Synthesis of L-2-Amino-4-methoxy-*trans*-but-3-enoic Acid

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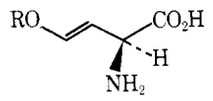
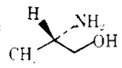
The naturally occurring amino acid, L-2-amino-4-methoxy-*trans*-but-3-enoic acid (**1a**), was synthesized starting from the aspartic semialdehyde derivative **2**. Formation of the enol ether moiety was accomplished by conversion of acetal **3a** to the hemiacetal ester **3b**, followed by pyrolysis of **3b** to yield a mixture of trans and cis enol ethers **4**. A resolution of the synthetic material was achieved by selective enzymatic hydrolysis of the *N*-acetyl group with hog kidney acylase I. This procedure provides the pure natural product **1a**, as well as partially racemized D-amino acid **8**.

In recent years, there have been found in nature a number of amino acids **1** which are unusual in that they each contain an enol ether function. The first two compounds, **1a** and **1b**,

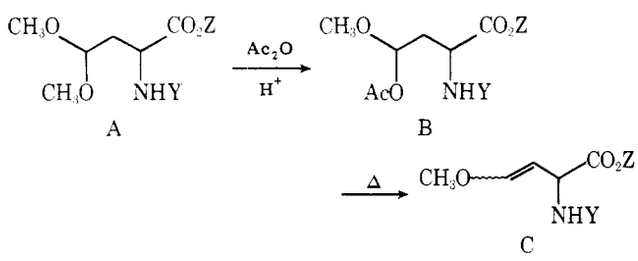


1a, R = CH_3
b, R = $CH_2CH_2NH_2$
c, R = 

were isolated and characterized by Scannell, Pruess, and co-workers.[1,2] The methyl enol ether **1a** was obtained from *Pseudomonas aeruginosa*,[1] while the aminoethyl enol ether **1b** was produced by an unidentified species of *Streptomyces*.[2] Rhizobitoxine (**1c**), the most complex member of this new group of amino acids, was isolated from *Rhizobium japonicum* and its structure determined by Owens and co-workers.[3,4] More recently, we have assigned the absolute configuration shown in structure **1c** to rhizobitoxine[5] and confirmed that assignment by synthesis.[6]

All three of these compounds were shown to strongly inhibit the production of ethylene by plant tissue.[2,7] Since ethylene plays a vital role in controlling certain plant life processes, this activity could be economically important.[8] In order that this area of study might be more fully explored, we have developed two potentially general methods for synthesizing members and analogues of this new class of amino acids. The first method is presented in the current paper and is illustrated by a synthesis of the methyl enol ether **1a**. The second is discussed in an accompanying paper and is illustrated by a synthesis of the racemic modification of the aminoethyl enol ether **1b**.[9]

In considering the synthesis of L-2-amino-4-methoxy-*trans*-but-3-enoic acid (**1a**), we felt that the critical steps would involve generation of the enol ether moiety in the presence of the potentially reactive amino acid portion of the molecule. We present as a solution to this problem a mild two-step sequence for the synthesis of enol ethers. The method



utilizes the conversion of an acetal A to a hemiacetal ester B, followed by pyrolysis of B under neutral conditions to yield an enol ether C. The pyrolysis of hemiacetal esters to yield enol ethers has been discussed previously in the literature.[10] Until now, however, there has not been a convenient way to make the starting esters B. In the present case, the easy and efficient production of B makes the overall sequence viable.

Aldehyde **2**, which is available by a published procedure,[11] served as our starting material (see Scheme I). Acetalization to give **3a** was accomplished in 77% yield by heating **2** at reflux temperature in methanol/trimethyl orthoformate with ammonium chloride catalyst. The dimethyl acetal **3a** seemed like a suitable precursor for the enol ether function. It was found, however, that the conditions required for the acid-catalyzed elimination of methanol from **3a** were so severe that the engendered enol ether did not survive.

