isolation of the resulting lignan glycoside acetals, the reaction mix was dild with $CHCl_3$ and the catalyst was removed by filtration or washing out with H_2O. After removal of excess carbonyl compd the reaction product was obtd in pure form after column chromatogr or by direct crystn. Noncryst products were purified by pptn from a suitable solvent.

Method A. 4′-Demethyl-1-*O*-[4,6-*O*-(2-thenylidene)-β-D-glucopyranosyl]epipodophyllotoxin (19).—Dried 4′-demethylepipodophyllotoxin β-D-glucopyranoside[1] (6.8 g) was suspended in 68 ml of freshly distd 2-thiophenecarboxaldehyde, 3.4 g of anhyd $ZnCl_2$ was added, and the mixt was shaken under N_2. The course of the condensation was followed by tlc. After 3–4 hr, the yellow sol was dild with 300 ml of $CHCl_3$ and 300 ml of H_2O. The org layer was sepd and the H_2O phase was extd twice with $CHCl_3$. The combined $CHCl_3$ exts were washed several times with H_2O and then concd *in vacuo* to 50–70 ml. The remaining colorless oil was dropped into 1000 ml of pentane with stirring, whereby an oily ppt formed. After decanting the solvent the ppt was taken up in 40 ml of $CHCl_3$, and this soln was added dropwise to 1500 ml of pentane. The ppt was filtered off (6.5 g) and crystd from EtOH: mp 246–255°; $[\alpha]^{20}D$ −108.6° (*c* 0.5, $CHCl_3$–MeOH, 9:1); ir (Nujol) 3490, 3375 (OH); 1780 (γ-lactone); 1605, 1514, 1504, 1487 (arom C=C); nmr (DMSO-d_6) δ 8.24 (1 H, s), H, phenol OH, 7.7–6.8 (4 H, m), H of thiophene ring and H at C-8, 6.56 (1 H, s), H at C-5, 6.22 (2 H, s), H at C-2′ and H at C-6′, 6.04 (2 H, s) CH_2O_2, 5.88 (1 H, s), acetal H of the thenylidene group, 3.64 (6 H, s), 2 CH_3O.

4′-Demethyl-1-*O*-[4,6-*O*-(isopropylidene)-β-D-glucopyranosyl]epipodophyllotoxin (42).—To a suspension of 8 g of 4′-demethylepipodophyllotoxin β-D-glucopyranoside in 160 ml of CH_3NO_2 was added 4 g of anhyd $ZnCl_2$ and 32 ml of acetone dimethyl ketal, and the mixt was stirred for 0.5 hr at 20° with exclusion of moisture. The clear sol was then dild with 500 ml of $CHCl_3$ and washed 3 times, with 50-ml portions of H_2O. The org layer was dried (Na_2SO_4) and evapd to dryness. The residue was chromatographed on 150 g of kieselgel using $CHCl_3$–MeOH (98:2) as eluant. The tlc-pure fractions were crystd from MeOH yielding 5.11 g: mp 210–212°; $[\alpha]^{20}D$ −108° (*c* 1, $CHCl_3$); ir (CH_2Cl_2) 3580, 3527 (OH); 1775 (γ-lactone); 1618, 1515, 1503, 1484 (arom C=C); nmr ($CDCl_3$) δ 6.85 (1 H, s), 6.56 (1 H, s), 6.27 (2 H, s), H, C-8, H, C-5, and 2 H, C-2′, C-6′, 5.99 (2 H, s), CH_2O_2, 5.52 (1 H, s), H, phenol OH, 3.76 (6 H, s), 2 CH_3O, 1.52 (3 H, s) and 1.44 (3 H, s) H isopropylidene.

Method B. 4′-Demethyl-1-*O*-[4,6-*O*-(ethylidene)-β-D-glucopyranosyl]epipodophyllotoxin (5).—To a suspension of 1.5 g of 4′-demethylepipodophyllotoxin β-D-glucopyranoside in 30 ml of CH_3NO_2 was added 6 ml of acetaldehyde dimethyl acetal and 150 mg of *p*-TsOH, and the mixt was stirred under N_2 for 1 hr at 20°. The soln was dild with 400 ml of $CHCl_3$ and washed 3 times with H_2O (25-ml portions). The org phase was dried (Na_2SO_4) and evapd *in vacuo* yielding 1.74 g of crude product. Chromatog on 100 g of kieselgel using $CHCl_3$–MeOH (95:5) as eluant afforded 1.24 g of pure material which was crystd from MeOH: mp 236–251°; $[\alpha]^{20}D$ −110.5° (*c* 0.6, $CHCl_3$); ir (CH_2Cl_2) 3578, 3527 (OH), 1776 (γ-lactone), 1610, 1515, 1503, 1484 (arom C=C); nmr (DMSO-d_6) δ 8.23 (1 H, s), H of the phenolic OH, 7.03 (1 H, s), 6.55 (1 H, s), 6.21 (2 H, s), H at C-8, C-5, C-2′ and C-6′, 6.04 (2 H, s) H CH_2O_2, 3.62 (6 H, s), 2 CH_3O, 1.25 (3 H, d, J = 5 Hz) CH_3CH.

Method C. 4′-Demethyl-1-*O*-[4,6-*O*-(cyclopentylidene)-β-D-glucopyranosyl]epipodophyllotoxin (44).—A mixt of 2 g of dried 4′-demethylepipodophyllotoxin β-D-glucopyranoside in 40 ml of cyclopentanone and 4 g of Dowex WX2 ion exchanger was stirred for 2 hr at 20° with exclusion of moisture. The catalyst was filtered, and the filtrate was dild with 500 ml of $CHCl_3$. The soln was washed several times with H_2O, dried (Na_2SO_4), and evapd. The residue was chromatographed on 120 g of kieselgel using $CHCl_3$–MeOH (96:4) as eluant. A pure product was obtd after repeated chromatog of the main fractions. The anal. sample (400 mg) was crystd from EtOH–Et_2O: mp 176–182°; $[\alpha]^{20}D$ −105.8° (*c* 0.8, $CHCl_3$); ir (CH_2Cl_2) 3575, 3525 (OH), 1775 (γ-lactone), 1620, 1518, 1504, 1485 (arom C=C); nmr ($CDCl_3$) δ 6.83 (1 H, s), 6.53 (1 H, s), 6.24 (2 H, s), H at C-8, C-5, C-2′, and C-6′, 5.96 (2 H, s), CH_2O_2, 5.6–5.4 (1 H, m), H of phenolic OH, 3.74 (6 H, s), 2 CH_3O, 2.25–1.4 (8 H, m), H cyclopentylidene.

