Preparation of Spin-Labeled Analogs of the Antitumor Agents TEPA and Thio-TEPA 1, 2
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In recent years, a considerable amount of work has been done in order to elucidate the role of free radical intermediates in carcinogenesis3. Particularly, with the EPR technique a series of interesting but controversial results have been obtained6. As a consequence, to date, no conclusions can be made concerning the role of free radicals and other paramagnetic species during the induction and propagation of tumors5. However, there is hope that it might be possible to develop the EPR technique into a useful diagnostic tool for detection of tumors5.

Until present, the application of chemotherapy in treatment of cancer has been of modest success4. Nevertheless, in certain types of cancers a considerable progress has been achieved, and therefore, there exists a justifiable optimism that with the advance in better understanding of the mechanisms of initiation and propagation of tumors, more effective drugs can also be devised for treatment of cancers4.

Now we would like to report the synthesis of two new spin-labeled analogs (5, X = O and 5, X = S) of the well known antineoplastic drugs TEPA (6, X = O) and Thio-TEPA (6, X = S)5. It is believed that the new compounds will be of value in cancer research since they possess not only an easily measurable susceptibility but may also exhibit through the nitroxy moiety an inhibitory-sequenching effect on radicals and/or other paramagnetic species in tumor tissues4.

The synthesis of 5 (X = O) and 5 (X = S) was achieved by a "one-batch" process. Thus, the reaction of a benzene solution of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (1)6 with either phosphorus oxychloride (2, X = O) or thiochloride (2, X = S) in the presence of triethylamine resulted in the formation of intermediates 3 (X = O) and 3 (X = S), respectively.

Without isolating these intermediates were allowed further to react with ethyleneimine (4) in the presence of additional triethylamine to give the products 5 (X = O) and 5 (X = S).

3 + 2 HN=<(C2H5)3N/C6H5 □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ ...
0.5 hour, followed by a solution of ethyleneimine (1.72 g, 0.04 mole) in dry benzene (40 ml) which was added during 0.8 hour at 6 – 8 °C. After stirring at 10 °C for 1 hour, the mixture was stirred at room temperature for 7 days. The suspension was then filtered to remove the triethylamine hydrochloride (5.23 g, 95% of theory), and the filtrate was concentrated on a rotating evaporator at 40 °C (25 mm Hg). The remaining red oil was dissolved in benzene, and the solution chromatographed on an alumina column (1 g oil/10 g Al₂O₃) using a 4:1 (v/v) solution of benzene and ethyl acetate as eluent. Concentration of the eluted solution gave a red oil which solidified on storage in a refrigerator overnight to a red solid, 3.7 g (61%); m.p. 56 – 60 °C (dec.). EPR: 3 equidistant lines of equal intensity, ₐₑ = 15 G.

Analysis

C₁₃H₂₅N₃O₃P:
Calcd:  C 51.64 H 8.33 N 13.90 Mol. wt. 302.32,
Found: C 51.43 H 8.01 N 13.60 Mol. wt. 309.

0-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidyl)-N,N;
N,N'-bis(ethylene)-phosphorodiamidithioate
(5, X = S)

A solution of phosphorus thiochloride (3.39 g, 0.02 mole) in dry benzene (100 ml) was cooled to 6 – 8 °C, and vigorously stirred under anhydrous conditions. A solution of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (1, 3.44 g, 0.02 mole) and triethylamine (2.02 g, 0.02 mole) in dry benzene (100 ml) was then added during 1.0 hour. After stirring at 10 °C for 1.5 hour, the reaction mixture was allowed to warm up to room temperature, and the stirring continued for 24 days. The precipitated triethylamine hydrochloride was removed by filtration (2.75 g, 100% of theory). The filtrate containing O-(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl) phosphorodichloridithioate (3, X = S, 0.02 mole) in dry benzene (200 ml) was cooled to 5 – 8 °C and vigorously stirred. A solution of ethyleneimine (1.72 g, 0.04 mole) and triethylamine (4.04 g, 0.04 mole) in dry benzene (70 ml) was added during 1.5 hours. After stirring for 2 hours at 10 °C, the reaction mixture was allowed to warm up to room temperature, and the stirring continued for 6 days. The reaction mixture was then filtered to remove the triethylamine hydrochloride (5.28 g, 96% of theory). After removal of the solvent on a rotating evaporator at 40 °C (25 mm Hg), the remaining red oil was dissolved in dry benzene, and the solution chromatographed on a alumina column (1.0 g oil/10 g Al₂O₃) using a 4:1 (v/v) solution of benzene and ethyl acetate as eluent. Concentration of the eluted solution gave a red solid, 3.0 g (50%); m.p. 91 – 93 °C (dec.). EPR: 3 equidistant lines of equal intensity, ₐₑ = 15 G.

Analysis.

C₁₃H₂₅N₃O₂PS:
Calcd:  C 49.06 H 7.86 N 13.20 Mol. wt. 318.38,
Found: C 49.07 H 7.76 N 13.02 Mol. wt. 315.

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2 Mention of commercial or proprietary names in this paper does not constitute an endorsement or quality preference of these products over other commercial products of the same chemical composition by the authors, editors, or publishers.

3 H. M. Swartz, Advances Cancer Res. 15, 227 [1972], and references therein.


