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Note

Detection and quantitative analysis of sanguinarine in edible oils*

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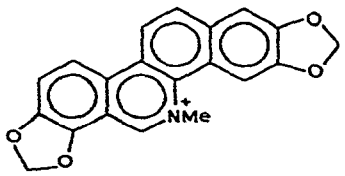
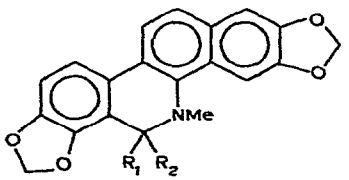
Since about 1886 widespread epidemics of dropsy have been recorded[1] in India, the last one occurring in Bombay as recently as 1966. An associated high-tension glaucoma was observed among some of the victims[2]. It has been established[3,4] that epidemic dropsy is caused by the adulteration of cooking oils with the seed oil of *Argemone mexicana* L., which grows prolifically across vast areas of India and other tropical countries. The two major alkaloids of this seed oil are[5] sanguinarine chloride (I) and dihydrosanguinarine (IIa), which together may be present in a concentration of 10 mg/ml. Sanguinarine is present in a large number of species of the Papaveraceae and Fumareaceae families[6] and it has been found[7] that sanguinarine appears in the milk and liver of cattle and sheep that graze upon pasture containing various species of poppies. It has been postulated[8] than an endemic glaucoma of various parts of the world may be caused by the innocent ingestion of very small amounts of sanguinarine over a period of time.

In order to test this hypothesis, and in order to screen cooking oils, especially in India, for the presence of *A. mexicana* seed oil it is important that a reliable method is available to detect and measure small amounts of sanguinarine. A method of detection involving paper chromatography (PC) has been described[6,7,9] but it is not completely reliable and the lower limit of detection is not known.



(I)



(IIa) $R_1 = R_2 = H$
(IIb) $R_1 = H$; $R_2 = OH$
(IIc) $R_1 + R_2 = O$
(IId) $R_1 = H$; $R_2 = CH_2COMe$

* This work was inspired by the enthusiasm of Dr. S. A. E. Hakim, who had for some years, devoted his attention to the possible relationship between the ingestion of sauguinarine and the incidence of glaucoma and cancer. Sohrab Hakim died of cancer in May 1976, and this paper is dedicated to his memory.

Hence, the aims of the present work were: (a) to improve on the method of detection of sanguinarine in certain foodstuffs and in biological samples such as urine, blood and liver, and to establish the lower limit of sensitivity and (b) to develop a method for the quantitative determination of small amounts of sanguinarine. Both procedures preferably should be capable of use in a rapid, routine method of screening by semi-skilled personnel and utilising the minimum of sophisticated equipment.

A thin-layer chromatographic (TLC) method for the detection of sanguinarine was found to be more sensitive, quicker, and more convenient than the PC and electrophoresis methods described by Hakim[6–9]. Silica plates were used, rather than alumina, since quaternary salts such as I can be transformed into pseudobases (*e.g.*, IIb) by the latter absorbant. Minor alkaloids present in the seed oil of *A. mexicana* (and identified here for the first time, see Experimental) do not interfere with the detection procedure. By using standard solutions of sanguinarine chloride in acidified ethanol, it was established that a spot applied to the TLC plate containing 10^{-9} g or more of sanguinarine could be detected.

The initial work on the quantitative determination of sanguinarine in the oils involved direct solvent extraction of I but this was thwarted by the peculiar solubility behaviour of the alkaloid and also by the readiness with which it undergoes a disproportionation reaction to give a mixture of dihydrosanguinarine (IIa) and oxysanguinarine (IIc). However, reduction of I with sodium borohydride gives IIa quantitatively and the latter, being a base, is easily extracted into dilute mineral acids (along with the other basic material present). Several methods were considered for the separation of IIa from this basic fraction, and for the quantitative measurement. PC and electrophoresis methods were quickly discarded, owing to slow development of the chromatograms and poor resolution. Gas–liquid chromatographic methods were also found to be unsatisfactory.

The TLC method developed for the detection of sanguinarine proved to be the best method. The spot of dihydrosanguinarine (IIa) present in the mixture of bases derived from the reduction procedure was well separated from other spots, and it could be easily and rapidly oxidised by air to sanguinarine by irradiating the plate with UV light. The sanguinarine, which appears as an orange spot under UV light, was quantitatively removed with a specially designed[10] micro "vacuum cleaner" and transferred to a standard volumetric flask. The concentration of sanguinarine in the spot was then derived from a measurement of the extinction coefficient at 330 nm of a solution in acidified ethanol. In this way reliable results were obtained with samples containing as little as 10^{-5} g sanguinarine in the spot applied to the TLC plate.

EXPERIMENTAL

TLC plates were prepared in the usual way by coating 20 × 20 cm glass plates with silica containing no indicator. Absorbances were measured using a Pye Unicam SP500 spectrophotometer in the null balance mode, or with the Perkin-Elmer 402. Developing solvents were distilled, and allowed to reach equilibrium in the developing chamber for 24 h before use.

