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THE EFFECT OF pH ON SANGUINARINE IMINIUM ION FORM

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Sanguinarine, a member of the benzophenanthridine alkaloids, has been shown to exhibit antimicrobial activity (1-5). Collagenase suppression and the inhibition of Na^+, K^+−ATP'ase and tubulin assembly have also been demonstrated (6-10).

The biological activity of sanguinarine has been associated with the iminium ion in a pH dependent equilibrium with the alkanolamine. Conversion from the iminium ion to the alkanolamine improves the lipophilicity of sanguinarine (Figure 1). This may enhance the antimicrobial activity by increasing the cellular availability of the alkaloid. Use of the sanguinarine alkanolamine as a prodrug has been reported (7, 11). The dynamic equilibrium between the alkanolamine and the iminium ion forms of sanguinarine may be of value in improving antimicrobial and other biological activities of sanguinarine. The stability of the iminium and alkanolamine forms will be critical to any prospective use of sanguinarine. It is the purpose of this paper to report on the equilibrium and stability of the sanguinarine iminium ion and alkanolamine forms.

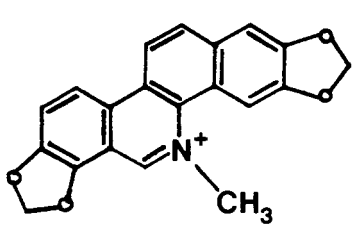
EXPERIMENTAL

MATERIAL PREPARATION.—Sanguinarine as the chloride salt was isolated from *Sanguinaria canadensis* L. (Papaveraceae) and purified at Vipont Laboratories. Purity was checked by melting point (278-279°) and hplc (12). A stock solution of 100 μg/ml sanguinarine chloride was prepared by dissolving 0.1 g of sanguinarine chloride in a 1000-ml volumetric flask with deionized H_2O-EtOH (80:20 v/v). Solutions of deionized H_2O-EtOH (80:20 w/v) with 1% citric acid and 0.5M NaOH, respectively, were also prepared. The citric acid and/or NaOH solutions were added dropwise to 100-ml aliquots of the stock solution producing a range of solutions between pH 3.5 and 11.9 (Table 1). The pH measurements were made using a Corning 125 pH meter calibrated to standard buffers pH 4 and pH 7. Concurrently, a second set of solutions was made identically for uv studies with citric acid, NaOH, and solvent (H_2O/EtOH).

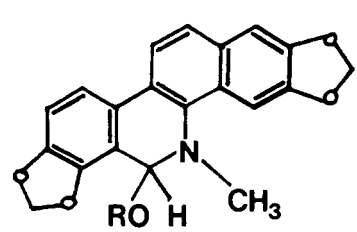
UV ANALYSIS.—A set of four standard dilutions of sanguinarine chloride was prepared as a standard curve in H_2O-EtOH (80:20) using 0.11060 g/liter, 0.07365 g/liter, 0.03681 g/liter, and 0.00249 g/liter sanguinarine, each buffered to pH 3.25 with 0.25 g/liter citric acid. A linear regression was plotted for the analysis of sanguinarine chloride in the test samples.

Uv absorption spectra were obtained using a Coleman 124 double beam spectrophotometer with deuterium lamp. Samples were diluted 0.5 ml in 3.0 ml corresponding reference solution and read at 273 and 328 nm. Standard curves at both wavelengths were established. Both absorbances are indicative of the sanguinarine in solution. Extinction coefficients were 39,300 at 273 nm and 30,700 at 328 nm. The alkanolamine forms of sanguinarine are insoluble in either H_2O or EtOH. Only the supernatant from the solutions was analyzed by uv after centrifugation of a 5-ml sample.

HPLC ANALYSIS.—Samples were analyzed by hplc using a Waters Radial-Pak 5CN 10μ column



Form I



Form II

FIGURE 1. Chemical structures for iminium ion and alkanolamine forms of sanguinarine.

TABLE 1. Percent Sanguinarine Iminium Ion versus pH as Analyzed by Hplc

pH	Day 1	Day 7	Day 49	Day 91	Day 120[a]
3.5	97.4	98.2	100.8	96.8	90.3
4.0	93.8	94.1	90.6	86.5	90.3
5.0	92.0	95.3	97.4	84.0	96.0
5.4	100.0	93.7	100.0	94.8	93.0
6.0	87.9	72.2	61.3	72.2	90.6
6.6	41.1	30.3	28.5	33.6	97.5
7.0	5.0	4.8	4.0	3.7	95.8
7.6	1.5	2.0	1.7	1.2	95.8
9.8	0.7	1.9	0.9	0.9	104.5
11.9	0.4	0.4	0.3	0.2	108.1

[a]Samples were acidified to pH 3.5 with H_3PO_4 and analyzed by the hplc method described.

with a mobile phase of MeOH-H_2O (84:16) with 5 mM triethylamine buffered to pH 5.4 with H_3PO_4 at a flow rate of 0.5 ml/min. A Waters 440 absorbance detector at 280 nm was used to analyze for sanguinarine. A 5-ml aliquot of the solution was removed and centrifuged. The supernatant from the pH solutions was removed and acidified with one drop of concentrated H_3PO_4. A 20-μl injection was used and quantitation was made by comparison with a sanguinarine standard solution.

