

Synthesis and Preliminary Pharmacological Evaluation of Asymmetric Chloroquine Analogues¹

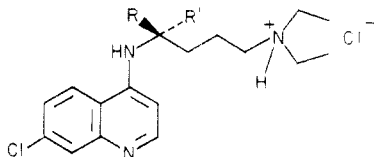
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Asymmetric chloroquine analogues (1-4) were prepared of known absolute configuration in order to assess stereochemical influences on selected biological activities. Since chloroquine has been shown to possess spasmolytic properties, analogues 1-4 were tested for similar pharmacological effects on smooth-muscle contraction. The (*S*)- and (*R*)-chlorochloroquine enantiomers (1 and 2, respectively) were more potent antispasmodics than the less lipophilic (*S*)- and (*R*)-hydroxychloroquines (3 and 4, respectively) when tested against KCl- or acetylcholine-induced contractions of the isolated mouse ileum. A membrane stabilizing mechanism of action for the chloroquine analogues is proposed, since neither cellular toxicity nor calcium antagonism plays a role in the spasmolytic action of these compounds. Although compounds 1-4 also inhibited PGF_{2α}-induced contractions of the ileum, 1 was significantly more potent than 2; the latter in turn was equipotent to 3 and 4. It is tentatively proposed that 1 may possess stereoselective affinity for the PGF_{2α} receptor in the ileum. This observation may be further exploited to obtain more selective profiles of biological activity through molecular manipulation.

Chloroquine is primarily an antimalarial drug, but is also used in rheumatoid arthritis, lupus erythematosus, porphyria cutanea tarda, photoallergic reactions, hepatic amebic infestations, myotonia, asthma, experimental shock, patent ductus arteriosus, and possibly melanoma.^{2,3} In the rat, chloroquine inhibits gastric motility by an anticholinergic mechanism.^{4,5} The spasmolytic effect of chloroquine is also demonstrable on the spontaneous and evoked movements of the rat portal vein.⁶ In the rat mesenteric vascular bed preparation, chloroquine blocks the vasoconstricting actions of a variety of agonists (nor-epinephrine, angiotensin, and potassium chloride) which require both prostaglandins and calcium ions for expression of their spasmogenic effects.^{2,7,8} It has been proposed that chloroquine blocks prostaglandin receptors,² although a calcium antagonistic mechanism or a membrane-stabilizing action^{3,9} cannot be ruled out. In fact, an analysis of the spasmolytic effect of chloroquine in the guinea pig ileum^{10,11} using a variety of agonists (acetylcholine, histamine, nicotine, and serotonin) supports the concept of a nonspecific membrane-stabilizing action of chloroquine.

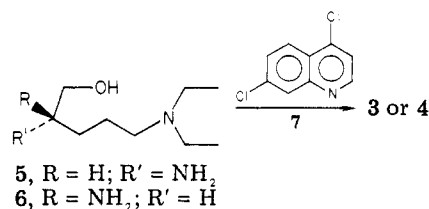
Asymmetric chloroquine analogues (1-4) were prepared



- 1 (*S*, dihydrochloride salt), R = H; R' = CH₂Cl
2 (*R*, dihydrochloride salt), R = CH₂Cl; R' = H
3 (*S*), R = H; R' = CH₂OH
4 (*R*), R = CH₂OH; R' = H

of known absolute configuration in order to assess stereochemical influences on selected biological activities. (*S*)-Chlorochloroquine and (*R*)-chlorochloroquine enantiomers (1 and 2, respectively) represent compounds predicted to be more lipophilic than chloroquine, whereas hydroxychloroquine (not to be confused with the generic hydroxychloroquine of different structure) enantiomers (3 and 4) are more hydrophylic than the parent drug. Hopefully, these compounds would serve to provide leads for exploiting stereoselective drug influences on biological activity.

Synthetic Aspects. Optically pure analogues 1-4 of known absolute configuration were prepared from (*S*)- or (*R*)-glutamic acid (8 or 9) via intermediates 5 and 6, respectively. Hydrophylic HO-substituted chloroquine enantiomers 3 and 4 were obtained via condensation of 5 and 6, respectively, with 4,7-dichloroquinoline (7).¹²

