# Synthesis and Preliminary Pharmacological Evaluation of Asymmetric Chloroquine Analogues[1]

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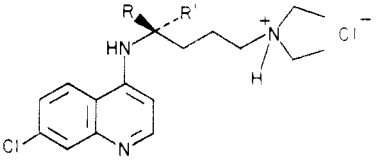
Asymmetric chloroquine analogues (1-4) were prepared of known absolute configuration in order to assess stereochemical influences on selected biological activities. Since chloroquine has been shown to possess spasmolytic properties, analogues 1-4 were tested for similar pharmacological effects on smooth-muscle 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 $PGF_{2\alpha}$-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 $PGF_{2\alpha}$ receptor in the ileum. This observation may be further exploited to obtain more selective profiles of biological activity through molecular manipulation.

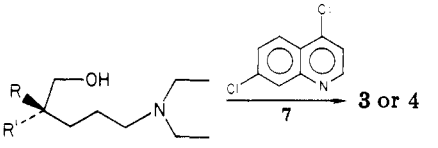
Chloroquine is primarily an antimalarial drug, but is also used in rheumatoid arthritis, lupus erythematosus, porphyria cutanea tarda, photoallergic reactions, hepatic amebic infestations, myotonia, asthma, experimental shock, patent ductus arteriosus, and possibly melanoma.[2,3] In the rat, chloroquine 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 (norepinephrine, 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 of known absolute configuration in order to assess stereochemical influences on selected biological activities. (*S*)-Chlorochloroquinine 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.



1 (*S*, dihydrochloride salt), R = H; R' = $CH_2Cl$
2 (*R*, dihydrochloride salt), R = $CH_2Cl$; R' = H
3 (*S*), R = H; R' = $CH_2OH$
4 (*R*), R = $CH_2OH$; R' = H

**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]



5, R = H; R' = $NH_2$
6, R = $NH_2$; R' = H

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 $Et_2O$ containing dicyclohexylamine was reported[15] to afford the corresponding $\alpha$-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

(1) Supported by U.S. Public Health Service Grant HD-14853.
(2) M. S. Manku and D. F. Horrobin, *Prostaglandins*, 12, 789 (1976).
(3) M. S. Manku and D. F. Horrobin, *Lancet*, 2, 115 (1976).
(4) E. Minker and Z. Matejka, *Acta Physiol. Acad. Sci. Hung.*, 53, 369 (1979).
(5) F. Varga, E. Fischer, and S. T. Szily, *Pharmacology*, 13, 401 (1975).
(6) E. Minker, T. Kádár, and Z. Matejka, *Acta Physiol. Acad. Sci. Hung.*, 55, 71 (1980).
(7) D. F. Horrobin, M. S. Manku, R. Karmali, B. A. Nassar, and P. A. Davies, *Nature (London)*, 250, 425 (1974).
(8) J. P. Mtabaji, M. S. Manku, and D. F. Horrobin, *Can. J. Physiol. Pharmacol.*, 54, 357 (1976).
(9) G. Weissmann, *N. Engl. J. Med.*, 273, 1143 (1965).
(10) J. P. Famaey, J. Fontaine, and J. Reuse, *J. Pharm. Pharmacol.*, 29, 761 (1977).
(11) J. P. Famaey, J. Fontaine, and J. Reuse, *Agents Actions*, 5, 354 (1975).
(12) A. R. Surrey and R. A. Cutler, *J. Am. Chem. Soc.*, 73, 2623 (1951).
(13) Z. Grzonka and B. Lammek, *Synthesis*, 661 (1974).
(14) R. Schwyzer, B. Iselin, H. Kappeler, B. Riniker, W. Rittel, and H. Zuber, *Helv. Chim. Acta*, 46, 1975 (1963).
(15) E. von Schroder and E. Klieger, *Liebigs Ann. Chem.*, 673, 196 (1964).

