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LIGAND-EXCHANGE CHROMATOGRAPHY OF ALKALOIDS

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SUMMARY

Alkaloids are separated by liquid chromatography on resins having functional carboxyl groups combined with copper(II) ions. The eluent is aqueous alcohol containing ammonia. Some resins retain alkaloids much better than others. Alkaloids studied included morphine, codeine, strychnine, atropine, papaverine, narcotine, cocaine, quinine, cinchonine and methadone.

INTRODUCTION

Considering that alkaloids are bases that form cations in acid solution, it is surprising that cation-exchange chromatography has not been used more often for the analysis of alkaloid mixtures. Cation exchange has been used to recover quinine on a commercial scale[1], and the fact that morphine, which has a phenolic function and is a weak acid, may be absorbed on anion-exchange resins has been known for a long time[2]. A column of SE-Sephadex C-25, a strongly acidic sulfoethyl dextran gel, gave successful separations of a dozen alkaloids and related drugs[3], but the separations took 10–15 h. Pellicular cation and anion exchangers, Zipax SCX and SAX, were used by Knox and Jurand[4], for morphine and its derivatives, also papaverine and other alkaloids. As eluents they used borate buffer, pH 9–10. In this pH range, morphine, with $pK_b = 6.1$, exists in solution almost entirely as the free base, and other alkaloids are primarily uncharged bases rather than cations. Thus the chromatographic mechanism is probably not cation exchange as such, but rather the absorption of uncharged alkaloid molecules by the resin polymer matrix. We have noted that at pH values of 8 and less, alkaloids are retained very strongly by a pellicular cation-exchange resin of the polystyrene type, and are eluted as broad bands with much tailing.

Wu *et al.*[5] separated alkaloid mixtures very successfully by high-speed liquid chromatography on polyethylene glycol held on a silica gel support, using the "dynamic coating" method. The solvent was an ethanol–heptane mixture partly saturated with polyethylene glycol. Tropane alkaloids were separated on silica, with tetrahydrofuran containing ammonia as eluent[6].

We have used ligand-exchange chromatography to separate alkaloids. The

stationary phase was a cation-exchange resin loaded with Cu(II) ions, the eluent a solution of ammonia in aqueous alcohol. Previously we separated phenethylamine drugs[7], ethanolamines and aziridines[8] and amino sugars[9] by this technique.

EXPERIMENTAL

Resins

The resins were obtained from Bio-Rad Labs. (Richmond, Calif., U.S.A.): Chelex-100, with iminodiacetate groups on a polystyrene matrix; Bio-Rex 70, with carboxyl groups on an aliphatic matrix; PC-20, with carboxyl groups on an aliphatic matrix, an acrylic resin supplied for special purposes, not listed in the Bio-Rad catalogue. Two batches of PC-20 were received and found to have different properties. The resin Poragel PT was obtained from Waters Ass. (Milford, Mass., U.S.A.). It is a non-ionic polar packing for liquid chromatography. It contains ester groups which we hydrolyzed by boiling with 10% alcoholic KOH for 1 h. This treatment converts the resin into a cation exchanger with functional carboxyl groups[10].

Poragel PT, as sold, has a nominal size range of 37–75 μm. We requested a smaller particle size, and received, as a gift, a few grams of finer-size material. Microscopic examination showed that most of this was in the 20- to 50-μm range (see Conclusion).

All resins were converted to the Cu(II) form before packing into columns. This was done by stirring and washing with an ammoniacal cupric sulfate solution, about 0.2 *M* in Cu(II), then washing with dilute aqueous ammonia until the washings were only pale blue. The packed columns were washed with influent solution (ammonia in aqueous alcohol) until the UV absorption became constant.

A few tests were made with hydrolyzed Poragel PT loaded with Ni(II) and Zn(II). The corrected retention volumes for nickel-loaded resin were about four-fifths of those found with Cu(II); those for zinc-loaded resin were slightly greater. Copper ions are retained more strongly by the resin than zinc ions, however; the concentration of copper in the effluent was well below 10^{-4} *M*. For this reason, copper-loaded resins were chosen for study.

