conformation when bound to DNA.

The large upfield shifts of the aromatic protons of **1S** and **1N** also agree with a model of intercalation of the nonfused rings with DNA. The small shift of the N-CH_3 (and other side chain) protons indicates that the cationic substituent, as expected, is not intercalated. Similar experiments with other intercalators have shown large differences in the aromatic ring proton chemical shifts that depend on the overlap of the intercalator and the DNA base pairs.[12] The similarity of the shifts for all protons on **1S** and all protons on **1N** indicates that both the thiophene and pyrimidine rings are intercalated in the complex and that both have very similar overlap with the DNA base pairs.[12] The slightly smaller shifts for **1N** are probably due primarily to the weaker binding of **1N** than **1S** to DNA.

An interesting result of this study is that even though **1S** and **1N** have similar conformations and both bind to DNA by intercalation, their strength of interaction with DNA is significantly different. The increase in DNA Tm is larger for **1S** than for **1N** and the equilibrium constant for **1S** binding to DNA more than 4 times the binding constant for **1N** with DNA under the same conditions. In a standard biological test with phleomycin, **1S** shows significantly higher activity than **1N**.[1] Since our NMR studies, using ligand and DNA ^1H and ^{31}P signals, have shown that the complexes of **1S** and **1N** with DNA base pairs are structurally similar, this difference in interaction strength was at first quite surprising. However, this difference can be explained, assuming that charge-transfer forces are important for the intercalation complexes of **1S** and **1N** with DNA. Thus, compound **1S** has the lower π-electron density, as reflected by INDO/S calculations, and it is expected to form the stronger complexes in which DNA bases act as electron donors. A similar interaction model, in which charge-transfer forces are believed to be important, has been employed to describe binding of nucleosides to riboflavin.[13] In addition, the dipole moment of **1S** is larger than for **1N**, but the dipole moments have the same relative orientation in the molecular systems. This would serve to orient the molecules in a similar manner with respect to the dipole moment of the DNA base pairs, as observed, but would give a stronger interaction of **1S** with the base pairs than **1N**, also as observed.

In summary, all of these experimental findings conclusively demonstrate that **1S** and **1N** bind to DNA by intercalation with their rings in an s-cis conformation. In agreement with the biological activity of the two compounds, the interaction of **1S** with DNA is significantly stronger than for **1N**, and these results strongly suggest that phleomycin amplification by these compounds may involve an interaction of the amplifier and phleomycin with DNA. More detailed studies of these compounds are in progress.

Acknowledgment. This work was supported by the American Cancer Society, by NIH Grant GM 30267 (W.D.W.), and by Grant 85-34 from the Milheim Foundation for Cancer Research (L.S.).

(12) Chandrasekaran, S.; Kusuma, S.; Boykin, D. W.; Wilson, W. D. *Magn. Res. Chem.*, in press.

(13) Ts'o, P. O. P. In *Basic Principles in Nucleic Acid Chemistry*; Ts'o, P. O. P., Ed.; Academic: New York, 1974; pp 526–562.

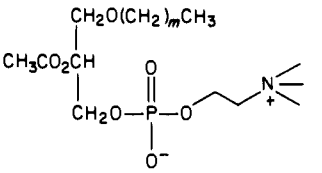
Analogues of Platelet Activating Factor. 5.[1] Multiple Oxygen Substitution of the Alkoxy Chain

A. Wissner,* C. A. Kohler, and B. M. Goldstein

Metabolic Disease Research and Cardiovascular Biological Research Sections, Medical Research Division, American Cyanamid Company, Lederle Laboratories, Pearl River, New York 10965. *Received November 18, 1985*

Racemic analogues of platelet activating factor (PAF) in which the alkoxy chain ($R = -O(CH_2)_{15}CH_3$) has been replaced with a chain containing multiple ether linkages near the end (17, $R = -O(CH_2)_8O(CH_2)_2O(CH_2)_2OCH_3$) or at the beginning (18, $R = -O(CH_2)_2O(CH_2)_2O(CH_2)_9CH_3$) of the chain have been prepared. Both compounds exhibit reduced hypotensive and platelet aggregation responses compared to racemic C_{16}-PAF (**1a**). This reduction in the biological activities is more apparent when the new oxygen atoms are located near the end of the chain. This substitution of additional oxygen atoms into the alkoxy chain does not result in any dramatic gains in selectivity of the biological responses.

Platelet activating factor (PAF) is an alkyl ether phospholipid of structure **1** composed primarily of the C_{16} and C_{18} homologues. It is a potent activator of various inflammatory cells such as platelets, neutrophils, and basophils and is one of the important mediators of anaphylaxis and inflammation. PAF is also a potent hypotensive agent.[2]



1a, $m = 15$
b, $m = 17$

Over the last few years a number of groups have reported the preparation and biological activity of PAF

(1) For the previous publication in the series see: Wissner, A.; Sum, P.-E.; Schaub, R. E.; Kohler, C. A.; Goldstein, B. M. *J. Med. Chem.* **1985**, *29*, 328.

