electron density; these two effects would provide a stronger hydrogen bond. Since hydrogen bonding tends to stabilize the conformation of a receptor, it would then compensate the effect caused by the hydrophobic interaction of the alkylene group; consequently, less stimulation would be observed.

Acknowledgment.—The authors express their thanks to Dr. Alan Burkhalter, Department of Pharmacology, University of California Medical Center, for helpful suggestions and to Dr. Corwin Hansch, Department of Chemistry, Pomona College, for providing the log P values of the phenylurea derivatives.

Stereochemical Studies on Medicinal Agents. VI.[1] Bicyclic Bases.[2] Synthesis and Pharmacology of Epimeric Bridged Analogs of Meperidine, 2-Methyl-5-phenyl-5-carbethoxy-2-azabicyclo[2.2.1]heptane[3]

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Epimeric 2-methyl-5-phenyl-5-carbethoxy-2-azabicyclo[2.2.1]heptanes were elaborated from N,O,O-tritosylhydroxyprolinol, and the stereochemistry was determined from physicochemical studies. The benzoquinone-induced writhing test indicated the *endo*-phenyl epimer to be twice as potent as meperidine and six times more potent than the *exo* isomer. In terms of brain concentrations, however, the *endo*–*exo* potency ratio is 3.7. Evidence is presented which quantitatively relates difference in brain levels between the epimers to their partition coefficients. The large difference in geometry between the *exo* and *endo* epimers suggests that their comparable activities are due to differing modes of interaction with analgetic receptors.

The importance of steric factors on the action of strong analgetics has received considerable attention.[4] Most of the research in this area has been focused on the relationship between absolute stereochemistry of conformationally mobile compounds and analgetic activity. While it is generally believed that differences in potencies which are observed with enantiomeric analgetics are a consequence of events at the receptor level, the role of conformational factors in influencing analgetic activity has remained controversial.[4,5]

The recognition[6] of the structural relationship between morphine and meperidine led to the postulate[7] that the axial-phenyl conformer of meperidine would be expected to "fit" the receptor surface better than the equatorial conformer. This conclusion was based on the known conformation[8] of the phenylpiperidine moiety in morphine, which contains an aromatic group that is held rigidly in an axial position relative to the piperidine ring.

Evidence consistent with this view[7] was obtained from the stereostructure–activity relationship of prodine diastereomers. As it was known[9] that β-prodine is more potent than the α isomer, it was concluded that this was because the conformational equilibrium for the former analgetic favors more of the axial species than the latter.

On the other hand, the work of Ziering, *et al.*,[9] suggested that the steric relationship of the phenyl group with respect to the piperidine ring is of minimal importance. This was supported by the fact that the 3-ethyl analogs show a reversal in the potency ratio when compared to the prodines. Since modification of the 3 substituent from methyl to ethyl to allyl should cause only minor changes in the conformational equilibria, this suggests that other overriding factors are responsible for the differences in potency between *cis* and *trans* racemates in the prodine series. This is supported further by a recent study[10] on the brain concentrations of α- and β-prodine in rats. It has been found that higher brain levels of β-prodine fully account for the observed difference in potency[9,11] between the α and β isomers.

Analysis of the stereostructure–activity relationship of other conformationally mobile, prodine-type[12–14] analgetics also suggested[4] that both equatorial- and axial-phenyl conformations have the ability to produce comparable analgetic activity.

The question of the identity of a favorable pharmacophoric conformation for the piperidine ring in 4-phenylpiperidine analgetics also remains unanswered. The work of Bell and Archer[15,16] on the analgetically active tropane analog (**1**) of meperidine suggests that this compound may exist primarily in the boat conformation.

Although the above analyses suggest that the conformational requirements of the aromatic group in 4-

(1) Previous paper: P. S. Portoghese, T. L. Pazdernik, W. L. Kuhn, G. Hite, and A. Shafi'ee, *J. Med. Chem.*, **11**, 12 (1968).

(2) Previous paper: P. S. Portoghese and A. A. Mikhail, *J. Org. Chem.*, **31**, 1059 (1966).

(3) (a) A preliminary report of some of this material was reported by P. S. Portoghese, H. J. Kupferberg, and A. A. Mikhail, *Pharmacologist*, **8**, 191 (1966). (b) Presented before the symposium on Newer Analgetics and Narcotic Antagonists of the Medicinal Chemistry Section, 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., April 9–14, 1967.

(4) P. S. Portoghese, *J. Pharm. Sci.*, **55**, 865 (1966), and references cited therein.

(5) P. S. Portoghese, *J. Med. Chem.*, **8**, 609 (1965).

(6) O. Schaumann, *Arch. Exptl. Pathol. Pharmakol.*, **196**, 109 (1940).

(7) A. H. Beckett and A. F. Casy in "Progress in Medicinal Chemistry," Vol. 4, G. P. Ellis and G. B. West, Ed., Butterworth and Co. Ltd., 1965, p 171, and references cited therein.

(8) M. Mackay and D. C. Hodgkin, *J. Chem. Soc.*, 3261 (1955).

(9) A. Ziering, A. Motchane, and J. Lee, *J. Org. Chem.*, **22**, 1521 (1957).

(10) H. J. Kupferberg and P. S. Portoghese, unpublished data.

(11) N. B. Eddy, H. Halbach, and O. J. Braenden, *Bull. World Health Organ.*, **14**, 363 (1956).

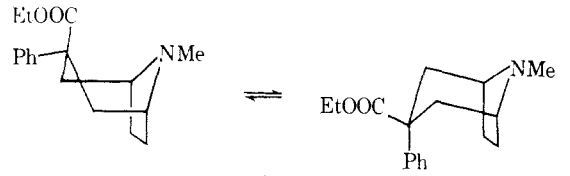
(12) I. N. Nazarov, N. S. Postakov, and N. I. Shvetson, *Zh. Obshch. Khim.*, **26**, 2798 (1956).

(13) N. S. Postakov, B. E. Zaitsev, N. M. Mikhailova, and N. N. Mikheeva, *ibid.*, **34**, 463 (1964).

(14) O. I. Sorokin, *Izv. Akad. Nauk SSSR*, 460 (1961).

(15) M. R. Bell and S. Archer, *J. Am. Chem. Soc.*, **82**, 4638 (1960).

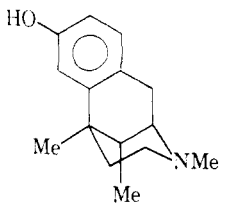
(16) M. R. Bell and S. Archer, *ibid.*, **82**, 151 (1960).



