glacial acetic acid, then spin-evaporated *in vacuo* on a hot water bath. The syrupy residue was partitioned between 25 ml. each of chloroform and water. The separated aqueous phase was extracted with an additional 25 ml. of chloroform. The combined organic extracts, dried with magnesium sulfate, were spin-evaporated *in vacuo*. Crystallization from ethyl acetate gave 0.586 Gm. (25%) of product, m.p. 158–161°. Recrystallization gave white crystals, m.p. 160–162°; $\nu_{max.}$ 2800–1700 (broad acidic OH); 1620 (C=O); 1590, 1530, 1500, 1490 (C=C); 760, 725, 695, 690 cm.$^{-1}$ (phenyl CH); $\lambda_{max.}^{pH\ 1}$ 252 mμ (ε 12,800); $\lambda_{max.}^{pH\ 7}$ 235 (ε 10,100), 345 mμ (ε 17,100); $\lambda_{max.}^{pH\ 13}$ 240 (inflection), 345 mμ (ε 19,500); δ 13.70, one chelated hydrogen.

Anal.—Calcd. for $C_{18}H_{17}NO_2$: C, 77.5; H, 6.10; N, 5.01. Found: C, 77.2; H, 5.95; N, 4.90.

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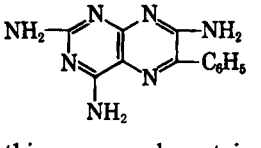
Phase Solubility Technique in Studying the Formation of Complex Salts of Triamterene

By LEWIS W. DITTERT, TAKERU HIGUCHI, and DAVIS R. REESE

Profiles of the apparent solubility (37°) of triamterene (2,4,7-triamino-6-phenylpteridine) as a function of pH were obtained in the presence of hydrochloric, nitric, sulfuric, phosphoric, and acetic acids. These diagrams permitted detection of complex salt species containing both protonated and unprotonated triamterene. The stoichiometries of the complexes were determined from the slopes of log ($S - S_o$) *versus* pH plots and were confirmed by elemental analysis of the solid phases. Equilibria which explain the pH-solubility profiles of the various acids are proposed. The utility of the phase solubility technique in detecting and studying complex salts is discussed.

SALT SPECIES, other than simple salts, are normally discovered accidentally or incidentally in other studies. Since these complex salts often have solubilities considerably different from normal salts, they may be important pharmaceutically from standpoints such as better *in vivo* availability, greater stability, ease of formulation, etc. In the present study, the phase solubility technique was used to detect complex salt species of triamterene in the presence of various acids. Although this study is limited to triamterene, it is apparent that the procedure is applicable to other systems.

Triamterene (2,4,7-triamino-6-phenylpteridine) is a pteridine diuretic with the structure



Although this compound contains three primary amine groups, it is only weakly monobasic, *e.g.*, toward perchloric acid in glacial acetic acid. Probably because of this weak basicity, most attempts to form classical salts have failed.

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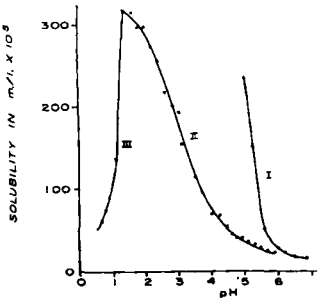

Fig. 1.—pH-solubility profile for triamterene in the presence of hydrochloric acid.

Triamterene is only slightly soluble in water (45 mcg./ml.). This study was initiated to determine to what degree the aqueous solubility of triamterene is increased in the presence of various acids.

EXPERIMENTAL

Analysis of Total Triamterene in Solution.—A clear portion of the solution in equilibrium with a suspension of triamterene at a given pH was obtained by withdrawing the solution into a 5 or 10 ml. Luer-Lok syringe[1] through a Swinney filter adapter[2] containing a type HA filter[2] and a pressed glass wool prefilter.[2] The Swinney adapter was removed, and the fluid was transferred to a small beaker. An aliquot of the solution was pipeted into a 100-ml. volumetric flask containing 4 ml. of concentrated reagent grade formic acid (88%). The contents of the flask were brought to volume with water, and the concentration of triamterene was determined using the absorbance maximum at 357.5 mμ.

pH-Solubility Profiles.—The experiments were conducted in a 600-ml. beaker suspended in a water bath maintained at 37°. The contents of the beaker were stirred by an overhead stirrer with a glass impeller. The pH was followed with a pH meter.

