Biological and Phytochemical Evaluation of Plants XI: Isolation of Aspidospermine, Quebrachidine, Rhazinilam, (−)-Pyrifolidine, and Akuammidine from *Aspidosperma quebracho-blanco* (Apocynaceae)

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Abstract □ A phytochemical investigation of the leaves of *Aspidosperma quebracho-blanco* (Apocynaceae) resulted in the isolation of rhazinilam, a new nonindole lactam alkaloid, in addition to the known bases, aspidospermine, quebrachidine, (−)-pyrifolidine, and akuammidine. Aspidospermine and quebrachidine were the major leaf alkaloids. Akuammidine and (−)-pyrifolidine were isolated from the leaves for the first time, and the isolation of the latter compound confirms its natural occurrence in this plant.

Key phrases □ *Aspidosperma quebracho-blanco* leaves—isolation, identification of aspidospermine, quebrachidine, rhazinilam, (−)-pyrifolidine, akuammidine □ Rhazinilam—new nonindole lactam alkaloid isolated from *Aspidosperma quebracho-blanco* □ Akuammidine—isolated from leaves of *Aspidosperma quebracho-blanco* □ (−)-Pyrifolidine—isolated from leaves of *Aspidosperma quebracho-blanco* □ Quebrachidine—major leaf alkaloid in *Aspidosperma quebracho-blanco* □ Medicinal plants—isolation, identification of constituents from *Aspidosperma quebracho-blanco*

Aspidosperma or quebracho, a drug composed of the bark of *Aspidosperma quebracho-blanco* Schlecht. (Apocynaceae), has been employed in Argentine and Chilean folk medicine for the treatment of fever, especially as an antiperiodic (1–3). The drug was included in USP VII and IX as well as in NF III, and the powdered drug, as well as the fluidextract and other extractives, was employed at the turn of the century as a remedy in cardiac and asthmatic dyspnea. It is said to be valuable as a respiratory stimulant in cases of emphysema, bronchitis, chronic pneumonia, and other pulmonary dysfunctions (3). The fluidextract of this drug is still commercially available[1].

DISCUSSION

Since the isolation of aspidospermine from *A. quebracho-blanco* by Fraude (1), in 1878, more than 25 alkaloids have been isolated or detected as constituents of the bark of this plant (1, 2, 4–11). The compounds so identified to date include: aspidospermine (1, 2, 4, 7, 8), aspidosamine (4), aspidospermatine (4, 7, 8), hypoquebrachine (4), quebrachamine (4, 7, 8), quebrachine (yohimbine) (5, 7, 8), quebrachacidine (6), deacetylaspidospermine (7, 8), N_a-methyldeacetylaspidospermine (7, 8), (−)-pyrifolidine (7, 8), N_a-acetylaspidospermatidine (8), aspidospermatidine (8), aspidospermidine (8), deacetylaspidospermatine (8), deacetylpyrifolidine (8), 1,2-dehydroaspidospermidine (8), 14,19-dihydroaspidospermatine (8), eburnamenine (8), N_a-methylaspidospermatidine (8), 1-methylaspidospermidine (8), 12-methoxyaspidospermatidine (8), eburnamonine (9), rhazidigenine N_b-oxide (10), rhazidine (11), and akuammidine (11). Additional nonalkaloid constituents of the bark have been identified as the triterpenes lupeol and α-amyrin (12).

The pharmacological actions of the quebracho alkaloids have been reported by a number of investigators. Aspidospermine, aspidosamine, quebrachine, and quebrachamine have been shown to be hypotensive (13). Quebrachamine and yohimbine have also been found to be sympatholytic agents (14). Aspidospermine has been reported to possess diuretic, peripheral vasoconstrictor, arterial hypertensive (15), respiratory stimulant (15, 16), and uterine sedative (16) actions, as well as having an inhibitory effect on the growth and mitosis of chicken heart fibroblasts (17). Pharmacological actions of akuammidine include local anesthetic (18), intestinal spasmogenic (19), hypotensive (20, 21), skeletal muscle relaxant, and sedative (21).

Although phytochemical and pharmacological studies on the bark of *A. quebracho-blanco* have been extensive, investigations of the leaves are limited to a single report describing the isolation of aspidospermine and quebrachidine (22). The latter alkaloid has been reported to be a psychosedative and adrenergic blocking agent (23).