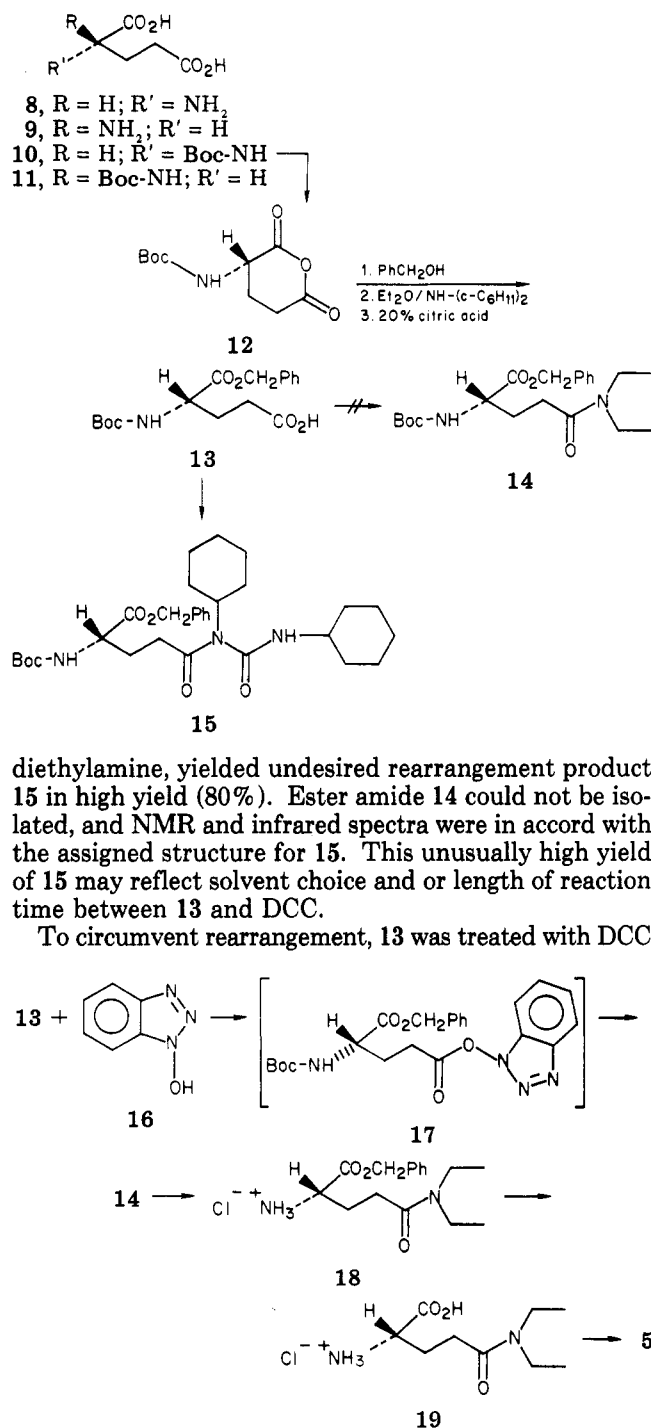


Initially, the *N*-(*tert*-butoxycarbonyl) protected glutamic acids 10 and 11 were prepared from (*S*)- or (*R*)-glutamic acid (8 and 9) and *tert*-butoxycarbonyl azide in the presence of triethylamine.¹³ Derivatization of the amino function utilizing the Boc group was deemed advantageous, since this function is easily removed under mildly acidic conditions.¹⁴

Dehydration of 10 with DCC afforded cyclic anhydride 12 as a viscous oil. Solvolysis of 12 with benzyl alcohol in dry Et₂O containing dicyclohexylamine was reported¹⁵ to afford the corresponding α-benzyl ester 13, which was isolated in 66% yield by fractional crystallization of the dicyclohexylammonium salt. Under similar conditions we obtained 13 in 63% yield. Free acid 13 was liberated upon treatment with 20% citric acid. Reaction with DCC in anhydrous THF at 0 °C (1 h), followed by addition of

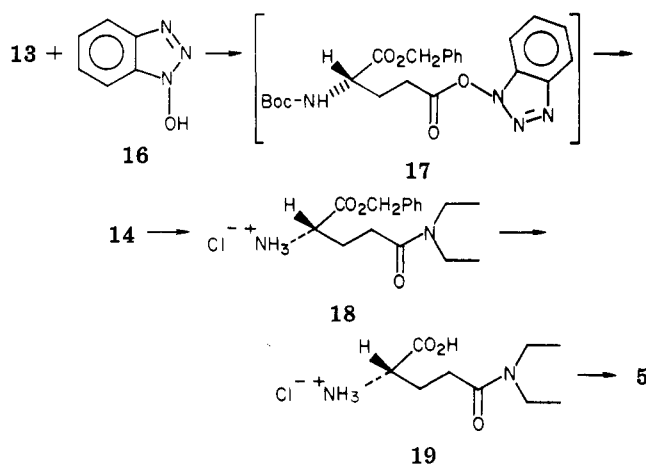
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diethylamine, yielded undesired rearrangement product 15 in high yield (80%). Ester amide 14 could not be isolated, and NMR and infrared spectra were in accord with the assigned structure for 15. This unusually high yield of 15 may reflect solvent choice and or length of reaction time between 13 and DCC.

To circumvent rearrangement, 13 was treated with DCC

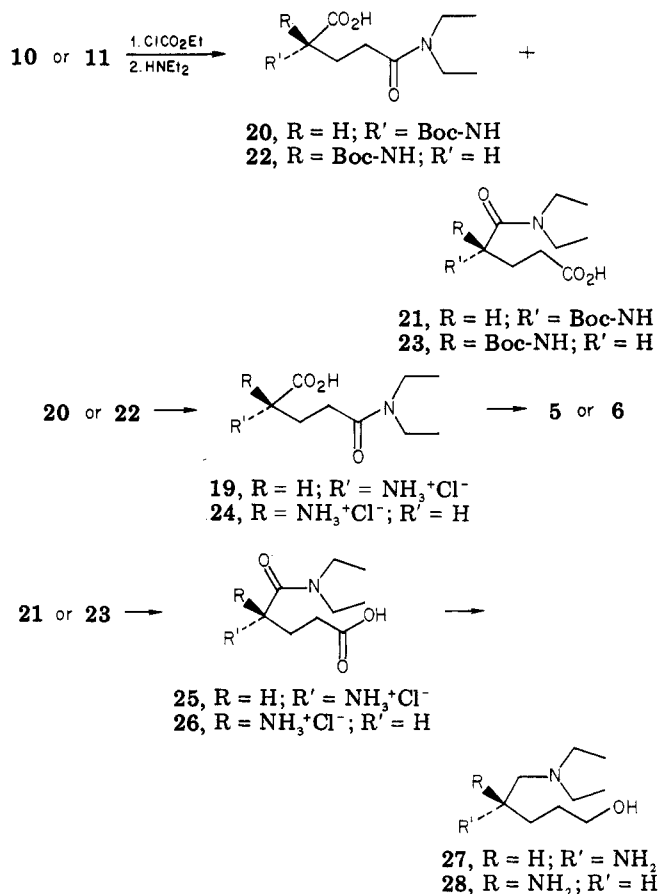


in the presence of 1-hydroxybenzotriazole (16), presumably affording highly reactive benzotriazole ester intermediate 17 in situ.¹⁶ Subsequent addition of 2 equiv of diethylamine afforded 14 as a viscous oil, which decomposed upon attempted distillation. Following chromatography, 14 was obtained as a colorless oil. Gaseous HCl treatment of 14 in ether cleaved the N-protecting group and afforded hydrochloride 18 as an oil, which could not be crystallized. Catalytic hydrogenolysis of benzyl ester 18 occurred rapidly (theoretical uptake of H₂ was complete within 10 min) and afforded the corresponding amino acid hydrochloride 19 in excellent yield. Potentiometric titration of 19 confirmed the position of the CO₂H group α to the amino function. The determined pK_a of 2.32 was in accord with the pK_a

of the α -CO₂H group (2.19) in glutamic acid hydrochloride.¹⁷ Thus, the diethylamido functionality in compounds 14, 18, and 19 must be in the γ position.

