combined GLC–mass spectrometry, reference sparteine and genisteine gave essentially the same mass spectrum.

Isolation of Sparteine—The entire alkaloid fraction (3 g) was dissolved in ethanol–chloroform (1:1) and streaked onto 12 silica gel PF_{254} plates (1 mm) using a previously described technique (11). The band corresponding with sparteine and genisteine was scraped from the plates, and the alkaloid material was eluted from the silica gel scrapings with three 30-ml portions of methanol. The eluates were combined and processed in the usual fashion (12) to give a light-brown oil. The IR spectrum of this material was the same as that recorded for reference sparteine. The oil was dissolved in benzene and treated with methyl iodide to give sparteine methiodide, mp 235–236° [lit. (13) mp 237–238°].

Quantitation of Alkaloids—The quantity of each identified alkaloid in the extract was determined as previously described (10). Based on the dry weight of the plant, the following levels of alkaloid were found: sparteine, 0.05%; β-isosparteine, 0.005%; Δ^5-dehydrolupanine, 0.04%; α-isolupanine, 0.60%; lupanine, 0.01%; thermopsine, 0.36%; and anagyrine, 0.01%.

DISCUSSION

Crooked calf disease is characterized by congenital malformations in calves born to cows that ingested certain *Lupinus* species during early gestation (9). Symptoms include arthrogryposis or torticollis and scoliosis and, occasionally, cleft palate (5). Keeler (6) suggested that the quinolizidine alkaloid anagyrine may be the teratogen responsible for crooked calf disease because of its high concentration in those species of *Lupinus* shown to induce the disease. In another report, Keeler (9) pointed out that *L. argenteus* is probably nonteratogenic because of a total lack of anagyrine; this report revealed lupanine as the only alkaloid identified in extracts of *L. argenteus*.

The present study of *L. argenteus* var. *stenophyllus* verifies the presence of lupanine in the species but also reveals low concentrations of anagyrine. According to Keeler (9), this situation would suggest nonteratogenicity. However, several alkaloids present in the title plant were apparently not studied by Keeler (6, 9). For instance, the epimer of anagyrine, thermopsine, may also be a potential teratogen.

Furthermore, Keeler (6) stated that the GLC peak associated with extracts of teratogenic lupines is really a mixture of anagyrine and α-isolupanine. Perhaps α-isolupanine is also teratogenic. In view of the large concentrations of α-isolupanine and thermopsine in *L. argenteus* var. *stenophyllus*, further biological testing with whole extracts and individual alkaloids appears necessary before concluding that this plant is nonteratogenic.

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Simultaneous Salt and Ethanol Removal from Human Serum Albumin

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Received April 8, 1977, from the *Department of Biochemical Development, Cutter Laboratories, Inc., Berkeley, CA 94710.* Accepted for publication June 28, 1977.

Abstract □ Diafiltration of salt and ethanol from human serum albumin was shown to be a competitive alternative to freeze drying. At least 99% of the original alcohol content could be removed in approximately five volume changes. Data on changes in ionic strength, bacteriological buildup, permeate flux, and dimer contents are presented.

Keyphrases □ Albumin, human serum—simultaneous salt and ethanol removal by diafiltration □ Diafiltration—simultaneous salt and ethanol removal from human serum albumin

Albumin is prepared commercially by variation of the low temperature–alcohol methods (1). Method 6 yields albumin as Fraction V upon lowering the pH to 4.7–4.9 at 40% ethanol. Salts are removed by redissolving Fraction V in 10% ethanol and reprecipitating at 40% ethanol.

Most pharmaceutical companies use freeze drying for the removal of ethanol from the albumin paste, but other routes such as gel filtration (2) and thin-layer evaporations under vacuum (3) also are feasible. An alternative process, diafiltration, is proposed for the simultaneous removal of salt and alcohol. The data obtained provide the basis for utilizing this method in the large-scale production of albumin preparations for clinical use.

EXPERIMENTAL

Fraction V is the starting material. An ultrafiltration thin-channel system[1] was employed in the preliminary study. This system streams the process fluid over the membrane (10,000 mol. wt. retentivity) in shallow channels, and smooth laminar flow is produced. The accompanying shear force is regulated by adjusting the fluid velocity with a reversible peristaltic pump. The system is connected with a fluid reservoir pressurized to 25 psig. A hollow fiber unit[2] also was studied for operations greater than

[1] Model TCF10, Amicon, Lexington, Mass.
[2] Model DC-30, Amicon, Lexington, Mass.

