$F_{1\alpha}$ methyl ester could be completely hydrolyzed by the undiluted rat plasma in less than 1 min.[14] Therefore, we do not expect any of the originally administered ester to survive the action of the hydrolases and appear in the urine.

The mechanism for the formation of the tetranor metabolite from the *cis*-$\Delta^4$-PG has been discussed by Green and Samuelsson.[11] They postulated that the reaction sequence included one step of $\beta$-oxidation followed by the hydration of the $\Delta^2$-cis double bond to form the 3-D(−)-hydroxyl compound. The 3-D(−) compound is then epimerized by the action of D(−)-$\beta$-hydroxyacyl CoA epimerase of the 1(+) antipode which, in turn, can enter another cycle of $\beta$-oxidation. This mechanism, however, cannot explain the presence of the dinorprostaglandin of the $F_1$ series in which the carboxylic side chain is fully saturated. Furthermore, in this study we could not detect any dinor metabolites with an unsaturated top side chain. All dinor metabolites found by Green and Samuelsson from the *cis*-$\Delta^4$-$PGF_{1\alpha}$ also lost the $\Delta^4$ double bond.

From the study of the substrates specificity of bovine liver acyl CoA dehydrogenase, Kunau[16] reported that polyunsaturated fatty acids with the $\Delta^4$ double bond were very poor substrates for the first enzyme of the $\beta$-oxidation system. The $\Delta^4$ double bond has to be reduced before further chain shortening can take place. An enzyme, 4-enoyl-CoA reductase, which catalyzed the hydrogenation reaction has been found in mammalian liver. Our results suggest that the $\Delta^4$-prostaglandins may also follow this metabolic pathway. It would be of interest to test whether $\Delta^4$-prostanoate is a substrate of the $\Delta^4$-enoyl-CoA reductase.

**Acknowledgment.** I thank R. Johnson, E. Nidy, E. W. Yankee, and J. Sih of The Upjohn Company for providing the necessary compounds for this study. I also thank J. C. McGuire for his invaluable assistance.

**Supplementary Material Available:** Figure 2 (mass spectra of the methyl ester, $Me_3Si$ ether derivatives of **2** and **3**) and Figure 3 (mass spectra of the methyl ester, $Me_3Si$ derivatives of **4** and **5**) (2 pages). Ordering information is given on any current masthead page.

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# *Notes*

## Antineoplastic Agents. Structure–Activity Relationship Study of Bis(substituted aminoalkylamino)anthraquinones

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A structure–activity relationship study was conducted on a number of bis(substituted aminoalkylamino)anthraquinones. These compounds were prepared by the condensation of substituted or unsubstituted leucoquinizarin with appropriate amines, followed by air oxidation. Both the position and the nature of the center nitrogen atom of the side chain are vital to the antineoplastic activity. The possible mode of action of these aminoquinones was discussed. 1,4-Dihydroxy-5,8-bis[[2-(hydroxyethyl)amino]ethyl]amino-9,10-anthracenedione (DHAQ) was found to possess potent inhibitory activity against both the P-388 leukemia system (T/C of 299 at 0.5 mg/kg with 4/6 cures) and the B-16 melanoma system (T/C of 503 at 1 mg/kg with 7/10 cures).

The mode of action of many chemotherapeutic agents, including certain antineoplastic drugs, has been claimed to be due to their ability to intercalate between the base pairs of the DNA double helix. The molecular complex formation was earlier postulated to explain the biological activity of chloroquine[1,2] and acridine.[3] Although some investigators consider drug–DNA intercalation just a late subterminal event rather than the prime mode of action, other workers accept it as a convenient working hypothesis and have used it to explain the activity of many anticancer agents, including actinomycin D,[4,5] daunorubicin,[6] adriamycin,[7] anthramycin,[8] and coralyne.[9,10] Among these agents, both daunorubicin and adriamycin are recognized as very promising drugs and the latter, particularly, shows good inhibitory activity against leukemia as well as many solid tumors.[11] However, these drugs (or their metabolites) cause severe and irreversible cardiotoxicity which could be fatal if the accumulated dose of these drugs exceeds a limited amount.[12] The amino sugar portion of these drugs has been proposed as responsible for this toxicity.[13] It was