Reduction of 19 with LiAlH₄ in refluxing THF afforded chiral diamino alcohol 5 in 75% yield. Nonaqueous workup (solid Na₂SO₄·10H₂O)¹⁸ proved valuable for product isolation owing to extreme water solubility. Diamino alcohol 5 is air and light sensitive and was stored under N₂ in the dark.

Alternatively, 5 was synthesized by treatment of 10 in



dry THF with 1 equiv of ethyl chloroformate. The mixture of anhydrides was not isolated but immediately converted to isomeric amides 20 and 21. Amide 21 could be separated from the reaction mixture via fractional crystallization from Et₂O. Amide 20 was isolated as an oil by chromatography. For 21, the methine proton resonance signal was downfield from the equivalent proton in 20 by 0.44 Hz. Under identical reaction conditions, enantiomer 11 was converted to isomeric amides 22 and 23. When cyclic anhydride 12 was treated with diethylamine in THF, similar yields of products 20 and 21 were obtained.

Mild acid treatment of amides 20–23 afforded amino acids 19 and 24–26 as their hydrochlorides. Whereas enantiomeric hydrochlorides 19 and 24 were stable and easily crystallized, isomeric derivatives 25 and 26 were deliquescent and difficult to isolate in the crystalline form. Again, potentiometric titration provided evidence for the position of the free CO₂H group. For 25, the determined pK_a of 3.95 is in accord with the assignment of the diethylamide function to the α position. Reduction of 19 and 24–26 with LiAlH₄, followed by nonaqueous workup

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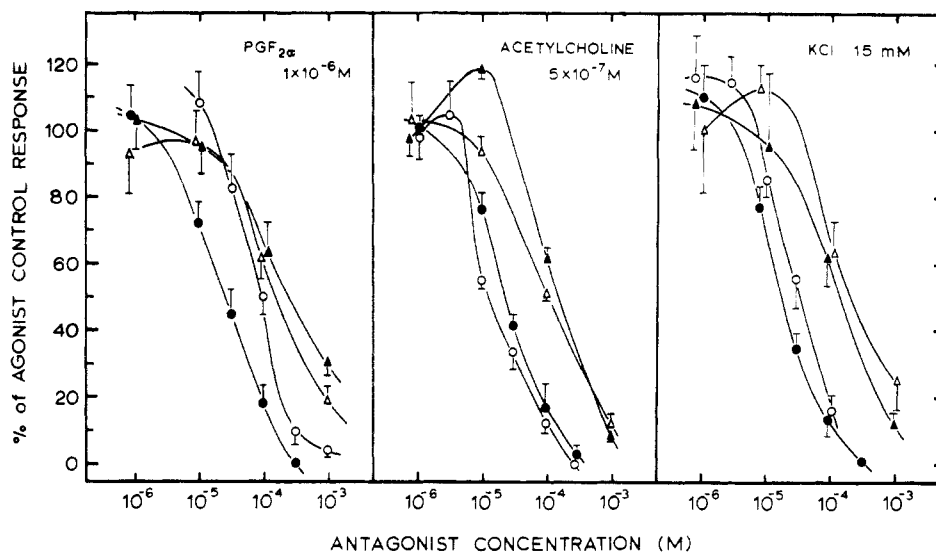
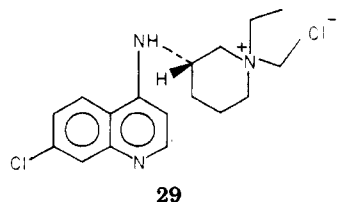


Figure 1. The effect of various concentrations of compounds 1-4 (x axis) on contractions induced by prostaglandin $F_{2\alpha}$, acetylcholine, and KCl in the isolated mouse ileum: (●-●) 1, (○-○) 2, (▲-▲) 3, (△-△) 4.

($\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$),¹⁸ afforded the chiral diamino alcohols 5, 6, 27, and 28.

Surrey and Cutler¹² reported that phenol was the solvent of choice for the preparation of chloroquine via the condensation of 2-amino-5-(diethylamino)pentane with 7. Their results suggested that phenol may first react with 4,7-dichloroquinoline to form 7-chloro-4-phenoxyquinoline, which then undergoes nucleophilic attack by the amine to form chloroquine. Heating either diamino alcohol 5 or 6 in phenol (2 equiv) containing 7 facilitated nucleophilic displacement at the 4 position of the quinoline ring, affording 3 or 4. Enantiomeric analogues 3 and 4 were isolated as white crystalline free bases. Reaction only proceeded when phenol was employed as solvent; 2 equiv yielded optimal results. A reaction temperature of 125 °C was required for optimal yield. No product formed at temperatures below 100 °C. Product was detectable after 8 h by TLC. Attempts to prepare 3 under conditions where the solvent was modified (EtOH and H^+ , Me_2SO , or 1,4-dioxane in the presence or absence of 1 equiv of phenol) proved unsuccessful and led only to isolation of starting material.

Dihydrochlorides 1 and 2 were prepared by refluxing 3 or 4 in CHCl_3 containing SOCl_2 . When a small amount of 1 was converted to its free base and allowed to stand at room temperature, the tertiary amine slowly underwent intramolecular conversion to the quaternary salt 29 tentatively identified by NMR spectroscopy.



Pharmacological Results and Discussion

The results of the preliminary pharmacological evaluation of compounds 1-4 are presented in Figure 1. Each of the four compounds was found to possess marked antispasmodic activity against all three spasmogens tested on the isolated mouse ileum preparation. When tested against either acetylcholine (AcCh) or KCl, compounds 1 and 2 were demonstrated to be more potent spasmolytics than compounds 3 and 4. In each case, the concentration-response curves for the more lipophilic chloro-

chloroquines were approximately 0.8 log dose unit to the left of the hydroxychloroquine concentration-response curves. With the exception of some apparent potentiating effect of compound 1 at the 10^{-5} M concentration on the spasmogenic activity of AcCh, there were no differences in potency between enantiomers of either the chloro or hydroxy derivatives against AcCh or KCl.

