Ergoline Alkaloidal Constituents of Hawaiian Baby Wood Rose, *Argyreia nervosa* (Burm. f.) Bojer

JEW-MING CHAO* and ARA H. DER MARDEROSIAN▲

Abstract □ Chemical analysis showed that the seeds of *Argyreia nervosa* contain the highest percentage of indole alkaloid constituents (0.5–0.9%) of the genera of the Convolvulaceae thus far studied. A total of 19 indole alkaloids were identified by thin-layer and paper chromatographic procedures. Of these, lysergene, festuclavine, setoclavine, isosetoclavine, agroclavine, elymoclavine, ergine, and isoergine were isolated by column chromatographic procedures and characterized by TLC and IR analyses. Penniclavine, chanoclavine-I, chanoclavine-II, ergometrine, ergometrinine, lysergic acid α-hydroxyethylamide, isolysergol, racemic chanoclavine-II, molliclavine, lysergol, and isolysergic acid α-hydroxyethylamide were identified by TLC only. Of these, lysergene, setoclavine, isosetoclavine, chanoclavine-II, racemic chanoclavine-II, isolysergol, and molliclavine were identified for the first time in species of the Convolvulaceae. Ergine (0.136%) and isoergine (0.188%) were found in the highest concentration. In addition, 11 unidentified indole alkaloids were detected, these being found in very low concentration. Although the pericarp showed the same alkaloid pattern as the seeds, the concentration was much lower (0.0015%). No alkaloids could be detected in the vegetative tissues of nonflowering specimens.

Keyphrases □ Alkaloidal constituents, *Argyreia nervosa* seeds—isolation, identification of 19 indole alkaloids □ *Argyreia nervosa* (Burm. f.) Bojer seeds—isolation, identification of 19 indole alkaloids □ Morning-glory seeds—isolation, identification of 19 indole alkaloids □ Ergoline alkaloids—isolation, identification from *Argyreia nervosa* (morning-glory)

Argyreia nervosa (Burm. f.) Bojer is a member of the Convolvulaceae (morning-glory family) and is characterized by heart-shaped leaves with dense, white, silky hairs beneath. The plant is believed to have its origin in India. Historically, its roots have been used by the Hindus as an alternative, tonic, antirheumatic and in the treatment of diseases of the nervous system (1). The leaves have been used as a local stimulant, rubefacient, and vesicant. Because the under surface of the leaf is covered by a thick layer of silky hairs, it has been used as a natural impermeable temporary covering and poultice for minor skin abrasions by natives in India. However, Dymock (2) reported the leaves to be ineffective for this purpose.

This species is commonly and widely cultivated as a garden plant in several tropical countries because of its showy flowers. The mature capsules are handsome and persistent and are widely used in ornamental dried-flower arrangements. To date, no significant pharmacological or phytochemical studies on *A. nervosa* have appeared in the literature.

Since Aztec times in the uplands of southern Oxaca in Mexico, the seeds of *Rivea corymbosa* (known as "Ololiuqui") and *Ipomoea violacea* (known as "badoh negro") have been used for divinatory and hallucinatory purposes during religious ceremonies. The native use of these seeds for ceremonial purposes was extensively reported by Schultes (3–5) and Wasson (6). Following these publications, there occurred a rash of use of morning-glory seeds for hallucinatory purposes in the United States.

Among the morning-glories that have become quite popular is *A. nervosa* (Hawaiian Baby Wood Rose); it is readily available from the southern parts of California and Florida as well as from Hawaii. The availability of this plant in the United States has led to its use by juveniles who seek hallucinatory experiences through ingestion of the seeds. Contributing to its popularity among juveniles is the potency of seeds of *A. nervosa* compared to seeds of ipomoea species. To achieve a psychotomimetic response, relatively few seeds of *A. nervosa* are needed as opposed to a few hundred of *I. violacea*. Hylin and Watson (7) reported that each gram of seeds contains approximately 3 mg. of total alkaloids, of which 0.36 mg. is represented by one of the psychoactive constituents, ergine. The results from the present study indicate that the seeds contain even higher concentrations of ergine and total indole alkaloid constituents.

Prior to 1960, ergoline-type alkaloids were known to occur only in certain lower fungi, particularly the genus claviceps. Hofmann and Tscherter (8) were the first to find and isolate these ergoline alkaloids from higher plants. Later, several investigators (9–37) showed a wider distribution of the ergoline-type alkaloids in the Convolvulaceae (morning-glory family). These currently include the genera rivea, ipomoea, argyreia, cuscuta, and stictocardia.

