N-Hydroxymethyl Derivatives of Nitrogen Heterocycles as Possible Prodrugs II: Possible Prodrugs of Allopurinol, Glutethimide, and Phenobarbital

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Abstract □ Solid samples of 1-hydroxymethyl- and 1,5-dihydroxymethylallopurinol, 1-hydroxymethylglutethimide, and 1-hydroxymethylphenobarbital were prepared, and equilibrium constants for their formation from formaldehyde and allopurinol, glutethimide, or phenobarbital were calculated. The *N*-hydroxymethyl derivatives had higher water solubilities and faster dissolution rates than the parent drugs, and they appear to be potentially useful as prodrugs.

Keyphrases □ Allopurinol—*N*-hydroxymethyl derivative synthesized, structure confirmed by NMR and elemental analyses, potential prodrug □ Glutethimide—*N*-hydroxymethyl derivative synthesized, structure confirmed by NMR and elemental analyses, potential prodrug □ Phenobarbital—*N*-hydroxymethyl derivative synthesized, structure confirmed by NMR and elemental analyses, potential prodrug □ Prodrugs, potential—*N*-hydroxymethyl derivatives of allopurinol, glutethimide, and phenobarbital synthesized, structures confirmed by NMR and elemental analyses

The potential usefulness of *N*-hydroxymethyl derivatives of heterocyclic amides and imides as prodrugs was discussed in a previous paper (1). This work was consistent with studies on the *N*-hydroxymethyl derivatives of hydantoins (2) and other amides and imides (3).

The present paper is concerned with the synthesis and properties of *N*-hydroxymethyl derivatives of allopurinol (I), glutethimide (II), and phenobarbital (III). These drugs have low water solubility and dissolve slowly. Hence, it is likely that their bioavailability and delivery (4) would be improved if appropriate prodrug forms could be developed.

RESULTS AND DISCUSSION

The interaction of formaldehyde with allopurinol (I), glutethimide (II), and phenobarbital (III) at 25° was studied by analyzing phase solubility diagrams (Figs. 1–3). These diagrams were constructed by plotting the total substrate concentration in solution $[S]_T$ *versus* the added formaldehyde concentration $[H_2CO]_T$. At least one solid phase was present in all solutions.

***N*-Hydroxymethylation of I**—Analysis, using arguments set out by Higuchi and Connors (5) of the double Bs-type phase solubility diagram for I (Fig. 1), led to the conclusion that the reactions shown in Scheme I took place. The A–E portion of the diagram is consistent with the formation of 1-hydroxymethylallopurinol (1-hydroxymethyl-4,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one, I*a*), and the E–I portion is consistent with the formation of 1,5-dihydroxymethylallopurinol {1,5-bis(hydroxymethyl)-4,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one, I*b*}. The B–C portion is believed to be caused by saturation of the solution with respect to I*a*.

Confirmation of these postulates comes from elemental analysis and NMR spectra (in dimethyl sulfoxide-d_6) of the solid phase at point E and in the portion H–I (Table I), from calculation of the stoichiometry of the reactions using portions B–D and F–G, and from equilibrium constants calculated from the slopes of the lines AB (K_{Ia}) and EF (K_{Ib}).

The NMR peak at 5.63 ppm in the spectrum of the solid isolated at point E and those at 5.40 and 5.63 ppm in the spectrum of the solid isolated in the portion H–I had similar chemical shifts to the N–CH_2–O protons in the *N*-hydroxymethyluracils (1). The stoichiometry of the solid isolated at point E calculated from the phase solubility diagram was 1 part of I to 1.03 parts of formaldehyde, and that of the compound isolated in the region H–I was 1 part of I to 2.06 parts of formaldehyde. The elemental analyses of the compounds were as expected for I*a* and I*b*.

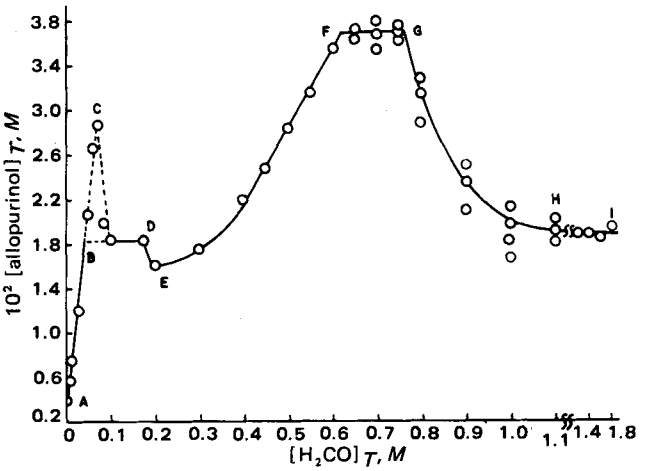
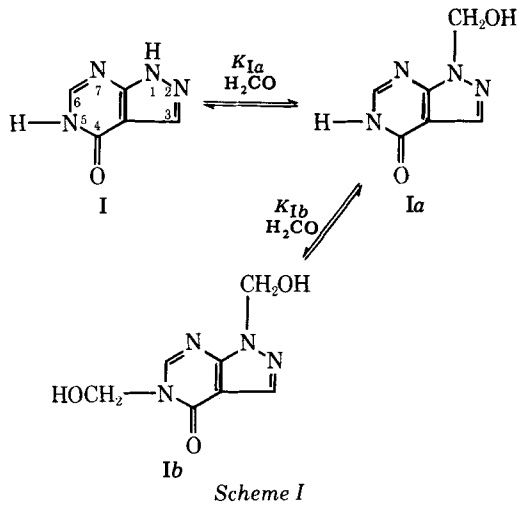