Whereas compounds 3 and 4 inhibited $\text{PGF}_{2\alpha}$ -induced contraction of the ileum to the same extent and in a concentration-dependent nonstereospecific manner, compounds 1 and 2 were found to exhibit some degree of stereoselectivity in their ability to antagonize $\text{PGF}_{2\alpha}$ -induced contractions. Compound 1 inhibited $\text{PGF}_{2\alpha}$ -induced contractions by 28% at a concentration of 10^{-5} M, while compound 2 at a similar concentration showed no such inhibition ($p < 0.025$ by Student's t test). At 3×10^{-5} M, compound 1 produced a 55% inhibition of the control $\text{PGF}_{2\alpha}$ response, whereas compound 2 inhibited this response by only 17% ($p < 0.01$). At 10^{-4} M concentrations, compound 1 inhibited $\text{PGF}_{2\alpha}$ -induced contractions by 32% more than did compound 2 ($p < 0.005$).

The antispasmodic effect of test compounds 1-4 was reversible, since the control responses to all three agonists were regained after washing the test compounds from the medium bathing the tissue. Furthermore, the antispasmodic effect of compounds 1-4 was independent of calcium concentrations in the medium over a range of 0.9-28.7 mM calcium. These findings indicate that the chloroquine analogues do not act as cellular poisons nor as selective calcium antagonists, since their antispasmodic actions were not reversed by calcium. That the antispasmodic action of the chloroquine analogues is not restricted to the smooth muscle of the ileum became apparent when (*S*)-chloroquine was tested in the isolated mouse uterus preparation¹⁹ and, at a concentration of 3×10^{-7} M, resulted in a 50% reduction in the contractile response of the estrous uterus to 3×10^{-8} M $\text{PGF}_{2\alpha}$ (data not shown in Figure 1).

Thus, the antispasmodic actions of the chloroquine analogues, 1-4, may be attributable to a nonspecific membrane-stabilizing property similar to that ascribed to the parent compound, chloroquine.⁹⁻¹¹ Membrane stabilization is expected to render the tissue less responsive to

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a variety of applied agonists which activate smooth-muscle contraction by increasing cation influx through the cell membrane (as is the case with KCl ,²⁰ AcCh ,²¹ and $\text{PGF}_{2\alpha}$)²² and would not be expected to be reversed by increasing the concentrations of extracellular calcium, since this cation further stabilizes cell membranes.²³ A tentative alternative mechanism of action can be proposed for (S)-chloroquine (1). Since this isomer was a significantly more potent inhibitor of $\text{PGF}_{2\alpha}$ -induced contraction of the ileum than the (R)-chloroquine (2), it may possibly possess stereoselective affinity for the prostaglandin receptor, as has also been proposed for chloroquine.² Interestingly, (+)-chloroquine has recently been shown to be a more potent antimalarial agent than the (–) isomer, whereas the latter exhibited greater toxicity than the former,²⁴ thus demonstrating stereoselective properties in the parent compound. It is possible that the observed stereospecificity of the chloroquine analogues, 1 and 2, may be further exploited to obtain more selective profiles of biological activity through molecular manipulation. Relative to membrane stabilization, this property of the chloroquine analogues appears not to be stereoselective.

Experimental Section

Chemistry. Elemental analyses were performed by Clark Microanalytical Laboratory, Urbana, IL, and Galbraith Laboratories, Knoxville, TN. Infrared spectra were recorded on a Perkin-Elmer Model 257 or Beckman IR 4230 spectrophotometer. Optical rotations were performed using a Perkin-Elmer polarograph 246. Nuclear magnetic resonance spectra were recorded on Varian A-60A and Bruker HX-90E spectrophotometers. All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected.

N-(tert-Butoxycarbonyl)-(S)-glutamic acid (10) was prepared according to the method of Grzonka and Lammek¹³ in 89% yield: mp 113–115 °C (lit.¹⁵ mp 110–112 °C); $[\alpha]_D^{25}$ –15.20° (c 1.0, MeOH) [lit.¹⁵ $[\alpha]_D^{25}$ –16.1° (c 1.0, MeOH)].

N-(tert-Butoxycarbonyl)-(R)-glutamic acid (11) was prepared according to previously described methods:¹³ yield 112.0 g (90%); mp 113–115 °C; $[\alpha]_D^{25}$ +13.4° (c 1.0, MeOH).

N-(tert-Butoxycarbonyl)-(S)-glutamic acid anhydride (12) was prepared according to the method of von Schroder and Klieger¹⁵ in 95% yield: mp 114–116 °C (lit.¹⁵ mp 115–116 °C); $[\alpha]_D^{25}$ –46.0° (c 1.0, AcOH) [lit.¹⁵ $[\alpha]_D^{25}$ –47.7° (c 1.0, AcOH)].

1-Benzyl N-(tert-Butoxycarbonyl)-(S)-glutamate (13) was prepared according to the method of Schroder and Klieger¹⁵ via the dicyclohexylamine salt: mp 171–172 °C (lit.¹⁵ mp 172 °C); $[\alpha]_D^{25}$ –18.4° (c 1.0, MeOH) [lit.¹⁵ $[\alpha]_D^{25}$ –19.2° (c 1.0, MeOH)]. The salt was converted to 13 (63% from 12) by stirring in a suspension of 150 mL of EtOAc and 150 mL of 20% citric acid for 0.5 h. The white crystalline product exhibited mp 92–94 °C (lit.¹⁵ mp 93–93.5 °C); $[\alpha]_D^{25}$ –28.9° (c 1.0, MeOH) [lit.¹⁵ $[\alpha]_D^{25}$ –30.2° (c 1.0, MeOH)]. Anal. ($\text{C}_{17}\text{H}_{23}\text{NO}_6$) C, H, N.

