The inotropic activity of the test compounds was assayed utilizing a guinea pig atrial preparation. Isolated guinea pig left atria (obtained from 200-g animals) were placed in a 50-ml muscle bath and isometric tension was measured with a strain gauge transducer (Statham μC3) and recorded with an oscillographic recording system (Sanborn 150). The bath temperature was maintained at 37 °C and the atria were paced at 60 beats/min at a voltage 10% above threshold utilizing platinum field electrodes. The resting tension was set at 300 mg. The bathing solution had the following composition—NaCl, 125 mM; $NaHCO_3$, 24 mM; KCl, 4 mM; NaH_2PO_4, 1.2 mM; $MgSO_4$, 0.6 mM; $CaCl_2$, 1.2 mM; glucose, 23 mM—and was continuously aerated with a mixture of 95% O_2–5% CO_2. The compounds were dissolved in propylene glycol such that 5 μl of the resulting digitoxigenin (**1b**) stock solution yielded a bath of 1×10^{-7} M, and, in the case of **7**, 1×10^{-5} M bath solutions. After prior stabilization for 30 min of the atria in the baths without test compounds, appropriate aliquots of the stock solutions were added with a micropipet at 30-min intervals so as to obtain cumulative dose responses. The force of contraction was continuously recorded throughout the experiment and the maximal response following each dose was measured. The percent change in contractile tension at each dose was calculated and standard errors were determined. Five experiments were performed for **1b** and five for **7**. In separate experiments, depression due to cumulative equivalent doses of propylene glycol was also determined and these results are quite similar to those reported by Thomas.[6] Digitoxigenin (**1b**) was studied over a range of 1×10^{-7}–1×10^{-6} M, and the 17β-unsaturated aldehyde **7** was studied from 1×10^{-6} to 2×10^{-5} M.

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Some Novel Potential Alkylating Agents Derived from Diethylstilbestrol

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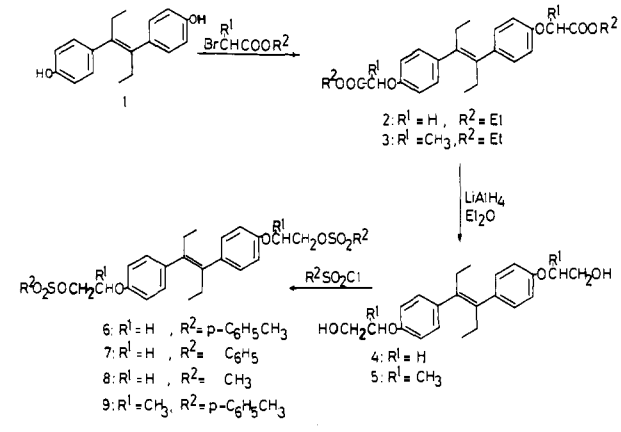
Several alkylating agents containing sulfonic esters, nitrogen mustards, and aziridine moieties attached to diethylstilbestrol through ethyl and isopropyl chains have been synthesized. The tests of some of the products for antileukemic activity in L1210 lymphoid leukemia indicated no significant activity over diethylstilbestrol.

In the course of studies aimed at developing alkylating agents that can be directed to specific target tissues, two series of steroidal alkylating agents were synthesized and pharmacologically tested for their anticancer activities. The compounds of the first series[1-4] included several steroidal nitrogen mustards derived from various steroids by replacement of their hydroxyl or ketonic functions with the nitrogen mustard unit $-N(CH_2CH_2Cl)_2$. The second[5-7] incorporated some steroidal esters of *p*-[*N*,*N*-bis(2-chloroethyl)amino]phenylacetic acid and the corresponding phenylbutyric acid, steroidal sulfides of *p*-[*N*,*N*-bis(2-chloroethyl)amino]thiophenol, and steroidal ethylenimine derivatives.

