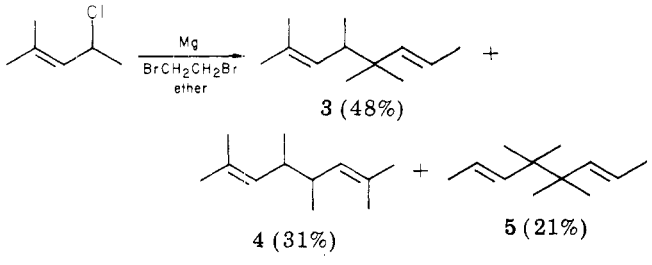
were at m/z 121 and 123! The peak at m/z 121 and a second at m/z 107 cannot correspond to any species containing one chlorine and both necessarily indicate the presence of more than six carbons. Furthermore, significant peaks appear at 149, 164, and 246. The small peak at 118 was found by high-resolution studies to have m/z 118.055 which corresponds only to $C_6H_{11}Cl$. The major group of low molecular weight ions can be accounted for as fragment peaks from the m/z 83 (82%) ion formed by loss of a chlorine atom. The presence of a significant radical cation at m/z 82 along with those at m/z 164 and 246 suggests strongly that **1** readily loses hydrogen chloride during introduction into the mass spectrometer and the diene forms dimers and trimers. Under different entry conditions, the intensities of these radical cation peaks varied considerably, and m/z 246 was often absent or of very low intensity.

With the chloride mixture in hand, we set out to prepare a Grignard reagent, but the chlorides were relatively unreactive unless the magnesium was preactivated with dibromoethane. Then reaction did occur, leading, however, only to a mixture of coupling products. The mixture was separated readily by GLC into a major (79%) and a minor (21%) fraction. The minor fraction was readily identified as 4,4,5,5-tetramethyl-2,6-octadiene (**5**), while the major



fraction contains a mixture of ca. 60% 2,4,5,5-tetramethyl-2,6-octadiene (**3**) and ca. 40% 2,4,5,7-tetramethyl-2,6-octadiene (**4**). In an attempt to limit the dimer formation we used Rieke magnesium.[9] The reaction mixture was treated with acetone but the expected tertiary alcohol constituted at best a trace constituent of the complex mixture. No pure compounds could be isolated. It is clear therefore that while **1** can be prepared, the great ease with which it loses hydrogen chloride has a strong influence on its chemistry.

Experimental Section[10]

4-Methyl-3-penten-2-ol. The alcohol was prepared by the procedure of Cain,[7] using a half-molar scale. The alcohol, bp 71 °C (44 mm), was obtained in 70% yield: IR (neat) 3430 (br), 1680; NMR (CCl_4) δ 1.12 (d, 3 H, J = 6 Hz), 1.55, 1.57 (2 d's, 6 H, $J \simeq 2$ Hz), 3.62 (OH), 4.43 (quintet, 1 H, J = 7 Hz), 5.15 (d of quintet, 1 H, J = 7.5, 1.5 Hz).

2,4-Dichloro-2-methylpentane. A sample, 2.0 g (0.02 mol), of the above alcohol was allowed to stand overnight in 25 mL of concentrated hydrochloric acid. The organic products were taken up in pentane, and the solution was washed with sodium bicarbonate and dried ($MgSO_4$). The main product was collected from GLC (2% SE-30 column at 77 °C); NMR (CCl_4) δ 1.57 (d, 3 H, J = 7 Hz), 1.66 (s, 6 H), 2.20 (d, 2 H, J = 5 Hz), 4.43 (near sextet, 1 H, $J \simeq 6$ Hz).

4-Chloro-2- (and -4-) methyl-2-pentene. The two isomeric chlorides were prepared by two procedures, but in all cases a mixture of the two in the ratio **1**/**2** of 84:16 was always obtained, and the two could not be separated.

