Approaches to Phenazine-Derived *N*-Isosteres of Anthracyclinones

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1-Hydroxyphenazine 5,10-dioxide showed antitumor properties against mouse leukemia P388. It also participated in biochemical mechanisms of quinoid antitumor agents, as indicated by inhibition of radiolabelled DNA-RNA precursors in cultured leukemia L1210 cells and by stimulation of oxygen consumption in mammalian microsomes. This suggests that the isosteric di-*N*-oxide system may be a biologically active substitute for 1,4-quinone, and that di-*N*-oxides of tetrahydrobenzo[*b*]phenazines can be explored as anthracyclinone *N*-isosteres. As potential synthetic intermediates, 7,8,9,10-tetrahydro-6,11-dihydroxybenzo[*b*]-phenazines have been prepared by 1) Diels-Alder addition of phenazine-1,4-quinone and 1-methoxy-3-(trimethylsilyloxy)-1,3-butadiene to give isolable but labile adducts and 2) condensation of 6,7-diamino-1,2,3,4-tetrahydro-2-hydroxy-5,8-dimethoxy-2-naphthoic acid with 3-methoxy-1,2-quinone. Attempts at *N*-oxidation gave instead oxidation of the 6,11-hydroquinone ring to quinone, regardless of hydroxyl protection. Despite previous literature indications, we have been unable to synthesize the 1,4-dihydroxyphenazine 5,10-dioxide system. We conclude that this hydroxyl substitution pattern (1,4) in an adjacent ring must be avoided in the redesign of anthracyclinone isosteres that have di *N*-oxide in place of quinone.

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Quinone structures occur in numerous natural anticancer agents (1), such as the mitomycins, streptonigrin, and the anthracyclines including doxorubicin and daunorubicin (**1a**, X = OH and H, respectively). Antitumor properties have also been observed for numerous synthetic quinones that have simpler structures (1,2). The quinone unit is an important site of biochemical action and has often been directly implicated in the antitumor properties of these molecules (2-5). Mechanisms have been proposed involving one-electron or two-electron reductions of the quinone. The one-electron process has been the more widely explored. It involves generation of free radicals (*e.g.*, semiquinone, superoxide, hydroxyl) and has been evidenced by enhancement of oxygen consumption upon adding the drug to microsomal systems (3,4,6), by DNA nicking following chemical prereduction of the drug (7,8), and by esr studies (4,6) that have included spin trapping techniques (9,10). The two-electron reduction process has been observed in the electrochemical studies (11,12) and has been associated with alkylating activity (2,5,7). These mechanisms may be related to other effects of the quinoid agents besides their antitumor properties. With the anthracyclines, current thinking tends to associate the generation of free radicals with the cardiotoxic side effects in particular, because cardiac tissue is deficient in protective enzymes (13). However, complex quinone-bearing molecules like the anthracyclines are no doubt capable of multiple mechanisms of action, at multiple biological sites. Thus, structural changes that alter reactivity of the quinone function may significantly alter the overall pattern of biological effects, although specific effects cannot be targeted based on current knowledge. Such changes offer an important approach to the development of analogs with improved properties, but so far analog development with quinoid drugs has usually been attempted through changes elsewhere in these molecules, and has rarely involved changes within the quinone ring.

Of special interest as novel quinone analogs are the di-*N*-oxides (**5** and **6**, respectively) of phenazines and quinoxalines, which are *N*-isosteres of anthraquinones (**2**) and naphthoquinones (**3**). These isosteric structures exhibit redox properties analogous to those of the quinones, as indicated by electrochemical studies, and by the esr and absorption spectra that have identified anion radicals from **5a** and **6** that correspond to semiquinones (14-16). Several quinoxaline di-*N*-oxides (**6** and derivatives) generated esr signals upon chemical or biological reduction, and the production of free radicals was suggested to explain the observed degradation of DNA and inhibition of DNA synthesis (17). Antitumor properties have been reported (18) for several phenazine 5,10-dioxides (**5** and derivatives) against Ehrlich ascites carcinoma in mice. The most active was the antibiotic iodinin (**5c**), in which the phenazine dioxide structure is hydroxylated in positions 1 and 6. This is suggestive of the hydroxyl substitution adjacent to the quinone in some anthracyclinones (*e.g.*, citromycinone). Perhaps the OH's in iodinin enhance the biological activity of the phenazine dioxide function. An obscure report (19) indicates that 1,4-dihydroxyphenazine 5,10-dioxide (**5d**) was as active as iodinin (**5c**) against sarcona 180 in mice. Recently we found that 1-hydroxyphenazine 5,10-dioxide (**5b**) (20) had borderline antitumor activity (T/C = 124% at 12.5 mg/kg, qd 1-9, average of 2 tests) against lymphocytic leukemia P388 in mice, the test employed by NCI as a primary screen (21). This compound (**5b**) inhibited the incorporation of radiolabelled DNA-RNA precursors in cultured lymphoid leukemia L1210 cells (22) at dose levels (ED_{50} 2.6 and 2.4 μM) comparable

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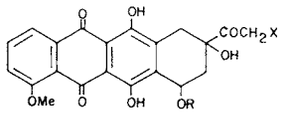
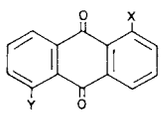
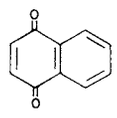


