extracted with chloroform (4 × 50 mL). The combined extracts were dried over sodium sulfate, filtered and then evaporated leaving an oil. The oil was chromatographed over 200 g of silica gel by using 9:1 hexane/ethyl acetate as eluent to give 2.78 g (68%) of **6** as a colorless oil; the NMR spectrum of this material shows it to be a mixture of its keto–enol forms: IR (neat) 1750 (CO_2-C_2H_5), 1720 and 1705 cm−1 (C=O).

Anal. Calcd for $C_{13}H_{22}O_6$: C, 56.92; H, 8.08; O, 35.00. Found: C, 56.55; H, 8.12.

4,4-Diethoxy-3-oxovaleronitrile (7). Dry tetrahydrofuran (20 mL) was placed in a three-necked 250-mL round-bottomed flask fitted with an addition funnel, thermometer, and septum. The flask was cooled to −70 °C under nitrogen, and 18.8 mL (30 mmol) of 1.6 M *n*-butyllithium was added over 5 min. To this mixture was added dropwise over 15 min a solution of 1.23 g (30 mmol) of acetonitrile in 20 mL of dry tetrahydrofuran. After addition was complete, the mixture was stirred at −70 °C for 1 h, and then a solution of 4.25 g (15 mmol) of **1** in 20 mL of dry tetrahydrofuran was added dropwise over 15 min. After addition was complete, the mixture was stirred at −70 °C for 0.5 h and then allowed to warm to room temperature. The mixture was treated with 40 mL of 1 N hydrochloric acid, and then the organic layer was separated. The aqueous phase was extracted with ether (2 × 50 mL), and the combined organic extracts were dried over sodium sulfate, filtered, and evaporated, leaving an oil. This was chromatographed over 150 g of silica gel by using 1.6:0.3:0.1 of chloroform/cyclohexane/ethyl acetate to afford 2.48 g (89%) of **7** as an oil: NMR ($CDCl_3$) 3.80 (s, 2 H), 3.51 (q, 4 H), 1.47 (s, 3 H), 1.23 (t, 6 H); IR (neat) 2260 (CN), 1745 cm^{-1} (C=O).

Anal. Calcd for $C_9H_{15}NO_3$: C, 58.36; H, 8.16; N, 7.56. Found: C, 57.90; H, 8.01; N, 7.21.

***p*-Nitrophenyl 2-Ethoxypropenoate (8).** A mixture of 7.0 g (60 mmol) of 2-ethoxypropenoic acid (**3**), 14.1 g (60 mmol) of *p*-nitrophenyl trifluoroacetate, and 40 mL of pyridine was stirred at room temperature for 24 h. The mixture was poured into 200 mL of water and extracted with ether (5 × 50 mL). The combined extracts were washed with 5% aqueous sodium hydroxide (5 × 25 mL), then dried (Na_2SO_4), filtered, and thoroughly evaporated, leaving a solid. Recrystallization from cyclohexane afforded 11.6 g (82%) of **8** as a white crystalline solid, mp 91–92 °C; NMR ($CDCl_3$) 8.30 (d, 2 H), 7.34 (d, 2 H), 5.52 (d, 1 H), 4.81 (d, 1 H), 3.91 (q, 2 H), 1.42 (t, 3 H).

Anal. Calcd for $C_{11}H_{11}NO_5$: C, 55.70; H, 4.67; N, 5.90. Found: 55.57, H, 4.71; N, 6.04.

2-Ethoxy-2-propenamide (9). A solution of 3.56 g (15 mmol) of **8** in 80 mL of tetrahydrofuran was stirred at room temperature, and gaseous ammonia was bubbled into the mixture for 0.5 h. The mixture was concentrated, and the residue was taken up into 80 mL of ethyl acetate. The organic solution was washed with 5% aqueous sodium hydroxide (4 × 30 mL), then dried (Na_2SO_4), filtered, and evaporated, leaving a white solid. Recrystallization from isopropyl ether afforded 1.12 g (65%) of **9** as a white crystalline solid, mp 68–69 °C; NMR ($CDCl_3$) 6.60 (br, 2 H), 5.33 (d, 1 H), 4.46 (d, 1 H), 3.84 (q, 2 H), 1.34 (t, 3 H).