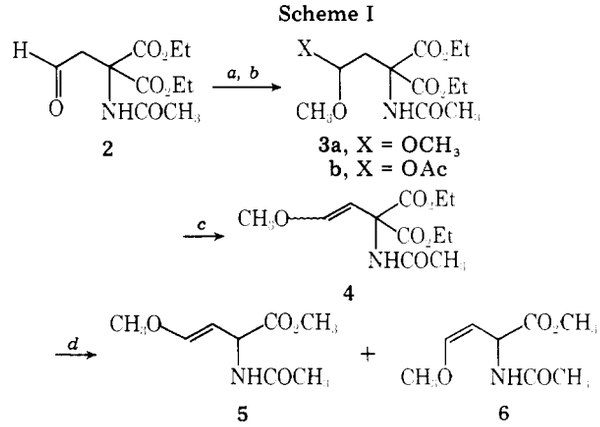
This problem was circumvented by resorting to the more labile hemiacetal ester **3b**. Thus, treatment of **3a** with acetic anhydride and dry cationic exchange resin at 65 °C cleanly effected the exchange of one methoxy group for an acetoxy group. Then, following the method of Erickson,[10] pyrolysis of the resultant hemiacetal ester **3b** at 185 °C (17 mm) gave a mixture of the trans and cis enol ethers **4** (79% from **3a**).

Dissolution of enol ethers **4** in methanolic sodium methoxide caused the removal of one ethoxycarbonyl group, resulting in a mixture of enol ethers **5** and **6**. The two compounds were separated by silica gel chromatography followed by crystallization to yield the pure trans and cis compounds **5** (39%) and **6** (12.9%).

Since the starting material **2** for the synthesis is achiral, the material in hand at this point, trans enol ether **5**, is racemic. The resolution of this substance, as well as the removal of its protecting groups, is described in the following section (see Scheme II).

Hydrolysis of the ester function in **5** with 1.05 equiv of lithium hydroxide gave the racemic lithium carboxylate salt. Incubation for 16 h at 39 °C of an aqueous solution of the salt and hog kidney acylase I effected selective hydrolysis of the L form of the *N*-acetylamino acid salt. The resultant mixture of L-amino acid **1a** and D-*N*-acetylamino acid **7** was separated by distribution between dilute hydrochloric acid (pH 2) and

Scheme I



[a] CH_3OH, $(CH_3O)_3CH$, NH_4Cl, reflux temperature. [b] Ac_2O H^+, 65 °C. [c] 185 °C (17 mm), 1.5 h. [d] $NaOCH_3$, CH_3OH.

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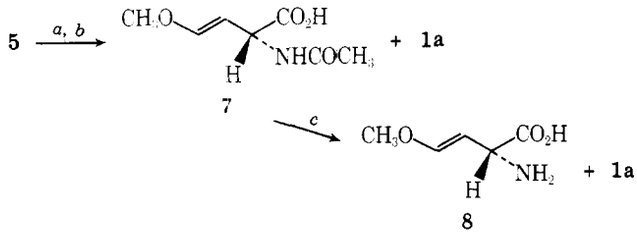
Scheme II



[a] LiOH. [b] Hog kidney acylase, 39 °C. [c] $NH_2NH_2 \cdot H_2O$ (85%), 70 °C.

ethyl acetate. The amino acid was isolated by cation exchange chromatography, followed by crystallization to give a substance (69.3% from **5**) which was shown to be identical with the naturally occurring amino acid **1a** by comparison with an authentic sample.[1,14]

The acetyl group of the D-*N*-acetylamino acid was removed by heating a solution of **7** in 85% hydrazine hydrate at 70 °C for 15 h. Removal of the hydrazine and trituration of the residue with ethanol gave an amino acid (46% yield from **5**) with the same gross structure as the methyl enol ether **1a**, as confirmed by spectral analysis. The specific rotation (see the Experimental Section) indicated that the substance consists of an 80% enantiomeric excess of the D-isomer **8**.

Experimental Section

General. Melting points were taken on a Kofler hot stage melting point apparatus (Reichert) and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer 621 or a Beckman IR-9. Ultraviolet spectra were recorded on a Cary Model 16 spectrophotometer. Rotations were measured on a Perkin-Elmer 141 automatic polarimeter. Proton NMR spectra were obtained on Varian HA-100 and XL-100 instruments and are reported in parts per million downfield from internal or external tetramethylsilane. Elemental analyses and amino acid analyses were carried out under the supervision of Dr. F. Scheidl of our Microanalytical Laboratory.