Figure 1—*Plot of I solubility as a function of formaldehyde concentration in aqueous buffer at pH 4.9, μ = 0.1* M, *and 25°.*

The equilibrium constants calculated from the phase solubility diagram were 120 M^{-1} for formation of I*a* (K_{Ia}) and 4 M^{-1} for the formation of I*b* (K_{Ib}). This value of K_{Ia} is similar to values reported (6) for the addition of secondary amines to formaldehyde (40–800 M^{-1}). The value of K_{Ib} is similar to that calculated for the addition of amino and imino groups in uracils to formaldehyde (1).

***N*-Hydroxymethylation of II**—The Bs-type phase solubility diagram in Fig. 2 was analyzed similarly to the one for *N*-hydroxymethylation of I. Elemental analysis and NMR spectral data (Table I) of the solid phase obtained at point D (from deuterium oxide solutions) indicated that it was 1-hydroxymethylglutethimide (1-hydroxymethyl-3-phenyl-2,6-piperidinedione, II*a*). Confirmation of the reactions shown in Scheme II was obtained from the facts that the phase solubility diagram indicated that the stoichiometry of the complex was 1 equivalent of II to 1.02 equivalents of formaldehyde and that the equilibrium constant for its formation, K_{IIa}, was 3.4 M^{-1}.



Scheme I

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Table I—NMR and Elemental Analysis Data for *N*-Hydroxymethyl Compounds

Compound	Chemical Shifts[a], ppm	Analysis, %	Calc.	Found
Ia[b]	5.63 bs	C	43.34	43.02
		H	3.61	3.70
		N	33.71	33.32
Ib[b]	5.63 bs, 5.40 bs	C	42.83	42.70
		H	4.08	4.01
		N	28.55	28.09
IIa[c]	5.40 d	C	67.94	67.57
		H	6.87	6.62
		N	5.66	5.41
IIIa[b]	5.30 bs	C	59.49	59.46
		H	5.34	5.34
		N	10.68	10.94

[a] Chemical shifts of protons not present in the parent compound. The area under the peak integrated for two protons, and bs and d represent broad singlet and doublet, respectively. [b] Spectra measured in dimethyl sulfoxide-d_6. [c] Spectrum measured in deuterochloroform.

Table II—Amounts of Substrate and Ligand Used in Phase Solubility Studies

Substrate	Ligand	Total Volume, ml	Equilibrium Time, days
I, 0.1 g	Formaldehyde, 0–2 *M*	5	15
II, 0.4 g	Formaldehyde, 0–2 *M*	5	10
III, 0.4 g	Formaldehyde, 0–2 *M*	20	10

Table III—Melting Points, Solubilities, and Initial Dissolution Rates[a] of Drugs and Their Prodrugs

Compound	Melting Point	Solubility[b], *M*	Initial Dissolution Rate, *M*/min × 10^5
I	360°	0.004	0.2
Ib	350°	0.019	1.2
II	85°	0.005	1.3
IIa	82°	0.022	2.2
III	178°	0.004	0.9
IIIa	99°	0.025	2.8

[a] Dissolution rates were obtained in pH 7 buffer at 25° and μ = 0.1 *M*. [b] Solubilities were obtained from phase solubility diagrams (Figs. 1–3).

Formaldehyde concentrations of >2 *M* caused the total amount of II in solution to increase, resulting in the separation of an oil (Table II). The reactions occurring in this region of the phase solubility diagram were not investigated further, but they probably are caused by addition of the >N–CH_2OH group to additional formaldehyde molecules to form polymers such as >N–$[CH_2O]_n$–. There is evidence (7) that formaldehyde polymerizes in concentrated aqueous solutions.