Equipment

Glass columns of 0.63 cm I.D. were used; columns, sample introduction valves, UV detectors and a Model CMP-2 pump were obtained from Chromatronix (Santa Clara, Calif., U.S.A.); a Model 6000 pump from Waters Ass. was also used. Because of the softness of some of the resins, pressures were kept below 450 p.s.i. (15 bars), although hydrolyzed Poragel PT tolerates higher pressures than this.

Alkaloid salts

The alkaloids were introduced as solutions of the salts, usually sulfates. Sometimes ammonia was added before injection to simulate the composition of the eluent, but adding ammonia encouraged atmospheric oxidation and, in some cases, hydrolysis, so this was not usually done. The alkaloid salts were converted to the free bases plus ammonium sulfate (or chloride) by the ammonia in the influent as soon as they entered the column. In all chromatograms an UV-absorbing peak appeared at the void volume, caused by the displacement of Cu(II) from the resin by the ammonium salt.

Peak identification was performed in some cases by collecting the effluent and

running the UV absorption spectrum with scale expansion in a Cary XVII spectrophotometer.

Ammonia uptake measurement

It was found that some resins retain alkaloids much more effectively than others. The alkaloid retention was correlated with ammonia binding, which was measured as follows. A batch of resin was saturated with cupric ions by washing with excess of cupric ammonia sulfate, followed by 0.5–1 *M* ammonia to remove dissolved copper salt. Excess liquid was removed from the resin by suction, and the resin was air-dried, allowing all the ammonia to evaporate. It now contained Cu(II) coordinated with water, but no ammonia. It was bottled to stabilize the water content. Weighed portions were analyzed to determine the copper content.

Other weighed portions, 0.5–1.0 g, were placed in 25-ml glass flasks. Known volumes of standardized aqueous ammonia solutions were introduced, with additional water to give total solution volumes of 7–10 ml. The flasks were stoppered and shaken for 1 h or more. Measured volumes of solution, free from resin particles, were removed and titrated with standard acid. After the acid titration the copper content was found by titration with EDTA. The copper concentration was never more than 0.004 *M* and usually much smaller. The amounts of ammonia and Cu(II) within the resin were calculated. Their ratio was plotted against the concentration of ammonia remaining in the solution (see Fig. 1).

RESULTS AND DISCUSSION

Effective and ineffective resins

Early trials were made with Chelex-100. This resin retained some alkaloids strongly, like cocaine and strychnine, but the peaks were very broad and unsymmetrical. This fact we attribute to the polystyrene matrix of the resin, which acts as a solvent

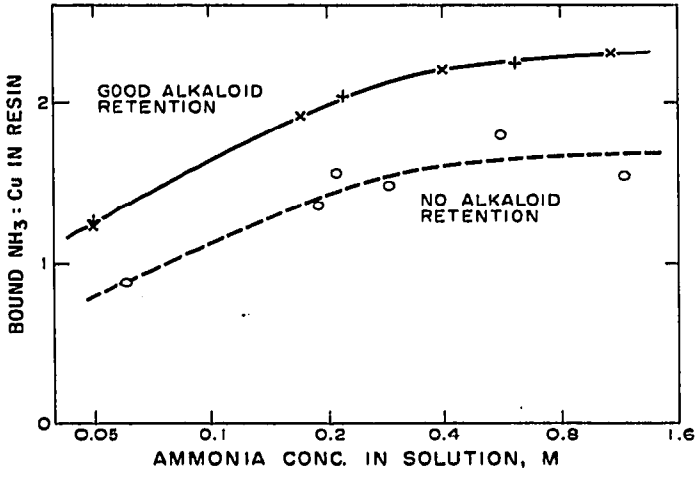

Fig. 1. Ammonia binding by copper-loaded resins. +, Hydrolyzed Poragel; ×, old PC-20 resin; ○, new PC-20 resin.

