SRI 62-834, an analog of the antitumor agent ET-18-OCH₃ in which the oxygen atom at carbon atom 2 has been incorporated into a five-membered heterocycle, has been prepared and evaluated as an antitumor agent. The compound exhibited good cytotoxicity in vitro against a variety of tumor cell lines and was as effective as ET-18-OCH₃ given orally in the mouse Meth A sarcoma model. SRI 62-834 was shown to be an inhibitor of platelet-derived growth factor (PDGF), possibly at the receptor level, and platelet-activating factor (PAF) at the receptor level.

**Lipids 22, 884-890 (1987).**

The ether phospholipid ET-18-OCH₃ (Fig. 1) has been shown to exhibit neoplastic activity in vitro and in vivo against a wide variety of tumor cell lines (1). Considerable preclinical studies suggest that the antitumor action of this agent may be attributed to generation of cytotoxic macrophages (2-4), direct cytotoxicity (5), diminished activity of the alkyl cleavage enzymes present in tumors (6-9), selective cell membrane interactions (10) and inhibition of a phospholipid cofactor of a phospholipid-sensitive Ca²⁺-dependent protein kinase (11). In addition, it has been shown that ET-18-OCH₃ can purge murine leukemia bone marrow, eliminate leukemic blasts and allow hematopoietic reconstitution to occur (12). Unlike most substances being used in the clinical treatment of tumors, ET-18-OCH₃ does not appear to have a direct effect on DNA synthesis or functions, and it is nonmutagenic (5,13). Clinical phase I and II studies with ET-18-OCH₃ against a variety of advanced solid tumors have recently appeared (1,14).

Our laboratories have been involved in the synthesis and evaluation of a variety of cyclic ether analogs of ET-18-OCH₃ as potential antitumor agents (Houlihan, W.J., Munder, P.G., Lee, M.L., Parrino, V.A., and Cheon, H., unpublished data). Here we present our findings with SRI 62-834, a tetrahydrofuran analog of ET-18-OCH₃ (cf Fig. 2), against a variety of tumor cell lines, platelet-derived growth factor (PDGF) and platelet-activating factor (PAF).

**MATERIALS AND METHODS**

**Chemical synthesis.** Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) data for H-NMR were taken on JEOL-FX-90 (90 MHz) or JEOL-FX-200 (200 MHz) spectrophotometers and are reported in δ (ppm) downfield from tetramethylsilane (TMS). ¹³C-NMR were determined at 22.5 MHz or 50.1 MHz on the JEOL instruments, respectively, with CDCl₃ (77.0) and TMS (0.0) as internal reference. ³¹P-NMR was measured at 80.76 MHz on a JEOL-FX-200 with H₃PO₄ as external reference. If not otherwise specified, chemicals and reagents were obtained from the Aldrich Chemical Co. (Milwaukee, Wisconsin). Solvents were of reagent grade and dried prior to use. Reaction progress and purity of final products were determined on E. Merck Silica Gel 60 chromatography plates. Column chromatography was carried out using E. Merck Silica Gel CH83 (0.06-0.20 mesh) with the indicated eluants. Eluants and RF values are reported where appropriate. The purity of SRI 62-834 was also assessed by high pressure liquid chromatography (HPLC) at 1500 psi on a Beckman 345 instrument with RI detection using a Lichrosorb Si 60 5 μ dp column eluted with methanol/chloroform/water (6:5:0.5, v/v/v) at 1.5 ml/min. Retention time is reported at a chart speed of 0.2 cm/min.

(±)-Methyltetrahydro-2-furoate. A suspension of 2-furoic acid (34.9 g, 0.31 mol), 95% ethanol (125 ml) and 10% palladium on carbon catalyst (1.6 g) in a pressure bottle was affixed to a Parr hydrogenation apparatus under 45 psi hydrogen and maintained at room temperature. After hydrogen uptake was complete (ca. 5 hr), the reaction mixture was filtered through a bed of celite at room temperature. The filter bed was washed with 95% ethanol, and the combined filtrates were concentrated in vacuo to give 30.0 g (77%) of a colorless oil. Anal. calc. C, 55.4; H, 7.7. Found C, 55.4; H, 7.7.

2,2-Bis-Hydroxymethyltetrahydrofuran. A stirred solution of methyltetrahydro-2-furoate (21.0 g, 0.16 mol) in anhydrous tetrahydrofuran (180 ml) under a nitrogen atmosphere was cooled to an internal temperature of -65 ± 5 °C and then treated dropwise with 25% diisobutylaluminum hydride solution in toluene (102 g, 0.17 mol) at such a rate that the internal temperature did not exceed -60 °C. The mixture was stirred for an additional 1.5 hr at -60 °C and then treated carefully with anhydr-
ous methanol (23 ml) such that the temperature did not exceed -50 C. The solution was allowed to warm to 5 C and then was added to a stirred solution of 37% aqueous formaldehyde (226 ml, 3.9 mol), sodium hydroxide (52 g, 1.3 mol) and water (170 ml) while maintaining the internal temperature at 10 ± 5 C. The mixture was treated with formic acid (6.8 ml) and then concentrated in vacuo. The residue was filtered, and the solid washed with methylene chloride (360 ml). The filtrate was concentrated with methylene chloride (90 ml) and stirred for ca. 0.25 hr. The resultant slurry was filtered, and the solid washed with methylene chloride (360 ml). The filtrate was concentrated in vacuo. The residue (11.0 g) was dissolved in water (100 ml) and extracted with diethyl ether (100 ml, three times). The ether layer was washed with saturated sodium chloride solution, dried with anhydrous magnesium sulfate, filtered and concentrated in vacuo. The residue (11.0 g) was chromatographed (chloroform/methanol) to give 5.8 g (50%) of a white solid; mp 58–60 C (lit. mp 51–53 C).

