Aromatic Amino Acid Hydroxylase Inhibitors. 2.[1] 3-Alkyl-α-methyltyrosines[2]

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3-Iodotyrosine is a potent inhibitor of tyrosine hydroxylase *in vitro*, but lacks significant *in vivo* activity due to deiodination and transamination. On the basis of previous studies with thyroxine analogs, a series of 3-alkyl-α-methyltyrosines was synthesized. A 3-Me, Et, or *i*-Pr substituent was found to have little effect on the tyrosine hydroxylase inhibitory property of α-methyltyrosine. A 3-*tert*-Bu group, however, caused a marked decrease in inhibitory activity.

Numerous attempts have been made to modify tissue catecholamine levels by use of inhibitors of catecholamine biosynthesis. While several enzymatic steps are amenable to pharmacological control, recent interest has focused on inhibitors of tyrosine hydroxylase since this enzyme represents the rate-limiting step in norepinephrine biosynthesis.[3]

In early studies, Udenfriend and coworkers[4] noted that α-methyltyrosine and its 3-halogenated derivatives were extremely potent competitive inhibitors of tyrosine hydroxylase. The relative activity of the 3-halogenated derivatives was I > Br > Cl > F. A similar order of inhibitory activity for the 3-halogenated α-methylphenylalanines also was reported from our own laboratories.[1,5] As would be expected, however, the phenylalanine analogs were much less active than the tyrosine analogs.

Normally competitive inhibitors of enzymatic reactions bear a close structural and steric relationship to the natural substrate. In the above cases, however, the most effective inhibitor in either the phenylalanine or tyrosine series was the analog bearing a bulky iodine atom at the 3 position. It was noteworthy that structure–activity studies with thyroxine analogs had revealed a similar importance for iodine at the 3′ position and that, in this case as well, the order of thyromimetic activity for the analogs was I > Br > Cl > F.[6] A subsequent ρ–σ–π analysis by Hansch and Fujita[7] revealed a positive correlation between thyroxine-like effects in rodents and the lipophilic and electronic character of the halogen substituents. They concluded, however, that halogens are not the best functions for optimum thyroxine-like activity and predicted that: "Allyl, propyl, or butyl groups should be more effective than iodine. The ideal group for increasing activity (assuming steric effects to be absent) would be the *t*-butyl."

A number of 3′-alkyl-3,5-diiodothyronine analogs have been synthesized and all possess potent thyromimetic activity.[8] The L-3′-*i*-Pr analog, for example, is the most potent antigoitrogenic compound known, being approximately twice as active as L-triiodothyronine. Two groups synthesized the 3′-*tert*-Bu analog and found it to possess activity greater than or equal to thyroxine.[9] These results indicate at least a qualitative relationship with Hansch's prediction.

These studies on alkyl thyroxine analogs took on added significance when it was reported that 3-iodotyrosine failed to inhibit tyrosine hydroxylase in animals. This lack of *in vivo* activity was attributed to rapid deiodination and transamination of the compound.[10] Since α-methylation of amino acids is known to retard decarboxylation[11] and transamination,[12] the synthesis and evaluation of a series of 3-alkyl-α-methyltyrosines seemed an obvious pursuit.[13]

A search of the literature revealed that Saari, *et al.*,[14] had described the synthesis of 3,α-dimethyltyrosine as part of a large series of tyrosine analogs. In their study, L-3-iodo-α-methyltyrosine and DL-3,α-dimethyltyrosine at 1×10^{-4} *M* caused 94 and 27% inhibition of tyrosine hydroxylase, respectively. On the other hand, these compounds were stated to differ in their mode of inhibition. Unlike the 3-I analog, the 3-Me derivative was found to be noncompetitive with substrate. This somewhat surprising finding reinforced our interest in the effect of 3-alkylation on the biological activity of α-methyltyrosine.