Two grams of triamterene and 400 ml. of water were stirred in the beaker. After approximately 15 minutes (the solution becomes saturated in less than 1 minute), the pH was recorded, and an aliquot of the saturated solution was withdrawn and analyzed as described above. Acid was added dropwise with stirring until the pH shifted about 0.3 units, and the stirring was continued for 15 minutes. A constant pH reading during this 15-minute period indicated that the system was at equilibrium. (Identical results were obtained when stirring times of 1 hour were allowed.) An aliquot was then removed and analyzed.

The pH was thus lowered stepwise and an aliquot removed and analyzed at each step, until the pH reached 1 or until all of the solid phase in the system dissolved. In some cases (*e.g.*, with hydrochloric acid) the pH initially fell, then rose upon further addition of acid. In these cases, sampling was discontinued until the pH remained constant with stirring. Sampling was then resumed at 0.3 pH unit intervals.

Elemental Analyses.—Suspensions of triamterene were adjusted to the proper pH with the acid under study and stirred at constant pH for 15 minutes. The suspensions were filtered through fine porosity sintered-glass Büchner funnels. The material remaining on the filter was dried over sulfuric acid for 3 days. The resulting solids were used for elemental analyses.

Solubility Product Constants.—A suspension of triamterene in a solution of a salt was adjusted to pH 1.3 with the appropriate acid. The suspension was stirred for 15 minutes at constant pH, then analyzed for triamterene in solution as previously described. The solubility product constants (K_{sp}) for four concentrations of each salt were calculated according to the K_{sp} equations shown in the tables below Figs. 2–5 and 7. (The concentration of anion in each case was calculated from the hydrogen ion, triamterene, and sodium salt concentrations.) The average of the four calculated constants is reported.

RESULTS AND DISCUSSION

Hydrochloric Acid.—The pH-solubility profile of triamterene in the presence of hydrochloric acid is shown in Fig. 1. After the solubility rose initially to a maximum at pH 5 (curve I), the pH became unsteady. Continued addition of a relatively large quantity of hydrochloric acid caused the pH to rise to about 6 and remain constant, while the solubility of triamterene fell near its initial level at pH 7. Further addition of acid resulted in the second rising curve (II), which ended in a precipitous drop in solubility (curve III) below pH 1.35.

The interaction of protons with triamterene in solution may be described by the equilibrium

$$\begin{matrix}\text{Triamterene} \\ \downarrow\ \uparrow \\ \text{Triamterene (s)}\end{matrix} + x\,H^+ \overset{K}{\rightleftarrows} \text{Triamterene}\cdot H_x^+ \qquad \text{(Eq. 1)}$$

where

$$K = \frac{[\text{Triamterene}\cdot H_x^+]}{[\text{Triamterene}]\,[H^+]^x} \qquad \text{(Eq. 2)}$$

K is the so-called stability constant of the tri-

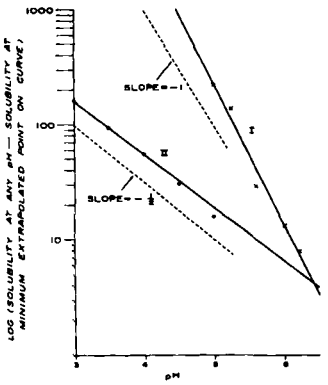

Fig. 2.—Log increase in solubility *vs.* pH plots for triamterene in the presence of hydrochloric acid. Key: x, high pH range (5.5–6.5); ●, medium pH range (3–5.5).

