noted by the disappearance of the iodine color and generation of a considerable amount of heat which necessitated occasional water cooling. Just at the equivalent, the amount of iodine was 8 g (0.062 mol). Vacuum distillation gave these fractions: bp 98–129° (4 mm), 12 g; 131–135° (4 mm), 10.6 g; and 150–165 (4 mm), 2 g. The first fraction was again treated with a dilute alkaline solution, extracted by ether and worked up by the usual procedure to give 7-iodo-*p*-menthene-1 (4). This substance was fairly unstable and changed to a brown color by liberation of free iodine and polymerized material after a few days. The second fraction was found to be tributyltin iodide from ir; its purity was determined as 98.6% by alkali titration. In the case of Hanus reagent, a further iodination of this tributyltin iodide to dibutyltin iodide and butyl iodide was recognized.

Cleavage Reaction with Iodine of the Limonene Adduct. 9-(Dibutyliodostannyl)-*p*-menthene-1 (5).—Iodine absorption by Hanus reagent was 4.16 equiv/mol. This was indifferent to the dilute iodine solution, but with iodine crystals it reacted at room temperature. To the limonene adduct, 20 g (0.05 mol), was added iodine crystals, 12.3 g (0.10 mol), in portions. Vacuum distillation gave three fractions: bp 129–136° (4 mm), 1.9 g, tributyltin iodide; 149–158° (4 mm), 7.6 g, the mixture of dibutyltin diiodide and tributyltin iodide (ratio, 44:56 wt %); 185–196° (4 mm), 12.3 g. In the trap 5.1 g of liquid was obtained. The third was redistilled, and alkali titration showed 98% purity as in 5. The trap condensate was redistilled (bp 128–129°, n^{25}D 1.4954, d^{25}_4 1.543) and found to be *n*-butyl iodide. This iodide (5) was again subjected to further iodination and obtained was *trans*-1-iodo-*p*-menthane (6) which was derived from the hydroiodination of *p*-menthene-1 or -1(7).

Attempted Preparation of 1:2 Adduct from the 1:1 Adducts.—β-Pinene adduct (5 g), tributyltin hydride (3.4 g), and AIBN (0.05g) were charged in the Pyrex tube and heated for 24 hr at 200°. After opening the tube, the contents were separated by vacuum distillation. Unreacted tributyltin hydride, bp 81–105° (33 mm), 2.8 g, and the adduct, bp 185–197° (3 mm), 5.5 g, were obtained, but no residue was found as in the 1:2 adduct.

Registry No.—1, 25828-13-1; 2, 25828-14-2; 3, 25828-15-3; 4, 25828-16-4; 5, 25828-17-5; 6, 25828-18-6; tributyltin hydride, 688-73-3.

The Synthesis of O-Acylamino Acids

Harland R. Hendrickson,[1a] John Giovanelli,[1b] and
S. Harvey Mudd

*Loboratory of General and Comparative Biochemistry,
National Institute of Mental Health,
U. S. Department of Health, Education and Welfare,
Public Health Service, National Institutes of Health,
Bethesda, Maryland 20014*

Presently available methods for the synthesis of *O*-acylamino acids are based on the reaction of an anhydride with the parent amino acid.[2,3] These methods obviously restrict the *O*-acylating agent to one which can be obtained in the form of an anhydride and, consequently, cannot be applied to the synthesis of, for example, pure *O*-oxalyl and *O*-malonyl derivatives. This paper describes a more general method for the synthesis of *O*-acylamino acids from the readily available acyl chlorides.

The following *O*-acylamino acids were synthesized with yields (as per cent of parent amino acid) shown in parentheses: *O*-oxalyl-L-homoserine (9%), *O*-mal-

onyl-L-homoserine (32%), *O*-malonyl-L-serine (39%), and *O*-succinyl-L-serine (19%). The properties of these preparations and the criteria used to assess their purity are listed below. The parent amino acid was the sole detectable organic impurity.

A. Acid Hydrolysis to the Parent Amino Acid and Organic Acid.—The preparations were hydrolyzed in 1 *M* HCl and then subjected to paper chromatography as described in the Experimental Section. The sole detectable products from these hydrolyses were the parent amino acid and organic acid.[4]

B. Loss of Ninhydrin Reaction under Alkaline Conditions.—Under alkaline conditions *O*-acylamino acids undergo a loss in ninhydrin reactivity, which has been ascribed to an "O → N acyl transfer."[3,5] Each *O*-acylamino acid was incubated in 2 *N* ammonium hydroxide at room temperature for 10 min and then lyophilized to dryness. The residue was dissolved in water and electrophoresed on paper. Comparison with untreated controls showed that the ninhydrin reaction observed with each *O*-acylamino acid was completely eliminated by preincubation with ammonium hydroxide.