The most widely used route to α-alkylamino acids is *via* the hydantoin of the appropriately substituted 2-propanone. The disubstituted 1-phenyl-2-propanones required for our study were synthesized from 2-alkylphenols (**1**) by two methods as shown in Scheme I. Bromination of **1** with dioxane dibromide in ether

(1) Paper 1, *J. Med. Chem.*, **13**, 1040 (1970).

(2) This work was supported by Grants HE-11274 from the National Institutes of Health and PRA-18 from the American Cancer Society.

(3) *Cf.* S. Udenfriend, *Pharmacol. Rev.*, **18**, 43 (1966).

(4) S. Udenfriend, P. Zaltsman-Nirenberg, and T. Nagatsu, *Biochem. Pharmacol.*, **14**, 837 (1965).

(5) P. S. Weinhold and V. B. Rethy, *ibid.*, **18**, 677 (1969).

(6) S. B. Barker in "Medicinal Chemistry" 2nd ed, A. Burger, Ed., Interscience, New York, N. Y., 1960, p 682.

(7) C. Hansch and T. Fujita, *J. Amer. Chem. Soc.*, **86**, 1616 (1964).

(8) E. C. Jorgensen, *Annu. Rep. Med. Chem.*, **1965**, 200 (1966).

(9) (a) E. C. Jorgensen and J. A. W. Reid, *J. Med. Chem.*, **8**, 533 (1965); (b) C. M. Buess, T. Guidici, N. Kharasch, W. King, D. D. Lawson, and N. N. Saha, *ibid.*, **8**, 469 (1965).

(10) (a) G. A. Johnson, E. G. Kim, W. Veldkamp, and R. Russell, *Biochem. Pharmacol.*, **16**, 401 (1967); (b) B. N. Lutsky and N. Zenker, *J. Med. Chem.*, **11**, 1241 (1968).

(11) C. A. Stone and C. C. Porter, *Advan. Drug Res.*, **4**, 76 (1967).

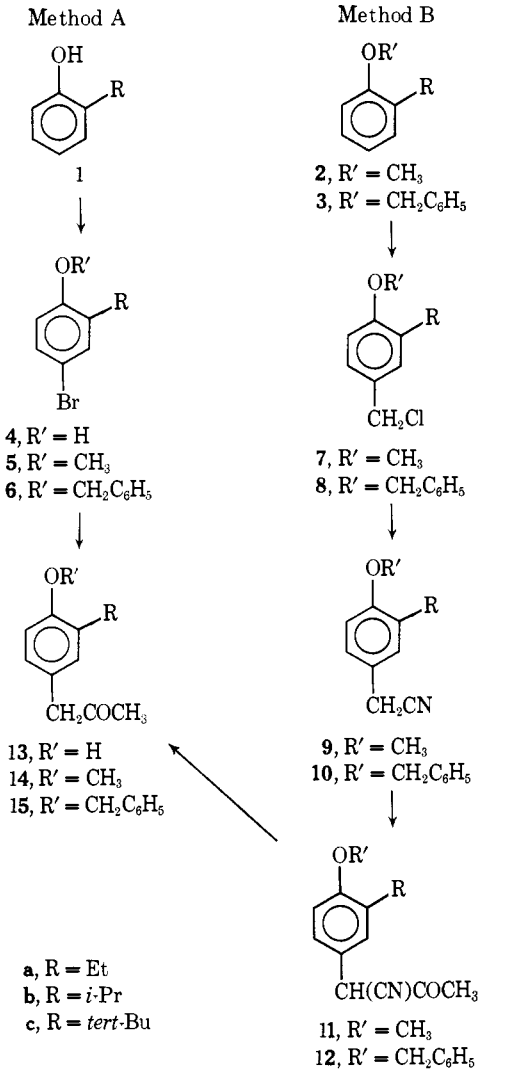
(12) A. Meister, "Biochemistry of Amino Acids," Vol. I, 2nd ed, Academic Press, New York, N. Y., 1965, p 399.

