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Antileukemic Agents. I. Some 2,5-Disubstituted *p*-Benzoquinones

HIDEO NAKAO and MASAO ARAKAWA

Central Research Laboratories, Sankyo Co., Ltd.[1]

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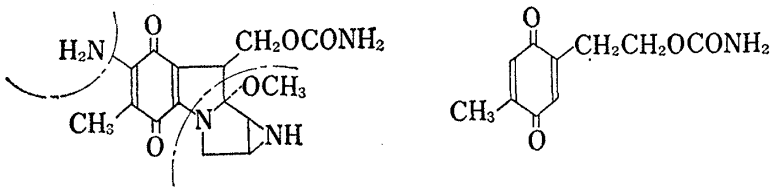
A series of *p*-benzoquinone derivatives having one or two carbamoyloxyalkyl groups in the 2 and/or 5-positions were synthesized and evaluated as antileukemic agents. Among these compounds 2,5-bis(1-aziridinyl)-3-(2-carbamoyloxyethyl)-6-methyl-*p*-benzoquinone (**21**) showed high activity against lymphoid leukemia L-1210 in BDF_1 mice.

It is well known that many quinone derivatives show antibacterial activity.[2] In addition a common *o*-aminoquinoid unit is found in some antitumor antibiotics,[3] *e.g.* streptonigrin, actinomycin and mitomycins. Particularly, the fact that mitomycin C (MMC) is in use clinically as an antitumor agent prompted us to synthesize *p*-benzoquinone derivatives having carcinostatic groups similar to those of MMC to find useful antitumor agents. The present paper describes the synthesis and antitumor activity of the title compounds.

MMC contains three carcinostatic groups–quinone, aziridine and urethane–in the structure. Thus, an initial attempt was made to synthesize 2-(2-carbamoyloxyethyl)-5-methyl-*p*-benzoquinone (**4**), which was regarded as a compound formally lacking two amino functions from the quinone moiety of MMC as shown in Chart 1.



MMC 4

Chart 1

The desired quinone (**4**) was readily obtained by nitric acid oxidation[4] of the corresponding hydroquinone dimethyl ether (**3**), which was prepared from 2,5-dimethoxy-4-methylphenethyl alcohol (**1**) as shown in Chart 2. Similarly, the N,N-dimethyl analog (**6**) of **4** was prepared. However, oxidation of the N-methyl analog (**7**) of 2,5-dimethoxy-4-methylphenethyl carbamate (**3**) with nitric acid gave 2-[2-(N-methyl-N-nitrosocarbamoyloxy)ethyl]-5-methyl-*p*-benzoquinone (**8**) accompanied with nitrosation. The structure of this compound was confirmed by the absence of NH band in the infrared spectra. A similar reaction sequence, starting from 2,5-dimethoxy-4-methylbenzyl alcohol (**9**) afforded the corresponding quinones (**12a—c**). Here again nitrosation was observed in the oxidation of the N-methyl analog (**11b**).

1) Location: *Hiromachi, Shinagawa-ku, Tokyo, 140, Japan.*
2) *a*) S. Petersen, W. Gauss, and E. Urbschat, *Angew. Chem.*, **67**, 217 (1955); *b*) W. Gauss and S. Petersen, *ibid.*, **69**, 252 (1957).
3) *a*) E. Jucker, "Fortschritte der Arzneimittelforschung Progress in Drug Research," Vol. 8, 1965, p. 431. ("On the Chemotherapy of Cancer"); *b*) K.V. Rao, K. Biemann, and R.B. Woodward, *J. Am. Chem. Soc.*, **85**, 2532 (1963).
4) H. Nakao, M. Fukushima, and T. Torizuka, *Ann. Sankyo Res. Lab.*, **22**, 90 (1970).