Carcinogenic and Adrenocorticolytic Derivatives of Benz[*a*]anthracene[1]

JOHN PATAKI,* CAROL DUGUID, PETER W. RABIDEAU, HOWARD HUISMAN, AND RONALD G. HARVEY*

The Ben May Laboratory for Cancer Research, The University of Chicago, Chicago, Illinois 60637

Received March 17, 1971

A series of dimethyl, trimethyl, hydroxymethyl, and formyl derivatives of benz[*a*]anthracene structurally related to the carcinogenic 7-methyl-, 12-methyl-, and 7,12-dimethylbenz[*a*]anthracene and to the adrenocorticolytic 7-hydroxymethyl-12-methylbenz[*a*]anthracene were synthesized. Me substitution outside the "*critical*" region (*i.e.*, the 6, 7, 8, and 12 positions) was shown to block sarcomagenic activity when in the 1, 2, 3, 4, and 5 positions (one exception noted), whereas introduction of Me groups elsewhere in the molecule was without apparent effect on the biological action.

Introduction of one, two, or three Me groups into the 6, 7, 8, or 12 positions of benz[*a*]anthracene dramatically transforms this biologically inert hydrocarbon into a highly potent carcinogen.[2] The position of substitution is critical in that Me groups in other sites fail to elicit these effects. 7,12-Dimethylbenz[*a*]anthracene (7,12-DMBA) appears unique in this series in its ability to also destroy the adrenal cortex of the rat.[3] 7-Hydroxymethyl-12-methylbenz[*a*]anthracene, formed *in vivo*, has been shown to be the active intermediate species,[4] and several other 7-hydroxyalkyl (and potential 7-hydroxyalkyl) derivatives of benz[*a*]anthracene have also been found active.[3b]

In connection with our continuing investigations of the relation between the structure and the carcinogenic[2] and andrenocorticolytic[3] activity of derivatives of benz[*a*]anthracene, we prepared a series of dimethyl, trimethyl, hydroxymethyl, and formyl derivatives of benz[*a*]anthracene including several active new compounds, whose synthesis we now report.

The trimethylbenz[*a*]anthracene (TMBA) isomers synthesized,† except for 6,8,12-TMBA, were members

(1) This investigation was supported by grants from the American Cancer Society, Jane Coffin Childs Memorial Fund, and Daisy Schwimmer Memorial Fund.

(2) (a) J. Pataki and C. B. Huggins, *Cancer Res.*, **29**, 506 (1969); (b) C. B. Huggins, J. Pataki, and R. G. Harvey, *Proc. Nat. Acad. Sci. U. S.*, **58**, 2253 (1967); (c) J. Pataki and C. B. Huggins, *Jerusalem Symp. Quantum Chem. Biochem.* **1**, 64 (1969).

(3) (a) J. Pataki, R. Wlos, and Y. Cho, *J. Med. Chem.*, **11**, 1083 (1968); (b) J. Pataki and C. B. Huggins, *Biochem. Pharmacol.*, **16**, 607 (1967); (c) C. B. Huggins, S. Morii, and J. Pataki, *Proc. Nat. Acad. Sci. U. S.*, **62**, 704 (1969).

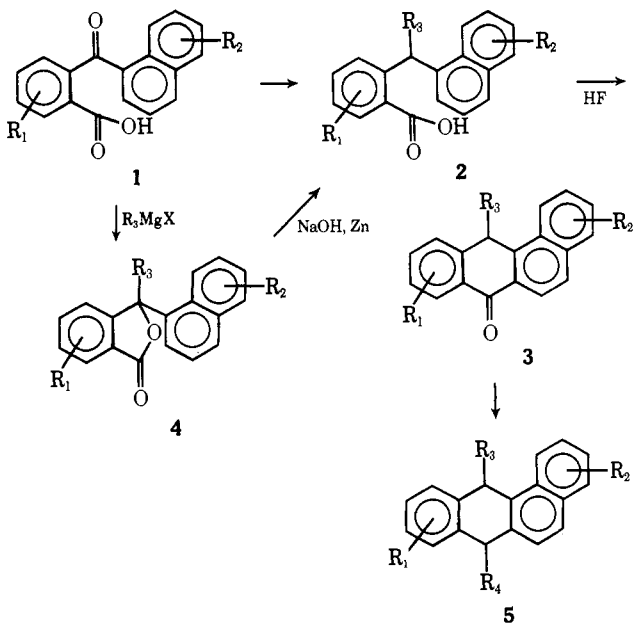
(4) D. N. Wheatley, I. R. Kernokan, and A. R. Currie, *Nature (London)*, **211**, 387 (1966).

† There are theoretically possible a total of 220 trimethylbenz[*a*]anthracene and 66 dimethylbenz[*a*]anthracene isomers; of the latter, 21 have one or both Me groups in the 7 or 12 positions.

of the series *x*,7,12-TMBA, specifically those where *x* = 2, 3, 5, 6, 9, and 10.[5] These molecules, distinguished by the presence of Me groups in both the *"critical"* region (*i.e.*, 6, 7, 8, and 12 positions) and elsewhere in the benz[*a*]anthracene structure, were of particular interest in connection with the question of whether methyl substituents outside the critical region play an active or a passive role. In other words, do these Me groups block biological action or merely fail to activate the molecule for biochemical transformation?

Scheme I depicts the approach adopted for the synthesis of the majority of the compounds reported herein. The starting materials were the keto acids **1** derived from interaction of phthalic anhydride or its Me derivative with the Grignard reagent from the appropriately substituted 1-bromonaphthalene. Thus, 5,7,12- and 6,7,12-TMBA **5a,b** were obtained through a sequence of transformations beginning with phthalic anhydride and the Grignard reagent derived from 1-bromo-4- and 1-bromo-3-methylnaphthalene, resp. The keto acids **1a,b** formed in the initial step underwent conversion upon treatment with MeMgBr to the methyl lactones **4a,b**; hydrolysis in alcoholic NaOH and reduction with Zn furnished the acid **2a,b**. Cyclization of the latter to the ketone **3a,b** was smoothly effected in liquid HF; methylation with MeLi and acid-catalyzed dehydration afforded **5a,b.**

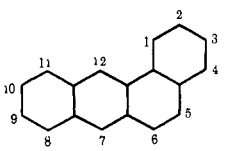
SCHEME I



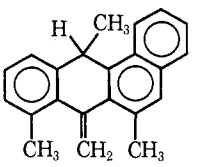
	a	b	c	d
R_1	H	H	8-CH_3	8-CH_3
R_2	5-CH_3	6-CH_3	6-CH_3	6-CH_3
R_3	CH_3	CH_3	CH_3	CH_3
R_4	CH_3	CH_3	H	CH_3

e	f	g	h	i
H	H	H	H	H
4-CH_3	5-CH_3	6-CH_3	4-CH_3	6-CH_3
CH_3	CH_3	CH_3	H	H
H	H	H	CH_3	CH_3

The numbering system utilized throughout corresponds to that of benz[*a*]anthracene:



A similar approach was utilized for the preparation of 6,8,12-TMBA[8] **5c** from 3-methyl phthalic anhydride and 1-bromo-3-methylnaphthalene, except that 6,8,-12-trimethylbenz[*a*]anthr-7-one **3c** was reduced instead of being methylated in the final stage. Reaction of **3c** with MeLi and dehydration of the product with $POCl_3$ afforded 7-methylene-6,8,12-trimethyl-7,12-dihydrobenz[*a*]anthracene **6** rather than the isomeric tetramethylbenz[*a*]anthracene **5d**. The structure assigned to **6** was supported by the nmr spectrum which



exhibited a pair of vinylic protons as an apparent singlet at δ 5.7, a benzylic proton as a quartet at 4.8 (J = 7 Hz), two Me singlets (6 H) at 2.6 and 2.7, and a Me doublet (3 H) at 1.5 (J = 7 Hz). Isomerization of **6** to **5d** could not be effected under acid or alkaline conditions, apparently due to steric inhibition of resonance.

Dimethyl derivatives of benz[*a*]anthracene bearing a Me group in either the 7 or 12 positions were readily synthesized by appropriate modification of this approach. Thus, the cyclic dimethyl ketones **3e,a,b**, upon reduction and dehydration afforded 4,12-, 5,12-, and 6,12-dimethylbenz[*a*]anthracene (**5e,f,g**) resp. Treatment of 4- and 6-methylbenz[*a*]anthr-7-one (**3h,i**) with MeMgBr and MeLi, resp, followed by acid-catalyzed dehydration furnished 4,7- and 6,7-dimethylbenz[*a*]anthracene (**5h,i**), resp.

An alternative synthetic approach was employed for the *x*,7,12-TMBA isomers for which *x* = 2, 3, and 10. These compounds were prepared from the corresponding *x*-methylbenz[*a*]anthracene *via* the sequence: (1) Li–NH_3 reduction; (2) methylation with *n*-BuLi and MeBr in liq NH_3; and (3) rearomatization with S (Scheme II).

Although 1-step reductive dialkylation in liq NH_3 is feasible,[9] the 2-stage approach was found advantageous in that it allowed more efficient removal by recrystallization of minor secondary products formed in the initial stage. Dimethylation of the purified dihydro derivatives, **7**, proceeded smoothly to furnish virtually quantitative yields of the corresponding *x*,7,12-trimethyl-7,12-dihydrobenz[*a*]anthracene compounds **8a–8c**. These were designated as cis on the basis of prior assignment of cis stereochemistry to the product

(5) Two additional isomers (*x* = 4 and 8) were prepared according to methods previously described.[6,7]

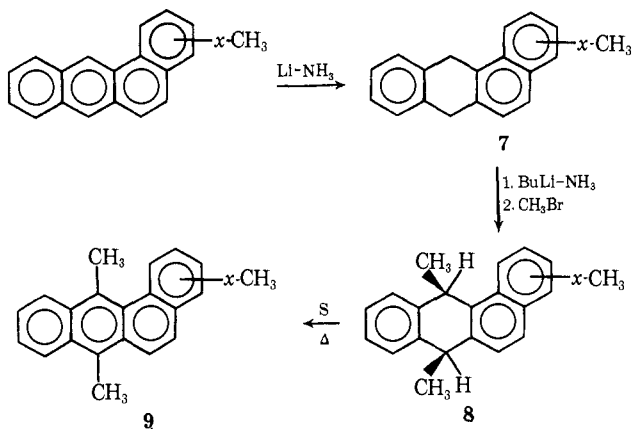
(6) C. Descamps and R. Martin, *Bull. Soc. Chim. Belg.*, **61**, 223 (1952).

(7) W. Bachmann and J. Chemerda, *J. Amer. Chem. Soc.*, **60**, 1023 (1938).

(8) During the preparation of this manuscript, Professor M. S. Newman, Ohio State University, informed us in a private communication of the synthesis of **5c** by Dr. W. Hung in his laboratory *via* an essentially identical approach; the mp of **5c**, as well as those of the intermediates, **2c**, **4c**, and **3c**, were in close agreement with the values reported herein.

(9) (a) R. G. Harvey and L. Arzadon, *Tetrahedron*, **25**, 4887 (1969); (b) R. G. Harvey and C. C. Davis, *J. Org. Chem.*, **34**, 3607 (1969).

SCHEME II



a, $x = 2$
b, $x = 3$
c, $x = 10$
d, $x = 11$

of analogous reaction of benz[*a*]anthracene; nmr chemical shift data in comparison with that reported earlier for the related cis[9] and trans[10] isomers of benz[*a*]anthracene was also consistent with this assignment. Dehydrogenation of **8a–8c** with sulfur afforded the fully aromatic trimethylbenz[*a*]anthracene derivatives **9a–9c**.

Attempted synthesis of 7,11,12-TMBA from 11-MBA by an analogous approach was frustrated by the resistance of the 7,12-dihydro intermediate **8d** to dehydrogenation with all reagents employed (S, DDQ, Pd/C, $AlCl_3$); decomposition ensued under more strenuous conditions. This resistance undoubtedly stems from the strong steric interaction expected between the Me group at C-12 and the substituents in the adjacent 1 and 11 positions of the aromatic product **9d**.

The remaining *x*,7,12-TMBA isomer, 7,9,12-TMBA, was obtained through treatment of 9-methylbenz[*a*]anthraquinone with MeMgI, utilizing a modification of the procedure of Sandin and Fieser.[11]

Introduction of the formyl group into the 7 position of 12-methylbenz[*a*]anthracene was previously achieved in low yield by Badger and Cook[12a] by the method of Bachmann.[12b] We found it more convenient to prepare the analogous 4,12- and 5,12-dimethyl-7-formylbenz[*a*]anthracene compounds **10a,b**, from **5e,f**, by treatment with oxalyl chloride and DMF in $(ClCH_2)_2$. Reduction of the formyl derivatives with $NaBH_4$ in pyridine proceeded smoothly at room temp to furnish the corresponding 7-hydroxymethyl derivatives **11a,b**.

Finally, 7,12-dicyanobenz[*a*]anthracene was synthesized from the dioxime of 7,12-diformylbenz[*a*]anthracene by treatment with Ac_2O at reflux temp.