1a R = daunosamine
1b R = H



2a X = Y = H
2b X = OH, Y = H
2c X = Y = OH



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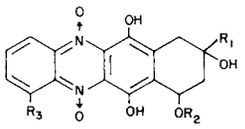
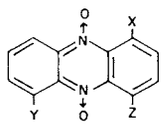
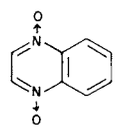


4a R_2 = daunosamine
4b R_2 = H



5a X = Y = Z = H
5b X = OH, Y = Z = H
5c X = Y = OH, Z = H (iodinin)
5d X = Z = OH, Y = H



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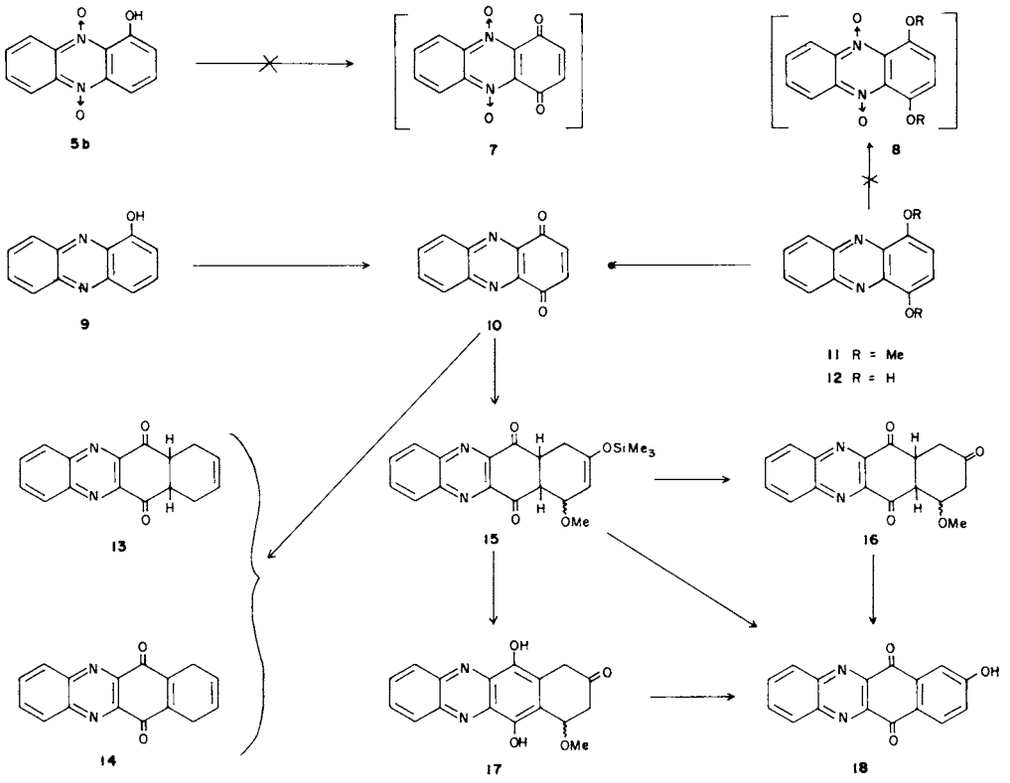
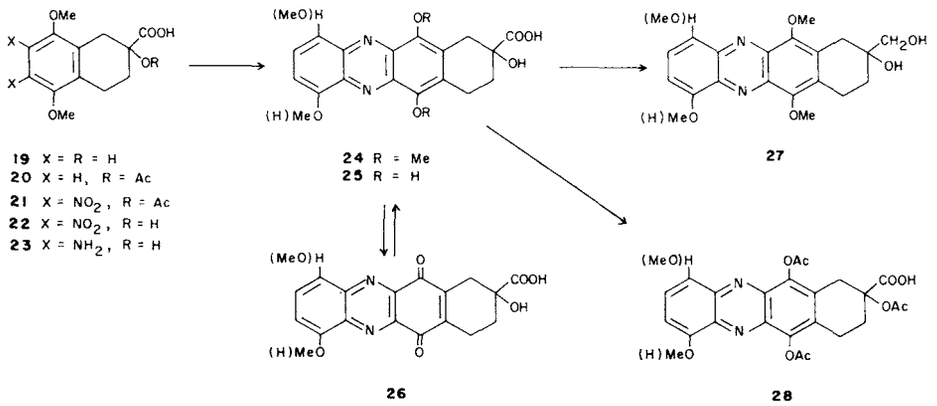
to those for doxorubicin (ED_{50} = 1.5 and 0.6 μM), but it had no effect on the thermal denaturation temperature of isolated helical DNA (22), suggesting a lack of binding to the DNA template. Addition of **5b** to mammalian microsomes markedly stimulated the consumption of oxygen, to attain 44% of the rate observed after adding doxorubicin (23) as reference. The di-*N*-oxide function was evidently essential to the activity observed with **5b**, because unoxidized 1-hydroxyphenazine (20) (**9**) was nearly 20 times less potent (ED_{50} = 41 and 44 μM) an inhibitor of DNA and RNA synthesis (22), and was 5 times poorer (8% of the doxorubicin rate) (23) in stimulating microsomal oxygen consumption (**9** was not submitted for *in vivo* tests). That the di-*N*-oxide structure of **5b** offers some advantage as an isostere of the quinone was suggested by the fact that 1-hydroxyanthraquinone (**2b**) was inactive when tested at high doses against three murine tumors, as was 1,5-dihydroxyanthrquinone (**2c**, the analog of iodinin) (1). Generally, quinones are more likely to be active if they bear amino-substituted side chains, which are believed to be required for efficient intercalation into helical DNA. The preliminary results with **5b** suggest that if the phenazine 5,10-dioxide structure were elaborated to afford tetracyclic *N*-isosteres **4b** of the anthracyclinones, which then could be coupled with daunosamine, the final products **4a** should be of considerable interest for evaluation of their antitumor properties and side effects. This report describes attempts to synthesize the isosteric anthracyclinones. Structure-activity relationships observed in the doxorubicin series (**1a**) show that the 4-methoxyl and the ketone side chain at C-9 can be altered or deleted (corresponding to R_3 and R_1 in **4**), and this should facilitate the design and synthesis of **4**.

