Synthesis of Diamagnetic Structural Analogues of Representative Doxyl, Proxyl, Piperidine, and Pyrroline Nitroxide Spin Labels

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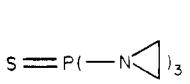
The objective of this work is the synthesis of diamagnetic close structural analogues of the more important doxyl **4**, proxyl **5**, piperidine **6**, and pyrrolidine **7** nitroxide spin labels which are extensively used in biophysical investigations. Our approach is based on the observation that a nitroxide group is similar both in size and polarity to a carbonyl group of a ketone. Diamagnetic doxyl nitroxide analogues **11**, **12**, and **13** were prepared by a series of reactions (**8** → **9** → **10** → analogue) which utilized the addition of 1-lithio-1-methoxyallene to the appropriate ketone as the key step. Diamagnetic proxyl nitroxide analogue **26** of 14-proxylstearic acid **27** was obtained from keto ester **17** via the reaction sequence outlined in Scheme II. The diamagnetic piperidine nitroxide analogues **29** and **33** were prepared from keto ketal **28**. Dibromination of **29** gave **35**, which served as the precursor for diamagnetic pyrroline nitroxide analogues **38**, **39**, and **40** through application of a Favorski ring contraction reaction sequence.

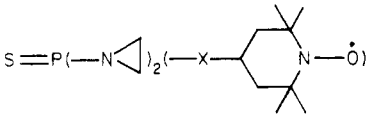
The study of biological systems by electron spin resonance (ESR) spectroscopy using stable nitroxide free radicals as spin labels continues to be a fruitful area of investigation.[1] The availability of *diamagnetic* close structural analogues of the nitroxides which are extensively used in these studies is important in several respects. For example, the question of the extent to which the presence of the bulky nitroxide sterically perturbs[2] the system under study can be approached by a study of the effect of increasing amounts of diamagnetic analogue on the ESR spectrum of the corresponding paramagnetic probe present at constant, low concentration. Use of the nitroxide itself is complicated by attendant ESR line broadening due to spin–spin interactions as the nitroxide concentration is increased above an upper limit ($\sim 10^{-3}$ M).

Spin-labeled lipids associated with membrane proteins show ESR spectra distinguishable from those due to the spin labels in a bilayer environment. It is thus possible to use ESR spin-labeling methods to approach the question of relative binding constants in the interaction of various lipids with membrane proteins.[3] The experimental design requires that the lipid binding sites on the membrane protein become saturated with the lipid in question, an approach which would be facilitated through use of diamagnetic analogues if line broadening problems ensue.

A third potential application of an appropriate diamagnetic analogue is to ascertain whether or not it is the nitroxide free radical moiety, per se, that is responsible for the enhanced antitumor activity of certain nitroxide spin labeled thiotriethylenephosphoramide (thio-TEPA) analogues as reported by Emanuel, Sosnovsky, and others.[4]

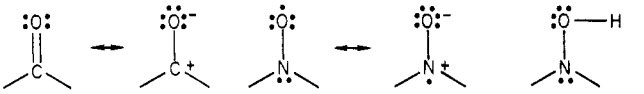


thio-TEPA



spin-labeled thio-TEPA (X = NH, O)

Our approach to the design and synthesis of the diamagnetic analogues is based on the observation that a nitroxide group is similar both in size and in polarity (but not chemical reactivity, of course) to that of a carbonyl group of a ketone. The oxygen atom in both groups is bonded to the adjacent atom (N or C) through a σ bond and a π bond. Among ketones, the average C=O bond length is about 1.23 Å, while the average C—CO bond length is about 1.52 Å.[5] By way of comparison, the N—O· bond length in doxylcyclohexane (**1**) is 1.26 Å, while the C—NO· value is 1.48 Å.[6] The atoms attached to the nitrogen atom of a nitroxide group tend to lie in a plane, or nearly so,[6] similar to the arrangement in a carbonyl group. The N—O· group, like a C=O group, is a H—bond acceptor but not a donor.



carbonyl nitroxide *N*-hydroxy derivative

The diamagnetic *N*-hydroxy derivative, obtained by a facile one-electron reduction of a nitroxide group (**1** → **2**) is not a satisfactory "diamagnetic analogue" of a nitroxide spin label for several reasons. *N*-Hydroxy compounds are highly susceptible to oxidation by air or other mild reagents back to the nitroxide.[7] The NOH group is both a H-bond acceptor and a H-bond donor; thus it is inherently of much greater polarity than that of a carbonyl group. The nitrogen atom of the *N*-hydroxy group is approximately sp^3 hybridized and therefore the atoms bonded to the nitrogen atom do not tend to lie in one plane, although nitrogen inversion can and does take place. Lastly, the *N*-hydroxy derivatives of doxyl nitroxides tend to undergo facile, irreversible hydrolysis to the corresponding ketone (e.g., **2** → **3**).[7]

Herein, we describe the synthesis of representative diamagnetic structural analogues of the four most important classes of nitroxide spin labels, namely, doxyl (**4**), proxyl (**5**), 2,2,6,6-tetramethylpiperidinyl-*N*-oxy (**6**), and 2,2,5,5-tetramethylpyrrolinyl-*N*-oxy (**7**) nitroxides.

(1) For a review, see "Spin Labeling: Theory and Applications", L. J. Berliner, Ed., Academic Press, New York, Vol. 1, 1976, and Vol. 2, 1979.

(2) For a different approach to the question of steric perturbation by a nitroxide spin label, see M. G. Taylor and I. C. P. Smith, *Biochim. Biophys. Acta*, **599**, 140–149 (1980). For a series of lipid nitroxide spin labels designed to minimize steric perturbation of the system, see T. D. Lee and J. F. W. Keana, *J. Org. Chem.*, **43**, 4226–4231 (1978).

(3) O. H. Griffith and P. C. Jost in "Cytochrome Oxidase", T. E. King, Ed., Elsevier/North-Holland Biomedical Press, Amsterdam, 1979, pp 207–218.

(4) N. Emanuel, N. Konovalova, and R. Djachkovskaya, *Can. Treat. Rep.*, **66**, 1605–1609 (1976); G. Sosnovsky and M. Konieczny, *Z. Naturforsch., Teil B*, **32**, 87–93 (1977).

(5) R. C. Weast, Ed., "Handbook of Chemistry and Physics", The Chemical Rubber Co., Cleveland, OH, 1967, pp F-145 and 146.

(6) For leading references, see J. F. W. Keana, R. S. Norton, M. Morello, D. van Engen, and J. Clardy, *J. Am. Chem. Soc.*, **100**, 934–937 (1978).

(7) J. F. W. Keana, *Chem. Rev.*, **78**, 37–64 (1978); J. F. W. Keana in "Spin Labeling: Theory and Applications", L. J. Berliner, Ed., Academic Press, New York, 1979.

