was triturated with acetone. The insoluble ammonium chloride was removed by filtration and evaporation of the filtrate gave the oily product; yield, 112 mg. The crude product was dissolved in ether and a small amount of methanol. Dry HCl was bubbled through the chilled solution, and the white salt was collected by filtration; yield, 65 mg. (32.4%); m.p. 180-183° (oil bath); λ_{max}, mμ ($\epsilon \times 10^{-3}$): pH 1, 254 (9.35); pH 7, 257 (9.35); pH 13, 263 (10.0); $\bar{\nu}$, cm.$^{-1}$ (KBr): 3400 (OH), 3000–2700 (NH_2^+), 1690 (C=N$^+$H), 1570 (C=C).

Anal. Calcd. for $C_8H_{12}ClN_5O$: C, 41.83; H, 5.26; Cl, 15.44. Found: C, 41.65; H, 5.05; Cl, 15.23.

Reagents and Assay Procedure.—Adenosine and adenosine deaminase were purchased from the Sigma Chemical Company. The general method of assay has been described by Kaplan[9] and involves measuring the rate of disappearance of the absorption band of adenosine at 265 mμ. All enzymatic reactions were performed in 0.05 *M* phosphate buffer at pH 7.6 and 25°. The substrate and the stock solutions of all reagents were prepared in 0.05 *M* phosphate buffer at pH 7.6. For the assay, the cell contained a total volume of 3.1 ml. which was 0.066 m*M* with respect to adenosine. To study inhibition, appropriate amounts of buffer were excluded from the cells and were replaced by an equal volume of a solution of the inhibitor in phosphate buffer.

Results and Discussion

Previously it was found[4] that V and VI were competitive inhibitors of adenosine deaminase with K_i values of 3.0×10^{-5} *M* and 9.8×10^{-5} *M*, respectively. Enzymatic evaluation of compounds VII–XI revealed that they were all essentially noninhibitory at concentrations 2–3 times that of the substrate. These results establish that adenosine deaminase has little bulk tolerance for groups on the 6-amino group of the purine nucleus. For example, the replacement of the 6-amino group by a 6-methylamino group increased the K_i by a factor greater than 3. Thus, it would appear that it will not be feasible to prepare active-site-directed irreversible inhibitors at the 6-position of the purine nucleus. It might be suggested that even though the enzyme has little bulk tolerance for branch chain groups at the 6-position of the purine nucleus, it might tolerate straight-chain groups. Therefore, the 6-*n*-propylamino analog (XII) was synthesized, and it, too, was essentially noninhibitory at concentrations 2–3 times that of the substrate. Consequently, adenosine deaminase has little bulk tolerance for either branched or unbranched groups on the 6-amino group of the purine nucleus. Finally, it was found that 7-(3-hydroxypropyl)-6-aminopurine (XIII) was noninhibitory against adenosine deaminase. This fact may be rationalized if it is assumed that the enzyme has little bulk tolerance for a group at the 7-position of the purine nucleus or that an essential binding group at the 9-position is absent. At the present time, however, it is not possible to answer this question unambiguously.

(9) N. O. Kaplan in "Methods in Enzymology," Vol. II, S. P. Colowick and N. O. Kaplan, Ed., Academic Press Inc., New York, N. Y., 1955, p. 473.

Analogs of Tetrahydrofolic Acid. XVII.[1,2] On the Mode of Binding of the *p*-Aminobenzoyl Moiety of N-(2-Amino-4-hydroxy-6-methyl-5-pyrimidylpropyl)-*p*-aminobenzoyl-L-glutamic Acid to Dihydrofolic Reductase

B. R. BAKER AND JOHANNES H. JORDAAN

Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo 14, New York

Received August 20, 1964

The binding of 2-amino-5-(3-anilinopropyl)-6-methyl-4-pyrimidinol (IV) to dihydrofolic reductase is tightened by introduction of carboxyl (V) or carboxyglycyl groups (VI) in the *para* position. Since the benzene ring of IV probably binds to the enzyme in a charge–transfer complex with the benzene ring being an electron acceptor, the electron-withdrawing *p*-carbonyl group of V and VI could tighten binding by making the benzene ring a better electron acceptor. Strong evidence to support this hypothesis has now been obtained by comparison of IV–VI and the *p*-(4-chloro-3-oxo-1-butenyl) (X) and *p*-(4-chloro-3-oxobutyl) (VIIIb) derivatives of IV as inhibitors of dihydrofolic reductase.

The pyrimidyl analog of tetrahydrofolic acid (III)[3] binds to folic reductase ($K_i = 2.0 \times 10^{-6}$) better than the substrate, folic acid ($K_m = 10 \times 10^{-6}$). Removal of the carboxy-1-glutamate residue as in IV[4] increases K_i to 63×10^{-6}; it can be calculated readily from $-\Delta F = RT \ln K$ that the loss in free energy of binding by removal of the carboxy-L-glutamate is only 22% of III. About one-half of the binding of the carboxy-L-glutamate residue is due to the *p*-carboxyl as shown by $K_i = 13 \times 10^{-6}$ for V.[4] The α-carboxyl of III would appear not to contribute to binding since the K_i of VI is also 13×10^{-6}; thus the other half of the binding of the carboxy-L-glutamate residue of III is due to the γ-carboxyl.[4] Evidence was presented that both the *p*- and γ-carbonyls were probably complexed to the enzyme by hydrogen bonding.[4]

It was pointed out[4] that it should be possible to obtain an active-site-directed irreversible inhibitor[5,6] of folic reductase if the proper type of functional group for formation of a covalent bond could be placed on III positioned where the *p*- or γ-carbonyl normally occur. Several chloromethyl ketones (VIII and X) were synthesized to evaluate this possibility, since halomethyl ketones have been previously used for

(1) This work was supported in part by Grants CA-05867 and CA-06624 from the National Cancer Institute, U. S. Public Health Service. J. H. Jordaan is indebted to the Atomic Energy Board of the Republic of South Africa for a fellowship.