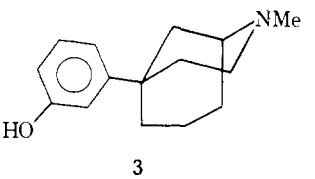
1

phenylpiperidine analgetics are minimal, it is conceivable that a preferred ground-state conformation could be transformed into an energetically unfavorable conformation on interacting with a receptor. For this reason, molecules having structural features which impart conformational homogeneity would be of value in determining whether 4-phenylpiperidines are capable of exerting their analgetic activity in more than one conformation.

May[17,18] has synthesized two structures (**2** and **3**) having the aromatic groups fixed in equatorial and in axial positions. Benzomorphan **2** is conformationally homogeneous by virtue of the methylene bridge which connects the axial aromatic group to the piperidine ring. The equatorial counterpart (**3**) owes its conformational restriction to the presence of the trimethylene bridge which prevents conformational inversion. These compounds are potent analgetics with comparable activity.[17-19] However, since the conformationally restricted compounds (**2** and **3**) which are being compared have skeletal differences, it could be argued that this is not a valid comparison.

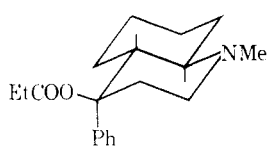


2

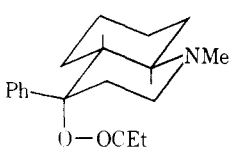


3

Recently, Smissman and Steinman[20] have attempted to shed light on this problem by synthesizing two epimeric, conformationally restricted prodine analogs (**4a**, **b**) and have found them to be equipotent on the basis of subcutaneous ED_{50} doses. It was suggested that no definitive conformational requirements of the phenyl group are required for analgetic action.

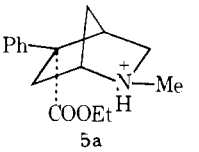


4a

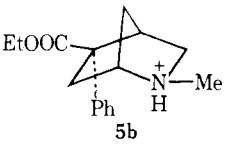


4b

All of the studies and correlations regarding the relationship between conformation and analgetic potency reported thus far are based on the assumption that diastereomeric compounds give comparable brain levels upon administration of equal subcutaneous doses. It is quite possible, as was found[10] with α- and β-prodine, that the brain levels of diastereomeric compounds may not be the same because of differences in physical properties. This would mean that the ratio of the subcutaneous ED_{50} doses for two diastereomers is not necessarily the same as the potency ratio based on brain concentration. To investigate this problem and the effects of conformation on analgetic potency we have prepared and tested diastereomeric, conformationally rigid,[21] meperidine analogs (**5a**, **b**) and have measured their brain levels in mice.



5a



5b

Chemistry.—Our approach to the synthesis of **5a** and **5b** involved the reaction of N,O,O-tritosylhydroxyprolinol[2] (**6**) with phenylacetonitrile anion. It was envisaged that displacement of the primary tosyloxy group of **6** by this anion would afford intermediate **7** which would subsequently undergo cyclization to **8** by internal S_N2 displacement of the secondary tosyloxy substituent. According to our proposed scheme for the elaboration of the 2-azabicyclo[2.2.1]heptane system, the ring-closure reaction can take place only if the substituents attached to the pyrrolidine ring are *trans* oriented. This factor, coupled with our previous success[2] in employing this pyrrolidine derivative (**6**) in the synthesis of a somewhat related bicyclic system, encouraged us to select this compound as a likely precursor for intermediate **8**.

When the cyclization reaction was carried out in the presence of 2 equiv of sodamide and 1 equiv of phenylacetonitrile in tetrahydrofuran, we were able to isolate one of the two possible isomeric products (**8a**) in very low yield. A substantial quantity of another product was obtained which was determined to be N-tosyl-2-methylpyrrole (**10**). One possible pathway leading to the formation of **10** could involve the elimination of both tosyloxy groups to yield intermediate **9** which would then undergo isomerization.

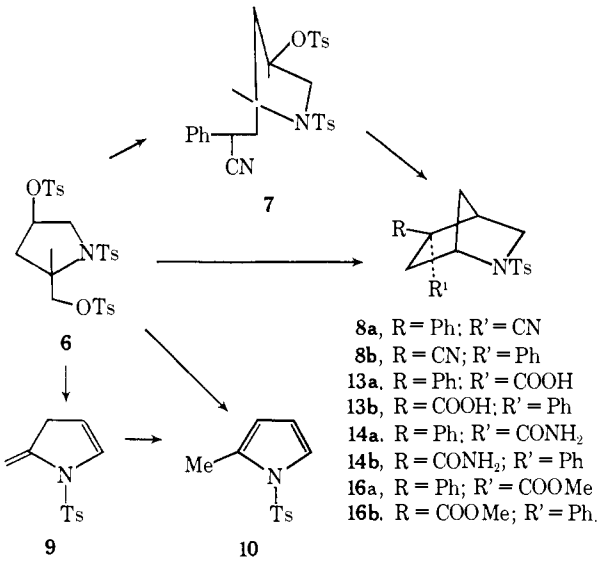
It was reasoned that the amide anion, by virtue of its small size and high basicity, would be more capable of effecting elimination of the tosyloxy substituents than would the much bulkier and less basic phenylacetonitrile anion. Accordingly, the reaction procedure was modified by using 4 equiv of sodamide and 5 of phenylacetonitrile, so that no free amide anion would be present. When this modification was employed, a mixture of the desired epimeric products (**8a**, **b**) was obtained in 75% yield, and only a trace of **10** could be detected.

It is noteworthy that neither **7** nor any other possible intermediate in the cyclization could be found in the reaction mixture, even when the reaction was not brought to completion. This suggests that displacement of the primary tosyloxy group in **6** is the rate-limiting step in the cyclization process.

The two isomeric bridged compounds (**8a**, **b**) were isolated in pure form by chromatography on acidic alumina, although it subsequently was found more expedient to effect a separation at a later stage in the synthesis. From the optical rotations of the pure

(17) E. L. May and J. G. Murphy, *J. Org. Chem.*, **20**, 1197 (1955).
(18) E. L. May and E. M. Fry, *ibid.*, **22**, 1366 (1957).
(19) N. B. Eddy, *Chem. Ind.* (London), 1462 (1959).
(20) E. E. Smissman and M. Steinman, *J. Med. Chem.*, **9**, 455 (1966).

(21) The term "rigid" in this case signifies that no conformational mobility of the piperidine ring is possible. The term "restricted" is relegated to compounds where some type of rotational isomerism is possible, but not probable.



isomers, the mixture of isomers derived from the cyclization reaction was found to consist of 60% **8a** and 40% **8b**.

