derivatization reactions and are also used extensively in organic synthesis (32), especially in the preparation of isoquinoline alkaloids (33).

REFERENCES

(1) T. Kametani, "The Chemistry of the Isoquinoline Alkaloids," American Elsevier, New York, N. Y., 1969.

(2) M. Shamma, in "Chemistry of the Alkaloids," S. W. Pelletier, Ed., Van Nostrand Rheinhold, New York, N. Y., 1970, pp. 31–84.

(3) H. O. Bernhard and V. A. Snieckus, in "The Alkaloids," Specialist Periodical Reports, vol. 2, The Chemical Society, Burlington House, London, England, 1972, pp. 97–184.

(4) E. Schlittler, in "The Alkaloids," Specialist Periodical Reports, vol. 1, The Chemical Society, Burlington House, London, England, 1971, pp. 463–491.

(5) A. Brossi, H. Besendorf, L. A. Pirk, and A. Rheiner, Jr., in "Analgetics," Monographs in Medicinal Chemistry, vol. 5, George de Stevens, Ed., Academic, New York, N. Y., 1965, pp. 281–330.

(6) E. Grunberg and H. N. Prince, *Antimicrob. Ag. Chemother.*, **1966**, 527.

(7) N. M. Larin, A. S. Beare, M. P. Copping, C. P. McDonald, J. K. McDougall, B. Roberts, and J. B. Smith, *ibid.*, **1967**, 646.

(8) S. E. Reed and M. L. Bynoe, *J. Med. Microbiol.*, **3**, 346 (1970).

(9) S. E. Reed, A. S. Beare, M. L. Bynoe, and D. A. J. Tyrrell, *Ann. N. Y. Acad. Sci.*, **173**, 760(1970).

(10) R. A. Moe, H. M. Bates, Z. M. Polkoski, and R. Banziger, *Curr. Ther. Res.*, **6**, 299 (1964).

(11) A. Scriabine, K. D. Booher, J. N. Pereira, W. K. Mc Shane, J. W. Constantine, R. C. Koch, and S. Miknius, *J. Pharmacol. Exp. Ther.*, **147**, 277(1965).

(12) M. M. Creighton, W. Leimgruber, and W. Wenner, U. S. pat. 3,207,759 (1965).

(13) E. Grunberg and H. N. Prince, *Proc. Soc. Exp. Biol. Med.*, **129**, 422(1968).

(14) E. Grunberg and H. N. Prince, *Ann. N. Y. Acad. Sci.*, **173**, 122(1970).

(15) B. R. Murphy and L. A. Glasgow, *ibid.*, **173**, 255(1970).

(16) F. E. Schenker, R. A. Schmidt, T. Williams, and A. Brossi, *J. Heterocycl. Chem.*, **8**, 665(1971).

(17) A. S. Leon, C. B. Coutinho, H. M. Solomon, and W. B. Abrams, *J. Newark Beth Israel Med. Center*, **21**, 22(1971).

(18) J. Knabe, *Arch. Pharm.*, **292**, 416(1959).

(19) D. E. Schwartz and J. Rieder, *Clin. Chim. Acta*, **6**, 453(1961).

(20) D. E. Schwartz, H. Bruderer, J. Rieder, and A. Brossi, *Biochem. Pharmacol.*, **13**, 777(1964).

(21) W. Leimgruber and F. E. Schenker, U. S. pat. 3,661,913 (1972).

(22) F. E. Schenker, R. A. Schmidt, W. Leimgruber, and A. Brossi, *J. Med. Chem.*, **9**, 46(1966).

(23) J. A. F. de Silva and J. Kaplan, *J. Pharm. Sci.*, **55**, 1278 (1966).

(24) J. A. F. de Silva and M. R. Hackman, *Anal. Chem.*, **44**, 1145 (1972).

(25) B. Z. Senkowski, M. S. Levin, J. R. Urbigkit, and E. G. Wollish, *Anal. Chem.*, **36**, 1991(1964).

(26) H. Oelschläger, J. Volke, G. T. Lim, and U. Bremer, *Arch. Pharm.*, **303**, 364(1970).

