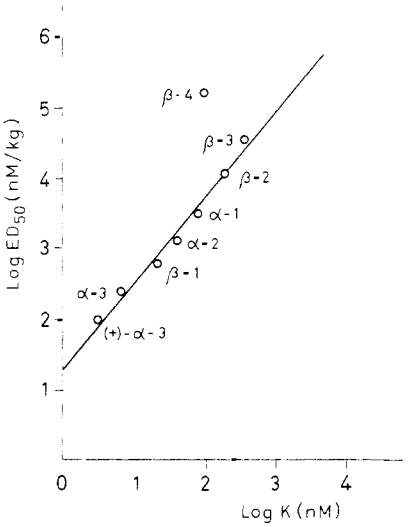

Figure 1. Correlation between analgetic activities and receptor affinities of α- and β-prodine-type compounds. The line was drawn from log y = 1.16 log x + 1.38; correlation coefficient r = 0.99. The value for β-4 was omitted in the calculation of the regression line because this compound showed a very poorly dose-related analgetic response.

(10 ml/g of brain) by the general procedure of Whittaker.[9] Osborne Mendel rats (150–200 g) from the NIH colony were used. The homogenates were centrifuged for 10 min at 1000g and the supernatant fraction was then centrifuged at 10000g for 20 min. This pellet (P_2) was resuspended in the original volume of 0.32 M sucrose and used within 24 h of storage at 0 °C or stored in 2-ml aliquots at −60 °C (at this temperature the pellet was stable for many months).

Binding experiments were routinely carried out as follows. P_2 fractions were diluted with 8 vol of 0.32 M sucrose–0.01 M Tris–HCl, pH 8.0. This suspension (900 μl), which contained approximately 0.5 mg of protein, was used in each polycarbonate assay tube which also contained the substance to be tested as competitor of [^{3}H]dihydromorphine binding. As the final concentration, 100 μl of 10^{-8} M solution of [^{3}H]dihydromorphine in 0.32 M sucrose was added to each tube. Two blank tests without inhibitor and 14 increasing concentrations of the inhibitor were used in each run. Samples were kept at 0 °C prior to incubation. After incubation at 37 °C for 10 min, the samples were centrifuged at 19000 rpm (45000g) for 10 min in a refrigerated Sorvall centrifuge. The supernatant fluid was carefully withdrawn with a syringe equipped with a blunt tipped No. 18 needle and the pellet was rinsed with 1 ml of 0.32 M sucrose and again carefully withdrawn with the syringe. The pellet was suspended in 500 μl of 1% Triton X-100 and transferred to a scintillation vial. The assay tube was rinsed with a second 500 μl of 1% Triton which was added to the same vial after which 7.5 ml of Triton–toluene scintillation fluid[10] was added and the radioactivity determined.

Specific binding is defined to include only that portion of [^{3}H]dihydromorphine binding which can be displaced by narcotic analgesics or antagonists and not by their inactive stereoisomers.[11] The proportion of the total binding, which is specific by this criterion, decreases with increasing concentration of [^{3}H]dihydromorphine. At 10^{-9} M [^{3}H]dihydromorphine, the concentration used in our experiments, specific binding is approximately one-half of the total binding.

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9-Hydroxy-1,2,3,4,5,6-hexahydro-1,5-methano-3-benzazocine Derivatives as Analgesics

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Three 1,3-dimethyl-9-hydroxy-1,2,3,4,5,6-hexahydro-1,5-methano-3-benzazocine derivatives (**7**–**9**) have been synthesized and tested as analgesics. The synthesis of these compounds involved conversion of 1-methyl-7-methoxy-β-tetralone (**1**) by Mannich reaction with $MeNH_2$ and HCHO to give the 11-ketone **2**, from which **7**, **8**, and **9**, respectively, were obtained. These compounds have analgesic activity, and **7** was found to be comparable to codeine.

