Synthesis and ESR and ENDOR Investigation of Phenalenyl Radical, a Useful ENDOR Reference Compound

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Efforts to establish electron nuclear double resonance spectroscopy (ENDOR) are underway at Hampden-Sydney College. To this end, phenalenyl radical (PNT), a useful reference ENDOR compound, was successfully synthesized and ESR samples of the compound were prepared. The compound was prepared via a two step procedure from perinaphthenone. The perinapthhenone was reacted with p-toluenesulfonylhydrazide and then the intermediate product was reduced to the radical by sodium borohydride and work-up was undertaken. The successful synthesis was confirmed by the acquisition of ESR and ENDOR spectra of PNT. This report outlines in detail the synthetic procedure employed and ought to be very useful for anyone attempting to produce their own PNT samples for use as an ENDOR reference compound or just to produce a clear ESR spectrum for teaching purposes.

INTRODUCTION

Phenalenyl or perinaphthenyl radical (PNT) is useful in ENDOR spectroscopy because the literature contains well resolved spectra and the ESR and ENDOR spectra resulting from phenalene are comparatively simple because of the high degree of symmetry in the molecule (1). PNT is used to establish that an ENDOR spectrometer is functioning properly. The compound is not commercially available unless bundled with a new spectrometer. Thus, PNT had to be synthesized on-site at Hampden-Sydney College. The reader is encouraged to refer to the existing literature for an exhaustive treatment of the theory and applications ESR and ENDOR since this paper's concern is the synthesis of PNT (2; 3; 4; 5).
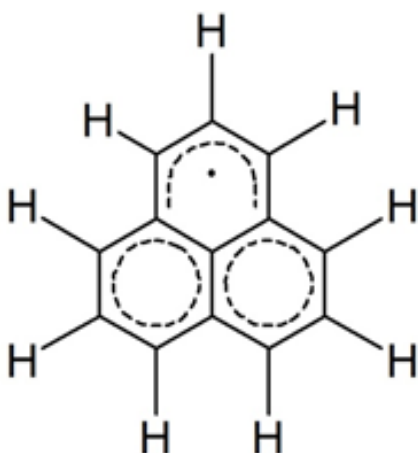

Fig1. The phenalenyl or perinaphthenyl radical (PNT), the end-product of the synthesis. Obtained in ca. 24% yield.

METHODS

The synthesis of PNT from perinaphthenone proceeded by a two-step process via a tosylhydrazide intermediate. Methanol was twice redistilled through a 50cm Vigreux column to reduce the water content (2). The reaction apparatus consisted of a three-neck 100mL round-bottom flask with an attached reflux condenser. The apparatus was flame-dried and flushed with nitrogen. The 50mL dried methanol were added. 1.10g perinaphthenone were added followed by 2.0g of p-toluenesulfonylhydrazide. Two drops of concentrated HCl were added and the mixture was refluxed under nitrogen for four hours. It was then let to cool slowly and after reaching room temperature was immersed in an ice-water bath. The product precipitated as a yellowish brown solid upon cooling.
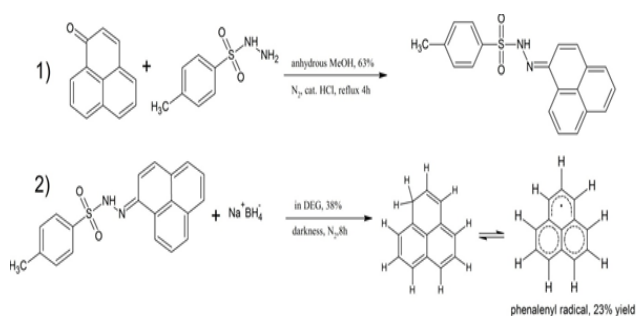

Fig 2. The two step synthesis as undertaken. Both reactions and the final workup had to take place under nitrogen and in the absence of water. The intermediate product was not especially air-sensitive and could be stored in a tightly closed vial for several weeks. The overall yield of the reaction was 24%.

The product, 4-methyl-N'-[(1Z)-1H-phenalen-1-ylidene] benzenesulfonohydrazide, was filtered and washed with ice-cold methanol three times. The

vacuum-dried product weighed 1.21g, which corresponds to a 63% yield. The product was found to have a melting point range from 177.4 – 178.2°C at a ramp rate of 2°C per minute and a melting point of about 183°C at a ramp rate of 10°C/min. Dr. Dwyer reported to the authors a melting point of 186-188°C at unspecified ramp rate in personal correspondence. The product was stored in a tightly sealed vial since it is only moderately air-sensitive. Our sample kept for several weeks but a two year old sample of the intermediate product from an unsuccessful earlier attempt at the synthesis had a black layer coating all the crystals