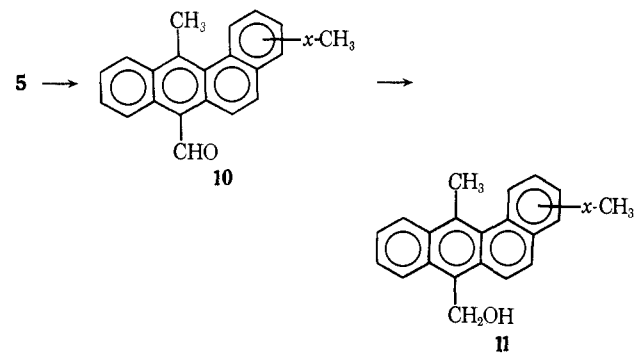
The sarcomagenic activity of the compounds reported herein are summarized in Table I. Included for the sake of completeness are data reported earlier[2a] for several of the compounds. Since the results of more complete biological evaluation are to be presented elsewhere, we shall only summarize briefly the salient features of these results. Me substitution outside the critical region in the 1, 4, and 5 positions of 7- and 12-methylbenz[*a*]anthracene appears to effectively abolish activity; whereas, introduction of Me groups into the 6, 8, or 11 positions of these same hydrocarbons is without effect on biological activity within the limits of the experimental method. The consequences of Me substitution in 7,12-DMBA are similar; activity is lost with methyl groups in the 2, 3, and 5 positions, and retained with Me groups in the 4, 6, 8, 9, and 10 positions. The only inconsistency between the two series is the high activity of 4,7,12-TMBA compared with the inactivity of 4,12-DMBA. Tentatively, therefore, it appears that Me substitution in positions 1 through 5 of carcinogenic benz[*a*]anthracene compounds can block biological action of these molecules, whereas methylation elsewhere is without effect is this respect. Although the significance of this finding is at present

TABLE I

SPINDLE-CELL SARCOMA EVOKED BY ALKYL DERIVATIVES OF BENZ[*a*]ANTHRACENE[a]

Compd	No. of Rats	No. with Sarcoma	Range, days
1,12-DMBA[b]	8	0	
4,7-DMBA	8	0	
4,12-DMBA	8	0	
5,12-DMBA	8	0	
6,7-DMBA[b]	8	8	70–126
6,12-DMBA[b]	7	7	36–153
7,8-DMBA[b]	7	7	82–163
7,11-DMBA	7	7	109–190
7,12-DMBA[b]	20	20	67–116
8,12-DMBA[b]	8	8	67–97
7-CH_2OH-4,12-DMBA	8	1	246
7-CH_2OH-5,12-DMBA[c]	8	0	
2,7,12-TMBA	16	0	
3,7,12-TMBA	8	0	
4,7,12-TMBA	8	8	52–97
5,7,12-TMBA	8	1	121
6,7,12-TMBA[b]	6	6	102–130
6,8,12-TMBA[b]	7	7	52–123
7,9,12-TMBA	8	8	46–94
7,10,12-TMBA	7	7	55–97
7,12-Dicyano-BA	12	0	

[a] Male Long–Evans rats, 25 days old were injected im with a solution of sesame oil, 0.5 ml, containing 2.5 mg of the compound. The experiment was terminated at 9 months. [b] Carcinogenic activity previously reported.[2a] [c] This compd is also inactive as an adrenocorticolytic agent despite the high activity of 7-hydroxymethyl-12-methylbenz[*a*]anthracene.[3]



a, $x = 4$
b, $x = 5$

(10) R. G. Harvey, L. Arzadon, J. Grant, and K. Urberg, *J. Amer. Chem. Soc.*, **91**, 4535 (1969).
(11) R. Sandin and L. Fieser, *ibid.*, **62**, 3098 (1940).
(12) (a) G. M. Badger and J. W. Cook, *J. Chem. Soc.*, 409 (1940); (b) W. E. Bachmann, *J. Org. Chem.*, **1**, 347 (1936).

unknown, an attractive hypothesis is that metabolic activation of the carcinogen occurs in this region.

Experimental Section[13]

2-[1-(4-Methylnaphthoyl)]benzoic Acid (1a).—A Grignard soln, prepd from 97.3 g (0.44 mole) of 1-bromo-4-methylnaphthalene and 12.9 g (0.53 g-atom) of Mg turnings in 250 ml of Et_2O and 90 ml of C_6H_6, was added within 25 min to a stirred warm soln of 71.60 g (0.48 mole) of phthalic anhydride in 750 ml of C_6H_6. The mixt was then refluxed for 2.5 hr and cooled, and 400 ml of ice-cold 10% HCl was slowly added. Conventional work-up and crystn from C_6H_6 afforded 92.21 g of **1a**, mp 150–152°; anal. sample, mp 153–155°. *Anal.* ($C_{19}H_{14}O_3$) C, H.

2-[1-Hydroxy-1-(4-methyl-1-naphthyl)ethyl]benzoic Acid Lactone (4a).—To a stirred soln of 50.00 g of **1a** in 750 ml of C_6H_6 and 375 ml of Et_2O, 170 ml of a 3 *M* MeMgBr soln was added slowly. The soln was then refluxed for 2 hr, cooled, and decomposed with 10% HCl soln. Work-up by conventional procedures gave 46.90 g of neutral fraction, which crystd from EtOH to provide 29.59 g of **4a**, mp 145–148°; anal. sample, mp 148.5–150°. *Anal.* ($C_{20}H_{16}O_2$) C, H.

2-[1-(4-Methyl-1-naphthyl)ethyl]benzoic Acid (2a).—A soln of 25 g of **4a** in 500 ml of a 5% NaOH soln in 85% EtOH was evapd to dryness under reduced pressure, and the solid residue was dissolved in 750 ml of H_2O. To this was added 75.0 g of Zn dust, and the mixt was stirred at reflux temp for 25 hr. The filtrate from the reaction mixt was acidified with HCl, and the resulting ppt was collected and dried. On crystn from MeOH–C_6H_6, 15.30 g of **2a**, mp 193–194°, and 5.74 g, mp 191.5–193°, were obtd; the anal. sample (MeOH) had mp 193–194°. *Anal.* ($C_{20}H_{18}O_2$) C, H.

5,12-Dimethylbenz[*a*]anthr-7-one (3a).—A soln of 18.20 g of **2a** in 120 ml of anhyd HF was allowed to evaporate overnight at room temp. The residue was dissolved in C_6H_6, washed with 5% aq Na_2CO_3 and H_2O, and evapd to dryness to afford 17.36 g of crude **3a** as a yellow foam.