Benzyl N²-(tert-Butoxycarbonyl)-N,N-diethyl-(S)-glutamate (14). To a solution of 5.0 g (0.015 mol) of 13 and 2.60 g (0.017 mol) of 1-hydroxybenzotriazole (16) in 50 mL of anhydrous THF (0 °C) was added dropwise with stirring 3.09 g (0.015 mol) of DCC in 20 mL of anhydrous THF. The reaction was monitored by TLC [CHCl_3 -MeOH-AcOH (9:1:3 drops)] until all acid was consumed (ca. 1 h). Subsequently, 2.48 g (0.034 mol)

of diethylamine in 30 mL of anhydrous THF was added with stirring. After the addition, the mixture was stirred at room temperature for 8 h. The resulting suspension was filtered and the filtrate concentrated under reduced pressure. The syrupy residue was dissolved in 100 mL of EtOAc and filtered. The filtrate was extracted with three 50-mL portions of cold 1 N HCl solution followed by extraction with three 50-mL portions of 10% NaHCO_3 solution and dried (Na_2SO_4). The solvent was removed under reduced pressure to yield a thick yellow liquid which decomposed on distillation and failed to solidify when triturated with petroleum ether (30–60 °C). The liquid was chromatographed on silica gel CHCl_3 -EtOAc (65:35), affording 4.6 g (78%) of a clear viscous oil, $[\alpha]_D^{25}$ –25.6° (c 1.0, MeOH). Anal. ($\text{C}_{21}\text{H}_{32}\text{N}_2\text{O}_5$) C, H, N.

N,N-Diethyl-(S)-glutamine Hydrochloride (19). Method A. Gaseous HCl (passed through a gas trap containing H_2SO_4) was bubbled through a solution of 20.0 g (0.066 mol) of N²-(tert-butoxycarbonyl)-N,N-diethyl-(S)-glutamine (20) in 500 mL of dry Et₂O until all of the starting material was consumed [0.5–1.0 h; monitored by TLC (CHCl_3 -MeOH-NH₄OH; 9:1:3 drops)]. After the solution cooled (ca. 4 °C) overnight, the solid precipitate was collected and washed with dry Et₂O, followed by immediate recrystallization (deliquescent) from absolute EtOH-anhydrous Et₂O, affording 14.9 g (95%) of white crystals: mp 154–156 °C; $[\alpha]_D^{25}$ +26.9° (c 1.0, MeOH); pK_a = 2.32; IR (neat) 1730 and 1650 cm^{-1} ; NMR (D_2O) δ 4.17 (t, 1 H, CH), 3.17–3.63 (2 overlapping q, 4 H, CH_2CH_3), 2.5–2.87 (m, 2 H, CH_2CO), 2.0–2.45 (m, 2 H, CH_2CH), 0.91–1.33 (2 overlapping t, 6 H, CH_3). Anal. ($\text{C}_9\text{H}_{19}\text{N}_2\text{O}_3\text{Cl}$) C, H, N, Cl.

Method B. Gaseous HCl (passed through a gas trap containing H_2SO_4) was bubbled through a solution of 14.5 g (0.037 mol) of benzyl N²-(tert-butoxycarbonyl)-N,N-diethyl-(S)-glutamate (14) in 250 mL of dry Et₂O until no starting material remained [0.5 h; monitored by TLC (CHCl_3 -EtOAc, 65:35)]. After the solution cooled (ca. 4 °C) overnight, the ether was decanted, the oily residue was washed (dry Et₂O), and the solvent was removed under reduced pressure (0.05 mm) to yield 10.0 g (85%) of crude benzyl N,N-diethyl-(S)-glutamate hydrochloride (18) as a yellow viscous oil, which was not further purified but was used as such for the preparation of 19. A mixture of 18 (10.0 g, 0.045 mol), using 10% Pd/C (0.5 g) in 100 mL of absolute EtOH, was hydrogenated at room temperature (40 psi) for 1 h. The reaction mixture was filtered (Celite) and the filtrate concentrated under reduced pressure. The residue was triturated with petroleum ether (30–60 °C), yielding a solid which was recrystallized from absolute EtOH-petroleum ether (30–60 °C), affording 6.9 g (90%) of 19 as white crystals: mp 151–154 °C; $[\alpha]_D^{25}$ +26.1° (c 1.0, MeOH).

N,N-Diethyl-(R)-glutamine Hydrochloride (24). Following method A, N²-(tert-butoxycarbonyl)-N,N-diethyl-(R)-glutamine (22) was converted to 24: yield 14.9 g (95%); mp 154–156 °C; $[\alpha]_D^{25}$ –26.8° (c 1.0, MeOH).

[N⁴-(tert-Butoxycarbonyl)amino]-N,N-diethyl-(S)-glutaramic Acid (21) and N²-(tert-Butoxycarbonyl)-N,N-diethyl-(S)-glutamine (20). Procedure A. To a solution of 88.5 g (0.36 mol) of N-(tert-butoxycarbonyl)-(S)-glutamic acid (10) in 450 mL of anhydrous THF was added dropwise with stirring 36.4 g (0.36 mol) of triethylamine in 150 mL of dry THF. After the solution cooled (–10 °C), 39.1 g (0.36 mol) of ethyl chloroformate in 100 mL of dry THF was slowly added dropwise with stirring. After the addition, the mixture was stirred at –10 °C for 1.5 h and filtered to remove triethylamine hydrochloride. Subsequently, 52.7 g (0.72 mol) of diethylamine in 100 mL of dry THF was added to the filtrate, and the resulting mixture was stirred for 3 h at room temperature. The mixture was extracted with H₂O, and the aqueous layers were collected, washed with Et₂O, acidified (pH 1–2) with cold 1 N HCl, and extracted with CHCl_3 . The CHCl_3 layers were collected and dried (Na_2SO_4), and the solvent was removed under reduced pressure to yield a viscous yellow residue. The residue was dissolved in 500 mL of Et₂O and the solution was cooled (ca. 4 °C) overnight. The crystalline product was removed by filtration and washed with Et₂O, air-dried, and recrystallized from CHCl_3 -Et₂O, affording 38.09 g (35%) of 21: mp 155–157 °C; $[\alpha]_D^{25}$ –41.4° (c 1.0, MeOH).