Anal. Calcd for $C_5H_9NO_2$: C, 52.15; H, 7.88; N, 12.16. Found: C, 52.61; H, 7.42; N, 12.01.

Ethyl 2-Carbethoxy-4-ethoxy-3-oxo-4-pentenoate (10). A slurry of 0.80 g (33 mmol) of sodium hydride in 60 mL of dry tetrahydrofuran was stirred at room temperature under nitrogen and 5.30 g (33 mmol) of diethyl malonate in 20 mL of dry tetrahydrofuran was added dropwise over 10 min. After addition was complete, the mixture was stirred at room temperature for 15 min, and then a solution of 3.56 g (15 mmol) of **8** in 30 mL of dry tetrahydrofuran was added dropwise over 15 min. After addition was complete, the mixture was stirred at room temperature for 4 h, then poured carefully into 200 mL of ice–water, and brought to pH 7 with 2 N hydrochloric acid. The aqueous mixture was extracted with chloroform (4 × 50 mL), and the combined extracts were dried over anhydrous sodium sulfate, filtered, and then evaporated, leaving an oil. This was chromatographed over 250 g of silica gel by using 19:1 hexane/ethyl acetate as eluent to give 1.98 g (51%) of **10** as an oil: NMR ($CDCl_3$) 5.28 (d, 1 H), 4.87 (s, 1 H), 4.40 (d, 1 H), 4.3–3.8 (m, 6 H), 1.26 (overlapping 9 H).

Anal. Calcd for $C_{12}H_{18}O_6$: C, 55.81; H, 7.03; O, 37.17. Found: C, 55.39; H.7.16

***N*,*N*-Dimethyl-*N*'-(2-ethoxypropenoyl)guanidine (11).** Sodium methoxide (1.12 g, 20.7 mmol) was added to 40 mL of *N*,*N*-dimethylformamide, and to this was added 3.84 g (20.7 mmol) of *N*,*N*-dimethylguanidinium sulfate. This mixture was warmed to 60 °C until a consistent slurry resulted, at which point 4.00 g (16.9 mmol) of **8** was added. The new yellow slurry was stirred at 80 °C for 1.5 h, then cooled, and filtered. Concentration of the filtrate afforded an oil, which was chromatographed over silica gel using 20:1 chloroform/methanol as eluent to give 1.70 g (54%) of **11** as a pale yellow solid, mp 148–150 °C: NMR ($CDCl_3$) 7.60 (br, 2 H), 5.48 (d, 1 H), 4.41 (d, 1 H), 3.82 (q, 2 H), 3.07 (s, 6 H), 1.37 (t, 3 H). HRMS, obsd M$^+$ *m*/*z* 185.1174, $C_8H_{15}N_3O_2$ calcd 185.1164.

Registry No. **1**, 108818-40-2; **2**, 25741-02-0; **3**, 32821-76-4; (*E*)-**4**, 108818-41-3; (*Z*)-**4**, 108818-47-9; **5**, 92845-55-1; **6**, 108818-42-4; **7**, 108818-43-5; **8**, 108818-44-6; **9**, 34068-59-2; **10**, 108818-45-7; **11**, 108818-46-8; pyruvic acid, 127-17-3; 2-oxobutanoic acid, 600-18-0.

Use of Tritium and Deuterium in Assigning Proton NMR Spectra: Reinvestigation of the Configuration of 5-Phenylpenta-2,4-dienoic Acid (mp 165 °C)

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Contradictory reports[1-3] concerning the configuration of the 5-phenylpenta-2,4-dienoic acid stereoisomer (Figure 1A) melting at 164–165 °C led us to make a thorough investigation of the NMR characteristics of this compound. Although this isomer has long been thought to have the 2*E*,4*E* configuration,[3] a recent NMR study has suggested that it is the 2*E*,4*Z* form.[1]

As it is known that the configuration of dienoic acids significantly affects their physiological properties, it is important to be certain of the stereochemistry of a particular isomer. There are four stereoisomers of 5-phenylpenta-2,4-dienoic acid, and since 1877 several methods[3-8] have been used to produce the isomer with melting point 164–165 °C. The physiological activity of several of the stereoisomers of the dienoic acid and their esters have been studied,[2,9] and a number of the esters were found to have bactericidal and fungicidal properties.

