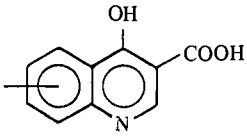
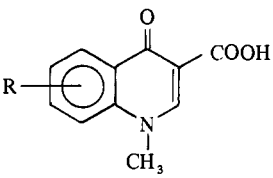
Table V. Physical Properties of R- 

No.	R[a]	Solvent[b]	Mp, °C	Yield, %	Formula[c]
3	6-C_4H_9-*n*	A	235-236 dec	89	$C_{14}H_{15}NO_3$
4	6-C_6H_5	B	288-289 dec	78	$C_{16}H_{11}NO_3$
5	6-$C_6H_5CH_2$	B	262-263 dec	42	$C_{17}H_{13}NO_3$
7	6-$C_6H_5CH_2O$	C	264-265 dec	66	$C_{17}H_{13}NO_4$
8	6-$C_6H_5(CH_2)_2O$	C	247-249 dec	63	$C_{18}H_{15}NO_4$
9	6-$C_6H_5(CH_2)_3O$	D	247-248 dec	72	$C_{19}H_{17}NO_4$
10	6-$C_6H_5O(CH_2)_2O$	D	235-237 dec	54	$C_{18}H_{15}NO_5$
11	6-$C_6H_5O(CH_2)_3O$	A	202-205	98	$C_{19}H_{17}NO_5$
12	6-$C_6H_5O(CH_2)_4O$	A	237-239	58	$C_{20}H_{19}NO_5$
13	6-$C_6H_5O(CH_2)_5O$	A	215-217	45	$C_{21}H_{21}NO_5$
14	7-C_6H_5	D	279-280 dec	10	$C_{16}H_{11}NO_3 \cdot 0.25H_2O$
15	7-$C_6H_5CH_2$	A	268 dec	37	$C_{17}H_{13}NO_3$
16	7-$C_6H_5O(CH_2)_3O$	A	218-221	63	$C_{19}H_{17}NO_5$
17	8-C_6H_5	A	247-248	58	$C_{16}H_{11}NO_3$
18	8-$C_6H_5CH_2$	C	258-259	33	$C_{17}H_{13}NO_3$
19	8-$C_6H_5O(CH_2)_3O$	C	247-248	56	$C_{19}H_{17}NO_5$

[a]Compds in Table IV saponified by previously described procedure.[2] [b]Recrystn solvents: A, EtOH; B, HOAc; C, 2-methoxyethanol; D, 2-methoxyethanol-H_2O. [c]*Anal.* C, H, N.

Table VI. Physical Properties of R- 

No.	R[a]	Solvent[b]	Mp, °C	Yield, %	Formula[c]
24	6-C_6H_5	A	251-252	82	$C_{17}H_{13}NO_3$
25	6-$C_6H_5CH_2$	B	238-240	40	$C_{18}H_{15}NO_3$
26	6-$C_6H_5CH_2O$	B	253-255	58	$C_{18}H_{15}NO_4$
27	6-$C_6H_5O(CH_2)_3O$	B	194-195	59	$C_{20}H_{19}NO_5$
28	7-$C_6H_5CH_2$	B	235-237	66	$C_{18}H_{15}NO_3$
29	7-$C_6H_5O(CH_2)_3O$	A	177-179	16	$C_{20}H_{19}NO_5$
30	8-C_6H_5	B	278-280	63	$C_{17}H_{13}NO_3$
31	8-$C_6H_5CH_2$	A	191-215[d]	40	$C_{18}H_{15}NO_3$
32	8-$C_6H_5O(CH_2)_3O$	A	172-175	46	$C_{20}H_{19}NO_5$

[a]By alkylation and saponification of compds in Table IV according to the previously described procedure.[2] [b]Recrystn solvents: A, EtOH; B, 2-methoxyethanol. [c]*Anal.* C, H, N. [d]Retains this mp after addl recrystns.

give 2.75 g (56%), mp 196-197°. *Anal.* ($C_{16}H_{19}NO \cdot HCl$). See Table II for other compounds prepared in this manner.