Minor alkaloids of the seed oil of Argemone mexicana L.

Seed oil (200 g) was extracted by digestion with 95% ethanol at room temper-

ature over five days. The layers were separated and the ethanol evaporated under reduced pressure. The residue was dissolved in chloroform (200 ml) and extracted with 2 *M* hydrochloric acid (5 × 100 ml). The combined acid solutions were basified with ammonia and extracted with chloroform (5 × 100 ml). The organic solution was washed with water (2 × 100 ml), dried ($MgSO_4$), and evaporated under reduced pressure to leave a residue (600 mg; 0.3%) which was chromatographed on thick (1 mm) silica gel plates using benzene–methanol (6:1). The upper 80% of the orange sanguinarine band was removed and discarded. The remainder of the plate was extracted with ethanol, concentrated, and subjected to TLC on silica gel, using (A) benzene–methanol (6:1); (B) benzene–methanol (3:2), and (C) benzene–acetone–methanol (7:2:1).

Authentic samples of chelerythrine, berberine and protopine were also run on the same plates and all of the spots were visualised under UV light. Three spots were thus identified as chelerythrine (R_F values in solvents A, B, and C: 0.76, 0.72, and 0.67, respectively), protopine (R_F values in solvents A, B, and C: 0.35, 0.27, and 0.25, respectively), and berberine, which remained on the origin. Using the micro "vacuum cleaner"[10] the spots were removed, eluted and the UV spectra recorded to confirm identity. A further spot was tentatively identified as norsanguinarine [R_F value in benzene–chloroform (3:1): 0.2] but insufficient material precluded measurement of the UV spectrum. Two other minor alkaloids remained unidentified.

Detection of sanguinarine

A spot of the neat oil (*A. mexicana* L. or edible oil) was applied to a thick (750 μm) layer of silica gel G on a 20 × 20 cm plate in the usual way. The spot was developed in the first direction with benzene, and then at right angles with benzene–methanol (6:1). Sanguinarine appears, under UV light, as an orange spot (R_F values: 0.00 and 0.7–0.8 in the first and second direction, respectively). (Although the R_F value in the first solvent is zero, it was found that better resolution of the spots occurred in the second solvent if this procedure was adopted.)

Quantitative determination of sanguinarine

The sample of oil (*A. mexicana* seed oil or cooking oil) (5–30 g, as appropriate) was dissolved in petroleum ether (b.p. 40–60°) and a solution of sodium borohydride (500 mg) in ethanol (10 ml) was added with stirring. After stirring for 15 min the turbid liquid was treated with 2 *M* hydrochloric acid. The resultant lower acid layer was collected and the upper organic phase was extracted twice with 2 *M* HCl. The combined acid layers were basified ($NaHCO_3$ solution) and extracted into chloroform. The dried chloroform extract was evaporated, the residue was dissolved in ethanol, and the volume was adjusted to 1.00 ml in a standard flask.

Samples (2 μl) of this ethanol solution were spotted onto a 20 × 20 cm plate coated with silica gel G (250 μm) in the usual way. The spot runs were then scored and the chromatogram was developed over 10 cm with benzene–methanol (6:1). The plate was dried (air blast for 5 min), then irradiated with long-wavelength UV light for 15–20 min to effect oxidation of dihydrosanguinarine to sanguinarine. The latter appeared as an orange spot at R_F 0.78. This was removed from the plate with the micro vacuum cleaner[10], dissolved in ethanol containing one drop conc. HCl and the solution made up to 5.00 ml in a volumetric flask. The absorbance of this solution at 330 nm was measured and the concentration of sanguinarine computed from a calibration

curve of concentration of pure sanguinarine chloride *vs.* extinction at 330 nm. Usually ten samples of the test solution were processed on one plate and the average concentration was calculated.

RESULTS

The Beer–Lambert law was verified using solutions of sanguinarine chloride in acidified ethanol over the concentration range 0–4.0 mg per 100 ml. Measurements of absorbance were made at 330 nm.

Within a plate, using ten spots of sanguinarine chloride solution, the reproducibility of the R_F value was $\pm 3\%$ of the mean value provided that the plates were pre-saturated with solvent and the spot runs were scored. By developing plates spotted with the same sample solution in different chambers, the R_F value was $\pm 4\%$ above a mean value. The R_F value was found to be very sensitive to methanol concentration and to the method used for drying the plates after development.

A comparison of the concentration of sanguinarine chloride in ethanol solution after plating, eluting and absorbance measurement with the concentration of the original solution measured directly showed that an efficiency of $92 \pm 5\%$ was achieved in the analytical procedure.

The analysis was also tested using (a) *A. mexicana* seed oil containing a known concentration of dihydrosanguinarine and (b) mixtures of cooking oils to which known amounts of *A. mexicana* seed oil had been added.

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