The compounds described in Table I were prepared and tested as analgesics because the closely related 2,3,4,5,-6,7-hexahydro-1,6-methano-1*H*-3-benzazonine[1] and 2,3,-4,5,6,7-hexahydro-1,6-methano-1*H*-4-benzazonine derivatives,[2] ring C enlarged benzomorphans, have been found to possess analgesic activity. The substituents chosen were those which often enhance this activity of 6,7-benzomorphans.[3]

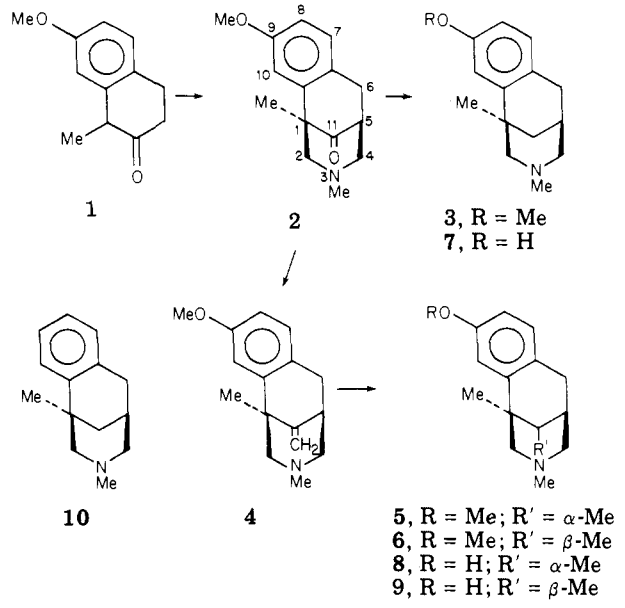
Chemistry. Conversion of 1-methyl-7-methoxy-3,4-dihydro-2(1*H*)-naphthalenone (**1**)[4] to the desired framework **2** was achieved by Mannich reaction with $MeNH_2$ and HCHO in one step.[5] The carbonyl group in **2** was reduced to methylene by Wolff–Kishner reaction, giving compound **3**. On the other hand, ketone **2** was treated with $Ph_3P{=}CH_2$ to give the 11-methylene compound **4**. Hydrogenation of **4** over Pt catalyst in EtOH gave 11α-methyl

Table I. Analgesic Activities of 1,2,3,4,5,6-Hexahydro-1,5-methano-3-benzazocine Derivatives

Compd	ED_{50}, mg/kg sc
7·HBr[a]	8.4 (7.2–9.9)[d]
8·HBr[a]	28.0 (22.4–35.0)
9·HBr[a]	32.8 (26.9–40.0)
10[b]	>50
Codeine[c]	9.2 (8.1–10.4)

[a] Administered in saline. [b] Administered as hydrochloride in saline; see ref 5. [c] Administered as phosphate in saline. [d] Confidence interval (95%).

isomer **5**, while the hydrogenation in AcOH–$HClO_4$ gave a mixture of the 11β-methyl isomers **6** and **5** (ratio, 7:1).[6] The stereochemistry of **5** and **6** was established by comparison of the chemical shifts of C-11 methyl protons of **5** (δ 0.82) and **6** (δ 1.11).[7]



O-Demethylation of compounds **3**, **5**, and **6** by refluxing with hydrobromic acid afforded the final compounds **7**, **8**, and **9**, respectively.

Pharmacology. In Table I are given analgesic activities (method of pressure stimuli on mouse tail[8]) of compounds **7**, **8**, and **9** as well as the parent compound, 1,3-dimethyl-1,2,3,4,5,6-hexahydro-1,5-methano-3-benzazocine **10**.[5] Comparative data for codeine are also presented. Groups of ten albino male mice, dd strain, were tested at five dose levels. ED_{50} values were calculated by the Lichfield–Wilcoxon method.[9] Activity of compound **7** is comparable to that of codeine or 2′-hydroxy-2-methyl-6,7-benzomorphan[3] and surpasses those of the 11-methyl derivatives **8** and **9**. A phenolic hydroxyl at the 9 position distinctly increases the activity.

It is noteworthy that structural change from the 6,7-benzomorphans to the 1,2,3,4,5,6-hexahydro-1,5-methano-3-benzazocine framework decreases but still retains analgesic activity and that the effect upon analgesic activity of methyl group on the bridge methylene (C-11) in 1,2,3,4,5,6-hexahydro-1,5-methano-3-benzazocine is different from that of the 6,7-benzomorphans[3] and homobenzomorphans.[2]

Experimental Section

Melting points were taken with a micro melting point apparatus (Yanagimoto) and are uncorrected. IR spectra were recorded on a Japan Spectroscopic IR-E spectrometer. NMR spectra were taken on a JEOL PMX-60 (Me_4Si) spectrometer. Mass spectra were obtained on a JEOL JMS-01SG spectrometer.