(27) G. Dryhurst and P. J. Elving, *Talanta*, **16**, 855(1969).

(28) B. Lovrecek and D. Cipris, *J. Electroanal. Chem.*, **11**, 44 (1966).

(29) T. Fujinaga, K. Izutsu, and K. Takaoka, *ibid.*, **12**, 203 (1966).

(30) C. Parkanyi and R. Zahradnik, *Bull. Soc. Chim. Belg.*, **73**, 57(1964); through *Chem. Abstr.*, **60**, 10520(1964).

(31) M. Maturova, V. Preininger, and F. Santavy, *Abh. Deut. Akad. Wiss. Berlin, Kl. Chem. Geol. Biol.*, **1**, 85(1964); through *Chem. Abstr.*, **62**, 7620(1965).

(32) T. R. Evans, in "Energy Transfer and Organic Photochemistry," vol. XIV, of Techniques of Organic Chemistry Series, A. A. Lamola and N. J. Turro, Eds., Interscience, New York, N. Y., 1969, pp. 297–348.

(33) T. Kametani and K. Fukamoto, *Accounts Chem. Res.*, **5**, 212(1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 10, 1972, from the *Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche, Inc., Nutley, NJ 07110*

Accepted for publication February 21, 1973.

The authors thank Dr. M. A. Schwartz and Dr. F. Schenker for their permission to publish on 1-isoquinolineacetamide (I-A); Dr. R. Pocelinko for conducting the clinical study on 3,4-dihydro-1-isoquinolineacetamide hydrochloride (I-HCl) at the Special Treatment Unit, Newark City Hospital (Martland Memorial), Newark, N. J.; Dr. B. A. Koechlin and Mr. F. Rubio for helpful discussions on the analysis of I-HCl; Dr. S. A. Kaplan and Mr. S. Cotler for conducting the dog experiments on 8-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (II-HCl); and Mr. T. Daniels and Mr. R. McGlynn for the drawings of the figures presented.

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Serum Protein Binding of Erythromycin, Lincomycin, and Clindamycin

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Abstract □ Serum protein binding determinations for erythromycin, lincomycin, and clindamycin utilizing ultrafiltration of serum containing antibiotic in a concentration of 5 mcg./ml. and tube dilution techniques revealed a high degree of binding: erythromycin base, 73%; erythromycin propionate, 93%; lincomycin, 72%; and clindamycin, 94%.

Keyphrases □ Erythromycin and erythromycin propionate—serum protein binding □ Lincomycin—serum protein binding □ Clindamycin—serum protein binding □ Antibiotics—serum protein binding of erythromycin, lincomycin, and clindamycin □ Serum protein binding—erythromycin, lincomycin, and clindamycin

Serum protein binding of antibiotics is of potential clinical significance since bound antibiotic has been found to have no antibacterial activity and it is probable that the level of free antibiotic in the tissues is no greater than the peak level in the blood (1). The extent of protein binding of erythromycin, lincomycin, and

Table I—*In Vitro* Determination of Human Serum Protein Binding of Erythromycins Using Ultrafiltration and Bioassay (100% Serum)

Erythromycin Propionate			Erythromycin Base		
Serum, mcg./ml.	Ultrafiltrate, mcg./ml.	Percent Bound	Serum, mcg./ml.	Ultrafiltrate, mcg./ml.	Percent Bound
5.68	0.343	93.96	5.20	1.29	75.19
5.68	0.422	92.57	5.20	1.23	76.34
5.68	0.400	92.95	5.20	1.46	71.92
6.60	0.420	93.63	5.08	1.36	73.23
6.60	0.457	93.07	5.08	1.48	70.87
6.08	0.388	93.61	5.08	1.32	74.02
6.08	0.359	94.09	5.26	1.47	72.05
6.08	0.425	93.00	5.26	1.46	72.24
7.70	0.740	90.38	5.26	1.35	74.33
7.70	0.795	89.67			73.35
7.70	0.700	90.90			
6.40	0.420	93.43			
6.40	0.440	93.13			
		92.64			