EQUILIBRIA IN THE HYDROCHLORIC ACID SYSTEM

pH Range	Equilibrium
5–7	Triamterene + $H^+ \rightleftharpoons$ triamterene$\cdot H^+$
About 5	Triamterene$\cdot H^+$ + triamterene + $Cl^- \rightleftharpoons$ (triamterene)$_2\cdot$HCl (ppt. 1)
2–5	(Triamterene)$_2\cdot$HCl + $H^+ \rightleftharpoons$ 2 triamterene$\cdot H^+$ + Cl^-
Below 1.35	Triamterene$\cdot H^+$ + $Cl^- \overset{K_{sp}}{\rightleftharpoons}$ triamterene$\cdot$HCl (ppt. 2)

$K_{sp} = 1.5 \times 10^{-4}$

[1] Becton-Dickenson and Co., Rutherford, N.J.
[2] Millipore Filter Corp., Bedford, Mass.

TABLE I.—ELEMENTAL ANALYSES OF TRIAMTERENE COMPLEXES

Complex	Structure	Element	Theoretical %	% Found[a]
Hydrochloric acid				
Precipitate 1	(Triamterene)$_2$·HCl	Cl	6.5	6.2
Precipitate 2	(Triamterene)·HCl	Cl	12.3	11.5*
Nitric acid				
Precipitate 1	(Triamterene)$_3$·HNO$_3$	N	37.5	38.1*
Precipitate 2	(Triamterene)·HNO$_3$	N	34.3	33.5*
Sulfuric acid				
Precipitate 1	(Triamterene)$_4$·H$_2$SO$_4$	S	2.9	2.8
Precipitate 2	(Triamterene)$_2$·H$_2$SO$_4$	S	5.3	5.1
Phosphoric acid				
Precipitate 1	(Triamterene)$_2$·H$_3$PO$_4$	P	5.1	5.0
Precipitate 2	(Triamterene)·H$_3$PO$_4$	P	8.8	9.9*

[a] When attempts were made to wash or recrystallize the complexes, they decomposed to triamterene base and the corresponding acid. The complexes were prepared for analysis without washing. Therefore, some of the elemental analyses (indicated by an asterisk) are outside the usual acceptable limits of error for well-defined compounds. The results are closer to the theoretical per cents shown that to those for any other triamterene:acid ratios.

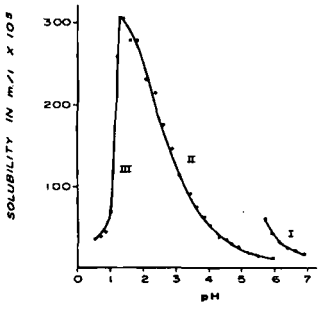

Fig. 3.—pH-solubility profile for triamterene in the presence of nitric acid.

EQUILIBRIA IN THE NITRIC ACID SYSTEM

pH Range	Equilibrium
5.7–7	Triamterene + H$^+$ $\rightleftharpoons$ triamterene·H$^+$
About 5.7	Triamterene·H$^+$ + 2 triamterene + NO$_3^-$ $\rightleftharpoons$ (triamterene)$_3$·HNO$_3$ (ppt. 1)
2–6	(Triamterene)$_3$·HNO$_3$ + 2H$^+$ $\rightleftharpoons$ 3 triamterene·H$^+$ + NO$_3^-$
Below 1.25	Triamterene·H$^+$ + NO$_3^-$ $\overset{K_{sp}}{\rightleftharpoons}$ triamterene·HNO$_3$ (ppt. 2)
	$K_{sp} = 7.6 \times 10^{-5}$

amterene·H$_x^+$ complex, and x is the number of protons required to solubilize one molecule of triamterene. In this expression, [triamterene] is the saturation solubility of the solid phase in the pH region covered by a particular curve. For example, triamterene base is the solid phase in the region of curve I, and the insoluble complex, (triamterene)$_2$·HCl, is the solid phase in the region of curve II. The solubility of the solid phase is a constant for each curve; the numerator term, [triamterene·H$_x^+$], is the increase in solubility of triamterene in the region of that curve. Equation 2 can be transformed into a form (Eq. 3) similar to that used by Higuchi *et al.* (1) to describe the solubility behavior of weak acids at various pH levels. In this equation, the term $(S - S_o)$ is the increase in solubility of triamterene as the pH is lowered. Plots of the hydrochloric acid data according to Eq. 3 are shown in Fig. 2. Values for S_o were determined by extrapolating curves I and II to their minima at the high pH ends. The slopes of the plots in Fig. 2 show that in the pH range of curve I, one proton solubilizes one molecule of triamterene; in the pH range of curve II, one proton solubilizes two molecules of triamterene.