The assignment of stereochemistry for the two diastereomers (**8a**, **b**) was based on the product ratio, their relative affinities for alumina, and other physicochemical data obtained from compounds derived from **8a** and **8b** (Table I).

TABLE I

CRITERIA EMPLOYED IN THE STEREOCHEMICAL ASSIGNMENT OF 5-SUBSTITUTED 2-AZABICYCLO[2.2.1]HEPTANE EPIMERS

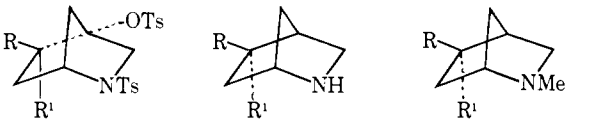
Compd	Criterion	Epimer a	Epimer b
8	Product yield, %	60	40
8	R_f value[a]	0.69	0.56
16	Hydrolytic rates,[b] hr	74	52
5	Basic pK_a[c]	8.35	8.19
15	Acid pK_a[c]	5.32	5.08

[a] Obtained on neutral alumina with C_6H_5–$CHCl_3$ (10:3) as solvent. [b] Expressed as half-life. [c] Determined on the HCl salt in MeOH.

The isomer which was obtained in greater yield was assigned the *exo*-phenyl configuration (**8a**). The *exo*-phenyl isomer should be favored if one makes the reasonable assumption that the transition state more closely resembles the product than the starting material. Since the steric bulk of the phenyl group is much greater than that of the nitrile substituent,[22] transition state **11a** should be favored over **11b** because the *endo* is more hindered than the *exo* position.[23]

In support of this assignment, **8a** was found to possess a higher R_f value on both neutral and acidic alumina with a variety of solvents. Since the nitrile substituent is more polarizable than the phenyl group, the difference in adsorptive capacity between the two isomers can be attributed to the greater accessibility of the former substituent. The compound **8a** containing the sterically more hindered nitrile group, therefore, should have the higher R_f value.

Hydrolysis and detosylation of each of the pure isomers (**8a**, **b**) was carried out by refluxing in aqueous sulfuric acid. The *exo*-phenyl isomer afforded a crystalline product directly from the reaction mixture which was identified as the tosylate salt of **12a**. The fact that



the tosylate, rather than the sulfate salt, was isolated is most likely due to the greater solubility of the latter. Treatment of the *endo*-phenyl compound under the same conditions gave both the tosylate and sulfate salts of **12b**. The free amino acids were generated from the salts by ion-exchange chromatography.

It was found that solutions of the tosylate or sulfate salt of **12b** afforded the free amino acid as a crystalline precipitate upon treatment with pyridine. This procedure failed to generate the *exo*-phenyl acid (**12a**) from its salt, which was recovered unchanged. The different properties of these salts were utilized in separating the mixture of amino acids (**12a**, **b**) obtained by hydrolysis of the mixture of **8a** and **8b**. This procedure avoided the laborious chromatographic separation which was carried out prior to this discovery.

Since the hydrolysis of the N-tosylnitriles was conducted under vigorous conditions, it was necessary to ascertain whether the bicyclic system was still intact. This was accomplished by resynthesizing **8a** and **8b** in four steps from the corresponding amino acids (**12a**, **b**). Tosylation of each isomeric amino acid gave the N-tosyl acids (**13a**, **b**) which were converted to the amides (**14a**, **b**) by treatment with thionyl chloride and then ammonia. Dehydration of **14a** and **14b** afforded **8a** and **8b**, respectively. Thus, it is apparent that no skeletal changes had occurred during the hydrolytic step.

Treatment of amino acids **12a** and **12b** with formaldehyde–formic acid yielded the methylated derivatives (**15a**, **b**). The ethyl esters (**5a**, **b**) were prepared in the conventional fashion *via* the acid chloride.

For the purpose of obtaining further evidence for the stereochemical assignment of **8a** and **8b**, the methyl esters (**16a**, **b**) were prepared and their hydrolytic rates were determined. It was found (Table I) that **16a** was hydrolyzed at a slower rate than **16b**. This is expected in view of the more hindered[23] nature of the *endo*-carbomethoxy group in **16a**.

The dissociation constants (Table I) of **5a** and **5b** also are in accord with the proposed stereochemistry. The former isomer is a stronger base by 0.16 pK_a unit. According to current concepts,[24] the differences in basicity between certain diastereomeric amines are a reflection of the relative abilities of the conjugate acid to form intramolecular hydrogen bonds with a neighboring, proton-acceptor group. Presumably, internal hydrogen bonding stabilizes the protonated form of the amine, and this results in enhanced basicity. Steric factors may either prevent, weaken, or facilitate such hydrogen bonding. This principle has been employed

(22) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis," Interscience Publishers, Inc., New York, N. Y., 1965, p 44.

(23) For leading references, see E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 303.

(24) For leading references, see J. F. King in "Technique of Organic Chemistry," Part I, Vol. XI, Interscience Publishers, Inc., New York, N. Y., 1963, Chapter VI, p 318.

extensively in the stereochemical assignments of amines.[24,25] Internal hydrogen bonding of this type can occur with **5a** because of the *endo* relationship of the carbethoxy group. However, with isomer **5b**, the acceptor group is in the *exo* position, thereby rendering such intramolecular association impossible. Consequently, **5a** should be the stronger base and this is consistent with the assigned configuration.

The acid dissociation constants (Table I) of the N-methylamino acids (**15a**, **b**) show isomer **15a** to be a stronger acid than **15b** by 0.24 pK_a unit. This can be attributed to greater steric hindrance to solvation in the former isomer. A somewhat similar relationship has been reported[26] for compounds containing axial and equatorial carboxyl groups. An equatorial carboxyl has a lower pK_a value due to greater stabilization of the carboxylate anion by solvent. This relationship should also exist in the 2-azabicyclo[2.2.1]heptane system where the more hindered *endo*-carboxyl in **15a** should be less dissociated when compared with its diastereomer (**15b**). This is indeed what is observed.[27]

Pharmacology.—The bridged meperidine compounds (**5a**, **b**) were tested by a modification of the benzoquinone-induced writhing procedure.[28] Groups of five male mice weighing 25–30 g were injected with the meperidine analog, followed, after 30 min, by intraperitoneal administration of benzoquinone. After 5 min the number of writhes over a 10-min period were recorded. The same procedure was followed with control animals, except that saline was used in place of the drug. All testing was performed at 24°. The results show that **5b** was six times more potent (3 mg/kg) than **5a** (18 mg/kg) and twice as potent as meperidine (6 mg/kg).

The *exo* isomer displayed no toxicity at a dose level of 100 mg/kg, whereas the LD_{50} of the *endo* isomer was 100 mg/kg. The toxicity syndrome was very similar to that of meperidine and was characterized by CNS stimulation and convulsions.