The free amine was liberated by partitioning the HCl salt between 2 *N* NaOH and CH_2Cl_2. The org phase was dried ($MgSO_4$), spin-evapd and used in the next step.[2]

References

(1) B. R. Baker and R. R. Bramhall, *J. Med. Chem.*, 15, 233 (1972) (paper 190).
(2) B. R. Baker and R. R. Bramhall, *ibid.*, 15, 230 (1972) (paper 189).
(3) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967.
(4) B. Riegel, G. P. Lappin, B. H. Adelson, R. I. Jackson, C. J. Albisetti, Jr., R. M. Dodson, and R. H. Baker, *J. Amer. Chem. Soc.*, 68, 1264 (1946).
(5) R. E. Foster, R. D. Lipscomb, T. J. Thompson, and C. S. Hamilton, *ibid.*, 68, 1327 (1946).
(6) R. L. Shivalker and S. V. Sunthankar, *J. Sci. Ind. Res., Sect. B*, 18, 447 (1959); *Chem. Abstr.*, 54, 14252*d* (1960).
(7) Farbenfabriken Bayer, A.-G., British Patent 764,633; *Chem. Abstr.*, 51, 9170*e* (1957).
(8) N. P. Buu-Hoi, M. Gautier, and N. Dat Xuong, *Bull. Soc. Chim. Fr.*, 2154 (1962).
(9) C. G. Raison, A. G. Caldwell, and L. P. Walls, British Patent 770, 411; *Chem. Abstr.*, 51, 14806*b* (1957).
(10) G. J. Lourens, Ph. D. Thesis, University of California at Santa Barbara, Santa Barbara, Calif., 1968.

Irreversible Enzyme Inhibitors. 192.[†,1] Hydrophobic Bonding to Some Dehydrogenases with 5-Substituted-4-hydroxyquinoline-3-carboxylic Acids

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Fifteen derivatives of 4-hydroxyquinoline-3-carboxylic acid bearing aryl, aralkyl, aralkoxy, or aroxyalkoxy substituents at the 5 position and Cl, Me, or H at the 8 position were synthesized and evaluated as inhibitors of glutamate, lactate, malate, and glyceraldehyde phosphate dehydrogenases; good to excellent hydrophobic interaction was observed with the first three enzymes, the 8-Cl-5-$C_6H_5(CH_2)_6$ substituent (12) giving the best interaction. 12 was complexed to malate dehydrogenase 1100-fold more effectively than the parent 4-hydroxyquinoline-3-carboxylic acid (1) and 4300-fold more effective than L-malate; 12 complexed to glutamate dehydrogenase 250-fold better than the parent 1 and 500-fold better than L-glutamate. Furthermore, 12 was complexed to lactate dehydrogenase 65-fold better than the parent 1 and 300-fold better than the substrate, pyruvate.

The possible utility of inhibitors or glutamate, glyceraldehyde phosphate, lactate, and malate dehydrogenases for treatment of cancer cells in the resting phase (G_0) was discussed in a previous paper.[2] 4-Hydroxyquinoline-3-carboxylic acid (1) was selected[2] for further study to determine if potency could be increased by hydrophobic bonding with

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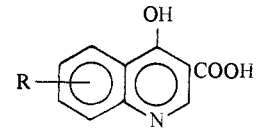


1, R = H
2, R = 1-$(CH_2)_4OC_6H_4NH_2$-*p*
3, R = 6-$C_6H_5O(CH_2)_5O$

hydrocarbon groups strategically placed on the carrier, **1**. Only poor hydrophobic bonding could be detected with a variety of substituents at the 1 position, although the 4 enzymes showed bulk tolerance[3] for large groups at this position as shown by inhibition of molecules such as **2**.[4] A study of hydrocarbon substituents at the 6, 7, or 8 positions on **1** revealed that good hydrophobic bonding to malate dehydrogenase could be achieved with **3**;[1] **3** showed poor to fair hydrophobic bonding to glutamate or lactate dehydrogenases, but none with glyceraldehyde phosphate dehydrogenase.[1]

5-Substituted-4-hydroxyquinoline-3-carboxylic acids cannot be synthesized by the general method[1,2] of cyclization of meta-substituted anilinomethylene malonic esters since cyclization occurs exclusively at the least hindered position to give 7-substituted derivatives of **1**; the cyclization can be forced to the more hindered position by blocking one ortho position by CH_3 or Cl to give 5-substituted derivatives of **1** with CH_3 or Cl at the 8 position, then removal of the Cl by hydrogenolysis (see Chemistry section). Thus a series of 5-substituted derivatives of **1** with H, Me, or Cl at the 8 position were synthesized for evaluation against the four dehydrogenases; some excellent inhibitors due to hydrophobic bonding were found and make up the subject of this paper.

Enzyme Results. Good to excellent hydrophobic bonding was observed with 5-aralkyl-8-Cl-4-hydroxyquinoline-3-carboxylic acids (**8–12**) to 3 of the 4 enzymes, the exception again being glyceraldehyde phosphate dehydrogenase. Activity maximized at $C_6H_5(CH_2)_4$ (**11**) and $C_6H_5(CH_2)_6$ (**12**). These 2 compounds (**11**, **12**) were complexed to malate dehydrogenase 500- and 1100-fold more effectively than the parent 4-hydroxyquinoline-3-carboxylic acid (**1**), and 2000- and 4300-fold more effectively than the substrate, L-malate.