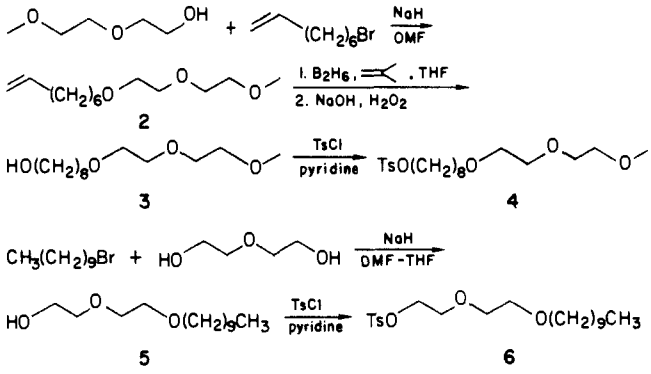
0022-2623/86/1829-1315$01.50/0

Table I. Blood Pressure and Platelet Aggregation Activities for PAF Analogues

compd	MABP[a]	platelet EC_{50}[b]	max aggregation resp[c]	ratio[d]
1a	1.25 (0.30, 4.98)	2.65×10^{-8} (n = 9)	71 (6.8×10^{-6})	0.47
17	80.5 (8.0, 877.4)	5.3×10^{-6} n = 1)	67 (1.9×10^{-4}	0.15
18	12.9 (4.8, 35.7)	1.3×10^{-7} (n = 1)	61 (1.9×10^{-4})	0.99

[a] Dose (μg/kg, iv) required to decrease mean arterial blood pressure (MABP) 50 mmHg. Values in parentheses are 95% confidence limits. [b] Molar concentration required to produce 50% of maximum aggregation. The n values are the number of experiments in which a dose–response curve was determined over the range of 10^{-4}–10^{-9} M with two to six replicates per dose level. [c] Maximum aggregation observed at the indicated molar concentration. [d] (MAPB/platelet EC_{50}) $\times 10^{-8}$.

Scheme I

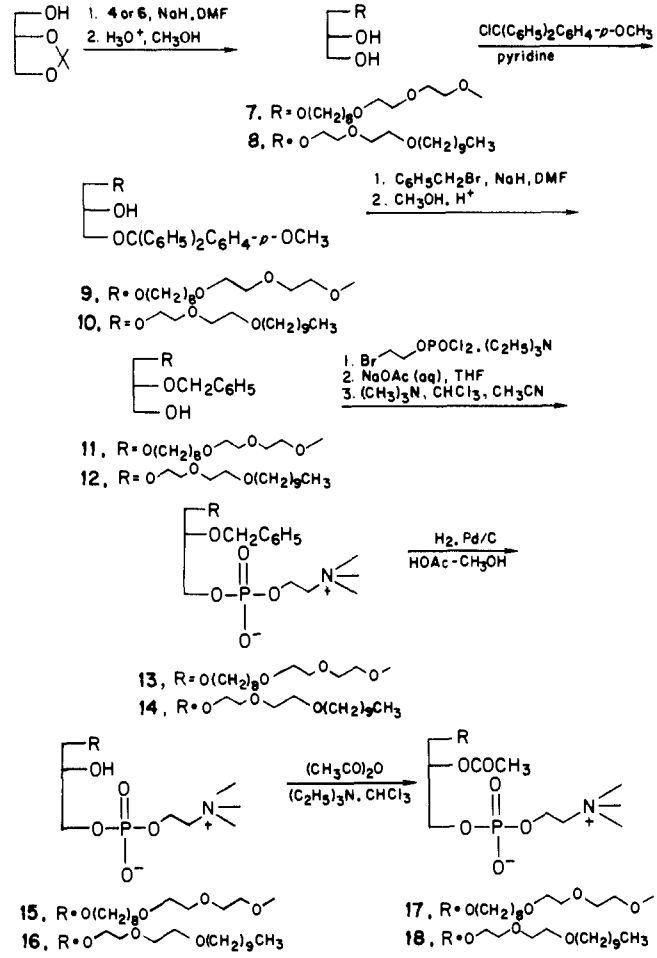


analogues that incorporate various modifications of the alkyl ether chain. In particular, we,[3] as well as others,[4] reported that removal of the ether oxygen results in a substantially decreased biological response. Another group has observed a similar effect by replacing the ether oxygen with a sulfur atom.[5] Increasing the degree of unsaturation in the alkoxy chain, however, has only minimal effects on the biological activity.[6] We have also shown that steric effects involving the alkyl ether chain are important.[3] In a continuation of these studies, we presently describe the preparation and biological activity of two additional PAF analogues in which the alkoxy chain now incorporates multiple ether oxygens. It was expected that this type of modification would affect the lipophilic properties of the alkoxy chain and thus may alter the blood pressure lowering and platelet aggregation responses.

Chemistry

It was of interest to prepare two racemic analogues **17** and **18**, each incorporating a group of three oxygen atoms interspersed by two ethylene units. In one compound (**17**), this group is placed near the end of the alkoxy chain, while in the other (**18**), it is placed at the beginning. These two analogues are prepared by an identical synthetic strategy that involves the initial preparation of tosylates **4** and **6** (Scheme I).

Scheme II



Alkylation of 2-(2-methoxyethoxy)ethanol with 8-bromo-1-octene using sodium hydride in DMF gives the olefin **2** in 80% yield. Hydroboration–oxidation of **2** gives the alcohol **3**, which is converted to the tosylate **4** by the usual procedure.