Plasma and brain levels (Tables II and III) of N-methyl-tritiated *exo* and *endo* compounds were determined from groups of four animals per time interval after subcutaneous administration. At various time intervals the mice were decapitated, and the blood was collected. The brains were removed, blotted to remove excess blood,[29] weighed, and homogenized. After making the homogenate alkaline and extracting with C_6H_6, an aliquot of the C_6H_6 phase was then counted in a liquid scintillation spectrometer. The same procedure was employed for plasma. The recoveries were found to be 100% (± 10%) for both brain and plasma.

Discussion.—As it was observed that the *exo–endo* plasma ratios (Table II) between the 5- and 45-min periods after administration were quite similar to the subcutaneous dose ratio, the sixfold difference in potency between the *exo* (**5a**) and *endo* (**5b**) isomers probably was not due to metabolism. During this same time period, the *exo endo* brain ratios (Table III) were between 3 and 4 and fairly constant within the limits of experimental error. The lower brain ratios, when compared to the plasma or dose ratios, suggest that the *endo* isomer is penetrating the brain with greater facility. Accordingly, the *endo* isomer is actually approximately 3.7 times more potent than the *exo* isomer and not six times as indicated from subcutaneous ED_{50} values.

The C_6H_6–pH 7.4 buffer partition coefficients of the *exo* and *endo* isomers were found to be 2.37 and 4.23, respectively. This suggested that the higher brain levels of **5b** (after correcting for the dose difference between **5a** and **5b**) are related to its lipid solubility. Further support for this was obtained by dividing the dose ratio, 20/3 = 6.7, by the average brain ratio (3.75) obtained during the analgetic testing period. The value obtained from this calculation is nearly identical with the *endo-exo* ratio (1.78) of the partition coefficients. If this is not fortuitous, the near equality of these ratios suggest a direct relationship between brain level and lipid solubility. The work of Brodie, *et al.*,[30] would suggest that the rate of passage through a boundary separating the blood from the brain may be an important factor in this case. The brain/plasma ratios (Table IV) of both the *exo* and *endo* isomers achieve values which are greater than unity after a relatively short time period. The fact that the brain/plasma ratio for the *endo* isomer exceeds unity in less

TABLE II

PLASMA CONCENTRATIONS OF *exo* AND *endo* ISOMERS OF 2-METHYL-5-PHENYL-5-CARBETHOXY-2-AZABICYCLO[2.2.1]HEPTANE

Time,[a] min	Plasma concn, μg/ml — *exo*-Phenyl (**5a**) (20 mg/kg)[b]	*endo*-Phenyl (**5b**) (3 mg/kg)[b]	Ratio *exo*–*endo*
5	3.15 ± 0.75	0.46 ± 0.11	6.9
15	3.59 ± 0.39	0.47 ± 0.03	7.4
30	2.43 ± 0.17	0.42 ± 0.08	6.1
45	1.70 ± 0.14	0.25 ± 0.02	6.7
60	1.36 ± 0.13	0.15 ± 0.11	9.0
120	0.38 ± 0.05	0.07 ± 0.03	5.5

[a] Time period after administration of compound. [b] Administered subcutaneously to mice.

TABLE III

BRAIN CONCENTRATIONS OF *exo* AND *endo* ISOMERS OF 2-METHYL-5-PHENYL-5-CARBETHOXY-2-AZABICYCLO[2.2.1]HEPTANE

Time,[a] min	Brain concn, μg/g wet wt — *exo*-Phenyl (**5a**) (20 mg/kg)[b]	*endo*-Phenyl (**5b**) (3 mg/kg)[b]	Ratio *exo*–*endo*
5	2.87 ± 0.75	0.86 ± 0.08	3.33
15	6.16 ± 0.61	1.95 ± 0.14	3.15
30	6.83 ± 0.63	1.83 ± 0.08	3.73
45	5.67 ± 0.49	1.50 ± 0.08	3.78
60	5.06 ± 0.32	0.98 ± 0.15	5.16
120	1.61 ± 0.12	0.31 ± 0.14	5.19

[a] Time period after administration of compound. [b] Administered subcutaneously to mice.

(25) H. S. Aaron, G. E. Wicks, Jr., and C. P. Rader, *J. Org. Chem.*, **29**, 2248 (1964); H. O. House and W. M. Bryant, III, *ibid.*, **30**, 3634 (1965); H. S. Aaron, C. P. Rader, and G. E. Wicks, Jr., *ibid.*, **31**, 3502 (1966).

(26) Reference 22, p 186; ref 23, p 324; J. Sicher, M. Tichý, and F. Sipös, *Collection Czech. Chem. Commun.*, **31**, 2238 (1966).

(27) The basic nitrogen in **15a** could possibly produce an effect opposite to those described, as a consequence of facilitating ionization of the *endo* carboxyl group. Apparently, steric shielding of solvation outweighs this effect, and this is not unexpected when one considers the large differences in acid dissociation[26] between axial and equatorial carboxyl groups.

(28) R. Okun, S. C. Liddon, and L. Lasagna, *J. Pharmacol. Exptl. Therap.*, **138**, 107 (1963).

(29) Since the amount of trapped blood in the brain substance is about 12 μl and the weight of the brain is in the range of 0.4–0.5 g, this would introduce only a very small error in the brain-level values.

(30) B. B. Brodie, H. Kurz, and L. S. Schanker, *J. Pharmacol. Exptl. Therap.*, **130**, 20 (1960); S. Mayer, R. P. Maickel, and B. B. Brodie, *ibid.*, **127**, 205 (1959).

TABLE IV

BRAIN–PLASMA RATIOS OF *exo* AND *endo* ISOMERS OF 2-METHYL-5-PHENYL-5-CARBETHOXY-2-AZABICYCLO[2.2.1]HEPTANE

Time,[a] min	Ratio (brain–plasma)	
	exo-Phenyl (**5a**)	*endo*-Phenyl (**5b**)
5	0.91	1.87
15	1.76	4.15
30	2.81	4.36
45	3.34	6.00
60	3.72	6.53
120	4.24	4.43

[a] Time period after subcutaneous administration of drug.

than 5 min, while the value for the *exo* isomer requires a longer period, supports the idea that the rate of passage into the brain substance is governed by the lipid solubility of the compound. Since brain/plasma ratios greater than unity occur quite quickly, it is not likely that metabolism of the drug in the plasma contributes significantly to the high ratios, although at later time intervals it is conceivable that this may be an important factor. In view of the high lipid content of the brain, one possible explanation for the high brain/plasma ratios may be related to the partitioning of the isomers into a lipoidal fraction of the brain. Another possibility would be the operation of an active transport system into the brain.