Table II—*In Vitro* Determination of Human Serum Protein Binding of Lincomycin and Clindamycin Using Ultrafiltration and Bioassay (100% Serum)

Lincomycin			Clindamycin		
Serum, mcg./ml.	Ultrafiltrate, mcg./ml.	Percent Bound	Serum, mcg./ml.	Ultrafiltrate, mcg./ml.	Percent Bound
5.85	1.62	72.30	5.88	0.400	93.20
5.85	1.60	72.64	5.88	0.365	93.80
5.85	1.66	71.62	5.88	0.373	93.70
6.10	1.95	68.03	4.25	0.270	93.64
6.10	1.88	69.18	4.25	0.267	93.71
6.10	1.88	69.18	4.25	0.272	93.60
6.10	1.48	75.73	6.35	0.397	93.74
6.10	1.48	75.73	6.35	0.410	93.54
6.10	1.47	75.90			93.62
5.85	1.65	71.79			
5.85	1.67	71.45			
5.85	1.78	69.57			
		71.93			

clindamycin has been variously reported in the literature as low (2–4) to high (5–7). The present study was carried out to help delineate the protein binding characteristics of these three antibiotics using a variety of methods.

EXPERIMENTAL

Three techniques were used to study binding of these antibiotics by human serum proteins to compare the results obtained and to investigate possible causes of variation in the results reported by other workers. The antibiotic standard powders used in these studies were: erythromycin base[1], erythromycin propionate[1], ^{14}C-erythromycin[1], lincomycin[2], and clindamycin hydrochloride[2].

Ultrafiltration Method—The precise method and equipment reported by Bennett and Kirby (8) was used in these protein binding determinations involving vacuum ultrafiltration of pooled human serum from healthy volunteers containing antibiotic added in a concentration of approximately 5 mcg./ml. For these studies, a single layer of cellophane membrane[3], previously sterilized by rinsing with 90% ethanol, was utilized. All experiments were performed in a manner simulating *in vivo* conditions in that the initial serum pH was adjusted to the physiological range by bubbling of carbon dioxide, and all experiments were carried out in a 37° incubator. Ultrafiltration required less than 45 min. in every case, and post-filtration pH determinations revealed that the pH remained in the physiological range throughout the tests.

A serum sample, corresponding to 100% serum because of concentration during filtration, was obtained for antibiotic assay at the midpoint of filtration. The principle and mathematics of this were explained in the original article (8). Antibiotic concentrations in the midpoint sample and in the ultrafiltrate sample were assayed by an agar well method (9) using *Bacillus subtilus* (ATCC 6633) as the test organism for erythromycin and *Sarcina lutea* (FDA, PCI 1001) for lincomycin and clindamycin. The percent binding by 100% human serum was calculated by dividing the difference between the midpoint serum and filtrate antibiotic concentrations by the serum antibiotic concentration and multiplying by 100. Modifications of this same formula were used throughout this study for determination of protein binding. Impermeability of the membrane to unbound antibiotic as a possible cause of apparent protein binding was investigated by ultrafiltration of buffered saline solutions of the drugs.

Tube Dilution Method—A good correlation between protein binding and decrease in antibacterial activity has been observed by a number of workers (10, 11). For the present studies the minimum inhibitory concentration in tryptose phosphate broth was compared to that obtained in a series of tubes containing a fixed concentration of serum (25, 75, 90, or 100%) and small increments of antibiotic (0.02 mcg./ml.), with a range from 0.005 to 2.0 mcg./ml.

[1] Eli Lilly.
[2] Upjohn.
[3] Union Carbide, D.M. 1-7/8.

