Stereochemical Studies on Medicinal Agents. 11.†,[1] Metabolism and Distribution of Prodine Isomers in Mice‡

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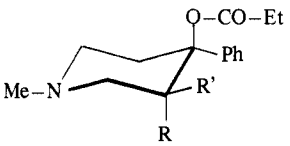
The metabolism and distribution of racemic and optically active prodines (**1**) and desmethylprodine (**2**) were investigated in mice. The (+)-antipodes of the analgetically more active prodines were demethylated more slowly *in vitro*. However, a significant difference between the *in vivo* rates of decline in plasma was observed only between β-prodine antipodes. It was found that O → N propionyl migration of the demethylated compounds (**4**, **8**, **13**) proceeds at a rapid rate at pH > 9.5 to yield the corresponding amides (**5**, **9**, **14**), and this was utilized as an analytical procedure to quantitate the amount of demethylation. Within 15 min after sc administration each isomer achieved steady-state brain/plasma ratios, suggesting rapid equilibration between these tissues. Ratios of >5 were attributed to the lipophilic nature of the prodines. The differences in affinities of the prodine isomers for plasma proteins are small. In no case were the differences in analgetic potency accounted for by distribution or metabolism. It is concluded that the large enantiomeric potency ratios for **1** are related primarily to stereoselective events at analgetic receptors.

Stereostructure-activity relationships of narcotic analgetics have been investigated extensively in attempts to probe the analgetic-receptor interaction.[3,4] The rationale for using chiral analgetics as receptor probes in such studies is based on the reasonable assumption that stereoisomers usually have very similar or identical partition coefficients and thus have near-equal access to the receptors. Although there is reason to believe this is true for antipodal analgetics,[5-8] this does not necessarily hold for diastereomers where differences in partition coefficients exist.[9]

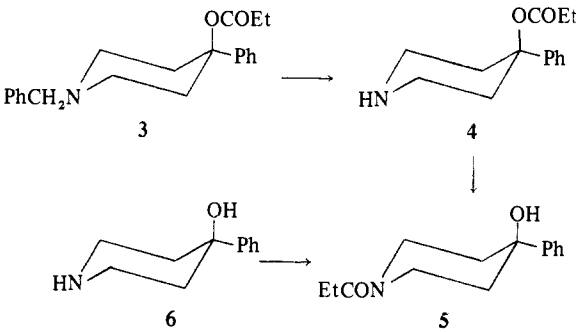
In connection with our stereochemical investigation of the optical isomers[10,11] of α- and β-prodine (**1a**, **1b**),[12] the distribution and metabolism of these compounds and the 3-desmethyl analog (**2**)[13] were studied in mice in order to determine whether any differences in their analgetic activities could be attributed to differential access to the receptors and/or to differences in metabolism.



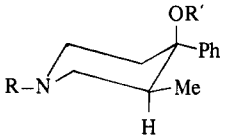
1a, R = H; R' = Me
1b, R = Me; R' = H
2, R = R' = H

Chemistry. The (±)-[^{3}H]prodine diastereomers (**1a**, **b**) and **2** were synthesized using [^{3}H]bromobenzene according to a modification of the method of Ziering and Lee.[12] Optically active α-[^{3}H]prodine (**1a**) and β-[^{3}H]prodine (**1b**) were prepared by fractional crystallization of the acid tartrate and acid dibenzoyltartrate salts[10,11] of the corresponding piperidinol diastereomers followed by esterification with propionyl chloride. Lability of ^{3}H was tested by heating the compounds with 4 *N* HCl and with 10% KOH. No exchange of tritium was detected.

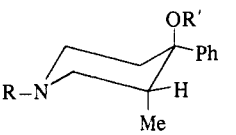
As demethylation might be of major importance in the metabolism of the prodines, synthesis of nor compounds **4**, **8**, and **13** was attempted. Catalytic hydrogenolysis of **3**·HCl afforded a product which showed an ester band at 1735 cm^{-1} as the only carbonyl chromophore. When the product (as the free base) was allowed to stand 24 hr at room temperature an additional absorption at 1640 cm^{-1} was observed, indicating amide formation. Work-up afforded amide **5** together with starting material and minor quantities of 4-phenylpiperidine. An improved yield of **5** was obtained when the hydrogenation reaction mixture was allowed to stand in aqueous base prior to work-up. The identity of **5** was confirmed by reaction of **6** with propionic anhydride. The formation of **5** is best explained by O → N acyl migration of **4** and similar migrations have been reported for propionoxy esters of other secondary amines.[14] Boggiano, *et al.*,[15] reported the presence of **5** among the products of catalytic hydrogenation of **3**·HCl, but were unable to reproduce this result.



Similar treatment of a mixture (**7**·HCl and **12**·HCl) yielded a mixture (9.5:1) of isomeric amides (**9**, **14**). Fractional crystallization afforded the trans isomer **9** whose properties agree with those of Casy[16] who obtained this compound through a different procedure. We were unable to effect purification of the cis isomer **14**, as repeated crystallization gave a 1:1 mixture of **9** and **14**.



7, R = $PhCH_2$; R^2 = COEt
8, R = H; R^1 = COEt
9, R = COEt; R^1 = H
10, R = H; R^1 = H
11, R = Me; R^1 = H



12, R = $PhCH_2$; R^1 = COEt
13, R = H; R^1 = COEt
14, R = COEt; R^1 = H
15, R = H; R^1 = H
16, R = Me; R^1 = H

In Vitro **Metabolism Studies.** The rates of N-demethylation of the racemates and optical isomers of prodine by mouse liver homogenate are summarized in Table I. The

†We gratefully acknowledge support of this work by Public Health Service Grant NS 05192. We wish to thank Hoffman-LaRoche, Inc. for a supply of (±)-α- and β-prodinol.

‡For a preliminary account of this work see ref 2.

Table I. Rates of *In Vitro* N-Demethylation for Prodine Isomers

Substrate[a]	V_{max}, μmole/g of liver per hr[b]	$K_m \times 10^5 M$[b]
(+)-α-Prodine·HCl	10.4 (±0.6)[c]	35.3 (±5.2)
(−)-α-Prodine·HCl	13.5 (±0.9)	15.4 (±2.9)
(±)-α-Prodine·HCl	14.1 (±1.1)	9.1 (±1.3)
(+)-β-Prodine·HCl	9.8 (±1.1)	33.0 (±4.0)
(−)-β-Prodine·HCl	18.1 (±2.0)	12.0 (±3.0)
(±)-β-Prodine·HCl	16.3 (±1.5)	7.7 (±1.5)
3-Desmethylprodine·HCl	14.0 (±1.7)	13.1 (±1.7)
Meperidine·HCl	8.8 (±1.4)	15.1 (±3.8)

[a] Substrate concentrations used were 0.336×10^{-4}, 0.672×10^{-4}, 1.344×10^{-4}, and $2.688 \times 10^{-4} M$. [b] The V_{max} and K_m values were determined by measuring the formaldehyde produced[17] at 15 min after incubation and calculated by the method of Wilkinson.[18] [c] Values in parentheses are standard errors.