If the meperidine analogs are true narcotic analgetics,[31] this means that there is a low conformational requirement for meperidine-type compounds. The distance between the center of the aromatic ring and the basic nitrogen in **5a** is about 6 Å, whereas in **5b** it is approximately 4 Å. From a thermodynamic viewpoint, a factor of 3 or 4 in potency is not great when one considers the large difference in geometry and distance between critical moieties in these isomers. The minimal conformational requirements for analgetic activity[31] can be rationalized in terms of differing modes of interaction[4,5] with analgetic receptors. In this connection it is important to point out that the apparent lack of conformational specificity does not necessarily imply that this will also be the case in highly potent analgetics where specific, short-range attractive forces between drug and receptor conceivably may be involved.

Experimental Section[32]

2-Tosyl-5-phenyl-5-cyano-2-azabicyclo[2.2.1]heptane (8a, b).—A solution of 58.5 g (0.5 mole) of phenylacetonitrile in 150 ml of peroxide-free THF was added over a 30-min period to $NaNH_2$ freshly prepared from 9.2 g (0.4 g-atom) of Na in 600 ml of liquid NH_3. This was carried out under N_2[33] as was the remainder of the procedure. The NH_3 was allowed to escape, and a solution of 58.0 g (0.1 mole) of **6**[2] in 550 ml of peroxide-free THF was added over a 20-min period. The mixture was heated slowly to remove the remaining NH_3 and then allowed to reflux for 18 hr. The cooled mixture was treated with 20 g of NH_4Cl followed by 10 ml of H_2O, then stirred and refluxed for 15 min and filtered hot. The filtrate was evaporated *in vacuo* to afford an oily residue which was dissolved in $CHCl_3$ and washed successively with 2 *N* NaOH, 2 *N* HCl, and H_2O. After drying (Na_2SO_4), the solvent was removed, and the unreacted phenylacetonitrile was distilled *in vacuo*. The residue was dissolved in a minimum of boiling MeOH, decolorized (activated charcoal), and cooled. The yield of the mixture of **8a** and **8b**, mp 145–168°, $[\alpha]^{25}D$ +13.9° (*c* 1.46, $CHCl_3$–EtOH, 1:1), was 26.65 g (75.5%).

The mixture was chromatographed on a column of 750 g of acidic alumina (Woelm, grade 1) using petroleum ether (63–68°) initially, and gradually changing the solvent polarity to a final mixture of 1:2 petroleum ether–Et_2O. The total volume of solvent was 139 l. collected in fractions at a rate of 1 l./25 min. Removal of solvent from fractions 33–68 and crystallization of the residue from MeOH yielded 4.79 g of **8a**: mp 168–170°; $[\alpha]^{25}D$ +37.1° (*c* 1.28, $CHCl_3$–EtOH, 1:1); λ_{max} 4.58 (CN), 6.20 (aromatic), 7.38 and 8.50 μ (NSO_2). The nmr spectrum showed no vinyl protons and the correct ratio of aromatic to saturated protons. *Anal.* ($C_{20}H_{20}N_2O_2S$) C, H, N, S, mol wt.

Removal of solvent from fractions 95–139 afforded a residue which was crystallized from MeOH to yield 2.52 g of **8b**: mp 175–177°; $[\alpha]^{25}D$ −20.9° (*c* 1.37, $CHCl_3$–EtOH, 1:1); λ_{max} 4.58 (CN), 6.20 (aromatic), 7.44 and 8.65 μ (NSO_2). The nmr spectrum showed the correct ratio of protons and no vinyl-proton absorption. *Anal.* ($C_{20}H_{20}N_2O_2S$) C, H, N, S, mol wt.

When the above reaction was carried out with 1 equiv of phenylacetonitrile and 2 equiv of $NaNH_2$, there was obtained N-tosyl-2-methylpyrrole (**10**), mp 93–94°, in 24% yield; λ_{max} 6.30 (aromatic), 6.40 (pyrrole), 7.42 and 8.58 μ (NSO_2); nmr ($CDCl_3$), pyrrole Me (2.33, singlet), Ar Me (2.42, singlet), pyrrole protons (H_2, 6.01, multiplet; H_3, 6.20, triplet; H_4, 7.33, doublet), aromatic (H_2 and H_4, 7.33, doublet; H_1 and H_3, 7.73, doublet). *Anal.* ($C_{12}H_{13}NO_2S$) H, N, S; C: calcd, 61.25; found, 61.75.

(1*S*:4*R*:5*S*)-5-Phenyl-5-carboxyl-2-azabicyclo[2.2.1]heptane (12a) from 8a.—To 1.50 g (0.00426 mole) of **8a**, 2.5 ml of cold 66% v/v H_2SO_4 was added, and the mixture was stirred at room temperature for 30 min. It was heated until a clear solution was obtained; 1 ml of H_2O was added dropwise. The mixture was stirred and refluxed for 8 hr during which time H_2O (9 ml) was added. The mixture was treated with activated charcoal, boiled for a few minutes, and filtered while hot. The filtrate was refrigerated overnight to yield 1.418 g (85.4%) of crude crystalline *p*-toluenesulfonate salt of the *exo*-phenyl amino acid (**12a**), mp 120–142°. It was dried in a vacuum desiccator overnight. Upon recrystallization from MeOH–EtOAc and drying in vacuum at 110°, the yield of pure crystals, mp 174–176°, was 1.287 g (77.8%). *Anal.* ($C_{20}H_{23}NO_5S$) C, H, N, S.

Amberlite IR-45 suspended in 1 *N* HOAc was packed in a column then washed with H_2O. A solution of 1 g of *p*-toluenesulfonate salt of **12a** in 250 ml of H_2O was passed through the column followed by 500 ml of 1 *N* AcOH. The eluate was evaporated *in vacuo*. When the last traces of H_2O were azeotropically distilled with absolute EtOH, 0.506 g (91%) of crystalline amino acid **12a** was obtained. It was recrystallized from MeOH–H_2O to give crystals: $[\alpha]^{25}D$ −20.0° (*c* 1.2, 1 *N* HCl–EtOH, 4:1); mp 360° dec; λ_{max} 2.94 (broad), 6.12 (H_2O of crystallization), and 6.38 μ (carboxylate). Tosylate bands were absent. *Anal.* ($C_{13}H_{15}NO_4 \cdot 0.25H_2O$) C, H, N.

