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Keyphrases

o-Azidobenzaldehyde—synthesis
2-Substituted indazoles—synthesis
Palladium-on-carbon—hydrogenation
Condensation reactions

Solubilization of Aminopteridines

By MILTON LAPIDUS

Aqueous solutions of a series of aminopteridines (5–10 mg./ml.) stable at physiologic pH's were prepared either by salt formation with hydrochloric acid or by complex formation with 10 percent deoxyribonucleic acid. Deoxyribonucleic acid was superior to ribonucleic acid, crystalline bovine serum albumin, human serum albumin fraction V, or gum acacia in solubilizing 4,7-diamino-2(*p*-chlorophenyl)-*N*-(2-diethylaminoethyl)-6-pteridinecarboxamide at pH 7. Potentiometric titration detected complex formation between deoxyribonucleic acid and the above aminopteridine.

In the course of preparing injectable solutions of a number of aminopteridines it was observed that very few in the series under investigation were appreciably soluble in water. The general lack of aqueous solubility of substituted pteridines has been reported as being due to substituted polar groups (OH, SH, and NH_2) (1). Pteridine (unsubstituted) was reported as being highly soluble (1 part in 7.5 parts of water at 20°).

Although classic salt formation has been used successfully to solubilize aliphatic amines, this method has limited applicability to weakly basic aminopteridines. The apparent acid solubility of triamterene (2,4,7-triamino-6-phenylpteridine) is reversed as the pH is raised to 7 (2).

Complex formation has been reported to increase the aqueous solubility of small molecules (3–5). Speculation that deoxyribonucleic acid, ribonucleic acid, serum proteins, and gum acacia may form aminopteridine complexes with enhanced solubility characteristics stimulated this study.

EXPERIMENTAL

The aminopteridines studied were synthesized by Osdene *et al.* (6, 7). Deoxyribonucleic acid-sodium (DNA-Na), ribonucleic acid-sodium (RNA-Na), and crystalline bovine serum albumin (BSA) were purchased from Nutritional Biochemical Corp., Cleveland, Ohio; human serum albumin (HA) fraction V from Pentex, Inc., Kankakee, Ill.; and USP gum acacia from S. B. Penick and Co., New York, N. Y. The standard reagents used were the best grades commercially available.

Analysis of Wy-4029—Wy-4029 [4,7-diamino-2-(*p* - chlorophenyl) - *N* - (2 - diethylaminoethyl) - 6-pteridinecarboxamide] in 0.1 *N* sulfuric acid fluoresces at 445 mμ when activated at 390 mμ. Using the Aminco Bowman spectrophotofluorometer, a linear response was obtained in the concentration range of 2–9 mcg./ml.

pH of Complete Solubility and Solubility Reversal—To 25 mg. of each of the aminopteridines suspended in 50 ml. of water increments of 1 *N* HCl were added until the soluble end point was reached. The pH was monitored with a Beckman pH meter, and pH equilibrium was established after the addition of each increment of acid. Back titration with 0.1 *N* sodium hydroxide resulted in the end point of solubility reversal.

pH Solubility Profile of Wy-4029—To a series of 500-mg. samples of Wy-4029 suspended in 20 ml. of water were added varying amounts of concentrated hydrochloric acid to obtain a constant pH of 3, 4, 5, 6, or 7. The contents of each beaker was stirred for 1 hr. at 25°. The mixtures were then filtered through a 0.45-μ Millipore filter (the standard method subsequently used) and the concentration of Wy-4029 in the filtrate was determined.

Potentiometric Evidence of Complex Formation—Solutions of Wy-4029 (50 mg./100 ml.), DNA-Na (1 Gm./100 ml.), and Wy-4029 (50 mg./100 ml.) plus DNA-Na (1 Gm./100 ml.) were adjusted to pH 3.5 with 1 *N* hydrochloric acid and titrated with 0.1 *N* sodium hydroxide, pH equilibrium being established after each addition of alkali.

Stability of Macromolecule–Aminopteridine Complex—To 25 mg. of each of the aminopteridines was added 50 ml. of a 1% solution of DNA-Na (pH 2.5), and the mixtures were triturated in a Potter-Elvehjem homogenizer until soluble (5 min.). Increments of 0.1 *N* sodium hydroxide were slowly added, pH equilibrium being established after the addition of each increment. The pH at which turbidity occurred was considered the end point of solubility reversal.

Effect of Concentration and pH of Macromolecules on Wy-4029 Solubility—To various concentrations of DNA-Na (pH 5.0), RNA-Na (pH 2.8), HA (pH 4.9), BSA (pH 5.1), and USP gum acacia (pH 4.4) in 20 ml. of water was added an excess (50–400 mg.) of Wy-4029. The mixtures were

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triturated in a Potter-Elvehjem homogenizer for 5 min., stirred an additional 2 hr., adjusted to pH 7, filtered, and the concentrations of Wy-4029 determined.