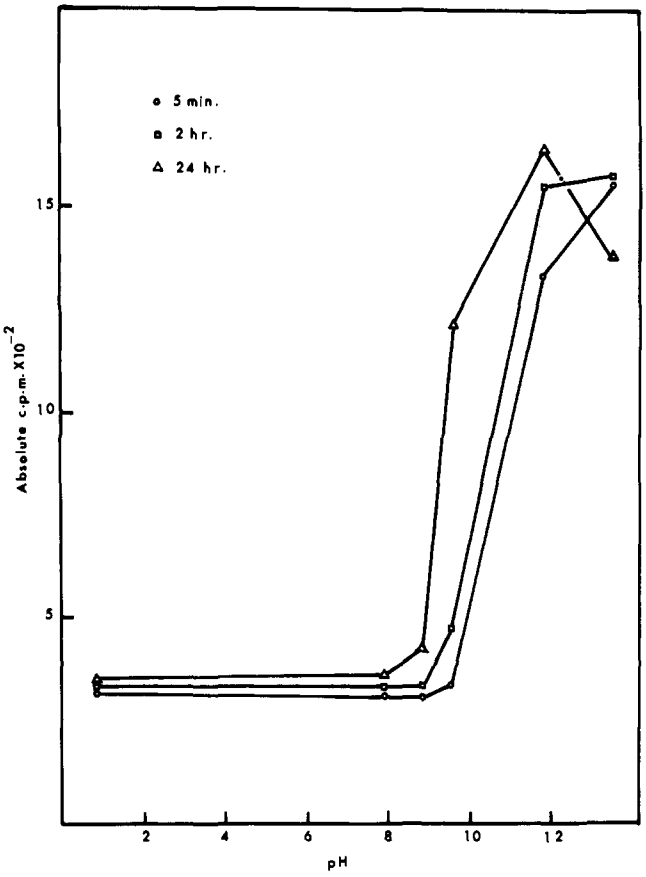


Figure 1. The effect of pH on the rearrangement of N-demethylated (±)-α-[^{3}H]prodine [(±)-[^{3}H]-**8**] produced by mouse liver homogenate. Radioactivity was identified as propionamide (±)-[^{3}H]-**9** and measured after incubation for 5 min (○), 2 hr (□), and 24 hr (△). Each point represents the mean of three experiments.

analgetically more potent (+)-antipodes[10,11] were demethylated significantly§ more slowly than the less potent (−) isomers. However, there was no significant difference in the rates of demethylation between racemates or between the racemates and 3-demethylprodine. It is of interest that, in contrast to our results, Axelrod[19] found that the analgetically more potent antipodes of morphinan derivatives and methadone were metabolized more rapidly.

In view of the finding that facile O → N migration occurs with the N-demethylated prodines, we were interested in characterizing the *in vitro* demethylation products. The total benzene-extractable radioactive material following 1-hr

§ All data in this paper were statistically evaluated at the 95% confidence level.

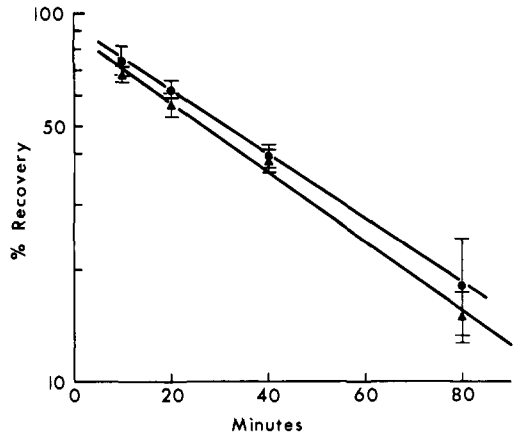


Figure 2. Recovery of unchanged drug from mouse homogenate after sc administration of 2.5 mg/kg of (±)-α-[^{3}H]prodine·HCl (○) and 0.7 mg/kg of (±)-β-[^{3}H]prodine·HCl (▲). Each point represents the mean observation on 3 animals ± the standard error.

incubation of (±)-α-[^{3}H]prodine with liver homogenate amounted to 89%. The nonbasic fraction contained radioactivity equivalent to 46% of the incubated substrate and on tlc possessed an R_f value identical with that of amide **9**. The basic fraction (43% of incubated substrate) showed tlc characteristics corresponding to substrate. When (±)-β-prodine was treated similarly, 81% of the total radioactivity was extracted; 36% as the unchanged drug and 45% as amide **14**.

Glc analysis further substantiated the identity of the neutral metabolites derived from **1a** and **1b**. In both cases only two peaks were observed; one corresponding to unchanged substrate and the other to the amide produced by O → N acyl migration of the N-demethylated substrate. None of the alcohols (**11**, **16**) or noralcohols (**10**, **15**) were detected.

As the extraction procedure in the preceding study involved strongly basic conditions, the pH at which facile propionyl migration occurs was evaluated. A kinetic-pH profile was obtained for the rearrangement of norester **8** obtained from the demethylation of **1a** by liver homogenate. At pH <9 virtually no amide **9** formation occurred, even after 24-hr incubation, while at pH ≥12 the rearrangement was complete within 5 min (Figure 1). The smaller amount of **9** present at 24 hr relative to that detected at earlier time periods (at pH 13) may be due to hydrolysis of **9**, as the secondary amine **10** produced under these conditions would not be extracted by benzene from acid solution. The results suggest that O → N acyl migration does not occur under the incubation conditions and is likely a result of the basic work-up procedure.

It is reasonable that O → N acyl migration takes place through the free base, and consequently a sharp acceleration in the rate of acyl migration would occur at a pH in the vicinity of the pK_a of 8. As pure **8** was not available, the pK_a of the corresponding alcohol **10** was determined. A pK_a of 9.4 is in the pH range where fast migration occurs and is consistent with the above assumption.

In order to ascertain whether acyl migration could be employed for the quantitative estimation of norester metabolite, the [^{3}H]prodine racemates were each incubated with liver homogenate and the amount of enzymatic N-demethylation was determined by assaying the formaldehyde produced and by measuring the radioactivity in the nonbasic fraction. There was no significant difference between demethylation rates determined by either method (Table II).