C. Quantitative Determination with the Hydroxylamine Assay.—Within the limits of accuracy of the assay (*ca.* ±10%), each preparation was judged to be pure.

D. Paper Chromatography.—Varying amounts of each preparation were chromatographed in solvents A, B, and C, and the organic acids and amino acids were visualized as described in the Experimental Section. The sole impurity detected was the parent amino acid. *O*-Oxalylhomoserine was estimated to contain *ca.* 10% homoserine; the other preparations contained a barely detectable proportion (less than 5%) of the parent amino acid.

E. Elemental Analysis.—The values determined differed from the calculated values by less than 0.3%, with the exception of *O*-malonyl-L-homoserine. With *O*-malonyl-L-homoserine the values for C and H differed by 1.50 and 0.37%, respectively. The observed analysis is consistent with that of a preparation containing *ca.* 4% water[6] in addition to the known trace of homoserine (see D above).

F. Nmr Spectra.—The nmr spectra were consistent with the proposed structures and exhibited no significant peaks other than those attributable to the *O*-acylamino acid. It was estimated that the parent amino acid would have to represent at least 20% of the total preparation to permit its detection.

In addition to the above *O*-acylamino acids, a small quantity of *O*-oxalyl-L-serine was synthesized. Several

(1) (a) Shell Development Company, Agricultural Research Division, Modesto Calif. 95352; (b) to whom correspondence should be addressed.

(2) S. Nagai and M. Flavin, *J. Biol. Chem.*, **242**, 3884 (1967).

(3) M. Flavin and C. Slaughter, *Biochem.*, **4**, 1370 (1965).

(4) An acid which was chromatographically distinct from the parent organic acid was commonly observed in the products of hydrolysis. This acid was obviously an artifact formed from the products of hydrolysis, since its formation could be reproduced when parent amino acid and organic acid were together (but not separately) subjected to the same procedure of acid hydrolysis and lyophilization described for the *O*-acylamino acids.

(5) To the best of our knowledge, the evidence that *O*-acylamino acids undergo an "O → N acyl transfer" is equally consistent with their conversion to a cyclic hemiacetal-like structure as proposed by the following: C. A. Grob and C. Wagner, *Helv. Chim. Acta*, **38**, 1699 (1955); G. Fodor and J. Kiss, *J. Amer. Chem. Soc.*, **72**, 3495 (1950); E. E. van Tamelen, *ibid.*, **73**, 5773 (1951); A. Nickon and L. F. Fieser, *ibid.*, **74**, 5566 (1952). In this note, the term "O → N acyl transfer" will be used to include both of the above possible reactions.

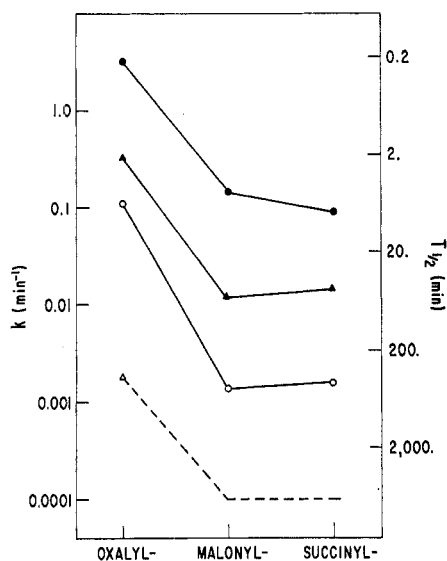(6) Elemental analyses were performed on samples that were dried overnight *in vacuo* at 50°.

