65.81; H, 6.85) was obtained in a 41% yield by treatment of the salt (II, R = $C_6H_5$) with β-carbethoxypropionyl chloride.

Benzoylation of the magnesium chelate formed from ethyl hydrogen malonate[10] and magnesium ethoxide led to the introduction of two benzoyl residues and the formation of ethyl dibenzoylacetate.[11]

The authors wish to acknowledge many helpful and stimulating discussions with Drs. R. M. Stiles and H. L. Finkbeiner.

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(12) Public Health Service Research of the National Heart Institute.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF MICHIGAN ROBERT E. IRELAND
ANN ARBOR, MICHIGAN JAMES A. MARSHALL[12]

RECEIVED APRIL 16, 1959

## THE "FLORY ($\theta$) TEMPERATURE" OF ATACTIC AND ISOTACTIC POLYPROPYLENE

*Sir:*

Identical intrinsic viscosity–molecular weight relationships for atactic and isotactic polystyrene *in thermodynamically good solvents* have been confirmed but real differences in the second virial coefficients were detected.[1,2,3]

According to theory, the second virial coefficient depends on (1) the unperturbed dimensions of the polymer chain, (2) the enthalpy and entropy parameters $\kappa_1$ and $\psi_1$ and (3) the partial specific volume of the polymer. Krigbaum, *et al.*, found (3) contributes only a minor effect and proposed the main effect arose through differences in (1).

The thermodynamic interaction parameters can be evaluated through phase equilibria studies in the liquid–liquid region where the critical temperature ($T_c$) is related to the thermodynamic parameters by the relationship[4]

$$1/T_c = 1/\theta[1 + (1/\psi_1)(1/x^{1/2} - 1/2x)] \qquad (1)$$

where

$$\theta = \kappa_1 T/\psi_1;\ x = \bar{M}_n \bar{v}_2/V_1$$

$V_1$ = molar volume of the solvent, $\bar{v}_2$ = specific volume of the polymer.

Phase diagrams of carefully characterized fractions[5] of atactic and isotactic polypropylene have been determined and binodials characteristic of *liquid–liquid* separations for both isomers obtain in phenyl ether at temperatures near the melting point of the isotactic polymer.

Reciprocals of the critical temperatures determined from the phase diagrams were plotted against the function $(1/x^{1/2} - 1/2x)$ according to equation (1) and the parameters $\psi_1$ and $\theta$ evaluated. Curves for atactic and isotactic polypropylene differ considerably in both slope and intercept and yield values for the parameters as shown in Table I.

Under these conditions the atactic polymer is less soluble than its isotactic counterpart, supporting the proposition that the atactic form is the more flexible.[3]

TABLE I
THERMODYNAMIC INTERACTION PARAMETERS IN PHENYL ETHER

| | $\theta$, °K. | $\psi_1$ |
|---|---|---|
| Isotactic | 418.4 | 1.414 |
| Atactic | 426.5 | 0.986 |
| Difference | 8.1 | 0.426 |

The data predict a reversal in precipitation order above the unique condition (intersection of the two curves) where the term $\psi_1(1 - \theta/T)$ for the two isomers is identical. The point of intersection depends on the solvent, the molecular weight and the temperature. A similar intersection appears in the data of Danusso and Moraglio[2] on the second virial coefficients of atactic and isotactic polystyrene and the unfortunate choice of a polymer near the critical molecular weight may have prevented Krigbaum, *et al.*,[3] from detecting differences in the unperturbed dimensions of polystyrene chains by viscosity ratios; however, their conjecture that the $\theta$ temperatures for the two isomers may differ is confirmed.

We finally conclude that both $\theta$ and $\psi_1$ as determined by phase studies may ultimately be used to reflect the degree of stereoregularity within polymer chains. As suggested by theory[6,7] intrinsic viscosity–molecular weight relationships may not be identical for conformational isomers in poor solvents where segment–segment interactions are magnified.

In a future paper we plan to reveal the unperturbed dimensions of the two isomers at their respective $\theta$ temperatures and will discuss the significance of these data in terms of structure and dilute solution behavior of polymeric stereoisomers.

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(8) This work was supported in part by a grant from the Research Corporation. The whole polymer samples were kindly provided by Hercules Powder Company, Wilmington, Delaware.

KEDZIE CHEMICAL LABORATORY
MICHIGAN STATE UNIVERSITY JACK B. KINSINGER[8]
EAST LANSING, MICHIGAN R. A. WESSLING

RECEIVED APRIL 6, 1959

## ON THE NATURE OF PROTEIN-BOUND LIPOIC ACID

*Sir:*

Previous work[1] indicated that lipoic acid, in its functional form in bacterial pyruvate dehydrogenation complexes, is bound to protein in covalent linkage through its carboxyl group. This communication presents evidence that lipoic acid is bound to the epsilon amino group of a lysine residue.

When *Escherichia coli* is grown aerobically in the presence of lipoic acid-$S_2^{35}$, radioactive lipoic acid is incorporated into the pyruvate dehydrogenation complex. A highly purified preparation[2] (176 mg.; sp. act. 678; 1.47 μg. radioactive lipoic acid per mg. protein) was treated with performic