(2) For the previous paper of this series, see B. R. Baker and B.-T. Ho, *J. Pharm. Sci.*, **53**, 1137 (1964).

(3) Papers VI and VII of this series: B. R. Baker and C. E. Morreal, *ibid.*, **51**, 596 (1962); *ibid.*, **52**, 840 (1963).

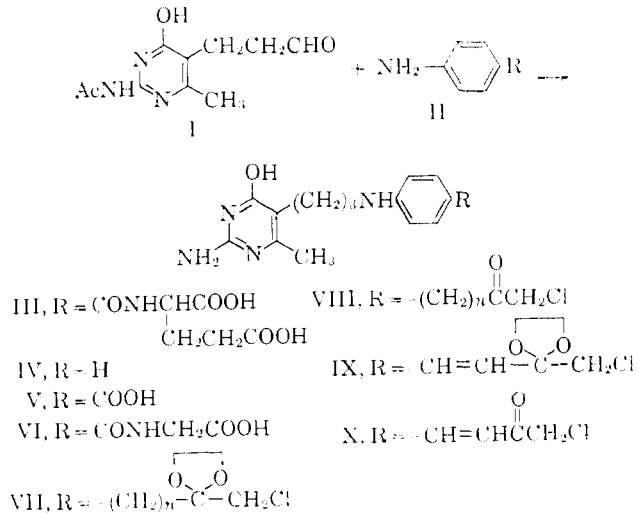
(4) Paper X of this series: B. R. Baker, D. V. Santi, P. I. Almaula, and W. C. Werkheiser, *J. Med. Chem.*, **7**, 24 (1964).

(5) B. R. Baker, *Cancer Chemotherapy Rept.*, **No. 4**, 1 (1959).

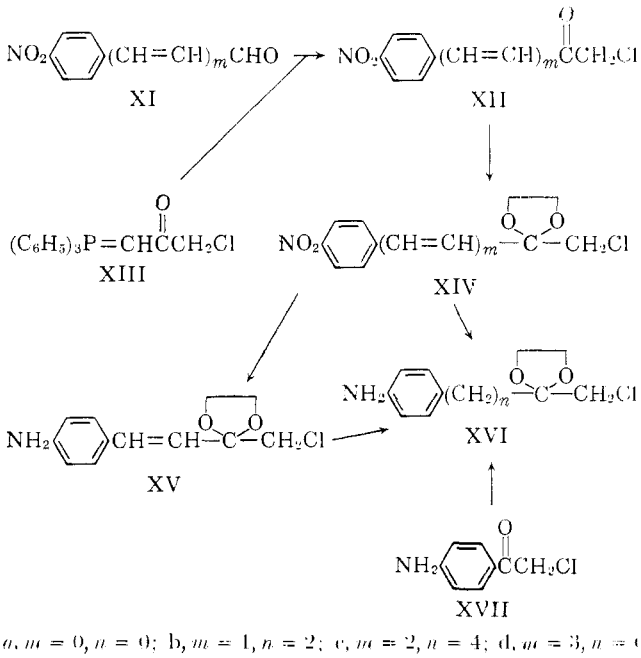
(6) B. R. Baker, *J. Pharm. Sci.*, **53**, 347 (1964), a review.

anchoring active-site-directed irreversible inhibitors.[7,8]

During the course of the above research problem it became apparent from other information that the anilino group of III and IV was not binding through the NH group as previously suggested,[4] but that the benzene ring was the site of binding, perhaps as an electron acceptor in a charge-transfer complex with the enzyme.[9] If such were the case, then the increased binding of V and VI compared to IV may well not have been by hydrogen binding of the *p*-carbonyl,[4] but by a net increase in binding of the benzene ring as an electron acceptor, due to the electron-withdrawing properties of the *p*-carbonyl group. Comparative enzyme evaluation of compounds IV–VI, VIIIa–d, and X as



a, $n = 0$; b, $n = 2$; c, $n = 4$; d, $n = 6$



a, $m = 0, n = 0$; b, $m = 1, n = 2$; c, $m = 2, n = 4$; d, $m = 3, n = 6$

reversible inhibitors of dihydrofolic reductase indicated that the increased binding in the charge-transfer complex is the better alternative to account for the tighter binding of V and VI compared to IV.

Chemistry.—Pyrimidyl analogs (III–VI) of tetrahydrofolic acid have been synthesized previously by the reductive condensation of an arylamine (II) with 2-acetamido-4-hydroxy-6-methyl-5-pyrimidylpropionaldehyde (I) by sodium borohydride.[3,4] In order to synthesize a chloromethyl ketone such as VIII, it would be necessary either to introduce the chloroacetyl group last in direct or indirect fashion or to block the ketone function so it could not be reduced by the sodium borohydride. There are many routes available for synthesis of chloromethyl ketones such as halogenation of a methyl ketone, treatment of an acid chloride with diazomethane, then hydrogen chloride, or treatment of chloroacetyl chloride with a dialkylcadmium; these methods would have questionable compatibility with structures such as IV and V. The recently described Wittig route to chloromethyl vinyl ketones by condensation of aldehydes with triphenyl chloroacetonyl phosphorane (XIII) appeared attractive,[10] providing the resultant vinyl group(s) could be reduced without affecting the ketone or halogen functions; the dioxolane blocking group, as in XIV, served admirably since the ketone was obviously blocked, but, less obvious, the halogen is tremendously deactivated and should survive steps such as XIV to XVI and I to VII.

The first member of the series (VIIIa) started with the commercially available 4-amino-α-chloroacetophenone (XVII). Conversion of XVII to the dioxolane (XVIa) was effected smoothly in boiling benzene in the presence of ethylene glycol and 1 equiv. of *p*-toluenesulfonic acid under constant water removal conditions; conversion to the free base gave pure crystalline dioxolane base (XVIa) in 79% yield. Reductive condensation of XVIa with I in methanol with sodium borohydride gave VIIa which crystallized from the reaction mixture in analytically pure form in 61% yield.