$$\log (S - S_o) = -x\,\mathrm{pH} + \log C \quad \text{(Eq. 3)}$$

Between the two curves the new solid phase, (triamterene)$_2$·HCl, is formed. This complex first forms as a supersaturated solution (at pH 5) which nucleates as additional hydrochloric acid is added, causing the precipitous drop in solubility and the fluctuating pH observed. After all the triamterene in the system has been converted to the new complex, each molecule of the complex is solubilized by one proton, causing the log plot of curve II (Fig. 2) to have a slope of $-1/2$.

The equilibria controlling the solubility behavior of triamterene in hydrochloric acid solutions are summarized in the table below Fig. 2. The final reaction is the solubility product equilibrium for triamterene hydrochloride. The solubility product constant for this reaction was 1.5×10^{-4}. Apparently the final drop in solubility (curve III, Fig. 1) is due to common ion suppression of the solubility product equilibrium.

The results of the elemental analyses of precipitates 1 and 2 are shown in Table I. These results are in agreement with the proposed equilibria. Precipitates 1 and 2 were solid phases in equilibrium

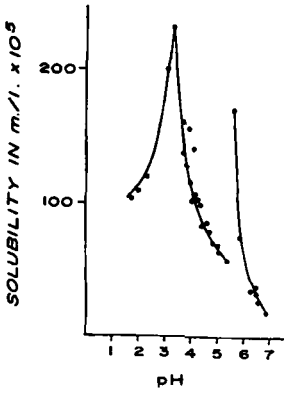

Fig. 4.—pH-solubility profile for triamterene in the presence of sulfuric acid.

EQUILIBRIA IN THE SULFURIC ACID SYSTEM

pH Range	Equilibrium
5.5–7	Triamterene + H$^+$ $\rightleftharpoons$ triamterene·H$^+$
About 5.5	2 Triamterene·H$^+$ + 2 triamterene + SO$_4^-$ $\rightleftharpoons$ (triamterene)$_4$·H$_2$SO$_4$ (ppt. 1)
3.2–5.5	(Triamterene)$_4$·H$_2$SO$_4$ + 2H$^+$ $\rightleftharpoons$ 4 triamterene·H$^+$ + SO$_4^-$
Below 3.2	2 Triamterene·H$^+$ + SO$_4^-$ $\overset{K_{sp}}{\rightleftharpoons}$ (triamterene)$_2$·H$_2$SO$_4$ (ppt. 2)
	$K_{sp} = 4.0 \times 10^{-5}$

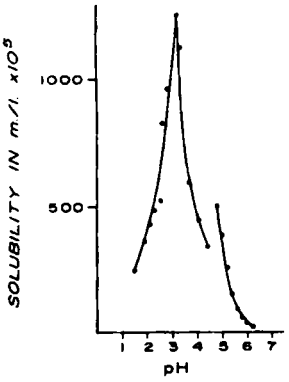

Fig. 5.—pH-solubility profile for triamterene in the presence of phosphoric acid.

EQUILIBRIA IN THE PHOSPHORIC ACID SYSTEM

pH Range	Equilibrium
4.8–7	Triamterene + $H^+ \rightleftharpoons$ triamterene·H^+
About 4.8	Triamterene·H^+ + triamterene + $H_2PO_4^- \rightleftharpoons$ (triamterene)$_2$·H_2PO_4 (ppt. 1)
3.2–4.8	(Triamterene)$_2$·H_2PO_4 + $H^+ \rightleftharpoons$ triamterene·H^+ + $H_2PO_4^-$
Below 3.2	Triamterene·H^+ + $H_2PO_4^- \overset{K_{sp}}{\rightleftharpoons}$ triamterene·H_2PO_4 (ppt. 2)

$K_{sp} = 8.7 \times 10^{-6}$

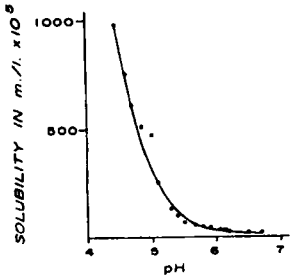

Fig. 6.—pH-solubility profile for triamterene in the presence of acetic acid.