(13) Subsequent to the near completion of this study, Y. H. Caplan and N. Zenker described the preparation and properties of 3-methyl and 3-isopropyl tyrosine following a similar rationale: 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, MEDI 032; Y. H. Caplan, N. Zenker, D. A. Blake, and E. M. Johnson, Jr., *J. Med. Chem.*, **14**, 405 (1971).

(14) W. S. Saari, J. Williams, J. F. Britcher, D. E. Wolf, and F. A. Kuehl, *ibid.*, **10**, 1008 (1967).

SCHEME I

SYNTHESIS OF DISUBSTITUTED 1-PHENYL-2-PROPANONES

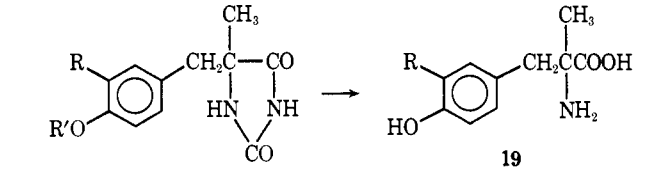


afforded **4** which was subsequently converted to the ethers **5** and **6** by standard methods. The Grignard reagent derived from these ethers was condensed with chloroacetone to give **14** and **15** in low yields (method A). A longer but superior method (B) involved chloromethylation of the phenol ethers **2** or **3** and conversion of the resulting benzyl chlorides (**7**, **8**) into the α-cyanopropanone derivatives (**11**, **12**) by standard methods. Hydrolytic decarboxylation of the latter gave the desired propanones (**14**, **15**) in good overall yield as indicated in Table III.

The 3-ethyl- and 3-isopropyl-α-methyltyrosines (**19a**, **b**) were conveniently prepared by simultaneous hydrolysis and demethylation of the hydantoins **17a** and **17b** by refluxing in 48% HBr (Scheme II). Similar treatment of **17c**, however, resulted in concommitant loss of the *tert*-Bu group to afford α-methyltyrosine. A similar loss of the 3′-*tert*-Bu group in the thyroxine series under these conditions was noted.[9a] All attempts to modify the hydrolytic conditions in an effort to retain the *tert*-Bu group were unsuccessful. Thus it became necessary to prepare **19c** by an alternate route. This was achieved by employing the benzyl group as the protective moiety in the initial synthetic steps. Hydrolytic decarboxylation of **12c** in HCl–AcOH resulted

SCHEME II

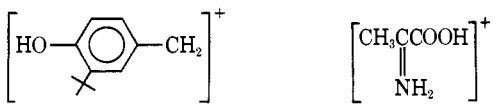
SYNTHESIS OF 3-ALKYL-α-METHYLTYROSINE



16, R′ = H
17, R′ = CH_3
18, R′ = $CH_2C_6H_5$
a, R = Et; **b**, R = *i*-Pr; **c**, R = *tert*-Bu

in simultaneous loss of the benzyl group to form **13c** in good yield. Formation of the hydantoin followed by hydrolysis in aq $Ba(OH)_2$ afforded the desired *tert*-butyl amino acid **19c**.

Nmr and other spectral properties of the amino acids were consistent with the assigned structures. In addition, the mass spectrum[15] of **19c** showed the molecular ion peak at *m/e* 251 and two major fragments at *m/e* 163 (M − 88) and *m/e* 88 (M − 163) corresponding to species i and ii, respectively.



i ii

Enzyme Inhibition Studies.—Tyrosine hydroxylase was prepared from beef adrenal medulla according to the procedure of Nagatsu, *et al.*[16] The conditions for the inhibition studies have been described previously.[5] The per cent inhibitions by the 3-alkyl-α-methyltyrosines at two different concentrations are shown in Table I.