In order to find appropriate conditions for removal of the dioxolane group of VIIa to give the chloro ketone VIIIa, a kinetic run was made in 0.1 *N* HCl; the reaction was readily followed by the appearance of a new peak of VIIIa at 349 mμ, which was complete in 1 hr. at 40°. Preparatively, the reaction was run with 0.025 *N* HCl in 75% aqueous ethanol at the boiling point for 1 hr., affording VIIIa in 93% yield. The compound exhibited no melting point below 300°, but was uniform on thin layer chromatography and gave proper combustion values.

p-Nitrobenzaldehyde (XIa), in which the aldehyde carbon is strongly electrophilic, reacted readily with triphenyl chloroacetonyl phosphorane (XIII) in refluxing benzene to give XIIb in 85% yield, as previously described.[10] Treatment of XIIb with ethylene glycol in benzene in the presence of a catalytic quantity of *p*-toluenesulfonic acid under constant water removal conditions afforded 81% of pure, crystalline dioxolane (XIVb). Selective hydrogenation of the nitro group of XIVb without concomitant reduction of the double bond was performed in ethyl acetate in the presence of Adams catalyst. Since 15 min. was required for reduction of the nitro group, but 48 hr. was required for complete reduction of the double bond, the crystalline *p*-aminostyryldioxolane (XV) was readily isolated

(7) B. R. Baker, R. P. Patel, and P. I. Almada, *J. Pharm. Sci.*, **52**, 1051 (1963).

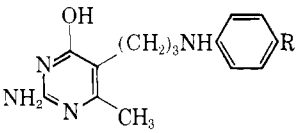
(8) G. Schoellman and E. Shaw, *Biochemistry*, **2**, 252 (1963).

(9) Paper XIII of this series; B. R. Baker, B.-T. Ho, and G. Cilurzo, *J. Heterocyclic Chem.*, **1**, 88 (1964).

(10) R. L. Hudson and P. A. Chopard, *J. Org. Chem.*, **28**, 2116 (1963).

TABLE I

INHIBITION OF (DIHYDRO)FOLIC REDUCTASE BY



Compd.	R	Inhibitor concn., mM	Dihydrofolic reductase[a] % inhibition	Dihydrofolic reductase[a] Inhibitor: substrate for 50% inhibition	Folic reductase[b] $K_i \times 10^6$
III	-CONHCHCOOH CH_2CH_2COOH	0.10	50	16	2.0
VI	$-CONHCH_2COOH$	0.16	50	27	13
XIX	$-CONH(CH_2)_3COOH$				4.8
V	-COOH	0.077	50	13	13
IV	H	0.60[c-e]	43	130	63
VIIIa	$-COCH_2Cl$	0.075[d,f]	29	30	
VIIIb	$-(CH_2)_2COCH_2Cl$	0.090[d,e]	43	20	
VIIIc	$-(CH_2)_4COCH_2Cl$	0.023[d]	50	3.8	
VIIId	$-(CH_2)_6COCH_2Cl$	0.10[d,e]	35	32	
X	$-CH{=}CHCOCH_2Cl$	0.018[d]	50	3.0	
XVIII	$-C_4H_9$-n	0.07[d,e]	0	>46	

[a] Dihydrofolic reductase from pigeon liver was a 45–90% saturated ammonium sulfate fraction prepared and assayed as previously described[12] by noting the rate of change in optical density at 340 mμ [M. J. Osborne and F. M. Huennekens, *J. Biol. Chem.*, **233,** 969 (1958)] with a Cary 11 recording spectrophotometer on a 0–0.1 optical density slide wire; cuvette concentrations of dihydrofolate [S. Futterman, *ibid.*, **228,** 1031 (1957)] and TPNH were 6 and 12 μM, respectively, in Tris Buffer pH 7.4. [b] Value previously reported[4] with rat liver folic reductase using folic acid as substrate at pH 6.1. [c] Previously reported.[12] [d] Cuvette also contained 10% N,N-dimethylformamide. [e] Near saturation concentration. [f] Maximum concentration allowing full light transmission.

pure in good yield. That the compound was the styryldioxolane (XV) and not the phenylethyldioxolane was shown readily by short acid hydrolysis to remove the dioxolane group. The resultant *p*-aminostyryl chloromethyl ketone showed a new peak of the conjugated system at 365 mμ; if the double bond had been reduced, no absorption peak at longer wave length than the 286-mμ peak of XV could be expected since the ketone and aminophenyl groups would not be conjugated. Reductive condensation of XV with I proceeded to crystalline IX in 80% yield; short acid hydrolysis of IX afforded the desired styryl chloromethyl ketone analog (X) which had no melting point below 350° but showed the conjugated carbonyl peak at 402 mμ.

When the hydrogenation of the nitrostyryldioxolane (XIVb) was continued after the reduction of the nitro group, a fourth mole was gradually absorbed over 48 hr. The resultant aminophenethyldioxolane (XVIb) was an oil that was uniform on thin layer chromatography and gave proper combustion values; it was reductively condensed with I to give the crystalline pyrimidyldioxolane (VIIb) in 30% yield. When VIIb was treated with hot 0.1 N HCl, the dioxolane blocking group was removed; although a long wavelength peak in the ultraviolet was not formed in the ketone of VIIIb due to lack of conjugation with the anilino group, that the reaction had proceeded could be shown by the loss of the dioxolane C–O–C band at 9.61 μ and generation of ketone C=O at 5.78 μ in the infrared.

Similarly, *p*-nitrocinnamaldehyde (XIb) and 4-(*p*-nitrophenyl)-2,4-pentadienal (XIc) were converted to crystalline XIIc and XIId, then to the desired pyrimidyl chloromethyl ketones, VIIIc and VIIId, respectively.

