n-BuOH:AcOH:H_2O (4:1:5 upper phase); R_f[2], *n*-BuOH:AcOH:H_2O:EtOAc (1:1:1:1); R_f[3], EtOH:H_2O (7:3).

pGlu-N^{im}-Bzl-His-Try-*O*-Bzl-Ser-Phe-Gly-Leu-N^G-Tos-Arg-Pro-Gly-NH_2 (1). *tert*-Butyloxycarbonylglycine resin (2.26 g, 0.63 mmol of Gly) (purchased from Schwarz Bio Research, Inc.) was added to the reaction vessel and, after deprotection and neutralization, each new amino acid (*tert*-Boc derivatives with the exception of pGlu) was coupled on successive days by a nine-cycle procedure described previously.[15] Amino acids (1.89 mmol) were coupled in the presence of equivalent amounts of DCI. *tert*-Boc-N^G-Tos-Arg, *tert*-Boc-N^{im}-Bzl-His, and pGlu were coupled in DMF, the remaining amino acids in CH_2Cl_2. Hydrogen chloride (1 *M*) in glacial AcOH was used for the removal of *tert*-Boc groups and, following the incorporation of *tert*-Boc-Try, 1% EtSH was included in this reagent.

After completion of the synthesis, drying *in vacuo* gave 2.98 g of protected decapeptide resin (86% incorporation based on initial butyloxycarbonylglycine content). Peptide resin (1.40 g) was suspended in dry MeOH (90 ml) which was saturated at −2° with dry NH_3. The mixture was stirred at room temperature in a stoppered flask (40 hr). NH_3 was partially removed *in vacuo* and, after filtration and extraction of the resin with DMF (three 15-ml portions), the combined filtrates were evaporated to dryness *in vacuo*.

Recrystallization of the residue (290 mg) from MeOH gave a white powder (176 mg, 40% based on initial Gly attached to resin): mp 162–164°; $[\alpha]^{26.5}D$ −25.3° (*c* 1.10, DMF); R_f[1] (silica) 0.46; single spot to Ehrlich, Pauly, and I_2–starch reagents. Amino acid analysis of acid hydrolysate: Try, 0.9; NH_3, 1.2; Arg, 1.0; Ser, 0.7; Glu, 1.0; Pro, 1.1; Gly, 2.0; Leu, 0.9; Phe, 0.9. *Anal.* ($C_{76}H_{93}N_{17}O_{14}S \cdot 3H_2O$) C, H, N.

pGlu-His-Try-Ser-Phe-Gly-Leu-Arg-Pro-Gly-NH_2 (2). The protected peptide **1** (145 mg) was dissolved in 250 ml of anhydrous, liquid ammonia. Sodium was added to the gently boiling, stirred solution from a small-bore glass tube until a faint, persistent blue color was observed. This was discharged immediately with 2 drops of dry AcOH and the NH_3 removed under anhydrous conditions. The residue was applied to a column (1.7 × 110 cm) of Sephadex G-15 and eluted in 50% AcOH. The peptide emerging close to the void volume of the column was recovered by lyophilization. This material was dissolved in water (6 ml) and loaded on a column (0.9 × 91 cm) of CM-cellulose equilibrated with 0.002 *M* NH_4Ac buffer (pH 4.6). After 40 ml had been collected, a pH and concentration gradient was begun by introducing 0.1 *M* NH_4Ac buffer (pH 7.0) through a 250-ml mixing flask containing starting buffer. [5-Phe]-LH-RH (**2**) was located between elution volumes of 575 and 650 ml by measurement of the optical density at 280 nm. The corresponding fractions were pooled and lyophilized. Relyophilization from 0.1 *M* AcOH to constant weight gave peptide **2** (41 mg, 35%): $[\alpha]^{26.5}D$ −66.0° (*c* 1.16, 10% AcOH); single spot to Ehrlich, Pauly, and I_2–starch reagents; R_f[1] (cellulose) 0.70, R_f[2] (silica) 0.56, R_f[3] (silica) 0.34; single component moving in direction of cathode after TLE in pyridine acetate buffers at pH 4.5 and 6.4. Amino acid analysis: Try, 1.2; His, 1.0; NH_3, 1.2; Arg, 0.9; Ser, 0.7; Glu, 1.0; Pro, 1.0; Gly, 2.2; Leu, 1.0; Phe, 0.8. *Anal.* ($C_{55}H_{75}N_{17}O_{12} \cdot 6CH_3COOH \cdot 3H_2O$) C, H, N.

