[1-β-Mercapto-β,β-diethylpropionic acid,4-leucine]-8-lysine-vasopressin and [1-β-Mercapto-β,β-diethylpropionic acid,4-leucine]oxytocin, Compounds Possessing Antihormonal Properties†

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The compounds [1-β-mercapto-β,β-diethylpropionic acid,4-leucine]-8-lysine-vasopressin and [1-β-mercapto-β,β-diethylpropionic acid,4-leucine]oxytocin have been synthesized by the stepwise *p*-nitrophenyl ester method, and their properties have been studied in the oxytocic, avian vasodepressor, rat pressor, natriuretic, antidiuretic, and anti-antidiuretic assays. The compounds incorporated alterations in both the 1 and the 4 positions of 8-lysine-vasopressin and of oxytocin. Both of the analogs retained, but in somewhat lower degree, the antioxytocic, antiavian vasodepressor, and antipressor properties previously established for the oxytocin and lysine-vasopressin analogs which have only the β-mercapto-β,β-diethyl substituent in the 1 position. In the renal function assays, the effects of [1-β-mercapto-β,β-diethylpropionic acid]oxytocin and [1-β-mercapto-β,β-diethylpropionic acid]-8-lysine-vasopressin were studied for the first time along with those of their leucine-substituted analogs. The 1- and the 1,4-substituted analogs of lysine-vasopressin exhibited low but definite antidiuretic activity of approximately 0.5 and 0.05 unit/mg, respectively. Neither showed natriuretic activity in the dose range having antidiuretic activity. The two analogs of oxytocin had only very low, residual antidiuretic and natriuretic activities. None of the four analogs showed inhibition of the antidiuretic activity of the USP Posterior Pituitary Reference standard.

The hormone analog [4-leucine]oxytocin,[2] which is obtained when a leucine residue is substituted for that of glutamine in position 4 of oxytocin (Figure 1), has been found to have marked natriuretic–diuretic activity in hydrated rats but none of the antidiuretic hormonal (ADH) activity exhibited by oxytocin. In addition, it is a strong inhibitor of the ADH activity of arginine-vasopressin (Figure 1). In other words, it possesses anti-ADH activity. [4-Leucine]oxytocin also possesses weak oxytocic, avian vasodepressor, and milk ejection activities (2.5, 9, and 16% of those of oxytocin, respectively) but exhibits a weak depressor effect in the rat instead of pressor activity. Rudinger and coworkers have reported that another 4-leucine analog, [4-leucine,8-isoleucine]oxytocin, possesses a diuretic rather than an antidiuretic effect together with natriuretic action.[3,4]

In contrast to [4-leucine]oxytocin, [4-leucine]-8-lysine-vasopressin ([4-Leu]-LVP)[5] retains very low ADH activity of 1–2 units/mg in the hydrated rat (compared to 240 units/mg for LVP) and has no anti-ADH activity. This compound shows a marked natriuretic effect when tested in rats maintained under mannitol diuresis to counteract its inherent ADH activity. [4-Leu]-LVP has low rat pressor activity§ (1.33 units/mg compared to 270 units/mg for LVP) but was found to inhibit the oxytocic and avian vasodepressor effects of oxytocin. [4-Leucine]-8-arginine-vasotocin[7] and a related series of 4-leucine hormone analogs containing arginine or lysine in position 8 have been found by Rudinger and coworkers to be strongly natriuretic–diuretic.[8] [4-Leucine]-8-arginine-vasotocin retains ADH activity of 3.2 IU/μmol (compared to 250 IU/mg for arginine-vasotocin).[7b]

It has recently been found that the substitution of a β-mercapto-β,β-diethylpropionic acid [β-Mpa(β-Et_2)] residue for the half-cystine residue in position 1 of oxytocin gives rise to a potent antioxytocic analog, [1-β-Mpa(β-Et_2)]oxytocin, the most potent of a series of structurally related analogs.[9] This analog also possesses antiavian vasodepressor[9] and antipressor activity.[10] A similar replacement in lysine-vasopressin gave [1-β-Mpa(β-Et_2)]-LVP, which is a highly potent inhibitor of the oxytocic and avian vasodepressor responses to oxytocin and of the pressor response to lysine-vasopressin.[10]