Method A. A solution containing 8.45 g (84.5 mmol) of 4-methyl-3-penten-2-ol in 30 mL of pentane was shaken with 80 mL of 12 N hydrochloric acid for 20 min. The pentane layer was separated, washed with sodium bicarbonate solution, and dried ($MgSO_4$). The pentane was removed under reduced pressure and the residue was distilled, bp 37.5 °C (51 mm) [lit.[5] bp 45–46 (40 mm)], giving 2.4 g (29%) of clear liquid: IR (neat) 3040, 1670, 1380, 970 (w), 840; NMR (CCl_4) δ 1.53 (d, 3 H, J = 7 Hz), 1.72, 1.74 (overlapping d's, 6 H, $J \simeq 1.5$ Hz), 4.72 (overlapping q's, 1 H, J = 10, 7 Hz), 5.29 (2 septets, 1 H, J = 10, ~1.5 Hz). The NMR spectrum contains, along with the above two additional patterns which were attributed to 4-chloro-4-methyl-2-pentene, δ 1.65 (s), 5.70 (apparent AB, $J \simeq 15$ Hz). The integration of the δ 5.70 vs. the δ 4.72 + 5.29 multiplets is 5.5 to 28.5 which corresponds to about 16% of the lesser isomer. A GLC analysis (4% SE-30 column at 68 °C) of the mixture shows two peaks with the ratio 80:20, but collection of the major peak indicates that reequilibration had occurred during passage through the detector and collection system; mass spectrum, m/z (relative intensity) 164 (2.4), 149 (3.7), 121 (14.5), 118 (2.4), 107 (10.3), 83 (81.9), 82 (36.1), 67 (50.3), 55 (54.8), 53 (16.4), 43 (24.6), 41 (66.4), 39 (47.5). Under different entry conditions the peak ratios shift notably with m/z 118 being half as intense as m/z 121 and either m/z 67 or 83 being the base peak.

Method B. The pentenol, 1.00 g (9.98 mmol), was allowed to stand for several days with 2.68 g (10.2 mmol) of triphenylphosphine in 3.6 mL of carbon tetrachloride. The precipitated triphenylphosphine oxide was removed and the product was distilled. The carbon tetrachloride codistilled with the mixture of chlorides. The product showed the same spectra as the chloride mixture obtained by method A.

Dimer Formation. Grignard magnesium, 1.24 g (51 mmol), in 30 mL of ether, was activated with 1,2-dibromoethane, and 360 mg (3.04 mmol) of the chloride mixture in 25 mL of ether was added dropwise. The mixture was stirred at room temperature for 2.5 h and 4 mL of water was added slowly. Enough 2 N sulfuric acid was added to dissolve the precipitate and the ether layer was separated and dried ($MgSO_4$). Ether was removed with a spinning-band column and the residue was separated by GLC (2% SE-30 column, 65 °C). Two products were collected, a major (79%) and a minor (21%) fraction. The main fraction was identified spectrally as 2,4,5,5-tetramethyl-2,6-octadiene apparently contaminated with some 2,4,5,7-tetramethyl-2,6-octadiene: NMR (CCl_4) δ 0.81, 0.82 (2 d, J = 6 Hz), 0.89, 0.91 (2 s), 1.60, 1.70 (2 d), 1.68 (d), 4.90 (m), 5.33 (m) (decoupling showed multiplets at δ 4.90 and 5.33 were coupled to the methyls at δ 1.60 and 1.70); IR (CCl_4) 3040, 1660, 1380, 1360, 968, 850; mass spectrum, m/z (relative intensity) 166 (2.6), 83 (100), 82 (6.4), 55 (30.8), 43 (14.8), 41 (18.0). Assuming the mixture contains solely the two isomeric dimers, the integration indicates 60% 2,4,5,5- and 40% 2,4,5,7-tetramethyl-2,6-octadienes.

The minor fraction was pure *trans,trans*-4,4,5,5-tetramethyl-2,6-octadiene: NMR (CCl_4) δ 0.90 (s, 12 H), 1.68 (d, 6 H, J = 5.5 Hz), 5.29, 5.51 (AB of ABX_3, J_{AB} = 15, $J_{AX} \rightarrow 0$, J_{BX} = 5.5 Hz); IR (CCl_4) 3035, 1380, 1365, 975 cm^{-1}; mass spectrum, m/z (relative intensity) 166 (0.8), 83 (100), 55 (33.2), 43 (11.9), 41 (19.5).

Registry No. 1, 21971-94-8; 2, 68318-00-3; 3, 75232-90-5; 4, 65164-65-0; 5, 75232-91-6; 2,4-dichloro-2-methylpentane, 33484-86-5; 4-methyl-3-penten-2-ol, 4325-82-0.

(9) Rieke, R. D.; Bales, S. E. *J. Am. Chem. Soc.* **1974**, *96*, 1775–1781.
(10) The HA 100 NMR spectrometer used in this work was purchased with the aid of an instrument grant from the NSF.