Synthesis.

A useful approach to the anthracyclinones has been the Diels-Alder reaction between quinizarin quinone and various dienes (24). Two limitations are the problem of



5b 7 8 9 10 11 R = Me 12 R = H 13 14 15 16 17 18



regioselectivity (which can be circumvented by deleting the 4-methoxyl in target structure **1**) and the competing addition of some dienes to the internal (Δ-4a,9a) as well as terminal (Δ-2,3) double bond of quinizarin quinone. We found no literature reference to the *N*-isostere **7** of quinizarin quinone, and our attempts to begin by synthesizing **7** were completely unsuccessful. Attempts to oxidize **5b** with Fremy's salt, potassium nitrosodisulfonate, or periodic acid (reagents for oxidizing phenols to quinones) (25) afforded either no reaction or decomposition. On the other hand, if the di-*N*-oxide function of **5b** was reduced, the resultant **9** was readily oxidized with these reagents to the phenazine 1,4-quinone (**10**) in moderate yields. Alternatively, quinone **10** was obtained (88% yield) by oxidation of 1,4-diol **12** (26) with lead tetraacetate (27), or directly from the 1,4-dimethoxy precursor **11** (26) with silver oxide in nitric acid (28) (40% yields). Successful oxidation of **12** (*via* the di-*O*-acetate) to the di-*N*-oxide (**8**, R = H) was reported (26), but we observed decomposition upon treatment of **11** with *m*-chloroperbenzoic acid in dichloromethane (*N*-oxidation of **11** would be identifiable in the ^{1}H nmr by a small downfield shift of the aryl protons (H-6 and H-9) peri to N→O). Similar treatment of **12** gave decomposition (although treatment of **12** with 30% hydrogen peroxide in acetic acid again gave quinone **10**). The quinone **10**, unoxidized at the nitrogen atom, proved to be a good dienophile with the reactive 1-methoxy-3-(trimethylsilyloxy)-1,3-butadiene (29), but reacted sluggishly with butadiene and gave little or no product under various conditions with chloroprene or 1-methoxybutadiene. The product from **10** and butadiene at 100° (sealed bomb) for 20 hours was a mixture of the expected tetracycle **13** with the products (**12** and **14**) of disproportionation, as evidenced by ^{1}H nmr analysis in comparison with carbocyclic analogs (30) of **13** and **14**. The vinyl H's at C-8 and C-9 (31) of **13** and **14** appeared at 5.77 and 5.89 δ, respectively, and the allylic H's at C-7 and C-10 of **13** and **14** appeared at 2.5 (broad singlet) and 3.40 δ (sharp singlet), respectively. Similar disproportionation products have been reported in the Diels-Alder reactions of quinoxaline-1,4-quinones (32). Reaction of **10** with 1-methoxy-3-(trimethylsilyloxy)butadiene occurred at room temperature to give the normal adduct **15** in 96% yield. The product was identified as **15** by appearance of a one-proton doublet at δ 5.20 that could be assigned to the single vinyl proton on C-8 (31), coupled to H-7 appearing as a quartet at δ 4.28. A two-proton multiplet at δ 3.58 could be assigned to the bridgehead H's at C-6a and C-10a. Complex one-proton multiplets centered at δ 3.28 and 2.35 were individually assigned to the two H's on C-10. Methoxyl and trimethylsilyloxy singlets appeared as expected. The infrared spectrum showed normal ketone bands at 5.81, and 5.88, and unsaturation at 6.03 μ. There was no evidence for a by-product from a possibly competing diene addition to the 4a,10a double bond of **10**. This is a notable advantage for this approach, as the Danishevsky diene (29) gave addition primarily (33) at the internal double bond of quinizarin quinone. However, in what is apparently the only Diels-Alder reaction reported (34) for anthracene-1,4-dione (isostere of **10**), no such by product was observed, so the absence of by-product from **10** is not surprising. The adduct **15** in cold dioxane or tetrahydrofuran could be hydrolyzed at the silyl enol ether function by brief treatment with dilute hydrochloric acid. Either the trione **16** or its enolized form, the keto hydroquinone **17**, could be obtained in good yield and good purity, after workup with water or bicarbonate solution, respectively. Both showed infrared carbonyl absorption at 5.80 μ, but **17** was distinguished by a strong OH band at 2.99 μ. In the ^{1}H nmr, **16** showed H-7 as a narrow multiplet at δ 4.34, which was shifted to δ 5.46 in **17**. In **17**, the only additional signals besides the aryl-H multiplets and OCH_3 singlet were a widely spaced quartet for the protons on C-10 and a quartet of doublets for the protons on C-8. The spectrum of **16** was more complex with the additional bridgehead protons on C-6a and C-10a appearing at δ 3.5-4.0. Compounds **15**, **16**, and **17** were sensitive molecules, prone to aromatization. Various attempts to

