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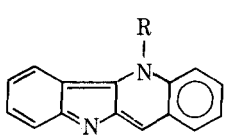
Received March 16, 1977, from the **Faculty of Pharmacy, University of Science and Technology, Kumasi, Ghana, West Africa*, and the ‡*Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261.* Accepted for publication June 22, 1977.

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.

Fractionation—The ethanol extract was partitioned between water (1 liter) and chloroform (1 liter) to give fractions of 4.3 (2.2%) and 6.0 (3.0%) g, respectively. The chloroform fraction was then partitioned between petroleum ether (bp 60–90°) (200 ml) and methanol–water (9:1) (200 ml) to yield fractions of 3.5 (18%) and 2.0 (1.0%) g, respectively.

Chromatography—The aqueous methanol fraction was chromatographed on silicic acid[4] (75 g, 2 × 50 cm) in chloroform.

Isolation of II—Elution with 1% methanol–chloroform afforded a residue (233 mg), which was dissolved in chloroform (25 ml) and partitioned with 1% hydrochloric acid (3 × 25 ml). The combined acid layers (75 ml) were made basic to pH 8 with concentrated ammonium hydroxide and partitioned with chloroform (3 × 75 ml). The combined chloroform layers were dried over anhydrous sodium sulfate and evaporated *in vacuo* at 40° to yield a yellowish residue (15 mg).

Repeated attempts at crystallizing quindoline failed, so data were obtained on the amorphous residue; UV: λ_{max} (methanol) 227 (log ϵ 4.30), 269 sh (4.45), 274 (4.46), 330 sh (3.82), and 345 (4.03) nm; λ_{max} (0.01 *N* ethanolic hydrochloric acid) 224 (log ϵ 4.27), 242 sh (3.94), 273 (4.36), 280 (4.38), 350 sh (4.03), and 368 (4.26) nm; IR: ν_{max} (potassium bromide) 1632, 1608, 1487, 1457, 1396, 1370, 1333, 1222, 1150, 1140, 1122, 1105, 1000, 875, 865, 845, 837, 813, 754, 745, 738, 710, and 604 cm^{-1}; mass spectrum (M^+): *m/e* 218 (100%), 217 (8), 190 (8), 109 (12), 95.5 (4), 90 (3), and 89 (5). The alkaloid was found to be identical with an authentic sample of quindoline[5] by direct comparison (UV, IR, and mass spectra and TLC).

Isolation of III—Elution with 2% methanol–chloroform afforded a residue (327 mg), which, when crystallized from methanol, gave III (105 mg), mp > 300°. The structural elucidation of this substance is currently in progress.

Isolation of I—Elution with 30% methanol–chloroform afforded I (300 mg), mp 167–168° dec. [lit. (1) mp 166–169°], hydrochloride mp 263–264° dec. [lit. (1) mp 263–265°], hydroiodide mp 285–287° dec. [lit. (4) mp 284–286°], *methiodide* mp 284–285° dec. [lit. (1) mp 285–288°]; UV: λ_{max} (methanol) 224 (log ϵ 4.11), 246 (3.87), 275 (4.41), 283 (4.43), 355 sh (4.02), 370 (4.33), 4.10 (3.28), and 433 (3.29) nm; λ_{max} (0.01 *N* ethanolic potassium hydroxide) 214 (log ϵ 4.40), 230 sh (4.01), 297 sh (4.38), 307 (4.48), 368 (3.50), and 386 (4.08) nm; IR: ν_{max} (potassium bromide) 1631, 1611, 1585, 1505, 1492, 1460, 1400, 1366, 1357, 1330, 1310, 1300, 1275, 1250, 1160, 1150, 1130, 1040, 900, 887, 875, 850, and 750 cm^{-1}; mass spectrum (M^+): *m/e* 232 (100%), 231 (12), 217 (26), 190 (10), 116 (14), and 89 (15); NMR (trifluoroacetic acid): δ 5.08 (s, 3H, NCH_3), 7.55–8.62 (m, 8H, aromatic), and 8.95 (s, 1H, aromatic) ppm.

[4] Mallinckrodt, 100 mesh.

[5] Reference sample provided by Professor Emery Gellert, Department of Chemistry, University of Wollongong, Wollongong, Australia.

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New Spectrophotofluorometric Assay for Probenecid

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Abstract □ A new spectrophotofluorometric assay for probenecid is presented based on conversion of the drug to a fluorescent anthranilic acid derivative. The assay is especially applicable with "clean" biological fluids such as cerebrospinal fluid and offers severalfold greater sensitivity than the commonly used UV method.

Keyphrases □ Probenecid—spectrophotofluorometric analysis in biological fluids □ Spectrophotofluorometry—analysis, probenecid in biological fluids □ Uricosuric agents—probenecid, spectrophotofluorometric analysis in biological fluids

Recently, several GLC procedures (1–4) and a new radioimmunoassay were developed (5) to measure low probenecid levels in biological fluids. Interest in such assays was stimulated by studies of the accumulation of cyclic adenosine monophosphate (6, 7) and metabolites of biogenic amines (8–11) in cerebrospinal fluid caused by probenecid and the well-known inhibitory effect of this drug on active transport systems in the brain (12).

Based on observations with a fluorescent amino derivative of probenecid (13), it was decided that a fluorometric assay would offer definite advantages over existing methodologies—greater sensitivity than the UV procedure (14) and less cost than GLC. In the present study, reaction conditions for quantitative conversion of probenecid to a fluorescent species were investigated and the fluorescent product was characterized. A method for quantitating probenecid in cerebrospinal fluid and plasma is proposed and evaluated.

EXPERIMENTAL

Probenecid[1], ring-labeled ^{14}C-probenecid[1] (specific activity of 0.8 mCi/mmole), and 2′-nitroprobenecid[1] were used as received. The side-chain metabolites were prepared as previously described (15). Radioactivity was measured in a liquid scintillation spectrometer[2] in a counting fluid prepared as described previously (5). Relative fluorescence intensity was measured in 1.0-cm cells in a spectrophotofluorometer[3].

TLC was carried out on silica gel G glass plates[4] (5 × 20 cm, 250 μm)

[1] Courtesy of Dr. John E. Baer, Merck Sharp and Dohme, West Point, Pa.

[2] Beckman LS-255; efficiency for ^{14}C = 90%.

[3] Aminco-Bowman, equipped with an EMI 9558 QC phototube, a Schoeffel microphotometer model 600 at 1200 v, and a magnetic arc stabilizer accessory; slits were set at 3 mm.

[4] Analabs, New England Nuclear, North Haven, Conn.