The detailed studies of the antitumor activities of these compounds[5-7] indicated that some of the esters, particularly 3β-hydroxy-5-cholestene-*p*-[*N*,*N*-bis(chloroethyl)amino]phenylacetate (phenesterin),[5] were potent in inhibition of solid tumors including Sarcoma 45, Walker carcinosarcoma, and alveolar liver carcinoma RS-1.

As a correlative study in the same field, we were interested in investigating the anticancer activities of a new series of alkylating agents in which the alkylating functions are attached to steroids or diethylstilbestrol through ether linkages. This part reports on the synthesis and antileukemic activities of some sulfonic esters, nitrogen mustards, and aziridine derivatives of diethylstilbestrol.

Scheme I



Chemistry. Diethylstilbestrol (1) was etherified with ethyl bromoacetate and ethyl α-bromopropionate as reported[8] and the products **2** and **3** were reduced with

Table I

Compd	Recrystn solvent	Yield, %[a]	Mp, °C	Formula	Analyses
6[b]	C_6H_6-petr ether	79	163-164	$C_{36}H_{40}O_8S_2$	C, H, S
7	EtOH	87	139-140	$C_{34}H_{36}O_8S_2 \cdot H_2O$	C, H, S
8	EtOH	97	131-132	$C_{24}H_{32}O_8S_2$	C, H, S
9	EtOH	82	157-158	$C_{38}H_{44}O_8S_2$	C, H, S

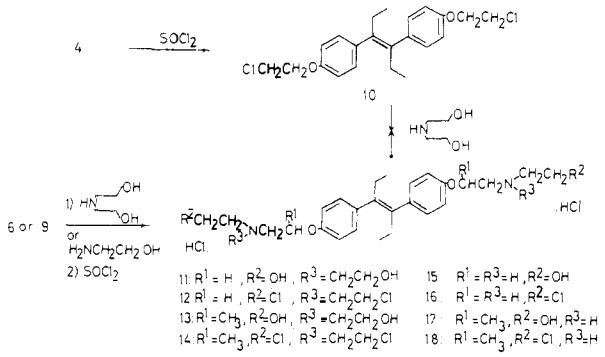
[a] Based on analytically pure sample. [b] Nomenclature (as example): α,α′-diethyl-4,4′-[bis(2-toluene-*p*-sulfonoxy)ethoxy]-stilbene.

Table II

Compd	Yield, % (as di-HCl)	Mp, °C	Formula	Analyses
11[a]	83	220	$C_{30}H_{48}N_2O_6Cl_2$	C, H, N, Cl
12	79	221-223	$C_{30}H_{44}N_2O_2Cl_6$	C, H, N, Cl
13	92	230-231	$C_{32}H_{52}N_2O_6Cl_2$	C, H, N, Cl
14	86	213-215	$C_{32}H_{48}N_2O_2Cl_6$	C, H, N, Cl
15	92	218-220	$C_{26}H_{40}N_2O_4Cl_2$	C, H, N, Cl
16	91	255-258 dec	$C_{26}H_{38}N_2O_2Cl_4 \cdot H_2O$	C, H, N, Cl
17	93	277-278	$C_{28}H_{44}N_2O_4Cl_2$	C, H, N, Cl
18	77	254-256 dec	$C_{28}H_{42}N_2O_2Cl_4$	C, H, Cl

[a] Nomenclature (as example): α,α′-diethyl-4,4′-bis[2-[bis(2-hydroxyethyl)amino]ethoxy]stilbene.

Scheme II

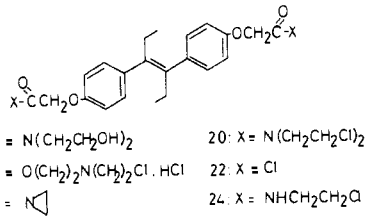


lithium aluminum hydride in ether to the alcohols **4** and **5**. These were then esterified with alkyl and arylsulfonyl chlorides to the desired sulfonic esters **6–9** (Scheme I, Table I).