5,7,12-Trimethylbenz[*a*]anthracene (5a).—To a stirred soln of 13.18 g of crude **3a** in 130 ml of Et_2O and 65 ml of C_6H_6, 100 ml of a 5% soln of MeLi was added, and the soln was refluxed for 2.5 hr under N_2. The soln was cooled, then decompd with ice-cold 6% HCl, and worked up in the usual way. The resulting yellow foam (14.90 g) was dissolved in 200 ml of C_6H_6 and stirred with 2.0 g of TsOH for 1 hr at reflux temp. The cold soln was washed until neutral with aq Na_2CO_3 and evapd to dryness, and the residue (13.64 g) was chromatogd over 420 g of alumina. Elution with 12% Et_2O–petr ether afforded 11.80 g of material which was crystd from hexane to give 10.57 g of **5a**, mp 127.5–129.5°; anal. sample, mp 129–131°. *Anal.* ($C_{21}H_{18}$) C, H.

2-[1-(3-Methylnaphthoyl]benzoic acid (1b) was prepd from 119.5 g of 1-bromo-3-methylnaphthalene[14] and 89.6 g of phthalic anhydride by essentially the same method employed for synthesis of **1a**. The yellow cryst product (121.1 g) was recrystd from C_6H_6 to furnish 79.00 g of **1b**, mp 189–192°, 19.47 g, mp 184–188°, and 9.32 g, mp 182–187°; anal. sample, mp 192–193°. *Anal.* ($C_{19}H_{14}O_3$) C, H.

2-[1-Hydroxy-1-(3-methyl-1-naphthyl)ethyl]benzoic Acid Lactone (4b).—To a stirred soln of 75 g of **1b** in 1900 ml of warm C_6H_6, 275 ml of a 3 *M* MeMgBr soln was added dropwise over 45 min. The mixt was then refluxed for 2 hr and worked up in the usual manner. The neutral portion recrystd from C_6H_6–EtOH gave 36.99 g of **4b**, mp 186–189°; anal. sample (EtOH, 2x) mp 188–189°. *Anal.* ($C_{20}H_{16}O_2$) C, H.

2-[1-(3-Methyl-1-naphthyl)ethyl]benzoic acid (2b) was prepd from 36 g of **4b** by the procedure utilized for **2a** except that refluxing with Zn (120 g) was contd for 48 hr. The crude material recrystd from C_6H_6 gave 32.09 g of **2b**, mp 185–186°; anal. sample, mp 186.5–187.5°. *Anal.* ($C_{20}H_{18}O_2$) C, H.

6,12-Dimethylbenz[*a*]anthr-7-one (3b).—A soln of 31.0 g of **2b** in 150 ml of anhyd HF was allowed to evap in a hood overnight. The residue was dissolved in EtOAc, washed with 5% aq Na_2CO_3 and H_2O, and dried. The evapn residue, 28.60 g, was a light yellow syrup.

6,7,12-Trimethylbenz[*a*]anthracene (5b).—Treatment of 15.10 g of crude **3b** with MeLi (6 hr) by the procedure for **5a** afforded an oil which crystd from CH_2Cl_2–hexane to give 7.15 g of **5b**, mp 177–178°; anal. sample, mp 178–178.5°. *Anal.* ($C_{21}H_{18}$) C, H.

2-[1-Hydroxy-1-(3-methyl-1-naphthyl)ethyl]-6-methylbenzoic Acid Lactone (4c).—Interaction of 30.20 g of **1c** (mp 189–191.5°; lit.[14] 182–183°) with MeMgBr by the method described for **4a** (addition 20 min; reflux 24 hr) afforded a neutral product fraction portion (28.39 g) which gave (from EtOH) 25.31 g of **4c**, mp 145–147°; anal. sample, mp 146.5–147°; lit.[15] 147–148°. *Anal.* ($C_{21}H_{18}O_2$) C, H.

2-[1-(3-Methyl-1-naphthyl)ethyl]-6-methylbenzoic Acid (2c).—The residue obtained from treatment of 25.0 g of **4c** with ethanolic NaOH in the usual manner was taken up in 750 ml of H_2O and 150 ml of concd NH_4OH; 75 g of Zn dust was added, and the mixt was stirred at reflux for 43 hr. The product was worked up as for **2a**. The acid portion, 22.46 g, was crystd from C_6H_6, affording 19.82 g of **2c**, mp 220–222°, and a second fraction of 2.13 g, mp 218–221°; anal. sample, mp 220.5–222°; lit.[15] 219–220°. *Anal.* ($C_{21}H_{20}O_2$) C, H.

6,8,12-Trimethylbenz[*a*]anthr-7-one (3c).—Treatment of 20.6 g of **2c** with 200 ml of anhyd HF as for **3a** afforded 19.44 g of a neutral product. Crystn from EtOAc–EtOH provided 16.76 g of **3c**, mp 165.5–167°, and 2.07 g, mp 162.5–165°; anal. sample (EtOAc), mp 166–167°; lit.[15] 165–166°. *Anal.* ($C_{21}H_{18}O$) C, H.

6,8,12-Trimethylbenz[*a*]anthracene (5c).—A soln of 4.00 g of **3c** in 32 ml of toluene was stirred under reflux with 100 ml of 10% NaOH soln and 20.0 g of Zn–Cu couple for 30 hr. The mixt was filtered, and the org layer sepd. The evapn residue (3.70 g) was dissolved in 200 ml of C_6H_6 and refluxed with 2.0 g of TsOH for 45 min. After the usual work-up, the crude product was crystd from Et_2O–hexane to give 2.30 g of **5c**, mp 131.5–135°. Recrystn from hexane gave the pure compd, mp 138–138.5°; lit.[15] 137–138°. *Anal.* ($C_{21}H_{18}$) C, H.

7-Methylene-6,8,12-trimethyl-7,12-dihydrobenz[*a*]anthracene (6).—To 6.00 g of **3c** dissolved in 100 ml of C_6H_6, 100 ml of a 5.16% MeLi soln in Et_2O was added. The soln was stirred at reflux for 6.5 hr under N_2, then cooled in an ice bath and decompd with HCl. The org layer was washed with H_2O and dried (Na_2SO_4), and the solvents were removed. The residue (7.13 g) was dissolved in 55 ml of anhyd pyridine, and 5 ml of $POCl_3$ was added to the soln at 0°. After standing 24 hr at room temp, the reaction mixt was worked up in the usual manner. The crude dehydration product (6.20 g) was chromatogd over 200 mg of alumina. Petr ether–C_6H_6 (1:1) eluted 4.26 g of a product which crystd from hexane to yield **6**, 1.784 g, mp 112–114°, and 1.14 g, mp 109–111.5; anal. sample, mp 113–114°. *Anal.* ($C_{22}H_{20}$) C, H.

2-[1-Hydroxy-1-(5-methyl-1-naphthyl)ethyl]benzoic Acid Lactone (4e).—To a well-stirred soln of 35.70 g of 1e[6] in 1100 ml of anhyd C_6H_6 and 300 ml of anhyd Et_2O, 120 ml of a 3 *M* MeMgBr soln was added over 35 min. The ppt which initially formed, dissolved at the end of the addn. The soln was refluxed with stirring for 2 hr, cooled, and decompd with 10% HCl. The org layer was washed with aq Na_2CO_3 and H_2O, and dried. Evapn of the solvent left 37.09 g of a reddish foam.