Considering the remarkable effects of the 4-leucine and 1-β-Mpa(β-Et_2) replacements on the pharmacological properties of oxytocin and lysine-vasopressin, we decided to study the behavior of the analogs of oxytocin and LVP in which both modifications have been incorporated into each of these hormones. The syntheses of [1-β-mercapto-β,β-diethylpropionic acid,4-leucine]oxytocin [[1-β-Mpa(β-Et_2),4-Leu]oxytocin] and [1-β-mercapto-β,β-diethylpropionic acid,4-leucine]-8-lysine-vasopressin [[1-β-Mpa(β-Et_2),4-Leu]-LVP] were therefore undertaken.

In the synthesis of [1-β-Mpa(β-Et_2),4-Leu]-LVP, Boc-Tyr(Bzl)-Phe-Leu-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2[5] was deprotected with anhydrous trifluoroacetic acid (TFA) and the resulting free amine salt was coupled with β-Mpa(β-Et_2)-ONp[10] in DMF to yield the protected polypeptide precursor. The blocking groups were removed with Na–NH_3[11] and the resulting disulfhydryl compound was subjected to oxidative cyclization with ICH_2CH_2I.[12] Purification of the LVP analog was accomplished by ion-exchange chromatography[13] on BioRex 70 followed by gel filtration chromatography[14] on Sephadex G25 in 0.2 *N* HOAc.

In the synthesis of [1-β-Mpa(β-Et_2),4-Leu]oxytocin, Z-Tyr(Bzl)-Ile-Leu-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2[2b] was deblocked with HBr–HOAc and the resulting free amine salt was coupled with β-Mpa(β-Et_2)-ONp to yield the protected precursor for the oxytocin analog. This compound was deblocked and ring closure was carried out as in the case of the LVP analog. [1-β-Mpa(β-Et_2),4-Leu]oxytocin was purified by preliminary desalting on Sephadex G15 in 50% HOAc[15] followed by gel filtration on Sephadex LH-20 in DMF.

Both [1-β-Mpa(β-Et_2),4-Leu]oxytocin and [1-β-Mpa(β-Et_2),4-Leu]-LVP were found to be devoid of oxytocic,

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§ [4-α-Aminobutyric acid]-LVP, which differs from [4-Leu]-LVP only by the absence of two γ-methyl groups, has 700 units/mg of ADH activity and 10 units/mg of pressor activity.[6]

Table I. Inhibitory Properties of Certain Analogs of Oxytocin and LVP

Analog	Antioxytocic $\times 10^7 \bar{M}$[a]	Antioxytocic pA_2[a]	Antiavian vasodepressor $\times 10^8 \bar{M}$	Antiavian vasodepressor pA_2	Antipressor $\times 10^7 \bar{M}$	Antipressor pA_2
[1-β-Mpa(β-Et_2)]oxytocin	0.58 (9),[b] σ = 0.12	7.24[b]	0.78 (8),[b] σ = 0.17	8.11[b]	5.73 (20),[c] σ = 2.72	6.24[c]
[1-β-Mpa(β-Et_2)]-LVP[c]	1.43 (7), σ = 0.37	6.84	2.34 (9), σ = 0.98	7.63	0.70 (11), σ = 0.12	7.15
[1-β-Mpa(β-Et_2),4-Leu]oxytocin	0.94 (16), σ = 0.21	7.02	4.57 (6), σ = 2.21	7.34	8.17 (7), σ = 4.13	6.08
[1-β-Mpa(β-Et_2),4-Leu]-LVP	2.04 (30), σ = 0.54	6.69	2.74 (10), σ = 0.47	7.56	1.22 (11), σ = 0.46	6.91