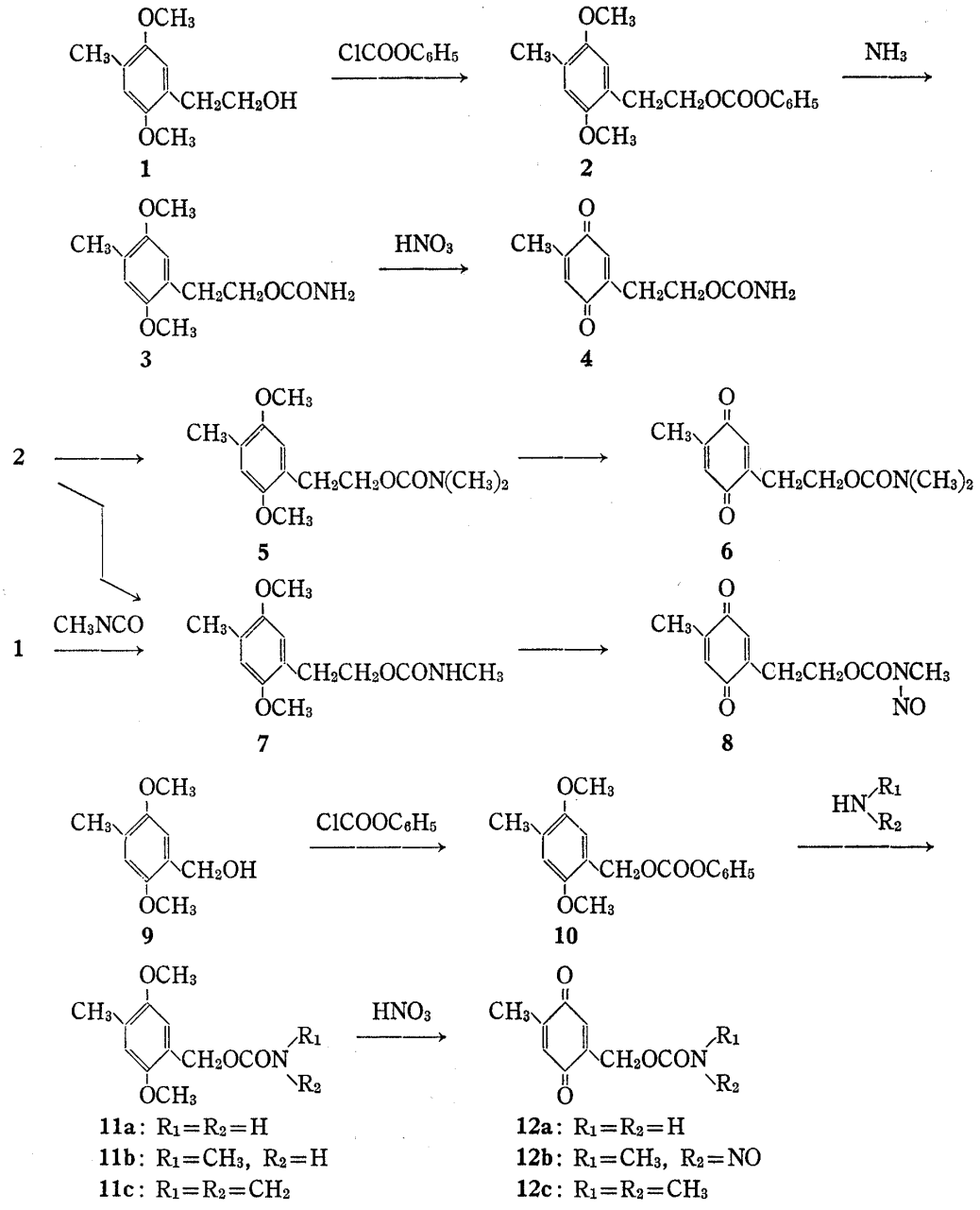


Chart 2

Although there are some monofunctional alkylating agents that have significant antitumor activity, the most active agents are bifunctional.[3a] Thus, the next aim was to synthesize 2,5-bis(2-carbamoyloxyethyl)-*p*-benzoquinones (**19a, b**) having two carbamoyloxy groups. The starting material, 2,5-dimethoxy-*p*-benzene diethanol (**16**) was prepared by $LiAlH_4$ reduction of the corresponding ester (**15**) which was obtained by hydrolysis followed by esterification of 2,5-dimethoxy-*p*-benzene diacetonitrile (**13**). Subsequently, by similar reactions mentioned above, the corresponding quinones (**19a, b**) were synthesized.