In Vivo **Metabolism.** The *in vivo* metabolism of (±)-α-

Table II. Rates of *In Vitro* N-Demethylation Determined by Measuring Formaldehyde and Propionamides (9, 14)

Substrate concn,[a] *M*	(±)-α-[^{3}H]Prodine·HCl		(±)-β-[^{3}H]Prodine·HCl	
	V^b (±SD)	V^c (±SD)	V^b (±SD)	V^c (±SD)
0.8×10^{-4}			0.909 (0.010)	0.912 (0.017)
1.6×10^{-4}	1.982 (0.092)	1.868 (0.002)	1.818 (0.042)	1.801 (0.024)
2.4×10^{-4}			2.526 (0.044)	2.488 (0.071)
3.2×10^{-4}	3.419 (0.154)	3.335 (0.045)	3.296 (0.05)	3.192 (0.066)

[a] Substrates were incubated for 15 min prior to analysis. [b] Velocity (μmole/g of liver per hr) determined by colorimetric assay[17] of formaldehyde. [c] Velocity (μmole/g of liver per hr) determined by measuring formation of propionamide as described in the Experimental Section.

Table III. Comparisons of the Analgesic ED_{50} Ratios with Brain Concentration Ratios

Prodine	(±)-α-	(+)-α-	(−)-α-	(±)-β-	(+)-β-	(−)-β-
3-Desmethyl-	1.74[a] (1.68–1.80)	1.17 (1.12–1.23)	25.47 (24.6–26.4)	2.24 (2.15–2.34)	2.95 (2.79–3.12)	3.56 (3.44–3.68)
	1.29[b] (1.04–1.72)	1.45 (1.17–1.86)	16.98 (13.7–21.4)	4.10 (2.74–8.28)	5.21 (3.86–7.08)	2.50 (1.48–4.35)
(−)-β-	2.04 (2.02–2.06)	4.17 (4.05–4.31)	7.16 (7.09–7.24)	7.97 (7.77–8.17)	10.50 (10.1–11.0)	
	1.94 (1.00–3.57)	3.62 (2.31–6.08)	6.79 (3.72–12.3)	10.26 (5.69–24.4)	13.1 (8.32–20.8)	
(+)-β-	6.73 (4.79–9.95)	2.52 (2.39–2.65)	78.2 (72.1–78.6)	1.32 (1.26–1.38)		
	5.14 (4.92–5.37)	3.60 (2.67–4.74)	88.5 (63.4–125)	1.27 (0.71–1.92)		
(±)-β-	3.90 (3.80–4.00)	1.91 (1.84–1.99)	57.1 (55.6–58.6)			
	5.30 (3.06–16.9)	2.83 (1.87–5.35)	69.7 (41.7–183)			
(−)-α-	14.63 (14.5–14.8)	29.9 (29.0–30.9)				
	13.2 (9.46–17.2)	24.6 (19.3–33.3)				
(+)-α-	2.04 (1.98–2.11)					
	1.87 (1.45–2.70)					

[a] Ratio of higher to lower brain levels (μmole/g) calculated at the ED_{50}, with 95% fudicial limits in parentheses. [b] Ratio of the greater to lesser analgesic ED_{50} (μmole) as determined at 15 min after sc administration[10,11] with 95% confidence limits in parentheses.

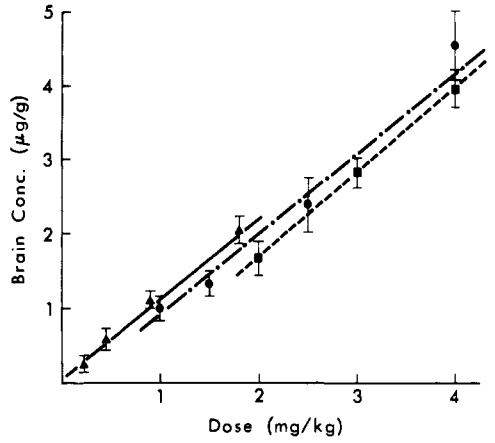


Figure 3. Brain levels at 15 min after sc administration of different doses of (±)-α-[^{3}H]prodine·HCl (●—·—·), (±)-β-[^{3}H]prodine·HCl (▲—), and 3-[^{3}H]desmethylprodine·HCl (■----). Each point represents the mean value of 5 animals ± the standard error.

(**1a**) and (±)-β-prodine (**1b**) was examined over a 120-min period. Mice were injected sc with equipotent doses of α- and β-prodine (2.5 mg/kg of **1a**·HCl or 0.7 mg/kg of **1b**·HCl) and were sacrificed at different time intervals. Equipotent doses were employed so that the compounds could be examined under conditions identical with those employed in analgetic testing. The whole animal plus any excretory products were homogenized and extracted with benzene. The balance of the injected dose not extracted with benzene was accounted for in the aqueous homogenate. Radioactivity of the basic fraction was determined by tlc to be unchanged drug. Figure 2 shows the per cent of injected radioactivity recovered as unchanged drug. The half-lives of racemic α- and β-prodine were 32–34 min and statistically indistinguishable.

Brain and Plasma Level Studies. Brain and plasma concentrations of [^{3}H]prodine isomers following sc administration in mice were examined as a function of dose and time. All of the compounds were found to exhibit reasonably good linear correlation between brain or plasma levels and injected dose, or between the log of the levels and time.

Table IV. Binding of Prodine Isomers to Mouse Plasma Protein

Isomer	% bound[a] (±SD)
(+)-α-Prodine·HCl	39.8 (±1.1)
(−)-α-Prodine·HCl	45.8 (±2.1)
(±)-α-Prodine·HCl	36.2 (±1.8)
(+)-β-Prodine·HCl	25.2 (±2.7)
(−)-β-Prodine·HCl	22.0 (±0.7)
(±)-β-Prodine·HCl	24.6 (±1.0)

[a] Average value for triplicate determinations at 0.25, 0.5, and 1.0 μg/ml.

The relationship between brain concentration and dose of the prodine racemates and 3-desmethylprodine is illustrated in Figure 3. At identical doses, all three compounds differ significantly in brain levels attained after 15 min, although the magnitudes of difference are not large. The analgetically more potent (±)-β-prodine gives rise to 12% higher brain concentrations than does (±)-α-prodine, which in turn achieves 11% higher levels than 3-desmethylprodine. Comparison of the ratio of ED_{50} values [(±)-**1a**/(±)-**1b**, (±)-**1a**/**2**, (±)-**1b**/**2**] with their brain level ratios (Table III) gives rise to a statistically significant difference only between (±)-β-prodine [(±)-**1b**] and 3-desmethylprodine (**2**). Thus the greater brain concentration of (±)-β-prodine at identical doses represents ~45% of the potency difference between the two compounds. However, the brain level ratios and ED_{50} ratios, (±)-**1a**/(±)-**1b** and (±)-**1a**/**2**, were not significantly different, and therefore the differences in brain levels between (±)-**1a** and (±)-**1b** or between (±)-**1a** and **2** do not statistically account for the actual potency differences. Interestingly, the prodine isomers differed in the extent of protein binding by ≤10% (Table IV), and it seems improbable that this could make a significant contribution to the observed differences in brain levels.