[a] pA_2 values [H. O. Schild, *Brit. J. Pharmacol.*, **2**, 189 (1947)] represent here the negative log to the base 10 of the average molar concentration ($\bar{M}$) of an antagonist which will reduce the response of the uterine horn, the chicken, or the rat to $2x$ units of pharmacologically active compound (agonist) to the response to x units of the agonist. The number of individual determinations is given in parentheses and σ is the standard deviation. Specific details of these assays are described by Vavrek, *et al.*,[9] and by Dyckes, *et al.*[10] Synthetic oxytocin was the agonist used in the antioxytocic and antiavian vasodepressor assays and synthetic LVP in the antipressor assays. Concentrations of the antagonists were calculated on the basis of a 10-ml tissue bath (oxytocic) or assumed blood volumes of 150 ml in the chickens and 6.7 ml/100 g in the rats. [b] Vavrek, *et al.*[9] [c] Dyckes, *et al.*[10]

avian vasodepressor, and pressor activities.& On the other hand, both compounds inhibit the effects of oxytocin in the oxytocic and avian vasodepressor assays and the effect of LVP in the pressor assay. pA_2 values and other data are given in Table I in comparison with corresponding data for [1-β-Mpa(β-Et_2)]oxytocin and [1-β-Mpa(β-Et_2)]-LVP. In each instance the analog containing both the 1-β-Mpa(β-Et_2) residue and the 4-leucine residue has less inhibitory potency than does the corresponding analog containing only the 1-β-Mpa(β-Et_2) residue.

The doubly substituted analogs of oxytocin and LVP were also tested for ADH, natriuretic, and anti-ADH activities in water-loaded rats.& For comparative purposes the 1-β-mercapto-β,β-diethylpropionic acid analogs of oxytocin and of LVP[9,10] were tested in the same assay system.

[1-β-Mpa(β-Et_2)]-LVP and [1-β-Mpa(β-Et_2),4-Leu]-LVP exhibited a low but definite ADH potency of approximately 0.5 and 0.05 unit/mg, respectively. Neither showed natriuretic activity in the dose range having ADH activity.

[1-β-Mpa(β-Et_2)]oxytocin and [1-β-Mpa(β-Et_2),4-Leu]oxytocin had no demonstrable ADH activity and no consistent natriuretic activity when given in single injections at dose levels of 0.5–0.7 μg/100 g. However, when these analogs were infused intravenously for 20–30 min at 0.1 μg/100 g/min after a priming dose of 0.5 μg/100 g, an ADH effect and an increase in Na excretion were observed. This indicates that the oxytocin analogs had low "residual" ADH and natriuretic activities.

In the anti-ADH assay, the two oxytocin analogs were tested either by the simultaneous infusion technique as described for [4-Leu]oxytocin[2c] or by single injections (up to 1.0 μg/100 g) of the analog combined with a predetermined dose of the USP Posterior Pituitary Reference standard. The two LVP analogs were tested only by the single injection technique. None of the four analogs showed inhibition of the ADH activity of the USP standard.

Thus we have found that the introduction of two alkyl groups on the β carbon in position 1 of [4-leucine]oxytocin abolishes the potent anti-ADH effect exhibited by that analog. Similarly, the substitution of a leucine residue for that of glutamine in the 4 position of [1-β-Mpa(β-Et_2)]oxytocin or [1-β-Mpa(β-Et_2)]-LVP results in a decrease in the potent antioxytocic, antiavian vasodepressor, and antipressor effects of those compounds.

It is interesting to note that β-Mpa(β-Et_2) substitution of oxytocin and LVP or of [4-leucine]oxytocin and [4-leucine]-LVP had different effects on activities depending on the type of target tissue involved. In target cells of smooth muscles (uterus and vascular muscles), the β-Mpa(β-Et_2)-substituted analogs had no intrinsic activity but retained a relatively high receptor affinity to become effective antagonists to the natural hormones. On the other hand, in target cells governing antidiuretic and natriuretic hormonal activity, both intrinsic activity and receptor affinity were reduced or abolished. Thus none of these β-Mpa(β-Et_2)-substituted analogs possessed more than very slight antidiuretic activity, and none had any natriuretic or anti-ADH activity.