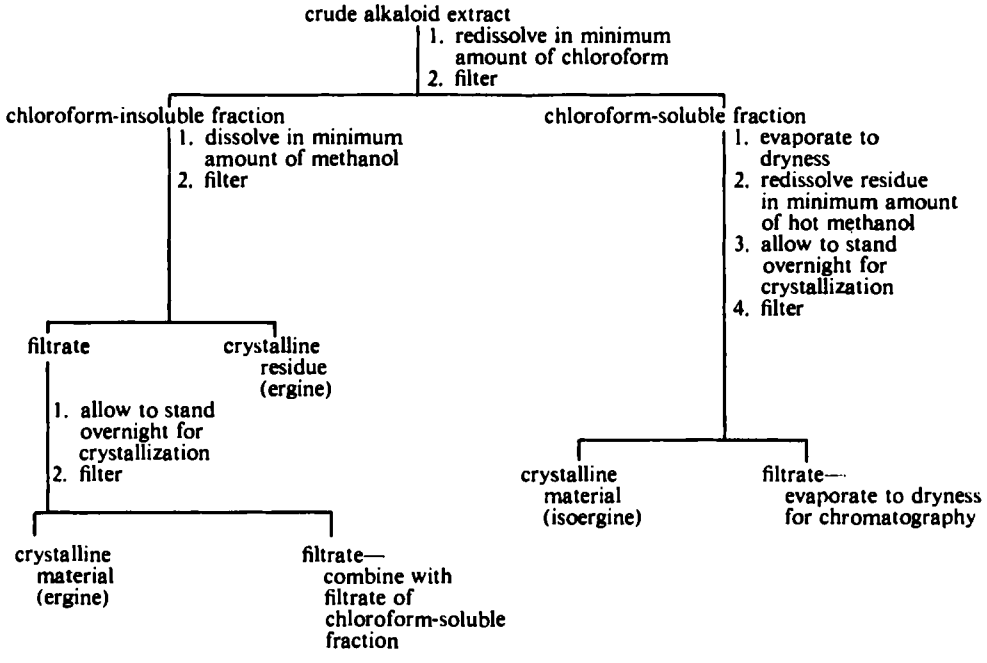
Noteworthy among the genera is argyreia because it possesses very high concentrations of a large number the ergoline alkaloids and until now it has not been subjected to extensive chemical studies. Only two compounds—*viz.*, ergine (lysergic acid amide) and isoergine (isolysergic acid amide), have been identified in *A. nervosa*, by Hylin and Watson (7) using TLC. Recently these two compounds were confirmed by McJunkins *et al.* (35) and isolated by Miller (37).

EXPERIMENTAL

Extraction of Crude Alkaloids and Isolation of Ergine and Isoergine—The total alkaloid was extracted by using a modification of the method of Genest (27). Due to the high concentrations of ergine and isoergine, they could be easily separated first without involving the chromatographic procedure. The diagrammatic isolation procedure is shown in Scheme I.

TLC—The following solvent systems were used for TLC: 1, silica gel plates, methanol–chloroform (20:80); 2, silica gel plates, diethylamine–chloroform (10:90); 3, silica gel plates, methanol–chloroform–concentrated ammonia (20:80:0.2); 4, silica gel plates, ethyl acetate–ethanol–dimethylformamide (86:7:7); 5, alumina plates, ethanol–chloroform (4:96); and 6, alumina plates, chloroform–benzene–glacial acetic acid (45:45:10).

For two-dimensional TLC, the method of Agurell (38) was used. A sample was spotted in the left-hand corner of the plates, 1.5 cm. from each side. The chromatograms were developed first in Solvent



Scheme I—Procedure for isolation of ergine and isoergine

System 1 and, after air drying for 5 min., were run in the second direction in Solvent System 2.

The fluorescent alkaloids were detected using both short- and longwave UV light. Ehrlich's spray reagent was used to locate all indole alkaloids. Dragendorff's reagent was used for general alkaloid detection.

Paper Chromatography—The method reported by Pöhm (39) was used for paper chromatography. The R_A value was calculated by using the R_f value of agroclavine as R_A 100. The solvent system is abbreviated as FBP.