In view of the limited studies on the alkaloids of the leaves and the large number of pharmacologically interesting alkaloids found in the bark of this plant, the current study was initiated in an attempt to isolate new biologically active alkaloids.

EXPERIMENTAL[2]

Extraction and Separation of Alkaloid Fractions—Fifty-five kilograms of dried, milled leaves of *A. quebracho-blanco* was extracted by percolation, using 350 l. of methanol. *In vacuo* concentration of the percolate resulted in a separation of 1.14 kg. of nonalkaloid precipitate, which was removed by filtration; the filtrate was then mixed with 14 l. of 5% acetic acid. Removal of the methanol from the mixture was followed by vacuum filtration, and the filtrate was set aside. The residue was redissolved in methanol and again mixed with acetic acid (6 l.); then the methanol was removed, and the resulting solution was filtered. This process was repeated twice to obtain a total volume of 32 l. of acid filtrate and 2.6 kg. of insoluble residue. The acid solution was made alkaline with 28% ammonium hydroxide and extracted with a total of 125 l. of chloroform. The combined chloroform extracts were dried over anhydrous magnesium sulfate, filtered, and taken to dryness *in vacuo* to yield 952 g. of Fraction A.

The post-Fraction A aqueous alkaline solution was extracted with 22 l. of *n*-butanol. This solution was dried over anhydrous magnesium sulfate and evaporated *in vacuo* to yield 370 g. of resinous alkaloid, Fraction B.

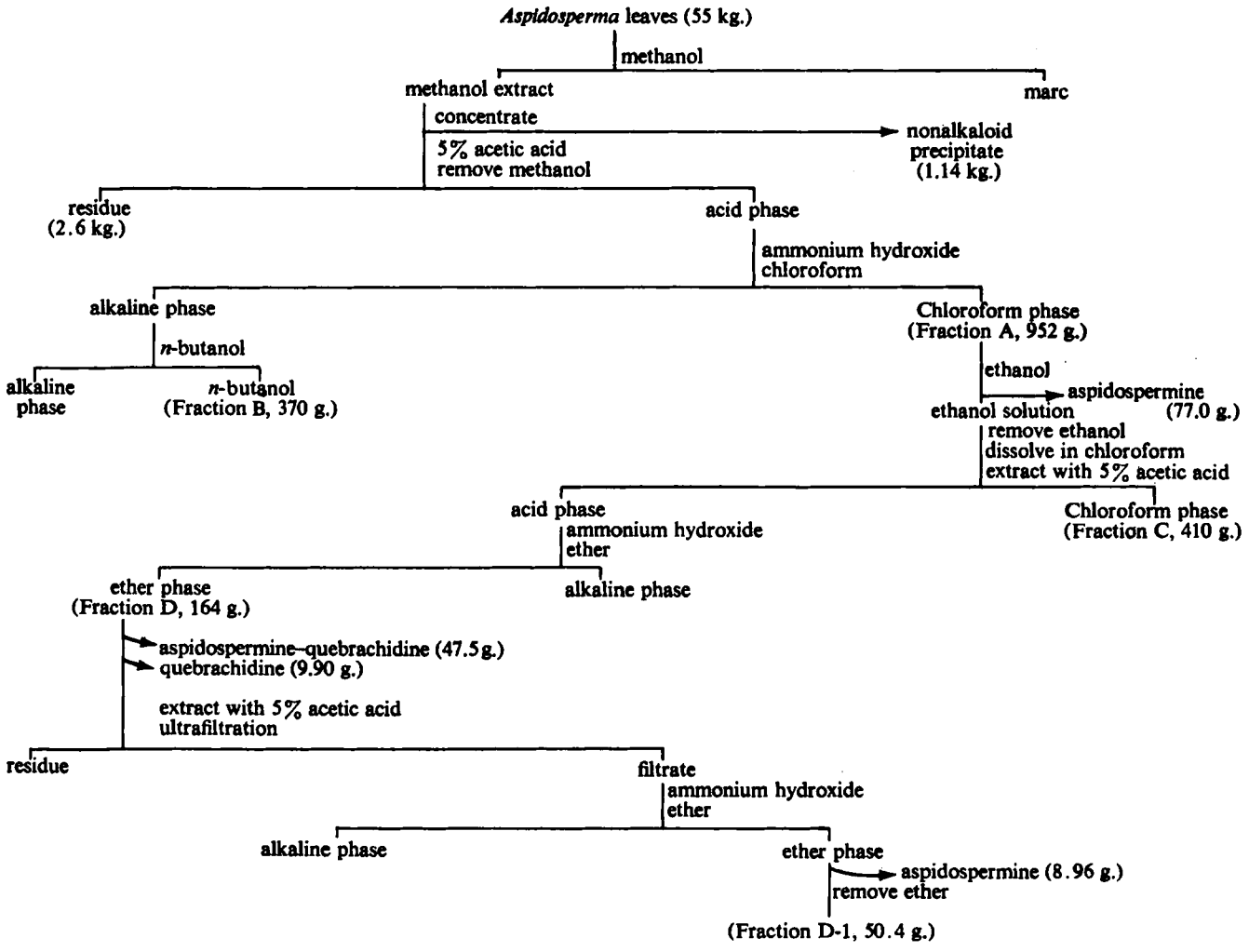
Treatment of Fraction A (952 g.) with hot ethanol, followed by filtration and cooling to room temperature, yielded 77.0 g. of a crystalline alkaloid, which was subsequently identified as aspidospermine. The ethanolic mother liquor was taken to dryness, dissolved in chloroform (3 l.), and extracted four times with 1.5-l. portions of 5% acetic acid solution. *In vacuo* evaporation of the chloroform phase yielded 410 g. of alkaloid Fraction C. The acid phase was rendered alkaline to pHydrion paper and extracted with 30 l. of anhydrous ether. The ethereal extracts were dried over anhydrous magnesium sulfate, filtered, and taken to dryness to yield 164 g. of alkaloid Fraction D. The alkaloid-free alkaline mother liquor was discarded.