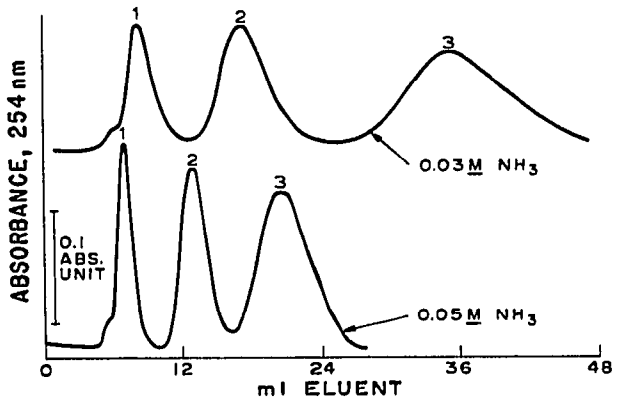

Fig. 2. Elution of alkaloids from copper-loaded PC-20. Column, 47 × 0.63 cm; solvent, 25% ethanol plus ammonia as indicated; flow-rate, 12 ml/h; room temperature. 1 = Morphine sulfate (25 μg); 2 = codeine sulfate (250 μg); 3 = strychnine sulfate (50 μg).

for aromatic compounds in general. We therefore tried Bio-Rex 70, a polymer whose matrix is largely aliphatic. This resin performs well for phenethylamine derivatives and amino sugars[7,9], but the retention of alkaloids was weak.

The next resin we tried was PC-20, a gift of the Bio-Rad Labs. (see above). This gave retention volumes (corrected) which in some cases were several times those found with Bio-Rex 70, and the elution bands were narrow enough for good chromatographic separations. We had only a small sample of this resin, and so we requested another batch. The second batch gave poor retention.

Ammonia uptake measurements, described above, showed that the first batch of PC-20 resin, loaded with copper, bound considerably more ammonia than did the second. Hydrolyzed Poragel PT bound ammonia well and retained alkaloids well. The ammonia uptakes are shown in Fig. 1. There is a clear correlation between the ability of a copper-loaded carboxylic resin to hold ammonia and its ability to hold alkaloids. It probably reflects subtle differences in the polymer network structure.

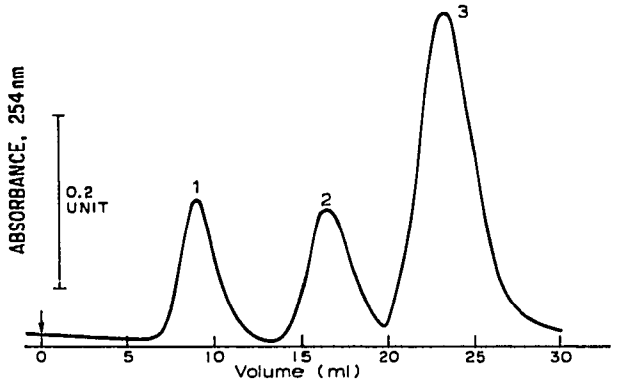

Fig. 3. Elution of alkaloids from copper-loaded Poragel. Column, 47.5 × 0.63 cm; solvent, 0.2 *M* ammonia in 33% ethanol; flow-rate, 24 ml/h; temperature, 50°. 1 = Morphine (0.012 mg); 2 = codeine (0.12 mg); 3 = strychnine (0.05 mg).

Eluent composition and temperature

Most alkaloid bases are sparingly soluble in water, and it was necessary to use eluents containing alcohol. Ethanol was a better solvent than methanol. Strychnine, for example, had a retention volume on Poragel in 33% methanol which was double the retention volume in 33% ethanol, both solutions being 0.2 *M* in ammonia. Most experiments were therefore made with ethanol–water mixtures. Retention volumes (corrected for void volume) in 25% ethanol were about double those in 33% ethanol, and were inversely proportional to the ammonia concentrations (see Fig. 2). Higher ammonia concentrations were needed with Poragel than with PC-20 (*cf.* Figs. 2 and 3).

Raising the temperature reduces the elution volumes and sharpens the bands. The overall effect is to increase the resolution, but the effect is not large. Where hydrolysis occurs, as with cocaine (see below), high temperature is a disadvantage.