MMC possesses a methyl group as well as an amino group on the quinone moiety, therefore the efforts were directed at the syntheses of some 2,5-diamino-*p*-benzoquinone derivatives having a methyl and a carbamoyloxyethyl groups at the 3 and 6 positions, respectively. The reaction of the quinone (**4**) with methylamine, ethylamine or 2-diethylaminoethylamine afforded the corresponding aminoquinones (**20a—c**), similarly the reaction of **4** with aziridine gave 2,5-bis(1-aziridinyl)-3-(2-carbamoyloxyethyl)-6-methyl-*p*-benzoquinone (**21**) as shown in Chart 4.

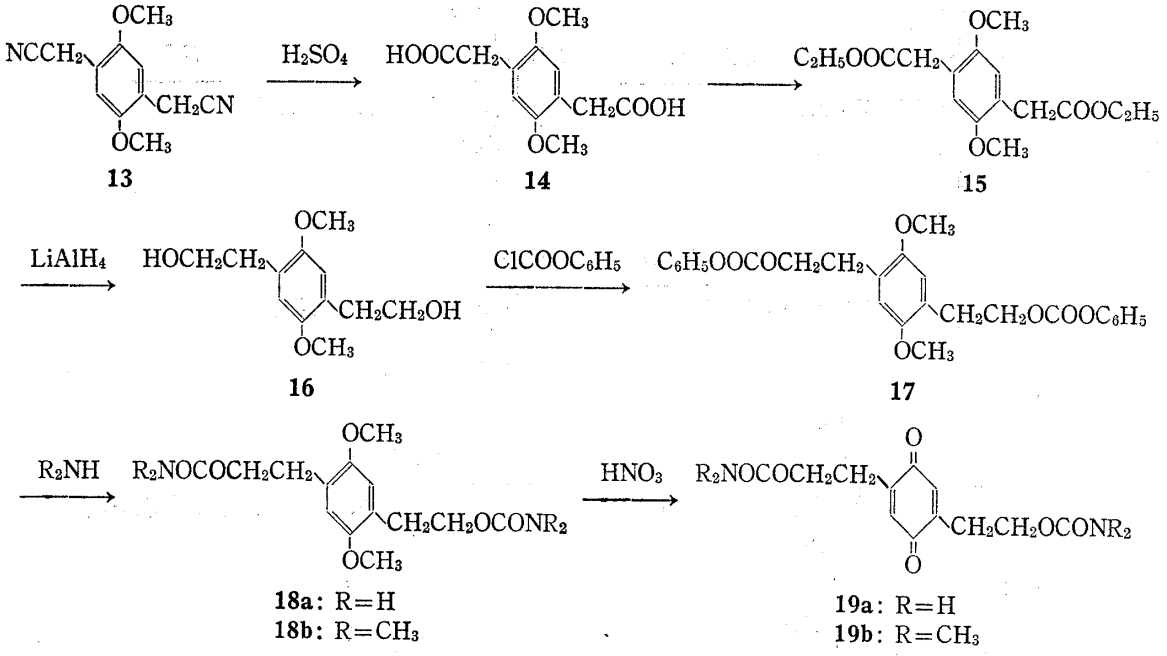


Chart 3

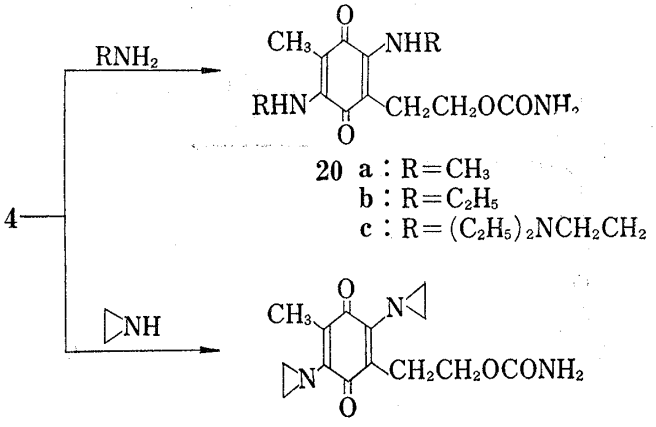
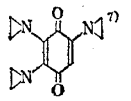
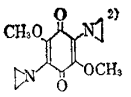
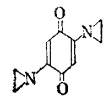
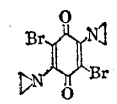