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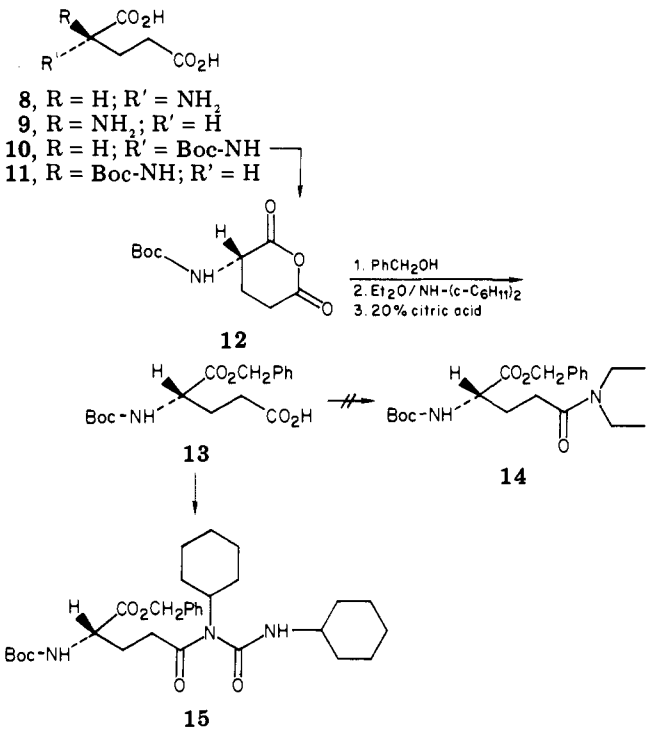
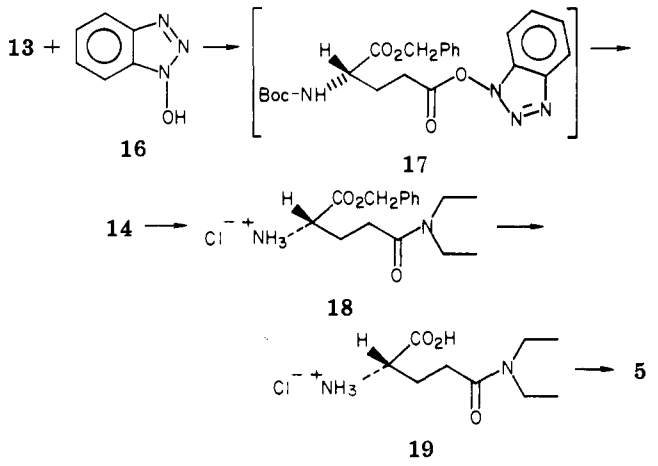



diethylamine, yielded undesired rearrangement product **15** in high yield (80%). Ester amide **14** could not be isolated, and NMR and infrared spectra were in accord with the assigned structure for **15**. This unusually high yield of **15** may reflect solvent choice and or length of reaction time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

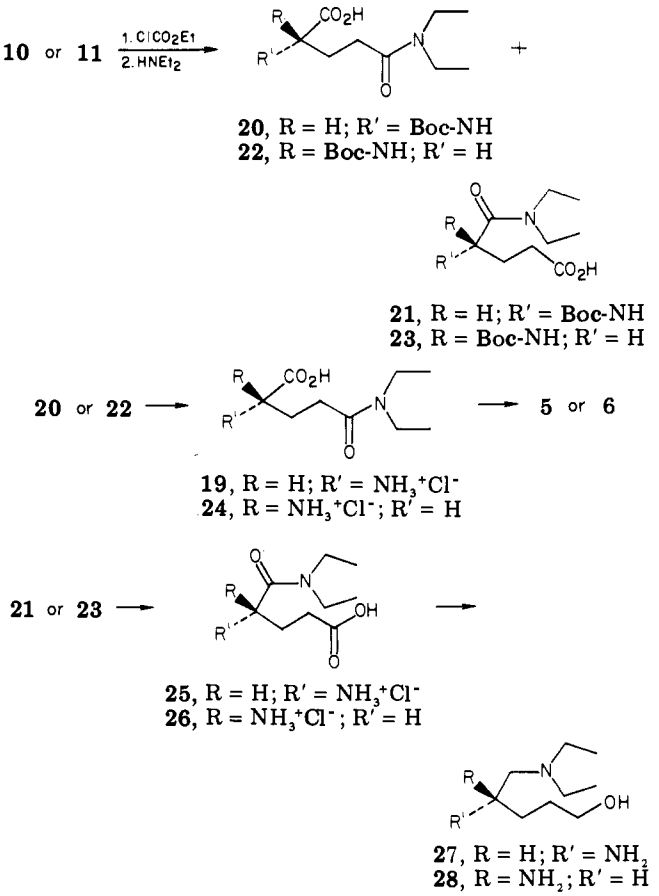
To circumvent rearrangement, **13** was treated with DCC