1,3-Dimethyl-9-methoxy-1,2,3,4,5,6-hexahydro-1,5-methano-3-benzazocin-11-one (2). A mixture of 1[4] (28.2 g, 0.142 mol), $MeNH_2$·HCl (13 g, 0.193 mol), and formalin (43.5 ml, 0.54 mol) in AcOH (305 ml) was refluxed for 3.5 h. After evaporation, the residual syrup was dissolved in 5% HCl (300 ml) and washed with $CHCl_3$. The aqueous layer was basified with 20% NaOH, extracted with $CHCl_3$, washed with H_2O, and dried (K_2CO_3). The residue of the extract (27 g) was chromatographed on a silica gel (150 g) column. Elution with $CHCl_3$ gave 8.0 g of **2**, which was distilled to give 7.2 g (20.6%) of pure sample: bp 140–162 °C (0.35 mmHg); IR (neat) 2760 (NMe), 1740 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.33 (3 H, s, C-1 Me), 2.16 (3 H, s, NMe), 3.76 (3 H, s, OMe), 6.67 (1 H, d, J = 2.5 Hz, C-10 H), 6.69 (1 H, double d, J = 9.0 Hz, J' = 2.5 Hz, C-8 H), 6.98 (1 H, d, J = 9.0 Hz, C-7 H); mass spectrum m/e 245 (M^+). The picrate had mp 203–205 °C (from MeOH). Anal. ($C_{15}H_{19}NO_2$·$C_6H_3N_3O_7$·CH_3OH) C, H, N.

1,3-Dimethyl-9-methoxy-1,2,3,4,5,6-hexahydro-1,5-methano-3-benzazocine (3). Compound **2** (1.5 g, 6.1 mmol), NH_2NH_2·H_2O (2.5 ml, 50 mmol), KOH (2.0 g, 36 mmol), and diethylene glycol (20 ml) were heated at 190–200 °C for 4 h. The cooled mixture was diluted with H_2O, extracted with Et_2O, and dried (K_2CO_3). The solvent was evaporated and the residue was distilled to give 1.0 g (70.7%) of **3**: bp 120–130 °C (2 mmHg); NMR ($CDCl_3$) δ 1.27 (3 H, s, C-1 Me), 2.06 (3 H, s, NMe), 3.75 (3 H, s, OMe), 6.62 (1 H, double d, J = 8.0 Hz, J' = 2.5 Hz, C-8 H), 6.78 (1 H, d, J = 2.5 Hz, C-10 H), 6.96 (1 H, d, J = 8.0 Hz, C-7 H). The picrate had mp 211–214 °C (from MeOH). Anal. ($C_{15}H_{21}NO$·$C_6H_3N_3O_7$) C, H, N.

1,3-Dimethyl-9-methoxy-11-methylene-1,2,3,4,5,6-hexahydro-1,5-methano-3-benzazocine (4). To a stirred solution of n-C_4H_9Li (20% in hexane, 7.5 ml, 23 mmol) in dry Et_2O (45 ml) was added $Ph_3P(Me)Br$ (7.6 g, 19 mmol) over 10 min under N_2 and ice cooling. Stirring was continued for 4 h. Compound **2** (1.0 g, 4.1 mmol) in THF (10 ml) was added to this solution over 10 min at room temperature. The mixture was stirred at room temperature for 16 h, refluxed for 2 h, diluted with Et_2O, and filtered. The solvents were evaporated, and the residue was dissolved in $CHCl_3$ and extracted with 2% H_3PO_4. The aqueous extract was basified with 12 M NH_4OH, extracted with Et_2O, and dried (K_2CO_3). The residue from the ethereal solution was distilled to give 0.8 g (80.6%) of 4: bp 125–130 °C (0.3 mmHg); IR (neat) 1655 cm^{-1} (C=C); NMR ($CDCl_3$) δ 1.42 (3 H, s, C-1 Me), 2.06 (3 H, s, NMe), 3.72 (3 H, s, OMe), 4.70 (1 H, s) and 4.77 (1 H, s) (C-11 =CH_2), 6.57 (1 H, double d, J = 8.0 Hz, J' = 2.2 Hz, C-8 H), 6.73 (1 H, d, J = 2.2 Hz, C-10 H), 6.90 (1 H, d, J = 8.0 Hz, C-7 H). The picrate had mp 198–208 °C (from MeOH). Anal. ($C_{16}H_{21}NO$·$C_6H_3N_3O_7$) C, H, N.