TABLE I

INHIBITION OF TYROSINE HYDROXYLASE BY 3-ALKYL-α-METHYLTYROSINES

Compd	3-Substituent	% inhibition, 2×10^{-4} *M*	% inhibition, 2×10^{-5} *M*
α-Methyltyrosine	H	87	44
3,α-Dimethyltyrosine	CH_3	72.7	
19a	CH_2CH_3	95.8	38
19b	$CH(CH_3)_2$	88.4	19.4
19c	$C(CH_3)_3$	0	0

The results indicate that alkylation of α-methyltyrosine at the 3 position has very little effect on the inhibitory properties of the parent compound until the *tert*-Bu group is substituted, and this causes a sharp drop in inhibitory activity. Moreover, since DL-3-iodo-α-methyltyrosine causes 50% inhibition at 3×10^{-7} *M*, it is clear that replacement of I with an alkyl group does not provide the same retention of activity that was observed for thyroxine analogs. Although the desired enhancement in inhibitory activity was not achieved, it is noteworthy that the enzyme can accommodate cer-

(15) This data was kindly provided by Dr. R. L. Foltz of the High Resolution Mass Spectrometry Center, Battelle Memorial Institute, Columbus, Ohio.

(16) T. Nagatsu, M. Levitt, and S. Udenfriend, *J. Biol. Chem.*, **239**, 2910 (1964).

TABLE II

4-BROMO-2-ALKYLPHENOLS AND ETHERS

Compd	R	R′	% yield	Bp (mm) or mp, °C	Formula	Analyses
4a	Et	H	96	122–132 (8–10)	C_8H_9BrO	*a*
4b	*i*-Pr	H	85	82 (0.35)	$C_9H_{11}BrO$	C, H
4c	*tert*-Bu	H	86	90–91 (0.4)	$C_{10}H_{13}BrO$	*b*
5a	Et	CH_3	96	118–119 (10)	$C_9H_{11}BrO$	C, H
5b	*i*-Pr	CH_3	70	123–128 (9)	$C_{10}H_{13}BrO$	*c*
6c	*tert*-Bu	$CH_2C_6H_5$	94	87–88	$C_{17}H_{19}BrO$	C, H

[a] Reported bp 110° (3 mm), E. Klarmann, L. W. Gates, V. A. Shternov, and P. H. Cox, Jr., *J. Amer. Chem. Soc.*, **55**, 4657 (1933). [b] Reported bp 128–130 (6 mm), H. Hart, *ibid.*, **71**, 1966 (1949). [c] Reported bp 107–110 (7 mm), D. Nasipuri and M. Guha, *J. Sci. Ind. Res., Sect. B*, **21**, 96 (1962).

tain functionality at the 3 position without adverse affects.

In contrast with the findings of Saari, *et al.*,[14] Lineweaver–Burk plots obtained with varying concentrations of **19a** revealed a pattern expected for competitive inhibition.

Preliminary studies with **19a** in rats made hypertensive with desoxycorticosterone acetate showed it to produce a significant lowering of arterial blood pressure at a dose of 25 mg/kg.[17] Further studies are in progress. Since one of the drawbacks of α-methyltyrosine in the clinic is its slow metabolism to α-methyldopa and α-methylnorepinephrine,[18] **19a** or a close relative may offer some advantage in this regard since it would not be expected to be metabolized to a catecholamine.

Experimental Section[19]

4-Bromo-2-alkylphenols (4). General Method.—To a soln of 2-isopropylphenol (**1b**, 27.2 g) in Et_2O (100 ml) was added freshly prepd dioxane dibromide (50 g). The reaction mixt was stirred and maintained at 5–10° during the addn which required 30 min. The mixt was allowed to come to room temp before washing consecutively with NaCl soln, 10% $NaHCO_3$, and NaCl soln. The ether phase was dried ($MgSO_4$) and evapd to dryness. The residue was distd *in vacuo* to furnish **4b** as a colorless liquid (36.0 g, 83.7%), bp 82° (0.35 mm) (see Table II).