Microbiological Preparation of (*S*)-(+)-2,3-Dihydroxy-3-methylbutanoic Acid by Syn Dihydroxylation of 3-Methylcrotonic Acid[1]

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I report the preparation of (2*S*)-2,3-dihydroxy-3-methylbutanoic acid (**1a**) by microbiological di-

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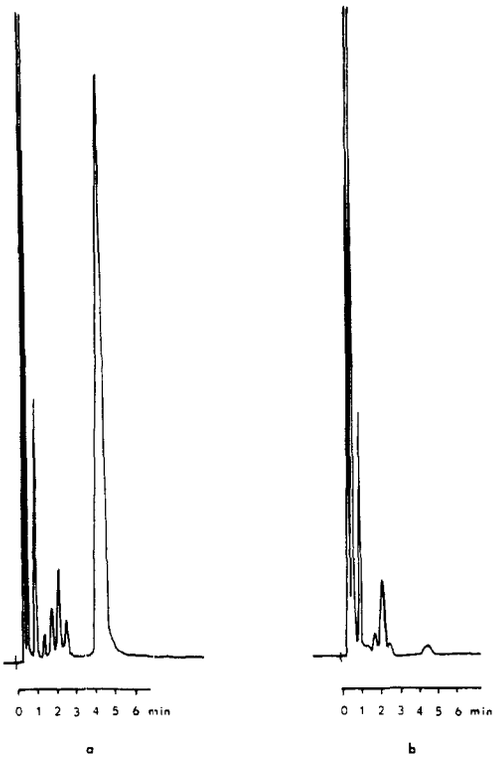
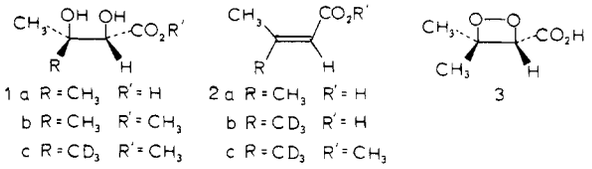


Figure 1. Gas chromatograms of CH_2N_2-treated extracts from incubations of 3-methylcrotonate with *Ps. putida*: (a) product from incubation *without* added glucose; (b) product from incubation *with* added glucose.

hydroxylation of 3-methylcrotonic acid (**2a**). Enantiomer **2a** becomes readily available on a multigram scale by this procedure.[2] I also report the overall stereochemistry of this dihydroxylation reaction.

Pseudomonas putida (ATCC 21244) cells were pregrown on the "Medium 2" used by Goodhue and Schaeffer[3] for the efficient production of L(+)-β-hydroxyisobutyric acid from isobutyric acid, with the substitution of isovaleric acid for the isobutyric acid as sole carbon source. The cells, collected by centrifugation, were then resuspended in 0.2% K_2HPO_4 (approximately equivalent to Goodhue and Schaefer's[3] Medium 3, *but with the omission of glucose*) to which was added 3-methylcrotonic acid, **2a** (5 g/L, adjusted to pH 7.4 with KOH). Incubation was then continued with efficient aeration for 24 h at 28 °C. After centrifugation, concentration to one-tenth of the original volume, acidification, and saturation with NaCl, the medium was extracted to yield the crude product as a viscous oil (ca. 20%) consisting mainly of **1a**. GC examination of the methylated (CH_2N_2) product (Figure 1a) revealed the presence of mainly **1b**, accompanied by minor unidentified impurities. The major peak, obtained by preparative GC, was identical in NMR and mass spectra with methyl (±)-2,3-dihydroxy-3-methylbutyrate (**1b**) prepared by hydroxylation of **2a** with $OsO_4/NaClO_3$[4]. Kugelrohr distillation of the crude methyl ester gave essentially pure **1b** (ca. 15% from **2a**). This product was not formed in significant yield when the normal Medium 3, with added glucose, was used, Figure 1b. From the specific rotation of the isolated product, $[\alpha]^{25}_D$ +26° ($CHCl_3$), the absolute configuration was assigned as 2*S*.[5] The product **1a** is thus the enantiomer of the (2*R*)-2,3-dihydroxyisovaleric acid which is an intermediate in valine biosynthesis.[5]