Chart 4

Table I. Effect of Compound 21 and Other Known Aziridine Derivatives on the Survival Time of Mice Bearing Leukemia L-1210 following Continuous Intranperitoneal Therapy[a)]

Compound	Dose (mg/kg/day)	Average life-span T/C(days)	increase(%)
21	0.5	19.2/8.0	140
Thio-TEPA[6)]	5.0	15.2/8.2	85
MMC	2.0	15.2/8.2	85
	0.15	17.5/8.2	114
	1.7	15.5/8.2	90
	inactive		
	inactive		

a) L-1210 ascites cells (10^5) were inoculated intraperitoneally. Treatment began 24 hr after implant, and continued for 12 days except on Sunday.

Antitumor Activity

The 2,5-disubstituted *p*-benzoquinones prepared in this work were evaluated for their antileukemic activity against lymphoid leukemia L-1210 in BDF_1 mice according to the method of CCNSC.[5)] Neither series of compound possessed outstanding antileukemic activity except the aziridinylquinone (**21**) which showed a high degree of activity. A number of aziridine derivatives have been recognized as antitumor agents. Among them thio-TEPA,[6)] MMC and some aziridinylquinones[2,7)] were selected for a comparison of their activity with that of **21**. As shown in Table I, **21** showed the highest activity.

5) Cancer Chemotherapy National Service Center, *Cancer Chemotherapy Rept.*, **17**, 1 (1962).
6) Tris(1-aziridinyl)phosphine sulphide.
7) *a*) W. Gauss and S. Petersen, *C.A.*, **60**, 2877a; *b*) "Merck Index," 8th Edition, 1065.

Experimental

2,5-Dimethoxy-4-methylphenethyl Phenyl Carbonate (2)——To an ice-chilled, stirred solution of 3 g of 2,5-dimethoxy-4-methylphenethyl alcohol[8] (1) in 15 ml of pyridine was added dropwise 3 ml of phenyl chloroformate. The resulting mixture was stirred at room temperatue for 2.5 hr and then diluted with water to separate oily substance, which was extracted with ether. The extract was washed with water, dried (Na_2SO_4) and evaporated to give 5 g of crude oily substance, which was used for following reaction without purification. For analysis a part of the product was distilled under reduced pressure, bp 190° (0.7 mmHg). *Anal.* Calcd. for $C_{18}H_{20}O_5$: C, 68.34; H, 6.37. Found: C, 68.48; H, 6.23. IR $\nu_{max}^{liq.}$ cm^{-1}: 1760 (C=O).

2,5-Dimethoxy-4-methylphenethyl Carbamate (3)——A mixture of 3.5 g of **2**, 20 ml of 28% aq. ammonia and 40 ml of EtOH was refluxed for 3 hr. The resulting mixture was concentrated under reduced pressure to one-third the volume. After cooling, to the mixture was added 15 ml of 5% aq. NaOH and the separated crystals were collected, washed with water and recrystallized from EtOH to give 1.5 g of colorless crystals, mp 142°. *Anal.* Calcd. for $C_{12}H_{17}O_4N$: C, 60.24; H, 7.16; N, 5.85. Found: C, 60.06; H, 7.34; N, 5.78. IR ν_{max}^{Nujol} cm^{-1}: 3450, 3250 (NH_2), 1680 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 290 (3.67).

2-(2-Carbamoyloxyethyl)-5-methyl-*p*-benzoquinone (4)——To a solution of 0.5 g of carbamate (3) in 4 ml of AcOH was added dropwise 0.3 ml of 60% HNO_3 with stirring at 15—20°. After stirring at room temperature for 1.5 hr, the mixture was poured into 15 ml of water and then the separated yellow crystals were collected, washed with water and recrystallized from EtOH to give 250 mg of yellow needles, mp 167°. *Anal.* Calcd. for $C_{10}H_{11}O_4N$: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.27; H, 5.41; N, 6.64. IR ν_{max}^{Nujol} cm^{-1}: 3470, 3320 (NH_2), 1730 (carbamoyl C=O), 1650 (quinone C=O). UV λ_{max}^{EtOH} mμ (log ε): 252 (4.22), 258 (4.19, sh.)