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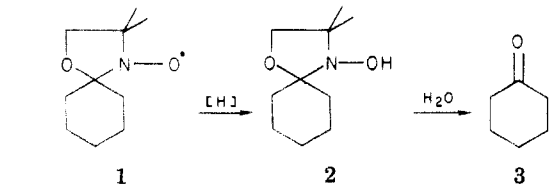
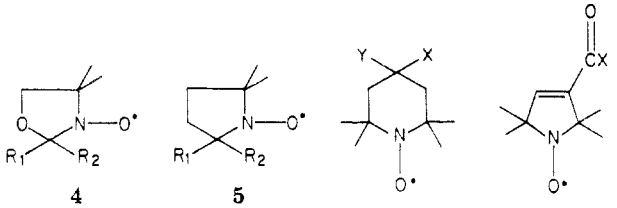


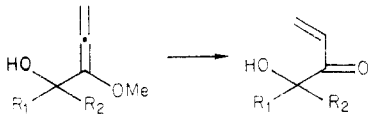
1 2 3



4 5 6 7

Results and Discussion

Diamagnetic Doxyl Nitroxide Analogues. Our synthetic approach (Scheme I) to these substances involves a modification and an extension of the general synthesis of dihydrofuran-3(2*H*)-ones introduced by Hoff et al.[8] and later exploited by Gange and Magnus.[9] Preliminary experiments revealed that 12-oxostearic acid (**8B**) underwent selective addition at the ketone group when treated with excess 1-lithio-1-methoxyallene in tetrahydrofuran (THF) at −78 °C. However, despite care during the workup we were unable to prevent hydrolysis of the desired adduct **15** to the corresponding vinyl ketone **16**,[10] undoubtedly owing to the presence of the carboxyl group in **8B**.

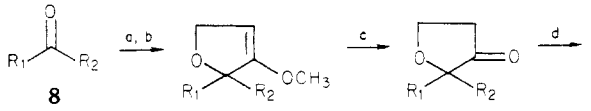


15 16

Conditions were therefore developed which allowed synthesis of dihydrofuran **9** from **8** without isolation of the intermediate **15**. This was accomplished by a quench of the initial organolithium adduct with 1 equiv of *tert*-butyl alcohol, replacement of most of the solvent by dimethyl sulfoxide (Me_2SO),[11] and subsequent addition of potassium *tert*-butoxide followed by a heating period of 3 h at 60 °C. In this way 3-heptanone (**8A**) and acid **8B** directly afforded the corresponding cyclic enol ethers **9A** and **9B**, which were immediately hydrolyzed to the ketones **10A** (65%) and **10B** (62%), respectively.

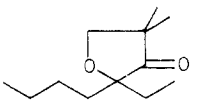
Ketone **10A** was next converted to the dimethyl derivative **11** (76%) by treatment with excess sodium hydride and methyl iodide in dimethoxyethane (DME) at 50 °C. At reflux temperature reduction of the carbonyl group by the sodium hydride[12] became a significant side reaction, leading to the corresponding α,α,O-trimethyl derivative after methylation. Under similar conditions, acid **10B** afforded 12-doxylstearic acid analogue **12** (66%) together with some of the corresponding methyl ester **12a** (10%). Alkaline hydrolysis of **12a** smoothly afforded acid **12** (70%).

Scheme I[a]



8 9 10

[a] A, $R_1 = n\text{-}C_4H_9$, $R_2 = C_2H_5$; B, $R_1 = n\text{-}C_6H_{13}$, $R_2 = (CH_2)_{10}CO_2H$; C, R_1 and R_2 = C_2 and C_4 of cholestan-3-one



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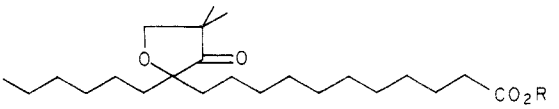
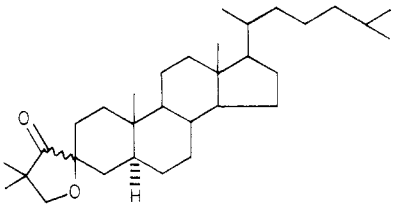
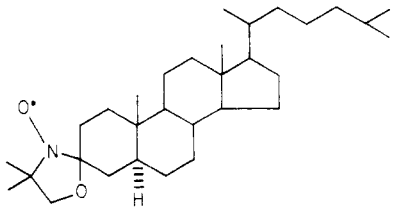


12, R = H
12a, R = CH_3



13



14

[a] a, $CH_3OCH{=}C{=}CH_2$, BuLi, THF, −78 °C; b *t*-BuOK, Me_2SO-THF; c, *t*-BuOH, 6 N H_2SO_4; d, NaH, MeI, DME.

Spin-labeling studies with 3-doxylcholestane (**14**),[13] the first of the nitroxide spin labeled cholesterol analogues,[14] have provided valuable information about the role of steroids in certain biological systems.[15] We have applied the above sequence of reactions to cholestan-3-one (**8C**), affording diamagnetic doxyl analogue **13** in good yield via intermediates **9C** and **10C**. Analogue **13** was obtained as an epimeric mixture of isomers (1:2), which were not separated in this present study.

Diamagnetic Proxyl Nitroxide Analogues. Scheme II summarizes our synthetic route to the diamagnetic analogue **26** of the representative proxyl nitroxide, 14-proxylstearic acid **27**.[16] Methyl 2-oxocyclopentanecarboxylate (**17**) was alkylated with bromobutane in the

(8) S. Hoff, L. Brandsma, and J. F. Arens, *Recl. Trav. Chim. Pays-Bas*, 87, 1179–1184 (1968); 88, 609–619 (1969).

(9) D. Gange and P. Magnus, *J. Am. Chem. Soc.*, **100**, 7746–7747 (1978); D. Gange, P. Magnus, L. Bass, E. V. Arnold, and J. Clardy, *ibid.*, **102**, 2134–2135 (1980).

(10) Methoxyallene derivatives are known to undergo facile hydrolysis to α,β-unsaturated ketones in acidic media. See J. C. Clinet and G. Linstrumelle, *Tetrahedron Lett.*, 1137–1140 (1978); Y. Leroux and C. Roman, *ibid.*, 2585–2586 (1973). Diagnostic spectral properties for **16** ($R_1 = n\text{-}C_6H_{13}$; $R_2 = (CH_2)_{10}CO_2H$): NMR δ 6.75–6.55 (m, 2), 5.89–5.74 (m, 1); IR (CCl_4) 1690, 1610 cm^{-1}. Because of the convenience of the sequence **8** → **9** → **10**, cyclization of intermediates **16** was not investigated.

(11) Yields were much lower when *tert*-butyl alcohol[9] was used as the solvent.

(12) For other examples, see J. S. McConaghy, Jr., and J. J. Bloomfield, *J. Org. Chem.*, **33**, 3425–3428 (1968).