Since the methyl group resonances of **1b** had been assigned,[4a] the stereochemistry of hydroxyl addition at C-3 of 3-methylcrotonate, relative to C-2, could be determined. The substrate, (*E*)-[4,4,4-$^2H_3$]-3-methylcrotonic acid, **2b**, was synthesized by treatment of methyl tetrolate with lithium dimethyl-d_6 cuprate,[4a] followed by saponification of the product, **2c**. Incubation of **2b** as above gave, after methylation, **1c** showing in the NMR spectrum a methyl singlet at δ 1.28 with only a trace of absorption (ca. 0.1 H) at δ 1.20 (resulting from a small degree of nonstereospecific labeling in the precursor). It follows that the metabolite is labeled as shown in **1c**. Thus the dihydroxylation proceeds stereospecifically in a syn manner on the *si,si* face of the carbon–carbon double bond. Such syn stereochemistry suggests the involvment of an endoperoxide intermediate **3** in the transformation.[6,7]

Experimental Section

Pseudomonas putida ATCC 21244 was obtained from the American Type Culture Collection, Rockville, MD, and was maintained on Difco nutrient agar plates. Incubations were carried out at 28 °C in a Lab-line incubator containing a New Brunswick Scientific Co. gyrotory shaker. NMR spectra were recorded on a Varian EM-360 instrument. Mass spectra were recorded by Dr. Thomas A. Wittstruck of this institution on a Nuclide 12-90-G mass spectrometer equipped with a Nuclide DA/CS 1.2 data acquisition system. Gas chromatographic analyses (GC) were performed by using a Varian Model 720 gas chromatograph with a 5 ft × 0.25 in. stainless steel colunm at 140 °C packed with 5% Carbowax 20M on 80/100 Supelcoport. Tetrolic acid was obtained from Farchan Research Laboratories, Willoughby, OH.

Incubation of 3-Methylcrotonate with *Ps. putida*. Individual colonies of *Ps. putida* from nutrient agar plates were transferred to two 250-mL Erlenmeyer flasks containing 100 mL of Goodhue and Schaeffer's[3] Medium 2 with the omission of isobutyric acid. Isovaleric acid (0.5 g in 5 mL of H_2O, adjusted to pH 7.4 with KOH; sterilized separately) was added to each flask, and the mixture was incubated aerobically on a gyrotory shaker at 28 °C, 250 rpm, for 2–3 days. The resultant yellow cell suspensions were transferred to two 2.8-L Fernbach flasks each containing 1 L of the above medium, and incubation was continued at 28 °C for 24 h (ca. 150 rpm). The cells were collected by centrifugation and were resuspended in 1800 mL of sterilized 0.2% K_2HPO_4 in two Fernbach flasks. To each flask was added 3-methylcrotonic acid (5 g, in 50 mL of H_2O, adjusted to pH 7.4 with KOH; this solution was not sterilized), and the mixtures were incubated at 27 °C as before for 24 h. The supernatant obtained

(2) This enantiomer of 2,3-dihydroxy-3-methylbutyric acid has previously been obtained by resolution of the racemic acid: F. B. Armstrong, U. S. Miller, J. B. Reary, D. Whitehouse, and D. H. G. Crout, *Biochim. Biophys. Acta*, **498**, 282–293 (1977).

(3) C. T. Goodhue and J. R. Schaeffer, *Biotechnol. Bioeng.*, **13**, 203–214 (1971).

(4) (a) R. K. Hill, S. Yan, and S. M. Arfin, *J. Am. Chem. Soc.*, **95**, 7857–7859 (1973); (b) G. Buchi, E. Demole, and A. F. Thomas, *J. Org. Chem.*, **38**, 123–126 (1973); (c) E. A. Cioffi, K. J. Shaw, W. F. Bailey, and C. M. Berg, *Anal. Biochem.*, **104**, 485–488 (1980).

(5) R. K. Hill and S. Yan, *Bioorg. Chem.*, 1, 446–456 (1971).

(6) Attempts to confirm the involvement of endoperoxide **3** in the transformation by carrying out the incubation in a closed system containing a mixture of $^{16}O_2$ and $^{18}O_2$ were not successful. The yield of **1a** in these incubations was quite low and the product contaminated with impurities, normally present in only trace amounts, which precluded a reliable determination of the mass spectrum. Very efficient aeration is essential to the high-yield preparation of **1a** from **2a**.

(7) No significant accumulation of a dihydroxy acid resulted from similar incubations of the bacteria with tiglic acid or *trans*-2-pentenoic acid, although the bacteria grew well on the saturated analogues, (±)-2-methylbutyric acid and *n*-pentanoic acid.