Direct crystallization of Fraction D from anhydrous ether yielded two crops of an alkaloid mixture, A (47.5 g), and 9.9 g. of crude quebrachidine. Removal of ether from the mother liquor was followed by the dissolution of the residue in 900 ml. of 5% acetic acid and subsequent purification by ultrafiltration. This was accomplished by passing the solution through a 0.22-μ membrane[3]

[1] Fluidextract quebracho bark USP IX (aspidospermatis), Sherwood Laboratories, Cleveland, Ohio.

[2] The leaves of *A. quebracho-blanco* Schlecht. used in this investigation were obtained from the Meer Corp. Voucher samples of the plant material are deposited at the Meer Corp., North Bergen, NJ 07047

[3] GSWP 142000, Millipore Corp., Bedford, Mass.



Scheme I—Flow diagram for the extraction and fractionation of A. quebracho-blanco *leaf alkaloids*

with the aid of nitrogen delivered at a pressure of 20 p.s.i. to remove resinous and fine particulate matter. The filtrate was then passed through a membrane[4] at 40 p.s.i. nitrogen to remove high molecular weight, nonalkaloidal compounds. Basification of the final filtrate with ammonium hydroxide was followed by extraction with five 900-ml. portions of ether. Concentration of the pooled, magnesium sulfate-dried, ether extract afforded two crops of crude aspidospermine in yields of 3.8 and 5.78 g., respectively. Complete removal of ether resulted in 50.4 g. of alkaloid residue, designated as Fraction D-1.

A flow diagram for this extraction and fractionation scheme is presented in Scheme I.

Identification of Aspidospermine—A 2.0-g. sample of the crude crystalline isolate obtained from the hot ethanol treatment of Fraction A was repeatedly recrystallized from ethanol to yield 0.86 g. of fine, colorless needles having m.p. 206–209° (Kofler hot stage, uncorr.); $[\alpha]_D^{30}$ −96° (concentration 1.0 ethanol); λ_{max}^{MeOH}: 208 (log ϵ 4.59), 256 (4.09), and a shoulder at 290 (3.42) nm., indicating a dihydroindole alkaloid. A molecular weight of 354 was obtained by mass spectrometry; the fragmentation pattern, showing other significant peaks at *m/e* 339, 326, 311, 174, 160, 152, and 124, was identical with that reported in the literature for aspidospermine (8). The IR spectra of the isolate and reference aspidospermine were superimposable, and a mixture melting-point determination showed no depression, thus confirming the identity of the alkaloid.