Figure 1.—Rate of "O → N acyl transfer" with various esters. The ordinate on the left-hand side represents pseudo-first-order rate constants, and that on the right hand side the corresponding half-times, for "O → N acyl transfer." Closed symbols (●, ▲) represent incubations in 0.2 M potassium borate, pH 8.8; open symbols (O, △) represent those in 0.2M potassium phosphate, pH 7.4. Serine derivatives are represented by circles, homoserine derivatives by triangles. The lower limit of reliability of the assay method is represented by the horizontal broken line.
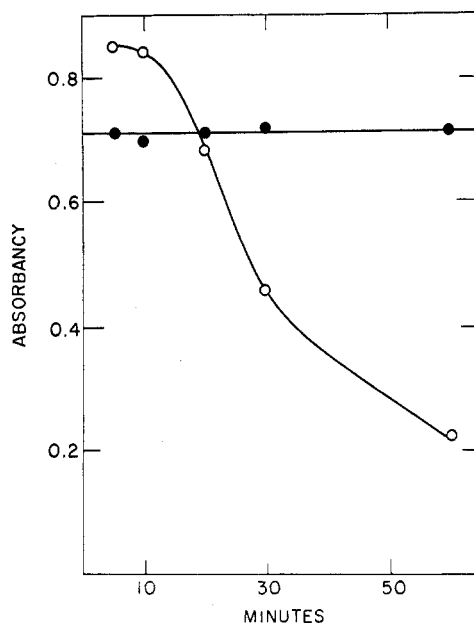

Figure 2.—Effect of time of incubation with bromine on hydroxylamine assay. Each O-acylamino acid was incubated with bromine for the times shown, before assay with hydroxylamine. Values for 2 μmol of O-malonyl-L-serine are represented by open circles, those for 1 μmol of O-acetylserine are represented by closed circles.

criteria show that the purity of this preparation was inferior to that of the preparations described above. *Anal.* Calcd for $C_5H_7NO_6$: C, 33.89; H, 3.98; N, 7.91. Found: C, 33.05; H, 4.16; N, 8.16.

Quantitative determination with the hydroxylamine assay indicated a purity of approximately 60%. Paper chromatography and electrophoresis demonstrated that the major component was O-oxalylserine and the contaminants predominantly serine and oxalate. O-Oxalylserine lost its reactivity toward ninhydrin by preincubation with ammonium hydroxide and yielded oxalic acid and serine as sole products of hydrolysis in 1 M HCl.

Since each compound was purified by elution from an anion exchange column with formic acid, the possibility was considered that the final products existed in the form of the formate salt. The elemental analyses reported agree with those calculated for the internal salts. However, the differences between the calculated elemental analyses for the internal and formate salts are small and, hence, do not provide a sensitive method for determining the relative proportion of the two salt forms. The following observations demonstrate that the products exist predominantly, if not exclusively, as the internal salt.

A. Samples of the compounds were mixed with a tenfold excess of radioactive formic acid and lyophilized to dryness. The samples were dissolved in water and again lyophilized to dryness; this process was then repeated. Less than 0.4% of the original radioactive formic acid remained in the lyophilized residue.

B. O-Malonylserine (2 mmol) was recrystallized from water–ethanol–ether (45:45:10) containing 2 mmol of radioactive formic acid. After collecting the crystals and washing with ethanol, less than 0.2% of the radioactive formic acid remained in the crystals.

Since it is planned to study the metabolism of O-acylamino acids in enzyme systems, it was of interest to determine the stability of these compounds to hydrolysis and "O → N acyl transfer." The latter reaction was of special interest since it forms the basis of a sensitive assay of enzymes that catalyze the acylation of serine and homoserine.[2] Figure 1 shows the pseudo-first-order rate constants and corresponding halftimes for "O → N acyl transfer" at pH 7.4 and 8.8 of the compounds synthesized. Both ordinates are represented on a logarithmic scale. No hydrolysis was detected under the conditions described. The rates of "O → N acyl transfer" for O-malonylhomoserine and O-succinylhomoserine at pH 7.4 were too low to be assayed reliably. The lower limit of reliability of the assay method is represented by the horizontal broken line in Figure 1.

The value of the hydroxylamine assay of O-malonylserine decreased unexpectedly after 10-min incubation with bromine (Figure 2). By contrast, the corresponding value for all other compounds remained constant between 5 and 60 min, as illustrated with O-acetylserine. This anomalous reaction of bromine with O-malonylserine has not been further investigated.

The method described has the advantages that it is relatively simple and can be applied to the synthesis of a wide range of O-acylamino acids in addition to the ones described. The products, with the exception of O-oxalylserine, were obtained in reasonable purity and yield. Even in the case of O-oxalylserine, a useful preparation was obtained which was approximately 60% pure, and contained predominantly serine and oxalic acid as the major impurities. The purity and yield of O-oxalyl-L-homoserine could probably be improved by avoiding overloading of the column during anion exchange chromatography. The relative chemical reac-

tivity (Figure 1) of the *O*-oxalyl derivatives may also have contributed to their low yields.