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Novel Analgetics and Molecular Rearrangements in the Morphine–Thebaine Group. 29.[1] Aryl and Arylalkyl Tertiary Alcohols in the 6,14-*endo*-Ethenotetrahydrothebaine Series

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In the homologous series of C-19 tertiary alcohols **1** derived from the thebaine–methyl vinyl ketone adduct, analgetic activity is maximal when *n* is 2 or 3 and thereafter becomes smaller.[2] The effect of increasing chain length is more pronounced in the analogous series **2** (also C-19) in which a phenyl group is placed at the end of the alkyl chain (Table II).[3] Related series of C-7 tertiary alcohols **3** and **4** have now been prepared in which the hydrocarbon side chain is directly attached to C-7.

6,14-*endo*-Etheno-7-oxotetrahydrothebaine (**5**)[1] reacted with methylmagnesium iodide to give a mixture of epimeric alcohols from which the 7α-methyl epimer **3** (*n* = 0) was isolated by repeated crystallization. The specificity of the reaction increased with the size of the Grignard reagent (Table I); 7α-benzyl-6,14-*endo*-etheno-7β-hydroxytetrahydrothebaine (**4**, *n* = 1) was obtained in best yield (64%). Assignment of structure to the isolated products was made from the position of the C-5β proton signal in the nmr spectrum. This appeared at δ 4.5[4] and is attributed to 1,3 deshielding by the 7β-hydroxyl group.[5] Attack of the Grignard reagents from the α face of ring C is preferred on steric grounds; approach from the β face is hindered by the C-5 and C-15 β-hydrogen atoms.

Structure–Activity Relationships. The analgetic potencies of the new tertiary alcohols in the rat tail pressure test,[6] when administered intraperitoneally, are shown in Table II. The α-alkyl alcohols **3** (*n* = 0, 2) are somewhat less potent than morphine; these levels are very similar to those of their isomers **6** and **7** in which the hydroxyl group is at C-19 instead of C-7.[2] In the homologous series **4** there are tenfold increases in potency between phenyl

Table I

Compd	Mp, °C	% yield	Formula[d]
3 (n = 0)	152–154[a]	11	$C_{22}H_{27}NO_4$[e]
3 (n = 2)	144–146[b]	14	$C_{24}H_{31}NO_4$
4 (n = 0)	215–216[a]	25	$C_{27}H_{29}NO_4$
4 (n = 1)	213–217[c]	64	$C_{28}H_{31}NO_4 \cdot HCl$
4 (n = 2)	240–243[c]	57	$C_{29}H_{33}NO_4 \cdot HCl$
4 (n = 3)	200–205[c]	51	$C_{30}H_{35}NO_4 \cdot HCl \cdot H_2O$
4 (n = 4)	230–234[c]	53	$C_{31}H_{39}NO_4 \cdot HCl \cdot H_2O$

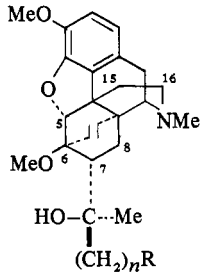
Recrystallization solvent: [a]EtOH; [b]MeOH; [c]EtOH–Et_2O. [d]Analyses for C, H, N. [e]C: calcd, 71.52; found, 71.04.