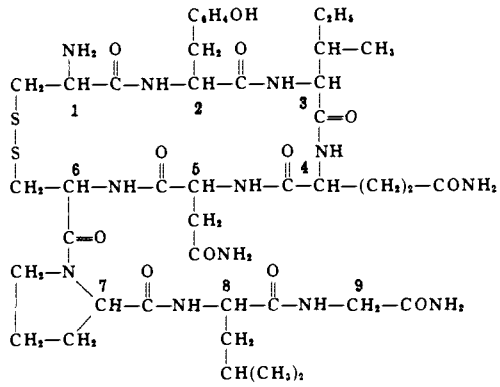

Figure 1. Structure of oxytocin with numbers indicating the position of the individual amino acid residues. In lysine-vasopressin, the isoleucine residue in position 3 is replaced with a phenylalanine residue and the leucine residue in position 8 is replaced with a lysine residue. In arginine-vasopressin, the lysine residue is replaced with an arginine residue.

Experimental Section

Precoated plates of silica gel F-254 (0.25 mm, E. Merck) were used for thin-layer chromatography (tlc) of 5–20-μg samples of compound and were developed with the solvent systems: (A) BuOH–HOAc–H_2O (3:1:1) and (B) BuOH–pyridine–H_2O (20:10:11). Spots were visualized by chlorination followed by KI-starch spray. Melting points were determined in open capillaries and are corrected. Amino acid analyses were performed in a

&Oxytocic assays were performed on isolated uteri from virgin rats in natural estrus according to the method of Holton,[16] as modified by Munsick,[17] with the use of Mg-free van Dyke–Hastings solution as the bathing fluid. Avian vasodepressor assays were performed on conscious chickens by the method of Coon,[18] as modified by Munsick, Sawyer, and van Dyke.[19] Pressor assays were carried out on anesthetized male rats as described in the U. S. Pharmacopeia.[20] ADH assays were performed on male rats by the method of Jeffers, Livezey, and Austin[21] as modified by Sawyer.[22] Anti-ADH and natriuretic studies were performed using the techniques of Chan, *et al.*[2a,c]

Beckman 116 analyzer on samples hydrolyzed for 24 hr in degassed 6 *N* HCl at 110°.[23] Cysteine was determined as cysteic acid on a sample hydrolyzed identically after a performic acid oxidation by the method of Moore.[24]

β-Mpa(β-Et$_2$)(Bzl)-Tyr(Bzl)-Phe-Leu-Asn-Cys(Bzl)-Pro-Lys-(Tos)-Gly-NH$_2$ (1). Boc-Tyr(Bzl)-Phe-Leu-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH$_2$[5] (1.79 g, 1.30 mmol) was dissolved in TFA (5 ml) at 0°. After 20 min the solution was warmed to 25° and let stand 1 hr. The solvent was removed *in vacuo* and the residual oil was triturated under Et$_2$O. The resultant white powder was collected on a glass frit, washed with Et$_2$O, and dried *in vacuo* to yield the deprotected amine salt (1.79 g, 99%).

A solution (0°) of this salt (1.08 g, 0.78 mmol) in DMF (3.0 ml) was treated with *i*-Pr$_2$NEt (0.14 ml, 0.8 mmol) and then with *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-diethylpropionate[10] (0.35 g, 0.94 mmol). Quantitative determination of the extent of reaction by means of the Kaiser test[10,25] indicated that the reaction had slowed considerably after 5 hr (42% reaction). An additional aliquot of *i*-Pr$_2$NEt (0.06 ml, 0.3 mmol) and 1,2,4-triazole catalyst[26] (54 mg, 0.78 mmol) were added. A Kaiser test on the solidified reaction mixture at 48 hr showed more than 94% reaction. The reaction mixture was diluted with 95% EtOH (15 ml) and the precipitate was triturated, collected, and washed with EtOH and EtOAc. Drying *in vacuo* yielded 0.93 g (79%) of product homogeneous by tlc (A, 0.65; B, 0.67). This product was reprecipitated from 80% EtOH (52% recovery): mp 226–227°; $[\alpha]^{25}D$ −42.1° (*c* 0.5, DMF). *Anal.* ($C_{79}H_{101}N_{11}O_{13}S_3 \cdot 2H_2O$) C, H, N.