(8) S. Yan, Ph.D. Dissertation, University of Georgia, 1973, pp 124–126.

after centrifugation was lyophilized. The residue was redissolved in 150 mL of H_2O, saturated with NaCl, acidified to pH 2 with concentrated HCl, filtered, and extracted continuously with ether for 24 h. The extract was evaporated under reduced pressure to a viscous oil, 2.34 g, consisting mainly of **1a** (by NMR analysis). The crude product was methylated (CH_2N_2) and distilled in a Kugelröhr apparatus, bp 110 °C (0.2 mm). The product showed only traces of impurities by GC. A portion of the main product separated by preparative GC had NMR ($CDCl_3$) and mass spectra identical with the spectra of **methyl (±)-2,3-dihydroxy-3-methylbutyrate.**[5]

Synthesis of (*E*)-[4,4,4-$^2H_3$]-3-methylcrotonic Acid and Its Incubation wtih *Ps. putida*. Methyl tetrolate [bp 52–53 °C (22 mm)] was prepared by treatment of tetrolic acid with excess CH_2N_2/ether. The product (6.78 g, 0.069 mol) was converted to methyl (*E*)-[4,4,4-$^2H_3$]-3-methylcrotonate, **2c**, 7.0 g (0.060 mol), by treatment with $Li(CD_3)_2Cu$, following a procedure closely analogous to that described[4a,8] for the preparation of ethyl (*Z*)-[4,4,4-$^2H_3$]-3-methylcrotonate. The NMR spectrum of **2c** had δ ($CDCl_3$) 1.90 (trace, ca. 0.1 H), 2.15 (3 H, d, J = 1.5 Hz), 3.63 (3 H, s), 5.54 (1 H, q, J = 1.5 Hz).

The product (7.0 g) was saponified by treatment with 2 N NaOH (50 mL), stirring at 25 °C for 40 h. Upon acidification (HCl), the precipitate was filtered, washed with cold H_2O, and air-dried, giving **2b**: 4.48 g; NMR δ ($CDCl_3$) 1.90 (ca. 0.1 H, d, J = 1.5 Hz), 2.18 (ca. 2.8 H, d, J = 1.5 Hz), 5.69 (1 H, q, J = 1.5 Hz), 11.08 (1 H, br s, D_2O exchangeable). An additional 1.15 g of **2b** was obtained by ether extraction of the aqueous filtrate.

The product (1.0 g in 10 mL of H_2O, adjusted to pH 7.4 with KOH) was incubated as previously described by *Ps. putida* cells from two 100-mL cultures, and the crude product (400 mg) was isolated as before. After purification by Kugelröhr distillation of the methylated product, **1c** (295 mg) was obtained. After removal of trace impurities by preparative GC, the product had NMR ($CDCl_3$ + D_2O) δ 1.22 (ca. 3 H, s), 1.30 (ca. 0.1 H, s), 3.75 (3 H, s), 4.93 (1 H, s).

Registry No. 1a, 63903-90-2; **1b**, 75347-92-1; **1c**, 75365-50-3; **2a**, 541-47-9; **2b**, 75347-93-2; **2c**, 75347-94-3; isovaleric acid, 503-74-2; methyl tetrolate, 23326-27-4.

Communications

Oxygen Functionalization in Cyclooctatetraene via Singlet Oxygenation: Synthesis and Transformations of *anti*-7,8-Dioxatricyclo[4.2.2.0^{2,5}]deca-3,9-diene, the Endoperoxide of the Bicyclic Valence Tautomer of Cyclooctatetraene

Summary: The novel endoperoxide of the bicyclic valence tautomer of cyclooctatetraene is prepared, characterized, and transformed into a series of new and valuable synthetic intermediates derived from the bicyclo[4.2.0]octa-2,4,7-triene skeleton via base-catalyzed isomerization and MnO_2 oxidation, thermolysis and mCPBA oxidation, triphenylphosphine deoxygenation, and exhaustive diimide reduction.

Sir: The fact that cyclooctatetraene (**1**) is inert toward singlet oxygenation, either via photosensitized[1] or chemical[2] generation of singlet oxygen, obliges indirect strategies for the synthesis of the desirable endoperoxides **2** (Scheme I). In the case of the synthetically more challenging endoperoxide **2b**, derived from bicyclic valence tautomer **1b**, the indirect sequence **1a** → **3** → **4** → **2b** (Scheme I) has also failed[3] since so far it has not been possible to debrominate the known[4] endoperoxide **4** under sufficiently mild conditions at which the peroxide linkage is preserved. Presently we report the preparation of the endoperoxide **2b** via the sequence **1a** → **3** → **1b** → **2b** (Scheme I) and its chemical transformations into a number of useful difunctionalized oxygen derivatives of the bicyclo[4.2.0]-octa-2,4,7-triene skeleton.