1,3,11α-Trimethyl- (5) and 1,3,11β-Trimethyl-9-methoxy-1,2,3,4,5,6-hexahydro-1,5-methano-3-benzazocine (6). (A) Hydrogenation of 4 (0.5 g, 2.1 mmol) over PtO_2 (0.2 g) in EtOH (5 ml) for 6 h gave 0.5 g (98.2%) of **5** as a colorless oil: bp 123–135 °C (0.2 mmHg) (bath temperature); NMR ($CDCl_3$) δ 0.82 (3 H, d, J = 7.0 Hz, C-11 Me), 1.25 (3 H, s, C-1 Me), 2.05 (3 H, s, NMe), 3.75 (3 H, s, OMe), 6.60 (1 H, double d, J = 8.5 Hz, J' = 2.5 Hz, C-8 H), 6.73 (1 H, d, J = 2.5 Hz, C-10 H), 6.95 (1 H, J = 8.5 Hz, C-7 H). The picrate had mp 210–213 °C (from MeOH). Anal. ($C_{16}H_{23}NO$·$C_6H_3N_3O_7$) C, H, N.

(B) Hydrogenation of 4 (1.0 g, 4.1 mmol) over PtO_2 (0.2 g) in 60% $HClO_4$ (1 ml) and AcOH (10 ml) for 7 h gave a residual syrup which was dissolved in H_2O, made alkaline with 20% NaOH, extracted with Et_2O, and dried (K_2CO_3). Evaporation of the Et_2O gave 620 mg of colorless oil, which was converted to the picrate and recrystallized from Me_2CO to give 845 mg (43.3%) of **6**·picrate: mp 245–247 °C. Anal. ($C_{16}H_{23}NO$·$C_6H_3N_3O_7$) C, H, N. The free base (**6**) had bp 135–145 °C (0.2 mmHg) (bath temperature): NMR ($CDCl_3$) δ 1.11 (3 H, d, J = 7.0 Hz, C-11 Me), 1.21 (3 H, s, C-1 Me), 2.05 (3 H, s, NMe), 3.76 (3 H, s, OMe), 6.63 (1 H, double d, J = 8.0 Hz, J' = 2.5 Hz, C-8 H), 6.84 (3 H, d, J = 2.5 Hz, C-10 H), 6.98 (1 H, d, J = 8.0 Hz, C-7 H).

The mother liquor was evaporated to dryness, and the residual solid was recrystallized from MeOH to give 136 mg (7%) of **5**·picrate.

1,3-Dimethyl- (7), 1,3,11α-Trimethyl- (8), and 1,3,11β-Trimethyl-9-hydroxy-1,2,3,4,5,6-hexahydro-1,5-methano-3-benzazocine (9). A mixture of **3** (0.5 g, 2.2 mmol) and 48% HBr

(5 ml) was refluxed for 0.5 h. Evaporation and recrystallization from MeOH–Me_2CO gave 0.35 g (54.3%) of 7·HBr as colorless crystals: mp >300 °C. Anal. ($C_{14}H_{19}NO$·HBr) C, H, N.

Compound 8·HBr and 9·HBr were obtained by similar O-demethylation of 5 and 6 with 48% HBr in 85 and 80% yield, respectively. 8·HBr had mp >300 °C (from MeOH). Anal. ($C_{15}H_{21}NO$·HBr) C, H, N. 9·HBr had mp 294–295 °C (from MeOH). Anal. ($C_{15}H_{21}NO$·HBr) C, H, N.

