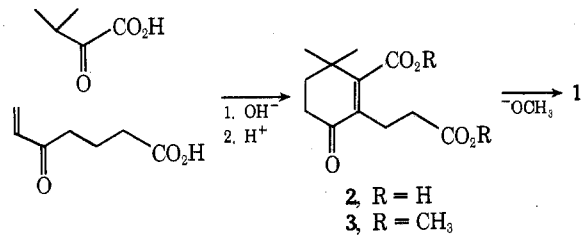
keto ester 1, which was the key intermediate in the first synthesis of strigol.[3]

Our synthesis of the β-keto ester 1 is outlined below. Two known compounds, dimethylpyruvic acid[5] and 5-oxo-6-heptenoic acid,[6] are condensed in aqueous base to give 70–80% yields of the dibasic acid 2, which is converted to the methyl ester in quantitative yield by diazomethane. Diester 3 is cyclized to the nicely crystalline β-keto ester 1 in 98% yield by the action of sodium methoxide in methanol.



2, R = H
3, R = CH_3

This route to the hydrindan portion of strigol is much shorter than those previously reported and the yields are quite good. Moreover, the starting materials, dimethylpyruvic acid and 5-oxo-6-heptenoic acid, are easily obtained. Dimethylpyruvic acid may be conveniently prepared by an azlactone synthesis as described many years ago.[5] We prepared 5-oxo-6-heptenoic acid in modest yield by acylating ethylene with glutaric anhydride and aluminum chloride, although it and its esters have been prepared by other routes.[6-9] The crude material from the Friedel–Crafts acylation is quite satisfactory for the condensation with dimethylpyruvic acid.

Experimental Section[10]

5-Oxo-6-heptenoic Acid. A mixture of aluminum chloride (66.7 g, 0.5 mol), glutaric anhydride (28.5 g, 0.25 mol), and methylene chloride (1 l.) was placed in a 2-l. three-necked flask equipped with a gas inlet tube, a drying tube, and a mechanical stirrer. Ethylene (44 g, 1.57 mol) was bubbled in during 4.5 hr with vigorous stirring, after which the reaction mixture was poured over a mixture of 5% hydrochloric acid (900 ml) and ice. The organic layer was separated and the aqueous portion was extracted once with ether (300 ml). The organic extracts were separately washed with water and evaporated under reduced pressure. The combined residues were warmed on the steam bath for 10 min with 100 ml of 10% potassium carbonate solution which resulted in a bright yellow suspension. This mixture was washed with ether until a colorless ether extract was obtained. The aqueous portion was acidified with hydrochloric acid and extracted with ether. The dried (Na_2SO_4) ether solution was evaporated to leave an orange oil (5.2 g, 14%) which crystallized on storage. A similar run gave a 34% yield of material with satisfactory spectroscopic properties. The material was triturated with carbon tetrachloride and collected. A sample of the crystalline material was purified by short-path distillation to produce a clear oil which gave crystalline material, mp 44–46°, after exposure to air (lit.[6] mp of the hydrate 45–46.5°): ir ν_{max} ($CHCl_3$) 1710, 1685, 1618 cm^{-1}; ^1H NMR 1.97 (p, 2 H, J = 7 Hz), 2.44 (t, 2 H, J = 7 Hz), 2.70 (t, 2 H, J = 7 Hz), 5.8–6.6 ppm (m, 3 H).