Table III—*In Vitro* Determination of Human Serum Protein Binding Using ^{14}C-Erythromycin Base (100% Serum)

Ultrafiltration			Centrifugation		
Serum, c.p.m.	Filtrate, c.p.m.	Percent Bound	Serum, c.p.m.	Filtrate, c.p.m.	Percent Bound
123,262.5	38,890.0	71.7	143,880.0	44,030.0	69.3
121,647.5	34,625.0	71.5	143,880.0	47,362.0	67.0
145,797.0	42,535.0	70.8	131,263.0	43,462.5	66.8
146,295.0	42,392.0	71.0			67.7
132,130.0	31,445.0	76.2			
129,948.0	26,440.0	79.6			
		73.5			

The test organism was a group A beta hemolytic streptococcus (clinical isolate), and hemolysis of human group O red blood cells was used as the bacteriostatic end-point. Minimal bactericidal concentrations were determined by subculture to antibiotic-free agar to establish an end-point where the hemolytic end-point was not clear cut. Percent binding was determined by dividing the difference between the minimum inhibitory concentration in serum and in tryptose phosphate broth by the minimum inhibitory concentration in serum and multiplying by 100.

Radioactive Assay Method—As an additional check on the accuracy of protein binding results, and as another way to study the possibility of membrane trapping of free antibiotic, a series of experiments was performed using labeled erythromycin. The ultrafiltration technique was modified so that counting of disintegrations per minute rather than bioassay was used to determine antibiotic concentrations. Experiments were also carried out using the centrifuge[4] cone technique, which is an ultrafiltration method using centrifugation rather than suction to effect separation of protein bound from free antibiotic. With this method, 5 ml. of serum containing the labeled antibiotic (5 mcg./ml.) was placed in a commercially prepared porous cone lined with an inner synthetic membrane and centrifuged at $100 \times g$ for 20 min. at 37°, yielding 1 ml. of protein-free filtrate. The antibiotic used in this series of experiments was ^{14}C-labeled erythromycin[5] at a specific activity of 9.45 μc./mg. All specimens were counted[6] for 20 min. in a liquid scintillation spectrometer[7] using appropriate internal standards. The concentration of erythromycin in the serum was compared with that in the filtrate or centrifugate by counting as already described, and the percent binding was calculated by dividing the difference between the counts per minute in serum and centrifugate or filtrate

[4] CF50A, Amicon Corp., Lexington, Mass.
[5] Lilly Research Laboratories.
[6] In Scintisol solution, Isolab, Akron, Ohio.
[7] Beckman.

Table IV—*In Vitro* Determination of Human Serum Protein Binding of Erythromycin Propionate, Lincomycin, and Clindamycin Using Tube Dilution Technique

Percent Serum	Erythromycin, Minimum Inhibiting Concentration			Lincomycin, Minimum Inhibiting Concentration			Clindamycin, Minimum Inhibiting Concentration		
	Broth, mcg./ml.	Serum, mcg./ml.	Percent Bound	Broth, mcg./ml.	Serum, mcg./ml.	Percent Bound	Broth, mcg./ml.	Serum, mcg./ml.	Percent Bound
25	0.06	0.08	25.0	0.40	0.8	50.0	0.04	0.10	60.0
	0.04	0.12	66.6	0.40	0.8	50.0	0.04	0.08	50.0
			45.8			50.0			55.0
75	0.02	0.12	83.3	0.40	1.8	77.7	0.008	0.08	90.0
	0.10	0.60	83.3	0.40	1.8	77.7	0.04	0.60	93.3
			83.3			77.7	0.04	0.40	90.0
									91.1
90	0.02	0.10	80.0	0.60	2.4	75.0	0.06	0.60	90.0
	0.03	0.08	62.5	0.10	0.4	75.0	0.025	0.40	93.7
			71.3			75.0			91.9
100	0.06	0.60	90.0	0.60	2.0	70.0	0.05	0.40	87.5
	0.10	0.60	83.3	0.09	0.4	77.5	0.03	0.60	95.0
			86.7			73.8			91.3

by the number of counts per minute in the serum and multiplying by 100.

Impermeability of the membranes to free antibiotic as a possible cause of high protein binding values was also studied by ultrafiltration and centrifugation of buffered saline solutions rather than serum. Possible membrane trapping was also checked in another way: the membranes used in two of the experiments with serum were carefully removed from the chambers, rinsed lightly with distilled water, and, after air drying, were burned to release carbon dioxide in a sample oxidizer[8]; radioactivity representing erythromycin adherent to the membrane was then counted.