The second step of the procedure is the reduction of the intermediate product to phenalene, which is oxidized to phenalenyl radical, with which it exists in equilibrium, by trace amounts of oxygen present. The reaction apparatus consisted of a three-neck 100mL round-bottom flask with a powder addition funnel and a regular addition funnel attached. The third neck was furnished with a nitrogen gas inlet. The glassware was flame dried and flushed with nitrogen gas. The powder addition funnel was charged with 1.0g of $NaBH_4$ and the liquid addition funnel was charged with 15mL H_2O. Note that the authors recommend adding the water by syringe rather than by addition funnel since there were observed minute leaks from the funnel over the extensive stirring period that contaminating the reaction mixture with at least two drops of water. 0.5g of the intermediate product are dissolved in 30mL DEG in the round-bottom flask. The mixture was stirred under nitrogen for 30min. The reaction mixture was cooled to 0°C in an ice water bath with some NaCl added to expedite the process. The temperature was monitored via an IR thermometer from the outside. After the mixture had cooled the $NaBH_4$ was added slowly over 45min under vigorous stirring. Foaming was observed whenever the addition proceeded too rapidly. An orange precipitate begins to form. The reaction mixture is shielded from incident light with aluminum foil and is allowed to warm to room temperature slowly and more hydrogen gas evolution is observed. The mixture was left to stir in darkness overnight for 18h (only 8h are necessary). 15mL of H_2O were added slowly and the product mixture turned blackish changing to dark-green.

The work-up of the radical in DEG solution has to be conducted under the exclusion of oxygen as well. The stopcock on the nitrogen inlet was closed and the reaction flask was moved to a glove bag that had been flushed three times with nitrogen gas. All solvents used in the glove bag had been bubbled with nitrogen to minimize the exposure of the product to dissolved oxygen. The product mixture was extracted three times with 25mL hexane. The combined extracts were washed three times with 25mL H_2O. The hexane solution was dried with Na_2SO_4. The concentration of the radical in solution was measured via UV/Vis spectroscopy at an absorption maximum of 320nm at which the extinction coefficient is 7900. After determining the concentration, appropriate amounts of the hexane solution to produce samples of 0.5mM and 0.25mM concentration were added to ESR sample tubes filled with toluene or mineral oil via micro syringe. The ESR tubes are closed off with their stopcocks and removed from the glove bag. The samples underwent three cycles of freeze-pump-thaw degassing on a high vacuum line to remove trace oxygen which causes signal broadening and leads to the slow decay of the radical over time.

ESR spectra of the samples were easily obtained on a JEOL RE1X (Peabody, MA) spectrometer equipped with a TE-011 cylindrical cavity. The spectra were recorded with LabView by The National Instruments Company (Austin, TX) software that has been customized in house on a standard Windows PC. Analysis and simulation of spectra was conducted with the most up-to-date WinSim software as of may 2010, which is freely available on the NIEHS website. Figures were prepared with Corel Draw 12 by Corel (Toronto, ON) and Microsoft Excel 2007 (Microsoft, Redmond, WA). The conditions were varied but a microwave frequency of 9.43GHz, a center field of 3408G, a sweep width of 50G, a field modulation of 0.025G, a gain of 200, while conducting 1 hour-long scan will give a very good spectrum.

The ENDOR spectrum was obtained using the same apparatus but in addition an ENI (Rochester, NY) A-300 RF power amplifier, a Philips (Eindhoven, Netherlands) PM5193 programmable synthesizer / function generator, and a JEOL ENDOR ES-LHC3X cavity was used. The parameters for the acquisition of the ENDOR spectrum were as follows. B_0 was 3474.4G, the phase was set at 100.05°, the sweep range was twenty MHz, the RF amplitude was 0.6, the ENDOR frequency range was 14.79MHz from 4.79 to 24.79 MHz, each frequency step was 19.53kHz and 5 scans were conducted with 1024 points recorded.