Effect of Initial pH of the Macromolecule Solution on Solubility of Wy-4029—To 20 ml. of 5% solutions of DNA-Na, RNA-Na, gum acacia, and BSA adjusted with 1 *N* hydrochloric acid or 1 *N* sodium hydroxide to pH's varying from 2 to 8 was added an excess (100–400 mg.) of Wy-4029. The mixtures were triturated in a Potter-Elvehjem homogenizer for 5 min., stirred an additional 2 hr., adjusted to pH 7, filtered, and the concentrations of Wy-4029 determined.

Preparation of Wy-4029–DNA Solution—This solution as prepared by adding 10 Gm. of DNA-Na to 90 ml. of water, adjusting to pH 3.5 with dilute hydrochloric acid, making up to 100 ml., and clarifying by centrifugation at 6,000 r.p.m. for 15 min. Wy-4029 (1 Gm.) was added and complete solubility was attained by trituration in a Potter-Elvehjem glass homogenizer. Each prepared solution was vigorously stirred and slowly adjusted to pH 7 with dilute sodium hydroxide, then either sterile-filtered through a 0.45-μ Millipore filter and stored in ampules or lyophilized and stored as a powder. This powder was readily reconstituted by the addition of water.

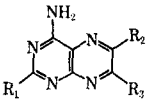
RESULTS AND DISCUSSION

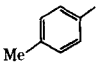
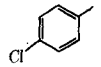
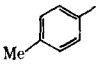
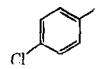
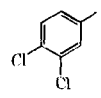
The aminopteridines investigated were all solubilized by classic salt formation (0.5 mg./ml.) with 0.1 *N* hydrochloric acid. By allowing pH equilibrium after the addition of each increment of acid, it was possible to arrive at the pH at which aqueous solubility was achieved. The compounds varied considerably in solubility pH (Table I). The individual values probably reflect the relative basicity: the weaker this basicity, the greater the difficulty of forming classic salts and the lower the pH required for solubilization.

Reversal of the apparent acid solubility of the aminopteridines, accomplished by the addition of sufficient 0.1 *N* sodium hydroxide, also occurred over a rather wide pH range (Table I). A number of aminopteridines remained soluble at 0.5 mg./ml. and pH 7.4, and for these compounds it was predicted that higher concentrations would be stable. Solutions of aminopteridines (10 mg./ml.) were then prepared by solubilization with hydrochloric acid and adjustment to pH 7.4 with alkali (Table I, footnote *c*).

To determine the effect of DNA on aminopteridine solubility, a 1% solution of DNA-Na (pH 2.5) containing 0.5 mg./ml. of an aminopteridine in solution was titrated with 0.1 *N* sodium hydroxide until solubility reversal occurred. In every example the rever-

TABLE I—EFFECT OF DNA ON "EXTENDING" pH SOLUBILITY OF AMINOPTERIDINES



Wy-	R_1	R_2	R_3	Soluble HCl Salt,[a] pH	Reversal of Salt Solubility, pH	Reversal of DNA Solubility,[b] pH
1841[c]		—CONHCH$_2$CH$_2$CH$_2$NEt$_2$	—NH$_2$	4.4	9.1	10.1
1843[c]		—CONHCH$_2$CH$_2$NEt$_2$	—NH$_2$	4.2	8.4	—
3519[d]	—NH$_2$		—NH$_2$	4.4	5.4	9.9
3648[d]		—CONHCH$_2$CHCH$_3$HMe$_2$	—NH$_2$	3.9	7.7	9.0
3665[c]		—CONHCH$_2$CH$_2$CH$_2$NEt$_2$	—NH$_2$	4.6	8.5	9.9
3873[c]		—CONHCH$_2$CH$_2$CH$_2$NMe$_2$	—NH$_2$	5.2	9.1	9.8
3876[d]		—CONHCH$_2$CH$_2$NMe$_2$	—NH$_2$	4.8	7.9	9.3
4027[c]	—NHCH$_2$CH$_2$CH$_2$NEt$_2$	—CONHCH$_2$CH$_2$CH$_2$NEt$_2$	—NH$_2$	8.5	10.8	—
4029[e]		—CONHCH$_2$CH$_2$NEt$_2$	—NH$_2$	4.1	7.7	9.4
4196[d]		—CONHCH$_2$CH$_2$NEt$_2$	—NH$_2$	4.3	7.0	9.0

(*Continued on next page*)

TABLE I—(*Continued*)