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(7) The difference in the C-methyl chemical shift between the α-methyl and β-methyl isomers would be the result of a change in environment of the C-methyl from a position above the aromatic ring to one away from the aromatic ring.
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Synthesis and Inhibition Analysis of 2(4)-Imino-4(2)-amino-2,4-dideoxyriboflavin, a Dual Antagonist of Riboflavin and Folinic Acid[1a]

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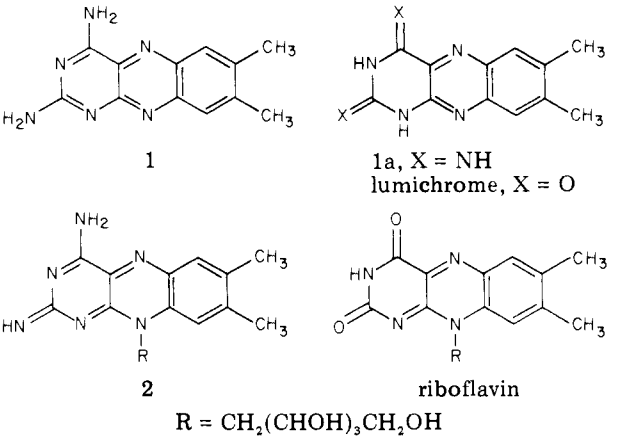
The synthesis of the 2,4-diamino analogue of riboflavin is described. Inhibition analysis in a microbial assay system indicated that this compound has a weak antifolate activity that could be overcome with a minimal amount of folinic acid, but at higher concentrations both folinic acid and riboflavin were required for the reversal of its inhibitory effect.

Earlier work in this laboratory, directed at the synthesis of antimetabolites designed to incorporate some of the structural features of two vitamin analogues into a single molecule,[2] led to the synthesis of 2,4-diamino-7,8-dimethylbenzo[*g*]pteridine[3] (1) which (in one of its unstable tautomeric forms, **1a**) may be regarded as the 2,4-diimino analogue of lumichrome, the aglycon of riboflavin. This compound was found to be a potent growth inhibitor of *Lactobacillus leichmannii*,[4] with an I_{50} of 0.1–0.2 μg/ml. The inhibition could be reversed competitively with folinic acid alone over a 200-fold concentration range, while at higher concentrations of the inhibitor, both folinic acid and riboflavin were required for reversal.[5] Thus, compound 1 appeared to act as a typical 2,4-diaminopyrimidine-fused heterocyclic inhibitor of dihydrofolate reductase[2,4] but, at high concentrations, also as an antimetabolite of vitamin B_2. Unfortunately, the poor solubility and tissue absorption properties of compound 1 severely limited its in vivo evaluation as a chemotherapeutic agent, although preliminary tests using various transplanted tumors in mice indicated that 1 may possess significant antitumor activity.[6,7]

It was felt that introduction of alkyl or hydroxyalkyl side chains in the N_{10} position of 1 might lead to compounds with better solubility and/or membrane transport properties. Such compounds, having flavin-type structures (e.g., 2), would bear a greater structural resemblance to riboflavin, while, on the other hand, they would be less analogous to the 2,4-diaminopteridine-type antifolates; therefore, they might be expected to act as more effective antagonists of vitamin B_2 but to show perhaps less activity as inhibitors of dihydrofolate reductase. Initially, we attempted to synthesize the desired N_{10}-substituted derivatives of 1 by various modifications of our general method[3] for the synthesis of 2,4-dideoxyalloxazines (including 1), i.e., the condensation of appropriately substituted 5,6-diaminopyrimidines with dimeric 4,5-dimethyl-*o*-benzoquinone. Although this method was later successfully employed in the synthesis of 2-imino-2-deoxyflavins (as well as in a new synthesis of riboflavin) by other investigators,[8,9] its application to the synthesis of 2(4)-imino-4(2)amino-2,4-dideoxyflavins invariably led to condensation products which were difficult to purify. Attempts to introduce an alkyl side chain at the N_{10} position of compound 1 by direct alkylation resulted in substitution at the N_1 position.[10] Subsequently, a series of 6-(*N*-alkyl-*N*-arylamino)pyrimidines was prepared as "open chain" analogues and potential synthetic intermediates of the desired N_{10}-substituted 2,4-dideoxyisoalloxazines.[11] A method for the conversion of such compounds derived from uracil to the corresponding isoalloxazines, including riboflavin, was recently reported by Yoneda et al.[12,13]



We found that the latter method, with some modifications, could be applied to the synthesis of 2(4)-imino-4(2)-amino-2,4-dideoxyriboflavin (**2**) (see Scheme I). Thus, 2,4-diamino-6-chloropyrimidine was allowed to react with