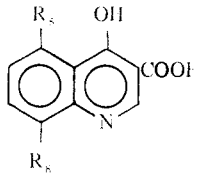
The binding increments to glutamate dehydrogenase were less dramatic, but still potent; **11** and **12** were complexed 120- and 250-fold more effectively than the parent **1** and 400- and 800-fold more effectively than the substrate, L-glutamate. Binding to lactate dehydrogenase by $C_6H_5(CH_2)_4$ (**11**) and $C_6H_5(CH_2)_6$ (**12**) was increased 65-fold over the parent **1**, and 300-fold over the substrate, pyruvate.

The 5-phenoxyalkyloxy derivatives (**13**, **14**) were not as good inhibitors as the 5-phenylalkyl derivatives (**11**, **12**). Furthermore, replacement of the 8-Cl with H or Me gave a 2-fold loss in binding as can be seen by comparing **1** *vs.* **4**, **16** *vs.* **14**, and **17** *vs.* **13**. Methylation at the 1 position reduced binding by a factor of about 4 as shown by comparison of **18–21** with the corresponding unmethylated quinoline-3-carboxylic acids (**7**, **13**, **17**, **12**).

The dehydrogenase inhibitors in this paper and the previous papers[1,2,4] were chosen to inhibit enzymes in the energy pathway from glucose to CO_2 in order to attack cancer cells in the G_0 resting phase;[5] these compounds should also inhibit cells in the growth phase if they are effective. Several of the more potent compounds were evaluated as growth inhibitors of L1210 cells in culture;‡ **3**, **5**, **12**, and **21** had ED_{50} = 25, 25, >70, and >70 μM respectively, thus indicating that the first 2 compounds could penetrate the L1210 cell wall, but the second 2 were poor.

‡We wish to thank Dr. Florence White of the CCNSC for these data measured by Dr. P. Thayer of Arthur D. Little, Inc.

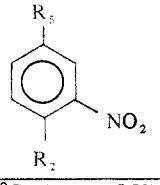
Table 1. Inhibition[a] of Four Dehydrogenases by



No.	R_8	R_5	I_{50},[b] μM Glu-DH	GDPH	LDH	MDH
1[c]	H	H	600	>1600[d]	440	520
4[c]	Cl	H	330	750	300	410
5[c]	Me	H	2100[e]	4700	340	2100[e]
6[c]	Cl	Me	110	150	140	120
7	Cl	C_6H_5	37	400	60	93
8	Cl	$C_6H_5CH_2$	13	410	150	5.9
9	Cl	$C_6H_5CH_2CH_2$	11	310	69	22
10	Cl	C_6H_5CH=CH	5.2	170	24	4.7
11	Cl	$C_6H_5(CH_2)_4$	5.2	140	6.7	1.0
12	Cl	$C_6H_5(CH_2)_6$	2.4	>40[d]	6.8	0.46
13	Cl	$C_6H_5O(CH_2)_3O$	54	340	73	63
14	Cl	$C_6H_5O(CH_2)_4O$	30	430[e]	69	17
15	Me	$C_6H_5O(CH_2)_2O$	150	>800[d]	80	>200[d]
16	Me	$C_6H_5O(CH_2)_4O$	77	>160[d]	59	38
17	H	$C_6H_5O(CH_2)_3O$	100	430	99	120
18	Cl	C_6H_5-1-Me	230	320	100	>240[d]
19	Cl	$C_6H_5O(CH_2)_3O$-1-Me	70	340	85	240
20	H	$C_6H_5O(CH_2)_3O$-1-Me	130	220	55	>360[d]
21	Cl	$C_6H_5(CH_2)_6$-1-Me	10	>40	30[e]	>10[d]

[a]The technical assistance of Nancy Middleton, Pauline Minton, and Diane Shea with these assays is acknowledged. [b]I_{50} = concn for 50% inhibition of Glu-DH = glutamate dehydrogenase, GDPH = glyceraldehyde phosphate dehydrogenase, LDH = lactate dehydrogenase, MDH = malate dehydrogenase; the assays were run as previously described[2] with 2 m*M* L-glutamate, 0.25 m*M* glyceraldehyde phosphate, 2m*M* pyruvate, and 2 m*M* L-malate. [c]Data from ref 2. [d]No inhibition at 0.25 this concn, the max solubility. [e]Estimated from V_0/V_1 observed at max solubility, which is less than I_{50}.