We have employed a combination of synthetic and analytical methods to extract the NMR data for the isomer of 5-phenylpenta-2,4-dienoic acid with melting point 164–165 °C. This multifaceted approach is an illustration of some generally applicable techniques, which we think may be useful in the elucidation of spectral assignments of peptides and other biomolecules.

The 300-MHz ^{1}H NMR spectrum of 5-phenylpenta-2,4-dienoic acid (mp 164–165 °C) is shown in Figure 1B.

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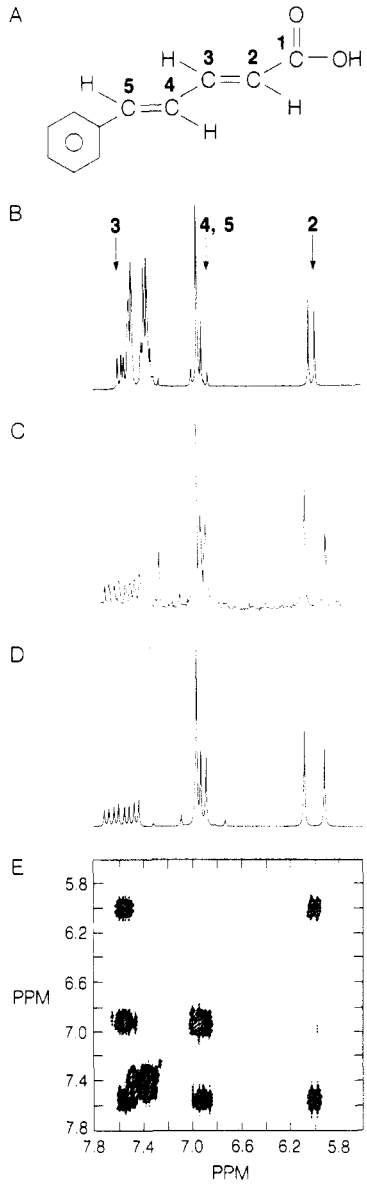

Figure 1. Proton NMR spectra of (2*E*,4*E*)-5-phenylpenta-2,4-dienoic acid: (A) structure of the substrate; (B) 300-MHz ^{1}H NMR spectrum; (C) 90-MHz ^{1}H NMR spectrum of 5-phenyl[$^2H_5$]penta-2,4-dienoic acid; (D) simulated 90-MHz ^{1}H NMR spectrum of 5-phenyl[$^2H_5$]penta-2,4-dienoic acid; (E) contour plot of the 300-MHz ^{1}H NMR phase-sensitive COSY spectrum of the title acid. The transformed matrix was 2K by 2K, and sine-bell window functions were applied in both dimensions.

Integration of the signal intensities indicates that the lowest field multiplet represents six protons and the next multiplet two protons, while the doublet centered at 6.00 ppm represents one proton, with splitting J = 15.00 Hz. The strategy taken in resolving this spectrum was as follows.

Tritiation at the 5-position gave the chemical shift of that position and the coupling constant $J_{4,5}$. The proton-coupled ^{3}H NMR spectrum showed a doublet centered at δ 6.90 for the 5-position, with a splitting of 15.3 Hz. Using the relationship $J_{HH} = J_{HT}\gamma_H/\gamma_T$,[10] the proton coupling constant was easily derived ($J_{4,5}$ = 14.25 Hz).

Synthesis of the compound with tritium at the 2- and 4-positions gave the chemical shifts of these protons, as well as making it obvious that the chemical shifts of the 4- and 5-positions are very similar (Table I). The clean doublet centered at 6.00 ppm in Figure 1B is due to H-2, with $J_{2,3}$ = 15.00 Hz. Combining the information so far shows that the 3-proton is part of the multiplet with intensity six centered at δ 7.5.