Elution sequences

Figs. 2–4 shows the order of elution of various alkaloids (note the different column lengths in Figs. 4a and 4b). From many experiments the sequence of elution was established which is shown in Table I. The sequence seemed to be the same for PC-20 resin as for hydrolyzed Poragel. The actual elution volumes found in a particular experiment depend, of course, on solvent composition and temperature.

Hydrolysis

Fig. 5 shows how cocaine dissolved in aqueous–alcoholic ammonia hydrolyzes with time. A little hydrolysis is evident from the first, for the residence time in the column was about 1 h. After 44 h the only UV-absorbing substance left in the solution was the benzoate ion.

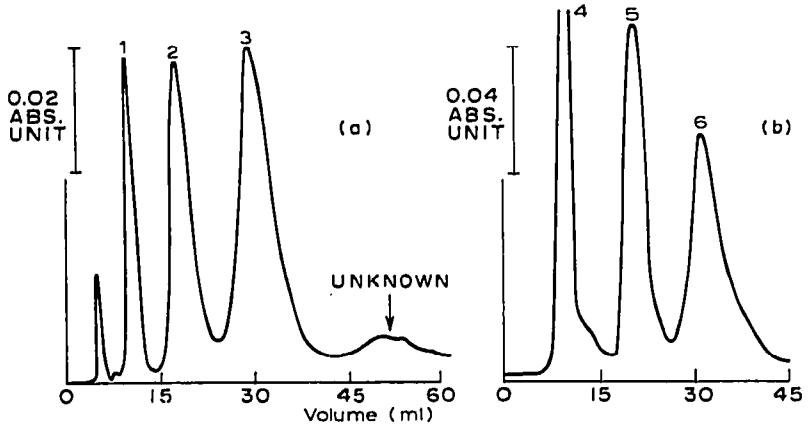

Fig. 4. Elution of alkaloids from copper-loaded Poragel of different particle size. (a) Column, Poragel "fines" (*cf.* text), 25 × 0.63 cm; solvent, 0.2 *M* ammonia in 33% ethanol; flow-rate, 30 ml/h; temperature, 60°. Micrograms injected: (1) papaverine·HCl, 15; (2) quinine sulfate, 100; and (3) cinchonine sulfate, 200. (b) Column, Poragel 37–75 μm, 47.5 × 0.63 cm; solvent, 0.2 *M* ammonia in 33% ethanol; flow-rate, 24 ml/h; temperature, 50°. Micrograms injected: (4) morphine sulfate, 25; (5) papaverine·HCl, 37; and (6) narcotine sulfate, 250.

TABLE I

ELUTION SEQUENCE OF ALKALOIDS ON HYDROLYZED COPPER-LOADED PORAGEL PT

The numbers are multiples of the bulk column volume and refer to room temperature. The eluent was 0.06 *M* ammonia in 33% alcohol, except for the last three alkaloids, where more concentrated ammonia was used; the volumes are reduced to those expected for 0.06 *M*. With methadone, 50% alcohol was used to prevent precipitation; again, the volume estimated for 33% alcohol is given. The numbers have qualitative significance only.

Alkaloid	*Elution volume*	*Alkaloid*	*Elution volume*
Morphine	1.0	Cocaine	4.5
Nicotine	2.0	Atropine	5.0
Codeine	2.25	Narcotine	6.8
Ethyl morphine	2.5	Quinine	9
Papaverine	3.0	Methadone	12
Strychnine	3.5	Cinchonine	16

Hydrolysis of esters is unavoidable in the ligand-exchange technique, where a strongly alkaline eluent is used. Atropine and methadone are esters, and subject to hydrolysis. In their chromatography the temperature, ammonia concentration and residence time in the column must be as low as possible. With methadone, for