With regard to the optical antipodes, only the brain levels of (+)- and (−)-β-prodine differed significantly with respect to each other at identical doses 15 min after administration (Figure 4). This amounted to a 25% higher brain concentra-

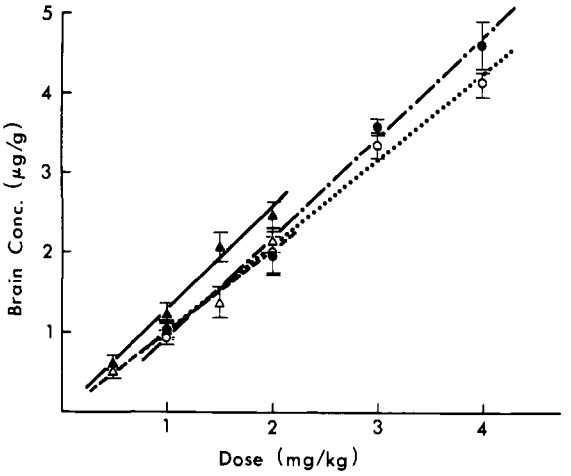


Figure 4. Relationship between brain concentration and dose of [^{3}H]prodine·HCl antipodes at 15 min after sc administration in mice [(+)-α-, ●—·—·; (−)-α-, ○·····; (+)-β-, ▲——; (−)-β-, △---]. Each point represents the mean observation of 5 animals ± the standard error.

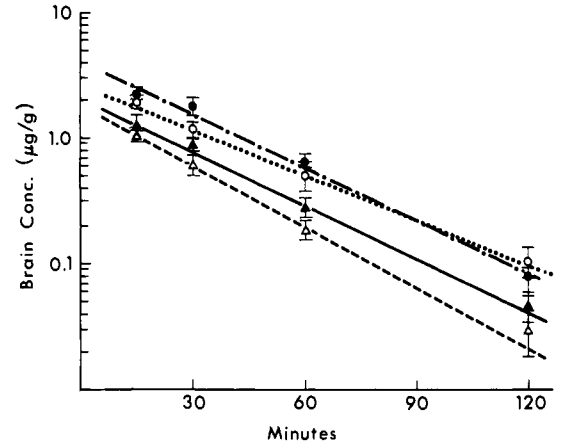


Figure 5. Decline of brain levels with time after sc administration of 2.0 mg/kg of α-[^{3}H]prodine·HCl antipodes [(+)-α-, ●—·—·; (−)-α-, ○·····] and 1.0 mg/kg of β-[^{3}H]prodine·HCl antipodes [(+)-β-, ▲——; (−)-β, △---] in mice. Each point is the mean observation on 5 animals ± the standard error.

tion for (+)-**1b**. However, principally due to the large variance associated with the ED_{50} values, the (+)-**1b**/(−)-**1b** ratio of brain levels calculated at their ED_{50} doses (Table III) was found not to differ significantly from their potency ratio. Hence, the possible contribution of the differences in brain levels to their potency difference is not supported statistically. In order to determine whether significant differences in brain levels between (+)- and (−)-**1b** were maintained at longer time intervals, their rates of decline were determined (Figure 5). Although the rates did not differ significantly with respect to each other, levels of (+)-**1b** were significantly greater than (−)-**1b** over the time period. This is in harmony with the observation (Figure 4) that levels of **1b** antipodes differed significantly from each other at different doses.

The linear regressions defining the relationship between dose and plasma levels of optical isomers 15 min after sc administration (Figure 6) indicate that the antipodes did not differ significantly although (+)-**1b** levels were consistently higher than those of (−)-**1b**. It is probable that a real difference between β-antipodes may have been overshadowed by a 5-fold greater intragroup variance of (−)-**1b**. Moreover, the time-course plasma levels for all optical isomers except (−)-**1b** were similar (Figure 7). The latter isomer differed

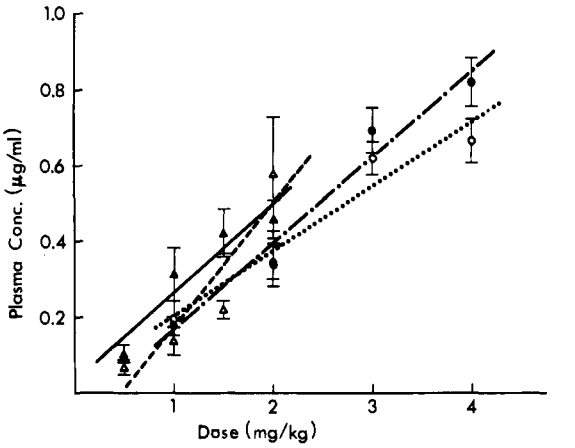


Figure 6. Relationship between plasma concentration and dose of [^{3}H]prodine·HCl antipodes at 15 min after sc administration in mice [(+)-α-, ●—·—·; (−)-α-, ○·····; (+)-β-, ▲——; (−)-β-, △———]. Each point represents the mean value from 5 animals ± the standard error.

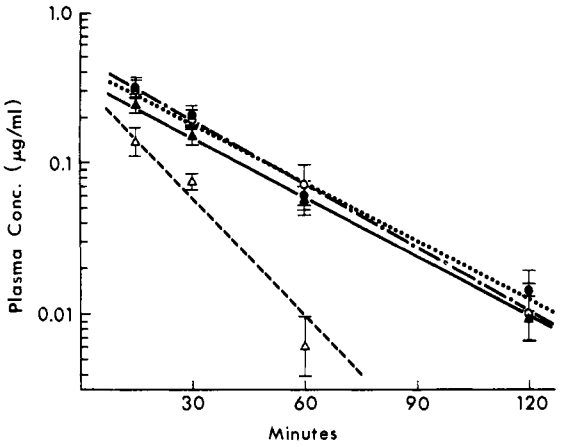


Figure 7. Decline of plasma levels with time after sc administration of 2.0 mg/kg of α-[^{3}H]prodine·antipodes [(+)-α-, ●—·—·; (−)-α-, ○·····] and 1.0 mg/kg of β-[^{3}H]prodine·HCl antipodes [(+)-β-, ▲——; (−)-β-, △———] in mice. Each point represents the mean observation on 5 animals ± the standard error.