Experimental Section[7]

Material.—*O*-Succinyl-DL-homoserine was prepared according to the method of Flavin and Slaughter;[3] *O*-acetyl-L-serine was obtained from Yeda Research and Development, Rehovoth, Israel. Oxalic acid-1,2-[14]C (Tracerlab), malonic acid-2-[14]C (Calbiochem), and succinic acid-1,4-[14]C (Tracerlab) were used without further purification. Sodium formate-[14]C (New England Nuclear) was purified before use by addition of a large excess of formic acid followed by lyophilization and collection of the volatile formic acid-[14]C. All other reagents were prepared with analytical grade chemicals. The analytical grade anion exchange resin, AG1-X10, 100–200 mesh, was obtained from BioRad Laboratories. The resin was supplied as the chloride form and was converted before use via the hydroxide form to the formate form.

An authentic sample of potassium oxalylmonohydroxamate was prepared as previously described[8] and had the following elemental analysis. *Anal.* Calcd for $KC_2H_2NO_4$: K, 27.31; C, 16.78; H, 1.41; N, 9.79. Found: K, 27.24; C, 16.03; H, 1.38; N, 9.80. Potassium malonylmonohydroxamate was similarly prepared, except the hydroxylamine reagent was adjusted to pH 10. The following elemental analysis of potassium malonylmonohydroxamate was obtained. *Anal.* Calcd for $KC_3H_4NO_4$: K, 24.88; C, 22.91; H, 2.57; N, 8.91. Found: K, 24.70; C, 23.51; H, 2.78; N, 9.06. These samples were used to determine an ϵ 540 mμ of 730 ± 40 and 710 ± 40 for oxalyl and malonyl derivatives, respectively, in the hydroxylamine assay. Absorbancies for the hydroxylamine assay were determined 1 min after addition of the ferric chloride reagent.

Analytical Procedures.—The hydroxylamine assay described by Delavier-Klutchko and Flavin[9] was used, except that it was necessary to reduce the incubation time with bromine from 30 to 10 min. The shorter incubation time was adequate for all *O*-acylamino acids and avoided the marked decrease in the color yield from *O*-malonylserine resulting from incubation times greater than 10 min (Figure 2).

The relative rates of "O → N transfer" and hydrolysis of *O*-acyl derivatives were determined as follows. The combined rate of "O → N transfer" and hydrolysis of the *O*-acyl group was determined from the decrease in the hydroxylamine assay. The rate of "O → N acyl transfer" was determined by loss of ninhydrin reactivity.[10] No significant difference was observed in the rate of decrease in these two assays, indicating that the major reaction of the *O*-acylamino group under the conditions studied is an "O → N acyl transfer," rather than hydrolysis. Pseudo-first-order rate constants of "O → N acyl transfer" were calculated from a minimum of four time periods, during which the reaction had proceeded to at least 30% completion. However, reliable rates with *O*-malonyl- and *O*-succinylhomoserine could not obtained at pH 7.4, since no significant difference in the hydroxylamine or ninhydrin assay was observed even after 12-hr incubation.

Paper Chromatography.—Descending chromatography was performed on Whatman No. 1 paper in the following solvents: A, ethanol–water (77:23, v/v); B, phenol–water (75:25, w/v); C, isopropyl alcohol–water (70:30, v/v); D, 1-butanol–acetic acid–water (24:6:10, v/v); E, *tert*-butyl alcohol–2-butanone–90% formic acid–water (40:30:15:15, v/v).

Acid Hydrolysis of *O*-Acylamino Acids.—Each *O*-acylamino acid was hydrolyzed in 1 *M* HCl at 100° for 20 min. The hydrolyzate was lyophilized to dryness; the residue was dissolved in a minimum amount of water and subjected to paper chromatography in solvents B, D, and E. All acids (organic and amino) were first located by dipping the paper in a solution of Bromocresol green.[11] The contrast between acid spots and the background was improved by steaming the paper for 3 min prior to

dipping in the bromocresol green solution. Amino acids were then located by dipping the same paper through a 0.5% solution of ninhydrin in acetone.