2-[1-(5-Methyl-1-naphthyl)ethyl]benzoic Acid (2e).—Conversion of the crude lactone **4e** (37.00 g) to the acid **2e** was achieved by the method employed for synthesis of **2c**, except the reaction was maintained at reflux for 27 hr only. The filtered soln was acidified with concd HCl, and the resulting ppt was taken up in C_6H_6–Et_2O (1:2). The soln was extd 3 times with 2.5% aq Na_2CO_3, the exts were again acidified with concd HCl, and the ppt was filtered off and washed well with H_2O. After drying, the soln was concd until incipient crystn to yield 13.75 g of **2e**, mp 189–191, and 6.89 g, mp 188–190.5°; anal. sample, mp 189.5–191.5°. *Anal.* ($C_{20}H_{18}O_2$) C, H.

4,12-Dimethylbenz[*a*]anthr-7-one (3e).—Cyclization of 20.5 g of **2e** in anhyd HF by the method employed for **3b** afforded, after the usual work-up (EtOAc solvent), a residue, 17.78 g, which was crystd from EtOAc–EtOH to afford 15.76 g of **3e**, mp 119.5–121°; anal. sample, mp 120–121°. *Anal.* ($C_{20}H_{16}O$) C, H.

4,12-Dimethylbenz[*a*]anthracene (5e).—To a soln of 15.50 g of **3e** in 240 ml of diglyme, 5.0 g of $NaBH_4$ was added, and the soln was allowed to stand for 22 hr at room temp. Conventional work-up gave a residue, 18.02 g, which was refluxed in 300 ml of C_6H_6 with 3.0 g of TsOH for 1 hr, cooled, and washed with aq

(13) All mps were determined on a Büchi capillary mp apparatus and are uncor. The structures of all compds were supported by ir and nmr spectra, obtained on a Perkin-Elmer Model 137 infracord spectrometer and a Varian A-60 spectrometer, resp. Where analyses are indicated only by symbols of the elements, anal. results obtained for these elements were within ±0.4% of the theor values.

(14) M. S. Newman and S. Blum, *J. Med. Chem.*, **7**, 466 (1964).

(15) M. S. Newman and W. Hung, private communication.

Na_2CO_3 and with H_2O. The dried soln was poured on a column of 200 g of alumina, eluted with **1** l. of Et_2O, and evapd. The residue, 13.45 g, crystd from Me_2CO–hexane to afford **5e**, 8.88 g, mp 114.5–117°, second crop, 1.91 g, mp 113–115.5°; anal. sample, mp 116–118°. *Anal.* ($C_{20}H_{16}$) C, H.

5,12-Dimethylbenz[*a*]anthracene (5f).—Reduction of 8.96 g of crude **3a** in PhMe–10% aq NaOH with 34.0 g of Zn–Cu couple for 24 hr, according to the procedure for **5c**, gave 8.67 g, of 5,12-dimethylbenz[*a*]anthr-7-ol. Dehydration was accomplished by refluxing in 100 ml of C_6H_6 with 1.0 g of TsOH for 1 hr. After the usual work-up, the crude product was crystd from hexane to afford 5.75 g of **5f**, mp 89–91°; lit.[16] mp 93–93.5.

6,12-Dimethylbenz[*a*]anthracene (5g).—A soln of 4.77 g of crude **3b** in 100 ml of glacial AcOH was stirred at reflux temp with 15.0 g of Zn–Cu couple for 24 hr. The mixt was filtered, the filtrate was reduced to *ca.* 50 ml *in vacuo*, and H_2O was added. The resulting pale yellow ppt was dissolved in Et_2O, and the soln was washed with 5% aq Na_2CO_3 and with H_2O. The soln was dried and evapd to furnish a residue which was crystd from hexane to give 2.90 g of **5g**, mp 75–76.5°; lit.[17] mp 75.1–75.5°.

2-{1-(5-Methylnaphthyl)methyl]benzoic Acid (2h).—A soln of 12 g of **1e**[6] in 280 ml of a 10% NaOH soln was stirred with 24 g of Zn dust at reflux for 30 hr, filtered, cooled, and acidified with 15% HCl. The resulting ppt was filtered, washed well with H_2O, and dried. The crude acid (10.66 g) was crystd from C_6H_6 to provide **2h**, 8.40 g, mp 196.5–198°; second crop, 1.21 g, mp 196–197°; anal. sample, mp 197–198°. *Anal.* ($C_{19}H_{16}O_2$) C, H.

4-Methylbenz[*a*]anthr-7-one (3h).—Cyclization of 9.2 g of **2h** in anhyd HF by the method employed for **3b** afforded after the usual work-up ($CHCl_3$ solvent) a residue (8.79 g) which was crystd from EtOAc to afford **3h**, 6.69 g, mp 174–175.5° dec, and 1.14 g, mp 173.5–175.5° dec; anal. sample, mp 175–176° dec. *Anal.* ($C_{19}H_{14}O$) C, H.

4,7-Dimethylbenz[*a*]anthracene (5h).—To a soln of 10 ml of 3 *M* MeMgBr in 75 ml of C_6H_6 was added **3h** (5.0 g) all at once. The resulting soln was stirred at ambient temp for 3.5 hr, then chilled, and decompd by addn of 10% HCl. Conventional work-up provided a residue (5.02 g) which was dissolved in 100 ml of C_6H_6 and heated under reflux with 1.00 g of *p*-TsOH for **1** hr. The usual work-up followed by filtration through a column of 100 g of alumina (eluted with C_6H_6) provided 3.41 g of **5h**. Recrystn from CH_2Cl_2–hexane gave 2.40 g, mp 154–155°, and 0.82 g, mp 153–154°; anal. sample, mp 154–155°; lit.[18] mp 154–154.5°. *Anal.* ($C_{20}H_{16}$) C, H.

2-[1-3-Methylnaphthyl)methyl]benzoic Acid (2i).—To a soln of 25.00 g of **1b** in 625 ml of 10% NaOH was added 50 g of Zn–Cu couple, and the mixt was stirred at reflux temp for 36 hr. Conventional work-up procedure gave the crude acid (22.32 g) which was crystd from C_6H_6 to afford 19.38 g of **2i**, mp 183–184°, and 1.53 g, mp 181–184°; anal. sample, mp 184–185°. *Anal.* ($C_{19}H_{16}O_2$) C, H.