The nitrogen mustards **12**, **14**, **16**, and **18** were synthesized by reacting the sulfonic esters **6** and **9** with diethanolamine and ethanolamine, respectively, in absolute ethanol and then treating the products with thionyl chloride in chloroform. An attempt to use the chloro derivative **10** instead of the sulfonic ester **6** with diethanolamine to prepare compound **11** was unsuccessful (Scheme II, Table II).

On the other hand, treatment of the ester **2** or the acid chloride **22** with diethanolamine in ethanol gave α,α′-diethyl-4,4′-stilbenedioxybis[*N*,*N*′-bis(2-hydroxyethyl)]acetamide (**19**). Treatment of **19** with thionyl chloride in dry chloroform and recrystallization of the product gave a rearranged monofunctional nitrogen mustard **21**, containing an ester group in the side chain, instead of the required bifunctional nitrogen mustard **20**. The same product **21** was obtained when the acid chloride **22** was treated with β,β′-dichlorodiethylamine in cold, dry chloroform (Chart I). Such rearrangement has been reported[9] to occur in the presence of traces of water or hydrogen chloride during the reaction or crystallization. Accordingly, the reaction of the acid chloride **22** with β,β′-dichlorodiethylamine was repeated in anhydrous ether containing triethylamine to obtain a good yield of the required α,α′-diethyl-4,4′-stilbenedioxybis[*N*,*N*-bis(2-chloroethyl)]acetamide (**20**). When the product **20** was warmed in chloroform containing a drop of water or traces of hydrogen chloride gas, it underwent rearrangement and gave

Chart I



the monofunctional nitrogen mustard **21**.

Furthermore, the synthesis of α,α′-diethyl-4,4′-bis(1-aziridinylcarbonylmethoxy)stilbene (**23**) could not be achieved by reacting the acid chloride **22** with aziridine in cold dry chloroform. The product was found to be α,α′-diethyl-4,4′-stilbenedioxybis[*N*-(2-chloroethyl)]-acetamide (**24**) as confirmed by elemental analysis, mass spectra, and superimposability in the infrared spectra with the authentic sample of **24** prepared from the acid chloride **22** and β-chloroethylamine. When the reaction of **22** with aziridine was repeated in anhydrous ether containing triethylamine, a good yield of the desired aziridine derivative **23** was obtained. Treatment of **23** with dry hydrogen chloride in chloroform opened the aziridine ring and produced the nitrogen mustard **24**.

Biological Data. Compounds **6–8**, **12**, **20**, **21**, and **23** were evaluated in the L1210 lymphoid leukemia and assays were performed according to the specifications of Drug Evaluation Branch, National Cancer Institute, Bethesda, Md. 20014. The tests were made on BDF_1 mice. The compounds dissolved in saline were given as intraperitonial injections 48 h after tumor implantation. The injections were repeated 4 days later using multiple dose assays and a three-mouse assay procedure. Antitumor activities were evaluated on day 30 of tumor implantation. Control animals were injected with saline but otherwise similarly treated. No antileukemic activity was found in the compounds tested. Evaluation of the same compounds in the Walker 256 carcinosarcoma screen is in progress.

Experimental Section

All melting points are uncorrected. Ir spectra were measured as Nujol mulls on a Perkin-Elmer 237B grating infrared spectrophotometer. The petroleum ether used in all the experiments has bp 40–60°. Analytical data indicated as symbols for the elements were within ±0.4 of the theoretical values.

α,α′-Diethyl-4,4′-bis(2-hydroxyethoxy)stilbene (4). The ester **2** (2 g) was placed in the thimble of a Soxhlet extractor and

allowed to dissolve and drop gradually into a stirred suspension of $LiAlH_4$ (0.6 g) in refluxing anhydrous Et_2O (60 ml). When the addition was completed, the mixture was heated under reflux for 2 h and cooled (ice) and the excess $LiAlH_4$ was decomposed with cold H_2O (2 ml). The ether layer was filtered, dried (Na_2SO_4), and evaporated, and the residue was crystallized from ligroine giving colorless needles: mp 128–129°; yield 95%. Anal. ($C_{22}H_{28}N_4$) C, H.