Fractionation of Crude Alkaloids Using Column Chromatography—In these experiments, a minimum of 1.5 g. of crude alkaloid mixture (after ergine and isoergine were isolated) was redissolved in 50 ml. of a chloroform–benzene (1:1) mixture (containing 2% of methanol) and then transferred to a 100 × 2.5-cm. glass column. The column was previously packed with 90 cm. of a thick slurry of an alumina[1] and chloroform–benzene (1:1) mixture. Elution was started with 200 ml. of 50% chloroform in benzene and then developed by increasing the polarity (increasing the percentage of chloroform) in the solvent system. The procedure used was to increase the chloroform percentage by 5% for every 200 ml. of eluent collected until the solvent system contained no benzene. Methanol was then used to increase the polarity of chloroform by increasing its concentration at a rate of 1% for every 200 ml. of solvent collected until the solvent system reached 16% methanol in chloroform. At this point, pure methanol was used to elute the column. Ten-milliliter fractions were collected at a rate of 1 ml./min. until 580 fractions were obtained. All fractions were analyzed using TLC (Solvent System 1) as previously described.

Quantitative Analysis of Indole Alkaloid—The assay for total indole alkaloid was a modification of the procedure of Michelon and Kelleher (40). Isoergine was used as a reference compound. For individual alkaloids, an aliquot of crude alkaloid mixture was separated on a two-dimensional TLC plate as previously described. The individual alkaloids were marked under UV light. Each spot was scraped off the plate and then assayed using the same procedure as for the total indole alkaloid.

RESULTS

Fractionation and Separation of Crude Alkaloids by Column Chromatography—The fractions collected after separation by column chromatography were pooled according to similarity of alkaloid patterns as determined by TLC monitoring of the eluent. A total of 580 fractions was collected, and 30 alkaloids were isolated by this procedure. Nineteen of them were identified by IR or various chromatographic systems.

Thin-Layer and Paper Chromatographic Results (R_f and R_A Values) of Isolated Ergoline-Type Alkaloids—The R_f and R_A values of ergoline-type alkaloids isolated from the seeds of *A. nervosa* in various chromatographic systems are shown in Table I. A diagrammatic map of the two-dimensional TLC system (using Solvent System 1 first and Solvent System 2 second) is given in Fig. 1. Thirty compounds were found by this two-dimensional TLC system.

The crude alkaloidal extract from the pericarp showed the same alkaloidal pattern as the seeds.

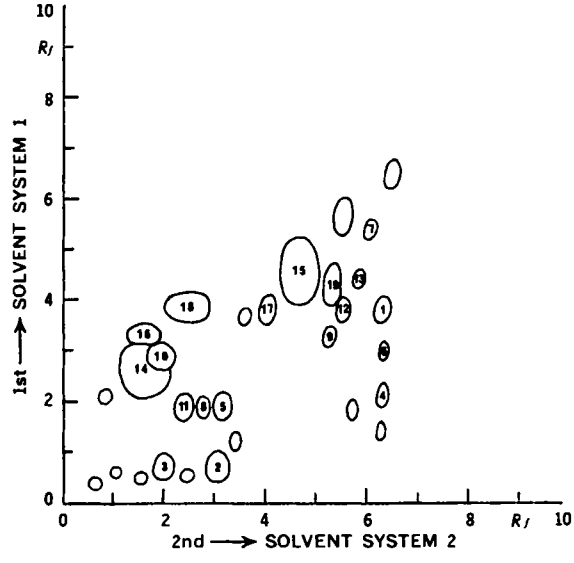


Figure 1—*Two-dimensional TLC of crude alkaloid mixture from the seeds of* A. nervosa *(Burm. f.) Bojer. Key: 1, agroclavine; 2, chanoclavine-I; 3, chanoclavine-II; 4, racemic chanoclavine-II; 5, elymoclavine; 6, festuclavine; 7, lysergene; 8, lysergol; 9, isolysergol; 10, molliclavine; 11, penniclavine; 12, setoclavine; 13, isosetoclavine; 14, ergine; 15, isoergine; 16, ergometrine; 17, ergometrinine; 18, lysergic acid α-hydroxyethylamide; 19, isolysergic acid α-hydroxyethylamide; and* O, *unidentified.*

[1] Alcoa.