convert **15**, **16**, or **17** to a 9-ketone cyanohydrin (toward elaboration of a 9-side chain) gave the aromatized elimination product **18**, evidenced by ^{1}H nmr resonance for aryl H's (δ 7.3-8.4) only. As expected, H-10 and H-8 (adjacent to aryl OH) were upfield (δ 7.61 d and 7.32 q, respectively) from the other protons. Infrared quinone absorption was observed at 5.92 μ. Other attempts to reduce the silyl enol ether of **15** or the 9-ketone of **17** to the 9-alcohol by catalytic hydrogenation also gave aromatization to **18**. Hydrogenation of **16** gave reduction of the diaza ring, but treatment of **16** with sodium cyanoborohydride also produced **18**. Tendencies toward aromatization have also been observed (35) with 9-keto anthracyclinones related to **17**, but a successful ketone reduction to the 9-alcohol using sodium cyanoborohydride has been reported (36). Because of the obvious lability of **15-17**, we explored a completely different approach.

5,8-Dimethoxy-2-hydroxy-1,2,3,4-tetrahydro-2-naphthoic acid (**19**) (37) was acetylated at the OH and, without isolation of **20**, was converted with cupric nitrate to the 6,7-dinitro derivative **21**, which was deacetylated with acid because base treatment caused decomposition. Hydrogenation of the resultant dinitro acid **22** (81% yield from **19**) afforded the diamine **23**. The sensitivity to air oxidation of such diamines has been noted (38), and diamine **23** without isolation was condensed (39) with freshly prepared 3-methoxy-1,2-quinone to generate the desired 7,8,9,10-tetrahydrobenzo[*b*]phenazine system. The product **24** (29% yield from **22**) was readily separated from neutral by-products and impurities because presence of the carbonyl function permitted extraction into aqueous bicarbonate solution, as one step during work-up. Although **24** was analytically pure and homogeneous by tlc, it was revealed as the expected (39) mixture of regioisomers by the multiplicity of methoxyl singlets in the ^{1}H nmr spectrum. Attempts to avoid regioisomerism by condensing diamine **23** with *o*-quinone under unoptimized conditions gave yields that were always less than **10%**. Attempts at *N*-oxidation of **24** gave only an unexpectedly facile oxidative demethylation to the 6,11-quinone **26**. These conditions included treatment with *m*-chloroperbenzoic acid in dichloromethane and with 30% hydrogen peroxide in acetic acid, which proved to be as effective as deliberate conversion to **26** using ceric ammonium nitrate (40). Yields were 60-90%. Use of trifluoroacetic acid with hydrogen peroxide only decreased the yield of **26**. There seemed to be no tendency to form the *N*-oxides of either **24** or **26**. Although resistance to *N*-oxidation has been encountered and attributed to steric hindrance in such systems (41), these results are in contrast to the reported bis *N*-oxidation of 5-hydroxy-8-methoxyquinoxaline (42). If oxidation was attempted directly on quinone **26** with 30% hydrogen peroxide in trifluoroacetic anhydride, there was only decomposition, as might be expected with this more reactive substrate.

Because of the reportedly (26) successful oxidation of 1,4-dihydroxy phenazine to the 5,10-dioxide **5d**, apparently through the 1,4-di-*O*-acetate, we alternatively tried *N*-oxidation of the 6,11-di-*O*-acetate **28** (acetylated at the 9-OH also). The best conversion of **24** to **28** was via the quinone **26**, which was reduced with aqueous dithionite to the hydroquinone **25** in 85% yield, and acetylated. The yield of **28** was 68% overall from **24**, whereas a reductive acetylation of the quinone **26** with zinc, acetic anhydride and triethylamine gave **28** in only 38% yield. Treatment of **28** with *m*-chloroperbenzoic acid in dichloromethane gave quinone **26** (as the 9-*O*-acetate) in yields up to 50% and, again, no evidence for *N*-oxide. The general inability to form *N*-oxides that we have observed in the 1,4-dioxy phenazine system is also in contrast to the well-documented *N*-oxidation of 1,6-dimethoxyphenazine (43). Despite the earlier reports (26,42), the hydroquinone → quinone oxidation apparently cannot be prevented by blocking the hydroquinone OH's and occurs to exclusion of the desired *N*-oxidation. We conclude that the target structures **4** should be redesigned with only one OH in either of the rings adjacent to the bis *N*-oxide.

Some exploratory experiments were undertaken toward introduction of a 7-oxy function in the hydroxy acid **24**, using bromination procedures developed for daunomycinone (**1b** X = H). The carboxyl function was a useful water solubilizing group for processing reaction products in this series, but it made **24** difficultly soluble in common bromination solvents. Consequently, we observed little reaction when **24** was treated (37) with bromine in carbon tetrachloride or tetramethylammonium tribromide in benzene. The products (yields about 10%), after treatment (37) with sodium trifluoroacetate and methanolysis intended to produce the 7-OH, appeared unexpectedly in a neutral fraction containing a lactone between the 7-OH and the carboxyl. This was indicated by infrared absorption at 5.57 μ, and by conversion to a mono trimethylsilyl derivative which had a mass spectrum consistent with the lactone structure. These difficulties with **24** could be avoided by reducing the 9-COOH prior to bromination. Treatment of **24** with borane-tetrahydrofuran afforded a nearly quantitative yield of the 9-hydroxymethyl analog **27**. This side chain at C-9 should be ultimately acceptable in targets such as **4**, but the 7-bromination and glycosidation of **27** was not pursued, because of the need to redesign the hydroxyl substitution pattern in **4** so as to permit *N*-oxidation.