4-Bromo-2-alkylanisoles (5). General Method.—4-Bromo-2-ethylphenol (**4a**, 25.0 g) was dissolved in 5 *N* NaOH (25 ml) and H_2O (30 ml). Me_2SO_4 (12 g) was added dropwise at 100°. Heating was contd for 30 min and more 5 *N* NaOH (20 ml) and Me_2SO_4 (11 g) added as above. This procedure was repeated a second time. The reaction mixt was cooled and extd with Et_2O The ether layer was washed with dil NaOH and H_2O, dried ($MgSO_4$), and evapd to dryness. The residue was distd to afford 4-bromo-2-ethylanisole (**5a**) as a colorless liquid (25.5 g, 95.7%) bp 118–119° (10 mm) (see Table II).

2-Benzyloxy-5-bromo-*tert*-butylbenzene (6c).—A mixt of 4-bromo-2-*tert*-butylphenol (**4c**, 22.9 g), benzyl chloride (14 g), and K_2CO_3 (15 g) in DMF (50 ml) was heated at 90° for 2 hr. The reaction mixt was poured into ice water, and the resulting ppt was collected. The product was washed with 2% NaOH and H_2O and recrystd from 95% EtOH (200 ml). This gave the pure benzyl ether as colorless prisms (30 g, 94%), mp 87–88°. *Anal.* ($C_{17}H_{19}BrO$) C, H.

3-Alkyl-4-methoxybenzyl Chlorides (7). General Method.—A mixt of 2-*tert*-butylanisole (**2c**, 16.5 g), paraformaldehyde (10.5 g), and glacial AcOH satd with HCl (150 ml) was stirred and heated at 60° for 5 hr. HCl gas was continuously bubbled into the reaction mixt throughout the time period. The addn of HCl gas was discontd, and the soln was heated to 80° for 1 hr. The soln was allowed to cool, poured into H_2O, and extd with $CHCl_3$. The organic phase was washed with H_2O and dried ($MgSO_4$), and the solvent was removed *in vacuo*. Distn of the oily residue under reduced pressure afforded **7c** as a colorless oil (16 g, 75%), bp 107–110° (0.3 mm). *Anal.* ($C_{21}H_{17}ClO$) C, H. The prepn of **7a** differed only in that the reaction mixt was heated at 35–40° for 5 hr before quenching. Distn afforded pure **7a** (54%), bp 102° (0.4). *Anal.* ($C_{10}H_{13}ClO$) C, H.

4-Benzyloxy-3-*tert*-butylbenzyl chloride (8c) was prepd from 2-benzyloxy-*tert*-butylbenzene (**3c**, 32 g) by treating with paraformaldehyde (14 g) and glacial AcOH (200 ml) satd with HCl gas as described above. This gave **8c** (19 g, 50%) as a colorless oil, bp 170–175° (0.35 mm), which solidified on cooling. Recrystn from petr ether and CH_2Cl_2 afforded **8c** as colorless needles, mp 71–72°. *Anal.* ($C_{18}H_{21}ClO$) C, H.

3-Alkyl-4-methoxybenzyl Cyanide (9). General Method.—A soln of NaCN (0.74 g) in H_2O (1 ml) was added to DMF (20 ml), and the mixt was heated to 50° with stirring. A soln of 3-*tert*-butyl-4-methoxybenzyl chloride (**7c**, 2.1 g) in DMF (10 ml) was added dropwise over a period of 30 min. Stirring and heating was contd for 3 hr, and the mixt was allowed to cool. H_2O (50 ml) was added, and the mixt was extd with Et_2O. The ext was washed with dil HCl and H_2O and dried ($MgSO_4$), and the solvent was removed. The oily residue was distd *in vacuo* to give **9c** (1.7 g, 87%) as a colorless oil, bp 122–125° (0.25 mm). *Anal.* ($C_{13}H_{17}NO$) C, H. Compd **9a**, prepd similarly, distd as a pale yellow oil (90% yield), bp 102° (0.1 m). *Anal.* ($C_{11}H_{13}NO$) C, H.