Nmr Spectra.—Proton magnetic resonance spectra were obtained on a Varian A-60 spectrometer. Spectra were determined on compounds dissolved in D_2O containing 0.1 *M* potassium phosphate buffer, pD 6.5. Line positions were measured with respect to 3-(trimethylsilyl)propane sulfonic acid sodium salt as an internal reference.

Anion Exchange Chromatography.—Optimal conditions for separation of the *O*-acyl amino acid product from its parent amino acid were determined in pilot experiments in which the reaction mixture was subjected to column chromatography under various conditions, and column fractions analyzed by paper electrophoresis. Paper electrophoresis was performed at 1000 V for 1 hr at 0° in either 0.1 *M* sodium acetate, pH 4.75 (*O*-oxalyl derivatives), or 0.1 *M* sodium phosphate, pH 6.7 (*O*-malonyl and *O*-succinyl derivatives). Under these conditions the *O*-acylamino acid derivatives have a net negative charge and readily separate from their parent amino acid which has no net charge. Complete separation of the fractions containing *O*-acylamino acid from those containing the parent dicarboxylic acid was demonstrated by the use of the appropriate radioactive dicarboxylic acid added to the reaction mixture immediately before application to the column.

Synthesis of *O*-Acyl Derivatives. A. *O*-Oxalyl-L-homoserine. —Oxalylchloride (40 ml, 0.472 mol) was added to 90 ml of acetone and cooled to 25°. To this solution was added 2.4 ml of 60% perchloric acid, followed immediately by 2.38 g (0.02 mol) of finely powdered L-homoserine. During these additions and subsequent incubation for 15 min, the reaction mixture was stirred vigorously and maintained at 25° by use of an ice bath when necessary. The reaction mixture was then cooled to 0° and subsequently maintained at this temperature. Ice was slowly added until a vigorous reaction was no longer evident. Water was added to a final total volume of 200 ml, and the solution was extracted twice, each time with 400 ml of ether. The aqueous phase was titrated to pH 5.0 with 1 *M* KOH and then filtered to remove the precipitate of potassium perchlorate. The filtrate was diluted with water to a total volume of 1.4 l. and applied to a column (5 × 51 cm) of AG1-X10 (100–200 mesh) in the formate form. Subsequent experience with other derivatives (see below) indicated that this column was overloaded. The column was washed with 1.5 l. of 0.1 *M* formic acid and then eluted with 2 *M* formic acid. The fraction eluting between 1.0 and 1.6 l. of 2 *M* formic acid contained *O*-oxalyl-L-homoserine. This fraction was lyophilized to dryness, and the resulting powder crystallized from about 20 ml of water to yield 330 mg of product (9% yield from homoserine) which had mp 173.5–175.4° after recrystallization from ethanol–water–ether (45:45:10): nmr δ 2.41 (m, *J* = 6 Hz, CHCH$_2$), 4.07 (t, *J* = 6 Hz, CH), 4.43 (t, *J* = 6 Hz, CH$_2$O). The low solubility of oxalylhomoserine precluded a determination of the number of H corresponding to each peak. *Anal.* Calcd for $C_6H_9NO_6$: C, 37.68; H, 4.75; N, 7.33. Found: C, 37.75; H, 4.92: N, 7.46.

B. *O*-Malonyl-L-homoserine.—Malonyl chloride (30 ml, 0.27 mol) was added to 120 ml of acetone and cooled to 25°. To this solution was added 4 ml of 60% perchloric acid, followed immediately by 2.38 g (0.02 mol) of finely powdered L-homoserine. During these additions and subsequent incubation for 10 min, the reaction mixture was stirred vigorously and maintained at 25° by use of an ice bath when necessary. The reaction mixture was then cooled to 0° and subsequently maintained at this temperature. Water was added to a total volume of 250 ml and the solution extracted twice, each time with 500 ml of ether. The aqueous phase was titrated to pH 5.0 with 1 *M* KOH and the precipitate of potassium perchlorate removed by filtration. The filtrate was diluted to a total volume of 19 l. and applied overnight to a column (9.5 × 44 cm) containing AG1-X10 (100–200 mesh) in the formate form. The column was washed with 6 l. of 0.1 *M* formic acid and then eluted with 2 *M* formic acid. The fraction eluting between 3.5 and 4.25 l. of 2 *M* formic acid contained *O*-malonyl-L-homoserine. This fraction was lyophilized to dryness and the resulting powder crystallized from 20 ml of water to yield 1.32 g of product (32% yield from homoserine) which had mp 149–153° after recrystallization from water–ethanol–ether (45:45:10): nmr δ 2.30 (m, 1.95, *J* = 7 Hz, CHCH$_2$), 3.95 (t, 0.94, *J* = 7 Hz, CH), 4.35 (t, 2.10, *J* = 6 Hz, CH$_2$O). *Anal.* Calcd for $C_7H_{11}NO_6$: C, 40.96; H, 5.41; N, 6.83. Found: C, 39.46; H, 5.78; N, 7.02.