The acquisition of higher quality ENDOR spectra has continued after preparation of this report.
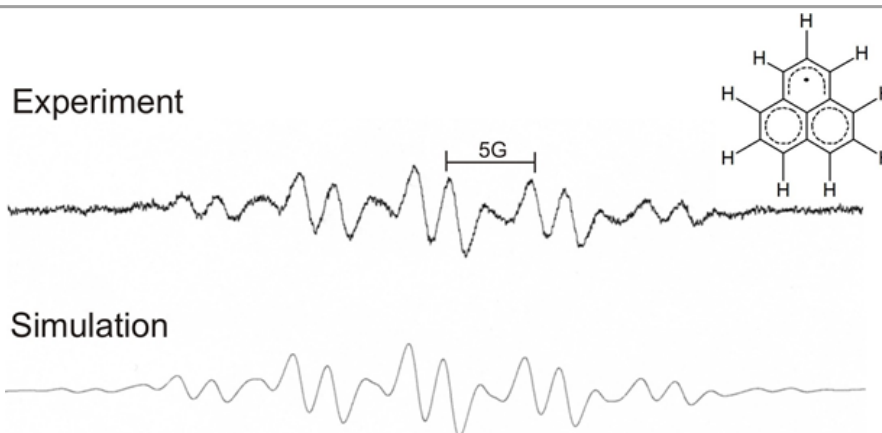
Figure 3. ESR spectrum of 0.5mM Phenalenyl radical in Toluene. The Simulation shows a 0.983 correlation with the experimental data. The septet split due to the 6 equivalent ortho hydrogen atoms can be observed, however, the outer quartet is too faint to distinguish from the noise. The Quartet splitting due to the three equivalent ipso hydrogen atoms can be observed in the central region of the spectrum. Toluene was found to be inferior to mineral oil as a solvent and this mineral oil was used in all subsequent experiments. The experimental conditions were: 1mW MW power, 9.400 GHz MW freq., 3407.9G center, 50G sweep width, 0.05 modulation amplitude, 50 gain, 0.03 sec time constant, 8192 data points were plotted, and 1 4 minute long scan was run
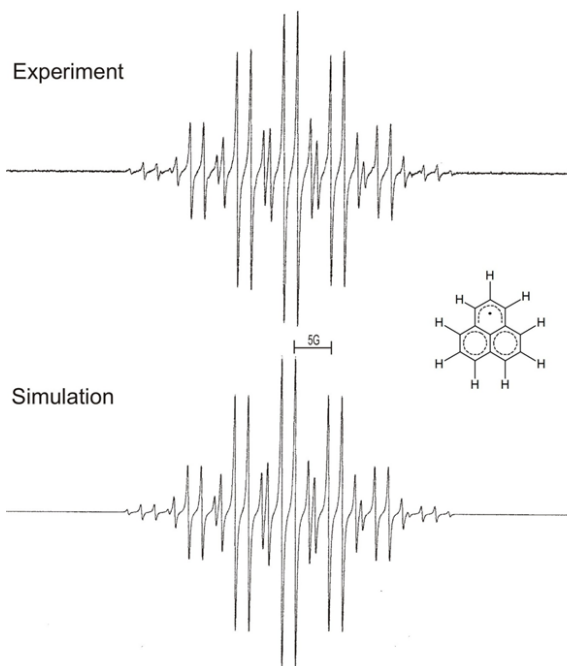