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acid[3] and the oxidized protein was partially hydrolyzed (12 *N* HCl, 105°, 3 hours). The hydrolysate was passed through a column of Dowex 50 ×8 (15 ml.) and the effluent was subjected to paper electrophoresis in 1 *N* acetic acid.[4] All of the radioactive material migrated toward the anode. It was eluted and subjected to descending paper chromatography (53 hours) with butanol–acetic acid–water (2:1:1). Radioautographs showed two major bands. The faster moving band was identified as 6,8-disulfoöctanoic acid by comparison with an authentic sample. The slower moving band was ninhydrin-positive and accounted, in several experiments, for 47–60% of the original radioactivity. It appeared to be homogeneous as indicated by paper chromatography and electrophoresis. Paper chromatography of a hydrolyzed sample (6 *N* HCl, 105°, 18 hours) showed a single ninhydrin-positive spot, which was identified as lysine by comparison with an authentic sample. The isolated radioactive material was allowed to react with 2,4-dinitrofluorobenzene and the DNP derivative was hydrolyzed (6 *N* HCl, 105°, 18 hours). A single DNP amino acid was detected and identified as α-DNP lysine by paper electrophoresis.[5] Microbiological assay[6] of a hydrolyzed sample of the isolated material indicated the presence of L-lysine in a 1:1 molar ratio with radioactive 6,8-disulfoöctanoic acid. These data indicate that the isolated material was ε-N-6,8-disulfoöctanoyl-L-lysine.

ε-N-DL-Lipoyl-L-lysine[7] was synthesized by reaction of lipoic–isobutyl carbonic anhydride with the copper-chelate complex of L-lysine; m.p. 225–229° (dec.), $\lambda_{max.}^{0.1N\ NaOH}$ 330 mμ (ε 116) (*anal.* Found: C, 50.21; H, 8.10; N, 8.33). A sample was oxidized with performic acid and the product was shown to exhibit identical behavior with the isolated radioactive material by paper chromatography and electrophoresis. With highly purified *E. coli* dihydrolipoic dehydrogenase[9] the synthetic material was reduced by DPNH 2 to 3 times as fast as DL-lipoamide.

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(6) We are indebted to Dr. Joanne M. Ravel for the assays.
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(10) Rosalie B. Hite Postdoctoral Fellow, 1958–1959, while on leave from Takeda Pharmaceutical Industries, Osaka, Japan.

CLAYTON FOUNDATION BIOCHEMICAL INSTITUTE AND DEPARTMENT OF CHEMISTRY
UNIVERSITY OF TEXAS
AUSTIN, TEXAS
HAYAO NAWA[10]
WILLIAM T. BRADY
MASAHIKO KOIKE
LESTER J. REED

RECEIVED MARCH 13, 1959

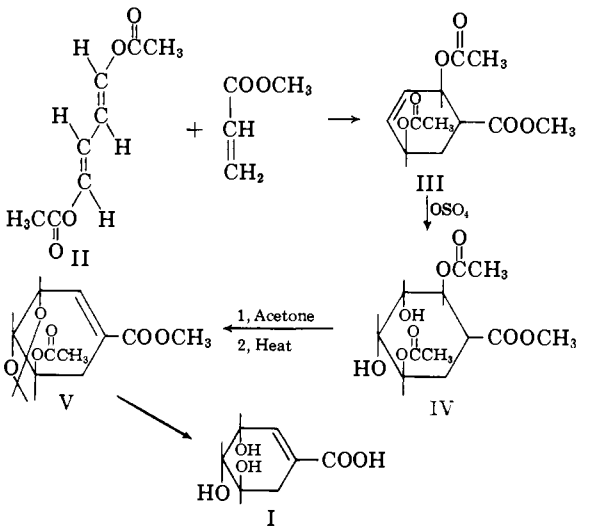
## A STEREOSPECIFIC SYNTHESIS OF *dl*-SHIKIMIC ACID[1]

*Sir:*

We wish to report the first total chemical synthesis of shikimic acid, I. This acid has been shown to be an important link in aromatic biosynthesis[2] and with this in mind we devised a scheme whereby we could introduce $C^{14}$ into specific positions of the molecule.

The structure determination of this metabolic intermediate was reported in 1937[3] but no total synthesis previously has been accomplished.

To perform the synthesis, the *trans,trans*-1,4-diacetoxybutadiene[4,5] II was allowed to react with methyl acrylate to produce methyl *cis*-3,6-diacetoxycyclohexene-4-carboxylate, III, in 93% yield, b.p. 152–155° (3 mm.), $n^{25}D$ 1.4680. *Anal.* Calcd. for $C_{12}H_{16}O_6$: C, 56.37; H, 6.32. Found: C, 56.24; H, 6.29. The olefinic bond was *cis* hydroxylated utilizing osmium tetroxide to give methyl β-2,β-5-diacetoxy-α-3,α-4-dihydroxycyclohexylcarboxylate, IV, in 67% yield, m.p. 161–162°, which was converted to the 3,4-acetonide in 33% yield, m.p. 143–144°. *Anal.* Calcd. for $C_{15}H_{22}O_8$: C, 54.53; H, 6.71. Found: C, 54.52; H, 6.65. The acetonide was pyrolyzed to give a 70% yield of methyl *dl*-3-acetyl-4,5-isopropylideneshikimate, V. We synthesized the comparable derivative from the natural (−)-shikimic acid by a previously reported method[6] in order to compare the infrared spectra in solution. The infrared spectra of the synthetic and the naturally derived compounds were identical in chloroform solution. Fischer and Dangschat previously have converted the acetylacetonide of shikimic acid to shikimic acid. Our *dl*-acetylacetonide was prepared as a viscous oil and was not obtained in a crystalline state; however, it had the identical ultraviolet absorption maximum ($E_{214\ mu}^{MeOH}$ 9000) reported for the natural derivative. *Anal.* Calcd. for $C_{13}H_{18}O_6$: C, 57.80; H. 6.66. Found: C, 58.20; H, 6.86. This material, V, was hydrolyzed by the method of Fischer and Dangschat, allowing the acetyl acetonide to be heated in 60% acetic acid for 3 hours, removing the acetic acid *in vacuo* and then treating with 0.1




(1) This work was supported by the Research Corporation and the National Science Foundation, Grant No. G4328.
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