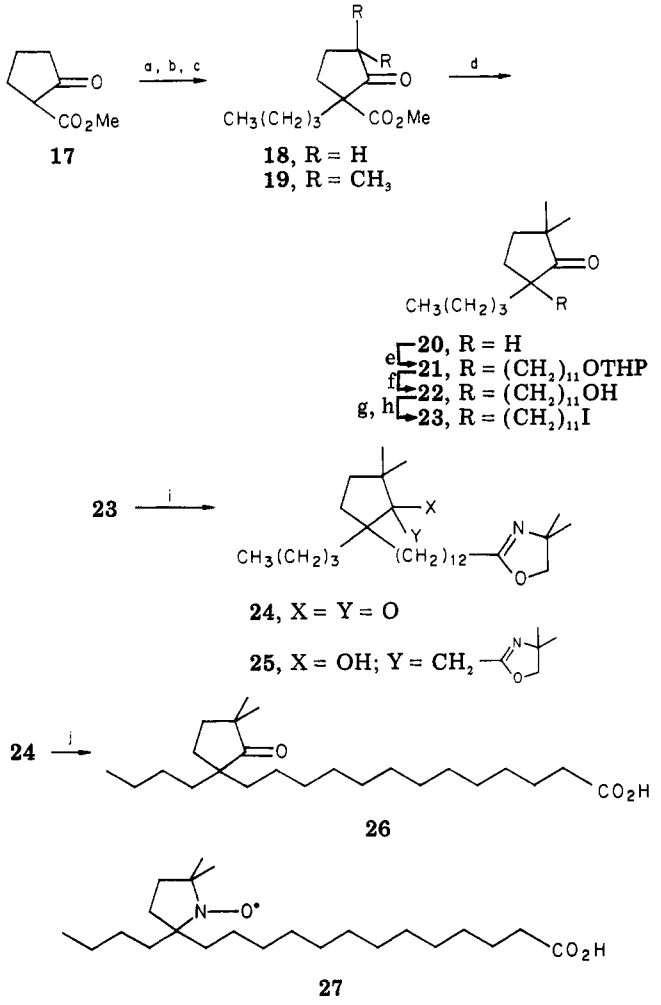
(13) J. F. W. Keana, S. B. Keana, and D. Beetham, *J. Am. Chem. Soc.*, 89, 3055–3056 (1967).

(14) A nitroxide spin labeled steroid which mimics cholesterol much better than **14** does has been recently described. See J. F. W. Keana, T. Tamura, D. A. McMillen, and P. C. Jost, *J. Am. Chem. Soc.*, **103**, 4904–4912 (1981).

(15) For leading references, see T. B. Marriott, G. B. Birrell, and O. H. Griffith, *J. Am. Chem. Soc.*, **97**, 627–630 (1975).

(16) J. F. W. Keana and S. A. Boyd, *J. Labelled Compd. Radiopharm.*, 18, 403–406 (1981); J. F. W. Keana, S. A. Boyd, D. A. McMillen, and P. C. Jost, *Chem. Phys. Lipids*, in press.

Scheme II[a]



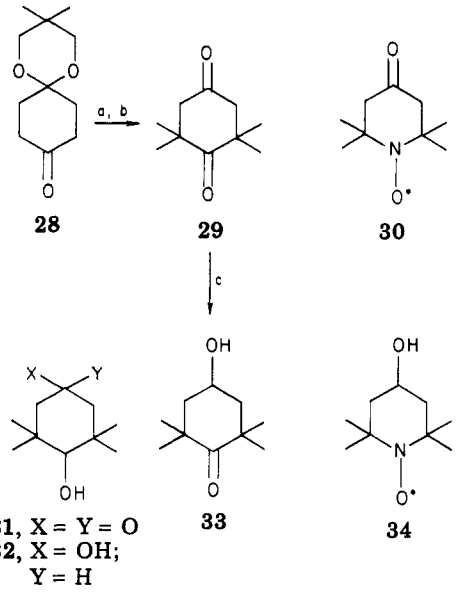
[a] a, Na, toluene; b, *n*-BuBr, reflux; c, NaH, MeI, DME, 55 °C; d, LiI, collidine, reflux; e, $Br(CH_2)_{11}OTHP$, NaH, DME, 50 °C; f, 3 N HCl, MeOH; g, $MeSO_2Cl$, Et_3N, CH_2Cl_2, 25 °C; h, NaI, MEK, reflux; i, 2,4,4-trimethyloxazoline, BuLi, THF, -78 °C; j, 3 N HCl, reflux.

usual fashion to give ester **18**[17] (68%), which was then methylated with sodium hydride and methyl iodide to give ester **19** (90%). Decarboxylation of **19** with lithium iodide[18] gave ketone **20** (89%), which was alkylated with the tetrahydropyranyl ether of 11-bromoundecanol to give ketone **21** (74%). Acid-catalyzed hydrolysis of **21** gave alcohol **22** (93%), which was then converted into iodide **23** (96%) via the corresponding methanesulfonate.

Reaction of **23** at 25 °C with an excess of the anion of 2,4,4-trimethyloxazoline[19] produced mainly alcohol **25** (86%), the product of diaddition. With 1 equiv of the anion at -78 °C, **23** was converted into the ketone **24** (84%). Hydrolysis of **24** with hot hydrochloric acid gave the desired acid **26** (83%).

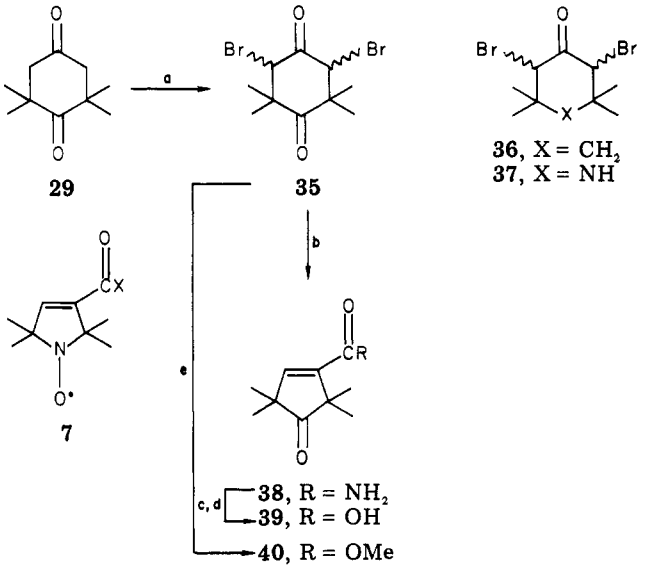
Diamagnetic 2,2,6,6-Tetramethylpiperidinyl-*N*-oxy Nitroxide Analogues. The mono-protected cyclohexanedione (**28**, Scheme III) was methylated and then hydrolyzed to 2,2,6,6-tetramethylcyclohexane-1,4-dione (**29**, 80%), a diamagnetic analogue of the versatile nitroxide

Scheme III[a]



[a] a, NaH, MeI, DME, 25 °C; b, H_3O^+-SiO_2, CH_2Cl_2; c, Raney Ni, H_2, MeOH.