Table I—R_f and R_A Values of Ergoline-Type Alkaloids Isolated from Seeds of *A. nervosa* (Burm. f.) Bojer in Various Solvent Systems

Alkaloids	FBP (R_A)	Solvent System[a] 1	2	3	4	5	6
				($R_f \times 100$)			
Agroclavine	100	38	63	69	21	95	34
Reference compound	100	38	62	68	21	95	34
Chanoclavine-I	6	7	30	20	3	33	16
Reference compound	6	7	30	20	3	33	16
Chanoclavine-II	6	8	19	18	3	41	16
Reference compound	—	—	—	—	—	—	—
Racemic chanoclavine-II	54	20	63	50	7	95	20
Reference compound	—	—	—	—	—	—	—
Elymoclavine	30	19	30	45	9	34	9
Reference compound	30	19	30	45	9	35	9
Festuclavine	82	32	63	63	18	91	25
Reference compound	82	32	63	63	18	91	25
Lysergene	118	54	60	77	46	91	32
Reference compound	119	54	60	77	46	91	32
Lysergol	32	19	27	—	—	—	—
Reference compound	32	19	27	—	—	—	—
Isolysergol	65	34	53	65	24	92	14
Reference compound	65	34	53	65	24	93	14
Molliclavine	5	29	19	45	30	18	3
Reference compound	—	—	—	—	—	—	—
Penniclavine	22	20	23	48	12	24	15
Reference compound	22	20	23	48	12	24	15
Setoclavine	84	39	55	67	38	92	16
Reference compound	84	39	55	68	38	92	16
Isosetoclavine	95	44	58	69	55	75	15
Reference compound	95	44	58	69	55	75	15
Ergine	12	27	15	48	15	22	18
Reference compound	12	27	15	48	15	22	18
Isoergine	48	47	45	68	35	55	5
Reference compound	48	47	45	68	35	55	5
Ergometrine	15	33	16	50	14	20	12
Reference compound	15	33	16	50	14	22	12
Ergometrinine	52	39	40	74	30	52	5
Reference compound	52	39	40	75	30	52	5
Lysergic acid α-hydroxyethylamide	19	38	24	56	17	17	12
Reference compound	19	38	24	56	17	17	12
Isolysergic acid α-hydroxyethylamide	54	41	53	—	—	—	—
Reference compound	—	—	—	—	—	—	—

[a] R_A values were calculated by using the R_f value of agroclavine as R_A 100. R_f values were calculated on the basis of a solvent front of 15 cm. and multiplied by 100. —: compounds were not available, the values were not determined.

The FBP solvent system is effective for separating most clavine-type alkaloids except chanoclavine-I and chanoclavine-II; however, it is ineffective for separating mixtures of both clavine- and lysergic acid-type alkaloids. None of the six TLC systems could separate the crude alkaloidal mixture from the seeds of *A. nervosa*. To obtain good separation, the two-dimensional TLC system was used for this mixture. Chanoclavine-I and chanoclavine-II showed very poor resolution in most systems but were separated well in Solvent System 2. Pennicalvine, lysergol, and elymoclavine were not separated by Solvent System 1 but were well separated by Solvent System 2. Agroclavine, festucalvine and racemic chanoclavine-II were well separated by most systems except 2 and 5.

Because of the limited amount of materials isolated, the R_f values of lysergol and isolysergic acid α-hydroxyethylamide were not obtained in Solvent Systems 3–6.

The R_f values represent the average of three chromatograms except for isolysergic acid α-hydroxyethylamide and lysergol; only one chromatogram was run for these compounds.

Quantitative Determination of Total and Individual Alkaloids—Various batches of seeds from different sources showed a 0.5–0.9% range of total indole alkaloid constituents. The results of one typical detailed assay (Table II) indicate the total percent of alkaloid to be 0.60, with isoergine (0.189%) and ergine (0.136%) being found in the highest concentrations in the seeds. These are followed, in order of decreasing indole alkaloid content, by ergometrine, lysergic acid α-hydroxyethylamide, isolysergic acid α-hydroxyethylamide, elymoclavine, ergometrine, chanoclavine-I, and agroclavine.

Table II—Major Alkaloid Content in the Seeds of *A. nervosa* (Burm. f.) Bojer

Alkaloids	Values Expressed as Percent of Total Alkaloid	Values Expressed as Percent of Dry Seed Weight
Agroclavine	1.09	0.006
Chanoclavine-I	2.65	0.016
Elymoclavine	3.62	0.022
Ergine	22.68	0.136
Isoergine	31.36	0.188
Ergometrine	8.20	0.049
Ergometrinine	1.81	0.011
Lysergic acid α-hydroxyethylamide	5.79	0.035
Isolysergic acid α-hydroxyethylamide	3.98	0.024
Tailing, minor, and unidentified alkaloids	18.82	0.113
Total	100.00	0.600

The tailing and minor alkaloids were scraped from the TLC plate and combined in the assay to give a total percentage of 0.113%.