(1*S*:4*R*:5*R*)-5-Phenyl-5-carboxyl-2-azabicyclo[2.2.1]heptane (12b) from 8b.—One gram (0.00284 mole) of intermediate **8b** was hydrolyzed according to the procedure used for the preparation of **12a**. After the reaction was completed, the mixture was decolorized with activated charcoal and cooled to yield 0.600 g of a crude product. Recrystallization from H_2O gave 0.310 g (41%) of the sulfate salt of **12b** which decomposes without melting at 300–320°. When the mother liquors of crystallization and of the reaction were combined, concentrated, and cooled, 0.372

(31) Although the induced-writhing procedure has been employed extensively for evaluating analgetic activity, it has been reported (H. F. Fraser and L. S. Harris, *Ann. Rev. Pharmacol.*, **7**, 277 (1967), and references cited therein) that protection against writhing is not limited to narcotic analgetics. For this reason, it cannot be stated unequivocally that **5a** and **5b** are true narcotic analgetics. Unfortunately, the supply of **5a** and **5b** became exhausted, and this precluded further testing to firmly establish the nature of the protection against benzoquinone-induced writhing.

(32) Melting points (Thomas–Hoover capillary melting point apparatus) are corrected. The ir spectra were obtained with a Perkin-Elmer 237B spectrophotometer, mull or KBr disk. The nmr data (ppm) were obtained with a Varian A-60 spectrometer using $CDCl_3$ as solvent and TMS as internal standard. Specific rotations were determined with a Perkin-Elmer 141 polarimeter. Molecular weights were determined with a Hitachi HMU-6D mass spectrometer. Radioactivity was measured on a Packard 3214 liquid scintillation spectrometer. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. The ir and nmr data of all of the compounds were consistent with the proposed structures.

(33) It was noted that substantial quantities of NaCN and polymeric material were formed during the reaction with low yields of product if air was present in the reaction flask.

g (33.5%) of the *p*-toluenesulfonate salt of **12b**, mp 292–295° dec, was obtained. The ir spectrum of the sulfate salt exhibited bands at 5.86, 5.94 (COOH), 6.22 (phenyl), and 8.82 μ (SO_4^{2-}). The *p*-toluenesulfonate salt showed bands at 5.78 (COOH), 6.25 (aromatic), 8.42, 9.60, and 9.88 μ (tosylate). The free amino acid (**12b**) was obtained from the above salts either by employing an ion-exchange resin treatment similar to that applied in generating **12a** from its *p*-toluenesulfonate salt or treating a very concentrated H_2O solution of the salt with pyridine followed by the addition of 95% EtOH, boiling, filtering, and cooling. In either case, recrystallization from EtOH–H_2O produced crystals of the amino acid, $[\alpha]^{25}D$ −133.5° (*c* 1.1, 0.1 *N* HCl–EtOH, 4:1), which decomposed at 325–350° without melting; λ_{max} 2.94 and 6.12 (H_2O of crystallization), and 6.36 μ (COO^-). Tosylate and SO_4^{2-} bands were absent. *Anal.* ($C_{13}H_{23}NO_2 \cdot 0.5H_2O$) C, H, N.

5-Phenyl-5-carboxyl-2-azabicyclo[2.2.1]heptane Epimers (12a and 12b) from a Mixture of 8a and 8b.—The mixture of **8a** and **8b** (18 g) obtained in the cyclization procedure was hydrolyzed as described for the pure material, using 30 ml of 66% v/v H_2SO_4 and 120 ml of H_2O. On cooling the filtered solution in an ice–salt bath, 16.43 g of the *p*-toluenesulfonate and sulfate salts of **12a** and **12b** were obtained. The mixture was dissolved in hot H_2O (35 ml), 150 ml of pyridine, and 200 ml of 95% EtOH. The solution was boiled, filtered, and left at 0°, whereupon 3.54 g of crystalline **12b** separated. The mother liquor was evaporated to dryness *in vacuo* and the residue was dissolved in 2 l. of H_2O. This solution was passed through an HOAc-treated, Amberlite IR-45 column followed by 1 l. of 1 *N* HOAc. The eluate was evaporated *in vacuo*. The residue was dissolved in 200 ml of hot 70% EtOH, decolorized with activated charcoal, and maintained at 3° for 24 hr; additional crystals (0.469 g) of **12b** separated. The mother liquor was evaporated to dryness *in vacuo*, the residue was dissolved in MeOH–H_2O, and the solution was decolorized and cooled. Compound **12a** was obtained as dense crystals (3.84 g). The mother liquor was treated with anhydrous Et_2O and cooled to give another crop (1.95 g) of **12a**.

(1*S*:4*R*:5*S*)-2-Tosyl-5-phenyl-5-carboxyl-2-azabicyclo[2.2.1]heptane (13a).—A solution of 1.167 g (0.003 mole) of the *p*-toluenesulfonate salt of **12a** in 6.6 ml of 6% NaOH (0.0099 mole) was shaken for 5 hr at room temperature with a solution of 0.629 g (0.0033 mole) of *p*-toluenesulfonyl chloride in 8 ml of Et_2O. The aqueous layer was separated, washed with 2 ml of Et_2O, acidified with 4 *N* HCl to pH 1.5, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O and dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue was crystallized from C_6H_6–petroleum ether to yield 0.957 g (86%) of crude **13a**, mp 184–188°. Recrystallization from Et_2O–MeOH produced crystals, mp 190.5–192°, $[\alpha]^{23}D$ +12.4° (*c* 2, EtOH). *Anal.* ($C_{20}H_{21}NO_4S$) C, H, N.

(1*S*:4*R*:5*R*)-2-Tosyl-5-phenyl-5-carboxyl-2-azabicyclo[2.2.1]heptane (13b).—Amino acid **12b** (0.65 g, 0.003 mole) was treated according to the procedure employed for the preparation of **13a**. The crude product was purified on a silicic acid–$CHCl_3$ column and crystallized from C_6H_6 to yield 0.795 g (71.5%) of **13b**, mp 197–198°, $[\alpha]^{25}D$ −102.8° (*c* 1.2, EtOH). *Anal.* ($C_{20}H_{21}NO_4S$) C, H, N.

(1*S*:4*R*:5*S*)-2-Tosyl-5-phenyl-5-carboxamide-2-azabicyclo[2.2.1]heptane (14a).—To 1 ml of $SOCl_2$ cooled in an ice–salt bath was added 0.074 g (0.0002 moles) of **13a** with stirring. The mixture was warmed slowly until the acid dissolved, refluxed for 1 hr, cooled, and poured dropwise with stirring into 4 ml of cold, concentrated NH_4OH (stirring for 1 hr). The amide separated as a white solid, mp 168–185°, 0.060 g (81%). On recrystallization from C_6H_6–MeOH, 0.052 g (70%) of **14a**, mp 185–187°, was obtained. *Anal.* ($C_{20}H_{22}N_2O_3S$) C, H.

