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.

(3) E. Schram, *et al.*, *Biochem. J.*, **57**, 33 (1954).
(4) J. R. Kimmel, *et al.*, *J. Biol. Chem.*, **217**, 151 (1955).
(5) I. M. Lockhart and E. P. Abraham, *Biochem. J.*, **62**, 645 (1956).
(6) We are indebted to Dr. Joanne M. Ravel for the assays.
(7) It is to be noted that this substance is strikingly similar in structure to biocytin (ref. 8).
(8) L. D. Wright, *et al.*, *Science*, **114**, 635 (1951).
(9) L. J. Reed and M. Koike, *Federation Proc.*, **18**, 308 (1959).
(10) Rosalie B. Hite Postdoctoral Fellow, 1958–1959, while on leave from Takeda Pharmaceutical Industries, Osaka, Japan.

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RECEIVED MARCH 13, 1959

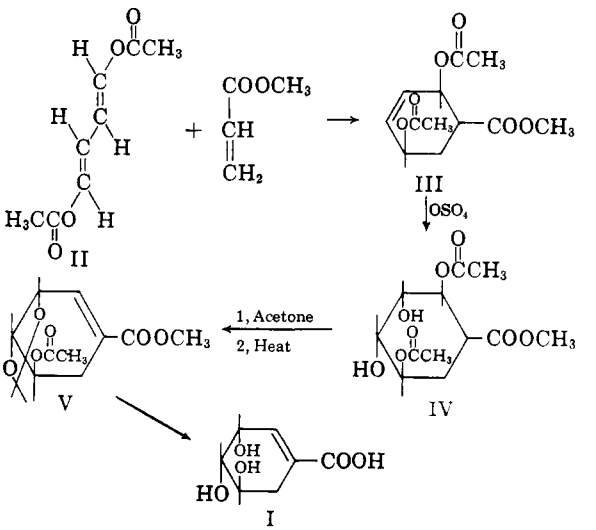
A STEREOSPECIFIC SYNTHESIS OF *dl*-SHIKIMIC ACID[1]

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.
(2) B. D. Davis, *J. Biol. Chem.*, **191**, 315 (1951); *J. Bacteriol.*, **64**, 729, 749 (1952).
(3) H. O. L. Fischer and G. Dangschat, *Helv. Chim. Acta*, **20**, 795 (1937).
(4) W. Reppe, O. Schlichting, K. Klager and T. Toepel, *Ann.*, **560**, 1 (1948).
(5) H. H. Inhoffen, J. Heimann-Trosien, H. Muxfeldt and H. Kramer, *Chem. Ber.*, **90**, 187 (1957).
(6) H. O. L. Fischer and G. Dangschat, *Helv. Chim. Acta*, **17**, 1200 (1934); **18**, 1211 (1935); **20**, 708 (1937).

N alcoholic sodium hydroxide for 15 hours at room temperature. The material was recovered by the use of an IR-120 column and the resulting white powder was assayed using an *Escherichia coli* mutant 81–3 which requires shikimic acid and it was found to be active. The infrared spectra of *dl*-shikimic acid and natural *l*-shikimic acid taken in KBr were compared and found to be almost identical. The melting point of *dl*-shikimic acid was found to be 193–195° and *l*-shikimic 190–191°. On mixed melt of the *dl* and *l* acids the melting point was 188–190°.

Acknowledgment.—We are indebted to Dr. Max E. Rafelson, Department of Biochemistry, University of Illinois Medical School, for the biological assays and comparison of our final product with natural shikimic acid.

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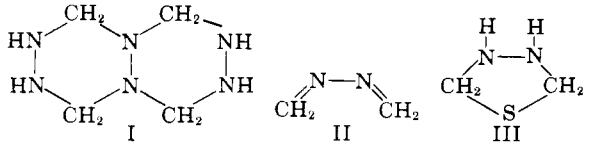
RECEIVED DECEMBER 6, 1958

MONOMERIC FORMALDAZINE—SYNTHESIS OF 1,3,4-THIADIAZOLIDINE—A NEW HETEROCYCLE

Sir:

The reaction of aqueous formaldehyde with hydrazine leads only to "tetraformaltrisazine" I[1] or a white amorphous polymer[2] instead of the expected formaldazine II.



I II III

However, by thermally decomposing[3] the polymer over a small flame in a nitrogen atmosphere (5–20 mm. pressure) and collecting the distillate in a Dry Ice trap we have isolated (50% yield), purified by low temperature distillation and characterized monomeric formaldazine II.