The tosylate **6** is prepared by alkylation of diethylene glycol with 1-bromodecane using sodium hydride in a mixture of DMF and THF followed by the reaction of the resulting alcohol **5** with tosyl chloride in pyridine. The monoalkylated product **5** is easily separated from the dialkylated product by distillation. The tosylates **4** and **6** are then converted to the racemic PAF analogues **17** and **18** as shown in Scheme II.

Alkylation of either **4** or **6** with solketal[7] using sodium hydride in DMF followed by acid-catalyzed solvolysis of the ketal group furnishes the diols **7** and **8** in 44 and 86%

(2) For recent reviews of the biological properties of PAF see: (a) Pinckard, R. N.; McManus, L. M.; Hanahan, D. J. *Adv. Inflammation Res.* **1982**, *4*, 147. (b) Snyder, F. *Ann. Rep. Med. Chem.* **1982**, *17*, 243. (c) Vargaftig, B. B.; Chegnord, M.; Benveniste, J.; Lefort, J.; Wal, F. *Ann. N.Y. Acad. Sci.* **1981**, *370*, 119. (d) O'Flaherty, J. T.; Wykle, R. L. *Clin. Rev. Allergy* **1983**, *1*, 353. (e) Vargaftig, B. B.; Benveniste, J. *Trends Pharmacol. Sci.* **1983**, *4*, 341.

(3) Wissner, A.; Sum, P.-E.; Schaub, R. E.; Kohler, C. A.; Goldstein, B. M. *J. Med. Chem.* **1984**, *27*, 1174.

(4) (a) Nakamura, N.; Miyazaki, H.; Ohkawa, N.; Koike, H.; Sada, T.; Asai, F.; Kobayashi, S. *Chem. Pharm. Bull.* **1984**, *32*, 2452. (b) Broquet, C.; Teulade, M.; Borghero, C.; Heymans, F.; Godfroid, J.; Lefort, J.; Coeffier, E.; Pirotzky, E. *Eur. J. Med. Chem.* **1984**, *19*, 229.

(5) Hillmar, I.; Muramatsu, T.; Zollner, N. *Z. Physiol. Chem.* **1984**, *365*, 33.

(6) Surles, J. R.; Wykle, R. L.; O'Flaherty, J. T.; Salzer, W. L.; Thomas, M. J.; Snyder, F.; Piantadosi, C. *J. Med. Chem.* **1985**, *28*, 73.

(7) It should be pointed out that both of the individual optical isomers of solketal are readily available: Jung, M. E.; Shaw, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 6304. As a consequence of this, the syntheses reported herein can be used to prepare not only the racemic analogues but either of the optical isomers desired.

yields, respectively. The primary hydroxyl groups of **7** and **8** are selectively protected as the *p*-methoxytrityl derivatives **9** and **10**, respectively, which, without purification, are converted to the respective benzyl ethers **11** and **12** by alkylation with benzyl bromide and sodium hydride in DMF followed by removal of the *p*-methoxytrityl groups with acidified methanol.

The phosphocholine groups are then introduced using 2-bromoethyl phosphorodichloridate by a modification of the method of Hirt.[8] Thus, the reaction of **11** or **12** with an excess of the phosphorus reagent and triethylamine followed by hydrolysis in aqueous sodium acetate gives the bromoethyl phosphates, which, without additional purification, are converted to phosphocholines **13** and **14**, respectively, by refluxing in a solution of chloroform–acetonitrile containing a large excess of anhydrous trimethylamine. The phosphocholines **13** and **14** are obtained in 58 and 54% yields, respectively, after purification by silica gel chromatography. The benzyl ether protecting groups can then be removed in excellent yield (95–99%) by hydrogenolysis using palladium on charcoal in a mixture of methanol and acetic acid. The resulting lyso compounds **15** and **16** are then acetylated with an excess of acetic anhydride and triethylamine in refluxing chloroform to give the desired polyether analogues **17** and **18**.

Biological Results and Discussion

Table I presents the hypotension and platelet aggregation data we obtained for the polyether analogues **17** and **18** as well as for racemic C_{16}-PAF, our standard compound. Platelet aggregation was measured in rabbit platelet-rich plasma (PRP) as described previously.[3,12] The data are expressed as the molar concentration of the compound required to obtain 50% of its maximum aggregation response (EC_{50} value, a measure of potency) and as the maximum aggregation response of the compound obtained at the indicated minimum molar concentration (a measure of efficacy).

For blood pressure studies we use the spontaneously hypertensive rat (SHR) as previously described.[3] The hypotensive data are given as the intravenous dose needed to reduce the mean arterial blood pressure (MABP) 50 mmHg as extrapolated from the dose–response curve.

We find it also of interest to measure the degree of separation of the two biological properties for a particular compound. Consequently, we calculate the ratio of the blood pressure and platelet aggregation values. A value of the ratio that is significantly smaller than that obtained for our standard compound, racemic C_{16}-PAF, is an indication of selectivity in favor of the hypotensive effect, while a ratio larger than that of the standard suggests selectivity in favor of platelet aggregation.

It is clear from the data presented in Table I that incorporation of multiple ether linkages into the alkoxy chain of PAF leads to a significant reduction of both biological responses compared to **1a**. Furthermore, it is of equal interest that this effect is more apparent when the oxygen atoms are substituted near the end of the alkoxy chain then when substituted at the beginning. For example, while **18** is about 10 times less potent than **1a** with respect to blood pressure lowering, **17** is about 64 times less potent. Similarly, with respect to the platelet aggregation EC_{50} values, it is apparent that while **18** is only about 5 times less potent than **1a**, **17** is 200 times less potent.