Scheme IV[a]



[a] a, Br_2, HOAc; b, NH_3, CH_2Cl_2; c, NaOH, H_2O, MeOH; d, H_3O^+; e, NaOMe, MeOH, CH_2Cl_2.

4-oxo-2,2,6,6-tetramethylpiperidinyl-*N*-oxy (Tempone, **30**).

Diketone **29** may serve as the key intermediate for the synthesis of a variety of diamagnetic analogues of nitroxide spin labels[1,7,20] obtainable from nitroxide **30**. For example, selective catalytic hydrogenation of **29** over alkaline Raney nickel[21] afforded alcohol **33** (100%), a diamagnetic analogue of the nitroxide 4-hydroxy-2,2,6,6-tetramethylpiperidinyl-*N*-oxy (Tempol, **34**), itself an important precursor for numerous nitroxide spin labels.[1,7,20]

While reduction of **29** with 1 equiv of L-Selectride also afforded **33** (68%) as the sole product, 1 equiv of sodium borohydride in MeOH or isopropyl alcohol at 0 °C gave the isomer **31** as the major product,[22] together with lesser

(17) A. E. Greene, A. Cruz, and P. Crabbé, *Tetrahedron Lett.*, 2707–2708 (1976).
(18) F. Elsinger, J. Schreiber, and A. Eschenmoser, *Helv. Chim. Acta*, **43**, 113–118 (1960); F. Elsinger, "Organic Syntheses", Collect. Vol. 5, Wiley, New York, 1973, pp 76–80.
(19) A. I. Meyers, D. L. Temple, R. L. Nolen, and E. D. Mihelich, *J. Org. Chem.*, **39**, 2778–2783 (1974).
(20) E. G. Rozantsev, "Free Nitroxyl Radicals", Plenum Press, New York, 1970.
(21) Raney Ni also exhibited selectivity in the catalytic hydrogenation of 2,2,6-trimethyl-1,4-cyclohexanedione; H. G. W. Leunberger, W. Boguth, E. Widmer, and R. Zell, *Helv. Chim. Acta*, **59**, 1832–1849 (1976).

amounts of **33** and a mixture of cis,trans diol isomers (**32**) (isomer a, mp 145–145.3 °C; isomer b, mp 145.8–146.1 °C). Reduction of **29** with diisobutylaluminum hydride gave **33** (47–57%) as the major product, accompanied by some diol **32** (40–30%).

Diamagnetic 2,2,5,5-Tetramethylpyrrolinyl-*N*-oxy Nitroxide Analogues. Through a series of reactions paralleling the conversion of nitroxide ketone **30** into a family of pyrroline nitroxides **7**,[1,7,20,23] dione **29** was converted into the diamagnetic analogues **38**, **39**, and **40** (Scheme IV). Thus, treatment of **25** with bromine in acetic acid at 25 °C gave dibromide **35** as a mixture of cis,trans isomers (45:55). The minor isomer could be obtained in pure form as white needles, mp 182–186 °C, by crystallization from dichloromethane.

The CHBr protons of this crystalline isomer appeared at δ 4.89, while those protons in the other isomer appeared at δ 5.13 in the NMR spectrum. A consideration of the chemical shifts of the bromomethine protons for several conformationally constrained α,α'-dibromocyclohexanones led Baretta et al.[24] to the observation that among cis,trans isomers, the axial protons always appear downfield with respect to the equatorial protons. For example, the cis isomer of cyclohexanone **36** shows the CHBr protons at δ 4.56 and the trans isomer at δ 4.78. The X-ray crystallographically confirmed cis isomer of amine dibromide **37** shows these protons at δ 4.54.[25] Based on the above considerations, we assign the cis configuration to our crystalline dibromide **35**.

The crude mixture of dibromide isomers **35** was used for subsequent steps. A Favorski ring contraction reaction initiated by ammonia in dichloromethane readily led to amide **38** (82%, based on ketone **29**). Basic hydrolysis of **38** readily afforded acid **39**, the diamagnetic analogue of the versatile nitroxide acid spin label **7** (X = OH). Alternatively, treatment of dibromide **35** with sodium methoxide in MeOH gave the ring contracted ester **40** (87%, based on **29**).

In summary, flexible synthetic routes have been developed to diamagnetic analogues of representatives of all the major nitroxide spin labels currently in usage. Furthermore the synthesis of deuterated analogues for use in deuterium NMR studies can be readily envisaged through use of CD_3I. Several of the potential applications cited in the Introduction are now being pursued.

Experimental Section

Melting points were obtained in a Thomas-Hoover apparatus and are uncorrected. NMR spectra were recorded in $CDCl_3$ on a Varian XL-100 spectrometer. Chemical shifts are expressed in δ units with Me_4Si as an internal standard. Infrared spectra (IR) were obtained on a Beckman IR10 infrared spectrophotometer. Only diagnostic NMR and IR absorptions are reported. Analytical TLC utilized Merck 60 F-254 precoated silica gel plates. Spots were visualized under ultraviolet light or by H_2SO_4 spray followed with charring. Elemental analyses and mass spectra were determined at the University of Oregon by Dr. R. Wielesek.

2-Butyl-2-ethyldihydrofuran-3(2*H*)-one (10A). A modification of the method of Brandsma et al.[8] and Magnus et al.[9] was employed. To a stirred solution of 18.8 mL (30 mmol) of *n*-butyllithium (1.6 M solution in hexane) and 15 mL of dry THF at −78 °C was added 2.1 g (30 mmol) of methoxyallene[26] dropwise (10 min). The resulting solution was stirred at −78 °C for 40 min and treated with 1.14 g (10 mmol) of 3-heptanone in 10 mL of THF dropwise (12 min). This was then stirred at −78 °C for 3 h,[27] allowed to warm up to 25 °C, and concentrated to about 30 mL. To this were added 35 mL of dry Me_2SO, 0.94 mL (30 mmol) of *tert*-butyl alcohol, and 2.8 g (25 mmol) of potassium *tert*-butoxide. The reaction mixture was then heated at 60 °C for 3 h, cooled to 25 °C, neutralized, and extracted with ether/pentane (1/2). The organic layers were combined, washed with brine, dried ($MgSO_4$), and concentrated to afford 1.93 g of crude oily 2-butyl-2-ethyl-3-methoxy-2,5-dihydrofuran (**9A**): NMR δ 3.69 (s, 3), 4.62 (d, 2, OCH_2CH=), 4.68 (t, 1, OCH_2CH=); IR (film) 1653 cm^{-1} (C=C). The crude oil was dissolved in 15 mL of *tert*-butyl alcohol, shaked with 6 N H_2SO_4 (10 mL) for 10 min, and extracted with ether. The extract was washed with water and brine, dried ($MgSO_4$), and concentrated. The residue was distilled, affording 1.109 g (65%) of **10A**: bp 46 °C (0.5 mm); NMR δ 0.86 (t, 3), 0.87 (t, 3), 1.20–1.75 (m, 8 H), 2.51 (t, 2, OCH_2CH_2C=O), 4.20 (t, 2, OCH_2CH_2C=O); IR (film) 1751 cm^{-1} (C=O). Anal. Calcd for $C_{10}H_{18}O_2$: C, 70.55; H, 10.65. Found: C, 70.33; H, 10.65.