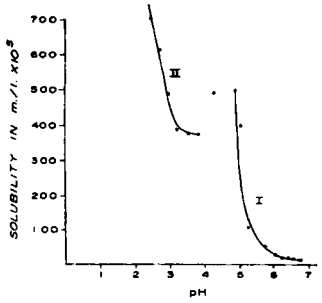

Fig. 7.—pH-solubility profile for triamterene in acetic acid solutions containing 6×10^{-3} *M* sodium acetate.

EQUILIBRIA IN THE ACETIC ACID–SODIUM ACETATE SYSTEM

pH Range	Equilibrium
4.8–7	Triamterene + $H^+ \rightleftharpoons$ triamterene·H^+
About 4.5	Triamterene·H^+ + triamterene + $OAc^- \rightleftharpoons$ (triamterene)$_2$·HOAc
2–4	(Triamterene)$_2$·HOAc + $H^+ \rightleftharpoons$ 2 triamterene·H^+ + OAc^-

with solutions at pH values 4 and 1, respectively. They are assumed to be composed of one molecular specie.

Nitric, Sulfuric, and Phosphoric Acids.—The pH solubility profiles and equilibria governing the behavior of triamterene in nitric, sulfuric, and phosphoric acid systems are shown in Figs. 3–5. In the nitric acid case, the slope of the log plot of curve II is $-2/3$, suggesting that the insoluble complex that precipitates near pH 5.7 has the composition triamterene$_2$·HNO_3. The elemental analysis of precipitate 1 (Table I) is in agreement with this hypothesis.

The sulfuric and phosphoric acid profiles differ from the nitric and hydrochloric acid profiles in that the final drops in solubility due to common ion suppression of the solubility product equilibrium occur at somewhat higher pH's in the former two systems. Also, the solubilities achieved with phosphoric acid are somewhat higher than those achieved with the other inorganic acids. With both sulfuric and phosphoric acid, elemental analyses of the precipitates removed from the systems agree with the proposed equilibria.

Acetic Acid.—The pH-solubility profile for triamterene in the presence of acetic acid is shown in Fig. 6. In this system the solubility of triamterene rises continuously following a single curve. Solubilities higher than those observed with any of the inorganic acids were achieved with acetic acid. The experiment was discontinued at pH 4.5 when all the triamterene in the system dissolved.

When the acetic acid experiment was repeated in the presence of 6×10^{-3} *M* sodium acetate, the resulting pH-solubility profile (Fig. 7) showed the familiar two-curve plot. Log $(S - S_o)$ plots of the data have slopes of -1 (curve I) and $-1/2$ (curve II), as in the hydrochloric acid case. There is no final drop in solubility due to solubility product effects at lower pH's with acetic acid, probably because the low pH's required cannot be reached with acetic acid and also because at low pH's acetic acid exists largely in the undissociated form. The equilibria which describe the solubility behavior of triamterene in solutions of acetic acid are shown in the table below Fig. 7.

CONCLUSIONS

The phase solubility technique has been used to determine the solubility behavior of triamterene in the presence of several acids. The pH-solubility profiles for triamterene revealed (*a*) the formation and stoichiometries of complex salt species in solution, (*b*) the formation and stoichiometries of insoluble complex salts, and (*c*) the controlling equilibria in each pH range. Phosphoric acid and acetic acid produced solubilities of triamterene higher than hydrochloric, nitric, or sulfuric acid. Acetic acid formed complexes more soluble than the inorganic acids.

The experimental method employed in studying triamterene might also be used for the following types of studies:

(*a*) Determination of the pH-solubility characteristics of salts of a drug without actual synthesis and and isolation.

(*b*) Detection and study of complexes of a drug under pharmaceutically significant conditions, *i.e.*, saturated solutions.

(*c*) Detection and isolation of pharmaceutically interesting complexes.

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