Analogous to Vogel et al.,[5] the dibromide **3** was debrominated with *n*-BuLi in diethyl ether at −60 °C. The resulting solution of the sufficiently stable bicyclic valence tautomer **1b** was submitted to photosensitized oxygenation at −30 °C for 2 h with tetraphenylphorphyrin (TPP) as the sensitizer and a 150-W sodium lamp as a light source. The endoperoxide **2b** was obtained quantitatively (mp 75–76 °C, recrystallized from a CH_2Cl_2/*n*-C_6H_{14} mixture; 85% yield).[6,7]

The chemical transformations of the novel endoperoxide **2b** are summarized in (Scheme II). For example, via pathway **2b** → **5** the known dienedione **5** was prepared in an overall 85% yield (mp 50–51 °C, after silica gel chromatography; lit.[4] mp 51–52 °C); however, our sequence **1a** → **3** → **1b** → **2b** → **5** entails considerably less work.[8] On the other hand, when a benzene solution of **2b** is heated[3b] at 100 °C for 1 h, the new diepoxide **6** was obtained quantitatively[6,9] (mp 105–107 °C, recrystallized from a CH_2Cl_2/*n*-C_6H_{14} mixture; 85% yield). Treatment with *m*-chloroperbenzoic acid (mCPBA) in CH_2Cl_2 led to the intriguing trioxide **7** in 70% yield (mp 209–210 °C, recrystallized from a CH_2Cl_2/n-C_6H_{14} mixture),[6,10] representing the first of a total of six trioxide isomers of the bicyclo[4.2.0]octa-2,4,7-triene valence isomer of cyclooctatetraene.[3b] An X-ray analysis of **7** (Dr. K. Peters, Max-Planck-Institut für Festkörperforschung, Stuttgart, for the X-ray determination; complete details will be disclosed in a full paper on this subject) reveals that the epoxy oxygens on the cyclohexane ring are syn to one another, but anti with respect to the cyclobutane ring, and that the epoxy oxygen on the cyclobutane ring is syn with

(1) (a) Gollnick, K. *Adv. Photochem.* **1968**, *6*, 1. (b) Matsuura, T.; Horinaka, A.; Nakashima, R. *Chem. Lett.* **1973**, 887.

(2) Adam, W.; Cueto, O.; De Lucchi, O.; K.-H. Hill, unpublished results. The reaction of cyclooctatetraene with chemical singlet oxygen sources such as 1,4-dimethoxy-9,10-diphenylanthracene 1,4-endoperoxide at elevated temperature (ca. 100–120 °C) were so far unsuccessful.

(3) (a) Adam, W.; Balci, M.; De Lucchi, O., unpublished results. (b) Adam, W.; Balci, M. *Tetrahedron* **1980**, *36*, 833. Cf. eq 19, p 839, and ref 36 therein.

(4) Oda, M.; Kayama, Y.; Kitahara, Y. *Tetrahedron Lett.* **1974**, 2019.

(5) Vogel, E.; Kiefer, H.; Roth, W. R. *Angew. Chem.* **1964**, *76*, 432; *Angew. Chem., Int. Ed. Engl.* **1964**, 3, 442.

(6) All new compounds exhibited satisfactory elemental composition by combustion analysis.

(7) For **2b**: ^1H NMR ($CDCl_3$, Me_4Si) δ 3.20 (q, 2 H), 4.59 (q, 2 H), 5.91 (s, 2 H), 6.20 (dd, 2 H); IR (CCl_4) 3120, 3060, 2970, 1375, 1300, 950, 920 cm^{-1}.

(8) Adam, W.; Balci, M.; Rivera, J. *Synthesis* **1979**, 807.

(9) For **6**: ^1H NMR ($CDCl_3$, Me_4Si) δ 3.10 (d, 2 H, J = 3 Hz), 3.30 (s, 2 H), 3.45 (d, 2 H, J = 3 Hz), 6.20 (s, 2 H); IR (KBr) 3130, 3000, 2915, 1580, 1420, 1060, 950, 860 cm^{-1}.

(10) For **7**: ^1H NMR ($CDCl_3$, Me_4Si) δ 2.81 (s, 2 H), 3.13 (m, 2 H), 3.45 (m, 2 H), 4.10 (s, 2 H); IR (KBr) 3000, 2980, 2940, 980, 940, 860 cm^{-1}.

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