To a stirred solution of (±)-2-Hydroxymethyl-2-octadecyloxytetrahydrofurran (9.9 g, 0.075 mol) in dimethyl sulfoxide and tetrahydrofurran. To a stirred solution of 2,2-bis-hydroxymethyltetrahydrofurran (11.0, 100 ml) was added at room temperature powdered potassium hydroxide (4.2 g, 0.075 mol) followed by dropwise addition of n-octadecylbromide (8.4 g, 0.025 mol) in tetrahydrofurran (30 ml). The reaction mixture was stirred for ca. 0.25 hr and then was added to a stirred solution of (±)-2-{Hydroxy[tetrahydro-2-(octadecyloxyl)methyl]phosphinyloxy} -N,N,N-trimethylethanol (16) (1.4 g, 9.8 mmol). The reaction mixture was stirred for 16 hr at room temperature and then concentrated in vacuo. The residue was treated with water (100 ml) and extracted with diethyl ether (100 ml, three times). The ether layer was washed with saturated sodium chloride solution, dried with anhydrous magnesium sulfate, filtered and concentrated in vacuo. The residue (11.0 g) was chromatographed (1:1 methyl t-butylether/hexane followed by 3:1 methyl t-butylether/hexane) to give 5.8 g (33%) of a white sticky solid, Rf 0.52 C. (±)-2-Hydroxymethyl-2-octadecyloxymethyltetrahydrofurran (33%) of a white sticky solid, Rf 0.52 C.

Calc C, 74.1; H, 12.4. Found: C, 74.0; H, 12.5.

To a stirred solution of 2,2-bis-hydroxy-5-[3H]thymidine was obtained from Amersham and Buchringer (20). Cultivation was effected by placing a suspension of 3 × 10^6 bone marrow cells/50 ml DMEM, 10% FCS, 5% horse serum and 5% supernatant of L929 fibroblast culture containing stimulating factor into ethylene oxide sterilized 30 × 5 cm Teflon bags (Biofolie, Heraeus, Hanau, FRG) as previously described (18). The cells are cultivated on the hydrophobic side of polymeric fluorocarbon film, and in 8–12 days the precursor cells develop into mature macrophages (19). Macrophages are brought into single cell by rolling the cultivation suspension side of the bag slightly without pressure between two fingertips. After 12 min, the moderately attached macrophages came off. After sterilizing, the cell suspension was centrifuged on a Ficoll hypaque layer at 500 g for 30 rain, and the mononuclear cells were washed, first at 400 g for 10 min and then at 250 g for 10 min. Biochemicals and radiochemicals. ET-18-OCH3 (rac-1-octadecyl-2-methoxy-3-phosphorylcholine) was obtained from R. Berchthold (Biochemicals Laboratory, Bern, Switzerland); human PDGF (300,000–500,000 units/mg where one unit is defined as the amount of PDGF needed to stimulate the incorporation of ^3H]thymidine into 3T3 fibroblast DNA to the same extent as 5% calf serum (20)) was obtained from Cellular Products, Buffalo, NY; ^3H]thymidine was obtained from Amersham and Buchler (Braunschweig, FRG) or ICN (Irvine, CA); ^125I-PDGF was prepared by Dr. Larry D. Witte (Columbia University, College of Physicians and Surgeons, New York City, NY) using the method outlined by Bowen-Pope and Ross (21); ^3H-PAF (9,10-1-O-octadecyl-2-H) was prepared in the

**Cell lines.** The tumor cell line Abelson-8.1 was obtained from A.W. Harris (Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia) and the YAC-1 from G. Klein (Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden). The cells were grown in stationary suspension culture in Dulbecco Modified Eagle’s Medium (DMEM) and 10% heat inactivated fetal calf serum (FCS) supplemented with 50 µM 2-mercaptoethanol, 100 units penicillin and 1 µg streptomycin. The P815 and L1210 cells were from the Max-Planck-Institut fuer Immunobiologie cell line collection and were passed intraperitoneally (ip) in vivo every week. The MethA fibrosarcoma cells were originally induced in BALB/C mice by administering methylcholanthrene according to the procedure of Old et al. (17).

The human neuroblastoma cells were obtained by dissociating and culturing a freshly explanted tumor specimen. The cells were used after weekly passage for four weeks in DMEM and supplements.

Human foreskin fibroblasts (AG1523) were obtained from the Institute of Medical Research (Carmen, New Jersey) and propagated from frozen stock in DMEM (pH 7.8) supplemented with 20% FCS, 1% nonessential amino acids (Gibco Cat. No. 320-1140), 1% essential amino acids (Gibco Cat. No. 320-1135), 1% vitamins (Gibco Cat. No. 320-1120), 6 mM glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin. The cells (passages 6 or 7) were maintained at 37 C under a humidified atmosphere of 95% air/5% CO₂.