The ratios of the hypotensive dose and the platelet aggregation EC_{50} values observed for **17** and **18** span a somewhat narrow range and are not very different from that observed for **1a**. Consequently, it appears that the incorporation of additional oxygen atoms into the alkoxy chain does not result in any dramatic gains in selectivity.

Evidence that at least some of the biological effects of PAF are the result of a receptor interaction include the stereospecificity of action of PAF,[9] the discovery of specific antagonists,[10] and the demonstration of specific PAF binding sites.[11] While, on the basis of data presented herein, it is difficult to draw any definite conclusions about the nature of the interaction of the alkoxy chain of PAF with the PAF receptor(s), it does appear that this receptor interaction is not only sensitive to steric effects, as has been observed in our earlier study,[3] but is also sensitive to the lipophilic character of the alkoxy chain as well.

Experimental Section

General Methods. Unless otherwise stated, the following are implied. The nuclear magnetic resonance (NMR) spectra were recorded on either a Varian EM-390, Varian FT-80, or a Nicolet-300 spectrometer and chemical shifts in parts per million (ppm) are reported with tetramethylsilane (Me_4Si) or chloroform as internal references. Infrared spectra (IR) were recorded on a Nicolet FT-7000 spectrophotometer. Electron-impact (EI) mass spectra were determined on a Finnegan-MAT Model CH-7 mass spectrometer. The field desorption (FD) and fast atom bombardment (FAB) mass spectra were obtained on a Kratos MS-50 mass spectrometer. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical value.

Biological Assays. The methods used to measure platelet aggregation and blood pressure lowering have been described in detail previously.[3,12]

8-[2-(2-Methoxyethoxy)ethoxy]-1-octene (2). To a stirred suspension of prewashed (hexanes) NaH 50% mineral oil dispersion (32.6 g, 0.68 mol) in 300 mL of DMF containing 8-bromo-1-octene (100 g, 0.52 mol) was added dropwise under argon a solution of 2-(2-methoxyethoxy)ethanol (72.3 g, 0.60 mol) in 100 mL of DMF over a 1-h period. After stirring an additional 3 h, the mixture was poured into H_2O and extracted with ether. The organic solution was washed with brine and dried ($MgSO_4$). Solvent was removed, and the residue was distilled in a Kugelrohr apparatus [80–90 °C (0.2 mm)] to give 97 g (80%) of **2** as a colorless liquid: NMR ($CDCl_3$) δ 6.10–4.83 (m, 3 H, $CHCH_2$), 3.85–3.36 (m's, 10 H, CH_2O), 3.37 (s, 3 H, OCH_3), 2.25–1.20 (m, 10 H, $(CH_2)_5$). Anal. ($C_{13}H_{26}O_3$) C, H.

8-[2-(2-Methoxyethoxy)ethoxy]-1-octanol (3). To a stirred solution of 1 M borane in THF (564 mL) at 0 °C under argon was added dropwise 2-methyl-2-butene (79.2 g, 1.13 mol) over a 0.5-h period. After the mixture was stirred an additional 0.5 h, **2** (65 g, 0.28 mol) was added dropwise. After 1 h, TLC of an aliquot indicated incomplete hydroboration. Another 700 mL of 1 M borane in THF was added. The mixture was stirred an additional 2 h. Excess borane was destroyed by the slow addition of H_2O. The mixture was cooled in an ice bath as 150 mL of 3 N NaOH was added followed by 150 mL of 30% H_2O_2. After stirring for 0.5 h, the mixture was extracted with ether. The ether layer was washed first with brine and then with a saturated solution of $NaHSO_3$. The organic solution was then dried ($MgSO_4$). Solvent was removed, and the residue was distilled in a Kugelrohr apparatus [160–180 °C (0.2 mm)], giving 41.3 g of **3** as a colorless

(8) Hirt, R.; Berchtold, R. *Pharm. Acta Helv.* **1958**, *33*, 349.

(9) (a) Wykle, R. L.; Miller, C. H.; Lewis, J. C.; Schmitt, J. D.; Smith, J. A.; Surles, J. R.; Piantadosi, C.; O'Flaherty, J. T. *Biochem. Biophys. Res. Commun.* **1981**, *100*, 1651. (b) Tence, M.; Coeffies, E.; Polonsky, J.; Benveniste, J. *Biochim. Biophys. Acta* **1983**, *735*, 526.

(10) (a) Terashita, Z.-i., Tsushima, S.; Yoshioka, Y.; Nomura, H.; Inada, Y.; Nishikawa, K. *Life Sci.* **1983**, *32*, 1975. (b) Kornecki, E.; Erhlich, Y. H.; Lenox, R. H. *Science (Washington, D.C.)* **1984**, *226*, 1454.

(11) (a) Valone, F. H.; Coles, E.; Reinhold, V. R.; Goetzl, E. J. *J. Immunol* **1982**, *129*, 1637. (b) Valone, F. H.; Goetzl, E. J. *Immunology* **1983**, *48*, 141. (c) Hwang, S.-B.; Lee, C.-S.; Cleah, M. J.; Shen, T. Y. *Biochemistry* **1983**, *22*, 4756.