EXPERIMENTAL

Solutions in organic solvents were dried over sodium sulfate and filtered. Evaporations were carried out *in vacuo* on a rotary evaporator. Melting points are uncorrected. Ir spectra in Nujol mull were routinely recorded on a Perkin-Elmer 137 spectrometer and generally showed the

expected bands, *e.g.*, alcohol OH, carboxyl OH, NO_2; only selected diagnostic bands are reported. Proton nuclear magnetic resonance (nmr) was determined at 90 MHz with a Varian EM390 spectrometer on solutions as noted with TMS (δ 0.0) internal reference; the signals are described as s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad); integrated peak heights were as predicted from the structures; we thank Dr. K. F. Kuhlmann and L. Garver for the spectra. Mass spectra were recorded on an LKB Model 9000 spectrometer at 12 eV, or on a CEC-21-110B high-resolution spectrometer to verify the compositions of **25** and **27**; we thank Dr. D. W. Thomas for the spectra and interpretations. Thin-layer chromatography (tlc) was carried out on 2 × 8-in glass plates coated with 0.25-mm layers of silica gel GF; R_f values are given for products purified to homogeneity. Several of the benzophenazines, even after drying *in vacuo*, were retentive of small amounts of organic solvent, as observed both in the nmr spectra and in elemental analyses.

1,4-Phenazinedione (**10**).

To a stirred solution of 1.06 g (5.0 mmoles) of 1,4-dihydroxyphenazine (**12**) (26) in 400 ml of chloroform was added dropwise, over a 10-minute period, a solution of 2.66 g (6.0 mmoles) of lead tetraacetate in 21 ml of chloroform-acetic acid (20:1). The mixture was stirred at 23° for 15 minutes, treated with 0.3 ml (5.4 mmoles) of ethylene glycol and stirred at 23° for 5 minutes. The reaction mixture was decanted from a gummy precipitate, and the precipitate was triturated with 50 ml of chloroform. The combined chloroform solution was washed with water (2 × 100 ml), dried, and evaporated to afford 0.922 g (88%), mp > 300° (lit (27) dec 240-248°); ir 5.98 μm (C=O); nmr (deuteriochloroform): δ 8.50 (m, H-6, H-9), 8.04 (m, H-7, H-8), 7.37 (s, H-2, H-3); ms: m/e 210 (M^+, 100), 182 (29), 82 (71), 54 (35); tlc in chloroform-methanol (99:1), R_f 0.35.

6a,7,10,10a-Tetrahydro-7-methoxy-9-trimethylsilyloxy-6,11-benzo[*b*]-phenazinedione (**15**).

A stirred solution of 0.841 g (4.0 mmoles) of the quinone **10** in 700 ml of dichloromethane was treated with 2.0 ml (9.2 mmoles) of 1-methoxy-3-(trimethylsilyloxy)-1,3-butadiene (29). After stirring at 23° for 18 hours, the solution was evaporated and the residue was triturated with 10 ml of cyclohexane to afford 1.470 g (96%); ir 5.81, 5.88 (C=O), 6.03 (C=C-O); 9.30 (SiO); 11.42, 11.83 μm ($Si(CH_3)_3$); nmr (deuteriochloroforml): δ 8.37 (m, H-1, H-4), 7.92 (m, H-2, H-3), 5.20 (d, J = 6 Hz, H-8), 4.28 (dd, J = 3, 6 Hz; H-7), 3.58 (m, 2, H-6a, H-10a), 3.28 (m, H-10B), 2.88 (s, OCH_3), 2.37 (m, H-10A), 0.30 (s, $Si(CH_3)_3$); ms: m/e 350 ($M-CH_3OH$, 100). The analytical sample was dried *in vacuo* at room temperature.

Anal. Calcd. for $C_{20}H_{22}N_2O_4Si \cdot 0.1CH_2Cl_2$ (evidenced by nmr singlet at δ 5.27): C, 61.75; H, 5.72; N, 7.16. Found: C, 61.80; H, 5.75; N, 7.19.

Decomposition occurred upon further drying at 78° of this labile compound, as evidenced by high values for C and N and low values for H.

Anal. Calcd. for $C_{20}H_{22}N_2O_4Si$: C, 62.79; H, 5.80; N, 7.32. Found: C, 63.24; H, 5.17; N, 7.77.

6a,7,8,10a-Tetrahydro-7-methoxy-6,9(10*H*),11-benzo[*b*]phenazinetrione (**16**).