2-Butyl-2-ethyl-4,4-dimethyldihydrofuran-3(2*H*)-one (11). To a stirred suspension of 336 mg (7.0 mmol) of sodium hydride[28] in 20 mL of dry DME (N_2) was added 1 mL of dry methyl iodide. The mixture was then treated with 510 mg (3.0 mmol) of **10A** in 5 mL of dry DME dropwise (20 min). The resulting solution was heated at 50 °C[29] for 5 h, cooled, and poured into cooled water (35 mL). The usual workup with ether afforded a residue which was distilled to give 452 mg (76%) of **11**: bp 48 °C (2 mm); NMR δ 0.86 (t, 6), 1.12 (s, 6), 1.20–1.75 (m, 8 H), 3.89 (s, 2); IR (film) 1753 cm^{-1} (C=O). Anal. Calcd for $C_{12}H_{22}O_2$: C, 72.68; H, 11.18. Found: C, 72.43; H, 11.25.

2-Hexyl-2-(10-carboxydecyl)dihydrofuran-3(2*H*)-one (10B). The title compound was prepared from 1.00 g (3.35 mmol) of 12-oxostearic acid[30] by the same procedure as described for the preparation of compound **10A** except for use of excess *n*-BuLi (20 mmol), methoxyallene (20 mmol), *tert*-butyl alcohol (20 mmol), and potassium *tert*-butoxide (10 mmol). The crude product was chromatographed on silica gel. Elution with ether/pentane/1% acetic acid gave 736.3 g (62%) of **10B** as a waxy oil: NMR δ 0.87 (t, 3), 1.16–1.75 (m, 28 H), 2.38 (t, 2), 2.52 (t, 2, OCH_2CH_2C=O), 4.20 (t, 2, OCH_2CH_2C=O), 8.30 (br s, 1); IR ($CHCl_3$) 1752, 1717 cm^{-1}. Anal. Calcd for $C_{21}H_{38}O_4$: C, 71.15; H, 10.80. Found: C, 71.32; H, 11.14.

4,4-Dimethyl-2-hexyl-2-(10-carboxydecyl)dihydrofuran-3(2*H*)-one (12) and 4,4-Dimethyl-2-hexyl-2-[10-(carbomethoxy)decyl]dihydrofuran-3(2*H*)-one (12a). By the same procedure as described for the preparation of **11**, 500 mg of keto acid **10B** was methylated. After workup, the residue was chromatographed on silica gel. Elution with ethyl acetate/hexane/1% acetic acid gave 356 mg (66%) of **12** as a waxy oil: NMR δ 0.87 (t, 3), 1.13 (s, 6), 1.16–1.74 (m, 28 H), 2.35 (t, 2, CH_2CO_2H), 3.87 (s, 2), 10.95 (br s, 1); IR (film) 1753, 1715 cm^{-1}; silica gel TLC (1% acetic acid/30% ethyl acetate/69% hexane) R_f 0.44. Anal. Calcd for $C_{23}H_{42}O_4$: C, 72.21; H, 11.07. Found: C, 71.94; H, 11.08. Another fraction of the chromatography provided 55 mg (10%) of ester **12a** as a clear oil: NMR δ 0.87 (t, 3), 1.13 (s, 6), 1.16–1.75 (m, 28 H), 2.32 (t, 2, $CH_2CO_2CH_3$), 3.66 (s, 3), 3.88 (s, 2); IR (film) 1753, 1744 cm^{-1}; silica gel TLC (1% acetic acid/30% ethyl acetate/69% hexane) R_f 0.89. Anal. Calcd for $C_{24}H_{44}O_4$: C, 72.68;

(22) (a) For a review on the stereochemistry and mechanism of cyclohexanone reductions by hydride reagents, see D. C. Wigfield, *Tetrahedron*, **35**, 449–462 (1979). (b) For a study on kinetics, stereochemistry, and mechanism of the sodium borohydride reduction of alkyl-substituted cyclohexanones, see B. Rickborn and M. T. Wuesthoff, *J. Am. Chem. Soc.*, **92**, 6894–6904 (1970).

(23) See H. O. Hankovszky, K. Hideg, and L. Lex, *Synthesis*, 147–149 (1981), and references cited therein.

(24) A. Baretta, J. P. Zahra, B. Waegell, and C. W. Jefford, *Tetrahedron*, **26**, 15–26 (1970).

(25) N. W. Alcock, B. T. Golding, P. V. Ioannou, and J. F. Sawyer, *Tetrahedron*, **33**, 2969–2980 (1977).

(26) S. Hoff, L. Brandsma, and J. F. Arens, *Recl. Trav. Chim. Pays-Bas.*, **87**, 916–924 (1968).

(27) At this point the adduct, 4-ethyl-3-methoxy-1,2-octadien-4-ol, can be isolated by employing the usual workup:[8,9] NMR δ 2.01 (s, 1, OH, exchanged with D_2O), 3.42 (s, 3), 5.58 (s, 2); IR (film) 1948 cm^{-1}.

(28) Oil was removed from the 50% sodium hydride oil dispersion by washing (3×) with dry hexane and then dry DME.

(29) When the reaction was carried out at reflux temperature, some of the reduction product, 2-butyl-4,4-dimethyl-2-ethyl-3-methoxytetrahydrofuran (26%), was obtained: NMR δ 0.78–1.74 (m, 14 H), 1.04 (s, 3), 1.14 (s, 3), 3.14 (s, 1), 3.43 (s, 5); IR ($CHCl_3$), no C=O; mass spectrum, *m/e* 214.194 (calcd for $C_{13}H_{26}O_2$, 214.193).

(30) A. S. Waggoner, T. J. Kingzett, S. Rottschaefer, O. H. Griffith, and A. D. Keith, *Chem. Phys. Lipids*, **3**, 245–253 (1969).

H, 11.08. Found: C, 72.39; H, 10.92. Ester **12a** was hydrolyzed to acid **12** (70%) by treatment with 0.1 N solution of sodium hydroxide in MeOH at 50 °C for 2 days followed by the usual workup.