Identification of Quebrachidine—The first two crops of alkaloid crystals (47.5 g.) from the ether treatment of Fraction D proved to be a mixture (A) of the alkaloids aspidospermine and quebrachidine, as shown by TLC. Silica gel G thin-layer chromatograms were developed with four solvent systems: A, ethyl acetate–absolute ethanol (3:1); B, *n*-butanol–acetic acid–water (4:1:1); C, methanol; and D, benzene–ethyl acetate–95% ethanol (4:4:1). The alkaloid mixture was revealed by chromogenic reactions with the ceric ammonium sulfate and modified Dragendorff's reagents (24) to be composed of two alkaloids. One of these compounds gave R_f values of 0.67, 0.17, 0.65, and 0.77, which corresponded with those values obtained for aspidospermine when developed in Solvent Systems A–D, respectively. The second alkaloid in the mixture gave R_f values identical with those found for quebrachidine (0.46, 0.34, 0.55, and 0.58 in Solvent Systems A–D, respectively). Furthermore, fractional crystallization from methanol afforded 0.78 g. of an alkaloid whose physical constants were identical with those cited for aspidospermine.

The third crop of alkaloid crystals (9.9 g.) obtained from the ether solution was found to be predominantly that of the lower R_f alkaloid in the TLC solvent systems. It was readily recrystallized from methanol, yielding an analytical sample of alkaloid prisms having m.p. 271–273° dec.; $[\alpha]_D^{28}$ + 38° (concentration 0.5 in chloroform); λ_{max}^{MeOH}: 207 (log ϵ 4.62), 242 (3.90), and 291 (3.58) nm. The IR and mass spectral data for the isolate were identical with those obtained for reference quebrachidine, and no depression was found with a mixture melting-point determination of the isolate and reference quebrachidine.

Chromatographic Separation of Fraction D-1 Alkaloids—The alkaloids of this fraction were found to be best resolved on silica gel G TLC plates developed using a solvent mixture of benzene–ethyl acetate–95% ethanol (4:4:1). Consequently, the alkaloids were separated over a column of silica gel PF_{254}[5], employing the same developing solvent system according to a detailed procedure to be described elsewhere. Two kilograms of the adsorbent was

[4] Millipore PSAC 14205.

[5] E. Merck, Darmstadt, Germany.

Table I—Chromatographic Data for *A. quebracho-blanco* Leaf (D-1) Fraction (50.4 g.)

Fraction[a]	Fraction Weight, g.	Alkaloid Isolated	Isolate Weight, g.
1–37	—	—	—
38–42	1.34	Rhazinilam	0.188
43–46	1.93	Aspidospermine	0.574
47–49	7.36	Aspidospermine	1.394
50–55	16.64	Aspidospermine	2.424
56–60	5.00	(−)-Pyrifolidine	0.619
61–66	2.62	Akuammidine	0.052
67–69	1.61	Quebrachidine	0.252
70–84	11.55	Quebrachidine	2.163
85–91	0.88	—	—
92–99	1.50	—	—
100–115	1.11	—	—
116–121	1.19	—	—
122–123	0.77	Rhazinilam	0.128
124–142	1.98	—	—

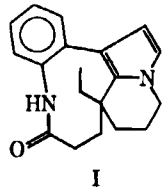
[a] Each fraction was 100 ml. with the eluting solvent being a mixture of benzene–ethyl acetate–ethanol (4:4:1).

first dried at 110° for 24 hr., followed by deactivation with 200 ml. of distilled water. A 25% slurry of the deactivated adsorbent in the solvent mixture of benzene–ethyl acetate–ethanol was prepared; after standing for 24 hr., the slurry was poured into a flat-bed column (8 × 150 cm.). The adsorbent was allowed to settle over 36 hr., with the solvent being passed through the column at a rate of 60 ml./hr. The alkaloid fraction (50.4 g.) was dissolved in 250 ml. of the developing solvent and added to the top of the packed column. Fractions of 100 ml. each were collected and monitored by means of silica gel G TLC and the ceric ammonium sulfate and modified Dragendorff's spray reagents. Those consecutive fractions containing alkaloids of similar R_f value and chromogenic response were combined for further workup. The results are presented in Table I.