RESULTS

Using the ultrafiltration method with 100% serum and bioassay of the specimens, binding of the four antibiotics was found to be as follows: erythromycin base, 73.4%; erythromycin propionate, 92.6%; lincomycin, 71.9%; and clindamycin, 93.6% (Tables I and II). Ultrafiltration of buffered saline solutions of the antibiotics revealed that less than 10% was trapped by the cellophane membrane, *i.e.*, did not pass through into the filtrate.

These ultrafiltration results correlated well with binding as determined by measurements of the decrease in antibacterial activity in serum by the tube dilution technique (Table III). With the use of small increments of antibiotic (0.02 mcg./ml.) from one tube to the next and bactericidal end-points, particularly for the higher serum concentrations, there was good agreement of serum binding figures obtained with the two methods.

Table IV summarizes the results of protein binding studies using ^{14}C-labeled erythromycin base. In these experiments, the mean binding of erythromycin by the ultrafiltration method was 73.5%, very close to the 73.4% observed with bioassay. Filtration of buffered saline solutions of the drug demonstrated little evidence of membrane trapping (retention <4%), again a result similar to that reported with bioassay of the specimens. Studies of membrane trapping carried out by burning the membrane after ultrafiltration of serum and counting the labeled carbon dioxide released showed that only 0.05 mcg. or less of the erythromycin was retained by the membrane. Centrifugation studies, performed with the cone technique as an alternative method of checking protein binding of ^{14}C-erythromycin, yielded a binding figure of 67.7%. With centrifugation of buffered saline solutions of the labeled antibiotic, 11.7% of the radioactivity was present in the cone.

DISCUSSION

The low degree of serum protein binding commonly attributed to erythromycin (2), lincomycin (3), and clindamycin (4) has been based chiefly upon tube dilution studies demonstrating little or no increase in minimum inhibiting concentration in serum as compared with broth. However, these measurements are subject to a large error when twofold dilutions are used and when the concentration of serum employed, often 50%, is far below that occurring in the blood. Other technical factors that may cause erroneous binding determinations include use of very high concentrations of antibiotic, far above those seen under *in vivo* circumstances, or the use of serum of different species which has been found to give binding results different for some drugs from those of human serum (1). A widely quoted ultrafiltration study said to demonstrate 18% binding of erythromycin by human serum was actually done with serum protein fractions rather than whole serum (12). A lincomycin dialysis study using 50% horse serum demonstrated no evidence of binding, while a similar study showing 20% binding did not specify the concentration of antibiotic used (3).

Recently reported high degrees of binding of erythromycin and erythromycin estolate carried out at antibiotic concentrations in the range usually found in patients and using the ultrafiltration technique revealed binding values of 81.4 and ≥95.7%, respectively (13). Other recent studies have also shown quite high binding figures for lincomycin (80–90%) (6) and clindamycin (83%) (7).

The presently reported studies, carried out in conjunction with the various controls described earlier, also indicate a high degree of serum protein binding for erythromycin and erythromycin propionate, which is the free base of erythromycin estolate. The difference in binding noted for the two erythromycins is apparently not solely due to the difference in molecular weights, since the percent binding for erythromycin base (mol. wt. 733.92) is about 20% less than that of erythromycin propionate (mol. wt. 789.96), while there is only a 7% difference in their molecular weights.

[8] Packard Tri-Carb model 305.

REFERENCES

(1) G. N. Rolinson, in "Recent Advances in Medical Microbiology," A. P. Waterson, Ed., Little, Brown, Boston, Mass., 1967, pp. 260, 272.

(2) T. M. Haight and M. Finland, *Proc. Soc. Exp. Biol. Med.*, **81**, 175(1952).

(3) "Lincomycin," W. E. Herrell, Ed., Modern Scientific Publications, Chicago, Ill., 1969, pp. 34, 49, 56.

(4) B. R. Meyers, K. Kaplan, and L. Weinstein, *Appl. Microbiol.*, **17**, 653(1969).

(5) W. Scholtan and W. Schmid, *Arzneim.-Forsch.*, **13**, 288 (1963).

(6) C. M. Kunin, *Ann. Intern. Med.*, **67**, 151(1967).