6-Methylbenz[*a*]anthr-7-one (3i).—Cyclization of **2i** (20 g) in liquid HF (*ca.* 150 ml) under essentially the same conditions employed for the prepn of **2h** gave **3i**, 15.28 g, mp 147–151°; anal. sample, mp 151–152°. *Anal.* ($C_{19}H_{14}O$) C, H.

6,7-Dimethylbenz[*a*]anthracene (5i).—To a well-stirred soln of 6.00 g of **3i** in 250 ml of C_6H_6, 100 ml of a 5.16% soln of MeLi in Et_2O was added. The soln was refluxed under N_2 for 6 hr, chilled, and decompd by dropwise addn of 10% HCl. Conventional work-up and passage through a column of 150 g of alumina (eluted with 700 ml of C_6H_6) gave an oil (4.52 g) which crystd from CH_2Cl_2–MeOH to furnish **5i**, 3.11 g, mp 112–114°; lit.[6] mp 114–114.4°.

2-Methyl-7,12-dihydrobenz[*a*]anthracene (7a).—2-Methylbenz[*a*]anthracene (5.4 g) underwent smooth reduction to **7a** with Li in NH_3 in the presence of $FeCl_3$ according to the method previously described for benz[*a*]anthracene.[19] Recrystn from EtOH provided pure **7a**, 4.42 g (82%), mp 99°. *Anal.* ($C_{19}H_{16}$) C, H.

2,7,12-Trimethyl-7,12-dihydrobenz[*a*]anthracene (8a).—Dimethylation of **7a** (4.0 g) with *n*-BuLi and CH_3Br in NH_3, according to the general method reported earlier from this laboratory,[9] provided almost pure (by nmr) **8a** as a syrup. Crystn from EtOH afforded **8a**, 3.91 g (98%), mp 137–137.5°. *Anal.* ($C_{21}H_{20}$) C, H.

2,7,12-Trimethylbenz[*a*]anthracene (9a).—Dehydrogenation of **8a** was accomplished by heating 1.5 g of **8a** with 190 mg of S under N_2 at 270° for 15 min. Crystn of the crude product from EtOH gave **9a**, 1.07 g (72%), mp 103–103.5°; lit.[20] 107°.

3,7,12-Trimethylbenz[*a*]anthracene (9b) was prepd from 3-methylbenz[*a*]anthracene by essentialy the same synth sequence employed for **9a**. The analogous intermediates, **7b** and **8b**, were used as isolated without further purification. The crude oily product crystd in the cold from EtOH to furnish **9b**, mp 122°. *Anal.* ($C_{21}H_{18}$) C, H.

10-Methyl-7,12-dihydrobenz[*a*]anthracene (7c).—Reduction of 10-methylbenz[*a*]anthracene with Li in NH_3 in the presence of $FeCl_3$ by the usual method[19] furnished essentially quant **7c**, mp 139.5–140.5°. *Anal.* ($C_{19}H_{16}$) C, H.

7,10,12-Trimethyl-7,12-dihydrobenz[*a*]anthracene (8c).—Dimethylation of **7c** with *n*-BuLi and CH_3Br in NH_3 by the general method[9] utilized in related syntheses afforded **8c**, (86%), mp 134.5–135.5°. *Anal.* ($C_{21}H_{20}$) C, H.

7,10,12-Trimethylbenz[*a*]anthracene (9c).—Dehydrogenation of **8c** with S by the procedure employed for **9a,b** gave **9c** (*ca.* 70%), mp 140–141°; lit.[21] 139–140°.

11-Methyl-7,12-dihydrobenz[*a*]anthracene (7d).—Reduction of 11-methylbenz[*a*]anthracene[22] (2.3 g) by the method employed for **7a,b** gave **7d**, 2.30 g, mp 81.5–82.0°. *Anal.* ($C_{19}H_{16}$) C, H.

7,11,12-Trimethyl-7,12-dihydrobenz[*a*]anthracene (8d).—Dimethylation of **7d** utilizing the procedure employed for the analogous compds gave **8d** (96%), mp 131.5–132.5°. *Anal.* ($C_{21}H_{20}$) C, H.

7-Formyl-4,12-dimethylbenz[*a*]anthracene (10a).—To 4.38 g (4.64 ml; 0.06 mole) of DMF in 20 ml of $(CH_2Cl)_2$, 5.72 g (3.84 ml; 0.045 mole) of oxalyl chloride in 40 ml of $(CH_2Cl)_2$ was added dropwise, at 0° with stirring. After 27 min, a soln of 7.69 g (0.03 mole) of **5e** in 60 ml of $(CH_2Cl)_2$ was added. Stirring was contd for 5 hr at a bath temp of 60–62°. Then 200 ml of 5% aq NaOAc was added, and the org layer was separated, washed 4 times with H_2O, dried (Na_2SO_4), and evapd. The residue (8.02 g) was chromatogd on 300 ml of alumina. Initial fractions eluted with petr ether–Et_2O (1:1) contained starting material (5.05 g). The crude product (2.18 g) was eluted with 4% MeOH in Et_2O. Recrystn from Me_2CO gave 1.38 g of **10a**, mp 146–148°; anal. sample, mp 148–149°. *Anal.* ($C_{21}H_{16}O$) C, H.

7-Hydroxymethyl-4,12-dimethylbenz[*a*]anthracene (11a).—A soln of **10a** (1.25 g) and $NaBH_4$ (0.30 g) in 25 ml of pyridine was maintained at ambient temp for 22 hr, then dild with H_2O, and extd with EtOAc. The ext was washed with dil HCl and H_2O, dried, and concd to incipient crystn. There was obtd **11a**, 0.81 g, mp 202–204°, and 0.18 g, mp 200–202°; anal. sample, mp 204–205.5°. *Anal.* ($C_{21}H_{18}O$) C, H.

7-Formyl-5,12-dimethylbenz[*a*]anthracene (10b).—Formylation of **5f** (5.13 g) by the procedure employed for **10a** (reaction time 3 hr) furnished the crude product (5.46 g). Trituration with Et_2O gave an insol portion (3.63 g) which upon recrystn from Me_2CO–hexane, gave **10b**, 3.01 g, mp 118–120°; anal. sample, mp 119–120.5°. *Anal.* ($C_{21}H_{16}O$) C, H.

From the Et_2O-sol portion, 0.87 g of starting material **5f**, mp 89–91°, was recovered by crystn from hexane.

7-Hydroxymethyl-5,12-dimethylbenz[*a*]anthracene (11b).—The aldehyde (2.30 g) was reduced with $NaBH_4$ by the procedure employed for **11a**, and worked up in the same manner. Recrystn from EtOH gave 1.39 g of **11b**, mp 144–145°, and 0.33 g, mp 141.5–143°; anal. sample, mp 146–147°. *Anal.* ($C_{21}H_{18}O$), C, H.