(12) Kohler, C. A.; Zoltan, B. J. *J. Pharm. Methods* **1984**, *12*, 113.

liquid: NMR ($CDCl_3$) δ 3.85–3.36 (m, 12 H, CH_2O), 3.36 (s, 3 H, OCH_3), 1.85–0.90 (m, 12 H, $(CH_2)_6$); IR (neat) 3440 (OH) cm^{-1}. Anal. ($C_{13}H_{28}O_4$) H; C: calcd, 62.87; found, 63.95.

2-[2-(Decyloxy)ethoxy]ethanol (5). To a stirred suspension of prewashed (hexanes) NaH 50% mineral oil dispersion (52.1 g, 1.09 mol) in 500 mL of DMF was added dropwise under argon a solution of diethylene glycol (196.7 g, 1.85 mol) in 300 mL of THF over a 1.5-h period. After the mixture was stirred an additional 0.5 h, 1-bromodecane (200 g, 0.90 mol) was added over 10 min. The mixture was then stirred an additional 1.5 h and then diluted with H_2O. The mixture was extracted with hexane. The hexane solution was washed with H_2O and brine and then dried ($MgSO_4$). Solvent was removed, and the residue was distilled in a Kugelrohr apparatus; the fraction boiling at 160–165 °C (0.1 mm) was collected, giving 97 g (44%) of **5** as a colorless liquid: NMR ($CDCl_3$) δ 3.95–3.30 (m, 10 H, CH_2O), 2.50 (s, 1 H, OH), 1.75–1.17 (m, 16 H, $(CH_2)_8$), 0.87 (m, 3 H, terminal CH_3); IR (neat) 3435 (OH) cm^{-1}. Anal. ($C_{14}H_{30}O_3$) C, H.

Tosylate of 8-[2-(2-Methoxyethoxy)ethoxy]-1-octanol (4). A solution of **3** (40 g, 0.16 mol) and tosyl chloride (35.3 g, 0.19 mol) in 200 mL of dry pyridine was allowed to stand overnight at 5 °C. The mixture was then poured into H_2O and extracted with ether. The ether solution was washed with dilute HCl and then with a saturated solution of $NaHCO_3$. The ethereal solution was dried ($MgSO_4$), and the solvent was removed, giving 42 g (65%) of **4** as an oil that was used without additional purification.

By an identical method **5** (116 g, 0.47 mol) was converted to 146.3 g (76%) of tosylate **6**.

2,5,8,17-Tetraoxaeicosane-19,20-diol (7). To a stirred suspension of prewashed (hexanes) NaH 50% mineral oil dispersion (9.01 g, 0.19 mol) in 100 mL of DMF was added dropwise under argon solketal (22.06 g, 0.17 mol) over a 1-h period. The mixture was stirred an additional 15 min and then cooled in an ice bath. A solution of **4** (42 g, 0.1 mol) in 25 mL of DMF was then added all at once. The mixture was stirred overnight and then quenched with H_2O. The aqueous mixture was extracted with ether. The ethereal solution was dried ($MgSO_4$), and the solvent was removed. The residue was refluxed in a mixture of 170 mL of CH_3OH, 25 mL of H_2O, and 1.25 mL of H_2SO_4 for 1 h. The CH_3OH was removed at reduced pressure. The residue was saturated with NaCl and extracted several times with ethyl acetate. The organic extracts were combined and dried ($MgSO_4$). Solvent was removed, and the residue was purified via HPLC on silica gel, eluting with ethyl acetate to give 14.7 g (44%) of **7** as a colorless oil: NMR ($CDCl_3$) δ 3.80–3.40 (m, 19 H, CH_2O's, OCH, OH's), 3.40 (s, 3 H, OCH_3), 1.80–1.25 (m, 12 H, $(CH_2)_6$); IR (neat) 3435 (OH) cm^{-1}. Anal. ($C_{16}H_{34}O_6$) C, H.

3-[2-[2-(Decyloxy)ethoxy]ethoxy]-1,2-propanediol (8). This compound was prepared by a method identical with the above using NaH (21.0 g, 0.44 mol), solketal (72.3 g, 0.55 mol), and **6** (146 g, 0.36 mol) to give, after purification by distillation in a Kugelrohr apparatus [220–225 °C (0.05 mm)], 99 g (85%) of **8** as a colorless liquid: NMR ($CDCl_3$) δ 4.00–3.05 (m, 17 H, CH_2O's, OCH, OH's), 1.60–1.16 (m, 16 H, $(CH_2)_8$), 0.85 (m, 3 H, terminal CH_3); IR (neat) 3420 (OH) cm^{-1}. Anal. ($C_{17}H_{36}O_5$) C, H.