Enzymic Evaluation.—In Table I is listed a summary of the results of inhibition of dihydrofolic reductase from pigeon liver by the compounds in question, using dihydrofolate at pH 7.4 as the substrate; also listed are the results previously obtained with some of the compounds with a different assay, that is, folic reductase from rat liver using folic acid as substrate at pH 6.1. Except for the *p*-carboxy analog (V), the same order of effectiveness was noted for the two assays; V is relatively more potent as an inhibitor in the dihydrofolate assay which might be attributable to the pH difference since the carboxyl of V would be fully ionized at pH 7.4, but not at pH 6.1.

Comparison of the ketone VIIIb with the parent anilino compound (IV) shows that the $-(CH_2)_2CO-CH_2Cl$ group tightens the binding from 130 to 20 for the ratio of inhibitor:substrate (see Table I); the most likely explanation is that the CO group of VIIIb hydrogen bonds with the enzyme. A previous comparison[4] of the parent anilino compound (IV) with its *p*-carboxy (V) and *p*-carboxyglycyl (VI) derivatives indicated that the glycyl carboxyl did not contribute to binding since the latter two compounds had $K_i = 13 \times 10^{-6}$ in the folic acid assay at pH 6.1; however, in the dihydrofolic assay, these two repeatedly gave a twofold difference in binding with V being superior (Table I). When the results with IV, V, VI, and VIIIb are considered together, the best current rationalization is as follows. (a) Both the glycine carboxyl of VI and the ketone CO of VIIIb can hydrogen bond to the enzyme even though their chain lengths differ by one atom. (b) The *p*-carboxylate of V makes the anilino group a better electron acceptor than the *p*-carboxamido group of VI even though the Hammet σ-values[11] indicate the reverse when a *p*-NH is not present.

(11) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, pp. 184–207.

The current results clearly point out again one of the major difficulties in rationalizing the effect of a given structural change; only a net change is measured and, if two equal and opposing effects occur, one assumes that the structural change caused no effect. This phenomenon had been encountered earlier in the study of the binding of the pyrimidyl moiety of IV[12] and only becomes apparent when studies on additional compounds show an inconsistency, as in the present case.

Another example arises by comparing VIIIc, VIIId, III, and IV. Since VIIIc and VIIId bind better than IV, it would appear that the ketone group of VIIIc and VIIId hydrogen bond to the enzyme at the same place as the γ-carboxyl of III; the oxohexane VIIIc with its shorter side chain binds better than the oxooctane VIIId. The fact that the oxohexane VIIIc binds better than the prototype carboxy-L-glutamate inhibitor (III) can be interpreted by the assumption that binding by the three carbonyl groups of III is weak compared to a tight binding by the ketone group of VIIIc. That hydrophobic binding[13,14] by the methylene groups of VIIIc and VIIId was unimportant was shown by the poor binding of the *n*-butyl analog (XVIII) compared to VIIIc or VIIId. Also against hydrophobic binding being a factor of importance was the fact that the hexyl ketone (VIIIc) complexed to the enzyme about 8 times better than the octyl ketone (VIIId); if hydrophobic binding were a factor, the reverse order of binding (VIIId better than VIIIc) should have been observed.

The chloroacetyl side chain of VIIIa tightens binding, compared to the anilino compound IV, the binding of VIIIa being almost as good as the *p*-carboxy derivative V; thus, the COO^- and $COCH_2Cl$ groups have similar effects which might be direct hydrogen bonding or might be an electron-withdrawing effect that increases the effectiveness of benzene binding. That the latter explanation is more tenable can be gleaned by comparison of the acetyl (VIIIa), oxobutyl (VIIIb), and oxobutenyl (X) derivatives; of the three compounds, X is by far the most effective inhibitor and is, in fact, the most effective compound in Table I. Several possible explanations emerge for consideration.

(a) The unsaturated ketone (X) is a better inhibitor than either the oxobutane (VIIIb) or the acetyl derivative (VIIIa), indicating that the combined effects of both VIIIb and VIIIa are acting; that is, (1) the CO of X hydrogen binds to the enzyme in the same way that the CO of the oxobutane VIIIb, and (2) the C=CC=O group of X has better electron-withdrawing powers than the C=O of VIIIa, presumably increasing the binding of the benzene ring as an electron acceptor. (b) The electron-rich locus on the enzyme which binds the anilino group of IV could be an indole, which is nearly twice as large as the aniline residue; therefore, the total tighter binding of X compared to IV, VIIIa, VIIIb, and V may be due to a more favorable charge-transfer complex between indole and the $-C_6H_4C=C-C=O$ moiety than indole and the $-C_6H_4CH_2CH_2CO$ moiety.

Experiments will be attempted to distinguish between these two explanations, such as replacement of the $-C_6H_5$ group of IV with groups such as crotonylphenone, biphenyl, fluorenone, and acetonaphthone.

All the chloromethyl ketones in Table I were investigated as possible active-site-directed irreversible inhibitors of dihydrofolic reductase from both pigeon liver and *E. coli* B. A 45–90% ammonium sulfate fraction[12] in the 0.05 *M* Tris buffer of pH 7.4 was incubated for 1 hr. at 37°, then chilled overnight at 3–5°. The enzyme solution was filtered through a Celite pad. An aliquot of the filtrate containing dihydrofolic reductase was incubated at 37° with or without 24 μ*M* TPNH and 50 μ*M* of inhibitor for 1 hr. The dihydrofolic reductase content was measured from aliquots removed at 2 and at 60 min. No decrease in dihydrofolic reductase concentration was observed after 60 min., with or without 50 μ*M* concentration of the inhibitor.[15]

The failure of any of the four chloromethyl ketones to inactivate irreversibly the two folic reductases cannot be attributed to the inactivity of the halogens; these chloromethyl ketones reacted with thiosulfate at about the same order as 4-(iodoacetamido)salicylic acid, an irreversible inhibitor of lactic and glutamic dehydrogenases.[15a] Therefore, the failure of the chloromethyl ketones could logically be attributed to failure to bridge to a nucleophilic site on the enzyme[15b] or the wrong nucleophilic character of the nucleophilic site.[15c] Since (a) both the α- and γ-carboxyl of III apparently can hydrogen bond to some site on the enzyme which must have sufficient nucleophilic character for binding, and since (b) compounds VIIIa–c should have the proper bridging capacity to the sites binding the α- and γ-carboxyl, it follows that the hydrogen bonding sites may be of poor nucleophilicity for the chloromethyl group. For example, the serine or threonine OH group might be good for hydrogen bonding to $-COO^-$ or $-CONH_2$ groups, but would have poor nucleophilic character for reaction with a chloromethyl ketone. A side chain related to VIII with a sulfonyl fluoride or high-energy phosphoryl group might be a better choice and is being pursued synthetically.