The crude alkaloidal extract of the pericarp showed 0.0015% of total indole alkaloid constituents.

DISCUSSION

In comparison to other members of the Convolvulaceae investigated so far, *A. nervosa* apparently contains one of the largest concentrations of ergoline derivatives. Various batches of seeds have assayed from 0.5 to 0.9% of total indole alkaloid constituents. The various components of the total alkaloidal mixture, as isolated and characterized by chromatographic and IR spectral data, include lysergic acid amide (ergine), isolysergic acid amide (isoergine), agroclavine, elymoclavine, lysergene, festuclavine, setoclavine, isosetoclavine, and racemic chanoclavine-II (no nonindole alkaloids were detected). Those characterized by chromatographic data alone include penniclavine, isolysergol, molliclavine, lysergol, chanoclavine-I, chanoclavine-II, ergometrine, ergometrinine, and the isomeric pair of isolysergic acid and lysergic acid α-hydroxyethylamides. Limited quantities of the isolated clavine- and lysergic acid-type alkaloids have precluded further chemical studies on these constituents.

Nineteen identified ergoline alkaloids are present in the seeds of *A. nervosa*. Of these, lysergene, setoclavine, isosetoclavine, chanoclavine-II, racemic chanoclavine-II, isolysergol, and molliclavine have been identified for the first time in species of the morning-glory family.

The various unknown minor indole alkaloidal constituents are considered to represent *bona fide* minor clavine- or lysergic acid-type alkaloids or decomposition products. The inability in many cases to obtain reproducible R_f values for these tends to indicate the latter, although the former should not be entirely discounted. The possibility that certain of the alkaloids isolated or described are decomposition products has been minimized by careful experimental procedures (avoidance of light, rapid TLC separation, rapid analysis, *etc.*) and reproducibility of results.

The results of the tentative identification of several ergoline alkaloids of 13 other argyreia species and two closely related species, *Stictocardia tiliifolia* and *R. corymbosa*, will be given in a separate paper dealing with the chemotaxonomic implications of these indole alkaloids.

REFERENCES

(1) U. C. Dutt, "Materia Medica of the Hundus," Thacker, Spink and Co., Calcutta, India, 1887, p. 206.

(2) W. Dymock, "The Vegetable Materia Medica of Western India," part III, Bombay Educational Society's Press, Bombay, India, 1885, pp. 566–568.

(3) R. E. Schultes, "The Pharmaceutical Sciences," Third Lecture Series, part V, University of Texas, 1960, pp. 138–185.

(4) R. E. Schultes, *Harvard Rev.*, **1**, 18(1963).