19-(Phenylmethoxy)-2,5,8,17-tetraoxaeicosan-20-ol (11). A solution of **7** (13.7 g, 42.5 mmol) and *p*-anisylchlorodiphenylmethane (14.4 g, 46.7 mmol) in a mixture of 10 mL of pyridine and 40 mL of THF was allowed to stand overnight. The mixture was poured into H_2O and extracted with ether. The organic solution was dried ($MgSO_4$), and the solvent was removed, giving **9** which, without additional purification, was dissolved in 50 mL of DMF and added over a 0.5 h-period under argon with stirring, to a suspension of prewashed NaH 50% mineral oil dispersion (3.26 g, 67.97 mmol) in 40 mL of DMF containing benzyl bromide (10.9 g, 63.7 mmol). After stirring overnight, the mixture was poured into H_2O and extracted with ether. The ether layer was washed with H_2O and dried ($MgSO_4$). Solvent was removed, and the residue was dissolved in 200 mL of hot CH_3OH containing 10 g of Amberlyst-15 ion-exchange resin. After stirring for 1 h, the mixture was filtered and the solvent was removed. The residue was then chromatographed on silica gel via HPLC, eluting with hexane–ethyl acetate (13:7) to give 12.9 g (74%) of **11** as an oil: NMR ($CDCl_3$) δ 7.34 (m, 5 H, C_6H_5), 4.66 (ABq, 2 H, J = 11.8 Hz, $C_6H_5CH_2$), 3.82–3.36 (m, 17 H, CH_2O's, OCH), 3.38 (s, 3 H, OCH_3), 1.93 (s, 1 H, OH), 1.56 (m, 4 H, CH_2CH_2O) 1.30 (m, 8 H, $(CH_2)_4$); IR (neat) 3450 (OH) cm^{-1}. Anal. ($C_{23}H_{40}O_6$) C, H.

3-[2-[2-(Decyloxy)ethoxy]ethoxy]-2-(phenylmethoxy)-1-propanol (12). This compound was prepared by using the same method as described above, starting with **8** (50 g, 0.16 mol) to give 27.8 g of **12** as a colorless oil after purification via HPLC on silica gel, eluting with hexane–ethyl acetate (7:4): NMR ($CDCl_3$) δ 7.30 (m, 5 H, C_6H_5), 4.66 (s, 2 H, $CH_2C_6H_5$), 3.85–3.35 (m, 15 H, CH_2O's, OCH), 2.25 (s, 1 H, OH), 1.75–1.15 (m, 16 H, $(CH_2)_8$), 0.85 (m, 3 H, terminal CH_3); IR (neat) 3440 (OH) cm^{-1}; MS, m/z 410 (M^+). Anal. ($C_{24}H_{42}O_5$) C, H.

22-Hydroxy-*N*,*N*,*N*-trimethyl-19-(phenylmethoxy)-2,5,8,17,21,23-hexaoxa-22-phosphapentacosan-25-aminium 22-Oxide, Hydroxide, Inner Salt 13. To a stirred solution of **11** (11 g, 26.7 mmol) and triethylamine (4.1 g, 40 mmol) in 125 mL of CCl_4 was added 2-bromoethyl phosphorodichloridate[8] (9.7 g, 40 mmol). After 1.5 h, the mixture was filtered and the solvent was removed. The residue was stirred in a mixture of 300 mL of 0.5 M sodium acetate and 300 mL of THF for 3 h. Most of the THF was removed at reduced pressure. The aqueous residue was acidified with HCl and extracted with ether. The ether solution was dried ($MgSO_4$), and the solvent was removed. The residue was then refluxed in a mixture consisting of 200 mL of CH_3CN, 180 mL of $CHCl_3$, and 100 g of anhydrous $N(CH_3)_3$ for 3 h. The solvents were removed, and the residue was stirred, in 150 mL of CH_3OH containing 2 g of Ag_2CO_3 and 8 g of Amberlite IR 4B resin for 1.5 h. The mixture was filtered, and solvent was removed. The residue was purified by chromatography on silica gel (400-mL dry volume), eluting first with $CHCl_3$–CH_3OH (7:3) to remove the less polar impurities and then with $CHCl_3$–CH_3OH–H_2O (14:6:1) to elute 8.9 g (58%) of **13** as a colorless oil: NMR ($CDCl_3$–CD_3OD) δ 7.33 (m, 5 H, C_6H_5), 4.68 (ABq, 2 H, J = 11.7 Hz, $CH_2C_6H_5$), 4.18 (m, 2 H, $POCH_2$), 3.97 (m, 2 H, CH_2OP), 3.85–3.40 (m's, 17 H, CH_2O's, OCH, CH_2N), 3.38 (s, 3 H, OCH_3), 3.09 (s, 9 H, $N(CH_3)_3$), 1.56 (m, 4 H, CH_2CH_2O's), 1.30 (m, 8 H, $(CH_2)_4$); MS (FAB), m/z 578 (M + H). Anal. ($C_{28}H_{52}O_9PN\,H_2O$) C, H, N, P.

4-Hydroxy-*N*,*N*,*N*-trimethyl-7-(phenylmethoxy)-3,5,9,12,15-pentaoxa-4-phosphapentacosan-1-aminium, 4-Oxide, Hydroxide, Inner Salt (14). This compound was prepared in an identical manner from **12** (14 g, 34.1 mmol), triethylamine (5.18 g, 51.1 mmol), and the phosphorus reagent 12.4 g, 51.1 mmol) to give after silica gel chromatography 10.64 g (54%) of **14** as an oil: NMR ($CDCl_3$ + CD_3OD) δ 7.30 (m, 5 H, C_6H_5), 4.68 (ABq, 2 H, J = 12.0 Hz, $CH_2C_6H_5$), 4.19 (m, 2 H, $POCH_2$), 4.00 (m, 2 H, CH_2OP), 3.85–3.38 (m's, 15 H, CH_2O's, OCH, CH_2N), 3.10 (s, 9 H, $N(CH_3)_3$), 1.57 (m, 2 H, CH_2CH_2O), 1.26 (m, 12 H, $(CH_2)_7$), 0.90 (m, 3 H, terminal CH_3); MS (FAB), m/z 578 (M + H). Anal. ($C_{29}H_{54}O_8PN{\cdot}1.5H_2O$) C, H, P, N.