[1-β-Mercapto-β,β-diethylpropionic acid,4-leucine]-8-lysine-vasopressin. A sample of reprecipitated 1 (200 mg, 0.13 mmol) was dissolved in liquid NH$_3$ (200 ml) which had been freshly distilled from Na. The solution was treated with a fresh stick of Na enclosed in a 4-mm glass tube until the solution retained a deep blue coloration for 30 sec. The color was discharged with HOAc, and NH$_3$ was removed *in vacuo* until a few milliliters of solution remained. This was lyophilized. The residue was dissolved in 50% aqueous acetone (250 ml) and the solution was treated with ICH$_2$CH$_2$I[12] (37.5 mg, 0.13 mmol) in acetone (10 ml). The oxidation of the sulfhydryl groups was followed by means of the Ellman test.[27] When the reaction appeared to stop at 50% completion, another identical aliquot of ICH$_2$CH$_2$I solution was added. Oxidation was continued overnight and was complete before morning. Acetone was removed *in vacuo*, the product was desalted[28] on a column (1.2 × 12 cm) of IRC-50 (H$^+$), and the crude analog was recovered by lyophilization: wt 154 mg (108%).

A sample of the crude analog (75 mg, 0.069 mmol) was chromatographed on a column (1.06 × 116 cm) of Bio-Rex 70 in 0.5 *M* NH$_4$OAc (pH 5.85).[13] Fractions of 3.0 ml were collected at a flow rate of 8.5 ml/hr. Eluted materials were detected by their uv absorption at 280 nm. The product peak emerged at fraction number 43. Fraction no. 45–70 were pooled, avoiding the inclusion of a tailing portion of the peak, and the product was desalted as before. The recovered material (29.4 mg) was chromatographed on a column (2.8 × 67 cm) of Sephadex G-25 in 0.2 *N* HOAc, at a flow rate of 29 ml/hr. The product emerged as a symmetrical peak at 84% of the total column volume, well separated from a very small peak at 75% of the column volume: wt 16.9 mg (22.5%); mp 184–186°; $[\alpha]^{24}D$ −73.0° (*c* 0.5, 1 *N* HOAc); homogeneous by tlc (A, 0.46; B, 0.50). Amino acid analysis: Asp, 1.0; Pro, 1.1; Gly, 1.0; Cys, 1.0; Leu, 1.0; Tyr, 0.9; Phe, 1.0; Lys, 1.1; NH$_3$, 2.2. *Anal.* ($C_{51}H_{75}N_{11}O_{11}S_2 \cdot C_2H_4O_2 \cdot 4H_2O$) C, H, N.

[1-β-Mercapto-β,β-diethylpropionic acid,4-leucine]oxytocin. Z-Tyr(Bzl)-Ile-Leu-Asn-Cys(Bzl)-Pro-Leu-Gly-NH$_2$[2b] (0.266 g, 0.221 mmol) was dissolved in dry HOAc (3 ml) and treated with 40% HBr-HOAc (4 ml). After 1 hr at 25° the solution was diluted with Et$_2$O (50 ml). The white precipitate was collected on a glass frit, washed with Et$_2$O, and dried *in vacuo* (0.266 g).