α,α′-Diethyl-4,4′-bis(1-methyl-2-hydroxyethoxy)stilbene (5). This compound was similarly prepared by reduction of the ester **3** with $LiAlH_4$ in anhydrous Et_2O and obtained as transparent oil which was homogeneous on TLC: R_f 0.34 ($CHCl_3$–C_6H_6–EtOH, 1:1:0.2); yield 90%.

Preparation of the Sulfonic Esters 6–9. General Procedure. The alcohol **4** or **5** (1 mmol) in pyridine (5 ml) was treated with the alkyl- or arylsulfonyl chloride (3 mmol) and the mixture was stirred at 0° for 6 h. Cold H_2O (2 ml) was added dropwise (internal temperature kept at 0–5°) with vigorous stirring and the solution was diluted with more H_2O (5 ml) to separate the sulfonate esters **6–9** as fine crystals. After filtration, the products were washed with H_2O until free from pyridine, dried, and recrystallized from the proper solvents (Table I).

α,α′-Diethyl-4,4′-bis(2-chloroethyl)stilbene (10). Freshly distilled $SOCl_2$ (0.5 ml, 6 mmol) was added to the solution of α,α′-diethyl-4,4′-bis(2-hydroxyethoxy)stilbene (**4**, 1.06 g, 3 mmol) in a mixture of dry C_6H_6 (10 ml), dry pyridine (2 ml), and dry $CHCl_3$ (2 ml) (cooled in ice–salt bath) and the mixture was stirred for 3 h. Stirring was continued for an additional 3 h at room temperature and then the mixture was heated under reflux for 2 h. The solvent and excess $SOCl_2$ were removed under reduced pressure and the residue was treated dropwise with H_2O. The product was separated as a fine yellow powder which after recrystallization from 95% EtOH gave yellowish white needles: mp 131–133°; R_f 0.44 and 0.1 (C_6H_6–petroleum ether, 1:2). These were purified by chromatography on silica gel (15 g) (column 1.5 × 15 mm) and eluting with C_6H_6 and a petroleum ether mixture (1:2). The first eluate (four 2.5-ml fractions) was evaporated to give the pure product **10**: mp 135–136°; yield 50%. Anal. ($C_{22}H_{26}O_4Cl_2$) C, H, N.

Reactions of 10 with Diethanolamine. Na_2CO_3 (250 mg) and a few crystals of NaI were added to the solution of **10** (390 mg, 1 mmol) and diethanolamine (630 mg, 6 mmol) in absolute EtOH (10 ml). The mixture was heated under reflux for 100 h, but no reaction took place (TLC at intervals of 10 h). The solvent was evaporated to dryness and the residue dissolved in C_6H_6 and shaken with H_2O to remove diethanolamine. The chloro derivative **10** was recovered with removal of the solvent.

Reaction of the Sulfonic Esters 6 and 9 with Diethanolamine and Ethanolamine. General Procedure. Diethanolamine or ethanolamine (10 mmol) was added to the suspension of the sulfonic ester **6** or **9** (0.6 mmol) in absolute EtOH (10 ml) and the mixture was heated under reflux for 4–6 h (occasionally 48 h was needed to produce a clear solution). The cooled mixture was poured into a small volume of cold H_2O and the separated oil was extracted with Et_2O. Ether extracts were dried (Na_2SO_4) and distilled off leaving the desired products as oils. These were converted into their HCl salts by saturating their solution in dry Et_2O with dry HCl gas. Two recrystallizations of the products from absolute EtOH and a petroleum ether mixture usually gave the analytical sample of **11**, **13**, **15**, and **17** (Table I). The ir spectra of the products showed a broad band around 2600 cm^{-1} characteristic for $>N^+<$. The products were homogeneous on TLC (BuOH–H_2O–HCOOH, 6:3.5:1.5).