Table I. Observed and Calculated Shifts and Coupling Constants

		coupling constant, Hz			
position	δ	2	3	4	5
		Observed			
2	6.00		15.00	0.40	
3	7.56	15.00		7.31	3.22
4	6.90	0.40	7.31		14.25
5	6.96		3.22	14.25	
		Calculated			
2	6.00		15.00	−0.18	
3	7.56	15.00		9.92	0.58
4	6.90	−0.18	9.92		14.25
5	6.96		0.58	14.25	

Synthesis of the ring deuteriated compound gave a much simpler ^{1}H NMR spectrum, revealing the full coupling pattern of the side-chain protons (Figure 1C), and especially those of the 3-proton centered at 7.56 ppm. By analysis of this spectrum it is possible to extract values for $J_{3,4}$ and $J_{3,5}$. The experimental shifts and coupling constants are given in Table I. Combining the information obtained from the steps above, the spectrum of the four-spin system was simulated, and the parameters were varied until good agreement with the experimental spectrum (Figure 1D) was reached. The calculated shifts and coupling constants are given in Table I.

Two-dimensional correlated spectroscopy of the substrate was subsequently performed to check the validity of the suspected coupling pattern (Figure 1E). It is also possible to extract some coupling constants from phase-sensitive COSY spectra,[11] and these show good agreement with the calculated values given in Table I.

The full NMR assignment of the configuration of the title compound is trivial from the material presented above. The assignment dilemma was resolved by the observation of the $J_{4,5}$ coupling as 14.25 Hz. This is sufficiently different from the cis coupling of 11.5 Hz,[1,2] to confirm that the compound with melting point 165 °C is the 2*E*,4*E* geometrical isomer. However, several points need to be accentuated, with relevance to more complicated systems.

First, tightly coupled systems such as the $J_{4,5}$ multiplet in this compound are extremely difficult to resolve in any other fashion than that employed here, i.e., tritiation at one of the sites, and observation of the ^{3}H–^{1}H coupling in the ^{3}H NMR spectrum. Note that the use of correlated spectroscopy is ineffective in this regard, as the chemical shift differences are much less than the coupling constant. As has been reported previously,[12] use of proton-coupled ^{3}H NMR is a very accurate way to establish proton coupling constants.

Second, ^{1}H chemical shifts in complicated spectra are accessible by selective tritiation of compounds and routine accumulation of ^{3}H NMR spectra. Recent publications[13–15]

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have also shown the use of ^3H–^1H correlation and J-resolved spectroscopy experiments to aid in labelling studies and in spectral assignment. It should be noted that neither change of solvent nor progressive addition of paramagnetic shift reagent (Eu(fod)$_3$) resolved the 4- and 5-protons in the ^1H NMR spectra—these chemical shifts were readily obtained from the ^3H NMR spectra.

In the course of preparing labeled compounds for these studies, two other interesting phenomena were observed. The first was an isotope effect in the oxidation of [1-^3H]benzyl alcohol to benzaldehyde; the analogous deuterium isotope effect has been reported previously.[16] Secondly, the proposed preparation of the 2-^3H compound by decomposition of cinnamylidenemalonic acid in the presence of HTO and pyridine yielded tritium in both the 2- *and* 4-positions. This reaction is believed to have two mechanisms under the conditions used in this preparation.[17] The first is decarboxylation of the diacid to yield label in the 2-position. The second has been proposed as involving formation of a lactone, exchange of the allylic position (4-position in the product compound), followed by a concerted ring opening and decarboxylation step, to yield the dienoic acid.

By way of clarifying the chemical shifts and coupling relations in the 2*E*,4*E* isomer of 5-phenylpenta-2,4-dienoic acid we have illustrated the usefulness of tritium and deuterium labeling in resolving NMR anomalies.

Experimental Section

NMR. Proton NMR spectra were obtained at both 90 and 300 MHz. The 90-MHz spectra were obtained on a Bruker WH-90 spectrometer, in deuteriated solvent, with tetramethylsilane as internal standard. The spectra were acquired at 25 °C, with pulse widths of 1.5–3 μs, recycle delays of 1.6–3.4 s, and 8K data points over a sweep width of 1200 Hz. Tritium NMR spectra were recorded at 96 MHz on the same spectrometer under the same conditions, and triton shifts were measured from a ghost reference derived from the ^1H signal of the internal Me_4Si.[18]

The 300-MHz proton NMR spectra were obtained on an IBM Instruments AF-300 spectrometer, at 25 °C, using 8K data points, a sweep width of 3 kHz, and a pulse width of 6 μs. The data for the phase-sensitive COSY, with sampling of 400 t_1 values, were collected and processed with standard Bruker DISNMR software.