Spiro[5α-cholestane-3,2′-dihydrofuran]-3′(2′*H*)-one (10C). By modification of the method of Gange and Magnus,[9] 774 mg of 5α-cholestan-3-one was converted into 883 mg of crude carbonyl adduct: NMR δ 3.44 (s, 3), 5.56 (s, 2, C=C=CH_2); IR (film) 1954 cm^{-1}. The adduct was then dissolved in a solution of 400 mg (3.6 mmol) of potassium *tert*-butoxide in 20 mL of dry Me_2SO and 20 mL of dry THF. The resulting solution was heated at 90 °C for 4 h, cooled, neutralized, and extracted (4×) with ether/pentane (1/2). The extract was washed with brine, dried ($MgSO_4$), and concentrated to give 869 mg of crude **9C**: NMR δ 3.63 (s, 3), 4.48–4.58 (m, 3); IR (film) 1654 cm^{-1}. The crude **9C** was dissolved in 10 mL of ether and vigorously shaked with 10 mL of 6 N HCl for 5 min. The usual workup with ether gave a residue which was chromatographed on silica gel. Elution with ether/pentane afforded 603 mg (68%) of **10C** as a waxy oil (1/2 mixture of epimers): NMR δ 2.47 (t, 2) (isomer a), 2.55 (t, 2) (isomer b), 4.13 (t, 2) (isomers a and b); IR ($CHCl_3$) 1750 cm^{-1}. Anal. Calcd for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.49; H, 11.23.

4′,4′-Dimethylspiro[5α-cholestane-3,2′-dihydrofuran]-3′-(2′*H*)-one (13). By the same procedure as described for the preparation of **11**, 177 mg of **10C** was methylated. The usual workup gave an oil which was chromatographed on silica gel. Elution with ether/hexane afforded 151 mg (80%) of **13** as a waxy mixture of two isomers (1:2): NMR (major isomer) δ 1.15 (s, 6), 3.91 (s, 2), (minor isomer) 1.14 (s, 6), 3.88 (s, 2); silica gel TLC (1/1 ether/hexane) R_f 0.60 for major isomer and R_f 0.64 for minor isomer. Anal. Calcd for $C_{32}H_{54}O_2$ (isomer mixture): C, 81.64; H, 11.56. Found: C, 81.59; H, 11.96.

Methyl 1-Butyl-3,3-dimethyl-2-oxocyclopentanecarboxylate (19). To a stirred suspension of 3.6 g (75 mmol) of sodium hydride[28] in 40 mL of dry DME (N_2) was added 11.4 g (80 mmol) of dry methyl iodide. The mixture was then treated with a solution of 5.0 g (25 mmol) of keto ester 18[17,18,31] in 20 mL of dry DME dropwise over a period of 30 min. The reaction mixture was heated at 55 °C for 6 h, cooled, and poured into cooled water. The organic layer was separated and the aqueous layer extracted with ether. The combined organic layers were washed with 0.1 N sodium thiosulfite and dried ($MgSO_4$). Removal of the solvent and distillation of the residue under reduced pressure gave 5.10 g (90%) of ester **19** as a colorless liquid: bp 65 °C (0.2 mm); NMR δ 0.89 (t, 3), 1.06 (s, 3), 1.10 (s, 3), 1.15–2.64 (m, 10), 3.76 (s, 3); IR (film) 1743, 1724 cm^{-1}. Anal. Calcd for $C_{13}H_{22}O_3$: C, 68.99; H, 9.80. Found: C, 69.17; H, 9.55.

5-Butyl-2,2-dimethylcyclopentanone (20). The procedure of Elsinger et al.[18] were used to decarboxylate 3.60 g of β-keto ester **19**. After workup, the residue was distilled under reduced pressure to afford 2.361 g (89%) of **20** as a colorless liquid: bp 41 °C (0.5 mm); NMR δ 0.90 (t, 3), 0.98 (s, 3), 1.08 (s, 3), 1.16–1.98 (complex m, 10), 2.15 (m, 1, CH_2CHC=O); IR (film) 1734 cm^{-1}. Anal. Calcd for $C_{11}H_{20}O$: C, 78.51; H, 11.98. Found: C, 78.21; H, 12.15.

2-Butyl-5,5-dimethyl-2-[11-[(tetrahydropyranyl)oxy]undecyl]cyclopentanone (21). To a stirred suspension of 571 mg (11.9 mmol) of sodium hydride[28] in 50 mL of dry DME (N_2) was added 2.395 g (7.14 mmol) of the tetrahydropyranyl ether of 11-bromoundecanol in 10 mL of DME. This was then heated at 50 °C and treated with a solution of 1.00 g (5.95 mmol) of ketone **20** and 20 mL of DME dropwise over a period of 2 h. The reaction mixture was heated at 50 °C for 20 h, cooled, and poured into ice water. The usual workup with ether gave an oil which was chromatographed on silica gel. Elution with ethyl acetate/hexane afforded 1.87 g (74%) of ketone **21** as a clear oil: NMR δ 0.88 (t, 3), 3.29–4.08 (m, 4), 4.59 (t, 1); IR (film) 1734 cm^{-1}. Anal. Calcd for $C_{27}H_{50}O_3$: C, 76.72; H, 11.92. Found: C, 76.84; H, 12.26.

2-Butyl-5,5-dimethyl-2-(11-hydroxyundecyl)cyclopentanone (22). To a stirred solution of 1.35 g (3.2 mmol) of ketone **21** in 50 mL of MeOH was added 20 mL of 3 N hydrochloric acid. The solution was stirred at 25 °C for 4.5 h. The usual workup with ether gave an oil which was chromatographed on silica gel. Elution with ethyl acetate/hexane gave 1.006 g (93%) of alcohol **22** as a clear, viscous oil: NMR δ 0.88 (t, 3), 1.03 (s, 6), 1.76 (s, 4), 3.64 (t, 2); IR (film) 3400, 1730 cm^{-1}. Anal. Calcd for $C_{22}H_{42}O_2$: C, 78.05; H, 12.50. Found: C, 77.81; H, 12.31.

2-Butyl-5,5-dimethyl-2-(11-iodoundecyl)cyclopentanone (23). A stirred solution of 872 mg (2.58 mmol) of alcohol **22** in 40 mL of dry CH_2Cl_2 (N_2) was cooled to −25 °C and treated first with 392 mg (3.87 mmol) of triethylamine and then with 325 mg (2.84 mmol) of methanesulfonyl chloride. The solution was allowed to warm to 25 °C and stirring was continued for 1 h. The solution was washed with water, dried ($MgSO_4$), and concentrated to give 1.037 g of oil: NMR δ 0.88 (t, 3), 1.02 (s, 6), 1.75 (s, 4), 2.98 (s, 3), 4.22 (t, 2). The oil was added to a stirred solution of 464 mg (3.10 mmol) of anhydrous sodium iodide in 60 mL of dry methyl ethyl ketone and heated at reflux for 20 min (N_2). This was then cooled, diluted with hexane, washed with water, dried ($MgSO_4$), and concentrated. The viscous residue was chromatographed on silica gel. Elution with 5% ethyl acetate in hexane gave 1.106 (96%) of **23** as a clear oil: NMR δ 0.88 (t, 3), 1.03 (s, 6), 1.75 (s, 4), 3.17 (t, 2); IR (film) 1732 cm^{-1}. Anal. Calcd for $C_{22}H_{41}OI$: C, 58.92; H, 9.21. Found: C, 59.06; H, 9.10.