Experimental

Melting points were taken in capillary tubes in a Mel-Temp block; all melting points below 230° are corrected. Infrared spectra were determined in KBr disks with a Perkin-Elmer 137B spectrophotometer unless otherwise indicated. Ultraviolet spectra were determined with a Perkin-Elmer 202 spectrophotometer. Thin layer chromatograms were run on Brinkmann silica gel G.

2-(*p*-Aminophenyl)-2-chloromethyl-1,3-dioxolane (XVIa).—To a solution of 1.7 g. (10 mmoles) of 4-amino-α-chloroacetophenone in 50 ml. of benzene was added 0.70 g. (11 mmoles) of ethylene glycol and 1.9 g. of *p*-toluenesulfonic acid. The mixture was refluxed with magnetic stirring under a Dean-Stark trap for 5 hr. when no more water collected. The mixture was shaken with 120 ml. of ice-cold 0.1 *N* HCl. The separated benzene layer was washed with two 50-ml. portions of water, dried with $MgSO_4$, then spin evaporated *in vacuo*. The crystalline residue was recrystallized from methanol-water; yield,

(12) Paper XII of this series: B. R. Baker, B.-T. Ho, and T. Neilson, *J. Heterocyclic Chem.*, **1**, 79 (1964).

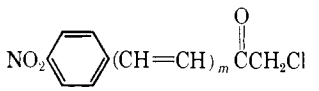
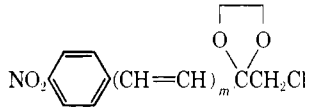
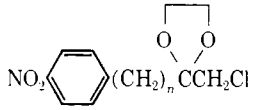
(13) R. A. Wallace, A. N. Kurtz, and C. Niemann, *Biochemistry*, **2**, 824 (1963).

(14) G. Nemethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).

(15) This type of incubation procedure has been previously employed for the study of irreversible inhibitors of lactic dehydrogenase and glutamic dehydrogenase; *e.g.*, (a) B. R. Baker, W. W. Lee, and E. Tong, *J. Theoret. Biol.*, **3**, 459 (1962); (b) B. R. Baker, *Biochem. Pharmacol.*, **11**, 1155 (1962); (c) B. R. Baker and R. P. Patel, *J. Pharm. Sci.*, **52**, 927 (1963).

TABLE II

PHYSICAL CONSTANTS OF AROMATIC PRECURSORS

Compd.	m	n	Method[a]	% yield	M.p., °C.	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N	λ_{max}^{EtOH}, mμ (ε × 10⁻³)
												
XIIb	1	...	A[b]	85	116–118[c]							306 (19.1)
XIIc	2	...	A	64	148–150	57.3	4.00	5.57	57.5	4.12	5.58[d]	224 (7.3), 345 (30)
XIId	3	...	A[e]	62	178–179	60.6	4.36	5.05	60.7	4.21	4.91	281 (7.2), 379 (46.4)
												
XIVb	1	...	B	81	155–156	53.4	4.49	5.19	53.5	4.61	5.13	222 (9.4), 302 (13.1)
XIVc	2	...	B	91	148–149	56.9	4.77	4.74	57.0	4.94	4.67	242 (16.4), 337 (18.8)
XIVd	3	...	B	61	146–147	59.7	5.01	4.35	60.1	5.22	4.47	280 (18.6), 372 (29.8)
												
XVIb	...	2	C	93	Oil[f]	59.6	6.67	5.79	59.8	6.89	5.61	238 (10.1), 276 (8.3)
XVIc	...	4	C	80	Oil[f]	62.3	7.47	5.19	61.7	7.21	4.75	241 (13.4), 274 (14.9)
XVId	...	6	C	94	Oil[f]	64.5	8.12	4.70	64.7	8.30	4.59	236 (9.8), 291 (1.5)

[a] See Experimental. [b] Refluxed 3 hr. in benzene. [c] Lit.[10] m.p. 116°. [d] Calcd: Cl, 14.1. Found: Cl, 14.1. [e] Refluxed 4 hr. in MeOH. [f] Moved as one spot on t.l.c. with 10% methanol in benzene.

1.70 g. (79%); m.p. 97–98°; λ_{max} 2.88 2.95, 3.04, 6.03 (NH), 6.22, 6.62, 12.03 (phenyl), 9.87 μ (C-O-C); λ_{max}^{EtOH} 242 mμ (ε 12,300).

Anal. Calcd. for $C_{10}H_{12}ClNO_2$: C, 56.2; H, 5.66; N, 6.55. Found: C, 56.1; H, 5.52; N, 6.29.

5-(4-Nitrophenyl)-2,4-pentadien-1-al (XIc).—A suspension of 6 g. (34 mmoles) of 4-nitrocinnamaldehyde (XIb) in 20 ml. of acetaldehyde was cooled in an ice bath at 0° and stirred magnetically. Then 0.5 ml. of 25% methanolic NaOH solution was added dropwise, and stirring continued for 30 min. The reaction flask was fitted with a downward condenser, 15 ml. of acetic anhydride was added, and the mixture was heated at 120° for 1 hr. It was cooled and 60 ml. of water carefully was added, followed by 15 ml. of 4.35 *N* HCl. The mixture was refluxed for 30 min., cooled, and left overnight. The product was filtered off, washed with 20 ml. of water, and recrystallized from ethanol–water; yield of yellow crystals, 6.10 g. (88%); m.p. 105–106°; λ_{max} 5.96 (C=O), 6.16 (C=C), 6.62 μ (NO_2); λ_{max}^{EtOH} 339 mμ (ε 13,940).