Table II. Physical Properties of



No.	R_2	R_5	Mp, °C	Solvent[a]	Yield, %	Formula[b]
42	Cl	$C_6H_5CH_2$[c]	*d*		95	
43	Cl	C_6H_5CH=CH[e]	90-92	B	94[f]	$C_{14}H_{10}ClNO_2$
44	Cl	$C_6H_5(CH$=$CH)_2$[e]	124-125[f]	A	42[f]	$C_{16}H_{12}ClNO_2$
45	Cl	$C_6H_5(CH$=$CH)_3$[e]	150-153[f]	A	41[f]	$C_{18}H_{14}ClNO_2$
46	Cl	$C_6H_5O(CH_2)_3O$[g]	71-73	B	83	$C_{15}H_{14}ClNO_4$
47	Cl	$C_6H_5O(CH_2)_4O$[g]	74-76	B	96	$C_{16}H_{16}ClNO_4$
48	CH_3	$C_6H_5O(CH_2)_2O$[g]	97-99	B	50	$C_{15}H_{15}NO_4$
49	CH_3	$C_6H_5O(CH_2)_4O$[g]	56-58	B	70	$C_{17}H_{19}NO_4$

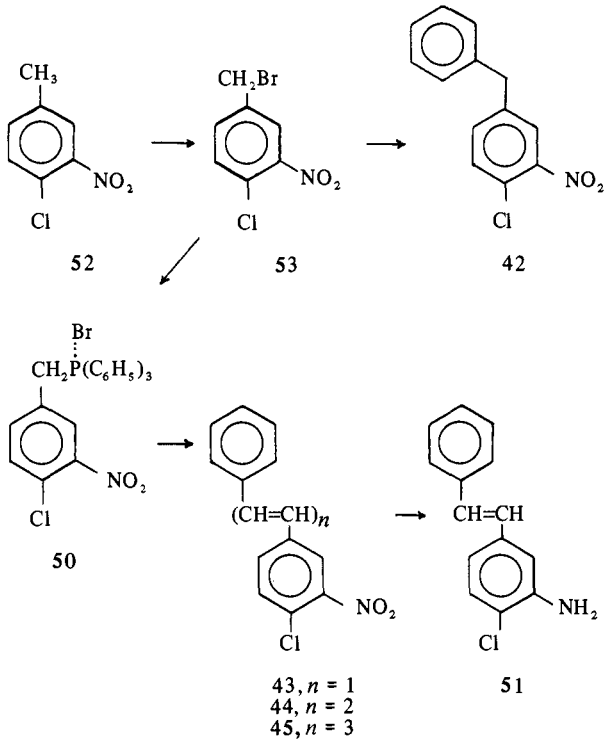
[a]Recrystallization solvents: A, MeOH; B, EtOH. [b]Analyzed for C, H, N. [c]For Friedel-Crafts procedure see ref 7. [d]Oil. [e]See Experimental Section for Wittig procedure. [f]Mp of all trans isomer; yield on crude total of useable isomers. [g]For alkylation procedure see ref 1.

Current studies in this laboratory are directed toward finding more potent 5- and 6-substituted-4-hydroxyquinoline-3-carboxylic acids by substitution on the phenethyl group of **9** and the phenylbutyl group of **11**. Further studies on inhibition of L1210 cell culture will also be performed since it would be desirable to have compounds that inhibit in the 1 μM range. Finally studies are needed on selective inhibition of these 4 enzymes from a tumor with minimal effect on these same enzymes in normal tissues.

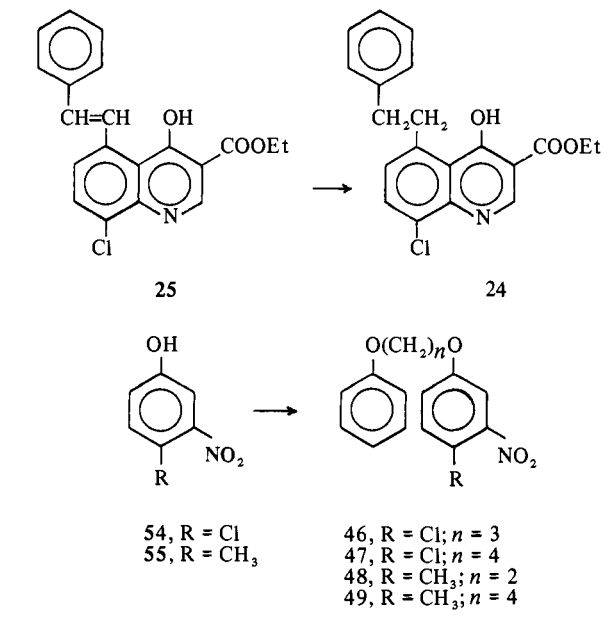
Chemistry. The procedure by which aromatic nitro com-

pounds are transformed into 4-hydroxy-3-quinolinecarboxylic acids was outlined in the first paper of this series.[2] The necessity of an ortho blocking group to direct the thermal ring closure into the more hindered direction was also mentioned. The nitro compounds required as intermediates are listed in Table II and were prepared by 3 different procedures.