4,4-Dimethyl-2-(2-carboxyethyl)cyclohex-2-en-1-one-3-carboxylic Acid (2). A solution of dimethylpyruvic acid (1.23 g, 0.0106 mol) and 5-oxo-6-heptenoic acid (1.509 g, 0.0106 mol) in 31.5 ml of 1.5 N aqueous potassium hydroxide was heated on the steam bath for 2 hr. The cooled solution was acidified with concentrated hydrochloric acid and the crystalline material (1.68 g) was collected by filtration. The ^1H NMR spectrum of this material was identical with that of the purified substance. The filtrate was extracted with ether to afford an additional 0.411 g of crude diacid 2 which was contaminated with ca. 50% by weight of dimethylpurivic acid as judged by its ^1H NMR spectrum. The total yield of crude diacid 2 (2.0 g) was 79%. The crude material was recrystallized from water to yield needles, mp 205–206.5°, with slight previous softening. Impure diacid is more conveniently recrystallized from ethyl acetate. The purified material showed ^1H NMR (Me_2SO-d_6–$CDCl_3$) 1.26 (s, 6 H), 1.95 (q, 2 H, J = 6 Hz), 2.2–2.7 ppm (m, 6 H); ir ν_{max} (KBr) 1710, 1635 cm^{-1}; mass spectrum m/e calcd for $C_{12}H_{16}O_5$, 240.100; found, 240.100.

4,4-Dimethyl-2-(2-carbomethyoxyethyl)-3-carbomethoxycyclohex-2-enone (3). A sample of dibasic acid 2 in tetrahydrofuran was treated with excess ethereal diazomethane. The solvent was evaporated and the residue was sublimed to give a quantitative yield of 3: mp 47–49°; ^1H NMR 1.23 (s, 6 H), 1.90 (t, 2 H, J = 7 Hz), 2.40–2.7 (m, 6 H), 3.65 ppm (s, 3 H); ir ν_{max} ($CHCl_3$) 1730, 1675, 1619 cm^{-1}; uv λ_{max} (EtOH) 237 nm (ε 12360); mass spectrum m/e calcd for $C_{14}H_{20}O_5$, 268.131; found, 268.129.

5,5-Dimethyl-8-carbomethoxybicyclo[4.3.0]non-1(6)-ene-2,7-dione (1). The diester 3 (0.20 g) was heated under reflux for 2 hr in a nitrogen atmosphere with 2 ml of 0.78 N sodium methoxide in methanol. The cooled solution was treated with 0.1 g of acetic acid and diluted with 1% hydrochloric acid. The crystalline material was collected by filtration and the aqueous portion was extracted with ether. The combined product was dried to give 0.173 g (98%) of 1. A sample was crystallized from methanol and sublimed to give an analytical sample, mp 136.8–141°. The material is clearly a mixture of tautomers (ca. 1:1) in chloroform solution as previously indicated: ^1H NMR ($CDCl_3$) 1.32, 1.38 (s, 6 H), 1.96 m, 4 H), 2.67 (q, 2 H, J = 7 Hz), 2.92 (m, 1 H), 3.28 (s, 1 H), 3.49 (m, 1 H), 3.78, 3.83 ppm (s, 3 H); ir ν_{max} ($CHCl_3$) 1740, 1715, 1680 sh, 1615, 1548 cm^{-1}; uv λ_{max} (EtOH) 222 nm (ε 9170), 256 (7590), 325 (6478); mass spectrum m/e calcd for $C_{13}H_{16}O_4$, 236.105; found, 236.103.

Registry No.—1, 51799-98-5; 2, 57304-91-3; 3, 57304-92-4; dimethylpyruvic acid, 759-05-7; 5-oxo-6-heptenoic acid, 6934-67-4; glutaric anhydride, 108-55-4; ethylene, 74-85-1; diazomethane, 334-88-3; sodium methoxide, 124-41-4.

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(10) Infrared spectra were measured with either Beckman IR-5A or IR-7 infrared spectrophotomers. Proton magnetic resonance spectra were determined at 100 MHz with a Varian Model XL-100 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane internal standard. In the presentation of the ^1H NMR spectra the following notations are used: s = singlet, d = doublet, t = triplet, q = quartet, p = pentuplet, and m = multiplet.