4-Benzyloxy-3-*tert*-butylbenzyl Cyanide (10c).—Treatment of **8c** (17 g) in a manner similar to that described for prepn of **9** afforded **10c** (12 g, 71%) as a yellow solid, mp 76–77°. Recrystn from dil EtOH furnished an anal. sample, mp 78–79°. *Anal.* ($C_{19}H_{21}NO$) C, H.

α-Acetyl-3-alkyl-4-methoxybenzyl Cyanide (11). General Method.—To a boiling soln of Na (1.5 g) in EtOH (25 ml) was added dropwise a mixt of **9c** (9 g) and EtOAc (8 g). The addn required 1 hr, then the reaction mixt was stirred under reflux for 16 hr. The reaction mixt was cooled, H_2O was added, and the soln was extd with Et_2O. The aq phase was acidified with AcOH and reextd with Et_2O. This ether ext was washed with H_2O and dried ($MgSO_4$), and the solvent was removed. Recrystn of the solid from dil EtOH gave pure **11c** (6.5 g, 59%), mp 93–94°. *Anal.* ($C_{15}H_{19}NO_2$) C, H. Compd **11a** was obtd in a similar manner (72% yield), mp 85–86°. *Anal.* ($C_{13}H_{15}NO_2$) C, H.

α-Acetyl-4-benzyloxy-3-*tert*-butylbenzyl cyanide (12c) was prepd from **10c** in a manner similar to that described for 11. This gave **12c** (56%) as a yellow solid, mp 89–90°. *Anal.* ($C_{21}H_{23}NO_2$) C, H.

1-(4-Hydroxy-3-alkyl)phenyl-2-propanone and Ethers (13–15). Method A.—To a soln of Grignard reagent derived from 4-bromo-2-ethylanisole (**5a**, 13.2 g) and Mg turnings (1.46 g) in anhyd Et_2O (20 ml) was added dropwise with stirring a soln of chloroacetone (5.6 g) in Et_2O (20 ml). The reaction mixt was maintained at 5–10° during the addn, and stirring was contd for 1 addnl hr. Et_2O was removed under reduced pressure, and the residue was suspended in anhyd xylene (50 ml). The mixt was heated at reflux for 40 min with stirring, cooled, and treated with dil HCl, and the product was extd with Et_2O. The ext was washed with H_2O, dried ($MgSO_4$), and evapd. The residue was fractionally distd *in vacuo* to afford **14a** as a colorless liquid

(17) This information was kindly provided by Drs. L. Rozek and P. D. Klimstra of G. D. Searle & Co.

(18) K. Engelman, E. Jequier, S. Udenfriend, and A. Sjoerdsma, *J. Clin. Invest.*, **47**, 568 (1968).

(19) Melting points were taken on a Fisher-Johns hot stage and are corrected. Ir spectra are recorded on a Perkin-Elmer 337 spectrophotometer. The nmr spectra were obtained with a Varian A-60A spectrometer (Me_4Si). Elemental anal. were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. Where anal. are indicated only by symbols of the elements, anal. results for those elements were within ±0.4% of the theoretical values.

TABLE III

4-HYDROXY-3-ALKYLPHENYL-2-PROPANONES AND ETHERS

Compd	R	R′	Method	Bp, °C (mm)	% yield	2,4-DNP,[b] mp, °C	Formula[c]
13c	*tert*-Bu	H	B	148–150 (0.25)	50	112–113	$C_{19}H_{22}N_4O_5$
14a	Et	CH_3	A, B	88–90 (0.15)	21, 77	102–103	$C_{18}H_{20}N_4O_5$
14b	*i*-Pr	CH_3	A	82 (0.1)	18	124–125	$C_{19}H_{22}N_4O_5$
14c	*tert*-Bu	CH_3	B	128–130 (0.45)	62	149–150	$C_{20}H_{24}N_4O_5$
15c	*tert*-Bu	$CH_2C_6H_5$	A	155 (0.1)	20[a]	151–152	$C_{26}H_{28}N_4O_5$

[a] THF was used as a solvent for reaction in place of Et_2O. [b] Recrystd from dil EtOH. [c] All compds were anal. for C, H.