(7) Melting points were determined in unsealed capillary tubes with a Thomas–Hoover "Uni-Melt" melting point apparatus and are uncorrected.

(8) J. Giovanelli, *Biochim. Biophys. Acta*, **118**, 124 (1966).

(9) C. Delavier-Klutchko and M. Flavin, *J. Biol. Chem.*, **240**, 2537 (1965).

(10) H. Rosen, *Arch. Biochem. Biophys.*, **67**, 10 (1957).

(11) I. Smith, "Chromatographic and Electrophoretic Techniques," Vol. I, Interscience, New York, N. Y., 1962, p 279.

C. *O*-Malonyl-L-serine.—Finely powdered L-serine (3.15 g, 0.03 mol) was added to a solution of 60 ml of acetone and 3.6 ml of 60% perchloric acid. The resulting solution was added over a 3-min period to a solution of 30 ml (0.27 mol) of malonyl chloride in 55 ml of acetone. During these additions and subsequent incubation for 10 min, the reaction mixture was stirred vigorously and maintained at 30° by use of an ice bath when necessary. The reaction mixture was then cooled to 0° and subsequently maintained at this temperature. Water was added to a total volume of 250 ml and the solution extracted twice, each time with 500 ml of ether. The subsequent procedure was the same as that described for *O*-malonylhomoserine. The product appeared at approximately the same elution volume as did *O*-malaonylhomoserine. The product was crystallized from water to yield 2.29 g (39% yield from serine) of product, which had mp 163–165° after recrystallization from water–ethanol–ether (45:45:10): nmr δ 4.15 (t, 0.80, *J* = 4 Hz, CH), 4.64 (d, 2.20, *J* = 4 Hz, CH₂O). *Anal.* Calcd for $C_6H_9NO_6$: C, 37.68; H, 4.75; N, 7.33. Found: C, 37.85; H, 4.84; N, 7.23.

D. *O*-Succinyl-L-serine.—To 100 ml of 1,4-*p*-dioxane containing 5 ml of 60% perchloric acid was added 3.15 g (0.03 mol) of finely powdered L-serine. This solution was added over a period of 10 min to 42 ml (0.38 mol) of succinyl chloride. During these additions and subsequent incubation for 1 hr, the reaction mixture was stirred vigorously and maintained at 30° by use of an ice bath when necessary. The reaction mixture was then cooled to 0° and subsequently maintained at this temperature. The relatively slow rate of hydrolysis of succinyl chloride permitted its efficient extraction with ether as follows. Ether (500 ml) was added to the reaction mixture, followed by 200 ml of water. This mixture was immediately shaken vigorously for 3 sec in a separatory funnel, and the aqueous phase was drawn off. Extraction of the ether phase with water was repeated. These extractions were performed rapidly so that most of the succinyl chloride could be extracted before it was hydrolyzed to succinic acid. The aqueous phases were then combined, extracted five times (each time with 500 ml of ether), and titrated to pH 6.5 with 1 *M* KOH. The subsequent procedure was the same as that described for *O*-malonylhomoserine with the following exception. The product was eluted with 0.75 *M* formic acid, appearing between 2.5 and 5.0 l. This fraction was lyophilized to dryness and the resulting powder crystallized from water–ethanol–ether (45:45:10) to yield 1.18 g (19% yield from serine), mp 156.5–157.5°.[12] The compound turned brown near its melting point: nmr δ 2.72 (s, 4.04, CH₂CH₂), 4.14 (t, 0.81, *J* = 4 Hz, CH), 4.60 (d, 2.15, *J* = 4 Hz, CH₂O). *Anal.* Calcd from $C_7H_{11}NO_6$: C, 40.96; H, 5.41; N, 6.83. Found: C, 41.10; H, 5.66; N, 6.91.