Table I—Changes in Ionic Strength *versus* Volume Changes

Volume Change	Ionic Strength Removed, %	Ionic Strength Predicted, %
1.4	77.0	75.0
2.0	86.0	86.5
2.6	91.0	92.6
4.0	96.0	98.2
5.0	97.0	99.3

0.5 liter. This system can utilize up to three hollow fiber cartridges, each with 9290 cm^2 of membrane surface area.

Protein concentration was determined by the biuret technique. Solution conductivity was monitored by a conductivity meter[3]. Based on dilutions, the percentage of initial ionic strength was related to the percentage of initial conductivity.

Ethanol[4] was assayed by GLC[5]. An internal standard of either distilled or spectroquality grade 1-propanol[6] was used. Ethanol concentration was calculated from both peak height and peak area ratios. Monomer and polymer contents of albumin were quantitated by the method of sodium lauryl sulfate–polyacrylamide electrophoresis (4).

RESULTS AND DISCUSSION

In the experiment illustrated in Fig. 1, diafiltration was carried out with a membrane filter[7] with a nominal molecular weight cutoff value of 10,000. It was freely permeable to low molecular weight solutes such as salt and ethanol but impermeable to albumin (molecular weight of 65,000). Ionic strengths and ethanol concentrations were determined for the samples taken from the retentate side of the membrane. The solid line depicts the theoretical line for zero rejection. It confirms that ethanol falls exponentially with time following a first-order decay curve. Salts, however, are slightly impeded, showing fractional binding with serum albumin.

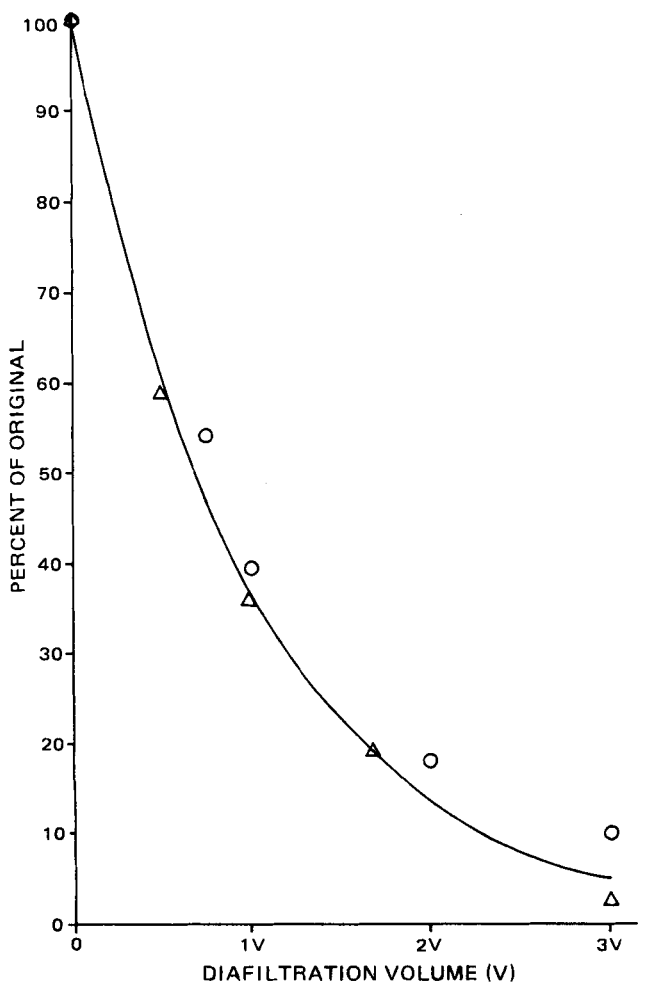

Figure 1—*Salt and alcohol levels during Fraction V diafiltration. Key:* ○, *ionic strength; and* △, *ethanol.*

[3] Radiometer, Copenhagen, Denmark.
[4] Shell, Houston, Tex.
[5] Model 3920, Perkin-Elmer, Norwalk, Conn.
[6] Mallinckrodt, St. Louis, Mo.
[7] PM10 membrane, Amicon, Lexington, Mass.