(1*S*:4*R*:5*R*)-2-Tosyl-5-phenyl-5-carboxamide-2-azabicyclo[2.2.1]heptane (14b).—Compound **13b** (0.074 g, 0.0002 mole) was transformed to the amide under the same conditions used in the above procedure. The amide was extracted from the reaction mixture with $CHCl_3$, washed with 0.1 *N* NaOH then with H_2O, and dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue, 0.059 g (80%), was recrystallized twice from H_2O–MeOH to yield, 0.040 g (54%), mp 112–115°. The product was used for the preparation of the nitrile without further purification.

(1*S*:4*R*:5*S*)-2-Tosyl-5-phenyl-5-cyano-2-azabicyclo[2.2.1]heptane (8a) from the Corresponding Amide (14a).—A solution of 0.037 g (0.0001 mole) of **14a** in 1 ml of $SOCl_2$ was refluxed for 25 hr, cooled, and excess $SOCl_2$ was removed *in vacuo*. The residue was treated with 2 ml of ice-cold H_2O and dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue was crystallized from MeOH to give 0.025 g (71%) of **8a**, mp 167–169°, $[\alpha]^{25}D$ +38.2°, mmp 167–170°. The ir spectrum was identical with **8a** prepared from the cyclization procedure.

(1*S*:4*R*:5*R*)-Tosyl-5-phenyl-5-cyano-2-azabicyclo[2.2.1]heptane (8b) from the Corresponding Amide (14b).—A solution of 0.026 g (0.00007 mole) of **14b** in 1 ml of $SOCl_2$ was treated according to the procedure described above. Recrystallization from MeOH produced 0.018 g (72.8%) of **8b**, mp 172–174°, $[\alpha]^{25}D$ −21.3°, mmp 173.5–176°. The ir spectrum was identical with that of **8b** prepared from the cyclization procedure.

(1*S*:4*R*:5*S*)-2-Methyl-5-phenyl-5-carboxy-2-azabicyclo[2.2.1]heptane (15a).—To a mixture of 2.5 ml of 37% CH_2O and 0.9 ml of HCO_2H was added 0.5 g (0.0023) of **12a** and the mixture was refluxed for 2 hr, then transferred to a crucible and heated on a H_2O bath until most of the unreacted CH_2O and HCO_2H had evaporated. The syrupy residue was treated with 0.5 ml of CH_2O solution and 1 ml of HCO_2H and reheated to dryness. The last traces of H_2O were azeotropically distilled with EtOH, then with C_6H_6. The residue was crystallized from EtOAc–MeOH. On recrystallization, the crude product, mp 304–307° dec, yielded 0.415 g (71%) of pure **15a**, mp 309–311° dec, $[\alpha]^{25}D$ −31.9° (*c* 1.05, 0.1 *N* HCl–EtOH, 4:1). *Anal.* ($C_{14}H_{17}NO_2$) C, H, N.

(1*S*:4*R*:5*R*)-2-Methyl-5-phenyl-5-carboxy-2-azabicyclo[2.2.1]heptane (15b).—Compound **12b** (0.5 g, 0.0023 mole) was treated under the same conditions and used in the preceding procedure. The dried residue was crystallized from EtOH–MeOH. The yield of **15b**, mp 325–328° dec, $[\alpha]^{25}D$ −144.3° (*c* 0.755%, 0.1 *N* HCl–EtOH, 4:1), was 0.389 g (73%). *Anal.* ($C_{14}H_{17}NO_2$) C, H, N.

(1*S*:4*R*:5*S*)-2-Tosyl-5-phenyl-5-carbomethoxy-2-azabicyclo[2.2.1]heptane (16a).—A solution of 0.742 g (0.002 mole) of **13a** in 50 ml of anhydrous MeOH was cooled to 0° and treated with an Et_2O solution of CH_2N_2 until a pale yellow color persisted. The reaction mixture was heated on a H_2O bath until the color disappeared; the solvent was removed *in vacuo*. The residue was crystallized from Et_2O–MeOH to yield 0.714 g (92.6%) of the Me ester, mp 126–127°, $[\alpha]^{25}D$ −24.4° (*c* 2, $CHCl_3$–EtOH). *Anal.* ($C_{21}H_{23}NO_4S$) C, H, N.

(1*S*:4*R*:5*R*)-2-Tosyl-5-phenyl-5-carbomethoxy-2-azabicyclo[2.2.1]heptane (16b).—Compound **13b**, 0.705 g (0.0019 mole), was esterified with CH_2N_2 under the same conditions used in the preceding procedure. The crude ester was crystallized from anhydrous MeOH. The yield of the pure, crystalline methyl ester, mp 145–146°, $[\alpha]^{25}D$ −94.6° (*c* 0.5, $CHCl_3$–EtOH, 1:1), was 0.658 g (90%). *Anal.* ($C_{21}H_{23}NO_4S$) C, H, N.

(1*S*:4*R*:5*S*)-2-Methyl-5-phenyl-5-carbethoxy-2-azabicyclo[2.2.1]heptane Hydrochloride (5a).—To 5 ml of ice-cold $SOCl_2$ was added with stirring 0.729 g (0.0032 mole) of **15a**. The mixture was warmed to room temperature and stirred for 30 min. The reaction mixture then was refluxed for 1 hr and cooled, and the excess $SOCl_2$ was removed *in vacuo*. The residue was treated immediately with 10 ml of absolute EtOH, and the reaction mixture was stirred at room temperature for 0.5 hr and refluxed for 2 hr. The mixture was then treated with activated charcoal, filtered hot, and concentrated *in vacuo* to about 0.5 ml. Anhydrous Et_2O was added to very slight turbidity, and the mixture was cooled to yield 0.790 g (84.5%) of HCl salt (**5a**), mp 189–190°, $[\alpha]^{25}D$ −23.4° (*c* 1.1, EtOH–H_2O, 1:4). *Anal.* ($C_{16}H_{22}ClNO_2$) C, H, N.

(1*S*:4*R*:5*R*)-2-Methyl-5-phenyl-5-carbethoxy-2-azabicyclo[2.2.1]heptane Hydrochloride (5b).—Compound **15b** was esterified under exactly the same conditions used for its isomer in the preceding procedure. The yield of **5b**, mp 187–189°, $[\alpha]^{25}D$ −108.8° (*c* 1.145, EtOH–H_2O, 1:4), was 90%. *Anal.* ($C_{16}H_{22}ClNO_2$) C, H, N.