RESULTS AND DISCUSSION

Analysis by uv absorption spectroscopy and hplc (Table 1) indicated that below pH 5.4 the iminium ion form of sanguinarine was the dominant form. Sanguinarine solutions at pH greater than 5.4 had decreased concentrations of the iminium ion. The conversion to the alkanolamine was essentially complete at pH 7.0. At pH 9.8, the concentration of the iminium ion was less than 1% of its concentration at pH 3.5. Solutions were analyzed at days 7, 49, and 91 and found to contain the iminium ion at the same concentration determined at day 1.

As the concentration of iminium ion decreased with increasing pH, a white precipitate was observed in the solutions. The quantity of the white precipitate in these solutions was correlated directly with the loss of iminium ion detected by uv spectroscopy and hplc. The white precipitate was identified by nmr in DMSO-d_6 as the alkanolamine by substitution at the α-carbon and was shown to be insoluble in either H_2O or EtOH. This is consistent with the formation of the alkanolamine reported (13). The alkanolamine could be either the 6-hydroxysanguinarine or the pseudoethanolate sanguinarine. The alkanolamine form is dependent on the solubility product of the compound in a solution of H_2O/EtOH.

At day 120, all samples were acidified to pH 3.5 with H_3PO_4 and analyzed by hplc (Table 1). Analysis of the solutions indicated almost complete recovery of the sanguinarine iminium ion concentrations at pH 3.5. Recovery of the iminium ion form of sanguinarine was 96.2±6%. Therefore, disproportionation of any alkanolamine in solution by oxygen to oxysanguinarine and dihydrosanguinarine at pH values above 6 was not observed in this system. These data confirm the results observed by Maiti on the stability of the alkanolamine (14).

Figure 2 is a plot of the concentration of sanguinarine iminium ion form versus pH at days 1 and 91. No significant loss of the iminium ion from these solutions was detected over this time period. The variations in the measured iminium ion concentrations were within the hplc experimental error.

Solutions of sanguinarine chloride in H_2O/EtOH without buffers analyzed as 100% iminium ion at pH 5.4. The observed shift in equilibrium to the alkanolamine occurred as the pH was in-

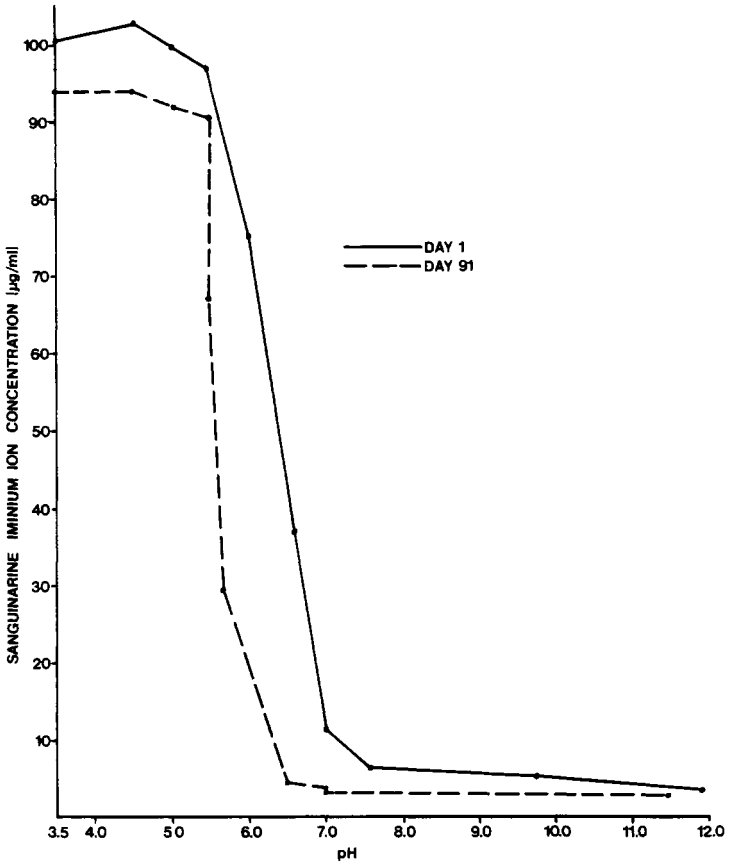


FIGURE 2. Concentration of sanguinarine iminium ion form versus pH at days 1 and 91 as analyzed by hplc.

creased with citrate and NaOH solutions. Near complete recovery of the iminium ion from solutions buffered above pH 5.4 was demonstrated when these H_2O/EtOH solutions were acidified to pH 3.5. These results indicate that sanguinarine is stable in a solution of H_2O/EtOH from pH 3.5 to 11.9.

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