Table II—Bacteriological Buildup during Processing

Sample	Counts per Milliliter D-2558	D-2561	D-2577
Initial time	17	70	63
After three volume changes	21	30	63
After five volume changes	53	80	84
Distilled water (control)	0	0	—

As a further test of the theoretical consideration, a 10-liter 5% albumin solution was diafiltered against pyrogen-free water in a hollow filter unit. Fluid was circulated through the hollow fibers. Salt and solvent were transported convectively through the walls by a pressure gradient. As shown in Table I, a good correlation was obtained between the experimental and the theoretical removal efficiency.

Ultrafiltration rates of the hollow fiber system are controlled by the average transmembrane pressure and the ambient temperatures. Figure 2 shows the flux of a 5% solution. The observed temperature dependence arises both from the increased diffusivity of the albumin and the lowered viscosity of the solution.

The microbiological aspect of diafiltration at +5° was studied to ascertain the feasibility for commercial fractionation. Pilot-scale preparations of 30–50 liters were attempted. Initial protein concentrations were maintained at 7.5 ± 0.5%. Retentate samples were collected for bacteria plate count, and average numbers for three runs are shown in Table II.

No more than a one order of magnitude increase in colonies was noted

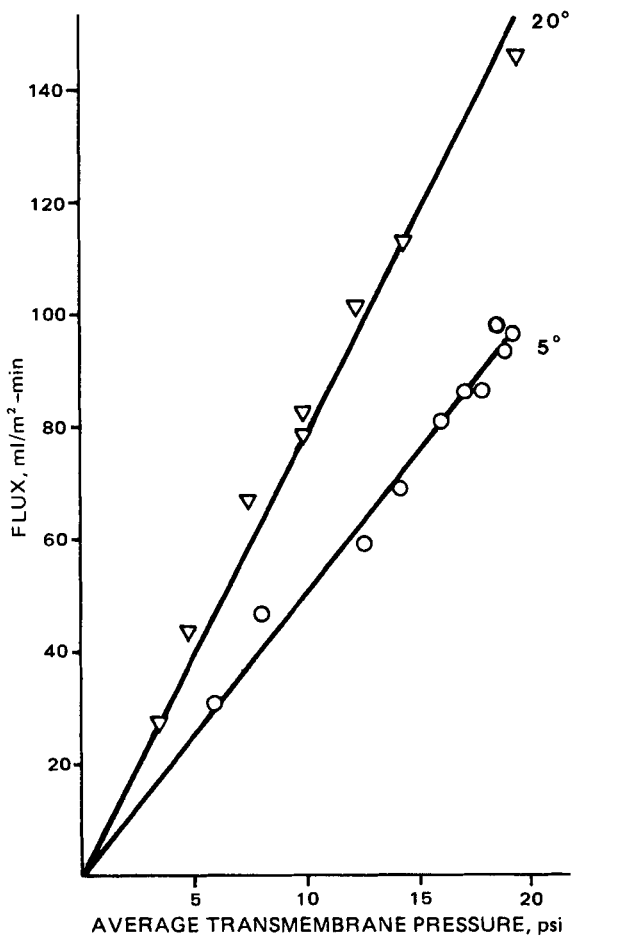

Figure 2—*Effect of pressure and temperature on the permeate flux through a hollow fiber cartridge (10,000 mol. wt. retentivity).*

Table III—Dimer Contents of Freeze-Dried (F) Albumin and Diafiltered (D) Albumin

Sample	Dimer, % (±1%)	Monomer, % (±1%)
F-93	3.8	96.2
F-2541	6.9	93.1
D-64	3.7	96.3
D-2558	3.8	96.2
D-2568	1.2	98.8
D-2573	0.6	99.4

at completion. The data showed that there was slight bacteriological buildup as the alcohol level dropped. The solution after five volume changes was sterile filtered. It passed the USP rabbit pyrogen test, suggesting that the bacteria counts were not dangerously high.