Anal. Calcd. for $C_{11}H_9NO_3$: C, 65.0; H, 4.46; N, 6.90. Found: C, 64.8; H, 4.46; N, 6.79.

Similarly, *p*-nitrobenzaldehyde was converted to *p*-nitrocinnamaldehyde (XIb) in 57% yield, m.p. 141–142°.

Chloromethyl 4-(4-Nitrophenyl)-1,3-butadienyl Ketone (XIIc).—A mixture of 5.32 g. (30 mmoles) of XIb and 10.59 g. (30 mmoles) of triphenyl chloroacetonyl phosphorane was dissolved in 50 ml. of methanol and heated at 50° with stirring for 36 hr. The mixture was cooled, and the crystalline product was collected on a filter and washed with 10 ml. of methanol. Recrystallization from benzene gave 4.82 g. (64%) of yellow crystals, m.p. 148–150°; λ_{max} 5.89 (C=O), 6.23 (C=C), 6.62, 7.46 μ (NO_2); λ_{max}^{EtOH} 224 mμ (ε 29,770).

Anal. Calcd. for $C_{12}H_{10}ClNO_3$: C, 57.3; H, 4.00; Cl, 14.1; N, 5.57. Found: C, 57.5; H, 4.12; Cl, 14.1; N, 5.58.

Other ketones prepared in this way are listed in Table II under method A.

2-Chloromethyl-2-(*p*-nitrostyryl)-1,3-dioxolane (XIVb).—A mixture of 2.9 g. (12.8 mmoles) of *p*-nitrostyryl chloromethyl ketone (XIIb). 20 ml. of benzene, 10 ml. of ethylene glycol, and 20 mg. of *p*-toluenesulfonic acid was refluxed overnight; the water formed in the reaction was continuously removed with a Dean and Stark trap. The cooled mixture was washed with two 50-ml. portions of water and dried ($MgSO_4$). Removal of the solvent *in vacuo* gave a yellow crystalline residue which was recrystallized from ethanol; yield, 2.8 g. (81%); m.p. 155–156; λ_{max}^{EtOH} 222 mμ (ε 9426), 302 mμ (ε 13,090); λ_{max} 6.29, 6.78 (phenyl), 6.68, 7.50 (NO_2), 9.7–9.8 μ (C-O-C).

Anal. Calcd. for $C_{12}H_{12}ClNO_4$: C, 53.4; H, 4.49; N, 5.19. Found: C, 53.5; H, 4.61; N, 5.13.

Other dioxolanes prepared in this way are listed in Table II under method B.

2-(*p*-Aminostyryl)-2-chloromethyl-1,3-dioxolane (XV).—A solution of 2.4 g. (8.9 mmoles) of XIVb in 200 ml. of ethyl acetate was shaken with hydrogen at 2–3 atm. in the presence of 60 mg. of platinum oxide catalyst. Approximately 3 moles of hydrogen per mole was consumed in 15 min.; the reaction then slowed down considerably and was stopped. The filtered solution was concentrated *in vacuo*. Recrystallization of the product from benzene–petroleum ether (60–110°) gave nearly white crystals; yield, 1.65 g. (77%); m.p. 94–96°; λ_{max} 2.88, 2.95, 3.06 (NH), 6.21, 6.60 (phenyl), 9.5–9.8 μ (C–O–C); λ_{max}^{EtOH} 286 mμ (ε 33,700), $\lambda_{max}^{pH\ 13}$ 280 mμ (ε 33,700).

Anal. Calcd. for $C_{12}H_{14}ClNO_2$: C, 60.0; H, 5.89; Cl, 14.8; N, 5.84. Found: C, 60.0; H, 5.73; Cl, 14.8; N, 5.83.

2-(*p*-Aminophenethyl)-2-chloromethyl-1,3-dioxolane (XVIb).—To a solution of 2 g. (7.47 mmoles) of 2-chloromethyl-2-(4-nitrostyryl)-1,3-dioxolane (XIVb) in 50 ml. of ethyl acetate and 150 ml. of ethanol was added 60 mg. of platinum oxide catalyst and the mixture was shaken with hydrogen at 2–3 atm. until hydrogen consumption ceased (about 48 hr.). The filtered mixture was concentrated *in vacuo*. The remaining oil was dissolved in 25 ml. of ethanol and refluxed with 1 g. of activated charcoal for 1 hr. The mixture was filtered and removal of the solvent *in vacuo* yielded a colorless oil which could not be crystallized; yield, 1.68 g. (93%); λ_{max}^{film} 2.86, 2.93, 3.08 (NH), 6.16, 6.61 μ (phenyl); λ_{max}^{EtOH} 238 mμ (ε 10,100), 276 mμ (ε 8300).

Anal. Calcd. for $C_{12}H_{16}ClNO$: C, 59.6; H, 6.67; N, 5.79. Found: C, 59.8; H, 6.89; N, 5.61.

Other reductions carried out in the same way are listed in Table II under method C.

N-[1-(2-Amino-4-hydroxy-6-methyl-5-pyrimidyl)-3-propyl]-*p*-amino-α-chloroacetophenone Ethylene Ketal (VIIa).—A mixture of 223 mg. (1 mmole) of XVIa and 213 mg. (1 mmole) of 2-acetamido-4-hydroxy-6-methyl-5-pyrimidylpropionaldehyde[3] was dissolved in 25 ml. of methanol and sodium borohydride (500 mg.) immediately was added in small portions to the stirred solution. Stirring was continued for 18 hr., then the precipitated product was collected on a filter. The product was recrystallized from ethanol–water; yield, 240 mg. (61%); m.p. 172–173°; λ_{max}^{EtOH} 253 mμ (ε 18,930); λ_{max} 2.9–3.5 (broad NH, CH, OH), 9.80 (C–O–C), 12.20 μ (*p*-C_6H_4).