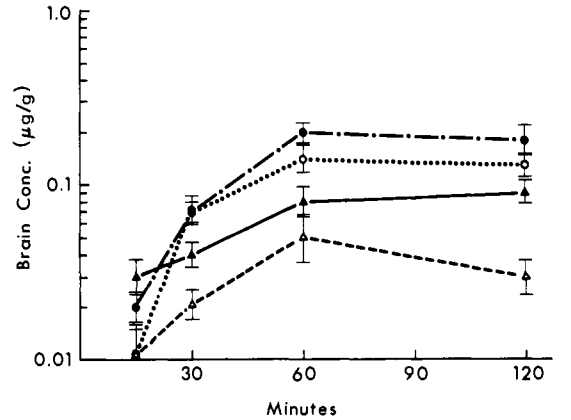


Figure 8. Time-course brain levels of *N*-desmethyl metabolites following sc administration of 2.0 mg/kg of α-[^{3}H]prodine·HCl antipodes [(+)-α-, ●—·—· and (−)-α-, ○·····] and 1.0 mg/kg of β-[^{3}H]prodine·HCl antipodes [(+)-β-, ▲— and (−)-β-, △———] in mice. Each point represents the mean observation on 5 animals ± the standard error.

both with respect to plasma levels and rate beyond the 15-min interval.

Peak brain levels of N-demethylated metabolites occurred at ≥60 min (Figure 8), which is after the time required for

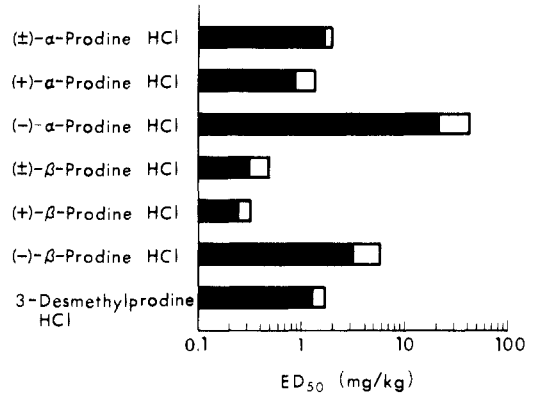


Figure 9. Comparison of the ED_{50} values of the prodine isomers as determined[10,11] 15 min (closed bar) and 30 min (open bar) after sc administration in mice.

Table V. Brain–Plasma Ratios of Prodine Enantiomers Following Subcutaneous Administration[a] in Mice

Isomer	Time, min	Ratio (95% limits)
(+)-α-Prodine·HCl	15	6.74 (5.61–8.40)[b]
	30	8.83 (6.10–12.23)
	60	10.47 (6.85–18.89)
	120	5.82 (3.11–27.22)
(−)-α-Prodine·HCl	15	6.42 (5.56–7.37)
	30	6.09 (5.29–7.08)
	60	6.87 (5.22–12.35)
	120	10.38 (6.38–22.02)
(+)-β-Prodine·HCl	15	5.25 (4.31–6.37)
	30	5.88 (4.51–7.65)
	60	4.83 (3.07–10.53)
	120	4.77 (2.18–96.2)
(−)-β-Prodine·HCl	15	7.58 (5.51–13.65)
	30	8.12 (5.51–11.21)

[a] Doses of (+)- and (−)-α-prodine·HCl, 2.0 mg/kg; (+)- and (−)-β-prodine·HCl, 1.0 mg/kg. [b] Fudicial limits calculated as described by Goldstein.[20]

peak biological activity (Figure 9). This suggests that the analgesic effect is due to the parent compound and not the metabolite.

It is noteworthy that brain/plasma ratios >5 were maintained throughout the time-course studies (Table V). Moreover, the ratios for each isomer at different time intervals did not differ significantly during this period, suggesting a rapid movement of drug between brain and plasma.

Conclusions

N-Demethylation is the primary metabolic pathway *in vitro* and *in vivo* for the prodine isomers in mice. Metabolites **4**, **8**, and **13** underwent facile O → N acyl migration in base to form amide alcohols **5**, **9**, and **14**, respectively. However, this rearrangement did not take place under physiological conditions, as a pH ≥9.5 was required for this reaction to proceed at a reasonable rate at 37°. The possibility that the prodines and their N-demethylated metabolites were hydrolyzed to the corresponding alcohols (**10**, **11**, **15**, and **16**) was investigated, but none of these potential metabolites were detected.

All of the prodine isomers exhibited first-order decay curves for brain and plasma levels during the 15- to 120-min interval following sc administration. This indicated that peak brain and plasma levels occurred prior to 15 min. By contrast, peak levels of the N-demethylated metabolite in brain and plasma were found at ≥60 min. Since the ED_{50} values determined at 15 min were greater than those at 30 min, it seems reasonable that analgetic activity resides with the parent compounds rather than with the major metabolites.

Within 15 min after sc administration each isomer achieved steady-state brain/plasma ratios, suggesting a rapid equilibration between brain and plasma. The rapid entry into brain and the large brain/plasma ratios (>5) for the prodines may be attributed to their lipophilic nature (octanol–pH 7.4 partition coefficient for each diastereomer is ~10), as it has been reported[9,21] that there is correlation between partition coefficient and the brain/plasma ratio.

In no cases were the differences in analgetic potency between isomers fully accounted for by metabolism or distribution. There were small but statistically significant differences between brain levels of racemic, desmethyl, and antipodal [(+)-**1b**, (−)-**1b**] prodines at identical doses, but only in the case of (±)-**1b** and **2** were the brain and potency ratios [(±)-**1b**/**2**] significantly different.

The (−)-prodine diastereomers were metabolized *in vitro* at significantly faster rates than the corresponding (+) isomers, but a significant difference between the rates of decline in plasma (*in vivo*) was observed only between β-prodine antipodes. This apparently is related to there being a greater difference in the rate of *in vitro* N-demethylation for the optical isomers in the β series (Table I).

It is likely that plasma binding does not play an important role in promoting stereoselective access of these analgetics into the CNS, as the difference in affinities of the prodine isomers for plasma proteins are relatively small.

The present study suggests that the large enantiomeric potency ratios [(+)/(−)] for α- and β-prodine (23 and 12, respectively) are related primarily to stereoselective events at receptors in the CNS and not to differences in distribution and metabolism. These findings support the view[3,4] that the use of antipodal ligands as probes for exploring analgetic receptors circumvents many of the complications associated with structure–activity relationship studies.