***N*-Hydroxymethylation of III**—The complex Bs-type phase solubility diagram for this system is shown in Fig. 3.

Based on the *N*-hydroxymethylation of uracil (1) and II, the reactions shown in Scheme III were expected. However, only one compound was

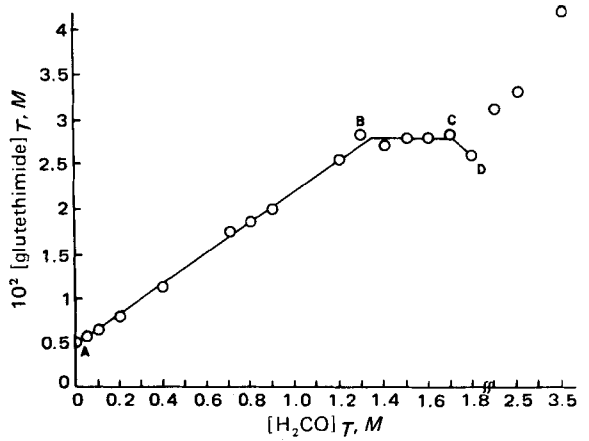
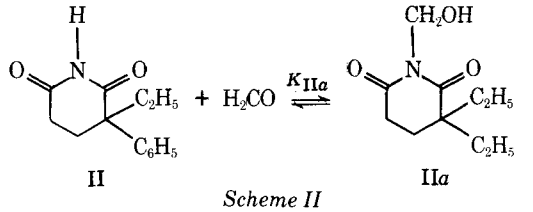


Figure 2—*Plot of II solubility as a function of formaldehyde concentration in aqueous buffer at pH 5.1, μ = 0.1 M, and 25°.*



II IIa

Scheme II

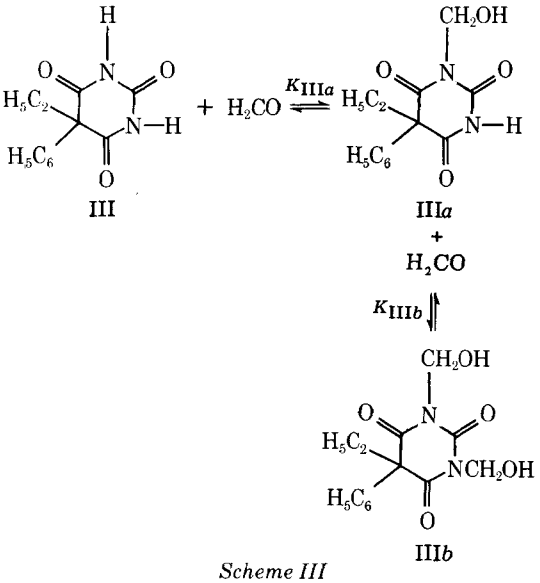
isolated from the system. The solid phase at point D had an elemental analysis and NMR spectrum consistent with it being 1-hydroxymethylphenobarbital (IIIa). Analysis of the portion B–C of the phase solubility diagram indicated that the compound had the required stoichiometric composition. Isolation of 1,3-dihydroxymethylphenobarbital was complicated by the fact that a viscous oil phase again separated out at formaldehyde concentrations of >1.6 *M*.

The shape and slope of the portion A–B of the phase solubility diagram would be a function of the equilibrium constants K_{IIIa} and K_{IIIb}. At low formaldehyde concentrations, the curve would have a limiting slope that would be related directly (5) to K_{IIIa}. The K_{IIIa} value was estimated to be 9 M^{-1}. Hence, equilibrium data are consistent with the proposed reaction scheme.

Aqueous Solubility and Dissolution Rates—Aqueous solubilities and relative dissolution rates in water of I, Ib, II, IIa, III, and IIIa are listed in Table III. The solubilities were obtained from the phase solubility diagrams (5), and dissolution rates were calculated as described under *Experimental*.

As expected (1), the change in intermolecular hydrogen-bonding possibilities in the solid state produced by *N*-hydroxymethylation of amines or imides increased the water solubilities of I–III. The increase in water solubility was reflected by an increase in the dissolution rate as required by the Noyes–Whitney model for dissolution (8, 9).

The *N*-hydroxymethyl derivatives were converted rapidly to the parent



III IIIa + H_2CO IIIb

Scheme III

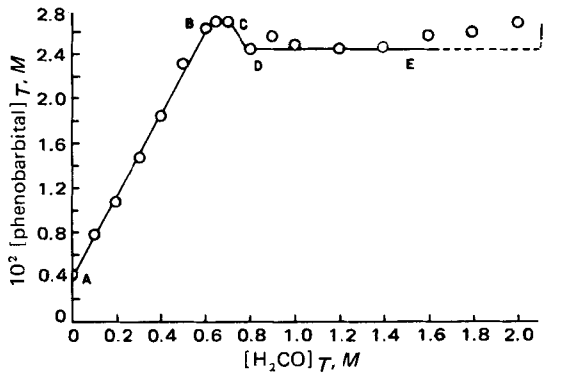


Figure 3—*Plot of III solubility as a function of formaldehyde concentration in aqueous buffer at pH 4.9, μ = 0.1 M, and 25°.*