Scheme I

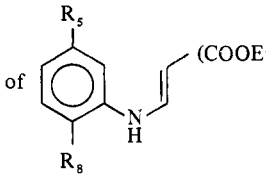


The substituted toluene (**52**) was converted into the benzyl bromide[6] (**53**) with NBS. The benzyl bromide was not isolated but carried on to the phosphonium bromide (**50**) which was characterized. The benzyl bromide (**53**) was also used in a Friedel-Crafts alkylation of benzene§ to give (**42**, Table II) a yellow oil that was characterized as the anilinomethylenemalonate, **34** (Table III).



§See ref 2 for the Friedel-Crafts procedure.

Table III. Physical Properties of



No.	R_8	R_5[a]	Mp, °C[b]	Yield, %	Formula[c]
33	Cl	C_6H_5[d]	123-124	52	$C_{20}H_{20}ClNO_4$
34	Cl	$C_6H_5CH_2$	80-82	46	$C_{21}H_{22}ClNO_4$
35	Cl	$C_6H_5CH{=}CH$	117-119	42	$C_{22}H_{22}ClNO_4$
36	Cl	$C_6H_5(CH_2)_4$	39-40	59	$C_{24}H_{28}ClNO_4$
37	Cl	$C_6H_5(CH_2)_6$	*e*	65	
38	Cl	$C_6H_5O(CH_2)_3O$	81-82	46	$C_{23}H_{26}ClNO_6$
39	Cl	$C_6H_5O(CH_2)_4O$	62-64	35	$C_{24}H_{28}ClNO_6$
40	CH_3	$C_6H_5O(CH_2)_2O$	84-85	84	$C_{23}H_{17}NO_6$
41	CH_3	$C_6H_5O(CH_2)_4O$	75-77	89	$C_{25}H_{31}NO_6$

[a]Prepared by previously described method[2] with a reaction time of 1 hr. [b]All recrystallized from EtOH. [c]Analyzed for C, H, N. [d]For preparation of starting amine see ref 8. [e]Oil.

The Wittig reaction was used to synthesize **43-45** from the phosphonium bromide and benzaldehyde, cinnamaldehyde, and 5-phenyl-2,4-pentadienal,[9] respectively. Compound **43** was reduced to **51** with Zn and NH_4Cl[10] to preserve the double bond. After the ring closure step, a portion of **25** (Table IV) was catalytically reduced to give **24**.

Alkylation of the phenols, **54**# and **55**, as by the method previously described[1] gave the ethers **46-49**. The 1-Me compounds (**18-21**, Table V) were also prepared by a previously described procedure.[2]

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncor. Each analytical sample had an ir spectrum compatible with its structure and was homogeneous on tlc on Brinkmann silica gel GF. All analytical samples gave combustion values for C, H, N within 0.4% of theoretical.

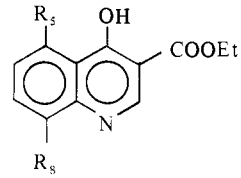
4-Chloro-3-nitrobenzyl Bromide (53). A well-stirred mixt of 60.0 g (0.35 mole) of **52**, 80.0 g (0.45 mole) of NBS, and 0.5 g of benzoyl peroxide in 600 ml of CCl_4 was irradiated with a bright sun lamp for about 18 hr. The succinimide was removed by filtration and the dark amber filtrate was passed through a 150-ml sintered glass funnel filled with silica gel. The silica was washed with an addl 200 ml of CCl_4. The CCl_4 solns were combined and evapd *in vacuo* to give 82.9 g (95%) of a light amber oil.

4-Chloro-3-nitrobenzyl Triphenylphosphonium Bromide (50). A mixt of 450 ml of toluene, 50 g (0.20 mole) of **53**, and 50 g (0.19) mole) of Ph_3P was refluxed for 1 hr. The reaction mixt was cooled, and the product was collected. The product was slurried in boiling PhH and collected again to give 45.7 g (47%) of a light yellow powder: mp 277-280° dec. This material was suitable for use in the subsequent Wittig reactions. A small portion was recrystd from EtOH to give pale yellow crystals: mp 281-284° dec. *Anal.* ($C_{25}H_{20}BrClNO_2P$) C, H, N.