The redistilled product collected as large colorless or white crystals, m.p. $-48 \pm 3°$, soluble in the cold in common polar organic solvents. On warming to room temperature (either neat or in solution) the material polymerized spontaneously, first to a viscous liquid and eventually to a white solid, *anal.*[4] Found: C, 42.61; H, 7.40; N, 50.06.

From the ratio of the intensities of the isotopic 57 to the parent 56 peak in the mass spectrometer (observed 3.05), the molecular formula was determined to be $C_2H_4N_2$ (theoretical 3.04).[5]

Of the many possible structures, the infrared spectrum in the vapor phase (52 mm., 10-cm. cell) was compatible only with structure II (bands at 3.26(m), 3.41(ms), 4.93(mw), 6.04(w), 6.12(w), 7.08(w), 7.16(m), 8.47(m), 8.64(w) and 9.81(s) microns). The n.m.r. spectrum (in carbon tetrachloride) showed four peaks (two doublets) centered at about +1 parts per ten million from benzene. Using the shift charts[6] one predicts +3 p.p.t.m. for the terminal methylene hydrogens.

The only non-polymeric derivative prepared to date has been assigned the interesting 1,3,4-thiadiazolidine structure III. Mixing ether solutions of an excess of hydrogen sulfide and formaldazine at −70° gave a precipitate which was sublimed at room temperature yielding beautiful white crystals with a slight sulfide-like odor, m.p. 86–91° (dependent on rate of heating, slight decomposition), *anal.* Found: C, 26.93; H, 6.85; N, 31.09; S, 35.63; mol. wt. 90 (mass spectrometer); 117 (Menzies).[4] The infrared spectrum showed the N–H band; the n.m.r. spectrum (in deuterated chloroform) showed two types of hydrogens in the ratio of 2.25 to 1 (theory 2 to 1). The methylene hydrogen resonance was at +30 p.p.t.m. from benzene (predicted[6] +33) and the amine hydrogen resonance was a broad band centered at +37.5 p.p.t.m. The material was stable in the cold under nitrogen, but decomposed slowly at room temperature with the release of hydrogen sulfide (odor). Other reactions of formaldazine are under investigation.

The kind assistance of F. C. Stehling, D. E. Nicholson, N. F. Chamberlain and O. G. Weir of the Humble Oil and Refining Company is gratefully acknowledged.

(1) K. A. Hofmann and D. Storm, *Ber.*, **45**, 1728 (1912).

(2) G. Pulvermacher, *ibid.*, **26**, 2360 (1893).

(3) The conversion was later found to be similar in principle to the method for preparing N-alkylmethylenimines, J. L. Anderson, U. S. Patent No. 2,729,679; see *C. A.*, **50**, P12097d (1956).

(4) Schwarzkopf Microanalytical Laboratories, Woodside, N. Y.

(5) Method described by H. E. Lumpkin, Meeting of Gulf Coast Spectroscopic Group, Corpus Christi, March, 1959.

(6) See N. F. Chamberlain, *Anal. Chem.*, **31**, 56 (1959).

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RECEIVED MARCH 25, 1959

IONIC POLYMERIZATION. THE EFFECT OF SOLVENTS ON THE COPOLYMER COMPOSITION IN CATIONIC CATALYZED POLYMERIZATION

Sir:

We wish to report an interesting and new observation in homogeneous cationic catalyzed polymerization. The object of this work was to study the effects of some reaction variables, particularly catalyst and solvent, on the monomer reactivity ratios in the *p*-chlorostyrene-isobutylene copolymer system catalyzed by cationic initiators. Such studies offer an effective method of obtaining the relative reactivities of monomers toward carbonium ions, and the system studied in this work is of particular value in this respect because of the differences in the reactivity and structure of the carbonium ions derived from the two monomers, and of the monomers themselves. This might be expected to lead to changes in reactivity ratios as the polarity of the solvent changes. In previous work,[1] only minor effects on monomer reactivity ratios were observed with monomer pairs of similar types (styrene, *p*-chlorostyrene) in homogeneous cases with mixed solvent system (CCl_4, $C_6H_5NO_2$). Here localized solvent effects from one of the solvents may play a role. With styrene and 3,4-

(1) C. G. Overberger, R. J. Ehrig and D. Tanner, THIS JOURNAL, **76**, 772 (1954).