A solution of the deprotected amine salt and *i*-Pr$_2$NEt (0.04 ml, 0.23 mmol) in DMF (3 ml) was treated with *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-diethylpropionate (0.123 g, 0.33 mmol). The reaction was followed to completion (2 days) by the Kaiser test. During this time additional aliquots of *i*-Pr$_2$NEt were added when needed as determined by the absence of amine in the atmosphere of the reaction flask (moist litmus).** The reaction mixture was diluted with 95% EtOH (20 ml) and the resultant gelatinous precipitate was collected, washed with 95% EtOH and EtOAc, and dried *in vacuo* (0.188 g). A solution of 152.4 mg of this material in liquid NH$_3$ (200 ml) was treated with Na, and the solvent was removed as described above. The residue was dissolved in 50% aqueous acetone (190 ml) and treated with ICH$_2$CH$_2$I (33 mg, 0.117 mmol) in acetone (7 ml). After reaction overnight the solution was stripped to dryness and the residue was dissolved in 50% HOAc (2 ml). The solution was filtered through a glass wool plug and desalted[15] by gel filtration chromatography on a column (97 × 1.1 cm) of Sephadex G-15 equilibrated to 50% HOAc. The hormone analog emerged as a symmetrical peak (47% of column volume) mostly separated from the dimer peak (41% of column volume).

The lyophilized product was dissolved in DMF (1 ml) and subjected to gel filtration on a column (2.82 × 48 cm) of Sephadex LH-20 in DMF. The product peak (44% of column volume) was well separated from the small dimer peak (37% of column volume). The pooled product peak was stripped to dryness and the residue was dissolved in HOAc (20 ml). The HOAc was removed by lyophilization to yield 65 mg (52%) of product: homogeneous to tlc (A, 0.70; B, 0.69); mp ~176°; $[\alpha]^{24}D$ −56.1° (*c* 1, DMF). Amino acid analysis: NH$_3$, 2.20; Asp, 1.02; Pro, 1.06; Gly, 1.00; Cys, 1.09; Ile, 1.02; Leu, 2.04; Tyr, 0.93. *Anal.* ($C_{48}H_{76}N_{10}O_{11}S_2 \cdot 4H_2O$) C, H, N.

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Amodiaquine Analogs. Synthesis of 6-[[3-(*N,N*-Diethylamino)methyl-4-hydroxy]anilino]-5,8-dimethoxy-2,4-dimethylquinoline and Related Compounds†

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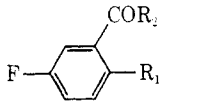
Oxidative amination of 2,4-dimethyl-5,8-quinolinedione (7) with 4-amino-α-diethylamino-*o*-cresol gave 6-[[3-(*N,N*-diethylamino)methyl-4-hydroxy]anilino]-2,4-dimethyl-5,8-quinolinedione (9), which was reduced to the corresponding 5,8-dihydroxyquinoline 13 with either borane or $Na_2S_2O_4$. Methylation of 13 with Me_2SO_4 gave mainly 6-[[3-(*N,N*-diethylamino)methyl-4-methoxy]anilino]-5,8-dimethoxy-2,4-dimethylquinoline (15), purified by column chromatography. Under controlled conditions, methylation of 13 with methyl iodide gave mainly 6-[[3-(*N,N*-diethylamino)methyl-4-hydroxy]anilino]-5,8-dimethoxy-2,4-dimethylquinoline (16). The structure of the latter was confirmed by further methylation to give 15 and also by the unambiguous synthesis of 16. The latter involved the oxidative amination of 7 with 5-amino-2-benzyloxy-*N,N*-diethylbenzamide (26) to give 2-benzyloxy-*N,N*-diethyl-5-[(2,4-dimethyl-5,8-dioxoquinolin-6-yl)amino]benzamide (28). Reduction of 28 with $Na_2S_2O_4$ gave the corresponding 5,8-dihydroxyquinoline (29), which was methylated to give 2-benzyloxy-*N,N*-diethyl-5-[(5,8-dimethoxy-2,4-dimethylquinolin-6-yl)amino]benzamide (31) and its 6-*N*-methyl derivative 32. Reduction of the amide moiety of 31 with a hydride gave 34, and the removal of the benzyl blocking group of 34 by hydrogenation over a palladium catalyst gave 16.