Preparation of the Nitrogen Mustards 12, 14, 16, and 18. General Procedure. Freshly distilled $SOCl_2$ (1–1.5 ml) was added to the solution of the hydroxyamino derivatives **11**, **13**, **15**, and **17** (1 mmol) in dry $CHCl_3$ (10 ml) and the mixture was heated under reflux for 2–5 h. The solvent and excess $SOCl_2$ were removed under reduced pressure and the solid residue was recrystallized from EtOH and a petroleum ether mixture to give the analytical sample of the products (as HCl salts, Table II). The products developed one spot on TLC (BuOH–H_2O–HCOOH, 6:3.5:1.5) and their ir spectra showed a broad band around 2600 cm^{-1} characteristic for $>N^+<$.

α,α′-Diethyl-4,4′-stilbenedioxybis[*N*,*N*-[bis(2-hydroxyethyl)]]acetamide (19). Method A. Diethanolamine (500 mg, 5 mmol) was added to the ester **2** (500 mg) in absolute EtOH (20 ml) and the mixture was heated under reflux for 6 h. After cooling, the separated product was filtered and crystallized from EtOH to give **19** as white prisms: mp 185–186°; yield 83%.

Method B. The solution of diethanolamine (20 mmol) in dry, cold $CHCl_3$ (10 ml) was added dropwise to the stirred solution of α,α′-diethyl-4,4′-bis(chlorocarbonylmethoxy)stilbene (**22**, 5 mmol), prepared by hydrolysis of the ester **2**, with 10% NaOH and treating the product with $SOCl_2$ in dry, cold $CHCl_3$ (10 ml). Stirring was continued while cooling (ice) for 3 h and the separated product was filtered, washed with H_2O, and dried. Crystallization from EtOH gave the product **19** which was the same as the sample of **19** prepared by method A (comparison of ir spectra and mixture melting point determination): yield 85%; R_f 0.76 (AcOH–H_2O, 7:3). Anal. ($C_{30}H_{42}N_2O_8$) C, H, N.

α,α′-Diethyl-4,4′-bis[[2-(2-chloroethylamino)ethoxy]carbonylmethoxy]stilbene Dihydrochloride (21). Method A. The hydroxyamide **19** (500 mg, 1 mmol) was suspended in dry $CHCl_3$ (10 ml), treated with freshly distilled $SOCl_2$ (1 ml), and heated under reflux for 3 h. The solvent and excess $SOCl_2$ were removed under reduced pressure and the oily residue was scratched with drops of $CHCl_3$ and Me_2CO to form a white powder. Crystallization from EtOH–petroleum ether mixture gave **21** as white prisms: mp 177–179°; yield 70%; R_f 0.73 ($CHCl_3$–EtOH, 7:1); ir 1775 (C=O) and 2600 cm^{-1} ($>N^+<$). Anal. ($C_{30}H_{42}N_2O_6Cl_4$) C, H, N, Cl.

Method B. A solution of the acid chloride **22** (800 mg, 2 mmol) in $CHCl_3$ (5 ml) was added dropwise to a cold, stirred solution of β,β′-dichlorodiethylamine hydrochloride (700 mg, 4 mmol) and triethylamine (800 mg, 8 mmol) in dry $CHCl_3$ (5 ml). Stirring was continued for 3 h at room temperature and the clear solution was washed successively with H_2O, 2 N aqueous HCl, saturated aqueous $NaHCO_3$, and H_2O. After drying (Na_2SO_4) the solvent was distilled off to leave an oily product which was dissolved in the minimum amount of EtOH and treated with excess petroleum ether to separate as white crystals (900 mg). These were found to be identical in all respects with the sample of **21** prepared by method A.