N,N-Dimethylcarbamate (5) of Alcohol (1)——A mixture of 2 g of carbonate (**2**), 10 ml of 40% aq. dimethylamine and 50 ml of EtOH was refluxed for 4 hr. The resulting mixture was concentrated to one-third the volume, diluted with water and then extracted with $CHCl_3$. The extract was washed with water, dried (Na_2SO_4) and evaporated. The residue was chromatographed on alumina with $CHCl_3$. The evaporation of $CHCl_3$ eluate gave colorless solid, which was recrystallized from cyclohexane yielding 0.9 g of colorless needles, mp 66°. *Anal.* Calcd. for $C_{14}H_{21}O_4N$: C, 62.90; H, 7.92; N, 5.24. Found: C, 63.17; H, 7.92; N, 5.07.

2-[2-(N,N-Dimethylcarbamoyloxy)ethyl]-5-methyl-*p*-benzoquinone (6)——To a solution of 2 g of **5** in 10 ml of AcOH was added dropwise 1.2 ml of 60% HNO_3 with stirring at 15—20°. After stirring at room temperature for 1 hr, the mixture was poured into 50 ml of water and the resulting mixture was extracted with ether. The extract was washed with water, dried and evaporated to give yellow liquid, which solidified by standing at refrigerator. Recrystallization from MeOH gave 0.5 g of yellow crystals, mp 65°. *Anal.* Calcd. for $C_{12}H_{15}O_4N$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.54; H, 6.55; N, 5.62. IR ν_{max}^{Nujol} cm^{-1}: 1725 (carbamoyl), 1650 (quinone C=O).

N-Methylcarbamate (7) of Alcohol (1)——A) A mixture of 1 g of **1** and 6 ml of methylisocyanate was refluxed for 6 hr and then concentrated to dryness. The resulting residue was recrystallized from cyclohexane to give 1.1 g of colorless needles, mp 102°. *Anal.* Calcd. for $C_{13}H_{19}O_4N$: C, 61.64; H, 7.56; N, 5.53. Found: C, 61.93; H, 7.70; N, 5.57. IR ν_{max}^{Nujol} cm^{-1}: 3400 (NH), 1700 (C=O).

B) To a solution of 2.5 g of crude carbonate (**2**) in 25 ml of CH_2Cl_2 was added 5 ml of liquid methylamine. The mixture was allowed to stand for two days at room temperature and then concentrated to dryness. The residue was extracted with $CHCl_3$ and the extract was washed with water, dried, concentrated to about 10 ml and chromatographed on alumina. Evaporation of $CHCl_3$ eluate gave 1.3 g of colorless crystals, mp 101°, which were identical with the product obtained by method A.

2-[2-(N-Methyl-N-nitrosocarbamoyloxy)ethyl]-5-methyl-*p*-benzoquinone (8)——To a solution of 3 g of carbamate (**7**) in 20 ml of AcOH was added dropwise 1.3 ml of 60% HNO_3 with stirring at 15°. After stirring at room temperature for 1.5 hr, the reaction mixture was poured into 140 ml of cold water. The separated crystals were collected, dried and recrystallized from benzene–cyclohexane (2:1) to give 0.8 g of yellow crystals, mp 75°. *Anal.* Calcd. for $C_{11}H_{12}O_5N_2$: C, 52.38; H, 4.80; N, 11.11. Found: C, 52.63; H, 4.97; N, 10.86. IR ν_{max}^{Nujol} cm^{-1}: 1760, 1640. UV λ_{max}^{EtOH} mμ (log ε): 250 (4.32).