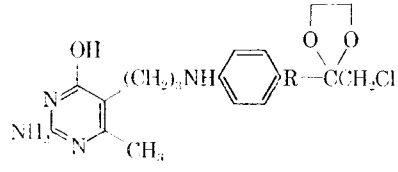
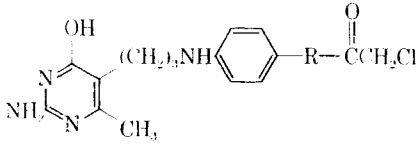
Anal. Calcd. for $C_{18}H_{23}ClN_4O_3$: C, 57.1; H, 6.12; N, 14.8. Found: C, 56.8; H, 5.96; N, 14.7.

Other related reductive condensations carried out in the same way are listed in Table III under method D.

N-[1-(2-Amino-4-hydroxy-6-methyl-5-pyrimidyl)-3-propyl]-*p*-aminochloroacetophenone (VIIIa).—To a solution of 0.76 g. (2 mmoles) of VIIa in 75 ml. of ethanol was added 25 ml. of 0.1

TABLE III

PHYSICAL CONSTANTS OF 2-AMINO-5-(3-ARYLAMINOPROPYL)-6-METHYL-4-PYRIMIDINOLS

Compd.	R	Method[a]	% yield	M.p., °C.	Calcd., %				Found, %				λ_{max}, mμ (ε × 10^{-4})		
					C	H	Cl	N	C	H	Cl	N	pH 1	pH 7	pH 13
															
VIIa	...	D	61	172–173	57.0	6.12		14.8	56.8	5.96		14.7		253 (18.9)	
VIIb	$-(CH_2)_2-$	D	30	217–218	59.0	6.69	8.71	13.8	58.9	6.87	8.50	13.9	265 (7.9)	238 (16.2) 292 (5.9)	240 (18.1) 281 (8.3)
VIIc	$-(CH_2)_4-$	D	65	139–141	60.7	7.18		12.9	60.8	7.30		12.8	265 (8.8)	241 (16.6) 292 (infl)	241 (18.1) 278 (9.2)
VIId	$-(CH_2)_6-$	D	45	168–169	62.3	7.62		12.1	62.2	7.67		12.1	265 (7.8)	241 (16.9) 293 (7.7)	240 (17.7) 281 (7.7)
IX	$-CH=CH-$	D	80[b]	170–172[b]	59.3	6.22	8.77	13.8	59.5	6.38	8.64	13.6	256 (28.2)	292 (27.7)	288 (27.7)
															
VIIIa	...	E	93	>300	57.4	5.70	10.6	16.7	57.2	5.92	10.5	16.5	249 (13.7)[c]		282 (9.5) 348 (22.5)
VIIIb	$-(CH_2)_2-$	F	64	>300	59.6	6.39	9.77	15.4	59.8	6.52	9.73	15.6	265 (7.1)		240 (12.8) 280 (6.8)
VIIIc	$-(CH_2)_4-$	F	65	139–141	61.5	6.96	9.07	14.3	61.5	6.96	8.97	14.4	265 (8.8)	241 (16.6)	241 (18.1) 278 (9.3)
VIIId	$-(CH_2)_6-$	F	84	128–129	63.1	7.46	8.46	13.4	63.2	7.46	8.56	13.9	265 (7.7)	239 (11.3)	279 (8.3)
X	$-CH=CH-$	F	60	>350	59.9	5.87	9.82	15.5	59.9	5.85	9.76	15.3	222 (16.0) 282 (20.3)	259 (9.8) 402 (18.9)	278 (10.2) 402 (18.7)

[a] See Experimental. [b] Recrystallized from aqueous 2-methoxyethanol. [c] pH 0.5.

N HCl. The mixture was refluxed for 1 hr., then diluted with 100 ml. of water, cooled in an ice bath, and made slightly alkaline with 0.1 *N* NaOH solution. Filter aid (3 g.) was stirred into the mixture and left for 0.5 hr. The precipitate was collected and washed well with water. The filter cake was then extracted with five 40-ml. portions of boiling ethanol. The alcohol was removed *in vacuo* and the yellow residue was recrystallized from aqueous ethanol; yield, 0.62 g. (93%); m.p. above 300° (dec.); λ_{max} 2.95–3.60 (broad OH, NH, CH), 12.30 (*p*-C_6H_4), 12.80 μ (C–Cl). See Table III for other data.

2-{N-[1-(2-Amino-4-hydroxy-6-methyl-5-pyrimidyl)-3-propyl]-4-aminophenyl}ethyl Chloromethyl Ketone (VIIIb).—A solution of 350 mg. (0.86 mmole) of XVIb in 25 ml. of 0.1 *N* aqueous HCl was heated on a steam bath for 1 hr. The pH of the solution was then adjusted to 9 with 0.1 *N* aqueous NaOH. The product was collected on a filter, washed with 10 ml. of water, recrystallized from ethanol–water, and gave light yellow crystals; yield, 200 mg. (64%); m.p. >300°; λ_{max} 2.90–3.40 (broad OH, NH, CH), 5.79 (C=O), no C–O–C band near 9.6 μ; $\lambda_{max}^{pH\ 13}$ 240 mμ (ϵ 12,700), 279 mμ (ϵ 6800); $\lambda_{max}^{pH\ 1}$ 217 mμ (ϵ 12,800), 265 mμ (ϵ 7100).

Other compounds prepared by a similar hydrolysis are listed in Table III under method F.