Isolation of Rhazinilam—The residue from the combined fractions 38–42 from the column was dissolved in 100 ml. of ether and extracted with four 30-ml. portions of 5% acetic acid to effect the separation of an ether-soluble weak base. The acid extracts were combined, made alkaline with ammonium hydroxide, and extracted with benzene, and the benzene-soluble alkaloids were set aside. The ethereal mother liquor was dried over anhydrous magnesium sulfate, filtered, and concentrated, yielding 0.188 g. of crystalline alkaloid. Repeated recrystallization from ether afforded large prisms, m.p. 214–216°; λ_{max}^{EtOH} 224 (log ϵ 4.68) with a shoulder at 264 nm. (4.13); $[\alpha]_D^{29}$ −432 (concentration 1.25 in methanol). An IR spectrum showed the presence of an amide NH (3230 cm.$^{-1}$), an amide carbonyl (1672 cm.$^{-1}$), and four adjacent hydrogens on an aromatic nucleus (755 cm.$^{-1}$). The NMR spectrum showed vinyl protons at 5.9 and 6.65 δ, four aromatic protons as a multiplet from 7.36 to 7.69 δ, terminal methyl protons as a triplet at 0.46 to 0.89 δ, CH_2 protons adjacent to a carbonyl as a triplet at 3.75 to 4.20 δ, and an NH proton at 6.84 δ. The compound gave an M^+ ion at *m/e* 294.1727 (calc. 294.1732) for a molecular formula of $C_{19}H_{22}N_2O$ by high-resolution mass spectrometry. This alkaloid exhibited a mass spectral fragmentation pattern similar to that of eburnamonine, showing significant peaks at *m/e* 265, 237, 224, and 209. Structure I was finally deduced for the isolate by X-ray analysis (25), and it was identified as rhazinilam, an alkaloid first isolated from *Rhazya stricta* by Banerji *et al.* (26).

A second crop of crystalline rhazinilam (0.128 g.) was obtained from later column fractions, 122–123, by similar workup.

Isolation of Aspidospermine—The pooled fractions 43–46, 47–49, and 50–55 from the column were dissolved in methanol. Concentration of the filtered methanolic solution yielded 0.574, 1.394, and 2.424 g. of aspidospermine from the respective fractions. Identification of the isolates was based on a comparison of the melting points, R_f values, and IR spectra with reference aspidospermine.



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Isolation of (−)-Pyrifolidine—By using ether as the crystallizing solvent, 0.619 g. of a crystalline alkaloid was realized from the residues of fractions 56–60 from the column. Recrystallizations from ethanol and a 1:1 mixture of acetone–water gave 0.085 g. of an analytical sample of fine needles, m.p. 144–146°; $[\alpha]_D^{20}$ − 98° (concentration 1.0 in chloroform). Literature (8) values of m.p. 148–150° and $[\alpha]_D$ − 93° have been reported for (−)-pyrifolidine. The UV spectrum (methanol) gave absorptions at λ 224 (log ϵ 4.61), 253 (4.06), and 289 (3.46) nm., indicating a dihydroindole alkaloid. The IR spectrum (KBr) showed absorption bands at ν_{max} 1655 [N—C(=O)CH_3], 2850 (aromatic —OCH_3), and 802 (two adjacent aromatic hydrogen) cm.$^{-1}$. The NMR ($CDCl_3$) spectrum revealed the presence of two aromatic protons (7.02 δ) (*AB* quartet), six methoxy protons (4.08 and 4.00 δ), and three protons [N—C(=O)-CH_3] (2.33 δ). Mass spectrometric analysis of the isolate indicated a molecular weight of 386, and its fragmentation pattern was found to be identical with that reported in the literature for (−)-pyrifolidine (8). Identification of the isolate was made on the basis of interpretation of the physical data obtained for the isolate and a comparison of the data reported in the literature for (−)-pyrifolidine.

Isolation of Akuammidine—Pooled fractions 61–66 from the column were reduced to dryness and dissolved in a mixture of methanol–ether (1:1), resulting in a yield of 0.052 g. of prismatic crystals. Recrystallization from methanol afforded an analytical sample, m.p. 243–245°. Identification of the isolate as akuammidine was made on the basis of mixture melting-point determination and superimposable IR, UV, and mass spectra with reference akuammidine.

Isolation of Quebrachidine—Pooled fractions 67–69 and 70–84 from the column were reduced to dryness, and each was taken up separately in minimum volumes of hot methanol. From the cooled solutions, 0.252 and 2.163 g. of crystalline prismatic crystals were obtained from the respective fractions. A comparison of the physical data for these isolates with those obtained earlier for quebrachidine revealed the compounds to be identical.

