of Mallinckrodt activated charcoal and 30 g. of palladium chloride in 43 ml. of concd. hydrochloric acid and 570 ml. of water was heated to 60°. To the mixture was added 31 g. of sodium hydroxide pellets at such a rate that the temperature did not exceed 80°. The mixture was then treated with 6.6 g. of solid sodium bicarbonate and stirred 12 hours. The catalyst was filtered, washed with 430 ml. of water and then with a mixture of 430 ml. of water and 8.6 ml. of glacial acetic acid. The catalyst was filtered, dried *in vacuo* at 65° and stored under nitrogen.

Preparation of α-Amino Acids. Hydrogenation of 2-Oxobutyric Acid and L-(−)-α-Methylbenzylamine.—The α-amino acids listed in Table I were prepared by essentially the same method. A cold solution containing 1.00 g. (9.79 mmoles) of 2-oxobutyric acid in 40 ml. of ethanol was treated with 2.374 g. (19.6 mmoles) of L-(−)-α-methylbenzylamine in 27 ml. of cold ethanol. The hydrogenation catalyst, in this case 1.5 g. of 10% palladium-on-charcoal, was added and the mixture reduced at 50 p.s.i. and 30°. After 10 hours, 1 mole of hydrogen was consumed. The catalyst was filtered, washed several times with hot water and the filtrate and combined washings concentrated to 20 ml. The concentrate was diluted with 50 ml. of 30% aqueous ethanol, 1.3 g. of palladium hydroxide-on-charcoal added and the hydrogenation repeated (50 p.s.i., 25°).

After hydrogen uptake ceased the catalyst was filtered, washed with hot water and the combined filtrate and washings concentrated to 5 ml. on the rotatory evaporator. Dilution with 30 ml. of ethanol afforded 0.766 g. (75.9%) of white plates, m.p. 285–292°, $[\alpha]^{26}D$ +13.0° (*c* 2.02 in 5 *N* HCl). The substance exhibited a single spot with ninhydrin reagent when chromatographed on Whatman No. 1 paper using 1-butanol–water–acetic acid (4:5:1). The R_f value of the sample was identical to that of authentic *d,l*-butyrine. The infrared spectrum of the preparation was also identical to the spectrum of authentic *d,l*-butyrine.

The hydrochloride derivative of "synthetic" butyrine was obtained in 63.7% yield, m.p. 265–266°, from ether–ethanol, $[\alpha]^{25}D$ +12.0° (*c* 2.1 in 5 *N* HCl); reported[20] $[\alpha]^{25}D$ +14.5°. The value of the specific rotation of "synthetic" butyrine corresponds to L-(+)-butyrine of 91.4% optical purity. However, some resolution of the hydrochloride salt may have occurred during crystallization.

Isolation of D-(+)-N-(α-Methylbenzyl)-alanine.—A solution containing 7.13 g. (0.04 mole) of benzyl pyruvate and 4.85 g. (0.04 mole) of D-(+)-α-methylbenzylamine in 40 ml. of benzene was refluxed under a water separator for 20 minutes. The benzene was removed *in vacuo* and the residue diluted with 60 ml. of absolute ethanol. Hydrogenation using 2.0 g. of 10% palladium-on-charcoal catalyst followed by filtration and evaporation afforded a white solid which was purified by sublimation, m.p. >275°, $[\alpha]^{25}D$ +87.5° (*c* 1.02 in 50% ethanol).

Anal. Calcd. for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.18; H, 7.80; N, 7.15.

Debenzylation of 0.508 g. (2.6 mmoles) of the material with 1.0 g. of palladium hydroxide-on-charcoal catalyst under the conditions described afforded 0.182 g. (78%) of alanine, $[\alpha]^{25}D$ −13.3° (*c* 1.49 in 6 *N* HCl). Samples of "synthetic" and authentic alanine exhibited identical R_f values when paper chromatographed in the 1-butanol–water–acetic acid system.

(13) Melting points and boiling points are uncorrected. Elemental analysis by Micro-Tech Laboratories, Skokie, Ill. Optical rotations were performed with a Rudolph polarimeter, model 80, equipped with a model 200 photoelectric attachment.

(14) A. H. Blatt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 506.

(15) H. Masson, *Compt. rend.*, **149**, 630 (1909).

(16) N. K. Ushenko and T. E. Gorizdra, *Ukrain. Khim. Zhur.*, **16**, 545 (1950); *C. A.*, **48**, 11391 (1954).

(17) (a) T. E. Gorizdra and S. N. Baranov, *Zhur. Obshchei Khim.*, **26**, 3092 (1956); (b) A. H. Blatt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 519.

(18) W. Tschelinzeff and W. Schmidt, *Ber.*, **62**, 2210 (1929).

(19) E. C. Horning, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 685.

(20) E. Fischer *Ber.*, **33** 2383 (1900).