***trans*-(4-Chloro-3-nitrophenyl)-4-phenylbutadiene (44).** A soln of 12.8 g (25.0 mmoles) of **50** in 125 ml of MeOH was added to a freshly prepd soln of NaOMe (from 0.58 g of Na in 100 ml of MeOH, 25 mmoles). A soln of 3.3 g (25 mmoles) of cinnamaldehyde in 25 ml of MeOH was added; the soln rapidly faded from red to a yellow color. After stirring overnight a small first crop of 0.79 g (11%) was collected: mp 124-125°; λ_{max} 348 in EtOH is consistent with the trans-trans isomer. *Anal.* ($C_{16}H_{12}ClNO_2$) C, H, N. On concn a second crop of 0.78 g (11%), mp 121-124°, was collected. The remaining mother liquors were evapd *in vacuo* to a yellow oil; PhH was added and the soln was passed through a column of 200 g of silica gel. The PhH eluant was concd to a yellow glass, which was crystd from EtOH to give an addl 1.46 g (20%) of useable material: mp 100-121°; total yield 42%.

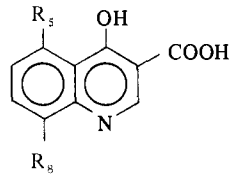
***trans*-3-Amino-4-chlorostilbene (51).** To 4.9 g (18.9 mmoles) of 43 and 1.6 g (30 mequiv) of NH_4Cl in 200 ml of 90% MeOH at 40-50° was added over 30 min 12.3 g (189 mg-atoms) of Zn dust.

#*m*-Nitrophenol was chlorinated to give 4-chloro-3-nitrophenol by the procedure of Henley and Turner.[11]

Table IV. Physical Properties of 

No.	R_8	R_5	Time, min[a]	Mp, °C	Solvent[b]	Yield, %	Formula[c]
22	Cl	C_6H_5	60	303 dec	A	42	$C_{18}H_{14}ClNO_3$
23	Cl	$C_6H_5CH_2$	120	224-226	*d*	32	$C_{19}H_{16}ClNO_3$
24	Cl	$C_6H_5CH_2CH_2$	*e*	175-177	B	15	$C_{20}H_{18}ClNO_3$
25	Cl	$C_6H_5CH{=}CH$	60	244-247	B	55	$C_{20}H_{16}ClNO_3$
26	Cl	$C_6H_5(CH_2)_4$	60	*f*		90	
27	Cl	$C_6H_5(CH_2)_6$	60	123-124	B	51	$C_{24}H_{26}ClNO_3$
28	Cl	$C_6H_5O(CH_2)_3O$	60	142-144	C	62	$C_{21}H_{20}ClNO_5$
29	Cl	$C_6H_5O(CH_2)_4O$	30	*f*		105	
30	CH_3	$C_6H_5O(CH_2)_2O$	45	183-184	B	65	$C_{21}H_{21}NO_5$
31	CH_3	$C_6H_5O(CH_2)_4O$	45	144-145[g]	B	55	$C_{23}H_{25}NO_5$
32	H	$C_6H_5O(CH_2)_3O$	*e*	152-153	D	59	$C_{21}H_{21}NO_5$

[a]Unless stated otherwise, time is for thermal ring closure; see ref 2. [b]Recrystallization solvents: A, 2-methoxyethanol; B, EtOH; C, acetone; D, EtOAc. [c]Analyzed for C, H, N. [d]Recrystallized 4 times from 2-methoxyethanol and once from EtOH. [e]24 and 32 prepared by hydrogenation of 25 and hydrogenolysis of 28 respectively; see Experimental Section. [f]Amber glass. [g]Mp 128-130°, solidifies 130-133°, remelts 144-145°.

Table V. Physical Properties of 

No.	R_8	R_5	Methods[a]	Mp, °C	Solvent[b]	Yield, %	Formula[c]
7	Cl	C_6H_5	A	286 dec	A	37	$C_{16}H_{10}ClNO_3$
8	Cl	$C_6H_5CH_2$	A	272-273 dec	B	22	$C_{17}H_{12}ClNO_3$
9	Cl	$C_6H_5CH_2CH_2$	A	274-276 dec	B	70	$C_{18}H_{14}ClNO_3$
10	Cl	$C_6H_5CH{=}CH$	A	276 dec	A	31	$C_{18}H_{12}ClNO_3 \cdot 0.25C_3H_8O_2$
11	Cl	$C_6H_5(CH_2)_4$	A	216-222	C	6[d]	$C_{20}H_{18}ClNO_3$
12	Cl	$C_6H_5(CH_2)_6$	A	199-200	B	69	$C_{22}H_{22}ClNO_3$
13	Cl	$C_6H_5O(CH_2)_3O$	A	201-203	D	76	$C_{19}H_{16}ClNO_5$
14	Cl	$C_6H_5O(CH_2)_4O$	A	233-235	A	41	$C_{20}H_{18}ClNO_5$
15	CH_3	$C_6H_5O(CH_2)_2O$	A	252-253 dec	A	89	$C_{19}H_{17}NO_5 \cdot 0.25C_3H_8O_2$
16	CH_3	$C_6H_5O(CH_2)_4O$	A	245-246 dec	A	81	$C_{21}H_{21}NO_5$
17	H	$C_6H_5O(CH_2)_3O$	A	218-219	B	48	$C_{19}H_{17}NO_5$
18	Cl	C_6H_5-1-CH_3	B,A	292-294	A	65[d]	$C_{17}H_{12}ClNO_3$
19	Cl	$C_6H_5O(CH_2)_3O$-1-CH_3	B,A	164-166	B	34[d]	$C_{20}H_{18}ClNO_5$
20	H	$C_6H_5O(CH_2)_3O$-1-CH_3	B,A	176-178	B	64[d]	$C_{20}H_{19}NO_5$
21	Cl	$C_6H_5(CH_2)_6$-1-CH_3	B,A	149-150	B	47[d]	$C_{23}H_{24}ClNO_3$