2-Butyl-5,5-dimethyl-2-[12-(4,5-dihydro-4,4-dimethyl-2-oxazolyl)dodecyl]cyclopentanone (24) and 2-Butyl-5,5-dimethyl-1-[(4,5-dihydro-4,4-dimethyl-2-oxazolyl)methyl]-2-[12-(4,5-dihydro-4,4-dimethyl-2-oxazolyl)dodecyl]cyclohexanol (25). A modification of the method of Meyers et al.[19] was used. To a stirred solution of 78 mg (0.69 mmol) of 2,4,4-trimethyl-2-oxazoline in 8 mL of dry THF at −78 °C was added 0.46 mL (0.69 mmol) of 1.5 M solution of *n*-butyllithium in hexane (N_2). The solution was then stirred at −78 °C for 5 min and treated with 310 mg (0.69 mmol) of iodide **23** in 4 mL of dry THF. After 3 h at −78 °C, the solution was poured into water. The usual workup with ether gave a residue which was chromatographed on silica gel. Elution with 20% ethyl acetate in hexane gave 250 mg (84%) of oxazoline **24** as a colorless oil: NMR δ 0.88 (t, 3), 1.02 (s, 6), 1.25 (s, 6), 1.75 (s, 4), 2.24 (t, 2), 3.90 (s, 2); IR (film) 1732, 1669 cm^{-1}. When the above procedure was modified by use of 1/3 molar ratio of **23** to 2,4,4-trimethyl-2-oxazoline and stirring the reaction mixture at 25 °C for 1 h before quenching, alcohol **25** (86%) was obtained: NMR δ 0.89 (t, 3), 1.03 (s, 6), 1.26 (s, 12), 2.24 (t, 2), 2.46 (s, 2), 3.88 (s, 4), 4.82 (br s, 1, OH, exchanged with D_2O); IR (film) 3400, 1668 cm^{-1}. Anal. Calcd for $C_{34}H_{62}N_2O_3$: C, 74.67; H, 11.43; N, 5.12. Found: C, 74.31; H, 11.26; N, 5.01.

2-Butyl-5,5-dimethyl-2-(12-carboxydodecyl)cyclopentanone (26). A stirred mixture of 237 mg (0.55 mmol) of **24** and 10 mL of 3 N HCl was refluxed for 30 min. The usual workup with CH_2Cl_2 gave an oil which was chromatographed on silica gel. Elution with ethyl acetate/acetic acid/hexane (25/1/74) gave 174 mg (83%) of acid **26** as an oil: NMR δ 0.88 (t, 3), 1.04 (s, 6), 1.76 (s, 4), 2.36 (t, 2), 8.15 (br s, 1); IR (film) 1732, 1716 cm^{-1}. Anal. Calcd for $C_{24}H_{44}O_3$: C, 75.74; H, 11.65. Found: C, 75.97; H, 11.99.

2,2,6,6-Tetramethyl-1,4-cyclohexanedione (29). To a suspension of 5.76 g (0.12 mol) of sodium hydride[28] in 50 mL of dry DME (N_2) was added 7.5 g (0.12 mol) of dry methyl iodide. The mixture was cooled to 0 °C and then treated with a solution of 4 g (0.02 mol) of 3,3-dimethyl-1,5-dioxaspiro[5.5]undecan-9-one (Aldrich Co.) in 30 mL of DME dropwise over a period of 30 min at 0 °C. The reaction mixture was allowed to warm to 25 °C (2 h) and then stirred for 33 h. The solution was poured onto ice and worked up in the usual manner with ether, affording 5.18 g of an oil. The crude oil was dissolved in 10 mL of CH_2Cl_2 and added to a slurry of 4.4 mL of 12% HCl, 30 g of silica gel (60–200 mesh), and 70 mL of CH_2Cl_2 while stirring.[32] The mixture was stirred at 25 °C for 1 h, neutralized with 5% aqueous $NaHCO_3$, and filtered. The silica gel was washed with more CH_2Cl_2. Concentration of the filtrate gave a white solid which was chromatographed on silica gel. Elution with ethyl acetate in hexane (5–15%) gave 2.69 g (80%) of **29**: mp 44–46 °C (hexane); NMR δ 1.21 (s, 12), 2.63 (s, 4), IR ($CHCl_3$) 1710 cm^{-1}. The C, H analysis of this compound (two attempts) indicated the presence of 0.25 mol of water. Sublimation at 40 °C (0.35 mm) afforded an anhydrous specimen: mp 44.5–46.5 °C. Anal. Calcd for $C_{10}H_{16}O_2$:

(31) R. Lukes and J. Plesek, *Collect. Czech. Chem. Commun.*, **20**, 1253–1255 (1955).

(32) This is a modification of the method of F. Huet, A. Lechevallier, M. Pallet, and J. M. Conia, *Synthesis*, 63–65 (1978).

C, 71.39; H, 9.59. Found: C, 71.32; H, 9.49.

4-Hydroxy-2,2,6,6-tetramethylcyclohexanone (33). A modification of the method of Leuenberger et al.[21] was used. A solution of 250 mg (1.49 mmol) of diketone **29** in 3 mL of MeOH was hydrogenated (1 atm) at 25 °C over 300 mg of Raney Ni.[33] After 30 min, the solution was decanted and the nickel residue was washed with MeOH. The MeOH was removed and the residue was dissolved in 10 mL of ether, dried ($MgSO_4$), and concentrated to afford 252 mg of alcohol **33** as white needles. Recrystallization from hexane afforded 242 mg (95%) of **33**: mp 61–61.7 °C; NMR δ 1.15 (s, 6), 1.23 (s, 6), 1.54–2.18 (complex m, 4), 1.76 (br s, 1), 4.32 (m, 1); IR (CCl_4) 3616, 3508, 1705 cm^{-1}. Anal. Calcd for $C_{10}H_{18}O_2$: C, 70.55; H, 10.66. Found: C, 70.33; H, 10.66.