To a stirred solution of 0.383 g (1.0 mmole) of **15** in 30 ml of dioxane cooled to 15° was added 3.0 ml of 0.1 *N* hydrochloric acid. After stirring at 15° for 10 minutes, the solution was poured into 75 ml of water and then extracted with dichloromethane (2 × 15 ml). The combined extracts were washed with water (3 × 50 ml) dried, and evaporated. The residue was triturated with 15 ml of dichloromethane and the insoluble by-product **18** of aromatization was removed by filtration. Evaporation of the filtrate afforded 0.250 g (81%) of the trione **16**; ir: 5.80 (C=O); nmr (deuteriochloroform): δ 8.37 (m, H-1, H-4), 7.97 (m, H-2, H-3), 4.34 (m, H-7), 3.3-4.0 (m, H-6a, H-10a, H-10B), 2.93 (s, OCH_3), 2.85-3.15 (m, H-10A, 2.67 (m, H-8B), 2.49 (m, H-8A); ms: m/e 310 (M^+, 7), 278 (100), 250 (47); tlc in chloroform-methanol (39:1), R_f 0.48 (with tailing). The analytical sample was dried *in vacuo* at room temperature.

Anal. Calcd. for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03. Found: C, 65.48; H, 4.39; N, 9.11.

7,8-Dihydro-6,11-dihydroxy-7-methoxy-9(10*H*)benzo[*b*]phenazinone (**17**).

To a stirred solution of 0.739 g (1.93 mmoles) of **15** in 55 ml of THF cooled in an ice bath was added 9.7 ml of 0.1 *N* hydrochloric acid. After stirring at 0° for 10 minutes, the solution was diluted with a solution of 0.162 g (1.93 mmoles) of sodium bicarbonate in 100 ml of water. The aqueous mixture was extracted with dichloromethane (2 × 30 ml). The combined extracts were washed with water (2 × 75 ml), dried and evaporated. The residue was triturated with 30 ml of dichloromethane and the insoluble aromatized quinone **18** was removed by filtration. Evaporation of the filtrate gave 0.444 g (70%) of the ketone **17**; ir 2.99 (OH), 5.80 (C=O); nmr (deuteriochloroform): δ 8.22 (m, H-1, H-4), 7.86 (m, H-2, H-3), 5.46 (m, H-7), 4.09 (d, J = 21 Hz, H-10B), 3.80 (d, J = 21 Hz, H-10A), 3.38 (s, OCH_3), 3.07 (dd, J = 3, 18 Hz; H-8B), 2.68 (dd, J = 3, 18 Hz; H-8A); ms: m/e 310 (M^+, 16), 278 (100), 250 (93); tlc in chloroform-methanol (39:1), R_f 0.60, with minor contaminants at R_f 0.20 (**18**) and 0.03.

9-Hydroxy-6,11-benzo[*b*]phenazinedione (**18**).

The isoluble by-product from preparation of **16** or **17** had mp > 300°; ir 3.00 (OH), 5.92 (C=O), 6.28, 6.37 μm (aryl); nmr (Me_2SO-d_6): δ 8.39 (m, H-1, H-4), 8.22 (d, J = 8 Hz, H-7), 8.11 (m, H-2, H-3), 7.61 (d, J = 3 Hz, H-10), 7.32 (dd, J = 3, 8 Hz; H-8); ms: m/e 276 (M^+, 100), 248 (42), 220 (46); tlc in chloroform-methanol (39:1), R_f 0.20.

Anal. Calcd. for $C_{16}H_8N_2O_3 \cdot 0.25H_2O$: C, 68.45; H, 3.05; N, 9.98. Found: C, 68.27; H. 3.15; N, 9.77.

2-Acetoxy-1,2,3,4-tetrahydro-5,8-dimethoxy-6,7-dinitro-2-naphthoic Acid (**21**).

A suspension of 4.83 g (19.1 mmoles) of 1,2,3,4-tetrahydro-2-hydroxy-5,8-dimethoxy-2-naphthoic acid (**19**) (37) in a mixture of 38 ml of acetic anhydride and 0.3 ml of pyridine was stirred at 23° for 18.5 hours. To the stirred mixture was then added, portionwise over a 1-hour period, 13.8 g (57.3 mmoles) of cupric nitrate trihydrate; the temperature was maintained at 45-50° by occasional cooling of the exothermic reaction mixture. After the addition was complete, the mixture was stirred at 23° for 2 hours, cooled in a water bath and diluted with 20 ml of water added dropwise. The reaction mixture was stirred at 23° for 2 hours, poured into 150 ml. of water and extracted with dichloromethane (4 × 75 ml). The combined extracts were washed with water (4 × 50 ml), dried, and evaporated to give 6.99 g (95%), mp 208-211°; ir 5.75 (C=O, ester), 5.86 (C=O, acid); nmr (Me_2SO-d_6): δ 3.89 (s, OCH_3), 3.85 (s, OCH_3), 3.35 (d, J = 18 Hz, H-1B), 3.28 (d, J = 18 Hz, H-1A), 2.89 (m, 4-H_2), 2.25 (m, 3-H_2), 2.00 (s, $OCOCH_3$); ms: m/e 324 ($M-CH_3CO_2H$, 100), 43 (54); tlc in chloroform-methanol (9:1), R_f 0.25.

Anal. Calcd. for $C_{15}H_{16}N_2O_{10}$: C, 46.88; H, 4.20; N, 7.29. Found: C, 46.90; H, 4.19; N, 7.08.

1,2,3,4-Tetrahydro-2-hydroxy-5,8-dimethoxy-6,7-dinitro-2-naphthoic Acid (**22**).