Ethyl 2-Acetamido-2-ethoxycarbonyl-4,4-dimethoxybutyrate (3a). A solution consisting of 55 g (0.212 mol) of ethyl 2-acetamido-2-ethoxycarbonyl-4-oxobutyrate (**2**),[11] 134 g (1.26 mol) of trimethyl orthoformate, 1.1 g (0.02 mol) of ammonium chloride, and 880 mL of methanol was heated with magnetic stirring at reflux temperature for 48 h. The solution was allowed to cool to room temperature and diluted with ether (1500 mL). The resultant solution was washed three times with 450-mL portions of saturated sodium bicarbonate/brine (1:1) and two times with 400-mL portions of brine. The ether phase was dried over sodium sulfate and concentrated in vacuo, giving an oil which was distilled through a 4 in vacuum-jacketed Vigreux column to yield 52.6 g (77%) of **3a:** bp 138–143 °C (0.04 mm); IR ($CHCl_3$) 1738, 1683, 1598 cm^{-1}; NMR ($CDCl_3$) δ 6.86 (broad, 1 H, NH), 4.31 (t, 1 H, J = 6 Hz, $-CH_2CH(-O)_2$), 4.21 (q, 4 H, J = 8 Hz, $2CH_3CH_2O-$), 3.21 (s, 6 H, $2CH_3O-$), 2.68 (d, 2 H, J = 6 Hz, $-CH_2CH(-O)_2$), 2.03 (s, 3 H, CH_3CO-), 1.24 (t, 6 H, J = 8 Hz, $2CH_3CH_2O-$); mass spectrum, *m/e* 305 (M^+), 274, 260, 232, 217, 200, 158, 130, 75.

Anal. Calcd for $C_{13}H_{23}NO_7$: C, 51.14; H, 7.59; N, 4.59. Found: C, 51.16; H, 7.62; N, 4.54.

***dl*-Ethyl 2-Acetamido-4-acetoxy-2-ethoxycarbonyl-4-methoxybutyrate (3b).** To a solution under argon consisting of 34.8 g (0.114 mol) of **3a** and 144 mL of acetic anhydride was added 8.7 g of dry AG 50W-X4 cation exchange resin (100–200 mesh; H^+ form).[12] The resultant suspension was stirred magnetically at 65 °C for 1.5 h. The resin was removed by filtration through a sintered glass funnel, and the filtrate was concentrated in vacuo to yield **3b** as a pale yellow oil. A small sample gave the following spectral and analytical data after prolonged drying under vacuum (0.1 mm, 60 °C, 15 h): IR ($CHCl_3$) 3425, 1738, 1680, 1490 cm^{-1}; NMR ($CDCl_3$) δ 6.82 (broad, 1 H, NH), 5.73 (t, 1 H, J = 6 Hz, $-CH_2CH(-O)_2$), 4.25 (q, 4 H, J = 8 Hz, $2CH_3CH_2O-$), 3.32 (s, 3 H, CH_2O-), 2.80 (m, 2 H, $-CH_2CH(-O)_2$), 2.04 (s, 6 H, $2CH_3CO-$), 1.25 (t, 6 H, J = 8 Hz, $2CH_3CH_2O-$); mass spectrum, *m/e* 274, 260, 200, 158, 144, 116, 75.

Anal. Calcd for $C_{14}H_{23}NO_8$: C, 50.45; H, 6.96; N, 4.20. Found: C, 50.55; H, 6.87; N, 4.19.