Wy-	R_1	R_2	R_3	Soluble HCl Salt,[a] pH	Reversal of Salt Solubility, pH	Reversal of DNA Solubility,[b] pH
4276[c]		—CONHCH$_2$CH$_2$NH$_2$	—NH$_2$	5.2	>10.1	—
4437[c]		—CONHCH$_2$(CH$_2$)$_2$CH$_2$NMe$_2$	—NH$_2$	7.6	9.2	—
4923[d]	 Me	—CONHCH$_2$CH$_2$CH$_2$NEt$_2$	—NH$_2$	4.4	8.5	9.6
5120[c]		—CONHCH$_2$CH$_2$NEt$_2$	—NHCH$_2$CH$_2$NEt$_2$	8.0	8.8	9.8
5121[c]		—CONHCH$_2$(CH$_2$)$_2$CH$_2$NEt$_2$	—NH$_2$	4.2	9.3	10.3
5250[c]		—CONHCH$_2$(CH$_2$)$_4$CH$_2$NMe$_2$	—NH$_2$	3.6	8.5	10.5
5256[f]		—CONHCH$_2$CH$_2$OMe	—NHCH$_2$CH$_2$OMe	2.5	6.1	6.9
5330[c]		—CONHCH$_2$(CH$_2$)$_3$CH$_2$NMe$_2$	—NH$_2$	4.9	8.4	10.0
5365[d]		—CONHCH$_2$(CH$_2$)$_3$CH$_2$NEt$_2$	—NH$_2$	3.2	8.7	9.9
5588[c]		—CONHCH$_2$CH$_2$NEt$_2$	—NH$_2$	4.8	8.5	9.7
6520[e]	 Cl	—CONHCH$_2$CH$_2$NEt$_2$	—NH$_2$	5.0	7.6	9.6
7037[c]	 Me	—CONHCH$_2$CH$_2$NEt$_2$	—NH$_2$	4.6	8.3	—
7038[c]	 Cl	—CONHCH$_2$(CH$_2$)$_2$CH$_2$NMe$_2$	—NH$_2$	5.6	8.5	10.4

[a] Aminopteridine, 25 mg./50 ml. water. [b] Aminopteridine, 25 mg./50 ml. of a 1% solution of DNA-Na. [c] Solubilized by salt formation, 10 mg./ml. pH 7.4. [d] Solubilized by complex formation, 5 mg./ml. in 10% DNA, pH 7.0. [e] Solubilized by complex formation, 10 mg./ml. in 10% DNA, pH 7.4. [f] Solubilized by complex formation, 5 mg./ml. in 10% DNA, pH 6.5.

sal occurred at a higher pH than had previously been determined for solutions prepared by classic salt formation (Table I). The 0.7–4.5 unit upward extension of the pH solubility range suggested the concomitant utility of complex formation by DNA as well as other macromolecules for preparing stable solutions of aminopteridines at higher concentrations than was previously possible. As shown in Table I, some aminopteridines cannot be solubilized at 10 mg./ml. and pH 7.4, probably because they are weakly basic and do not form stable hydrochlorides. Wy-4029, representative of this group, has limited solubility at pH 7 (Fig. 1). The concentration of Wy-4029 in solution was increased considerably by complex formation with macromolecules. Solutions of DNA-Na (pH 5), RNA-Na (pH 2.8), HA fraction V (pH 4.9), BSA (pH 5.1), and gum acacia (pH 4.4) at their respective natural pH's were effective in solubilizing Wy-4029 and in maintaining concentrations at pH 7 previously not considered possible (Fig. 2). Since the pH of the macromolecule solution is a variable in determining the amount of aminopteridine solubilized, a pH-solubility profile study of 5% solutions of DNA-Na, RNA-Na, BSA, and gum acacia was made. For each of the macromolecules an optimum pH for maximum solubility of Wy-4029 was determined (Fig. 3). The anticipated superiority of DNA-Na in solubilizing Wy-4029 was confirmed.

The potentiometric evidence of complex formation between Wy-4029 and DNA-Na was provided by three distinct alkali titration curves (Fig. 4) (8). Complex formation existed up to approximately pH 9. DNA-Na maintained the solubility of Wy-4029 above the pH at which solubility by salt formation failed.

A solution of Wy-4029 solubilized in 10% DNA-Na at pH 3.5 (10 mg./ml.) and then adjusted to pH 7.4 was found to be stable at 4° and 25° for a minimum of 3 months. A lyophilized powder prepared from a freshly made solution was readily reconstituted in water.