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 $H_2$ 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 $CO_2H$ group $\alpha$ to the amino function. The determined $pK_a$ of 2.32 was in accord with the $pK_a$ of the $\alpha$-$CO_2H$ group (2.19) in glutamic acid hydrochloride.[17] Thus, the diethylamido functionality in compounds **14**, **18**, and **19** must be in the $\gamma$ position.

Reduction of **19** with $LiAlH_4$ in refluxing THF afforded chiral diamino alcohol **5** in 75% yield. Nonaqueous workup (solid $Na_2SO_4 \cdot 10H_2O$)[18] proved valuable for product isolation owing to extreme water solubility. Diamino alcohol **5** is air and light sensitive and was stored under $N_2$ in the dark.

Alternatively, **5** was synthesized by treatment of **10** in




dry THF with 1 equiv of ethyl chloroformate. The mixture of anyhdrides 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 $Et_2O$. 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 deliquesent and difficult to isolate in the crystalline form. Again, potentiometric titration provided evidence for the position of the free $CO_2H$ group. For **25**, the determined $pK_a$ of 3.95 is in accord with the assignment of the diethylamide function to the $\alpha$ position. Reduction of **19** and **24–26** with $LiAlH_4$, followed by nonaqueous workup

(16) G. C. Windridge and E. C. Jorgensen, *J. Am. Chem. Soc.*, **93**, 6318 (1971).

(17) R. A. Day and A. L. Underwood, "Quantitative Analysis", 3rd ed., Prentice-Hall, Englewood Cliffs, NJ, 1974, p 145.

(18) A. M. Treasurywala, *Aldrichim. Acta*, **9**(2), 22 (1976).

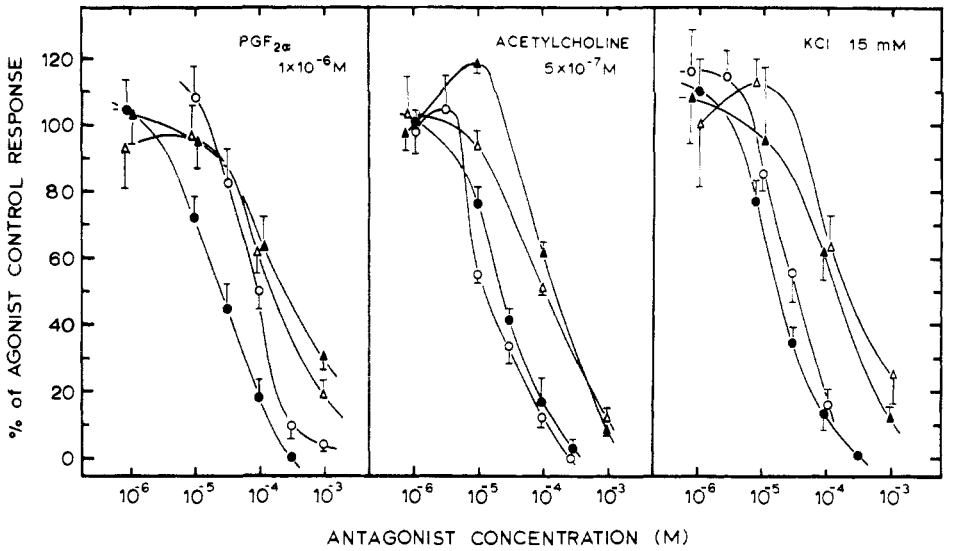
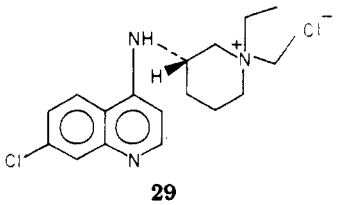



**Figure 1.** The effect of various concentrations of compounds 1–4 ($x$ axis) on contractions induced by prostaglandin $F_{2\alpha}$, acetylcholine, and KCl in the isolated mouse ileum: (●–●) 1, (O–O) 2, (▲–▲) 3, (△–△) 4.