The four-spin spectral simulation was calculated by the use of the PANIC program, which is also standard Bruker software for the AF series of spectrometer.

Syntheses: 5-Phenyl[2,4-$^3H_2$]penta-2,4-dienoic Acid. Cinnamylidenemalonic acid (300 mg), tritiated water (10 μL, 1850 GBq mL^{-1}), and pyridine (4 mL) were heated together under reflux for 8 h. Water (10 mL) was then added, the solution was acidified (3 N HCl), and the precipitate was recrystallized from benzene. The dienoic acid (110 mg; 6.66 GBq mmol^{-1}), mp 164–165 °C, had ^3H NMR [$(CD_3)_2SO$] δ 6.08 (s, 2-^3H) and 7.13 (s, 4-^3H).[17]

5-Phenyl[5-^3H]penta-2,4-dienoic Acid. This compound was prepared by the Reformatskii reaction of [1-^3H]benzaldehyde (95 mg) with ethyl γ-bromocrotonate (112 mg), followed by dehydration and alkaline hydrolysis. The [1-^3H]benzaldehyde (1.63 GBq mmol^{-1}) was prepared by oxidation of [1-^3H]benzyl alcohol (1.92 GBq mmol^{-1}) by CrO_3 in pyridine. The product of the Reformatskii reaction was dissolved in benzene and dehydration was effected by addition of a trace of *p*-toluenesulfonic acid and heating. Hydrolysis of the resultant ethyl ester was achieved by addition of a saturated solution of KOH in ethanol. The yield was 30 mg of material with specific activity 1.63 GBq mmol^{-1} and mp 165 °C. NMR analysis with broad-band proton decoupling yielded ^3H NMR [$CDCl_3$] δ 6.96 (s) and proton-coupled δ 6.90 (d, $J_{4^1H\text{-}5^3H}$ = 15.3 Hz).

5-Phenyl[$^2H_5$]penta-2,4-dienoic Acid. [$^2H_5$]Benzaldehyde (1.0 g) and ethyl γ-bromocrotonate (1.2 g) were used as the starting materials in the Reformatskii reaction. The product was isolated and purified as above, with a yield of 300 mg, mp 164–165 °C, M^+ 179. The ^1H NMR spectrum of the product is shown in Figure 1C, and the shifts and coupling constants are in Table I.

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syn- and *anti*-Dioxasesquinorbornenes. Singlet Oxidation of Exocyclic *s-cis*-1,3-Butadienes

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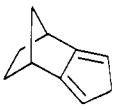
In the early 1980s a large amount of data was collected which demonstrated that isodicyclopentadiene (**1**) un-



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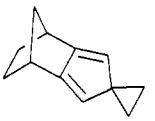
dergoes 4 + 2 cycloaddition preferentially on the bottom face.[1] Orbital tilting,[2] which results in severe closed shell repulsion when top approach is attempted, and a torsional/steric[3] argument were presented to explain this novel behavior. In 1980 Paquette and co-workers[4] reported that only moderate bottom selectivity was observed in the reaction of **1** with singlet oxygen. Endoperoxides, however, were not directly observed in the reactions of **1** and several of its alkylated derivatives[5] and the π-facial selectivity was inferred from examination of the decomposition products.

We report here that the endoperoxides from reactions of **1** and its spirocyclopropyl derivative **2** can be directly



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observed and the π-facial selectivity directly determined. In addition, we report the rates of singlet oxygen addition to both **1** and **2** and several exocyclic *s-cis*-1,3-butadienes.

The addition of singlet oxygen to **1** was accomplished by irradiation of an acetone-d_6 solution of **1** and rose bengal at −78 °C through at 0.5% potassium dichromate filter. The extent of reaction was monitored by ^1H and ^{13}C NMR at −80 °C. Upon completion of the reaction the proton-

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