SUMMARY

The isolation of rhazinilam in this study represents the first example of this new class of alkaloids to be found in the genus *Aspidosperma*. The attempt to elucidate the structure of this alkaloid by spectral means proved to be a formidable task. The IR and mass spectral data of this isolate resembled very closely those of eburnamonine, an alkaloid that Biemann and Watson (9) reported as being present in the bark of this plant on the basis of GC–mass spectrometric data. The UV absorptions, however, ruled out the isolate as belonging to the eburnamonine group of alkaloids. The structure (I) elucidated for rhazinilam by X-ray analysis was confirmed by the independent elucidation of this structure by DeSilva *et al.* (27) using chemical means.

(−)-Pyrifolidine was reported to occur in this plant by Biemann *et al.* (7, 8). Their isolation of the compound, however, was achieved by hydrolysis of an alkaloid separated from an acetylated alkaloid fraction of the bark. The isolation of this alkaloid from a chemically untreated leaf fraction in the present study confirms (−)-pyrifolidine to be a naturally occurring base.

The identification of akuammidine as one of the leaf alkaloids presented no difficulty, and the copious yields of aspidospermine and quebrachidine established them to be the major alkaloid constituents of *A. quebracho-blanco* leaves.

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Model Transport Studies Utilizing Lecithin Spherules II: Transport of 3-*O*-Methyl-^{14}C-D-glucose in D-Glucose Solution

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Abstract □ Recently, quantitative methods were developed for determining the permeability coefficient of solutes in lecithin spherules. The technique involved following (*a*) the direct release of solutes from the dispersions, (*b*) the release after prior dilution, and (*c*) the release from dispersions partly equilibrated with the solutes for a predetermined period. A quantitative evaluation of several physical models indicated that the models that assume that the spherules are equally spaced, multiconcentric bilayers of lecithin were in satisfactory agreement with the experimental release data. In the present study, this technique was applied to the transport of 3-*O*-methyl-D-glucose in liposome dispersions prepared from lecithin–dicetyl phosphate (10:1) and lecithin–dicetyl phosphate–cholesterol (10:1:1). The transport results for 3-*O*-methyl-D-glucose yielded a permeability coefficient that was 50 times larger than that for D-glucose. The dispersions prepared from lecithin–dicetyl phosphate containing 10% cholesterol yielded a permeability coefficient that was 2.4 times smaller than the dispersions prepared without cholesterol. The analysis of the results indicated that, for relatively large permeability coefficients as obtained in these studies, the dilution-release experiments show greater sensitivity in the determination of this parameter compared to the direct-release experiments.

Keyphrases □ Permeability coefficients, 3-*O*-methyl-D-glucose, radiolabeled—liposome dispersions, comparison of three methods □ D-Glucose solution—transport of 3-*O*-methyl-^{14}C-D-glucose, lecithin spherule dispersions, permeability coefficients □ 3-*O*-Methyl-D-glucose, radiolabeled—transport in D-glucose solution, lecithin spherule dispersions, permeability coefficients □ Lecithin spherules—model transport studies, 3-*O*-methyl-^{14}C-D-glucose in D-glucose solution, comparison of permeability coefficients determined by three methods □ Transport studies, model using lecithin spherules—3-*O*-methyl-^{14}C-D-glucose in D-glucose solution, comparison of permeability coefficients determined by three methods

Several physical models were evaluated (1) in determining the permeability coefficients for solutes in complex aqueous liposome dispersions to quantitate the transport of drugs across phospholipid membranes. The solute transport experiments were conducted using three different initial boundary conditions. The first (direct-release experiment) was the solute release from the dispersions in which the solute was fully equilibrated between the spherule interior and the external aqueous phases. In the second situation (dilution-release experiment), the dispersion was diluted by about a factor of 10 just prior to beginning the release run. In the third case (uptake-release experiment), the dispersion was first prepared without the radioactive solute and then the spherules were allowed to absorb the radioactive solute for a predetermined period just prior to the release run.

A careful evaluation of several physical models in conjunction with these three experimental procedures helped significantly in both the selection of the best models and the determination of the best set of values for the parameters. Simple physical models that assume monosize or multisize single membrane-controlled solute transport failed to provide reasonable agreement between the experimental data and the theory. The models assuming multiconcentric layers of equal thickness were generally found to be in good agreement with the experimental transport data. The introduction of