TABLE IV

SUBSTITUTED 5-BENZYL-5-METHYLHYDANTOINS

Compd	R	R′	Mp, °C	% yield	Formula[a]
16c	*tert*-Bu	H	120–121	55	$C_{15}H_{20}N_2O_3$
17a	Et	CH_3	196–197	68	$C_{14}H_{18}N_2O_3$
17b	*i*-Pr	CH_3	206–207	78	$C_{15}H_{20}N_2O_3$
17c	*tert*-Bu	CH_3	206–207	66	$C_{16}H_{22}N_2O_3$
18c	*tert*-Bu	CH_2–C_6H_5	226–227	47	$C_{22}H_{26}N_2O_3$

[a] All compds were anal. for C, H.

TABLE V

3-ALKYL-α-METHYLTYROSINES

Compd	R	R′	Method	Mp, °C	% yield	Formula[a]
19a	Et	H	B	>320	55	$C_{12}H_{17}NO_3$
19b	*i*-Pr	H	B	>320	62	$C_{13}H_{19}NO_3$
19c	*tert*-Bu	H	A	240–242 dec	47	$C_{14}H_{21}NO_3 \cdot H_2O$

[a] See Table IV, footnote *a*.

(see Table III). The 2,4-dinitrophenylhydrazone was recrystd from EtOH to give an anal. sample; mp 102–103°. *Anal.* ($C_{18}H_{20}N_4O_5$) C, H.

Method B.—A mixt of **11c** (6 g), glacial AcOH (100 ml), concd HCl (24 ml), and H_2O (24 ml) was stirred at reflux for 24 hr. The soln was concd to half the original vol, dil with H_2O, and extd (Et_2O). The ext was washed with H_2O and dried ($MgSO_4$), and the solvent was removed. The residual oil was purified by distn *in vacuo* to give **14c** (see Table III).

Substituted 5-Benzyl-5-methylhydantoins (16–18). General Method.—A mixt of **14a** (2.19 g), $(NH_4)_2CO_3$ (4.4 g), and KCN (1.55 g) in 50% EtOH (25 ml) was heated at 50–55° with stirring. The resulting ppt was collected, washed consecutively with H_2O and PhH, and recrystd twice from 75% EtOH. This gave pure **17a** (2.03 g, 68%) as colorless flakes; mp 196–197° (see Table IV).

α-Methyltyrosine Derivatives (19, 20). Method A.—A mixt of hydantoin **16c** (0.84 g), hydrated $Ba(OH)_2$ (4.0 g), and H_2O (50 ml) was heated in an oven at 165° in a sealed tube for 6 hr. The soln on cooling was acidified with 6 *N* H_2SO_4 and filtered. The filtrate was basified with NH_4OH and concd on a rotary evaporator until solid began to ppt. The flask was chilled in an ice–salt mixt, and the ppt was collected. Recrystn from NH_4OH gave **19c** (0.7 g) as a colorless solid, mp 240–242° dec (see Table V).

Method B.—A mixt of hydantoin **17a** (1.7 g) and 48% HBr (45 ml) was refluxed with stirring for 2 days under N_2. The reaction mixt was filtered, and the filtrate was concd to dryness. The residue was dissolved in H_2O and treated with excess NH_4OH. The soln was evapd to dryness, and the white residue was recrystd from H_2O twice to afford **19a** (0.72 g, 49.8%) as colorless prisms, mp > 320° (see Table V).