($Na_2SO_4 \cdot 10H_2O$),[18] afforded the chiral diamino alcohols **5**, **6**, **27**, and **28**.

Surrey and Cutler[12] 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.




**29**

## 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 lipophylic 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 $PGF_{2\alpha}$-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 $PGF_{2\alpha}$-induced contractions. Compound **1** inhibited $PGF_{2\alpha}$-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 $3 \times 10^{-5}$ M, compound **1** produced a 55% inhibition of the control $PGF_{2\alpha}$ response, whereas compound **2** inhibited this response by only 17% ($p < 0.01$). At $10^{-4}$ M concentrations, compound **1** inhibited $PGF_{2\alpha}$-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*)-chlorochloroquine was tested in the isolated mouse uterus preparation[19] and, at a concentration of $3 \times 10^{-7}$ M, resulted in a 50% reduction in the contractile response of the estrous uterus to $3 \times 10^{-8}$ M $PGF_{2\alpha}$ (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

(19) R. G. Rahwan, M. M. Faust, and D. T. Witiak, *J. Pharmacol. Exp. Ther.*, **201**, 126 (1977).

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 $PGF_{2\alpha}$[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*)-chlorochloroquine (**1**). Since this isomer was a significantly more potent inhibitor of $PGF_{2\alpha}$-induced contraction of the ileum than the (*R*)-chlorochloroquine (**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 chlorochloroquine 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 polaragraph 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 Lammek[13] in 89% yield: mp 113–115 °C (lit.[15] mp 110–112 °C); $[\alpha]^{25}_D$ −15.20° (c 1.0, MeOH) [lit.[15] $[\alpha]^{25}_D$ −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; $[\alpha]^{25}_D$ +13.4° (c 1.0, MeOH).

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

**1-Benzyl *N*-(*tert*-Butoxycarbonyl)-(*S*)-glutamate (13)** was prepared according to the method of Schroder and Klieger[15] via the dicyclohexylamine salt: mp 171–172 °C (lit.[15] mp 172 °C); $[\alpha]^{25}_D$ −18.4° (c 1.0, MeOH) [lit.[15] $[\alpha]^{25}_D$ −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); $[\alpha]^{25}_D$ −28.9° (c 1.0, MeOH) [lit.[15] $[\alpha]^{25}_D$ −30.2° (c 1.0, MeOH)]. Anal. ($C_{17}H_{23}NO_6$) C, H, N.

**Benzyl $N^2$-(*tert*-Butoxycarbonyl)-*N*,*N*-diethyl-(*S*)-glutaminate (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, $[\alpha]^{25}_D$ −25.6° (c 1.0, MeOH). Anal. ($C_{21}H_{32}N_2O_5$) 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 $N^2$-(*tert*-butoxycarbonyl)-*N*,*N*-diethyl-(*S*)-glutamine (**20**) in 500 mL of dry $Et_2O$ until all of the starting material was consumed [0.5–1.0 h; monitored by TLC ($CHCl_3$–MeOH–$NH_4OH$; 9:1:3 drops)]. After the solution cooled (ca. 4 °C) overnight, the solid precipitate was collected and washed with dry $Et_2O$, followed by immediate recrystallization (deliquescent) from absolute EtOH–anhydrous $Et_2O$, affording 14.9 g (95%) of white crystals: mp 154–156 °C; $[\alpha]^{25}_D$ +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. ($C_9H_{19}N_2O_3Cl$) 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 $N^2$-(*tert*-butoxycarbonyl)-*N*,*N*-diethyl-(*S*)-glutaminate (**14**) in 250 mL of dry $Et_2O$ 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 $Et_2O$), and the solvent was removed under reduced pressure (0.05 mm) to yield 10.0 g (85%) of crude benzyl *N*,*N*-diethyl-(*S*)-glutaminate 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; $[\alpha]^{25}_D$ +26.1° (c 1.0, MeOH).