Concentration of the mother liquor (ether filtrate) under reduced pressure afforded a viscous yellow oil which decomposed on distillation and failed to solidify when triturated with petroleum

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ether (30–60 °C). The oil was chromatographed on silica gel (CHCl₃–MeOH–AcOH, 9:1:3 drops), affording 65.3 g (60%) of **20** as a clear viscous oil: $[\alpha]^{25}_D -8.0^\circ$ (c 1.0, MeOH). Anal. (C₁₄H₂₆N₂O₅) C, H, N.

Procedure B. To a solution of 9.2 g (0.04 mol) of *N*-(*tert*-butoxycarbonyl)-(S)-glutamic acid anhydride (**12**) in 80 mL of anhydrous THF was added dropwise with stirring 6.0 g (0.08 mol) of diethylamine in 25 mL of dry THF. The reaction mixture was stirred overnight at room temperature and extracted with H₂O. The aqueous layers were collected, washed with Et₂O, acidified (pH 1–2) with cold 1 N HCl, and extracted with CHCl₃. The CHCl₃ layers were dried (Na₂SO₄), and the solvent was concentrated under reduced pressure to yield a yellow viscous oil, which was dissolved in Et₂O and refrigerated overnight. The crystalline product was filtered and washed with Et₂O, air-dried, and recrystallized from CHCl₃–Et₂O, affording 3.63 g (30%) of **21** as white crystals: mp 156–158 °C; $[\alpha]^{25}_D -40.0^\circ$ (c 1.0, MeOH). Concentration of the mother liquor (ether filtrate) under reduced pressure afforded a viscous yellow oil, which decomposed on distillation and failed to solidify when triturated in petroleum ether (30–60 °C). The oil was chromatographed on silica gel (CHCl₃–MeOH–AcOH, 9:1:3 drops), affording 7.2 g (60%) of **20** as a clear viscous liquid: $[\alpha]^{25}_D -8.0^\circ$ (c 1.0, MeOH).

[N⁴-(*tert*-Butoxycarbonyl)amino]-N,N-diethyl-(R)-glutamic Acid (23**) and N²-(*tert*-Butoxycarbonyl)-N,N-diethyl-(R)-glutamine (**22**).** Following procedure A, *N*-(*tert*-butoxycarbonyl)-(R)-glutamic acid (**11**) was converted to **23** [yield 38.0 g (35%); mp 157–158 °C; $[\alpha]^{25}_D +39.6^\circ$ (c 1.0, MeOH)] and **22** [yield 65.0 g (60%); $[\alpha]^{25}_D +8.1^\circ$ (c 1.0, MeOH)].

(S)-2-Amino-5-(diethylamino)pentan-1-ol (5**).** To a suspension of 10.0 g (0.042 mol) of *N,N*-diethyl-(S)-glutamine hydrochloride (**19**) in 125 mL of dry THF (freshly distilled from LiAlH₄) at 0 °C was added slowly with stirring 9.0 g (0.24 mol) of LiAlH₄. The resulting mixture was refluxed overnight. After the mixture cooled to 0 °C, finely powdered Na₂SO₄·10H₂O (prepared by recrystallization of anhydrous Na₂SO₄ from H₂O) was added slowly to the mechanically stirred reaction mixture until all the LiAlH₄ was decomposed. The resulting white suspension was filtered, and the precipitate was washed with hot EtOAc. The filtrate was concentrated under reduced pressure, yielding 5.2 g (75%) of **5** as a light yellow, air-sensitive oil. A small portion of **5** was converted to the respective dioxalate salt and recrystallized from absolute MeOH to afford crystals: mp 129–131 °C; $[\alpha]^{25}_D +2.2^\circ$ (c 1.0, MeOH); IR (neat) 3500, 3300 cm⁻¹; NMR (CDCl₃, free amine) δ 3.10–3.70 (m, 3 H, CHCH₂OH), 2.25–3.0 [m, 9 H, NH₂, OH exchangeable with D₂O, CH₂N(CH₂CH₃)₂], 1.29–1.81 (m, 4 H, CH₂CH₂), 1.0 (t, 6 H, CH₃, *J* = 7 Hz). Anal. (C₁₃H₂₆N₂O₉) C, H, N.

(R)-2-Amino-5-(diethylamino)pentan-1-ol (6**)** was prepared by identical methods with those employed for the preparation of the *S* isomer, except that **24** was used as starting material: yield was 5.21 g (75%) of dioxalate salt; mp 130–132 °C; $[\alpha]^{25}_D -2.6^\circ$ (c 1.0, MeOH).

(S)-4-Amino-5-(diethylamino)pentan-1-ol (27**).** Gaseous HCl (passed through a gas trap containing H₂SO₄) was bubbled through a solution of 20.0 g (0.066 mol) of [N⁴-(*tert*-butoxycarbonyl)amino]-N,N-diethyl-(S)-glutamic acid (**21**) in 500 mL of CHCl₃ until all the starting material was consumed [1.5–2.0 h; monitored by TLC (CHCl₃–MeOH–AcOH, 9:1:3 drops)]. After the solution cooled (ca. 4 °C) overnight, the oily suspension was extracted with H₂O, and the aqueous layers were collected and washed with CHCl₃. H₂O was removed under reduced pressure to yield 14.15 g (90%) of crude *N,N*-diethyl-(S)-glutamic acid hydrochloride (**25**) as a clear viscous oil, which was not further purified but was used as such for the preparation of **27**. Crude **25** (14.15 g, 0.059 mol) was dissolved in 250 mL of dry THF and cooled to 0 °C. LiAlH₄ (14.90 g, 0.40 mol) was added slowly with stirring to the solution. After refluxing for 24 h, the reaction slurry was cooled to 0 °C and finely powdered Na₂SO₄·10H₂O was added slowly to the mechanically stirred reaction mixture until all the LiAlH₄ was decomposed. The resulting white suspension was filtered and the precipitate was washed with hot EtOAc. The filtrate was concentrated under reduced pressure, yielding 7.80 g (76%) of **27** as a yellow, air-sensitive liquid. A small portion of **27** was converted to its dioxalate salt and recrystallized from absolute MeOH: mp 121–123 °C (softens 119 °C); $[\alpha]^{25}_{Hg578} +16.3^\circ$

(c 1.0, MeOH). Anal. (C₁₃H₂₆N₂O₉) C, H, N.