***cis*- and *trans*-Ethyl 2-Acetamido-2-ethoxycarbonyl-4-methoxybut-3-enoate (4).** The crude hemiacetal ester **3b,** obtained from the previously described reaction, was heated under vacuum (17 mm) with magnetic stirring at 185 °C for 1.5 h. The resultant pyrolyzate was distilled through a 4 in vacuum-jacketed Vigreux column to yield 24.8 g (79% from **3a**) of a mixture consisting of the cis and trans isomers **4:** bp 138 °C (0.06 mm); NMR ($CDCl_3$) δ 6.93 (broad, NH), 6.45 (d, J = 13 Hz, trans $-OCH{=}CH-$), 5.95 (d, J = 6 Hz, cis $-OCH{=}CH-$), 5.38 (d, J = 13 Hz, trans $-OCH{=}CH-$), 5.33 (d, J = 6 Hz, cis $-OCH{=}CH-$), 4.22 (q, J = 7 Hz, CH_3CH_2O-), 3.56 (s, CH_3O-), 2.05 (s, CH_3CO-), 1.27 (t, J = 7 Hz, CH_3CH_2O-).

***dl*-Methyl 2-Acetamido-4-methoxy-*trans*-but-3-enoate (5) and *dl*-Methyl 2-Acetamido-4-methoxy-*cis*-but-3-enoate (6).** The mixture of distilled cis and trans enol ethers **4** (24.8 g, 0.091 mol) was dissolved in 350 mL of anhydrous methanol containing 2 g (0.037 mol) of sodium methoxide. The resultant basic solution (pH 10 by moist pHydrion paper)[13] was stirred under argon at ambient temperature for 24 h. The reaction mixture was neutralized with acetic acid and concentrated under reduced pressure to give an oily slurry. Addition of ether to the slurry caused the precipitation of sodium salts which were removed by filtration through a pad of diatomaceous earth. The filtrate was concentrated in vacuo to yield 17 g of an oil which was applied in ether solution (minimum volume) to a column (59 mm i.d.) containing an intimate mixture of 400 g of silica gel 60 (70–230 mesh; E. Merck Reagent catalogue #7734) and 133 g of silica gel PF-254 (E. Merck Reagent catalogue #7747). The column was developed with ether/methanol (98:2), and 20-mL size fractions were collected. The eluent was monitored by thin-layer chromatography on silica gel plates [ether/methanol (96:4); visualization with I_2]. Fractions 155–206 were combined and concentrated in vacuo, and the resultant residue was crystallized from ether/petroleum ether to yield 5.45 g (32%) of **5:** mp 47.5–49.5 °C; IR ($CHCl_3$) 3425, 1737, 1670, 1500 cm^{-1}; NMR ($CDCl_3$) δ 6.64 (d, 1 H, J = 13 Hz, $-OCH{=}CH-$), 6.15 (broad, 1 H, NH), 4.8 (m, 2 H, $-OCH{=}CHCH<$), 3.66 (s, 3 H, CH_3O-), 3.55 (s, 3 H, CH_3O-), 2.01 (s, 3 H, CH_3CO-); mass spectrum, *m/e* 187 (M^+), 155, 144, 128, 84.

Anal. Calcd for $C_8H_{13}NO_4$: C, 51.33; H, 7.00; N, 7.48. Found: C, 51.40; H, 7.03; N, 7.44.

The mother liquor from the above crystallization was combined with fractions 137–154 and 207–218. The solution was concentrated and the residue chromatographed on a column similar to the one just described. The appropriate fractions were concentrated, and the residue was crystallized from ether/petroleum ether to yield 1.2 g (7.0%) of **5.** A total yield of 39% was realized for **5.**

Fractions 219–336 were concentrated in vacuo, and the residue was crystallized from ether/petroleum ether to yield 2.2 g (12.9%) of **6:** mp 120–123 °C; IR ($CHCl_3$) 3440, 1735, 1675, 1515 cm^{-1}; NMR ($CDCl_3$) δ 6.32 (broad, 1 H, NH), 6.10 (d, 1 H, J = 7 Hz, $-OCH{=}CH-$), 5.28 (t, 1 H, J = 9 Hz, $-OCH{=}CHCH<$), 4.50 (dd, 1 H, J = 7 and 9 Hz, $-OCH{=}CH-$), 3.65 (s, 3 H, CH_3O-), 3.60 (s, 3 H, CH_3O-), 2.01 (s, 3 H, CH_3CO-); mass spectrum, *m/e* 187 (M^+), 155, 144, 128, 86.

Anal. Calcd for $C_8H_{13}NO_4$: C, 51.33; H, 7.00; N, 7.48. Found: C, 51.32; H, 6.94; N, 7.51.

