Sanguinarine: A Simplified Method of Isolation

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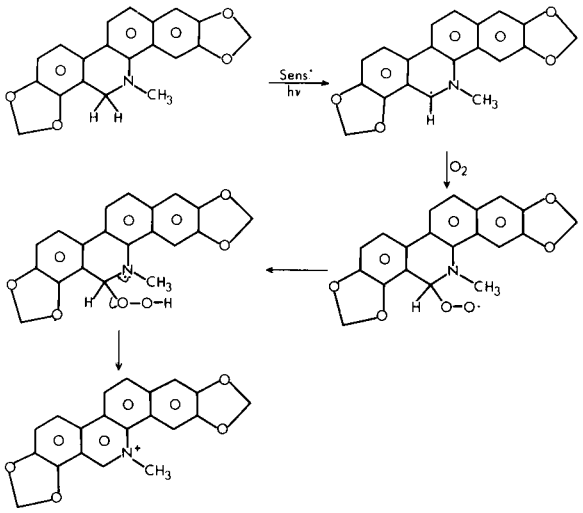
The isolation of sanguinarine (I) has been the subject of several papers including synthetic preparations (1-3) and chromatographic separations from both commerically available salts (4,5) and plant extracts (6,7). Recent work in this laboratory has led to a procedure for purifying this alkaloid in quantities sufficient for extensive biological studies. This procedure is easily adapted to large scale purification since chromatographic separations are not involved.

SCHEME I



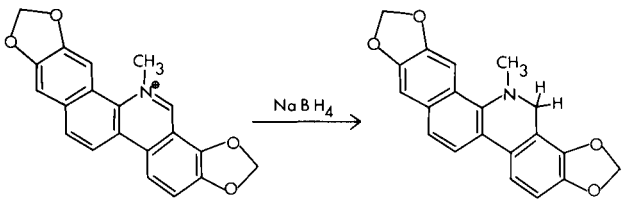
Commerically available sanguinarine sulfate (Pfaltz and Bauer) (8), after a preliminary wash, was dissolved in methanol and reduced with sodium borohydride. The light yellow solution of dihydrosanguinarine (II) was diluted with water and extracted continuously with pentane. The solvent was removed and the product recrystallized providing analytically pure II, whose colorless crystals fluoresce blue under ultraviolet radiation (365 nm). The compound was quite stable in the dark or under a nitrogen atmosphere, but in the presence of air and light was converted to the orange sanguinarine ion.

This photo-oxidation was studied for both mechanistic and preparative reasons. The photo-oxidation mechanism of amines is presently being reevaluated in several laboratories (9-13). Two mechanisms have been proposed to account for this reaction. The first involves interaction of triplet oxygen with a triplet excited state of sensitizer to provide excited state singlet oxygen which in turn reacts with the substrate to provide a hydroperoxide. The second involves hydrogen abstraction from the substrate by the electronically excited sensitizer. The radical thus formed reacts with triplet oxygen to give the observed products.

$$\text{R-H} \xrightarrow{\text{Sens}^*} \text{R}\cdot + \text{H}\cdot$$
$$\text{R}\cdot + \text{O}_2 \longrightarrow \text{R-OO}\cdot$$
$$\text{R-OO}\cdot + \text{R-H} \longrightarrow \text{ROOH} + \text{R}\cdot$$

In our studies external sensitizers were not added; therefore, dihydrosanguinarine apparently acts as both sensitizer and substrate. Recently Kearns, *et al.* (14) reported a novel method for determining the role of singlet oxygen in oxidation reactions. The life time of singlet oxygen in water has been measured at 2μ seconds (15) while in deuterium oxide it has a life time of 20 μ seconds (14). Thus a reaction involving singlet oxygen as an intermediate should be considerably faster in deuterium oxide than in water. Photo-oxidation studies of II indicate that it was oxidized at essentially the same rate in both water and deuterium oxide. If Kearns' theory is correct, then the photo-oxidation of (II) must proceed through hydrogen abstraction by the sensitizer followed by reaction of the radical with oxygen. Expulsion of the peroxide anion affords sanguinarine as outlined in Scheme I.