Figure 4. ESR spectrum of 0.5mM Phenalenyl radical in Mineral Oil. Simulation shows a 0.995 correlation with the experimental data. The septep of 6.4 G due to the 6 equivalent ortho hydrogen atoms, which is again split into 7 quartets of 1.8G by the 3 ipso hydrogen atoms, can be clearly observed. In addition, low intensity peaks arising from the nuclear spin of carbon 13 atoms can be observed. This spectrum was obtained after degassing three times by F-P-T. Compared with Figure 3 the line width has been markedly decreased. The experimental conditions were: 1mW MW power, 9,400 GHz MW freq., 3407.9G center, 80 G sweep width, 0.025 modulation amplitude, 200 gain, 0.03 sec time constant, 8192 data points were plotted, and 132 minute long scan was run.
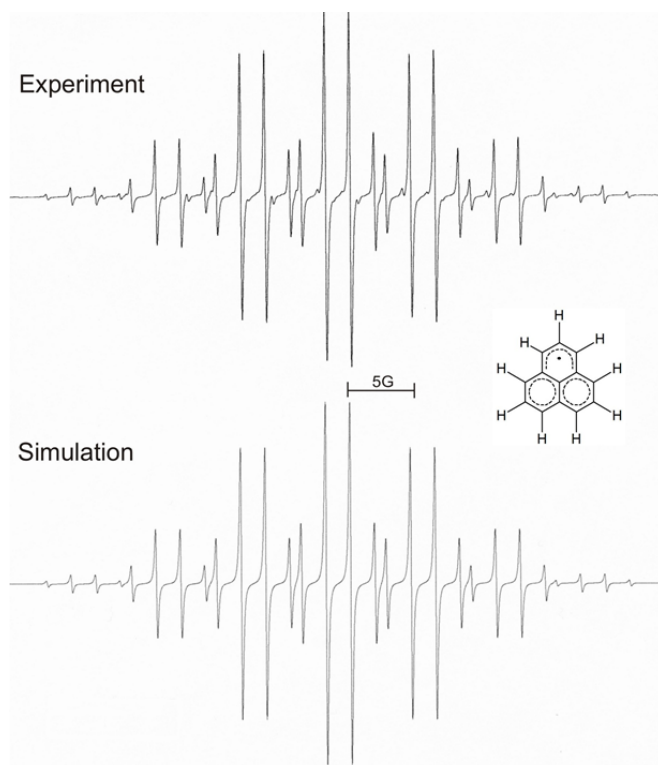
Figure 5. ESR spectrum of 0.5mM Phenalenyl radical in Mineral Oil. Simulation shows a 0.989 correlation with the experimental data. The septep of 6.4 G due to the 6 equivalent ortho hydrogen atoms, which is again split into 7 quartets of 1.8G by the 3 ipso hydrogen atoms, can be clearly observed. In addition, low intensity peaks arising from the nuclear spin of carbon 13 atoms can be observed. This spectrum was obtained after degassing three times by F-P-T. Compared with Figure 3 the line width has been markedly decreased. The experimental conditions were: 1mW MW power, 9,436 GHz MW freq., 3408.5G center, 50 G sweep width, 0.05 modulation amplitude, 100 gain, 1.0 sec time constant, 8192 data points were plotted, and 164 minute long scan was run.
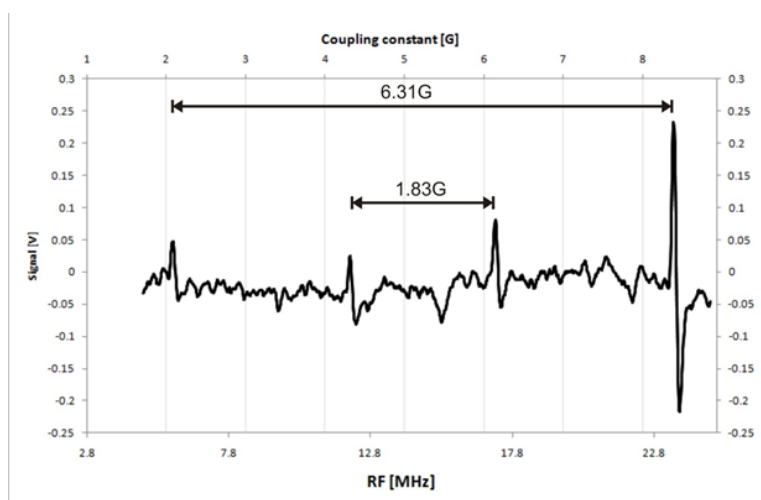

Fig 6. The first ENDOR spectrum of PNT that was acquired. The outer two lines clearly indicate the 6.3 G split due to the six equivalent ortho hydrogen atoms and the inner two lines represent the 1.8 G split due to the 3 equivalent ipso hydrogen atoms. The experimental conditions were a center RF frequency of 14.8 MHz and a 20 MHz sweep width, five five-minute-long scans were conducted, the RF amplitude was 0.6, the phase was 100.05 degrees and the magnetic center field was at 3474, and the microwave frequency was 9.63 GHz.

RESULTS AND DISCUSSION

The reaction was undertaken in two steps and each step was followed by work-up. The first step proceeded with 63% yield. The intermediate product was then reduced with to give the phenalenyl radical in approximately 38% yield. The overall yield of the synthesis was approximately 24%. This was still enough material for many more samples than needed to be prepared. It should be noted that the radical exists in equilibrium with the parent compound phenalene in solution and that therefore only about half of the compound may have been measured via spectroscopy, provided that the two compounds do not share an absorption maximum at 320nm,which would indicate a yield twice as high for the overall reaction. Four ESR sample tubes containing PNT in approximately 0.25mM and 0.50mM concentration in toluene and mineral oil containing a small amount of hexane were prepared. ESR spectra of the PNT solutions were obtained and it was decided that mineral oil is a more suitable solvent than toluene. We believe this is due to mineral oil's higher viscosity which limits tumbling of the radical and thus makes acquisition of spectra under depressed temperature unnecessary. The concentration of the sample did not influence the quality of the ESR spectra discernibly but the ENDOR spectra of the higher concentration samples were of higher resolution.

The ESR spectra clearly show the hyperfine splitting due to the six equivalent ortho hydrogen atoms that give rise to the 6.4G septet splitting. This septet is split once again by the three equivalent hydrogen atoms in the ipso positions to give rise to the 1.8G quartet splitting. In addition,small magnitude splitting due to ^{13}C atoms can be observed in the degassed sample of PNT in mineral oil solution.

The ENDOR spectrum, despite a thus far not satisfactory signal to noise ratio, clearly displays the two coupling constants in PNT. The outer pair of lines corresponds to the stronger (6.4G) splitting while the inner pair of lines corresponds to the weaker (1.8G) splitting. This spectrum illustrates the simplicity of the spectra obtained by ENDOR that makes the technique so attractive.

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