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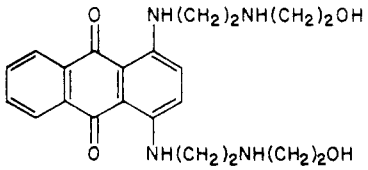
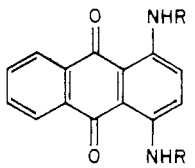
therefore suggested that replacement of the amino sugar portion of these drugs by a properly selected amino- or alkylamino-substituted side chain may eliminate the troublesome toxicity.[13-16] If the DNA intercalation postulation were indeed found true, properly designed amino, alkylamino, or aminoalkylamino side chains may stabilize the intercalated planer chromophore by interacting with the sugar and the phosphate units of DNA and modify its conformation and function. Model compounds of this type have been studied,[17] and binding of aminoalkylaminoanthraquinones to DNA was reported to compare favorably with several known intercalating agents.[18]

Recently, a bis(hydroxyethylaminoethylamino)anthraquinone (1) was found to possess good inhibitory

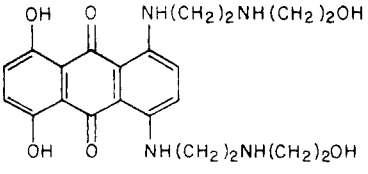


1

activity against leukemia P-388 (Decision Network Meeting, National Cancer Institute, April 18, 1977). This compound seems to conform to the aforementioned requirements for intercalation and the secondary stabilization action of the amino-substituted side chain. In connection with our continued investigation of various quinone derivatives as antineoplastic agents,[19-22] a structure-activity relationship study of these aminoanthraquinones, their minimum structural requirements for activity as well as the optimum activity, has been conducted in this laboratory through systematic molecular modification. This paper reports an initial study of compounds **2a-k** and **3**.



**2a**, R = $(CH_2)_2NH(CH_2)_2CH_3$
**b**, R = $(CH_2)_2NHCH_2CH_3$
**c**, R = $(CH_2)_2NHCH_3$
**d**, R = $(CH_2)_2NH_2$
**e**, R = $(CH_2)_2$-c-$NC_5H_{10}$
**f**, R = $(CH_2)_2$-c-$N(CH_2CH_2)_2NH$
**g**, R = $(CH_2)_2S(CH_2)_2OH$
**h**, R = $(CH_2)_5OH$
**i**, R = $(CH_2)_2$-$C_6H_3$-3,4-$(OCH_3)_2$
**j**, R = $(CH_2)_2NH(CH_2)_2NH(CH_2)_2OH$
**k**, R = $(CH_2)_3NH(CH_2)_2OH$



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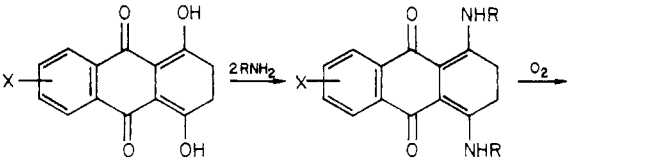
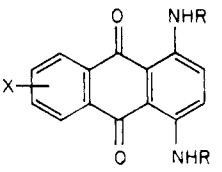
**Chemistry.** The method of preparation of the 1,4-bis(substituted alkylamino)anthraquinones was based on that of Greenhalgh and Hughes,[23] involving condensation of leucoquinizarins (4) with an excess amout of the appropriate amines at 50-55 °C, followed by air oxidation of the dihydro intermediates **5** to the desired products **6**.

Since the intermediates **5** and the products **6** have distinct and different UV absorption in EtOH (**5** are usually green, with $\lambda_{max}$ at 465 and 490 nm, whereas the



4 5



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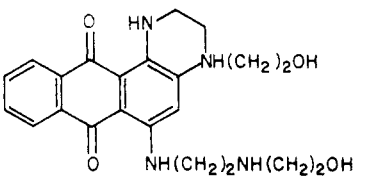
bright blue **6** have $\lambda_{max}$ at 580 and 630 nm), the absorption change of the reaction mixture was used to monitor the course of the reaction and to estimate the purity of the products. These products, which are soluble in water, strongly stain skin, fiber, and even plastic material. The intense, dark color of solutions of these compounds often affects purification processes.