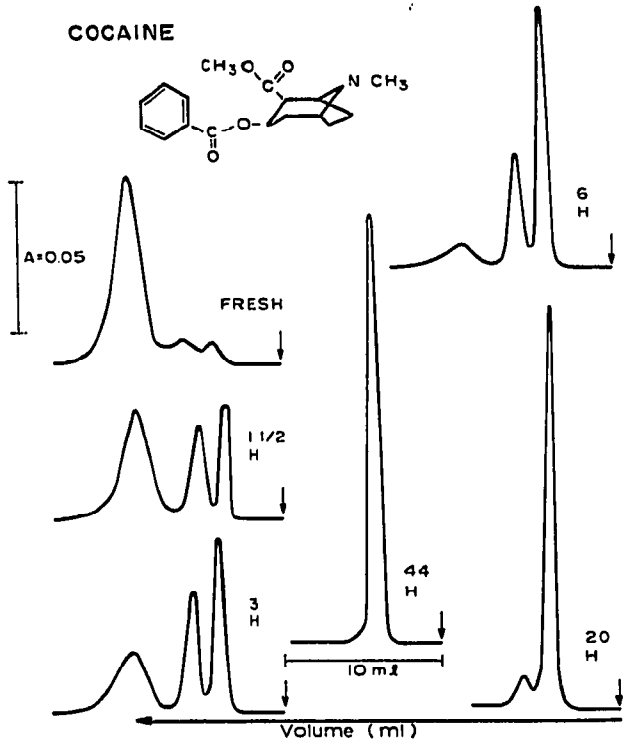

Fig. 5. Hydrolysis of cocaine. Column, copper-loaded PC-20, 12 × 0.63 cm; flow-rate, 12 ml/h; room temperature. Injected 0.5 ml of a solution containing 0.15 mg cocaine · HCl in 0.13 *M* ammonia in 33% ethanol when first prepared.

example, it is desirable to use a low ammonia concentration (say 0.05 *M*) and accelerate the elution by raising the alcohol concentration to 50%. The high alcohol concentration prevents methadone base from precipitating, which may otherwise happen, since the base is almost insoluble in water.

Plate numbers, resolution

The bands in Figs. 2–5 are sufficiently narrow to resolve the mixtures shown here, but they are not as narrow as one would like. An attempt to resolve an alcoholic extract of opium into its constituents was unsuccessful. Plate numbers and heights were calculated in the following way:

$$N = \left(\frac{\text{elution volume} - \text{void volume}}{\text{peak width at 0.606 of height}}\right)^2 \times 4$$

$$H = \text{column length}/N$$

The void volume is taken at the first sharp rise in absorbance. In Fig. 2, lower curve, the plate height, *H*, for codeine is 9 mm; in Fig. 3 it is 7.5 mm. In Fig. 4a, *H* for quinine is 2.5 mm, for cinchonine 2.0 mm; in Fig. 4b, *H* for papaverine and narcotine is 5.5 mm. The marked difference between Figs. 4a and 4b reflects the fact that the column used in Fig. 4a was packed with fine resin, most of whose particles were smaller than 40 μm, whereas the resin in Fig. 4b had the regular 37- to 75-μm size range.

CONCLUSION

We believe that the ligand-exchange method is competitive with published methods for the liquid chromatography of alkaloids. Its most serious drawback is the hydrolysis that occurs in the alkaline eluents. It is doubtful that the method would serve for heroin, for example, since this compound is rapidly hydrolyzed by alkali.

We noted the broad bands and large plate heights. We could not approach the plate heights of 0.1 mm and less that we have achieved with other compounds[11]. However, we could bring the plate height down to 2 mm, which is in the range that Knox and Jurand found with a pellicular resin[4]. The problem is that the large molecular size of alkaloids causes slow diffusion. The fast mass transfer that is essential to good chromatography can only be obtained by using a resin of fine particle size.

Our lowest plate heights were obtained with a small sample of Poragel PT, specially provided by the manufacturers from "fines" recovered from commercial production. Seen in the microscope, this material consisted of spherical particles of sizes ranging from 10 to 50 μm, with some irregular particles of 80 μm and larger. If the Poragel PT resin could be obtained in a reasonably uniform 10- to 20-μm size range, it should give much better chromatographic resolution. The resin is mechanically strong, and should permit high-pressure gradients and high flow-rates.

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