7,12-Diformylbenz[*a*]anthracene Dioxime.—A soln of 7,12-diformylbenz[*a*]anthracene (1.63 g) in 48 ml of dry pyridine and 20 ml of abs EtOH was refluxed with 2.23 g of $H_2NOH \cdot HCl$ for 90 min under N_2. The soln was cooled, dild with EtOAc, washed with several portions of 5% HCl, and with H_2O, dried, and evapd *in vacuo*. The cryst residue weighed 1.73 g. A sample recrystd from EtOAc–hexane melted at 234–236°. *Anal.* ($C_{20}H_{14}N_2O_2$) C, H, N.

7,12-Dicyanobenz[*a*]anthracene.—A soln of 1.70 g of the dioxime in 100 ml of Ac_2O was warmed at reflux temp for 45 min under N_2. The crystals deposited on cooling were filtered off and washed with glacial AcOH and MeOH to afford the dinitrile, 1.27 g, mp 272–273.5°. The latter, sparingly sol in all common organic

(16) L. Fieser and A. M. Seligman, *J. Amer. Chem. Soc.*, **61**, 136 (1939).
(17) L. Fieser and R. N. Jones, *ibid.*, **60**, 1940 (1938).
(18) B. M. Mikhailov and T. K. Kozminskaya, *Zh. Obshch. Khim.*, **23**, 1220 (1953).
(19) R. G. Harvey and K. Urberg, *J. Org. Chem.*, **33**, 2206 (1968).
(20) N. Defay and R. Martin, *Bull. Soc. Chim. Belg.*, **64**, 210 (1955).
(21) W. Bachmann and J. Chemerda, *J. Org. Chem.*, **6**, 36 (1941).
(22) L. Fieser and L. Johnson, *J. Amer. Chem. Soc.*, **61**, 168 (1939).

solvents, was recrystd from C_6H_6 and melted at 273–274°. *Anal.* ($C_{20}H_{10}N_2$) C, H, N.

Acknowledgments.—We are indebted to Professor M. S. Newman, Ohio State University, for generous donation of samples of several rare hydrocarbons, including 1-, 3-, and 11-methylbenz[*a*]anthracene, and for detailed description of the synthesis of 1-bromo-3-methylnaphthalene. We are also grateful to Professor Charles B. Huggins, The University of Chicago, for permission to cite the biological test data obtained in his laboratory.

Specificity in Enzyme Inhibition. 1. Synthesis of 4-(4-Imidazolyl)-3-amino-2-butanone, 4-(4-Imidazolyl)-3-acetamido-2-butanone, and 4-(4-Imidazolylmethyl)-2,5-dimethyloxazole for Assay as Inhibitors of Histidine Decarboxylase

EDWARD E. SMISSMAN* AND JAMES A. WEIS[1]

Department of Medicinal Chemistry, School of Pharmacy, The University of Kansas, Lawrence, Kansas 66044

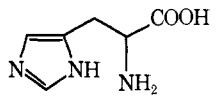
Received December 10, 1970

A general approach to specific enzyme inhibition is discussed. The synthesis and results of assay of 4-(4-imidazolyl)-3-amino-2-butanone (**2**), 4-(4-imidazolyl)-3-acetamido-2-butanone (**4**), and 4-(4-imidazolylmethyl)-2,5-dimethyloxazole are described.

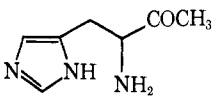
The receptor sites of many metabolic enzymes which utilize amino acids as substrates can be depicted as having 2 binding sites and 1 active site. The binding sites can be designated as specific and nonspecific. For example, if one views a model for the receptor site of a specific amino acid decarboxylase (Figure 1), the specific binding would be to the R group and would act to differentiate the amino acid to be used as a substrate. A nonspecific site would bind the amino group; the latter would act as the orienting function and would place the carboxyl group in juxtaposition to the site of chemical change, the active site.

On the basis of this model, an active-site-directed inhibitor of a decarboxylase enzyme should be capable of binding with the specific as well as the nonspecific sites of the enzyme but it should be incapable of undergoing the required chemical transformation, decarboxylation, at the active site. The same argument should apply to transaminases, aminopeptidases, aminesynthetases, certain oxidases, etc.

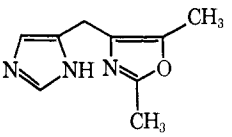
In order to investigate the applicability of this hypothesis, histidine was chosen as the substrate to be modified. L-Histidine decarboxylase is specific for the biosynthesis of histamine,[2] and a specific inhibitor of this enzyme should possess an imidazole ring which would approach and bind to the specific binding site, a basic N for binding to the nonspecific site, and a function incapable of decarboxylation to approach the active site. This phase of the investigation considered only reversible endo binding to the receptor.[3] In the initial study of the requirements of histidine analogs for decarboxylase inhibitor activity 3 compds were prepared. The α-amino ketone **2** would be expected to have the specific and nonspecific binding



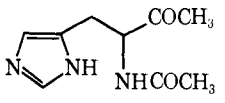
1



2



3



4

functions of histidine (**1**). The oxazole **3** would possess the basic nonspecific function and the specific imidazole ring but sterically might be less capable of endo binding to the receptor site. The *N*-acetyl-α-amino ketone **4** would have the specific binding function and the active-site-directed function which is incapable of decarboxylation, but it does not have the nonspecific binding group required for orientation of the Ac group to the active site.

It could be predicted that **2** would be an excellent and specific inhibitor, **3** would be specific but less active, and **4** would possess little or no activity as an inhibitor.

These compds were prepared from histidine·HCl (**1**) which underwent decarboxylative acetylation in pyridine and Ac_2O soln to provide 4-(4-imidazolyl)-3-acetamido-2-butanone·HCl (**4**) in 64% yield. Dakin and West[4] were unable to characterize this material, since they failed to obtain a crystalline product. In this study a crystalline product was obtained and spectral data support the proposed structure. The maximum yield was secured with mild reaction conditions.

The hydrolysis of the acetamido ketone **4** to produce 4-(4-imidazolyl)-3-amino-2-butanone·2HCl (**2**) was accomplished in 82% yield with 4 *N* HCl.

4-(4-Imidazolylmethyl)-2,5-dimethyloxazole·HCl (**3**) was obtained in 40% yield by refluxing the acet-

(1) Taken in part from the dissertation presented by J. A. Weis, Sept 1968, to the Graduate School of the University of Kansas, Lawrence, Kan., in partial fulfillment of the requirements for the Doctor of Philosophy Degree.

(2) G. Kahlson, E. Rosengren, and R. Thunberg, *J. Physiol. (London)*, **169**, 467 (1963).

(3) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967.

(4) H. D. Dakin and R. West, *J. Biol. Chem.*, **78**, 745, 757 (1928).