[a]Methods: A, saponification; B, alkylation, see ref 2. [b]Recrystallization solvents: A, 2-methoxyethanol; B, EtOH; C, MeOH; D, EtOH-H_2O. [c]Analyzed for C, H, N. [d]Yield over 2 steps.

After addn was complete the mixt was refluxed for 2.5 hr, then filtered hot. On cooling a yellow ppt (1.0 g) was obtd, which was recrystd from EtOH to give 0.44 g: mp 143-144°. *Anal.* ($C_{14}H_{12}ClN$) C, H, N.

The mother liquors were combined with the MeOH reaction mixt and evapd *in vacuo*. The resultant yellow paste was partitioned between $CHCl_3$ and H_2O, the $CHCl_3$ soln was dried ($MgSO_4$) and evapd to leave 2.9 g (67%) of an orange oily solid which had an ir that compared favorably with that of the above material and was suitable for further transformations by the previously described methods.[2]

Ethyl 8-Chloro-4-hydroxy-5-phenylethyl-3-quinolinecarboxylate (24). A mixt of 0.94 g (2.66 mmoles) of 25 (Table IV) was shaken with 50 mg of PtO_2 and H_2 at 2-3 atm until the tlc indicated that all of the starting material had been consumed. The catalyst and solvent were removed to leave an amber oil which was dissolved in 5 ml of PhH and placed on a 58-g silica gel column. The column was eluted with PhH, and tlc was run on each 150-ml fraction. The second band of material eluted from the column proved to be the desired product. All fractions contg that material were combined and PhH was removed. The residue was recrystd from EtOH to give 140 mg (15%) of a white solid: mp 175-178°. *Anal.* ($C_{20}H_{18}ClNO_3$) C, H, N.

Ethyl 4-Hydroxy-5-phenoxypropyloxy-3-quinolinecarboxylate (32). A soln of 1.20 g (2.99 mmoles) of 28 (Table IV) and 407 mg (2.99 mmoles) of NaOAc · $3H_2O$ in 100 ml of HOAc was shaken with 200 mg of 10% Pd/C in 2-3 atm of H_2 overnight. The catalyst and solvent were removed to leave an amber oil which was chromatogd over 50 g of silica gel. Elution with $CHCl_3$ removed several minor components as shown by tlc. The solvent was changed to EtOAc which eluted the desired product. The EtOAc soln was concd *in vacuo*. The residue was recrystd from EtOAc to give 0.44 g (40%), mp 152-153°, and a second crop of 0.20 g (19%), mp 153-155°. A small portion of the first crop was recrystd for analysis: mp 152-153°. *Anal.* ($C_{21}H_{21}NO_5$) C, H, N.

References

(1) B. R. Baker and R. R. Bramhall, *J. Med. Chem.*, 15, 235 (1972) (paper 191).
(2) B. R. Baker and R. R. Bramhall, *ibid.*, 15, 230 (1972) (paper 189).
(3) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967.
(4) B. R. Baker and R. R. Bramhall, *J. Med. Chem.*, 15, 233 (1972) (paper 190).
(5) J. A. Montgomery, T. P. Johnston, and Y. F. Shealy, "Medicinal Chemistry," Vol. 1, A. Burger, Ed., Wiley, New York, N. Y., 1970, p 759.
(6) P. Mamalis, L. Jeffries, S. A. Price, M. J. Rix, and D. J. Outred, *J. Med. Chem.*, 8, 684 (1965).
(7) Farbenfabriken Bayer, A.-G., British Patent 764, 633; *Chem.*

Abstr., 51, 9170e (1957).
(8) W. Blakey and H. A. Scarborough, J. Chem. Soc., 3000 (1927).
(9) R. Kuhn and A. Winterstein, Helv. Chim. Acta, 12, 439 (1929).
(10) J. I. DeGraw, M. Cory, W. A. Skinner, M. C. Theisen, and C. Mitoma, J. Med. Chem., 11, 225 (1968).
(11) R. V. Henley and E. E. Turner, J. Chem. Soc., 928 (1930).