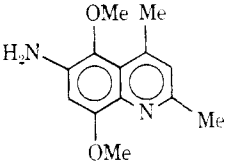
The aminoquinolines represent a group of drugs that possess broad antimalarial activity; the 4-aminoquinolines (chloroquine and amodiaquine) are effective against the plasmodia of erythrocytes but not those of tissue forms, whereas the 8-aminoquinolines (pamaquine and primaquine) act as gametocytocides against plasmodia in both man and mosquito and also act upon primary and secondary tissue schizonts. The 8-aminoquinolines are more toxic than the 4-aminoquinolines, the former showing marked toxicity in people having a deficiency of glucose-6-phosphate dehydrogenase in the erythrocytes.[1] Some 6-[(4-diethylamino-1-methylbutyl)amino]-5,8-dimethoxyquinolines were reported to be as well tolerated by mice and canaries as chloroquine and to be active against *Plasmodium vinckei* and the erythrocytic stages of *Plasmodium cathemerium* as primaquine.[2] In the search for more active and less toxic drugs, we undertook the synthesis of a 6-substituted amino analog of amodiaquine, since amodiaquine is less toxic than chloroquine.

Initially, introduction of the amodiaquine side chain was attempted by the alkylation route. The nitration of *m*-fluorobenzoic acid (1) gave the *o*-nitrobenzoic acid 2,[3] which was converted to the amide 4 *via* the benzoyl chloride 3. The alkylation of 5[4] with 4 in either refluxing xylene in the presence of an acid acceptor or hot DMF to give the corresponding 6-substituted aminoquinoline 6 was unsuccessful as was the fusion of 4 and 5 at 140°.

In another approach oxidative amination of the 5,8-quinolinedione 7[4] with 4-amino-α-diethylamino-*o*-cresol (8)[5] in ethanol containing acetic acid and cerous chloride to give the quinolinedione 9 occurred readily.‡ The preparation of the blocked quinoline 14 from 9 *via* the quinolinedione 10 and the dihydroxyquinoline 12 was attempted unsuccessfully when treatment of the latter with diazomethane in ether gave only 9 and a monomethylated derivative of 13. Also, a similar route using the tetrahydropyranyl blocking group was terminated when the conversion of 9 to 11 was effected in poor yield (mass spectrum).



1, $R_1 = R_2 = H$
2, $R_1 = NO_2$; $R_2 = H$
3, $R_1 = NO_2$; $R_2 = Cl$
4, $R_1 = NO_2$; $R_2 = NEt_2$



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Reduction of the 5,8-quinolinedione 9 was effected with both borane and $Na_2S_2O_4$ to give 13. The latter from the $Na_2S_2O_4$ reduction was confirmed by the mass spectrum. Methylation of this crude sample of 13 was attempted with Me_2SO_4, but the major product from this reaction was identified as the 5,8-quinolinedione 9. In contrast, the borane reduction product gave a weak mass spectrum, which was attributed to the presence of a boron complex. The surmise was confirmed by elemental analyses. Methylation of this product occurred with Me_2SO_4 but gave a mixture of 15, 17, and 19 from which the methyl ether 15 was obtained pure by column chromatography. The successful preparation of 16 involved the reduction of a solution of 9 in $CHCl_3$ with an aqueous solution of $Na_2S_2O_4$ in an oxygen-free drybox to give a solution of 13 in $CHCl_3$, which was evaporated to dryness to give 13 free of inorganic salts. Alkylation of a solution of 13 in DMAc containing sodium hydride with MeI for a shorter period of time than that used in the Me_2SO_4 reaction gave mainly the desired dimethylated product 16, purified by column chromatography. The isolation of 16 in good yield is remarkable as 31 methylated products are possible in this reaction. The structure of 16 was confirmed by methyl-

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‡The oxidative amination of 5,8-quinolinedione in the 6 position with both aliphatic and aryl amines has been reported; see ref 4 and 6.