4-Hydroxy-3,3,5,5-tetramethylcyclohexanone (31) and *cis*- and *trans*-2,2,6,6-Tetramethyl-1,4-cyclohexanediol (32). In general, mixtures of **31**, **32**, and **33** were obtained when diketone **29** was reduced with either sodium borohydride in MeOH or isopropyl alcohol, lithium tri-*sec*-butylborohydride (L-Selectride) in ether (gave essentially only **33**) or diisobutylaluminum hydride (Dibal-H) in benzene (see text). The following procedure is representative. To a stirred solution of 45 mg (0.27 mmol) of **29** in 3.0 mL of MeOH at 0 °C was added 2.5 mg (0.07 mmol) of solid sodium borohydride. After 30 min the solution was diluted with water and extracted with ether. The residue obtained from the extract was fractionated by preparative TLC (Whatman PK6F plates, 1/1 ethyl acetate/hexane) to give two major bands: 14 mg (31%) of **31**, mp 89.5–90 °C (hexane), R_f 0.65, NMR spectrum identical with that reported for **31**;[34] 4 mg (9%) of **33**, R_f 0.52 (see above). When a 5-fold molar excess of sodium borohydride was used, there was obtained **32** (35%, isomer a) and **32** (45%, isomer b). Isomer a: mp 145–145.3 °C (CCl_4); R_f 0.47; NMR δ 0.99 (s, 6), 1.04 (s, 6), 1.22 (t, 2), 1.43 (br s, 2), 1.84 (dd, 2), 3.04 (s, 1), 3.93 (m, 1); IR ($CHCl_3$), 3609, 3540–3340 cm^{-1}; mass spectrum, *m/e* 172.147 (calcd for $C_{10}H_{20}O_2$, 172.146). Isomer b: mp 145.8–146.1 °C (hexane); R_f 0.35; NMR δ 1.07 (s, 6), 1.10 (s, 6), 1.45–1.58 (m, 4), 1.48 (br s, 2), 2.96 (s, 1), 3.97 (m, 1); IR ($CHCl_3$) 3639, 3610, 3530–3380 cm^{-1}. Anal. Calcd for $C_{10}H_{20}O_2$: C, 69.72; H, 11.70. Found: C, 69.70; H, 11.60.

3,5-Dibromo-2,2,6,6-tetramethyl-1,4-cyclohexanedione (35). To a solution of 1.0 g of diketone **29** (5.9 mmol) in 10 mL of glacial acetic acid was added 0.60 mL (11.9 mmol) of bromine dropwise over a period of 12 min with stirring. The solution was then poured into water and extracted with CH_2Cl_2. The usual workup gave 2.03 g of a yellow solid. This was shown by NMR to be largely (>90%) the desired 3,5-dibromide **35** (45/55 mixture of cis, trans isomers), which was used in the next step without further purification.

The cis isomer can be separated from the crude dibromide by two recrystallizations from CH_2Cl_2: white needles, mp 182–186 °C; NMR δ 1.31 (s, 6), 1.45 (s, 6), 4.89 (s, 2); IR ($CHCl_3$) 1702, 1752 cm^{-1}. Anal. Calcd for $C_{10}H_{14}Br_2O_2$: C, 36.84; H, 4.33. Found: C, 36.89; H, 4.12. NMR of the trans isomer: δ 1.32 (s, 6), 1.41 (s, 6), 5.13 (s, 2).

4-Oxo-3,3,5,5-tetramethyl-1-cyclopentene-1-carboxamide (38). To a solution of 1.0 g of crude dibromide **35** in 55 mL of CH_2Cl_2 was bubbled ammonia gas over a period of 1 h while stirring. The mixture was filtered and the filtrate was washed with water, dried ($MgSO_4$), and concentrated to afford a solid. This was twice recrystallized from benzene to give 445 mg (82% based on **29**) of **38** as white needles: mp 156–158 °C; NMR δ 1.21 (s, 6), 1.36 (s, 6), 5.73 (br s, 2), 6.51 (s, 1); IR ($CHCl_3$) 3532, 3412, 1750, 1674, 1616, 1582 cm^{-1}. Anal. Calcd for $C_{10}H_{15}NO_2$: C, 66.27; H, 8.34; N, 7.73. Found: C, 66.34; H, 8.24; N, 7.55.

4-Oxo-3,3,5,5-tetramethyl-1-cyclopentene-1-carboxylic Acid (39). To a stirred solution of 150 mg (0.83 mmol) of the amide **38** in 5 mL of MeOH was added 5 mL of 10% aqueous sodium hydroxide. The solution was heated at reflux for 48 h. The cooled solution was acidified with 2 N HCl and extracted with ether. The usual workup gave a residue which was twice recrystallized from benzene to give 136 mg (90%) of **39** as white plates: mp 174–175 °C; NMR δ 1.23 (s, 6), 1.35 (s, 6), 7.13 (s, 1), 10.14 (br s, 1); IR ($CHCl_3$) 1750, 1691, 1618 cm^{-1}. Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.92; H, 7.74. Found: C, 65.74; H, 7.37.

Methyl 4-Oxo-3,3,5,5-tetramethyl-1-cyclopentene-1-carboxylate (40). To a solution of 500 mg of crude dibromide **35** in 6 mL of CH_2Cl_2 was added 6 mL of 1 M sodium methoxide in MeOH. After a 15-min stir at 25 °C, the solution was neutralized with 1 N H_2SO_4 and extracted with CH_2Cl_2. Distillation afforded 255 mg of (87% based on **29**) ester **40** as a viscous oil that subsequently solidified: bp 76 °C (2.5 mm); mp 49–50 °C; NMR δ 1.19 (s, 6), 1.31 (s, 6), 3.80 (s, 3), 6.96 (s, 1); IR ($CHCl_3$) 1751, 1713 cm^{-1}. Anal. Calcd for $C_{11}H_{16}O_3$: C, 67.32; H, 8.22. Found: C, 67.31; H, 8.16.

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(33) Raney Ni was generated from nickel–aluminum alloy (Grace, No. 2813) by following the procedure of R. Mozingo, "Organic Syntheses", Collect. Vol. 3, Wiley, New York, 1955, pp 181–183. However, instead of washing the nickel to the neutral point, the washing was continued until the pH of the washings was about 9. The nickel was then washed with absolute MeOH twice and stored under MeOH.

(34) M. M. Green and C. Djerassi, *J. Am. Chem. Soc.*, **89**, 5190–5198 (1967).

Notes

An Application of Molecular Mechanics to Structure Determination

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Recently, we have had occasion to react 1-acetoxybutadiene (**4**) with 2-(3,4-dichlorophenyl)-*N*-methylmaleimide (**3**) and have observed the formation of a single Diels–Alder product **5**. In principle, there are four possible regio- and stereoisomeric products, and distinguishing among them is not simple. Hückel calculations show that the LUMO of phenylmaleimide is nonbonding, implying high reactivity with electron-rich dienes. The LUMO, at least at the level of simple Hückel theory, has equal coefficients at the two olefinic carbons, and one can not predict by frontier molecular orbital theory the orientation of cycloaddition. The diene **4** is a mixture of geometric isomers[1] which further complicates the assignment of structure to the adduct **5**.

The NMR data demonstrate that the cyclohexene ring

(1) (a) Wichterle, O.; Hudlicky, M. *Collect. Czech. Chem. Commun.* **1947**, *12*, 564. (b) We find the commerical material to be a 2:1 mixture of *E* and *Z* isomers.

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