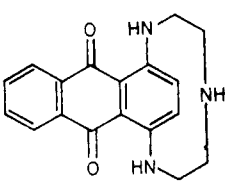
Oxidation of the dihydro intermediates **5** to the aminoanthraquinones **6** proceeded readily in most cases and sometimes can even be realized during recrystallization of the crude dihydro intermediates **5** or upon standing of **5** in solution. Hence, attempts to isolate the pure dihydro intermediates **5**, even under $N_2$, proved to be difficult. For example, attempted isolation of the dihydro intermediate of **1** gave an 80% yield of a crude compound, mp ~130-132 °C. It was, understandably, still contaminated with **1**. At higher reaction temperature (~100 °C), compound **1** readily cyclized to form **7**. For the prepa-



7

ration of many target compounds, therefore, the reaction temperature should be kept below 55 °C, preferably at 50 °C.

Some difficulties were encountered during the preparation of 1,4-bis(2-aminoethylamino)anthraquinone (**2d**) because of the presence of a primary amine function on the side chains and the insolubility of the intermediate **5** [X = H; R = $(CH_2)_2NH_2$] in the reaction solvents (EtOH and $CH_3CN$). The dihydro intermediate was eventually obtained but subsequent oxidation by air in $CH_3CN$ yielded a high-melting solid, mp 308-310 °C, which was only sparingly soluble in common organic solvents such as EtOH or $CHCl_3$. Elemental analysis indicated the presence of only three N atoms in the molecule and suggested **8** as the structure of the product. Apparently,



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compound **8** was formed by an intramolecular condensation of the two terminal chains with the elimination of $NH_3$. The desired compound **2d**, mp 174-176 °C, was

Table I. Antileukemic Activity of 1,4-Bis(substituted alkylamino)anthraquinones against Leukemia P-388[a]