Optimization of this process was considered elsewhere (5). Production of albumin solutions in final containers was attempted and was proven to be feasible. Test results showed all factors to be within specifications with regard to protein composition, pH, protein level, electrolytes, stabilizers, heme content, and heat stability. The monomer concentration of the heat-treated albumin was between 96.3 and 99.4% as measured by sodium lauryl sulfate–polyacrylamide electrophoresis. A comparison of samples prepared by diafiltration and freeze drying is shown in Table III. Statistically, there was no significant difference between the two groups.

Friedli and Kistler (2) showed that albumin containing 10.4 mg of ethanol/g of albumin could be pasteurized without obvious damage to the protein. Similar observations also were reported (6). Conventional freeze drying could presumably achieve a limit of 15 mg of ethanol/g of albumin in the final product (2). Starting from Fraction V, gel filtration effectively reduces ethanol from about 1000 mg/g of albumin to 10–15 mg/g of albumin. With diafiltration, less than 10 mg of ethanol/g of albumin could be obtained in approximately 4.6 volume changes. A constant albumin concentration was maintained throughout the entire process. This procedure has an obvious advantage over gel filtration, which requires acceptance of a more dilute eluate. The capital and running costs of freeze drying are prohibitively high. Diafiltration has the merit of much lower energy consumption since operation proceeds through the pumping of solutions. The process produces salt-poor albumin. Thus, the extra step of Fraction V reprecipitation can be avoided.

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Constituents of West African Medicinal Plants XX: Quindoline from *Cryptolepis sanguinolenta*

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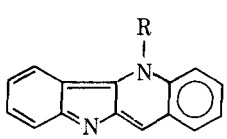
Received March 16, 1977, from the **Faculty of Pharmacy, University of Science and Technology, Kumasi, Ghana, West Africa*, and the ‡*Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261.* Accepted for publication June 22, 1977.

Abstract □ An ethanol extract of the roots of *Cryptolepis sanguinolenta* (Asclepidaceae) afforded, after partitioning and chromatography, the alkaloids quindoline and CSA-3. The structural elucidation of CSA-3 is currently in progress. Cryptolepine was also isolated from this species.

Keyphrases □ *Cryptolepis sanguinolenta*—ethanol extract of roots, quindoline and CSA-3 isolated □ Alkaloids—quindoline and CSA-3 isolated from ethanol extract of roots of *Cryptolepis sanguinolenta* □ Quindoline—isolated from ethanol extract of roots of *Cryptolepis sanguinolenta*

Cryptolepis sanguinolenta (Lindl.) Schlecter is a plant native to West Africa used in the treatment of malaria[1]. The indoloquinoline alkaloid, cryptolepine (I), previously was isolated from this species (1). This compound has reported hypotensive activity and causes a marked and prolonged fall in blood pressure in dogs as well as a lowering of body temperature (2, 3).

A systematic phytochemical investigation of this species resulted in the isolation of cryptolepine and two other alkaloids, quindoline (II) and CSA-3 (III), a new alkaloid whose structure will be reported later. Quindoline (II) was



I: R = CH_3
II: R = H

originally synthesized over 70 years ago (4, 5) and was since prepared from cryptolepine (1). This is the first report of the isolation of quindoline from a natural source.

EXPERIMENTAL[2]

Plant Material—Roots of *C. sanguinolenta* (Lindl.) Schlecter (Asclepidaceae) were used[3].

Extraction—Air-dried ground roots (200 g) were extracted by percolation with ethanol (6 liters). The extract was evaporated *in vacuo* at 40° to leave a dark-red syrup (11 g, 5.5%).

[1] Dr. Oku-Ampofo, Centre for Plant Medicine Research, Mampong-Akwapim, Ghana, personal communication.

[2] Melting points were determined on a Thomas-Hoover Uni-Melt melting-point apparatus and are corrected. IR spectra were run in potassium bromide using a Perkin-Elmer 257 spectrophotometer. UV spectra were run on a Perkin-Elmer 202 spectrophotometer. Mass spectra were recorded on an LKB-9000 spectrometer. NMR spectra were obtained on a Hitachi Perkin-Elmer R-24 spectrometer with tetramethylsilane as the internal standard.

[3] Identified by Mr. K. Obeng-Darko; a herbarium specimen is located at the Faculty of Pharmacy, University of Science and Technology, Kumasi, Ghana, West Africa.