L-2-Amino-4-methoxy-*trans*-but-3-enoic Acid (1a). A solution consisting of 5.0 g (0.027 mol) of **5,** 28 mL of 1 N lithium hydroxide, and 50 mL of methanol was allowed to stand at ambient temperature for 3 h. It was concentrated in vacuo, and the residue wash dissolved in 90 mL of deionized water. The pH was adjusted to 7.3 with 1 N hydrochloric acid, 18 mg of hog kidney acylase I (purchased from Sigma Chemical Co., catalogue #A-3010) was added, and the resultant solution was stirred magnetically at 39 °C for 16 h. The solution was then adjusted to pH 2 with 6 N hydrochloric acid and extracted with ethyl acetate. The aqueous phase was applied to an ion exchange column (AG 50W-X4; 100–200 mesh; pyridinium form; 250 mL of resin bed). The column was developed with water followed by 10% aqueous pyridine. The aqueous pyridine fraction was concentrated in vacuo, and the residue was crystallized from methanol to yield 1.2 g (69.3%) of **1a:** mp 225–235 °C dec; $[\alpha]^{25}_D$ +123° (*c* 0.7925, H_2O); NMR (D_2O) δ 7.33 (d, 1 H, J = 13 Hz, $-OCH{=}CH-$), 5.45 (dd, 1 H, J = 10 and 13 Hz, $-OCH{=}CH-$), 4.67 (d, 1 H, J = 10 Hz, $-OCH{=}CHCH<$), 4.11 (s, 3 H, CH_3O-); mass spectrum, *m/e* 86.

Anal. Calcd for $C_5H_9NO_3$: C, 45.80; H, 6.92; N, 10.68. Found: C, 45.65; H, 6.80; N, 10.89.

The synthetic material **1a** was shown by direct comparison to exhibit the same melting point behavior as the natural product,[1,14] and a mixture melting point was unchanged. The two substances have superimposable IR and NMR spectra and showed identical behavior when analyzed on an amino acid analyzer. A slight difference in the specific rotations of the natural ($[\alpha]^{25}_D$ +115°) and the synthetic ($[\alpha]^{25}_D$ +123°) materials is due to a trace impurity which is difficult to remove from the natural product.[1]

The ethyl acetate extract was concentrated in vacuo to yield 1.6 g (68.4%) of **7:** NMR (D_2O) δ 7.03 (d, 1 H, J = 12 Hz, $-OCH{=}CH-$), 5.1 (m, 2 H, $-OCH{=}CHCH$), 3.9 (s, 3 H, CH_3CO-).

D-2-Amino-4-methoxy-*trans*-but-3-enoic Acid (8). The crude

carboxylic acid **7** (2.24 g, 0.0129 mol) was dissolved in 35 mL of 85% hydrazine hydrate. The solution was heated at 70 °C for 15 h and concentrated in vacuo, and the resultant residue was dried in vacuo over concentrated H_2SO_4 to give a white solid. Trituration of the solid with ethanol gave 1.15 g (68%) of a substance which was 80% D-2-amino-4-methoxy-*trans*-but-3-enoic acid (**8**) and 20% racemate: mp 220 °C dec; $[\alpha]^{25}_D$ −98° (*c* 0.8370, H_2O); NMR (D_2O) δ 7.33 (d, 1 H, *J* = 13 Hz, -OC**H**=CH-), 5.45 (dd, 1 H, *J* = 10 and 13 Hz, -OCH-=C**H**-), 4.67 (d, 1 H, *J* = 10 Hz, -OCH=CHC**H**<), 4.11 (s, 3 H, CH_3O-); mass spectrum, *m/e* 86.

Anal. Calcd for $C_5H_9NO_3$: C, 45.80; H, 6.92; N, 10.68. Found: C, 45.90; H, 6.81; N, 10.88.

The specific rotation, $[\alpha]^{25}_D$ −98°, indicates that the D isomer is present in 80% enantiomeric excess.

Acknowledgment. We thank the staff of the Physical Chemistry Department of Hoffmann-La Roche Inc. for the determination of spectral and analytical data.