| Compd | Formula (analyses) | Mp, °C | Yield, % | Dose, mg/kg | Survival | Wt diff | T/C, % | Cures |
|---|---|---|---|---|---|---|---|---|
| 1[b] | $C_{22}H_{28}N_4O_4$ (C, H, N) | 156-158 | 47 | 32 | 6/6 | −5.9 | 81 | |
| | | | | 16 | 6/6 | −3.9 | 275 | 3/6 |
| | | | | 8 | 6/6 | −2.0 | 276 | 4/6 |
| | | | | 4 | 6/6 | 0 | 275 | 3/6 |
| 2a | $C_{24}H_{32}N_4O_2 \cdot H_2O$ (C, H, N) | 102-104 | 67 | 100 | 6/6 | −2.6 | 124 | |
| | | | | 50 | 6/6 | −1.7 | 125 | |
| | | | | 25 | 6/6 | −0.8 | 118 | |
| 2b | $C_{22}H_{28}N_4O_2 \cdot 2H_2O$ (C, H, N) | 118-120 | 56 | 25 | 11/12 | −4.8 | 132 | 1/12 |
| | | | | 12.5 | 5/6 | −2.8 | 168 | |
| | | | | 6.25 | 6/6 | −2.2 | 149 | |
| | | | | 3.13 | 6/6 | −2.3 | 142 | |
| 2c | $C_{20}H_{24}N_4O_2 \cdot 0.5H_2O$ (C, H, N) | 116-118 | 52 | 12.5 | 6/6 | −1.9 | 200 | 2/6 |
| 2d[c] | $C_{18}H_{20}N_4O_2$ (C, H, N) | 174-176 | 23 | 25 | 6/6 | −2.6 | 215 | |
| | | | | 12.5 | 6/6 | −1.1 | 174 | |
| | | | | 6.25 | 6/6 | −1.1 | 159 | |
| | | | | 3.13 | 6/6 | −0.9 | 107 | |
| | | | | 1.56 | 6/6 | −1.6 | 137 | |
| | | | | 0.78 | 6/6 | −1.3 | 150 | |
| 2e | $C_{28}H_{36}N_4O_2$ (C, H, N) | 126-128 | 71 | 100 | 12/12 | −4.5 | 128 | |
| | | | | 50 | 12/12 | −2.9 | 110 | |
| | | | | 25 | 6/6 | −3.6 | 104 | |
| 2f | $C_{26}H_{34}N_6O_2$ (C, H, N) | 168-170 | 85 | 50 | 10/12 | −2.7 | 134 | |
| | | | | 25 | 12/12 | −1.0 | 137 | |
| | | | | 12.5 | 12/12 | −1.1 | 117 | |
| | | | | 6.25 | 6/6 | −0.4 | 125 | |
| 2g | $C_{22}H_{26}N_2O_4S_2$ (C, H, N) | 176-178 | 82 | 100 | 6/6 | −2.2 | 94 | |
| | | | | 50 | 6/6 | −1.4 | 86 | |
| | | | | 25 | 6/6 | −1.6 | 95 | |
| 2h | $C_{24}H_{30}N_2O_4$ (C, H, N) | 144-146 | 42 | 100 | 6/6 | −4.0 | 88 | |
| | | | | 50 | 6/6 | −1.5 | 78 | |
| | | | | 25 | 6/6 | −1.4 | 88 | |
| 2i | $C_{34}H_{34}N_2O_6$ (C, H, N) | 128-130 | 90 | 400 | 6/6 | −2.9 | 91 | |
| | | | | 200 | 6/6 | −3.2 | 101 | |
| | | | | 100 | 6/6 | −1.7 | 86 | |
| 2j | $C_{26}H_{38}N_6O_4 \cdot 3C_4H_4O_4$[d] (C, H, N) | 160-162 | 42 | 100 | 15/18 | −2.6 | 120 | |
| | | | | 50 | 18/18 | −1.3 | 112 | |
| | | | | 25 | 18/18 | −1.6 | 110 | |
| 2k | $C_{24}H_{32}N_4O_4$ (C, H, N) | 110-112 | 67 | 100 | 11/12 | −2.6 | 98 | 1/12 |
| | | | | 50 | 12/12 | −2.1 | 133 | |
| | | | | 25 | 11/12 | −2.1 | 130 | |
| | | | | 12.5 | 6/6 | −1.4 | 117 | |
| | | | | 6.25 | 6/6 | 0.9 | 101 | |
| 3[e] | $C_{22}H_{28}N_4O_6$ (C, H, N) | 160-162 | 29 | 2 | 6/6 | −0.6 | 280 | 5/6 |
| | | | | 1 | 5/6 | −0.6 | 277 | 3/6 |
| | | | | 0.5 | 6/6 | −2.0 | 299 | 4/6 |
| | | | | 0.25 | 6/6 | −2.3 | 280 | 2/6 |
| | | | | 0.12 | 6/6 | −1.4 | 200 | |
| | | | | 0.06 | 6/6 | −1.7 | 208 | |
| 7 | $C_{22}H_{26}N_4O_4 \cdot 0.25H_2O$ (C, H, N) | 154-158 | 20 | 100 | 4/6 | −3.8 | 101 | |
| | | | | 50 | 5/6 | −3.5 | 163 | |
| | | | | 25 | 6/6 | −3.2 | 155 | |

[a] Ascitic fluid implanted in $BDF_1$ mice. Treatment started 24 h after implant. For the general screening procedure and data interpretation, cf. R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3, 1 (1972); Instruction Booklet 14, "Screening Data Summary Interpretation and Outline of Current Screen", Drug Evaluation Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md., 1977. [b] Against leukemia L1210: T/C 272, 227, and 156 at 25, 12.5, and 6.25 mg/kg, respectively. [c] Against B-16 melanoma: T/C 281, 280, and 280 at 16, 8, and 4 mg/kg, respectively; with cures 8/10, 6/10, and 6/10, respectively. [d] Trimaleate. [e] Against B-16 melanoma: T/C 503 and 466 at 1 and 0.5 mg/kg, respectively; with cures 7/10 and 4/10, respectively.

obtained in 23% yield by repeated recrystallizations of the dihydro intermediate **5** [X = H; R = $(CH_2)_2NH_2$] from $CH_3CN$ according to the method of Greenhalgh and Hughes.[23]

**Biological Activity and Discussion.** Preliminary screening results of 1,4-bis(substituted alkylamino)-anthraquinones (see Table I) revealed some interesting structure–activity relationships against leukemia P-388 in mice. These observations are summarized as follows.