2,5-Dimethoxy-4-methylbenzyl Phenyl Carbonate (10)——To an ice-chilled, stirred solution of 2.8 g of 2,5-dimethoxy-4-methylbenzyl alcohol[4] (**9**) in 15 ml of pyridine was added dropwise 2.5 ml of phenyl chloroformate. The resulting mixture was stirred at room temperature for 2.5 hr and then diluted with 25 ml of cold water to separate 5 g of colorless crystals. Recrystallization from cyclohexane gave 3.5 g of colorless prisms, mp 90°. *Anal.* Calcd. for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.57; H, 6.09. IR ν_{max}^{Nujol} cm^{-1}: 1760 (C=O).

Carbamate (11a) of Alcohol (9)——To a solution of 1 g of **10** in 20 ml of MeOH was saturated ammonia gas, then the resulting mixture was refluxed for 4 hr and evaporated to dryness *in vacuo*. The residue was

8) D. McHale, P. Mamalis, J. Green, and S. Marcinkiewicz, *J. Chem. Soc.*, **1958**, 1600.

recrystallized twice from EtOH to give 0.3 g of colorless leaflets, mp 144°. *Anal.* Calcd. for $C_{11}H_{15}O_4N$: C, 58.65; H, 6.71; N, 6.22. Found: C, 58.57; H, 6.48; N, 6.27.

N-Methylcarbamate (11b) of Alcohol (9)——Two g of **10** was reacted with 10 ml of 40% aq. methylamine in 50 ml of EtOH in a similar manner to that for **5** to give 1.2 g of **11b** as colorless crystals, mp 89°. *Anal.* Calcd. for $C_{12}H_{17}O_4N$: C, 60.24; H, 7.16; N, 5.85. Found: C, 60.15; H, 7.38; N, 5.84.

N,N-Dimethylcarbamate (11c) of Alcohol (9)——Two g of **10** was reacted with 10 ml of 40% aq. dimethylamine in 50 ml of EtOH in a similar manner to that for **5** to give 1 g of **11c** as colorless crystals (from petroleum benzin), mp 70°. *Anal.* Calcd. for $C_{13}H_{19}O_4N$: C, 61.64; H, 7.56; N, 5.53. Found: C, 61.67; H, 7.58; N, 5.37.

2-Carbamoyloxymethyl-5-methyl-*p*-benzoquinone (12a)——Zero point 5 g of carbamate (**11a**) in 10 ml of AcOH was oxidized with 0.3 ml of 60% HNO_3 in a similar manner to that for quinone **4** to give 0.3 g of **12a** as yellow needles (from EtOH), mp 133° (decomp.). *Anal.* Calcd. for $C_9H_9O_4N$: C, 55.38; H, 4.65; N, 7.18. Found: C, 55.24; H, 4.58; N, 7.16.

2-(N,N-Dimethylcarbamoyloxymethyl)-5-methyl-*p*-benzoquinone (12c)——Zero point 5 g of **11c** in 2 ml of AcOH was oxidized with 0.3 ml of 60% HNO_3 in a similar manner to that for **4** to give 0.2 g of **12c** as yellow needles (from MeOH), mp 111° (decomp.). *Anal.* Calcd. for $C_{11}H_{13}O_4N$: C, 59.18; H, 5.87; N, 6.28. Found: C, 59.07; H, 5.91; N, 6.25. IR ν_{max}^{Nujol} cm^{-1}: 1700 (carbamoyl C=O), 1660 (quinone C=O).

2-Methyl-5-(N-methyl-N-nitrosocarbamoyloxymethyl)-*p*-benzoquinone (12b)——Two point 1 g of **11b** in 10 ml of AcOH was treated with 1 ml of 60% HNO_3 in a similar manner to that for quinone **8** to give 0.7 g of **12b** as yellow crystals (from MeOH), mp 113°. *Anal.* Calcd. for $C_{10}H_{10}O_5N_2$: C, 50.42; H, 4.23; N, 11.76. Found: C, 50.66; H, 4.35; N, 11.57. IR ν_{max}^{Nujol} cm^{-1}: 1770, 1650. UV λ_{max}^{EtOH} $m\mu$ (log ε): 247 (4.15).

