Preparation of Spin-Labeled Analogs of the Antitumor Agents TEPA and Thio-TEPA [1,2]

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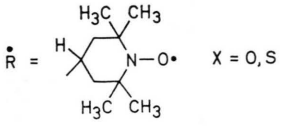
In recent years, a considerable amount of work has been done in order to elucidate the role of free radical intermediates in carcinogenesis [3]. Particularly, with the EPR technique a series of interesting but controversial results have been obtained [3]. As a consequence, to date, no conclusions can be made concerning the role of free radicals and other paramagnetig species during the induction and propagation of tumors [3]. However, there is hope that it might be possible to develop the EPR technique into a useful diagnostic tool for detection of tumors [3].

Until present, the application of chemotherapy in treatment of cancer has been of modest success [4]. 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 cancers [4].

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 by EPR-detectable label but may also exhibit through the nitroxyl moiety an inhibitory-scavening effect on radicals and/or other paramagnetic species in tumor tissues [6].

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.

$$\underset{\mathbf{1}}{\overset{\bullet}{\mathrm{ROH}}} + \underset{\mathbf{2}}{\mathrm{P(X)Cl_3}} \xrightarrow{(\mathrm{C_2H_5})_3\mathrm{N}/\mathrm{C_6H_9}} \underset{\mathbf{3}}{\overset{\bullet}{\mathrm{ROP(X)Cl_2}}}$$



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Without isolating them, 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).

$$\mathbf{3} + 2\,\mathrm{HN}\langle\!\rangle \xrightarrow{(\mathrm{C_2H_5})_3\mathrm{N}/\mathrm{C_6H_6}} \underset{\mathbf{5}}{\overset{\bullet}{\mathrm{ROP(X)}}}\left(\mathrm{N}\langle\!\rangle\right)_2$$

4

Both compounds are well defined red solids. The EPR spectroscopy of these compounds shows three equidistant lines of equal intensity with a hyperfine splitting a_N of 15 G. These spectra are characteristic of the mononitroxyl moiety [6].

An analogous spin-labeled compound (**7**) was independently prepared by Rozantsev and coworkers [7].

$$\underset{\mathbf{6}}{\mathrm{P(X)}\left(\mathrm{N}\langle\!\rangle\right)_3} \qquad \underset{\mathbf{7}}{\overset{\bullet}{\underset{\mathrm{H}}{\mathrm{RNP(S)}}}\left(\mathrm{N}\langle\!\rangle\right)_2} \qquad \underset{\mathbf{8}}{\overset{\bullet}{\mathrm{RNH_2}}}$$

However, the starting material, 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (**8**) is much more difficult to prepare than **1**. Therefore, the present procedure for the preparation of spin-labeled analogs of antineoplastic drugs seems to be the method of choice.

Experimental

*1-Oxyl-2,2,6,6-tetramethyl-4-piperidyl-N,N;N',N'-bis(ethylene)-phosphorodiamidate (**5**, X = O)*

A solution of phosphorus oxychloride (3.06 g, 0.02 mole) in dry benzene (100 ml) was cooled to 5–8 °C, and vigorously stirred under anhydrous conditions. A solution of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl [6] (**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 hour. After an additional 1 hour at 10 °C, the reaction mixture was allowed to warm up to room temperature (20 °C), and stirred overnight. The precipitated triethylamine hydrochloride was removed by filtration (2.70 g, 98% of theory). The filtrate containing 1-oxyl-2,2,6,6-tetramethyl-4-piperidyl-phosphorodichloridate (**3**, X = O, 0.02 mole) in dry benzene (250 ml) was cooled to 5–6 °C and vigorously stirred. A solution of triethylamine (4.04 g, 0.04 mole) in dry benzene (30 ml) was added during

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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 Al_2O_3) 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 strorage 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, $a_N = 15$ G.

Analysis

$C_{13}H_{25}N_3O_3P$:
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.

*O-(1-Oxyl-2,2,6,6-tetramethly-4-piperidyl)-N,N; N',N'-bis(ethylene)-phosphorodiamidothioate (**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[6] (**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) phosphorodichloridothioate (**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 Al_2O_3) 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, $a_N = 15$ G.

Analysis.

$C_{13}H_{25}N_3O_2PS$:
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 proprietory 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.

[4] C. G. Schmidt, Krebsforschung und Krebsbekämpfung, eds. H. E. Bock and U. Dold, **vol. 6**, p. 309, Urban & Schwarzenberg, München 1967, and references therein.

[5] The Merck Index, ed. P. G. Stecher, 8th edition, p. 1073, Merck & Co., Inc., Rahway, N.J., USA 1968.

[6] E. G. Rozantsev, Free Nitroxyl Radicals, pp. 185, 214, and references therein, Plenum Publ., New York 1970.

[7] A. B. Shapiro, A. A. Kropacheva, V. I. Suskina, B. V. Rozynov, and E. G. Rozantsev, Izv. Akad. Nauk SSSR, Ser. Khim. **1965**, 864.

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