Synthesis of a Useful Spin Labeled Probe, 1-Oxyl-4-carboxyl-2,2,6,6-tetramethylpiperidine

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In our continuing study of the cholinergic receptor we found it necessary to prepare a variety of spin labeled analogues of acetylcholine, one of which required the preparation of 1-oxyl-4-carboxyl-2,2,6,6-tetramethylpiperidine (1). Unfortunately, the preparation of 1 proved to be of great difficulty because the nitroxyl group is sensitive to many synthetically useful techniques.

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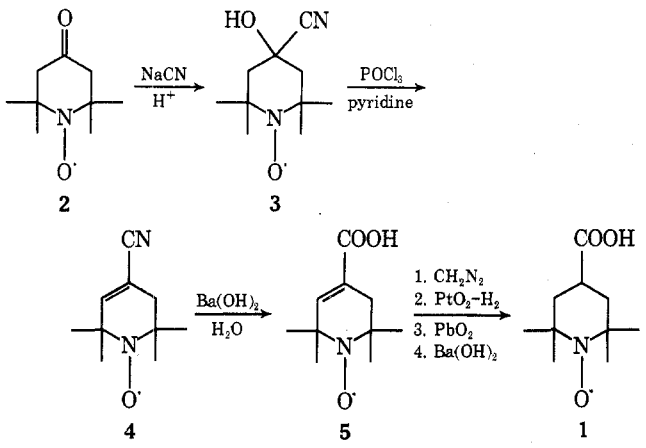
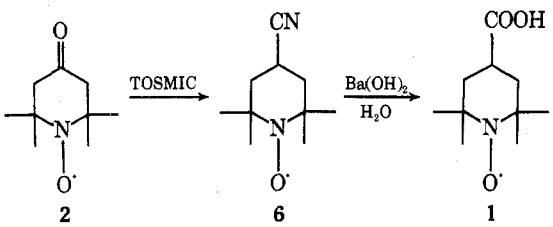
Scheme I



Recently Hsia et al.[1] reported the preparation of 1 as outlined in Scheme I. Using this reported procedure, we were unable to reduce 1-oxyl-4-carboxyl-2,2,6,6-tetramethyl-1,2,5,6-tetrahydropyridine (5) to the desired product (1). Palladium on charcoal, which we have found useful for other similar reductions, also failed to give a satisfactory yield of 1.

Toward this goal, we have devised an unequivocal synthesis of 5 using tosylmethyl isocyanide as originally reported by Van Leusen.[2] Treatment of 1-oxyl-2,2,6,6-tetramethyl-4-piperidone (2) with tosylmethyl isocyanide in the presence of base resulted in a high yield of 1-oxyl-4-cyano-2,2,6,6-tetramethylpiperidine (6). Hydrolysis of 6 to the



acid gave a product melting 20°C lower than that reported by Hsia et al.[1] It may be noted that the unsaturated acid, 5, has a reported melting point of 190–192°C, whereas the reduced (1) was reported to melt at 195–196°C. Furthermore, elemental analysis is unable to distinguish between the two compounds (1 and 5), given the generally accepted error of ±0.3%. To substantiate our findings, we performed a high-resolution mass spectral study of 1-oxyl-4-cyano-2,2,6,6-tetramethylpiperidine (6) and 1-oxyl-4-carboxyl-2,2,6,6-tetramethylpiperidine (1).

Field ionization mass spectra confirmed the molecular ions to be *m/e* 181 for 6 and *m/e* 200 for 1.

Experimental Section

Melting points were obtained on a Thomas-Hoover melting point apparatus and are corrected. The microanalysis was performed by M-H-W Laboratories, Garden City, Mich. Infrared spectra were recorded on a Perkin-Elmer Model 727 spectrophotometer. High-resolution mass spectra were recorded on a Varian CH-5 mass spectrometer.