2,5-Dimethoxy-*p*-benzenediacetic Acid[9] (14)——A mixture of 13 g of 2,5-dimethoxy-*p*-benzene diacetonitrile[10] (**13**), 90 ml of water and 75 ml of conc. H_2SO_4 was refluxed with stirring for 4 hr. The reaction mixture was poured into 300 ml of cold water and then separated crystals were collected, washed with water and recrystallized from 350 ml of EtOH to give 7.3 g of colorless needles, mp 246°. *Anal.* Calcd. for $C_{12}H_{14}O_6$: C, 56.69; H, 5.55. Found: C, 56.55; H, 5.58.

2,5-Dimethoxy-*p*-benzenediethanol[10] (16)——To a mixture of 2.9 g of $LiAlH_4$ and 40 ml of dry tetrahydrofuran (T.H.F.) was added dropwise a solution of 7.4 g of 2,5-dimethoxy-*p*-benzenediacetic acid diethyl ester[10] (**15**) in 100 ml of dry T.H.F. with stirring below 40°. The resulting mixture was refluxed for 1 hr. After cooling, to the mixture was added dropwise 5 ml of cold water followed by 40 ml of 50% H_2SO_4 and 40 ml of satd. aq. NH_4Cl to separate organic layer (upper), which was collected. The aqueous layer (under) was extracted with T.H.F. followed by ether. The combined extract and T.H.F. layer was evaporated to dryness. The resulting residue was recrystallized from H_2O–EtOH (10:1) to give 2.6 g of **16**, mp 131°.

Bis(phenoxyformate) (17) of Diethanol (16)——To an ice-chilled, stirred solution of 2.6 g of **16** in 17 ml of pyridine was added dropwise 4.7 ml of phenyl chloroformate. The resulting mixture was stirred at room temperature for 3 hr and then diluted with 50 ml of cold water to give crude products, which were recrystallized from EtOH yielding 4.5 g of colorless needles, mp 100°. *Anal.* Calcd. for $C_{26}H_{26}O_8$: C, 66.94; H. 5.62. Found: C, 66.99; H, 5.69. IR ν_{max}^{Nujol} cm^{-1}: 1760.

Dicarbamate (18a) of Diethanol (16)——A mixture of 4 g of **17**, 70 ml of EtOH and 35 ml of 28% aq. ammonia was refluxed for 4 hr. The separated crystals were collected and recrystallized from EtOH to give 1.4 g of **18a** as colorless crystals, mp 227°. *Anal.* Calcd. for $C_{14}H_{20}O_6N_2$: C, 53.84; H, 6.45; N, 8.97. Found: C, 53.76; H, 6.52; N, 8.53. IR ν_{max}^{Nujol} cm^{-1}: 3460, 3300, 1675.

Bis(N,N-Dimethylcarbamate) (18b) of Diethanol (16)——Four g of **17** was heated with 30 ml of 40% aq. dimethylamine in 70 ml of EtOH under reflux for 3 hr. The resulting mixture was concentrated *in vacuo* and diluted with water then extracted with $CHCl_3$. The extract was evaporated to dryness and the residue was recrystallized from cyclohexane–benzene to give 2 g of **18b** as colorless crystals, mp 131°. *Anal.* Calcd. for $C_{18}H_{28}O_6N_2$: C, 58.68; H, 7.66; N, 7.60. Found: C, 59.02; H, 7.72; N, 7.23.

2,5-Bis(2-carbamoyloxyethyl)-*p*-benzoquinone (19a)——To a suspension of 0.6 g of dicarbamate (**18a**) in 20 ml of AcOH was added dropwise 0.6 ml of 60% HNO_3 with stirring at room temperature. The resulting mixture was stirred for 2 hr and diluted with 30 ml of water. The separated crystals were collected, washed with water and recrystallized from dimethylformamide to give 0.2 g of yellow prisms, mp 230° (decomp.). *Anal.* Calcd. for $C_{12}H_{14}O_6N_2$: C, 51.06; H, 5.00; N, 9.93. Found: C, 51.06; H, 5.05; N, 10.19. IR ν_{max}^{Nujol} cm^{-1}: 3500, 3300, 1730, 1630.