drug. This result was indicated by the fact that the solid phase in a suspension of the *N*-hydroxymethyl derivatives (500 mg in 25 ml of pH 7 buffer) that had been shaken for 10 min had the same melting point and NMR spectrum in dimethyl sulfoxide as the parent drug. UV spectra of dilute solutions of the *N*-hydroxymethyl derivatives in pH 7 buffer were superimposable on those of the parent drug after the solutions had been shaken for 10 min.

All of these observations support the contention that *N*-hydroxymethyl derivatives of poorly soluble amides, imides, and amines are potentially useful prodrugs.

EXPERIMENTAL

Chemicals and Equipment—Allopurinol[1] (I), phenobarbital[1] (III), and glutethimide[2] (II) were used without further purification, as was formaldehyde[3] (37% aqueous solution).

Solutions containing formaldehyde were prepared fresh by appropriate dilution of the 37% formaldehyde solution. The succinic acid–sodium borate buffers (pH 3.69–6.9, μ = 0.1 *M*) and monobasic potassium phosphate–sodium borate buffers (pH 6.3–9.25, μ = 0.1 *M*) were prepared by the method described by Pitman (10). Sodium chloride was used to adjust the ionic strength (μ = 0.1 *M*).

3-(Trimethylsilyl)propanesulfonic acid was used as an internal reference in the NMR measurements.

Phase Solubility—A fixed quantity of substrate, in excess of its aqueous solubility, was weighed in a series of 25-ml polyseal-lined screw-capped vials to which were added fixed volumes of formaldehyde solutions of increasing concentration in the buffer (Table II). Vials were sealed, placed in a constant-temperature water bath at 25.0 ± 0.2°, and agitated with a rotating-action shaker until equilibrium was achieved. Aliquots of the supernatant liquid were removed using a pipet (whose tip had been wrapped with glass wool) or solutions were filtered using a sintered-glass funnel of medium porosity and analyzed spectrophotometrically.

Isolation of *N*-Hydroxymethyl Derivatives—*1,5-Dihydroxymethylallopurinol (Ib)*—One gram of allopurinol was dissolved in 50 ml of 2 *M* formaldehyde at pH 7. Agitation of this solution for 24 hr resulted in a white crystalline precipitate. The elemental analysis (Table I) and NMR spectrum of the solid (dried over calcium chloride) were consistent with the conclusion that the compound was *Ib*.

1-Hydroxymethylallopurinol (Ia)—Compound I*a* was isolated in a similar manner to I*b*, except 0.2 *M* formaldehyde was used in this case. The elemental analysis (Table I) and NMR spectrum of the dry solid were consistent with it being I*a*.

1-Hydroxymethylglutethimide (IIa)—Compound II*a* was prepared by agitating 1 g of glutethimide in 100 ml of 1.5 *M* formaldehyde in a pH 7 buffer for 24 hr. The NMR spectrum and elemental analysis of the resultant solid (dried over calcium chloride) were consistent with it being II*a* (Table I). However, at high concentrations of formaldehyde (10 *M*), a viscous oil was isolated. This oil was believed to be II*a* and the corresponding diformyl compound, which would be formed by a dimer of formaldehyde.

1-Hydroxymethylphenobarbital (IIIa)—Compound III*a* was prepared by agitating 1 g of phenobarbital in 20 ml of 1 *M* formaldehyde in a pH 5 buffer for 24 hr. The resultant white crystalline precipitate melted at 99° when drying over calcium chloride. The elemental analysis (Table I) and NMR spectrum of the solid were consistent with it being III*a* (Table I). However, at high concentrations of formaldehyde (10 *M*), a viscous oil was isolated.

Dissolution Rate—All dissolution studies were performed according to the USP dissolution test (11).

The solid whose dissolution rate was to be measured was reduced to a uniform particle size by sifting it through a 40-mesh sieve and collecting it in a 60-mesh sieve. Approximately 250 mg of this material then was compressed into a tablet in an evacuable potassium bromide die at 10,000 psi for 30 sec using a laboratory press. The dissolution medium (500 ml, pH 7 buffer solution) was placed in a 1-liter beaker immersed in a constant-temperature bath at 25°. One tablet was placed in a basket, and the basket was rotated at 100 rpm. The samples (5 ml) were withdrawn at appropriate times and analyzed spectrophotometrically.

[1] Sigma Chemical Co.
[2] Ciba-Geigy Co.
[3] J. T. Baker Chemical Co.

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