(5) R. E. Schultes, *Psychedelic Rev.*, **1**, 145(1963).
(6) R. G. Wasson, *Bot. Mus. Leafl. Harvard Univ.*, **20**, 161 (1963).
(7) J. W. Hylin and D. P. Watson, *Science*, **148**, 499(1965).
(8) A. Hofmann and A. Tscherter, *Experientia*, **16**, 414(1960).
(9) A. Hofmann, *Planta Med.*, **9**, 354(1961).
(10) A. Hofmann, *Bot. Mus. Leafl. Harvard Univ.*, **20**, 194 (1963).
(11) A. Hofmann, *Indian J. Pharm.*, **25**, 245(1963).
(12) A. H. Der Marderosian, R. L. Hauke, and H. W. Youngken, Jr., *Econ. Bot.*, **18**, 67(1964).
(13) *Ibid.*, **18**, 158(1964).
(14) A. H. Der Marderosian, A. M. Guarino, J. J. DeFeo, and H. W. Youngken, Jr., *Psychedelic Rev.*, **1**, 317(1964).
(15) A. H. Der Marderosian, Ph.D. thesis, University of Rhode Island, Kingston, R. I., 1964.
(16) A. H. Der Marderosian, A. M. Guarino, J. J. DeFeo, and H. W. Youngken, Jr., *Amer. J. Pharm.*, **137**, 24(1965).
(17) A. H. Der Marderosian, *Taxon*, **14**, 234(1965).
(18) A. H. Der Marderosian, *Lloydia*, **29**, 35(1966).
(19) A. H. Der Marderosian and R. H. Lockwood, paper presented at the 8th annual meeting of the American Society of Pharmacognosy, Ann Arbor, Mich., June 1967.
(20) A. H. Der Marderosian, *Lloydia*, **30**, 23(1967).
(21) A. H. Der Marderosian, *Amer. J. Pharm.*, **139**, 19(1967).
(22) W. A. Taber, L. C. Vining, and R. A. Heacock, *Phytochemistry*, **2**, 65(1963).
(23) W. A. Taber, R. A. Heacock, and M. E. Mahon, *ibid.*, **2**, 99(1963).
(24) D. Gröeger, K. Mothes, H. Floss, and F. Weygand, *Z. Naturforsch.*, **18**, 1123(1963).
(25) D. Gröeger, *Flora*, **153**, 373(1963).
(26) D. Gröeger, *Planta Med.*, **11**, 444(1963).
(27) K. Genest, *J. Chromatogr.*, **19**, 531(1965).
(28) K. Genest, *J. Pharm. Sci.*, **55**, 1284(1966).
(29) K. Genest and M. R. Sahasrabudhe, *Econ. Bot.*, **20**, 416 (1966).
(30) C. I. About-Charr and G. A. Digenis, *Nature*, **212**, 618 (1966).
(31) D. Stauffacher, H. Tscherter, and A. Hofmann, *Helv. Chim. Acta*, **48**, 1379(1965).
(32) D. Stauffacher, P. Niklaus, H. Tscherter, H. P. Weber, and A. Hofmann, *Tetrahedron*, **25**, 5879(1969).
(33) M. R. Gardiner, R. Royce, and B. Oidroyd, *Brit. Vet. J.*, **121**, 272(1965).
(34) R. Ikan, E. Rapaport, and E. D. Bergman, *Isr. J. Chem.*, **6**, 65(1968).
(35) S. P. McJunkins, J. I. Thornton, and D. J. Dillon, *J. Forensic Sci.*, **8**, 121(1968).
(36) T. Niwaguchi and T. Inoue, *J. Chromatogr.*, **43**, 510(1969).
(37) M. D. Miller, *J. Ass. Offic. Anal. Chem.*, **53**, 123(1970).
(38) S. Agurell, *Acta Pharm. Suecica*, **2**, 357(1965).
(39) M. Pöhm, *Arch. Pharm.*, **291**, 468(1959).
(40) L. E. Michelon and W. J. Kelleher, *Lloydia*, **26**, 192(1963).
(41) D. Stauffacher and H. Tscherter, *Helv. Chim. Acta*, **47**, 2186(1964).
(42) M. Abe and S. Yamatodani, *Bull. Agr. Chem. Soc. Jap.*, **19**, 161(1955).

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Clindamycin Dose–Bioavailability Relationships

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Abstract □ Analyses of dose-related changes in pharmacokinetic variables indicating bioavailability and of dosage equivalence were performed on data from single-dose studies on three preparations of the antibiotic clindamycin [7(*S*)-chloro-7-deoxylincomycin]: clindamycin hydrochloride hard-filled capsules, clindamycin palmitate flavored granules, and clindamycin phosphate sterile solution. The variables studied were serum clindamycin bioactivity and parameters calculated from a single-compartment pharmacokinetic model. In general, the changes with dose were linear but not proportional and the pharmacokinetic model fit the data well. There were, however, some suggestions of nonlinear kinetics, particularly in the clindamycin hydrochloride study. Within the dose ranges (150–450 mg. clindamycin hydrochloride, 150–600 mg. clindamycin palmitate, and 300–600 mg. clindamycin phosphate), the average response to a dose could be predicted well. An equivalent dose analysis for the three clindamycin preparations offered some insight into their absorption and disposition characteristics and the relative bioavailability of clindamycin from them. A single equivalent dose could not be computed; there were different equivalent doses for peak serum concentration, area under the serum concentration–time curve, and other variables.

Keyphrases □ Clindamycin formulations—absorption, disposition, and relative bioavailability of hydrochloride capsules, palmitate flavored granules, and phosphate sterile solution □ Dose–bioavailability relationships—clindamycin hydrochloride capsules, clindamycin palmitate flavored granules, clindamycin phosphate sterile solution □ Bioavailability—absorption, disposition of clindamycin capsules, flavored granules, and sterile solution □ Pharmacokinetics—absorption, disposition, and relative bioavailability of three clindamycin formulations

Two problems frequently confront researchers testing new drugs: prediction of response to a particular dosage and equivalence of formulations. In the case of antibiotics, it is often assumed that the desired response, eradication of pathogens, is closely related to the concentration of drug in the serum and, therefore, that