1-Oxyl-4-cyano-2,2,6,6-tetramethylpiperidine (6). To a stirred solution of 1-oxyl-2,2,6,6-tetramethyl-4-piperidone (2, 1.0 g, 5.9 mmol) and tosylmethyl isocyanide (1.17 g, 6.0 mmol, Aldrich Chemical Co.) in 40 ml of dimethoxyethane at 0°C was added 2 equiv (1.34 g) of potassium *tert*-butoxide dissolved in 20 ml of a 1:1 mixture of dimethoxyethane and *tert*-butyl alcohol. The mixture was stirred at 0°C for 45 min, the temperature was then raised to 20°C, and the mixture was stirred for an additional 1 hr. At that time, 100 ml of water was added and the mixture was extracted three times with 25-ml portions of ether. The ether extracts were combined and dried over anhydrous magnesium sulfate. The ether was then removed in vacuo to give approximately 0.9 g of a red powder. A portion of this product was twice recrystallized from ether, giving red needles: mp 146.5–147°C; ir 2250 cm^{-1} (–C≡N); *m/e* 181.1338.

Anal. Calcd for $C_{10}H_{17}N_2O$: C, 66.26; H, 9.45; N, 15.46. Found: C, 66.03; H, 9.63; N, 15.32.

1-Oxyl-4-carboxyl-2,2,6,6-tetramethylpiperidine (1). To a solution of 2 g of 1-oxyl-4-cyano-2,2,6,6-tetramethylpiperidine (6) in 25 ml of methanol was added a solution of 6 g of barium hydroxide and 0.5 g of sodium hydroxide in 100 ml of water. The mixture was refluxed for 24 hr, cooled, and extracted with chloroform. The remaining aqueous solution was cooled, acidified with 10% hydrochloric acid, and extracted exhaustively with chloroform. The chloroform solution was dried over anhydrous magnesium sulfate and evaporated in vacuo, giving 1.9 g of a red powder: mp 171–172°C from hexane–benzene (1:2); ir 1680 (–C=O), 3300–3100 cm^{-1} broad (–OH); *m/e* 200.1271.

Anal. Calcd for $C_{10}H_{18}NO_3$: C, 59.98; H, 9.06; N, 6.99. Found: C, 60.04; H, 9.19; N, 6.80.

Acknowledgment. This study was supported in part by a grant from NIH (NS 10823). We wish to thank Dr. Robert Harless for the mass spectral data.

Registry No.—1, 37149-18-1; 2, 2896-70-0; 6, 38078-71-6; tosylmethyl isocyanide, 36635-61-7.

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A Convenient Preparation of *S*-Adenosylhomocysteine and Related Compounds

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Since the discovery of *S*-adenosylmethionine,[2] a great variety of *S*-adenosylmethionine-dependent biological transmethylation reactions have been demonstrated.[3] A general feature of most *S*-adenosylmethionine-dependent methyltransferases is the inhibition produced by the demethylated product *S*-adenosyl-L-homocysteine. Because of its possible significance as a biological regulatory mechanism,[3b] this product inhibition has stimulated considerable research interest. Numerous compounds structurally related to *S*-adenosyl-L-homocysteine have been synthesized as potential inhibitors of this class of enzymes.[4]

Because of the high substrate specificity of the enzyme capable of synthesizing *S*-adenosylhomocysteine,[5] analogues of this compound have been prepared by chemical synthesis. The general route for the synthesis of *S*-adenosylhomocysteine analogues was modeled after the procedure first described by Baddiley and Jamieson.[6] This route involves (1) the synthesis of the parent nucleoside, if not commercially available; (2) protection of the 2′,3′-hydroxyl groups of the nucleoside using an isopropylidene protecting group; (3) activation of the nucleoside 5′ position by formation of the corresponding 5′-tosylate; (4) condensation of the intermediate 5′-tosylate with *S*-benzyl-L-homocysteine (or related compounds); and (5) removal of the isopropylidene protecting group using dilute acid. This standard procedure has proven quite successful for the synthesis of a broad spectrum of *S*-adenosylhomocysteine analogues.[4] A