α,α′-Diethyl-4,4′-stilbenedioxybis[*N*,*N*-bis(2-chloroethyl)]acetamide (20). A solution of β,β′-dichlorodiethylamine (4 mmol), prepared by neutralizing its HCl salt with Na_2CO_3 in Et_2O, in dry Et_2O (15 ml), and triethylamine (4 mmol) was added dropwise to an ice-cold, stirred solution of the acid chloride **22** (1 mmol). After stirring for 3 h, the product was filtered and the filtrate treated with petroleum ether until turbid and refrigerated to produce a solid product. Crystallization from Et_2O–petroleum ether mixture gave white prisms: mp 129–131°; R_f 0.41 ($CHCl_3$–absolute EtOH, 14:1); ir 1675 cm^{-1} (C=O); yield 83%. Anal. ($C_{30}H_{38}N_2O_4Cl_4$) C, H.

α,α′-Diethyl-4,4′-stilbenedioxybis[*N*-(2-chloroethyl)]acetamide (24). Method A. A solution of aziridine (0.5 ml, 4 mmol) in $CHCl_3$ (5 ml) was added dropwise to a cold stirred solution of the acid chloride **22** (400 mg, 1 mmol) in dry $CHCl_3$. After stirring for 3 h, the solvent was evaporated under reduced pressure (at room temperature) and the solid residue was washed with H_2O and dried. Crystallization from EtOH gave white crystals: mp 203–204°; R_f 0.7 (AcOEt); yield 88%; ir 1650 cm^{-1} (C=O); mass spectrum m/e (rel intensities) 506 (508 and 510 due to the presence of two chlorine atoms) (4% $M\cdot^+$), 470 (27), 434 (100), 334 (22), 321 (16), 266 (13), 265 (25), 251 (10), 237 (24), 85 (28), 36 and 38 (55). Anal. ($C_{26}H_{32}N_2O_4Cl_2$) C, H, N, Cl.

Method B. A solution of the acid chloride **22** (240 mg, 2 mmol) in anhydrous Et_2O (10 ml) was added dropwise to an ice-cold stirred solution of 2-chloroethylamine (300 mg, 4 mmol) in anhydrous Et_2O (15 ml). After stirring for 1 h, the solvent was evaporated and the residue was crystallized from EtOH, giving white crystals which were similar in all respects with the sample of **24** prepared by method A.

α,α′-Diethyl-4,4′-bis(1-aziridinylcarbonylmethoxy)stilbene (23). A solution of the acid chloride **22** (400 mg, 1 mmol) in anhydrous Et_2O (10 ml) was added dropwise to a cold (in ice–salt bath), well-stirred solution of aziridine (100 mg, 2 mmol) and triethylamine (240 mg, 2 mmol) in anhydrous Et_2O (10 ml). After stirring for 1 h, the separated product was filtered, washed with H_2O, and dried. Crystallization from a C_6H_6–petroleum ether mixture gave white plates: mp 163–165°; ir 1690 cm^{-1}; R_f 0.83

(AcOEt); yield 81%. Anal. ($C_{26}H_{30}N_2O_4$) C, H, N. When the solution of **23** in $CHCl_3$ was saturated with HCl and left at room temperature for 5 h, it was converted into compound **24** (TLC, ir, and melting point).

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Potential Bioreductive Alkylating Agents. 7. Antitumor Effects of Phenyl-Substituted 2-Chloromethyl-3-phenyl-1,4-naphthoquinones[1]

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Functional groups such as nitro, chloro, bromo, and methoxy were introduced in the meta and para positions of the phenyl ring of the antineoplastic agent 2-chloromethyl-3-phenyl-1,4-naphthoquinone. Tests for tumor-inhibitory potency of these derivatives against Sarcoma 180 ascites cells in mice indicated that the para-substituted methoxyphenyl, chlorophenyl, and bromophenyl derivatives possessed antitumor activity comparable to that of the parent compound 2-chloromethyl-3-phenyl-1,4-naphthoquinone, whereas meta-substituted nitro and bromo derivatives were either inactive or only weakly active anticancer agents in this system.