(R)-4-Amino-5-(diethylamino)pentan-1-ol (28**)** was prepared by methods identical with those employed for the *S* isomer, except that **23** was used as starting material: yield 7.82 g (76%) of dioxalate salt; mp 121–123 °C; $[\alpha]^{25}_{Hg578} -15.8^\circ$ (c 1.0, MeOH).

(S)-7-Chloro-4-[[1'-(hydroxymethyl)-4'-(diethylamino)-butyl]amino]quinoline (3**).** A 100-mL round-bottom flask containing 5.4 g (0.03 mol) of crude (*S*)-2-amino-5-(diethylamino)pentan-1-ol (**5**), 5.0 g (0.026 mol) of 4,7-dichloroquinoline (**7**), and 5.0 g (0.052 mol) of phenol was heated to 125 °C with stirring for 18–20 h. The black tarry residue was dissolved in CHCl₃ and extracted with 15% NaOH solution followed by 1 N HCl solution (5 × 50 mL). The acidic aqueous layers were washed with Et₂O, made basic with NaOH (pH 10–12), and extracted with CHCl₃. The combined CHCl₃ extracts were dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The resulting brown residue was chromatographed on silica gel (CHCl₃–MeOH–NH₄OH, 9:1:3 drops), affording a yellow solid which was recrystallized from acetone: yield 4.88 g (56%) of **3** as white crystals; mp 123.5–124.5 °C; $[\alpha]^{25}_{Hg578} -119.1^\circ$ (c 1.0, MeOH); IR (CHCl₃) 3600, 3420 cm⁻¹; NMR (CDCl₃) δ 8.42 (d, 1 H, aromatic H-2, *J* = 6 Hz), 7.92 (d, 1 H, aromatic H-8, *J* = 2 Hz), 7.71 (d, 1 H, aromatic H-5, *J* = 9 Hz), 7.23 (dd, 1 H, aromatic H-6, *J*₁ = 9 Hz, *J*₂ = 2 Hz), 6.41 (d, 1 H, aromatic H-3, *J* = 6 Hz), 5.67–5.97 (d, 1 H, NH, broad, exchangeable with D₂O), 5.35 (s, 1 H, OH, broad, exchangeable with D₂O), 3.40–4.05 (m, 3 H, CHCH₂OH), 2.30–2.81 [m, 6 H, CH₂N(CH₂CH₃)₂], 1.45–2.01 (m, 4 H, CH₂CH₂), 1.00 (t, 6 H, CH₃, *J* = 7 Hz). Anal. (C₁₈H₂₆ClN₃O) C, H, N, Cl.

(R)-7-Chloro-4-[[1'-(hydroxymethyl)-4'-(diethylamino)-butyl]amino]quinoline (4**)** was prepared by methods identical with those employed for the preparation of **3**, except that (*R*)-2-amino-5-(diethylamino)pentan-1-ol (**6**) was used as starting material: yield of **4** was 4.87 g (56%); mp 123.5–124.5 °C; $[\alpha]^{25}_{Hg578} +118.0^\circ$ (c 1.0, MeOH).

(S)-7-Chloro-4-[[1'-(chloromethyl)-4'-(diethylamino)-butyl]amino]quinoline Dihydrochloride (1**).** To a solution of 2.5 g (0.0075 mol) of **3** in 25 mL of CHCl₃ cooled to –10 °C (NaCl–ice bath) was added dropwise with stirring 3 mL of SOCl₂ (freshly distilled) in 10 mL of CHCl₃. After the addition was completed (0.25 h), the reaction mixture was refluxed for 1.0 h. The resulting yellow suspension was filtered, and the solid was washed with CHCl₃ and air-dried. The yellow solid was recrystallized twice from absolute EtOH, affording 2.9 g (98%) of **1** as white crystals: mp 258–261 °C dec; $[\alpha]^{25}_{Hg578} -56.4^\circ$ (c 1.0, MeOH); IR (Nujol) 3300 cm⁻¹; NMR (CDCl₃, free amine) δ 8.43 (d, 1 H, aromatic H-2, *J* = 6 Hz), 7.92 (d, 1 H, aromatic H-8, *J* = 2 Hz), 7.71 (d, 1 H, aromatic H-5, *J* = 9 Hz), 7.23 (dd, 1 H, aromatic H-6, *J*₁ = 9 Hz, *J*₂ = 2 Hz), 6.41 (d, 1 H, aromatic H-3, *J* = 6 Hz), 5.67–5.97 (d, 1 H, NH, broad, exchangeable with D₂O), 3.40–4.05 (m, 3 H, CHCH₂Cl), 2.30–2.81 [m, 6 H, CH₂N(CH₂CH₃)₂], 1.45–2.01 (m, 4 H, CH₂CH₂), 1.00 [t, 6 H, N(CH₂CH₃)₂, *J* = 7 Hz]. Anal. (C₁₈H₂₅Cl₂N₂·2HCl) C, H, N.

(R)-7-Chloro-4-[[1'-(chloromethyl)-4'-(diethylamino)-butyl]amino]quinoline dihydrochloride (2**)** was prepared by methods identical with those employed for the preparation of **1**, except (*R*)-7-chloro-4-[[1'-(hydroxymethyl)-4'-(diethylamino)-butyl]amino]quinoline (**4**) was used as starting material: yield 2.9 g (98%); mp 258–262 °C dec; $[\alpha]^{25}_{Hg578} +56.9^\circ$ (c 1.0, MeOH).

Pharmacological Methods. The experimental protocol was similar to that described in previous publications from our laboratories.^{19,25–27} Briefly, male albino mice (Cox ICR, 20–25 g) were sacrificed by cervical dislocation, and 1-cm sections of ileum were prepared for isometric contraction recording in 10-mL tissue baths containing a Krebs physiological solution maintained at 37 °C and composed of the following (g/L): NaCl, 6.87; KCl, 0.40; CaCl₂·2H₂O, 0.37; MgSO₄·7H₂O, 0.14; NaH₂PO₄·H₂O, 0.16; NaHCO₃, 2.10; dextrose, 2.0. Recordings were made using a Grass FT03 isometric transducer coupled to a Grass Model F polygraph re-

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corder (Grass Instruments Co., Quincy, MA). The bathing solution was aerated with 5% CO₂ in O₂. A 30-min equilibration period was allowed prior to all experiments.