Registry No.—**1a,** 35891-72-6; **2,** 14110-03-3; **3a,** 66966-87-8; **3b,** 66966-88-9; (*E*)-**4,** 66966-89-0; (*Z*)-**4,** 66966-90-3; **5,** 66966-91-4; **6,** 66966-92-5; **7,** 66966-93-6; **8,** 67010-40-6.

References and Notes

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(11) O. A. Moe and D. T. Warner, *J. Am. Chem. Soc.*, **74,** 2690 (1952).
(12) The cation exchange resin AG 50W-X4 (100–200 mesh; H^+ form) was purchased from Bio Rad Laboratories, Richmond, Calif. Before use, it was washed with several portions each of water, methanol, and ether. The resin was then dried over P_2O_5 under vacuum.
(13) Some acetic acid and acetic anhydride invariably codistill with the enol ethers. Thus, a greater amount of sodium methoxide may be necessary to achieve this pH.
(14) We thank Dr. J. Scannell of the Microbiology Department at Hoffmann-La Roche Inc. for a sample of the natural amino acid **1a.**

Synthesis of DL-2-Amino-4-(2-aminoethoxy)-*trans*-but-3-enoic Acid

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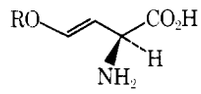
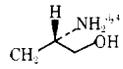
The racemic modification of the naturally occurring amino acid, L-2-amino-4-(2-aminoethoxy)-*trans*-but-3-enoic acid (**1b**), was synthesized starting from bis(2-chloroethyl) ether (**6**) and diethyl acetamidomalonate (**7**). The route included formation of the dehydroamino acid derivative **12,** followed by base-mediated isomerization of the double bond to form the critical enol ether linkage in **13.** Removal of the protecting groups from **13** then gave rise to the racemic amino acid **15** in an overall yield of 11%.

Recently, a new type of α-amino acid has been found in nature.[1-3] The members, **1,** of this class of compounds are

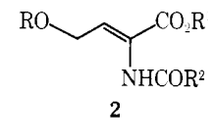


1a, R = CH_3[1]
b, R = $CH_2CH_2NH_2$[2]
c, R = 

distinguished both by having a centrally located enol ether function in the molecule and by their ability to inhibit the production of ethylene in plant tissue.[2,5] Since ethylene plays a vital role in controlling certain plant life processes, this activity is both intriguing and potentially economically important.[6]

We became interested in developing synthetic methods which would make these compounds and analogues of these compounds more readily available. One such sequence, described in the preceding paper, was used to make L-2-amino-4-methoxy-*trans*-but-3-enoic acid (**1a**).[7] The important steps of that synthesis are the generation of a hemiacetal ester followed by its pyrolysis to yield an enol ether. In this paper, we wish to describe a second route to these compounds and illustrate it with a synthesis of racemic 2-amino-4-(2-aminoethoxy)-*trans*-but-3-enoic acid (**1b**).

In our projected synthesis of **1b,** we intended to make the central enol ether function by isomerization of the double



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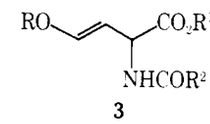

3

bond in a dehydroamino acid derivative **2** to form the enol ether **3.** The suggestion that this might be a fruitful approach comes from the studies of S. J. Rhoads and co-workers[8] and J. Hine and co-workers.[9] By studying the equilibration of methyl 4-methoxybutenoates **4,** both groups demonstrated that the double bond is stabilized more effectively by the methoxy group than by the ester. Thus, at equilibrium Hine found the mixture of olefins to be 99% **4b,** while Rhoads, under two different sets of equilibrating conditions, found the mixtures to be 92.5 and 96.9% **4b,** respectively.

$CH_3OCH_2CH{=}CHCO_2CH_3$
4a

$\rightleftarrows CH_3OCH{=}CHCH_2CO_2CH_3$
4b

In order to make the enol ether by this method, we required an appropriately substituted dehydroamino acid derivative. A recent publication by Shin and co-workers describes the synthesis of such compounds by the elimination of acetic acid from an *N,O*-diacetylhydroxyamino acid derivative.[10] This result suggested to us that dehydroamino acids could also be obtained by the elimination of HCl from a suitably disposed

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