Experimental Section#

(±)-α- and β-[^{3}H]Prodine·HCl [(±)-[^{3}H]-**1a**·HCl, **1b**·HCl]. Randomly labeled [^{3}H]bromobenzene (0.22 m*M*, 100 mCi/mmole) was mixed with freshly distd bromobenzene (0.65 g) and reacted with Li (0.1 g) in 50 ml of dry Et_2O under N_2 to afford [^{3}H]PhLi. The latter was reacted with 1,3-dimethyl-4-piperidone (0.51 g) to produce a mixt of (±)-[^{3}H]-**11** and (±)-[^{3}H]-**16** (0.42 g, sp act. 5.13 mCi/mmole). The alcohol (0.2 g) was added to pyridine (10 ml), treated with propionyl chloride (2 ml), and heated at 100° under N_2 for 12 hr to afford a crude mixt of labeled **1a** and **1b**. The crude product was mixed with cold **1a** (0.2 g) and **1b** (0.15 g) and chromatographed on a column of neutral alumina (Bio-Rad AG7, mesh 100–200) using anhyd Et_2O; yields, 0.33 g of (±)-**1a**·HCl (sp act. 2.67 mCi/mmole) and 0.155 g of (±)-**1b**·HCl (sp act. 0.39 mCi/mmole). The identity and radiochemical purity of the prodines were determined by tlc on alumina (EtOAc).

(±)-α-[^{3}H]Prodinol [(±)-[^{3}H]-**11**]. A mixt of (±)-[^{3}H]-**11** and (±)-[^{3}H]-**16** (0.22 g, sp act. 5.13 mCi/mmole) was recrystd (cyclohexane) to afford (±)-[^{3}H]-**11** (0.13 g) containing a trace of (±)-[^{3}H]-**16** as determined by tlc (alumina, Et_2O).

(±)-β-[^{3}H]Prodinol [(±)-[^{3}H]-**16**]. A soln of (±)-[^{3}H]-**1b** (0.150 g, 0.505 mmole) in 2.8% ethanolic KOH (3 ml) was refluxed under

#Melting points were determined in open capillary tubes with a Thomas-Hoover apparatus and are uncorrected. Microanalyses were performed by M-H-W Laboratories, Garden City, Mich. Radioactivity was measured with a Packard 3375 liquid scintillation spectrometer. Glc analysis was carried out on a Perkin-Elmer 900 instrument equipped with a flame ionization detector and a 0.25 × 72 in. column packed with 3% OV-17 on chromosorb W (80–100 mesh) using N_2 carrier gas. Nmr spectra were obtained in $CDCl_3$ with a Varian A-60D spectrometer using TMS as internal standard. The ir and nmr data of all compounds were consistent with the proposed structures.

N_2 for 23 hr, cooled, diluted with H_2O (5 ml), and the EtOH removed. The aqueous suspension was saturated with K_2CO_3, extd ($CHCl_3$), and washed (H_2O), and the $CHCl_3$ phase dried ($MgSO_4$). Evaporation of solvent afforded (±)-[^{3}H]-16 (0.101 g, 0.49 mmole).

(+)- and (−)-α-[^{3}H]Prodine·HCl [(+)-[^{3}H]-1a·HCl, (−)-[^{3}H]-1a·HCl]. (±)-α-[^{3}H]Prodinol (11) (0.10 g, 4.9 mmoles) in 10 ml of acetone was added to a soln of 0.74 g (4.95 mmoles) of (+)-tartaric acid in 1 ml of MeOH. The soln was filtered, diluted to 16 ml with acetone, seeded with (+)-11 (+)-acid tartrate,[10,11] and allowed to stand at room temp for 6 days. The isolated salt (0.068 g) was recrystd from MeOH–acetone (7:93) to give 0.042 g of (+)-[^{3}H]-11 (+) acid tartrate; mp 159–160°, [α]D +13° (*c* 1, H_2O). Treatment of this acid tartrate salt with NH_3, extraction ($CHCl_3$), drying ($MgSO_4$), and removal of $CHCl_3$ afforded 0.038 g of (+)-[^{3}H]-11. Labeled and unlabeled (0.040 g) (+)-11 were dissolved in 1.2 ml of toluene and refluxed under N_2 with 0.2 g of propionyl chloride for 5.5 hr. The cooled reaction mixt was diluted with Et_2O and extracted with 1 *N* HCl. The amine base was liberated with NH_3, partitioned into $CHCl_3$, and dried ($MgSO_4$), and the soln was concd to a small vol. Treatment with Et_2O–HCl and removal of Et_2O gave a residue which crystd from EtOH–Et_2O on standing (room temp) to yield 0.022 g of (+)-[^{3}H]-1a·HCl; recryst, mp 190–191°, [α]D +35.3° (*c* 1.1, saline), sp act. 1.33 mCi/mmole.

The solvent was removed from the mother liquor of the resolution of (+)-11·(+)-acid tartrate, and the residue was treated with aqueous NH_3. The liberated base was partitioned into $CHCl_3$ and dried ($MgSO_4$), and the solvent was removed. The residue was dissolved in 5 ml of acetone and added to a methanolic soln (0.7 ml) of (−)-tartaric acid (0.045 g, 0.3 mmole). Acetone was added to a total vol of 9.8 ml and the soln seeded with unlabeled (−)-11·(−)-acid tartrate.[10,11] After several days at room temp, 0.067 g of (−)-[^{3}H]-11·(−)-acid tartrate was isolated and recryst from MeOH–acetone (7:93); mp 156–157°, [α]D −12.7° (*c* 1, H_2O). The salt was converted to 0.026 g of (−)-[^{3}H]-11 by the procedure employed for the (+) isomer. Upon dilution with 0.26 g of unlabeled (−)-11 followed by the propionylation procedure described above, there was obtained 0.010 g of (−)-[^{3}H]-1a·HCl; mp 194–195°, [α]D −37.5° (*c* 0.74, saline), sp act. 2.35 mCi/mmole. Both labeled antipodes were identical in all respects with unlabeled material.[10,11]

(+)- and (−)-β-[^{3}H]Prodine·HCl [(+)-[^{3}H]-1b·HCl, (−)-[^{3}H]-1b·HCl]. (±)-β-[^{3}H]Prodinol (16) (0.101 g, 0.49 mmole) was dissolved in 2 ml of hot MeOH and treated with 0.185 g (0.49 mmole) of (−)-dibenzoyltartaric acid followed by 0.5 ml of H_2O. The resulting soln was seeded with the unlabeled (−)-dibenzoyltartrate salt of (+)-16,[10,11] and after standing several days at room temp there was obtained 0.062 g of (+)-[^{3}H]-16·(−)-dibenzoyltartrate; mp 162–163°, [α]D −45.0° (*c* 1, MeOH). The free base was generated by treating a MeOH soln of the salt with aqueous NaOH followed by $CHCl_3$ extraction and removal of solvent. The residue was refluxed in a mixt of toluene (1 ml) and propionyl chloride (0.2 ml) for 4.5 hr. Extn with 1 *N* HCl (5 ml), treatment with NH_3, extn with $CHCl_3$, drying ($MgSO_4$), and removal of $CHCl_3$ gave the base which was converted to (+)-[^{3}H]-1b·HCl; mp 185–186° (after cryst from Et_2O and drying at 80 *in vacuo*), [α]D +66.8° (*c* 1.3, saline), sp act. 0.42 mCi/mmole.