This laboratory has synthesized a variety of quinone derivatives with the potential to alkylate biological molecules following reductive activation and has demonstrated their antineoplastic activity against transplantable rodent tumors.[2-6] Studies on the biochemical mechanism of action of a representative member of this series, 2,3-bis(chloromethyl)-1,4-naphthoquinone, have indicated that this agent produces a variety of metabolic lesions including (a) inhibition of the biosynthesis of DNA, with lesser effects on the formation of RNA and protein; (b) inhibition of the coenzyme Q mediated enzyme systems, NADH oxidase and succinoxidase; (c) extensive and prolonged interaction with DNA, RNA, and protein; and (d) fragmentation of DNA.[7] Sodium borohydride reduction of 2,3-dimethyl-5,6-bis(acetoxymethyl)-1,4-benzoquinone in vitro provided chemical evidence for the generation of the proposed reactive *o*-quinone methide intermediate.[8] This finding coupled with evidence that the magnitude of the oxidation–reduction potential of these materials is important for antitumor activity[9] provides evidence in support of the concept of bioreductive alkylation by agents of this class.

2-Chloromethyl-3-phenyl-1,4-naphthoquinone appeared to be one of the best compounds of this series as an anticancer agent that we have synthesized to date;[6] thus, it appeared important to substitute the phenyl ring system in an effort to determine whether the therapeutic potential of this agent could be enhanced. To accomplish this we have introduced functional groups such as nitro, chloro, bromo, and methoxy onto the phenyl ring of 2-chloromethyl-3-phenyl-1,4-naphthoquinone.

Chemistry. The method of Kvalnes[10] for the synthesis of arylquinones was adapted to the preparation of arylnaphthoquinones **3a–d** (Scheme I). This was accomplished by coupling 1,4-naphthoquinone (**1a**) or 2-methyl-1,4-naphthoquinone (**1b**) to various diazotized anilines (**2a–d**). Attempts to couple diazotized 2-, 3-, and 4-aminopyridines to 1,4-naphthoquinone using this procedure were unsuccessful. Bromination of **3a** with NBS gave the desired bromomethyl derivative **4** in good yield. Chloromethylation of **3b,c,d**, using formaldehyde and hydrogen chloride, gave the appropriate final products **5a–c**, respectively, in moderate yields. Various demethylating reagents, such as BBr_3 and HBr, did not prove to be useful in attempts to demethylate the methoxy group of compound **4**.

Direct coupling of diazotized *m*-nitroaniline (**7**) to 1,4-naphthoquinone gave 2-(*m*-nitrophenyl)-1,4-naphthoquinone (**10**) in poor yield. Thus, an alternate route (Scheme II) was employed[11] for the preparation of **10** which involved the coupling of diazotized *m*-nitroaniline (**7**) to 1,4-benzoquinone (**6**) to give 2-(*m*-nitrophenyl)-1,4-benzoquinone (**8**). Treatment of **8** with butadiene gave the Diels–Alder adduct **9** which was then oxidized with chromic trioxide to give 2-(*m*-nitrophenyl)-1,4-naphthoquinone (**10**) in 60% yield. Chloromethylation of **10** produced the desired product 2-chloromethyl-3-(*m*-nitrophenyl)-1,4-naphthoquinone (**11**).

Antineoplastic Effects. The anticancer activities of phenyl-substituted 2-halomethyl-3-phenyl-1,4-naphthoquinones were assessed using mice bearing Sarcoma 180 ascites cells; the results obtained are shown in Table I. The para-substituted methoxyphenyl (**4**), chlorophenyl (**5a**), and bromophenyl (**5b**) derivatives possessed maximum antitumor activity comparable to that of the parent compound, 2-chloromethyl-3-phenyl-1,4-naphthoquinone, and the related agent, 2,3-bis(chloromethyl)-1,4-naphthoquinone, which was employed as a positive control,