Table II

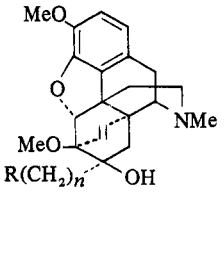
Structure	Analgetic[a] ED_{50}, mg/kg ip	Structure	Analgetic[a] ED_{50}, mg/kg ip
3 (n = 0)	2.8 (2.2–3.6)	2 (n = 0)	105
3 (n = 2)	6.6 (4.7–9.4)	2 (n = 1)	0.059 (0.02–0.11)
4 (n = 0)	38 (24–44)	2 (n = 2)	0.0028 (0.0012–0.0064)
4 (n = 1)	4.8 (3.3–7.0)	2 (n = 3)	0.047[b] (0.032–0.068)
4 (n = 2)	0.35 (0.16–0.77)	6	2.8 (1.8–4.2)
4 (n = 3)	0.38 (0.17–0.84)	7	4.6 (2.7–9.1)
4 (n = 4)	0.8 (0.61–1.04)		
Morphine	1.3 (0.92–1.79)		

[a]Rat tail pressure. [b]Previously reported as 2.1 × morphine.[2]

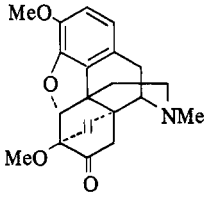
and benzyl and between benzyl and phenethyl; thereafter, marginal reductions are observed. The effect is similar to that found for the series of alcohols 1 and 2 and is in keeping with the postulate of a second lipophilic site on the analgetic receptor.[3]



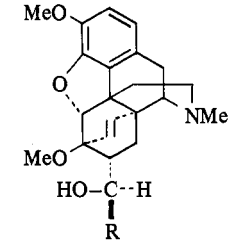
1, R = Me
2, R = Ph



3, R = Me
4, R = Ph



5



6, R = H
7, R = Et

Experimental Section

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, the results obtained for those elements were within ±0.4% of the theoretical values. The structures of all compounds were assigned on the basis of compatible ir and nmr spectra.

6,14-*endo*-Etheno-7β-hydroxy-7α-phenyltetrahydrothebaine (4, n = 0) (General Procedure for Tertiary Alcohols of Structures 3 and 4). 6,14-*endo*-Etheno-7-oxotetrahydrothebaine[1] (5, 30 g) in C_6H_6 was slowly added to a stirred, boiling ethereal solution of PhMgBr [from Mg (0.6 g) and PhBr (4.0 g)]. The mixture was set aside at room temperature for 18 hr and was then poured with vigorous stirring into saturated aqueous NH_4Cl. The organic layer was collected and the aqueous solution washed with C_6H_6. The combined extracts were dried (Na_2SO_4) and evaporated. Crystallization of the residue from EtOH afforded 4 (n = 0) (0.9 g), mp 215–216°. *Anal.* ($C_{27}H_{29}NO_4$) C, H, N.

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Penicillinase Inhibition†

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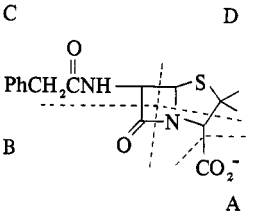
Penicillinase (EC 3.5.2.6), the enzyme catalyzing the hydrolysis of the β-lactam ring in penicillin, imparts resistance to the antimicrobial action of penicillin.[1] If a potent, yet safe, inhibitor of this enzyme could be found, it would be an effective adjunct in penicillin therapy against resistance common in many strains of staphylococci.

Many reports have dealt with penicillinase inhibition; notable among these are the early studies of Behrens and coworkers[2] and more recently those of Depue, *et al.*,[3] and Saz, *et al.*[4] Although these and other studies examined a wide range of compounds, no clinically significant inhibitors were uncovered. For this reason an analysis of the structural features important for penicillinase inhibition was undertaken.

The investigation of penicillinase inhibition described herein is directed toward delineation of the most important binding sites in the substrate, penicillin. Since penicillinase obtained from *Bacillus cereus* 569/H hydrolyzes benzylpenicillin, penicillin G was chosen as the model system for study. In Chart I, benzylpenicillin is dissected into regions

Chart I. Dissection of Benzylpenicillin into Possible Binding Sites



which might be expected to contribute to substrate binding.

Region A includes the carboxyl group. This moiety is included in all the compounds because (a) it is assumed to contribute substantially to the binding and (b) it imparts water solubility to all the test compounds.

Region B, the site at which hydrolysis actually occurs, comprises the lactam carbonyl group; the lactam nitrogen is not included in this region since it is not believed to

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