***N*,*N*-Diethyl-(*R*)-glutamine Hydrochloride (24).** Following method A, $N^2$-(*tert*-butoxycarbony)-*N*,*N*-diethyl-(*R*)-glutamine (**22**) was converted to **24**: yield 14.9 g (95%); mp 154–156 °C; $[\alpha]^{25}_D$ −26.8° (c 1.0, MeOH).

**[$N^4$-(*tert*-Butoxycarbonyl)amino]-*N*,*N*-diethyl-(*S*)-glutaramic Acid (21) and $N^2$-(*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 $H_2O$, and the aqueous layers were collected, washed with $Et_2O$, 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 $Et_2O$ and the solution was cooled (ca. 4 °C) overnight. The crystalline product was removed by filtration and washed with $Et_2O$, air-dried, and recrystallized from $CHCl_3$–$Et_2O$, affording 38.09 g (35%) of **21**: mp 155–157 °C; $[\alpha]^{25}_D$ −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

(20) Symposium, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **28**, 1624 (1969).
(21) M. R. Bennett, "Autonomic Neuromuscular Transmission", Cambridge University Press, London, 1972.
(22) S. Greenberg, P. J. Kadowitz, F. P. J. Diecke, and J. P. Long, *Arch. Int. Pharmacodyn. Ther.*, **205**, 381 (1973).
(23) T. Tomita, in "Smooth Muscle", E. Bulbring, A. F. Brading, A. W. Jones, and T. Tomita, Eds., Williams & Wilkins, Baltimore, MD, 1970, p 197.
(24) A. Haberkorn, H. P. Kraft, and G. Blaschke, *Tropenmed. Parasitol.*, **30**, 308 (1979).

ether (30–60 °C). The oil was chromatographed on silica gel ($CHCl_3$–MeOH–AcOH, 9:1:3 drops), affording 65.3 g (60%) of **20** as a clear viscous oil: $[\alpha]^{25}_D$ −8.0° (c 1.0, MeOH). Anal. ($C_{14}H_{26}N_2O_5$) 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 $H_2O$. The aqueous layers were collected, washed with $Et_2O$, acidified (pH 1–2) with cold 1 N HCl, and extracted with $CHCl_3$. The $CHCl_3$ layers were dried ($Na_2SO_4$), and the solvent was concentrated under reduced pressure to yield a yellow viscous oil, which was dissolved in $Et_2O$ and refrigerated overnight. The crystalline product was filtered and washed with $Et_2O$, air-dried, and recrystallized from $CHCl_3$–$Et_2O$, affording 3.63 g (30%) of **21** as white crystals: mp 156–158 °C; $[\alpha]^{25}_D$ −40.0° (*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 ($CHCl_3$–MeOH–AcOH, 9:1:3 drops), affording 7.2 g (60%) of **20** as a clear viscous liquid: $[\alpha]^{25}_D$ −8.0° (*c* 1.0, MeOH).

**[$N^4$-(*tert*-Butoxycarbonyl)amino]-*N*,*N*-diethyl-(*R*)-glutaramic Acid (23) and $N^2$-(*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° (c 1.0, MeOH)] and **22** [yield 65.0 g (60%); $[\alpha]^{25}_D$ +8.1° (*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 $LiAlH_4$) at 0 °C was added slowly with stirring 9.0 g (0.24 mol) of $LiAlH_4$. The resulting mixture was refluxed overnight. After the mixture cooled to 0 °C, finely powdered $Na_2SO_4{\cdot}10H_2O$ (prepared by recrystallization of anhydrous $Na_2SO_4$ from $H_2O$) was added slowly to the mechanically stirred reaction mixture until all the $LiAlH_4$ 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° (*c* 1.0, MeOH); IR (neat) 3500, 3300 $cm^{-1}$; NMR ($CDCl_3$, free amine) δ 3.10–3.70 (m, 3 H, $CHCH_2OH$), 2.25–3.0 [m, 9 H, $NH_2$, OH exchangeable with $D_2O$, $CH_2N(CH_2CH_3)_2$], 1.29–1.81 (m, 4 H, $CH_2CH_2$), 1.0 (t, 6 H, $CH_3$, $J$ = 7 Hz). Anal. ($C_{13}H_{26}N_2O_9$) 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 dioxolate salt; mp 130–132 °C; $[\alpha]^{25}_D$ −2.6° (c 1.0, MeOH).