The free base from the above resolution mother liquor was regenerated with aqueous NaOH, partitioned into $CHCl_3$, and the solvent removed. The residue was mixed with 0.110 g of (+)-dibenzoyltartaric acid in hot MeOH (1 ml) followed by addn of H_2O (0.3 ml). After standing 5 days at room temp, (−)-[^{3}H]-16·(+)-dibenzoyltartrate was collected and air-dried; mp 168–169°, [α]D +43.6° (*c* 1, MeOH). Treatment of this salt with aqueous NaOH, extraction ($CHCl_3$), and removal of solvent, yielded (−)-[^{3}H]-16 (0.020 g) which was refluxed in a mixt of toluene (1 ml) and propionyl chloride (0.2 ml) under N_2 for 5 hr. After removal of solvent, crystallization (Et_2O), and drying (*in vacuo*) there was obtained 0.012 g of (−)-[^{3}H]-1b·HCl; mp 190–191°, [α]D −71.0° (*c* 0.98, saline), sp act. 0.45 mCi/mmole. Both antipodes were identical in all respects with unlabeled material.[10,11]

3-[^{3}H]Desmethylprodine·HCl (3·HCl). Randomly labeled [^{3}H]bromobenzene (0.03 mmole, sp act. 100 mCi/mmole) was mixed with freshly distd bromobenzene (0.344 g) and reacted with Li (0.05 g) in 50 ml of dry Et_2O under N_2 to afford [^{3}H]PhLi. The latter was treated with 1-methyl-4-piperidone (0.228 g) and the reaction mixt was treated with H_2O (10 ml). The ether phase was separated and the solvent was removed under reduced pressure. The residue was dissolved in pyridine (10 ml), treated with propionyl chloride (2 ml), heated at 100° under N_2 for 12 hr, and diluted with H_2O (50 ml). The solvents were removed and the residue was dissolved in 1 *N* HCl (50 ml) and extracted with Et_2O. The aqueous phase was basified and extracted with successive portions of Et_2O. The combined Et_2O extracts were evapd under reduced pressure to afford a dark brown residue which was mixed with 50 mg of 3-desmethylprodine and dissolved in EtOAc (5 ml). Column chromatography on Bio-Rad Ag 10 basic alumina (EtOAc) yielded (after conversion to the HCl salt) 0.080 g of 3·HCl (sp act. 0.61 μCi/mmole). The identity and radiochemical purity of the product was determined by tlc on alumina and silica gel using EtOAc and EtOH, respectively.

1-Propionyl-4-phenylpiperidin-4-ol (5). (a) 4-Phenylpiperidin-4-ol (6) (0.35 g) was treated with a mixture of propionic anhydride (5 ml) and pyridine (5 ml) at 0° for 12 hr, poured on crushed ice, and extracted ($CHCl_3$), and the $CHCl_3$ was removed under reduced pressure. The residue was treated with MeOH (40 ml) and 10% aqueous K_2CO_3 (10 ml) for 24 hr, concd to remove MeOH, and extd with $CHCl_3$. Removal of $CHCl_3$ afforded 0.32 g of **5**, mp 172.5–174° after recrystn (EtOAc); reptd mp 173–174°.[15]

(b) 1-Benzyl-4-phenyl-4-propionoxypiperidine·HCl (3·HCl) (0.8 g) was dissolved in 95% EtOH (50 ml), treated with glacial AcOH (1 ml), and shaken with 5% Pd/C (0.2 g) under 40 psi of H_2 until the theoretical amt of H_2 was absorbed. The mixt was filtered and the solvent removed under reduced pressure. The residue was dissolved in H_2O (20 ml), basified by the addn of 2 *M* $KHCO_3$ soln, and immediately extd with $CHCl_3$. The $CHCl_3$ soln showed a band at 1735 cm^{-1} and no absorption at 1640 cm^{-1}. The soln was allowed to stand for 24 hr, whereupon strong absorptions at 1735 and 1640 cm^{-1} were observed. The $CHCl_3$ was removed and the residue was crystd (EtOAc) to afford **5**, mp 172–174°.

1-Propionyl-3-methyl-4-phenylpiperidin-4-ol (9, 14). A mixture of 7·HCl and 12·HCl (2.0 g) was dissolved in 95% EtOH (100 ml), treated with glacial AcOH (2 ml), and shaken with 5% Pd/C (0.5 g) at 40 psi of H_2 until the theoretical amount of H_2 was absorbed. The mixt was filtered and the solvent was removed under reduced pressure. The residue was treated with 5 *N* NaOH for 5 min, acidified with 5 *N* HCl, and extd with Et_2O. Removal of Et_2O afforded 0.82 g of a mixt of 9 and 14 in a ratio of 9.5:1 as shown by glc. Recrystn (cyclohexane–EtOAc) afforded 0.45 g of 9, mp 163.5–164.5° (reptd, 167°).[16] *Anal.* ($C_{15}H_{21}NO_2$) C, H, N.

Concn of the mother liquor from the previous crystn afforded an additional 0.12 g of 9. Removal of solvent afforded material which was crystd (cyclohexane) to give a mixt of 9 and 14 in a 1.1:1.0 ratio as shown by glc. *Anal.* ($C_{15}H_{21}NO_2$) C, H, N.

***In Vitro* Studies.** Adult male Swiss-Webster mice (25–30 g) were sacrificed by decapitation. The livers were immediately removed, washed with ice-cold 1.15% KCl, weighed, and homogenized in 3 volumes of ice-cold 1.15% KCl. The homogenate was centrifuged at 9000*g* (0–4°) for 30 min, and the supernatant was used as the source of metabolizing enzymes and NADPH-generating system. The incubation mixture (adjusted to pH 7.4) contained in 4 ml: 1 ml of the supernatant fraction (250 mg of liver), nicotinamide (20 μmoles), $MgCl_2·6H_2O$ (10 μmoles), semicarbazide·HCl (37.5 μmoles), NADP (2 μmoles), glucose 6-phosphate (20 μmoles), and Na_2HPO_4 (200 μmoles). The substrate to be studied was dissolved in 1 ml of KCl solution and added to the incubation mixture. Incubations were carried out in air at 37° in a Eberbach water bath shaker for the specified time and the reactions were stopped by the addition of 1 ml of 70% $HClO_4$. The formaldehyde produced was determined by the method of Cochin and Axelrod.[17] Alternatively, the reaction mixt was used for extraction of the metabolites as described under "extraction procedure" and "analysis of extracts."