E. *O*-Oxalyl-L-serine.—This compound proved to be the most difficult to synthesize, probably because of its instability (Figure 1). However, a small amount of the compound was synthesized by the same procedure as described for *O*-malonylserine.

Studies on Reactions of Isoprenoids.
XII.[1] The 1,4-Cycloaddition Reactions
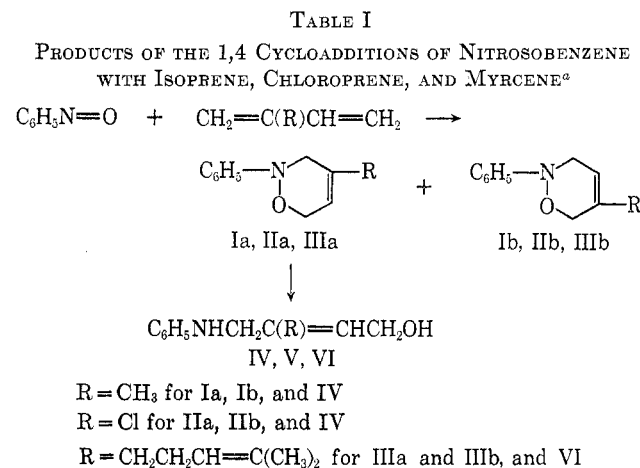## of Nitrosobenzene with Isoprene,
Chloroprene, and Myrcene

Tadashi Sasaki,* Shoji Eguchi,
Teruhiko Ishii, and Hiroto Yamada

*Institute of Applied Organic Chemistry,
Faculty of Engineering, Nagoya University,
Furo-cho, Chikusa-ku, Nagoya, 464, Japan*

Although 1,4-cycloaddition reactions of nitrosobenzene with 1-mono- and 1,4-disubstituted butadienes have been extensively studied,[2] those with 2-substituted butadienes seem to be still ambiguous with respect to the orientation; isoprene, 2-methoxybutadiene, and chloroprene have been known to react with nitrosobenzene to afford only one major cycloadduct, but the direction of the addition has not been adequately established or shown.[3] In a continuation of our study on the reactivity of myrcene, 7-methyl-3-methylen-1,6-octadiene, in 1,4-cycloaddition reactions, the reaction with nitrosobenzene was investigated in comparison with those of isoprene and chloroprene.[4]

The results are summarized in Table I. In the reaction with isoprene at 20°, only one product (Ia) was

TABLE I

PRODUCTS OF THE 1,4 CYCLOADDITIONS OF NITROSOBENZENE WITH ISOPRENE, CHLOROPRENE, AND MYRCENE[a]

$C_6H_5N{=}O$ + $CH_2{=}C(R)CH{=}CH_2$ ⟶

1,3-Dienes	Reaction temp, °C	Reaction time, hr	Products (yield, %)
Isoprene	20	72	Ia (42
Chloroprene	20	72	IIa (58)
Myrcene	20	72	IIIa (30) + IIIb (16)
Myrcene	80	1	IIIa (3) + IIIb (28)
Myrcene	0–5	24	IIIa (28) + IIIb (8)

[a] All reactions were carried out in benzene.

isolated as an oil. The direction of the addition was established by reduction of Ia with zinc powder and

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(12) A melting point of 173–174° has been reported by S. Guggenheim and M. Flavin [*J. Biol. Chem.*, **244**, 3722 (1969)], for *O*-succinyl-L-serine prepared by a method involving the reaction of *N*-benzyloxycarboxyl-L-serine with succinic anhydride. Drs. Guggenheim and Flavin kindly supplied a sample of their preparation of *O*-succinyl-L-serine which had been stored at −30° since its preparation in March 1967. In our hands, mp 157–158.5° was determined for their preparation; a mixture of *ca.* equal amounts of the two preparations had mp 157–157.5°.

* To whom correspondence should be addressed.
(1) Part XI of this series: T. Sasaki, S. Eguchi, and T. Oyobe, *Bull. Chem. Soc. Jap.*, **43**, 1252 (1970).
(2) G. Kresze and J. Firl, *Tetrahedron*, **24**, 1043 (1968), and references cited therein.
(3) J. Hamer and M. Ahmad, "1,4-Cycloaddition Reactions," J. Hamer, Ed., Academic Press, New York, N. Y., 1967, p 427.
(4) T. Sasaki, S. Eguchi, and T. Ishii, *J. Org. Chem.*, **34**, 3749 (1969).