1. Removal of the terminal hydroxyl group on the side chain of **1**, as in **2b**, retains the original activity, but at a lower level.

2. Compound **2k**, which contains an additional methylene unit between the two nitrogen atoms on the side chains of **1**, possesses only marginal activity, suggesting the importance of the distance between these nitrogen atoms.

3. Insertion of an additional ethylamino unit into the side chain of **1**, as in **2j**, drastically reduces the activity below that marginal level, indicating that additional basic centers and lengthening of the side chain are not desirable.

4. The nitrogen atom in the center of the side chain plays an important role in antileukemic activity. No activity is noted when this nitrogen atom is replaced by a methylene unit (compound **2h**, which still retains the original chain length). Replacement by a sulfur atom (compound **2g**) has the same deleterious effect. The inactivity of **2i** also substantiates this observation.

5. The importance of the aforementioned nitrogen atom to antileukemic activity is again demonstrated by changing the original secondary amino function to a tertiary amine function. Compound **2e** is only marginally active. The activity of compound **2f** is slightly above marginal, probably due to the presence of a binding terminal. On the other hand, compound **2d**, which contains primary amino groups at the end of both side chains, still retains good, although not as high as the original, antileukemic activity.

6. Cyclization of one of the side chains of **1** to a piperazine ring, as in **7**, still retains activity, but at a lower level. This suggests that cyclization of this type of molecular arrangement is probably not the preferred conformation for antileukemic activity.

7. Excellent antileukemic activity is obtained with compound **3** wherein two hydroxyl groups are substituted at positions 5 and 8 of the original compound **1**. This compound, which is much more soluble in water than **1**, requires less than one-tenth of the optimum dose than **1** to produce good activity. In addition, compound **3** shows outstanding activity against B-16 melanoma, with a T/C value of 503 at a dose of 1 mg/kg with 7/10 cures.

## Experimental Section

All melting points were taken on a Thomas-Hoover melting point apparatus. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

**1,4-Dihydroxy-5,8-bis[[2-(hydroxyethyl)amino]ethyl]-amino-9,10-anthracenedione (3, DHAQ).** To 10 g (0.036 mol) of 5,8-dihydroxyleucoquinizarin (leuco-1,4,5,8-tetrahydroxyanthraquinone), purified by continuous extraction with dioxane under $N_2$, was added dropwise, under $N_2$ with cooling and stirring, 38 g (0.36 mol) of 2-(2-aminoethylamino)ethanol. When a homogeneous paste was obtained, the reaction mixture was heated at 50–55 °C in an oil bath for 2 h. It was allowed to stir overnight. The mechanical stirrer (rinsed with 4 × 50 mL of EtOH, washings added to the reaction mixture) was replaced by a glass sparge tube and dry air (air passed through a tube containing Drierite) was bubbled through the reaction mixture while the entire system was under a slightly reduced pressure by connecting the top of the condenser to a water aspirator. This mild oxidation reaction was carried out at 55–60 °C for 3 h. The color of the syrup gradually changed from purple to bright blue. The mixture was then allowed to stand overnight at room temperature. The resulting dark blue solid was collected by filtration through a sintered glass funnel. The solid product was washed with EtOH (2 × 20 mL) and hexane (3 × 50 mL) and dried to give 4.6 g (29% yield) of **3**, mp 158–160 °C. An analytical sample was prepared by recrystallization of the crude product from a mixture of EtOH and hexane: mp 160–162 °C; $\lambda_{max}$ (EtOH) 244 nm (log $\epsilon$ 4.64), 279 (431), 525 (3.70), 620 (4.37), and 660 (4.38). Anal. ($C_{22}H_{28}N_4O_6$) C, H, N.

Compounds **2a–k** were prepared in a manner similar (see Table I) to the aforementioned procedure from commercially available 1,4,9,10-tetrahydroxyanthracene and ca. 10 times equivalent of the appropriate amines. Compound **7** was prepared in a similar fashion except that the reaction temperature was 80–100 °C and the reaction time was 8 h.

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