**(*S*)-4-Amino-5-(diethylamino)pentan-1-ol (27).** Gaseous HCl (passed through a gas trap containing $H_2SO_4$) was bubbled through a solution of 20.0 g (0.066 mol) of [$N^4$-(*tert*-butoxycarbonyl)amino]-*N*,*N*-diethyl-(*S*)-glutaramic acid (**21**) in 500 mL of $CHCl_3$ until all the starting material was consumed [1.5–2.0 h; monitored by TLC ($CHCl_3$–MeOH–AcOH, 9:1:3 drops)]. After the solution cooled (ca. 4 °C) overnight, the oily suspension was extracted with $H_2O$, and the aqueous layers were collected and washed with $CHCl_3$. $H_2O$ was removed under reduced pressure to yield 14.15 g (90%) of crude *N*,*N*-diethyl-(*S*)-glutaramic 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. $LiAlH_4$ (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 $Na_2SO_4{\cdot}10H_2O$ was added slowly to the mechanically stirred reaction mixture until all the $LiAlH_4$ 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° (c 1.0, MeOH). Anal. ($C_{13}H_{26}N_2O_9$) 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° (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 $CHCl_3$ and extracted with 15% NaOH solution followed by 1 N HCl solution (5 × 50 mL). The acidic aqueous layers were washed with $Et_2O$, made basic with NaOH (pH 10–12), and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried ($Na_2SO_4$), filtered, and concentrated under reduced pressure. The resulting brown residue was chromatographed on silica gel ($CHCl_3$–MeOH–$NH_4OH$, 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° (c 1.0, MeOH); IR ($CHCl_3$) 3600, 3420 $cm^{-1}$; NMR ($CDCl_3$) 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 $D_2O$), 5.35 (s, 1 H, OH, broad, exchangeable with $D_2O$), 3.40–4.05 (m, 3 H, $CHCH_2OH$), 2.30–2.81 [m, 6 H, $CH_2N(CH_2CH_3)_2$], 1.45–2.01 (m, 4 H, $CH_2CH_2$), 1.00 (t, 6 H, $CH_3$, $J$ = 7 Hz). Anal. ($C_{18}H_{26}ClN_3O$) 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° (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 $CHCl_3$ cooled to −10 °C (NaCl–ice bath) was added dropwise with stirring 3 mL of $SOCl_2$ (freshly distilled) in 10 mL of $CHCl_3$. 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 $CHCl_3$ 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° (c 1.0, MeOH); IR (Nujol) 3300 $cm^{-1}$; NMR ($CDCl_3$, 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), 6.41 (d, 1 H, aromatic H-3, $J$ = 6 Hz), 5.67–5.97 (d, 1 H, NH, broad, exchangeable with $D_2O$), 3.40–4.05 (m, 3 H, $CHCH_2Cl$), 2.30–2.81 [m, 6 H, $CH_2N(CH_2CH_3)_2$], 1.45–2.01 (m, 4 H, $CH_2CH_2$), 1.00 [t, 6 H, $N(CH_2CH_3)_2$, $J$ = 7 Hz]. Anal. ($C_{18}H_{25}Cl_2N_2{\cdot}2HCl$) 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° (c 1.0, MeOH).

**Pharmacological Methods.** The experimental protcol 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; $CaCl_2{\cdot}2H_2O$, 0.37; $MgSO_4{\cdot}7H_2O$, 0.14; $NaH_2PO_4{\cdot}H_2O$, 0.16; $NaHCO_3$, 2.10; dextrose, 2.0. Recordings were made using a Grass FT03 isometric transducer coupled to a Grass Model F polygraph re-

(25) D. T. Witiak, S. V. Kakodkar, T. P. Johnson, J. R. Baldwin, and R. G. Rahwan, *J. Med. Chem.*, **22**, 77 (1979).
(26) D. T. Witiak, S. V. Kakodkar, C. E. Brunst, J. R. Baldwin, and R. G. Rahwan, *J. Med. Chem.*, **21**, 1313 (1978).
(27) C. E. Akesson, R. G. Rahwan, R. J. Brumbaugh, and D. T. Witiak, *Res. Commun. Chem. Pathol. Pharmacol.*, **27**, 265 (1980).

corder (Grass Instruments Co., Quincy, MA). The bathing solution was aerated with 5% $CO_2$ in $O_2$. A 30-min equilibration period was allowed prior to all experiments.