19,22-Dihydroxy-*N*,*N*,*N*-trimethyl-2,5,8,17,21,23-hexaoxa-22-phosphapentacosan-25-aminium, 22-Oxide, Hydroxide, Inner Salt (15). A solution of **13** (7.9 g, 13.7 mmol) in 45 mL of CH_3OH and 45 mL of acetic acid containing 0.75 g of 5% Pd/C was shaken in an atmosphere of H_2 at an initial pressure of 40 psi. After 3.5 h, the mixture was filtered and solvent was removed at reduced pressure, giving 6.6 g (99%) of **15** as a colorless oil: NMR ($CDCl_3$–CD_3OD) δ 4.27 (m, 2 H, $POCH_2$), 3.93 (m, 2 H, CH_2OP), 3.80–3.35 (m's, 17 H, CH_2O's, OCH, CH_2N), 3.39 (s, 3 H, OCH_3), 3.21 (s, 9 H, $N(CH_3)_3$), 1.57 (m, 4 H, OCH_2CH_2's), 1.31 (m, 8 H, $(CH_2)_4$); IR (neat) 3260 (OH) cm^{-1}; MS (FAB), m/z 488 (M + H). Anal. ($C_{21}H_{46}O_9PN{\cdot}1.13H_2O$) C, H, P; N: calcd, 6.10; found, 6.64.

4,7-Dihydroxy-*N*,*N*,*N*-trimethyl-3,5,9,12,15-pentaoxa-4-phosphapentacosan-1-aminium, 4-Oxide, Hydroxide, Inner Salt (16). This compound was prepared in an identical manner as described above from **14** (9.6 g, 16.7 mmol) to give 7.7 g (95%) of **16** as a colorless oil: NMR ($CDCl_3$–CD_3OD) δ 4.30 (m, 2 H, $POCH_2$), 3.90 (m, 2 H, CH_2OP), 3.85–3.20 (m's, 15 H, CH_2O's, CHO, CH_2N), 3.24 (s, 9 H, $N(CH_3)_3$), 1.24 (m, 16 H, $(CH_2)_8$), 0.86 (m, 3 H, terminal CH_3); IR (neat) 3225 (OH) cm^{-1}; MS (FAB), m/z 486 (M + H). Anal. ($C_{22}H_{48}O_8PN{\cdot}1.38H_2O$) C, H, N; P: calcd, 6.07; found, 6.53.

19-(Acetyloxy)-22-hydroxy-*N*,*N*,*N*-trimethyl-2,5,8,17,21,23-hexaoxa-22-phosphapentacosan-25-aminium, 22-Oxide, Hydroxide, Inner Salt (17). A mixture of **15** (3.5 g, 7.2 mmol), acetic anhydride (18.3 g, 180 mmol), and triethylamine (7.3 g, 71.8 mmol) in 200 mL of $CHCl_3$ was refluxed for

4 h. Solvent and excess anhydride were removed at reduced pressure. The residue was chromatographed on silica gel (150-mL dry volume), eluting first with $CHCl_3$–CH_3OH (4:1) to remove less polar impurities and then with $CHCl_3$–CH_3OH–H_2O (140:60:9) to elute 3.6 g (95%) of 17 as a colorless oil: NMR ($CDCl_3$–CD_3OD) δ 5.12 (m, 1 H, OCH), 4.23 (m, 2 H, $POCH_2$), 4.00 (m, 2 H, CH_2OP), 3.75–3.34 (m's, 16 H, CH_2O's, CH_2N), 3.39 (s, 3 H, OCH_3), 3.22 (s, 9 H, $N(CH_3)_3$), 2.08 (s, 3 H, $COCH_3$), 1.56 (m, 4 H, OCH_2CH_2's), 1.29 (m, 8 H, $(CH_2)_4$); IR (neat) 1735 (C=O) cm^{-1}. Anal. ($C_{23}H_{48}O_{10}PN·H_2O$) C, H, P, N.

7-(Acetyloxy)-4-hydroxy-*N*,*N*,*N*-trimethyl-3,5,9,12,15-pentaoxa-4-phosphapentacosan-1-aminium, 4-Oxide, Hydroxide, Inner Salt (18). This compound was prepared by using the method described above from 16 (6.7 g, 13.8 mmol), acetic anhydride (35.2 g, 340 mmol), and triethylamine (14 g, 140 mmol) to give 6.5 g (89%) of 18 as a colorless gel: NMR ($CDCl_3$–CD_3OD) δ 5.14 (m, 1 H, HCO), 4.25 (m, 2 H, $POCH_2$), 4.00 (m, 2 H, CH_2OP), 3.70–3.30 (m's, 14 H, CH_2O's, CH_2N), 3.22 (s, 9 H, $N(CH_3)_3$), 2.08 (s, 3 H, $COCH_3$), 1.58 (m, 2 H, CH_2CH_2O), 1.26 (m, 14 H, $(CH_2)_7$), 0.87 (m, 3 H, terminal CH_3); IR (neat) 1727 (C=O) cm^{-1}; MS (FAB), *m/z* 528 (M + H). Anal. ($C_{24}H_{30}O_9P$-N·0.5H_2O) C, H, P. N.