2-Amino-5-(*p*-*n*-butylanilinopropyl)-6-methyl-4-pyrimidinol (XVIII).—A solution of 1.49 g. (10 mmoles) of *p*-*n*-butylaniline[16] and 2.23 g. (10 mmoles) of I in 100 ml. of methanol was allowed to react for 30 min. With magnetic stirring 2 g. of sodium borohydride was added in portions over a period of 30 min. After being stirred overnight, 20 ml. of 5% aqueous NaOH was added and the methanol was removed *in vacuo*. The solution was diluted with 100 ml. of water and the pH was adjusted to 9 with 5% HCl. The precipitated product was collected and recrystallized from methanol–water; yield, 2.3 g. (73%); m.p. 223–225°; λ_{max} 2.90–3.60 (broad OH, NH, CH), 6.17, 6.58 (NH, C=N, C=C), 12.38 μ (C_6H_5–); $\lambda_{max}^{pH\ 1}$ 265 mμ (ϵ 9300); $\lambda_{max}^{pH\ 7}$ 240 mμ (ϵ 15,600), 291 mμ (ϵ 6300); $\lambda_{max}^{pH\ 13}$ 240 mμ (ϵ 17,200), 280 mμ (ϵ 7900).

Anal. Calcd. for $C_{18}H_{26}N_4O$: C, 68.8; H, 8.33; N, 17.8. Found: C, 68.5; H, 8.50; N, 17.7.

(16) R. R. Read and D. B. Mullin, *J. Am. Chem. Soc.*, **50**, 1763 (1928).

Synthesis of Fatty Acids with Smooth Muscle Stimulant Activity

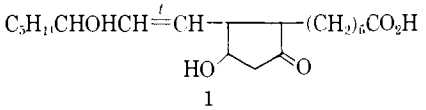
E. CRUNDWELL,[1] M. A. PINNEGAR, AND W. TEMPLETON

Miles-Ames Research Laboratories, Stoke Poges, Buckinghamshire, England

Received August 13, 1964

12-Hydroxyheptadec-*trans*-10-enoic acid has been synthesized by reduction of the corresponding acetylenic derivative with lithium in liquid ammonia at room temperature after pretreatment with lithium hydride to avoid hydrogenolysis. This acid, which represents a fragment of a prostaglandin, has been found to be three times as active as ricinelaidic acid in stimulating the isolated hamster colon. Homologs and other derivatives, including some containing an additional 6-oxo or 6-hydroxy group, have been synthesized and their activity has been determined. The hydroxyacetylenic acids were synthesized *via* chloroalkynols from alk-1-yn-3-ols and were converted into hydroxyoxoacetylenic acids *via* reaction of the acid chlorides with cycloalkenamines.

Interest has recently been renewed in the pharmacological potentialities of fatty acids largely because of the elucidation of the nature of the prostaglandins.[2] Consideration of the structure of prostaglandin E_1 (**1**) and of the pharmacologically active acids, notably ricinelaidic acid (**2**), among those examined for smooth muscle stimulant activity,[3,4] suggests that common features may include a hydroxyl group at position 12 and unsaturation or structural rigidity between positions 8 and 11 in an unbranched aliphatic acid.



1

$C_6H_{13}CHOHCH_2CH=^{t}CH(CH_2)_7CO_2H$

2

$RCHOHC\equiv C(CH_2)_nCO_2H$

3

Synthesis of acetylenic acids of type **3** was therefore investigated. One previous example (**3**, R = C_6H_{13}; n = 6) has been reported,[5] synthesized in unstated yield by a route involving the reaction of heptanal with lithium 8-chlorooctyne. From work with dimethylundecynamide (described below) there was reason to believe that higher alkynes might be unreactive so the following route was examined. This was

$$RCHO \longrightarrow \underset{\mathbf{4}}{RCHOHC\equiv CH} \longrightarrow \underset{\mathbf{5}}{RCHOPyC\equiv CH} \longrightarrow$$

$$\underset{\mathbf{6}}{RCHOHC\equiv C(CH_2)_nCl} \longrightarrow \underset{\mathbf{7}}{RCHOHC\equiv C(CH_2)_nCN} \longrightarrow$$

$$\underset{\mathbf{8}}{RCHOHC\equiv C(CH_2)_nCO_2Me} \longrightarrow$$

$$RCHOHCH=^{c}CH(CH_2)_nCO_2Me$$

9

Py = 2-tetrahydropyranyl

tested using the readily available hexynol (**4**, R = C_3H_7) to give the ester (8, R = C_3H_7; n = 6). Difficulties arose when the route was extended to use oct-1-yn-3-ol (**4**, R = C_5H_{11}). This had been previously prepared[6] by selenium dioxide oxidation of 1-octyne. This method is unsuitable for large-scale preparation so the familiar reaction of sodium acetylide with an aldehyde was used. However, it was found that with hexanal, particularly commercial samples, the yield and purity of octynol was very variable and frequently only higher boiling unsaturated hydroxy ketones were obtained. Similar difficulties were found with heptanal,[7] or when ethynylmagnesium bromide was used.

(1) School of Pharmacy, College of Technology, Park Road, Portsmouth, Hampshire, England.

(2) S. Bergström, R. Ryhage, B. Samuelsson, and J. Sjövall, *Acta Chem. Scand.*, **16**, 501 (1962).

(3) N. Ambache, *J. Physiol.* (London), **146**, 255 (1959); N. Ambache, M. Reynolds, and J. M. C. Whiting, *ibid.*, **166**, 251 (1963).

(4) M. S. Masri, L. A. Goldblatt, F. DeEds, and G. O. Kohler, *J. Pharm. Sci.*, **51**, 999 (1962).

(5) A. S. Bailey, V. G. Kendall, P. B. Lumb, J. C. Smith, and C. H. Walker, *J. Chem. Soc.*, 3027 (1957).

(6) R. Truchet, *Compt. rend.*, **196**, 708 (1933).

(7) L. Crombie, personal communication, 1963.