2,5-Bis[2-(N,N-dimethylcarbamoyloxy)ethyl]-*p*-benzoquinone (19b)——To a solution of 2.7 g of **18b** in 13 ml of AcOH was added dropwise 0.8 ml of 60% HNO_3 with stirring at room temperature. The resulting mixture was stirred for 1.5 hr and diluted with 70 ml of cold water to separate yellow crystals, which were collected, washed with water and dried to yield 1.7 g of **19b**, mp 97°. *Anal.* Calcd. for $C_{16}H_{22}O_6N_2$: C, 56.79; H, 6.55; N, 8.28. Found: C, 56.52; H, 6.68; N, 8.04. IR ν_{max}^{Nujol} cm^{-1}: 1700, 1630. UV λ_{max}^{EtOH} $m\mu$ (log ε): 253 (4.19), 258 (4.18 shoulder).

9) J.H. Wood, C.S. Colburn, L. Cox, and H.C. Garland, *J. Am. Chem. Soc.*, **66**, 1540 (1944).
10) J.H. Wood and R.E. Gibson, *J. Am. Chem. Soc.*, **71**, 393 (1949).

2,5-Bis(methylamino)-3-(2-carbamoyloxyethyl)-6-methyl-*p*-benzoquinone (20a)——To a solution of 0.2 g of quinone (**4**) in 30 ml of hot EtOH was added 1.5 ml of 25% ethanolic methylamine solution. The resulting mixture was allowed to stand overnight at room temperature to separate crystals, which were recrystallized from EtOH yielding 0.1 g of red purple crystals, mp 249° (decomp.). *Anal.* Calcd. for $C_{12}H_{17}O_4N_3$: C, 53.92; H, 6.41; N, 15.72. Found: C, 53.63; H, 6.51; N, 15.44. IR ν_{max}^{Nujol} cm^{-1}: 3450, 3280, 1725, 1570.

2,5-Bis(ethylamino)-3-(2-carbamoyloxyethyl)-6-methyl-*p*-benzoquinone (20b)——Zero point 8 g of **4** was reacted with 2 ml of 20% ethanolic ethylamine solution in 100 ml of EtOH in a similar manner to that for **20a** to give 0.3 g of purple crystals, mp 188° (decomp.). *Anal.* Calcd. for $C_{14}H_{21}O_4N_3$: C, 56.93; H, 7.17; N, 14.23. Found: C, 56.84; N, 7.21; N, 13.94. UV λ_{max}^{EtOH} mμ (log ε): 347 (4.39), 530 (2.47).

2,5-Bis(2-diethylaminoethylamino)-6-(2-carbamoyloxyethyl)-3-methyl-*p*-benzoquinone (20c)——To a solution of 0.5 g of **4** in 50 ml of hot EtOH was added a solution of 0.3 g of 2-diethylaminoethylamine in 4 ml of EtOH. After standing overnight at room temperature, the resulting mixture was concentrated *in vacuo* to about 5 ml volume and then allowed to stand for 3 days to separate crystals, which were recrystallized from benzene–cyclohexane to give 0.1 g of pink purple needles, mp 109°. *Anal.* Calcd. for $C_{22}H_{39}O_4N_5$: C, 60.38; H, 8.98; N, 16.01. Found: C, 60.33; H, 9.04; N, 15.74.

2,5-Bis(1-aziridinyl)-3-(2-carbamoyloxyethyl)-6-methyl-*p*-benzoquinone (21)——To a solution of 100 mg of quinone (**4**) in 25 ml of EtOH was added 0.3 ml of aziridine at room temperature. The resulting mixture allowed to stand for 3 days in refrigerator. The separated crystals were collected and washed with EtOH yielding 30 mg of orange needles, mp 196° (decomp.). *Anal.* Calcd. for $C_{14}H_{17}O_4N_3$: C, 57.72; H, 5.88; N, 14.43. Found: C, 57.54; H, 6.09; N, 14.50. IR ν_{max}^{Nujol} cm^{-1}: 3400, 3200, 1690, 1630, 1580. UV λ_{max}^{EtOH} mμ (log ε): 330 (4.20), 430 (2.74).

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