Methyl-Tritiated 2-Methyl-5-phenyl-5-carbethoxy-2-azabicyclo[2.2.1]heptane Hydrochloride (5a, b).—To a tube containing 0.0120 g (0.00043 mole) of tritiated paraformaldehyde was added 0.100 g (0.00046 mole) of amino acid (**12a** or **12b**) followed by 0.1 ml of HCO_2H and 0.1 ml of H_2O. The mixture was heated on a steam bath for 2 hr, and 0.042 g of cold paraformaldehyde was added. The mixture was heated for an additional hour, after which time all volatile material was removed by heating in a stream of N_2. The residue was crystallized from MeOH–Et_2O and converted to **5a** or **5b** by the procedure employed in the preparation of the cold material. The specific activities of **5a** and **5b** used in the physiological-distribution studies were 12.4 and 18.2 μcuries/mg, respectively.

Distribution Coefficients for 5a and 5b.—Ten milliliters each of C_6H_6 and pH 7.4 Krebs–Ringers phosphate were shaken for 1 hr with the tritiated compound (**5a** and **5b**). Each phase (1 ml) was counted in a liquid scintillation spectrometer. The counts per minute found in each phase were corrected to disintegrations per minute and the ratio was calculated.

Hydrolytic Rate Studies on 16a and 16b.—A 10-ml ethanolic 0.25 *N* NaOH solution containing 0.025 g of ester was maintained at 21.5°. Aliquots (1 ml) were drawn at intervals and titrated with 0.04 *N* HCl using phenolphthalein as indicator. A blank experiment was carried out.

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Experimentally Induced Phenylketonuria. II. Potential Inhibitors of Phenylalanine Hydroxylase

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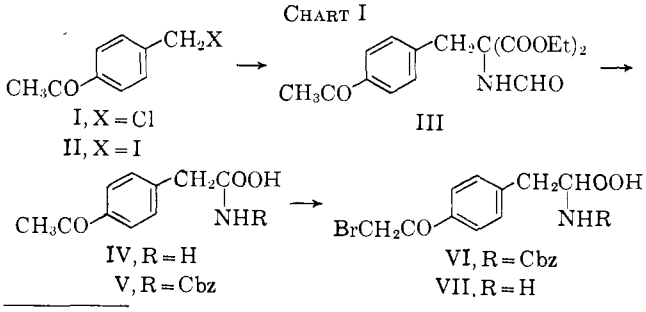
The preparation of a series of alkylating agents derived from phenylalanine is reported. The compounds were found to be ineffective as inhibitors of phenylalanine hydroxylase. Syntheses are described for 4-bromoacetyl-, 4-bromoacetamido-, 3-chloroacetamido-, and 4-fluoro-3-chloroacetamidophenylalanine.

In a previous publication[1] we reported an approach to the creation of a state of phenylketonuria (PKU) by inhibition of the enzyme, phenylalanine hydroxylase. We had confirmed earlier findings that 4-fluorophenylalanine[2] and esculetin (6,7-dihydroxycoumarin)[3] were good inhibitors of the enzyme *in vitro*. Our attempts to find a more potent inhibitor among a series of variously substituted phenylalanine derivatives were unsuccessful. However, we were able to conclude that alteration of the amino acid side chain and substitution of a group larger than fluorine in the 4 position was detrimental to activity. It appeared likely to us that irreversible inhibition of phenylalanine hydroxylase would more closely mimic the genetic defect responsible for PKU.

In the present work we have prepared some potential irreversible inhibitors in the form of alkylating agents derived from phenylalanine. The compounds were 4-bromoacetyl-, 4-bromoacetamido-, 3-chloroacetamido-, and 4-fluoro-3-chloroacetamidophenylalanine. As will be seen in the biological results, no significant degree of inhibition was realized with these compounds. Whether the lack of significant inhibition was due to the bulkiness of the haloalkyl substituents or insufficient reactivity of the alkylating functions is not known.

The synthesis of 4-bromoacetylphenylalanine started with 4-chloromethylacetophenone[4] (I) which was converted to the crude iodide (II), and this in turn was used to alkylate diethyl formamidomalonate (Chart I).

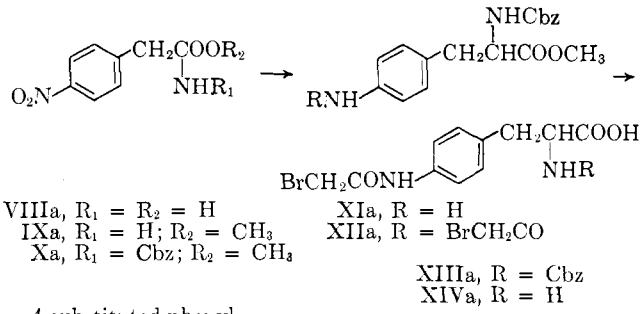
CHART I



Acid hydrolysis and decarboxylation of the intermediate III yielded 4-acetylphenylalanine (IV). An effort was made to brominate IV directly in acetic acid at 70° but no reaction with bromine was observed. The N-carbobenzoxy derivative V was then prepared and brominated readily with trimethylphenylammonium tribromide[5] in THF to afford the N-carbobenzoxybromoketone (VI) in 44% yield. Treatment of VI with HBr–AcOH smoothly cleaved the blocking group without disturbance of the bromine to yield 4-bromoacetylphenylalanine (VII) as the hydrobromide salt.

4-Nitrophenylalanine was converted to its N-carbobenzoxy methyl ester. The nitro group was reduced and the resulting amine (XIa) was converted to the bromoacetamido compound (XIIa) (Chart II). Mild

CHART II

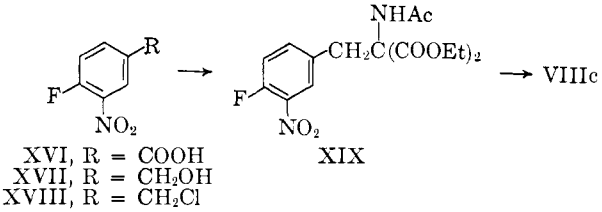


VIIIa, $R_1 = R_2 = H$
IXa, $R_1 = H$; $R_2 = CH_3$
Xa, R_1 = Cbz; $R_2 = CH_3$

XIa, R = H
XIIa, R = $BrCH_2CO$

XIIIa, R = Cbz
XIVa, R = H

a, 4-substituted phenyl
b, 3-substituted phenyl
c, 4-fluoro-3-substituted phenyl; chloroacetamides were prepared for the b and c series



XVI, R = COOH
XVII, R = CH_2OH
XVIII, R = CH_2Cl

XIX

exposure of XIIa to HBr–AcOH caused only partial removal of the methyl ester, while more vigorous conditions also caused cleavage of the bromoacetyl moiety.

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