A stirred solution of 6.98 g (18.2 mmoles) of **21** in 400 ml of 6*N* hydrochloric acid dioxane (1:1) was heated at 70-75° under nitrogen for 4 hours. The cooled reaction mixture was diluted with 200 ml of water and extacted with ethyl acetate (3 × 150 ml). The combined extracts were washed with brine (4 × 100 ml), dried, and evaporated. Crystallization of the residue from 75 ml of dichloromethane afforded 5.19 g (75%), mp 127-130°. The mother liquors gave an additional 0.75 g, mp 126-128° [total 5.94 g (85%)]; ir: 2.88 (alcoholic OH), 5.80 (C=O), 6.49 and 7.37 μm (NO_2); nmr (Me_2SO-d_6): δ 3.88 (s, OCH_3), 3.86 (s, OCH_3), 2.97 (m, 1-H_2, 4-H_2), 1.98 (m, 3-H_2); ms: m/e 342 (M^+, 83), 324 (100); tlc in chloroform-methanol-water (60:10:1), R_f 0.15.

Anal. Calcd. for $C_{13}H_{14}N_2O_9$: C, 45.62; H, 4.12; N, 8.18. Found: C, 45.62; H, 4.11; N, 7.88.

6,7-Diamino-1,2,3,4-tetrahydro-2-hydroxy-5,8-dimethoxy-2-naphthoic Acid (**23**).

A mixture of 3.83 g (11.2 mmoles) of **22** and 0.55 g of 5% palladium on carbon in 200 ml of acetic acid was reduced in a Parr hydrogenator at 45

psi at 23° for 6 hours. The reduction mixture was filtered, and the filtrate containing **23** was reacted *in situ* with 3-methoxy-1,2-benzoquinone.

3-Methoxy-1,2-benzoquinone.

A stirred solution of 4.06 g (16.5 mmoles) of tetrachloro-*o*-quinone in 80 ml of diethyl ether cooled to −25° was treated dropwise over a 5-minute period with a solution of 2.24 g (16.0 mmoles) of 3-methoxycatechol in 20 ml of ether. The reaction mixture was stirred at −25° for 2 hours. The resultant crystals were collected and washed with 5 × 10 ml of ether to yield 2.21 g (100%); ir: 6.01 (C=O), 6.18, 6.47 μm (aryl); nmr (deuteriochloroform): δ 7.03 (dd, J = 7.5, 10.5 Hz; H-5), 6.07 (d, J = 10.5 Hz, H-6), 5.92 (d, J = 7.5 Hz, H-4), 3.79 (s, OCH_3).

9-Carboxy-7,8,9,10-tetrahydro-9-hydroxy-4(1),6,11-trimethoxybenzo[*b*]-phenazine (**24**).

To a stirred solution of 11.2 mmoles of **23** in 145 ml of acetic acid, under nitrogen, was added 1.55 g (11.2 mmoles) of freshly prepared 3-methoxy-1,2-quinone over a 1 hour period. The reaction mixture was stirred at 23° under nitrogen for 17 hours and then evaporated. The residue was dissolved in 40 ml of methanol, the solution was diluted with 400 ml of ethyl acetate and then washed with 100 ml of water. The aqueous phase was back-extracted with ethyl acetate (3 × 50 ml), and the combined organic extracts were washed with water (5 × 50 ml). The organic phase was extracted with 0.5 *N* sodium bicarbonate solution (2 × 200 ml) and with 100 ml of water. The combined basic aqueous extracts were washed with dichloromethane (3 × 25 ml), acidified to *p*H 3.0 with 6*N* hydrochloric acid and then extracted with dichloromethane-methanol (9:1) (8 × 100 ml). The combined extracts were washed with water (2 × 75 ml), dried, and evaporated to a foamed glass (1.50 g). Crystallization from 15 ml of methanol gave 1.22 g (26%), mp 115-125°. The mother liquors afforded an additional 0.14 g (total yield 29%); ir: 5.81 μm (C=O); nmr (deuteriochloroform): δ 7.82 [d, J = 9 Hz, H-1(4)], 7.67 [t, J = 7.5, 9 Hz; H-2(3)], 6.98 [d, J = 7.5 Hz, H-3(2)], 4.21, 4.19, 4.14, 4.11 (4 singlets, OCH_3), 3.42 (br s, 10-H_2), 3.26 (m, 7-H_2), 2.19 (m, 8-H_2); ms: (as $[Si(CH_3)_3]_2$ derivative) m/e 528; tlc in chloroform-methanol-2*N* acetic acid (40:10:1), R_f 0.38.

Anal. Calcd. for $C_{20}H_{20}N_2O_6 \cdot 1.2CH_3OH$ (evidenced by nmr singlet at δ 3.48): C, 60.22; H, 5.91; N, 6.62. Found: C, 59.9; H, 5.56; H. 6.76.

Further drying required heating to 100° *in vacuo*, at which temperature decomposition occurred, as evidenced by darkening of the sample.

9-Carboxy-7,8,9,10-tetrahydro-9-hydroxy-4(1)-methoxy-6,11-benzo[*b*]phenazinedione (**26**).