In each experiment, control response to $PGF_{2\alpha}$ ($10^{-6}$ M bath concentration), acetylcholine (AcCh; $5 \times 10^{-7}$ 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. $PGF_{2\alpha}$, 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 $PGF_{2\alpha}$, 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 $PGF_{2\alpha}$, 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, $5 \times 10^{-7}$ M; KCl, 15 mM; $PGF_{2\alpha}$, $10^{-6}$ 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-Dimethyldihydromorphinones[1]

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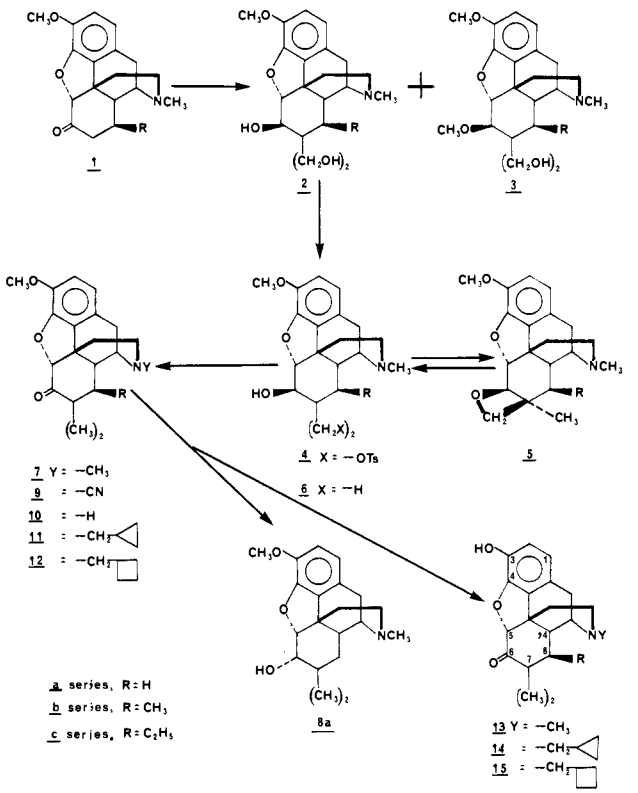
Treatment of dihydrocodeinone (**1a**) or the 8β-methyl (**1b**) or 8β-ethyl (**1c**) analogues with formaldehyde–$Ca(OH)_2$ in aqueous dioxane gave the corresponding 7,7-bis(hydroxymethyl)-6β-ols **2a–c**. Ditosylation of **2**, followed by $LiEt_3BH$ reduction, gave either the 7,7-dimethyl-6β-ol (**6a**) or 7α-methyl-6β,7β-oxetane compounds (**5b,c**). Compounds **5b** and **5c** were cleaved to **6b** or **6c** using $LiAlH_4$–$AlCl_3$. 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 Schulte[3] 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 area[4] 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 methanol–water as reported,[3] gave a bis(hydroxymethyl) derivative (Scheme I) which was isolated as the hydro-

Scheme I




(1) This paper has been presented in part, see "Abstracts of Papers", 181st National Meeting of the Americal Chemical Society, Atlanta, GA, April 1981; American Chemical Society: Washington DC, 1981; Abstr MEDI 48.
(2) (a) Kotick, M. P.; Polazzi, J. O.; Leland, D. L.; Schut, R. N. *J. Med. Chem.* **1980**, *23*, 166. (b) Polazzi, J. O.; Schut, R. N.; Kotick, M. P.; Howes, J. F.; Osgood, P. F.; Razdan, R. K.; Villarreal, J. E. *Ibid.* **1980**, *23*, 174. (c) Leland, D. L.; Kotick, M. P.; *Ibid.* **1980**, *23*, 1427.
(3) Mannich, C.; Schulte, K. *Arch. Pharm. (Weinheim, Ger.)* **1938**, *276*, 593.
(4) Leland, D. L.; Kotick, M. P. *Carbohydr. Res.* **1974**, *38*, C9.

0022-2623/81/1824-0717$01.25/0 