A toxicity evaluation of the aminopteridines and aminopteridine–DNA complexes (1 mg./mouse) demonstrated the protective effect of DNA in preventing skin lesions in mice at the subcutaneous site

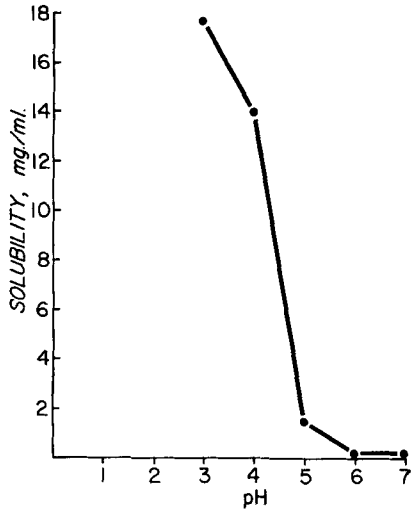


Fig. 1—pH Solubility profile of Wy-4029 in presence of hydrochloric acid.

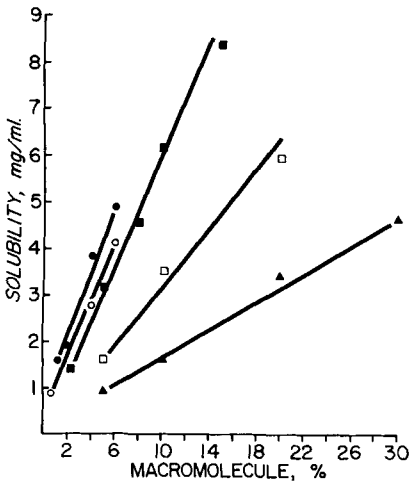


Fig. 2—Solubility at pH 7 of Wy-4029 complexed with each of five macromolecules. Complexes of Wy-4029. Key: ●, DNA-Na, (pH 5); ○, RNA-Na, (pH 2.8); ■, human albumin fraction V (pH 4.9); □, bovine serum albumin (pH 5.1); and ▲, gum acacia (pH 4.4). Wy-4029 was solubilized initially at the given natural pH (shown in parentheses) of each of the macromolecules.

of injection. Dissection of the subcutaneous site failed to reveal the depot of aminopteridine usually found after injecting an aqueous solution. It seems reasonable to assume that transport of the aminopteridine away from the site of injection is accelerated when it is complexed with DNA.

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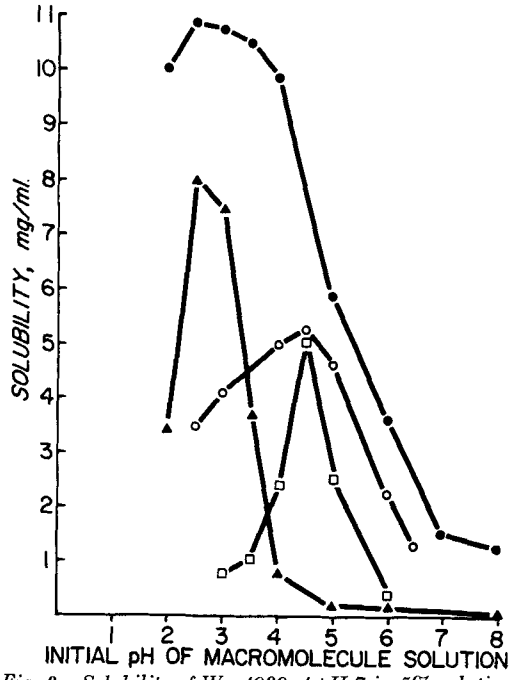

Fig. 3—Solubility of Wy-4029 at pH 7 in 5% solution of each of four macromolecules as a function of the solubilization pH. Key: ●, DNA-Na; ○, RNA-Na; ▲, gum acacia; and □, bovine serum albumin.

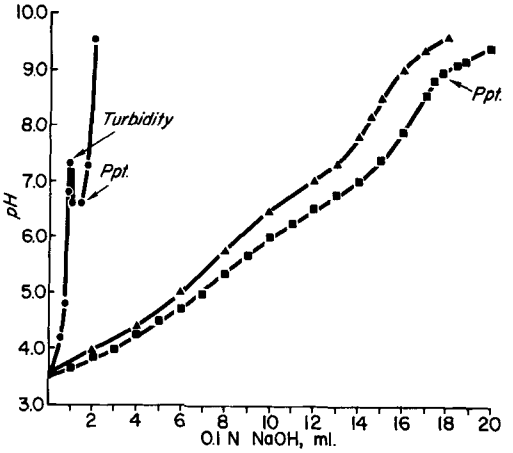

Fig. 4—Potentiometric detection of complex formation (25°). Key: ●, Wy-4029 (50 mg./100 ml.); ▲, DNA-Na (1 Gm./100 ml.); ■, Wy-4029 (50 mg./100 ml.) plus DNA-Na (1 Gm./100 ml.).

Keyphrases

Aminopteridines—solubilization
Complex formation—DNA–aminopteridines
Macromolecules—solubilizing effects
pH—Solubility profile
Toxicity—DNA–aminopteridines