Conformational Aspects of Systems Related to Acetylcholine. 3. Base-Catalyzed and Acetylcholinesterase-Catalyzed Hydrolysis of the Isomeric *dl*-3-Trimethylammonium-2-acetoxy-*trans*-decalin Halides and the Isomeric *dl*-1-Methyl-3-acetoxy-*trans*-decahydroquinoline Methiodides

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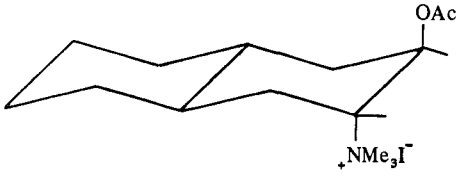
The catalytic rate constants for the base-catalyzed and AChE-catalyzed hydrolysis of 6 conformationally rigid ACh model substrates relative to the rate constants for ACh are reported as a function of solid state torsion angle τ (O-C-C-N^+). The acceleration of the enzymatic reaction over the nonenzymatic reaction relative to the acceleration for ACh (100%), (Rel k_{cat}'/K_m')/(Rel k_{OH}), is 17.5% when $\tau = 147°$, 9.12% when $\tau = 169°$, and <0.24% when $\tau = 60$–$74°$, demonstrating a preference by the enzyme for a transition-state geometry when $\tau \cong 150°$.

As part of an effort to determine the steric requirements of ACh receptor sites, 6 model compounds, in which the groups presumed responsible for attachment to the receptor site are conformationally rigid, *i.e.*, the isomeric *dl*-3-trimethylammonium-2-acetoxy-*trans*-decalin halides and the isomeric *dl*-1-methyl-3-acetoxy-*trans*-decahydroquinoline methiodides, have been synthesized and subjected to preliminary testing as previously reported.[1,2] Relative rates of hydrolysis of ACh in the presence of eel acetylcholinesterase (AChE) showed the trans diaxial isomer **1** to be the best substrate in the trans decalin series and the equatorial acetate **5** to be the better substrate in the trans decahydroquinoline series. This report presents the results of a more detailed investigation into the AChE-catalyzed hydrolysis as well as the nonenzymatic, base-catalyzed hydrolysis of ACh and the 6 model compounds **1-6**. Hydrolysis rates were measured by following the production of acid at 25° using a pH stat. The enzymatic hydrolyses were studied at pH 7.2; the base-catalyzed hydrolyses were studied at constant pH values ranging from 10 to 11.



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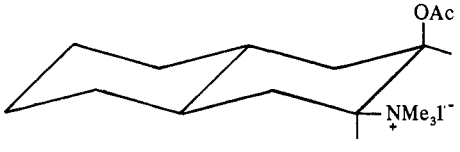

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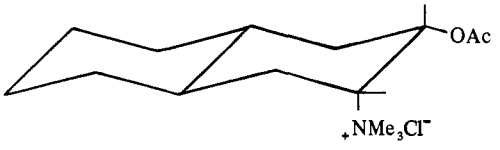

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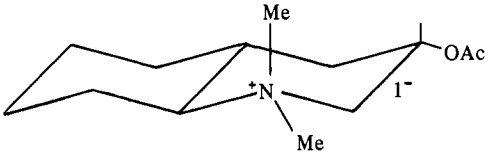

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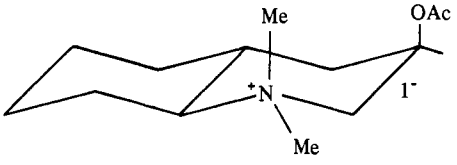

6

Results

The observed or estimated solid state torsion angles, τ, the Michaelis-Menten parameters, K_m and V_{max}, for the enzymatic hydrolyses, the second-order rate constants, k_{OH}, for the base-catalyzed hydrolyses, rate constants for the 2 reactions relative to ACh, as well as data and calculations used to determine the relative enzymatic acceleration of the nonenzymatic hydrolyses are given in Table I.

X-ray diffraction data show that the O-C-C-N^+ torsion angles (τ) of **1**, **5**, and **6** are 147°,[3] 169°,† and 74°,† respectively. Deviation from the "expected" angle of 180° for **1** can be assumed to arise from 1,3-diaxial interactions between the bulky Me_3N^+ group and the axial protons.[3] Deviation from the angle of 60° expected in **6** presumably arises from skew interaction of the functional groups as well as 1,3-interaction between the axial bridgehead proton and the axial AcO function. Compds **2** and **3** in which skew interactions of the functional groups and 1,3-diaxial

†E. Shefter, private communication.