Acknowledgment. We thank Dr. L. Gehrlein and staff for microanalytical determinations, Dr. M. Siegel and G. O. Morton and staff for spectral data, R. A. Gabel for his assistance with the hypotension assay, and D. R. Nytko for her assistance with the platelet aggregation assay.

Isolation, Synthesis, and Antitumor Evaluation of Spirohydantoin Aziridine, a Mutagenic Metabolite of Spirohydantoin Mustard

Robert F. Struck,* Marion C. Kirk, Louise S. Rice, and William J. Suling

Southern Research Institute, Birmingham, Alabama 35255-5305. *Received December 3, 1985*

Spirohydantoin mustard (SHM), a central nervous system directed nitrogen mustard with anticancer activity, was metabolized in the presence of mouse liver postmitochondrial supernatant (9000*g* fraction) to a nonpolar alkylating metabolite. The metabolite was isolated by thin-layer chromatography of chloroform or ethyl acetate extracts of incubation mixtures, and its structure was established by mass spectral analysis, synthesis, and cochromatography. The metabolite, spirohydantoin arizidine, was mutagenic for *Salmonella typhimurium* TA1535 in the Ames assay but inactive as an antitumor agent against P388 leukemia in vivo.

Spirohydantoin mustard (SHM, see Scheme I) was designed and synthesized as a central nervous system directed alkylating agent.[1] SHM is active against several experimental murine leukemias and solid tumors, including the ependymoblastoma mouse brain tumor. In preparation for possible clinical studies, preclinical toxicology studies have been completed, and phase I trials have been initiated. In previous studies SHM was observed not to be a direct-acting mutagen in the *Salmonella*/mammalian microsome mutagenicity assay (Ames assay) but is converted by mouse liver postmitochondrial supernatant (9000*g*, S9) to a mutagenic metabolite in the presence of NADPH.[2] The structural identity of this metabolite has not yet been established. Continuing investigations have demonstrated the production of an additional, direct-acting, mutagenic metabolite by mouse liver S9 fraction in the presence of NADPH. Isolation, identification, synthesis, and antitumor and mutagenic activities of this metabolite are reported here.

TLC of chloroform or ethyl acetate extracts of incubates of [^{14}C]-SHM with mouse liver S9 fraction indicated by radioscanning the presence of an alkylating metabolite of SHM of the following R_f values in the following solvents: chloroform–methanol (95:5) 0.26; chloroform–acetone (1:1), 0.17; chloroform–acetone (1:2), 0.15; and toluene–acetone (1:1), 0.27. The alkylating property of the metabolite was determined by its reaction with 4-(*p*-nitrobenzyl)pyridine on the TLC plates. The metabolite, which accounted for 5% of the extractable activity while SHM accounted for 40%, was purified further by TLC in one or more of these solvent systems. The isolated metabolite fraction was analyzed by fast atom bombardment (FAB) MS and yielded a molecular ion peak [(M + 1)$^+$] of *m/z* 238. Electron-impact (EI) MS yielded the following data:

m/z (rel abund)	possible struct
237 (50)	M^+
209 (100)	$[M - (CH_2)_2]^+$ or $[M - CO]^+$
182 (70)	$[M - (CH_2N(CH_2)_2 + H]^+$

The data are consistent with a structure in which the mustard group of SHM has been monodechloroethylated and the resulting (2-chloroethyl)amino group has cyclized to an aziridine, yielding spirohydantoin aziridine (SHAZ, see Scheme I). There is precedent for this proposed oxidative metabolism of the nitrogen mustard moiety leading to a monodechloroethylated product; cyclophosphamide, a leading anticancer and immunosuppressive drug, has been shown to be metabolized along an identical pathway by a purified cytochrome P-450 fraction from rat liver microsomes, leading to the corresponding structure with the nitrogen mustard moiety dechloroethylated to a (chloroethylamino) group.[3] Structural proof was obtained

Table I. Mutagenic Activity of SHAZ against *Salmonella typhimurium* TA1535

sample	amt, μg or μg SHM equiv/plate	induced His$^+$ revertants[a] expt 1	expt 2
metabolite	2	41	
SHAZ	1	15	21
SHAZ	10	227	238
SHAZ	100	1841	2000

[a] These values represent the difference between the observed number of revertants and the average number of spontaneous revertants, which was 13 ± 2 in these experiments, on four solvent control plates.

(1) Peng, G. W.; Marquex, V. E.; Driscoll, J. S. *J. Med. Chem.* **1975**, *18*, 846.
(2) Suling, W. J.; Struck, R. F.; Woolley, C. W.; Shannon, W. M. *Biochem. Pharmacol.* **1983**, *32*, 523.
(3) Struck, R. F.; Kari, P.; Kalin, J.; Montogomery, J. A.; Marinello, A. J.; Love, J.; Bansal, S. K.; Gurtoo, H. L. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 390.

0022-2623/86/1829-1319$01.50/0