***In Vivo* Studies.** Adult male Swiss-Webster mice (25–30 g) were injected sc with aqueous solns of the HCl salts of the labeled drugs in saline and placed in separate 1-l. beakers. The mice were stunned with a blow on the head at various times after injection, thoroughly homogenized together with any excretory products in a Waring blender, and then were strained through muslin. Sufficient 0.1 *N* HCl was added to give a total volume of 250 ml, and an aliquot of the homogenate was used for the determination of its radioactive contents using the methods described under "extraction procedure" and "analysis of extracts."

Extraction Procedure. The sample was adjusted to pH > 12, allowed to stand for 5 min, and mixed with twice its volume of C_6H_6. After shaking and centrifugation, an aliquot of the C_6H_6 phase was mixed with 0.1 *N* HCl, shaken, and centrifuged. The C_6H_6 phase after washing with 0.1 *N* HCl was used for analysis of "nonbasic fraction," The acidic phase was washed with C_6H_6, basified with NaOH, and extracted with C_6H_6. The C_6H_6 phase was used for the analysis of the "basic fraction."

Analysis of Extracts. The C_6H_6 soln to be counted was transferred to a glass counting vial and mixed with 10 ml of liquid scintillation cocktail consisting of 5.0 g of PPO and 0.5 g of dimethyl

POPOP in a liter of toluene. Controls with different known concentrations of the labeled drug were run concurrently with the experiment to serve as a check on the overall technique. All samples were counted for sufficient time to yield <2.5% error.

Extracts to be examined by glc were concentrated under reduced pressure and the residues were dissolved in acetone. The analysis was performed on a 6 ft × 0.25 in. od column packed with 3% OV-17 on chromosorb W (80–100 mesh) at 180–220° using N_2 as carrier gas.

Tlc was performed on basic alumina sheets (Eastman chromagram 6062) in one and two dimensions using EtOAc or Et_2O as solvent. The samples were mixed with authentic samples of nonlabeled carriers and applied to the plates. The spots were visualized with iodine reagent (0.5% I_2 in $CHCl_3$). The plates were divided into strips and the radioactivity on each strip was determined by scraping the adsorbent into a counting vial, adding toluene scintillation cocktail, and counting directly.

Brain and Plasma Levels. Adult male Swiss-Webster mice (25–30 g) were decapitated and exsanguinated at various times after sc administration of various doses of the labeled compounds. Blood was collected in beakers containing heparin sodium powder, transferred to tubes, and immediately centrifuged. Brains were removed immediately after decapitation, rinsed quickly with 3–5 ml of normal saline, blotted dry, and weighed. The weighed brains were then transferred to 15-ml glass homogenizer vessels, mixed with sufficient 0.01 *N* HCl to make a total volume of 5 ml, and homogenized. An aliquot of brain homogenate or plasma was adjusted to pH > 12, mixed with twice its volume of benzene, shaken, and centrifuged. An aliquot of the C_6H_6 phase was mixed with 0.1 *N* HCl, shaken, and centrifuged. The C_6H_6 phase, after washing with 0.1 *N* HCl, was used for analysis of the N-demethylated metabolite. The aqueous acid phase was washed with C_6H_6, basified, and extracted with C_6H_6. The C_6H_6 phase was used for the analysis of the unchanged drug. Controls with different known concentrations of the labeled drug were run concurrently to serve as a check on the overall technique, and a straight line was obtained when absolute counts per min were plotted against concentration. Percentage recoveries of labeled drugs were 90–95% from brains and plasma. The identity of the radioactive material in the benzene extracts was determined by tlc on basic alumina (EtOAc or Et_2O as solvents).

Plasma Protein Binding Studies. The per cent bound to mouse plasma protein was determined by equilibrium dialysis. Blood collected from 8–10 Swiss-Webster male mice was centrifuged at 9000*g* for 15 min. The plasma (0.4 ml) was placed inside a 0.5 × 3 cm tube of hydrated cellulose dialysis membrane which was stoppered at both ends with glass plugs. The filled tube then was placed in a test tube containing a sufficient vol of [^{3}H]prodine isomer in isotonic pH 7.4 phosphate buffer to make the internal and external liquid levels equal. The range of concentrations (0.25, 0.5, 1.0 μg/ml) of each [^{3}H]proiline isomer was in the vicinity of the peak plasma levels found *in vivo* at their ED_{50} doses. The dialysis assembly was immersed in a water bath (30°) which was agitated by an eccentric rotor for 15 hr (twice the time required for equilibrium). Dialysis at each concentration was performed in duplicate or triplicate. Analyses were carried out on 250-μl aliquots of the buffer and plasma phases by liquid scintillation spectrometry. Verification (tlc) of the radiolabeled material after dialysis indicated the absence of ester hydrolysis products. The per cent of protein-bound [^{3}H]prodines was calcd from the difference between the concentrations on the two sides of the dialysis membrane.

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Synthesis of α-[*p*-(Fluoren-9-ylidenemethyl)phenyl]-2-piperidineethanol, an Inhibitor of Platelet Aggregation

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Inhibition of blood platelet aggregation has been demonstrated among a number of pyridine- and piperidineethanols of benzyl- and benzylidenefluorene **1-15**. In preparation for clinical evaluation of some of these compounds, one of the diastereoisomers of α-[*p*-(fluoren-9-ylidenemethyl)phenyl]-2-piperidineethanol (**5**) was selected for further evaluation as an inhibitor of aggregation of human blood platelets. An improved synthesis of **5** is reported in which a novel Mannich-type reaction of 2,3,4,5-tetrahydropyridine trimer (**25**) and the complex **24** of 4′-(fluoren-9-ylidenemethyl)acetophenone (**19**) with magnesium methyl carbonate in dimethylformamide to give **6** is used.

As an extension of our work on pyridine- and piperidineethanols and Me ketones in the triphenylethane and triphenylethylene series,[1] we prepared **1** through **15** containing the benzyl- and benzylidenefluorene moiety.[2] Relatively early during the biological evaluation, **5** was found to prolong whole-blood clotting time in rats. It was subsequently found that at a concentration of 30 μg/ml **5** inhibits *in vitro* platelet aggregation (human citrated blood) induced by adenosine diphosphate (ADP), collagen, or thrombin. Compound **5** also inhibited clot retraction.[3,4] Still later, **5** was shown to antagonize ADP-induced thrombocytopenia in guinea pigs *in vivo* at 30 mg/kg po.[5] In this system, the two diastereoiso-