Application of this photo-oxidation was not fully exploited since an alternate chemical oxidation was developed. Dihydrosanguinarine has been oxidized to



I II

sanguinarine with mercuric acetate (4) and ferric chloride (1). We found this oxidation could be accomplished cleanly and in high yield by employing silver nitrate and 10% palladium on carbon in refluxing ethanol. Recrystallization of the product from methanol-chloroform provided pure sanguinarine.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage and are uncorrected. Infrared spectra were recorded on a Beckman IR-18A (potassium bromide). Elemental analyses were performed by a commercial analytical laboratory. Ultraviolet spectra were obtained on a Beckman DBG recording spectrophotometer.

Preparation of Dihydrosanguinarine (II).

Sanguinarine sulfate (1.000 g.) was dissolved in a refluxing solution of methanol (50 ml.) and 3*N* hydrochloric acid (350 ml.). Reflux was continued for one hour, the mixture was cooled, and sufficient methanol was added to produce a true solution which was extracted with ethyl acetate (4 x 100 ml.). The aqueous phase was adjusted to *p*H 3.0 with sodium hydroxide and repeatedly extracted with chloroform until the organic fraction was almost colorless. The combined chloroform extracts were evaporated to dryness and excess water was removed as an azeotrope by distilling with benzene and methanol. The bright red residue was dissolved in methanol (500 ml.). Under a nitrogen atmosphere sodium borohydride (1.68 g.) in methanol (100 ml.) was added rapidly from a dropping funnel to the stirred solution. The color rapidly changed from red to bright yellow during this step. Stirring was continued overnight. The solution was then poured into water (150 ml.) and the total solution extracted continuously with pentane for 30 hours under nitrogen. The course of the extraction was easily followed by observing the upper pentane layer in ultraviolet light (365 nm). The blue fluorescent color fades from the pentane layer as the reduced alkaloid is extracted. The pentane solution, protected from light, was evaporated to dryness. The resulting crystalline residue was recrystallized four times from benzene-hexane providing 138 mg. of analytically pure II [m.p. 190.5-193°; Lit. (4) 191-193°]; λ max (methanol) 325, 286, 239, 215 nm. (ϵ = 15,000; 34,000; 30,800; 18,000).

Anal. Calcd. for $C_{20}H_{15}NO_4$: C, 72.06; H, 4.54; N, 4.20. Found: C, 71.75; H, 4.48; N, 4.15.

Photo-oxidation of Dihydrosanguinarine.

To a 2.9×10^{-6} molar solution of (II) in methanol (10 ml.) were added glacial acetic acid (0.1 ml.) and water (10 ml.). A second solution was prepared in the same way but deuterium oxide was used in place of water. The two open beakers were exposed to ultraviolet radiation (365 nm). Aliquots were removed at 2.5, 5, 10, 20, 40 and 60 minutes, and the change in ultraviolet absorption noted. The two reactions proceeded at the same rate and were essentially complete after 20 minutes.

Chemical Oxidation of Dihydrosanguinarine.

Dihydrosanguinarine (26.55 mg.) was dissolved in refluxing 95% ethanol (25 ml.) and glacial acetic acid (1 ml.) under a nitrogen atmosphere. Palladium (10%) on charcoal (33.32 mg.) was added to the hot solution. Reflux was continued for 5 minutes. Silver nitrate (17.56 mg.) was then added and reflux continued for 30 minutes. Concentrated hydrochloric acid (1 ml.) was added to precipitate any unreacted silver ions, and the solution was filtered. The filter paper was carefully washed with chloroform and methanol. The solvent was evaporated under vacuum, and the precipitate dissolved in methanol (50 ml.) and water (250 ml.). Concentrated hydrochloric acid (5 ml.) was added and the solution extracted with ethyl acetate (4 x 50 ml.). The aqueous layer was treated with sodium hydroxide until basic (*p*H 10 or above) and then extracted with chloroform. The organic phase was washed with water and taken to dryness. Concentrated hydrochloric acid was added and the excess water removed as an azeotrope with benzene-ethanol to provide 28.46 mg. of sanguinarine chloride (97%).

Recrystallization from chloroform-methanol provided a product with a melting point of 284-289° dec. Crystallization from water gave sanguinarine monohydrate [m.p. 278-280°; Lit. (1) 278°]. A color change from red to off white was noted at about 200°. The infrared spectrum was identical when compared to a sample isolated by thin layer chromatography (5).

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