In each experiment, control response to PGF_{2α} (10⁻⁶ M bath concentration), acetylcholine (AcCh; 5 × 10⁻⁷ M bath concentration), or KCl (15 mM bath concentration) was obtained by first exposing the tissue to the particular agonist and then washing the tissue three times over a 15-min period. Control response to the agonist was elicited three times prior to incubation of the tissue with any of the test compounds (1-4), to ensure viability of the tissue and stability of the response. The test compound was then added to the bath and left in contact with the ileum for 3 min. PGF_{2α}, AcCh, or KCl was then added to the bath as before, and the resultant contraction was recorded. After 3 min, the bath was again washed three times and the control response to PGF_{2α}, AcCh, or KCl was reestablished. All values were calculated as percent of the average of the initial control responses. Control responses to the concentrations of PGF_{2α}, AcCh, and KCl

used represented, respectively, 45.7 ± 11.4, 53.6 ± 2.3, and 52.6 ± 9.4% of the maximum response obtainable with each spasmogen.

To determine if variation of the calcium concentration in the medium would affect the antispasmodic actions of compounds 1-4, the following method was used.¹⁹ The tissue was incubated for 10 min with the chosen concentration of calcium chloride to allow the spontaneous contractile activity to subside. The agonist (AcCh, 5 × 10⁻⁷ M; KCl, 15 mM; PGF_{2α}, 10⁻⁶ M) was added to the bath and a control contraction was recorded. The tissue was washed and reincubated for 10 min with the same concentration of calcium used to obtain the control response to the agonist. One of the test compounds (1-4) was then added and left in contact with the tissue for 3 min before the reintroduction of the agonist. The tissue was then washed and allowed to relax, and the entire procedure was repeated at a higher concentration of bath calcium (the concentrations of agonists and test compounds were kept constant).

Analgesic Narcotic Antagonists. 5. 7,7-Dimethyldihydrocodeinones and 7,7-Dimethyldihydromorphinones¹

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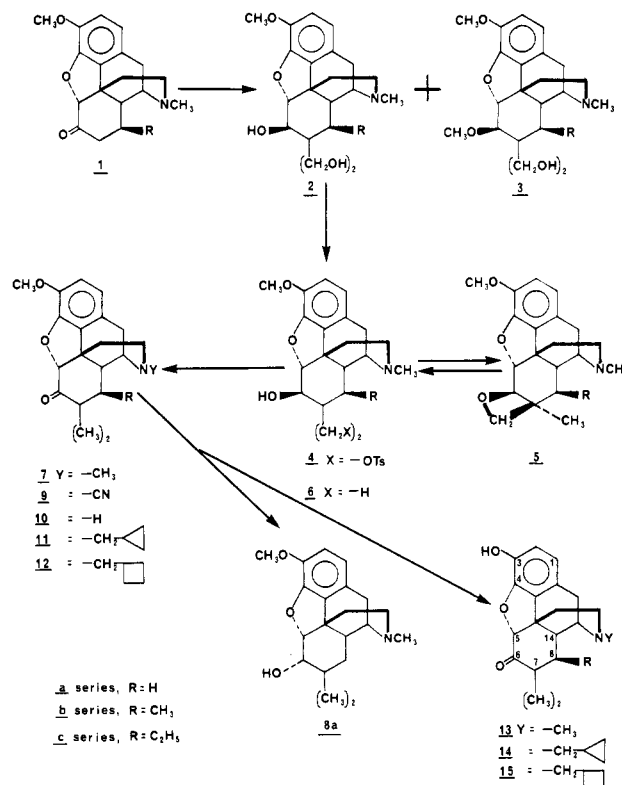
Treatment of dihydrocodeinone (1a) or the 8β-methyl (1b) or 8β-ethyl (1c) analogues with formaldehyde-Ca(OH)₂ in aqueous dioxane gave the corresponding 7,7-bis(hydroxymethyl)-6β-ols 2a-c. Ditosylation of 2, followed by LiEt₃BH reduction, gave either the 7,7-dimethyl-6β-ol (6a) or 7α-methyl-6β,7β-oxetane compounds (5b,c). Compounds 5b and 5c were cleaved to 6b or 6c using LiAlH₄-AlCl₃. The configuration of the C6-alcohol group of 6a was confirmed by an oxidation-reduction sequence which gave the 7,7-dimethyl-6α-ol 8a. Oxidation of 6 gave the C6-ketones 7a-c, which were converted to N-(cycloalkylmethyl) derivatives 11 and 12 and their corresponding 3-hydroxy compounds 14 and 15. The 3-methoxy-7,7-dimethyl-6-ones 7 were as active as dihydrocodeinone in agonist assays. One compound of this series, N-(cyclopropylmethyl)-7,7-dimethyldihydronorcodeinone (11a), was a potent mixed agonist-narcotic antagonist.

We have recently reported that the agonist and narcotic antagonist properties of 17-(cycloalkylmethyl)morphinan-6-ones can be modified by the introduction of short alkyl groups into the 7 and 8 positions of the C ring.² In order to further explore the effect of other modifications on pharmacological profiles, we sought additional methods for the formation of carbon-carbon bonds within this portion of the opiate nucleus.

Examination of the literature revealed that Mannich and Schulte³ reported in 1938 the facile aldol condensation-Cannizzaro reduction of dihydrocodeinone to give a 7,7-bis(hydroxymethyl)-6-hydroxy derivative. Our prior experience with a similar reaction in the carbohydrate area⁴ prompted us to explore this method for the preparation of intermediates for conversion to 7,7-dimethyl-N-(cycloalkylmethyl)dihydronorcodeinones. This paper reports the chemistry of the title compounds and the results of the pharmacological evaluation of these modified opiates.

Chemistry. Reaction of dihydrocodeinone (1a) with formaldehyde, in the presence of calcium hydroxide in 1:2

Scheme I



methanol-water as reported,³ gave a bis(hydroxymethyl) derivative (Scheme I) which was isolated as the hydro-

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