To a stirred solution of 0.845 g (2.0 mmoles) of **24** in 12 ml of acetic acid was added dropwise, over a 10 minute period, a solution of 2.41 g (4.4 mmoles) of ceric ammonium nitrate in 10 ml of water. After stirring at 23° for 35 minutes, the reaction mixture was diluted with 100 ml of water and extracted with 12 25-ml portions of chloroform-methanol (9:1). The combined extracts were washed with saturated aqueous sodium chloride (2 × 10 ml), dried, and evaporated. The residue was dissolved in 10 ml of chloroform-methanol (4:1), diluted with 20 ml of toluene and evaporated. Trituration of the residue with 25 ml of diethyl ether afforded 0.710 g (92%); ir: 5.82 (C=O, acid), 6.00 (C=O, quinone), 6.21, 6.42 μm (aryl); nmr (deuteriochloroform-perdeuteromethanol 4:1) δ 7.91 [d, J = 4.5 Hz, H-1(2), H-3(4)], 7.27 [t, J = 4.5 Hz, H-2(3)], 4.16 (s, OCH_3), 3.11 (br, s, 10-H_2), 2.99 (m, 7-H_2), 2.10 (m, 8-H_2); ms: m/e 336 (M-H_2O, 49), 321 (30), 308 (100), 293 (80); tlc in chloroform-methanol-2*N* acetic acid (40:10:1), R_f 0.18. Attempts to remove toluene of solvation required drying at 140° (0.1 mm), which caused decomposition evidenced by darkening of the sample.

9-Carboxy-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4(1)-methoxybenzo[*b*]-phenazine (**25**).

A stirred solution of 0.143 g (0.4 mmole) of **26** and 0.034 g (0.4 mmole) of sodium bicarbonate in 15 ml of water was treated, under nitrogen over a 3-minute period, with a solution of 0.278 g (1.6 mmoles) of sodium dithionite and 0.168 g (2.0 mmoles) of sodium bicarbonate in 4 ml of water. After stirring under nitrogen at 23° for 15 minutes, the reaction mixture was acidified to *p*H 3.0 with 1.0 *N* hydrochloric acid, and the resulting precipitate was collected and dissolved in 5 ml of methanol. The aqueous mixture was extracted with 3 × 15 ml of ethyl acetate; the extracts were combined with the methanol solution and washed with 3 × 5 ml of water. After drying, the extracts were filtered through Celite and evaporated to yield 0.130 g (85%); nmr (Me_2SO-d_6): δ 9.30 br s and 8.90 br s (11-OH and 6-OH), 7.79 [d, J = 4.5 Hz, H-1(2), H-3(4)], 7.21 [t, J = 4.5 Hz, H-2(3)], 4.08 (s, OCH_3), 3.18 (br s, 10-H_2), 3.02 (m, 7-H_2), 2.02 (m, 8-H_2); ms: m/e 356 (M^+, 9), 310 (100), at high resolution M^+ 356.0992 (calcd 356.1008 for $C_{18}H_{16}N_2O_6$); tlc in chloroform-methanol-2*N* acetic acid (40:10:1), R_f 0.13 (with tailing).

Anal. Calcd. for $C_{18}H_{16}N_2O_6$: C, 60.67; H, 4.53; N, 7.86. Found: C, 61.05; H, 4.30; N, 7.86.

Treatment of **25** with acetic anhydride-pyridine at room temperature afforded the triacetate **28**; nmr (deuteriochloroform): δ 7.74 [dd, J = 2, 9 Hz; H-1(4)] 7.67 [t, J = 7, 9 Hz; H-2(3)], 6.98 [dd, J = 2, 7 Hz; H-3(2)], 4.07 (s, OCH_3), 3.67 (d, J = 19 Hz, H-10B), 3.46 (d, J = 19 Hz, H-10A), 3.10 (m, 7-H_2), 2.60 s and 2.57 s (6-OAc and 11 OAc), 2.13-2.85 (m, 8-H_2), 2.02 (s, 9-OAc); tlc in chloroform-methanol (9:1), R_f 0.25.

7,8,9,10-Tetrahydro-9-hydroxy-9-hydroxymethyl-4(1),6,11-trimethoxybenzo[*b*]phenazine (**27**).

A stirred solution of 0.042 g (0.1 mmole) of **24** in 3 ml of dichloromethane was treated, under nitrogen over a 10 minute period, with 1.0 ml (1.0 mmole) of 1.0 *M* borane in tetrahydrofuran. After stirring at 23° under nitrogen for 29.5 hours, an additional 0.5 ml of 1.0 *M* borane was added to the mixture, and stirring was continued for 17 hours. The reaction mixture was treated with 1.0 ml of methanol added dropwise and then evaporated. A solution of the residue in 5 ml of dichloromethane was washed with 2 ml of water, 3 ml of 0.1 *N* aqueous sodium bicarbonate and 3 ml of water. After drying, the organic phase was filtered through Celite and evaporated. The residue was dissolved in 1 ml of dichloromethane, diluted with 3 ml of benzene and evaporated to afford 0.36 g (94%); nmr (deuteriochloroform): δ 7.83 [d, J = 9 Hz, H-1(4)], 7.68 [t, J = 7.5, 9 Hz; H-2(3)], 6.99 [d, J = 7.5 Hz, H-3(2)], 4.26, 4.24, 4.19, 4.15, 4.12 (5 singlets, OCH_3), 3.66 (br s, H-13), 2.8-3.4 (m, 7-H_2, 10-H_2), 1.6-2.2 (m, 8-H_2); ms: m/e 370 (M^+, 100), 339 (M-CH_2OH, 60), at high resolution M^+ 370.1510 (calcd 370.1529 for $C_{20}H_{22}N_2O_5$); tlc in chloroform-methannol (9:1), R_f 0.41.

Anal. Calcd. for $C_{20}H_{22}N_2O_5 \cdot 0.25\ H_2O$: C, 64.07; H, 6.05; N, 7.47. Found: C, 63.96; H, 6.09; N, 7.15.

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