(7) "Cleocin® Compendium," Upjohn Co., Kalamazoo, Mich., 1970.

(8) J. V. Bennett and W. M. M. Kirby, *J. Lab. Clin. Med.*, **66**, 721(1965).

(9) J. V. Bennett, J. L. Brodie, E. J. Benner, and W. M. M. Kirby, *Appl. Microbiol.*, **14**, 170(1966).

(10) W. M. M. Kirby, L. S. Rosenfeld, and J. Brodie, *J. Amer.*

Med. Ass., **181**, 739(1962).
(11) C. M. Kunin, *Clin. Pharm. Ther.*, **7**, 166(1967).
(12) H. H. Zinneman, W. H. Hall, L. Hong, and U. S. Seal, *Antimicrob. Ag. Chemother.*, **1962**, 637(1963).
(13) R. G. Wiegand and A. H. C. Chun, *J. Pharm. Sci.*, **61**, 425 (1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 7, 1972, from the *Department of Medicine, University of Washington, Seattle, WA 98195*

Accepted for publication January 31, 1973.

Supported by Public Health Service Training Grant A100146-12 from the National Institute of Allergy and Infectious Diseases and by grants from the Eli Lilly and Upjohn Companies.

The assistance of Dr. Patrick Goldsworthy, Dr. Gottfried Schmer, Miss Pat McMartin, and Mrs. Rita Carpenter in carrying out this study is gratefully acknowledged.

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Pharmacological Evaluation of Medicinal Plants from Western Samoa

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Abstract □ A total of 110 plant parts from 34 different species of medicinal and toxic plants was collected for pharmacological testing in or near the Lefaga District on the island of Upolu in Western Samoa. Voucher specimens of all species were also made. From the 110 plant parts, 211 different extracts were prepared and evaluated for antiviral, antibacterial, and anti-Ehrlich ascites tumor properties and for effects on blood pressure, heart rate, respiration, electrocardiogram, electroencephalogram, and gastric motility. Twelve extracts were effective *in vitro* as antibacterial agents and six inhibited Ehrlich ascites tumor growth in mice. Ninety-one extracts showed hypotensive properties, 31 for more than 30 min.

Keyphrases □ Medicinal plants—pharmacological evaluation of 34 species from Western Samoa □ Antiviral activity—34 plants from Western Samoa screened □ Antitumor activity—34 plants from Western Samoa screened □ Antibacterial activity—34 plants from Western Samoa screened □ Antihypertensive activity—34 plants from Western Samoa screened

As part of a broad program of ethnobotanical and pharmacological studies of medicinal plants and other biota from the Pacific basin, medicinal and poisonous plants of Western Samoa were collected for pharmacological and chemical evaluation. The ethnobotanical phase was centered in Lefaga District on the island of Upolu. The flora was studied intensively, and a large collection of herbarium specimens was obtained. Ethnobotanical data concerning the names, usage, and special significance of most of the woody and some of the herbaceous flora were gathered for native and introduced plants. This information, which included a large body of medicinal lore, was gathered from many people in Lefaga District. No prominent practitioner of native medicine was located in the District, so the data obtained on medicinal plants are not those of one outstanding individual but rather reflect that body of information widely known to many Samoans. Extensive botanical collections were also made in the interior of Savaii island and to a lesser extent at many coastal points on both islands. Whenever possible, ethnobotanical data were gathered at the same time from local people.

The 34 plants chosen for inclusion in the pharmacological studies are native or naturalized species, many of which also occur on adjacent island groups. Most are of therapeutic or toxicological interest to the Samoans today and many are noted in the literature concerning Samoa. Three plants, *Ochrosia oppositifolia*, *Barringtonia samoensis*, and *Terminalia samoensis*, were included because of their close botanical relationship to plants known for their physiological activity. Herbarium voucher specimens were made for all the plants studied[1]. The plant materials analyzed were air dried in Samoa in a large rack of screen trays heated gently by kerosene lanterns. The material was sealed in polyethylene for air shipment to Honolulu, Hawaii.

A direct evaluation under clinical conditions of the use of the plants by the local Samoans was not attempted. However, it was felt that the probability of physiological and chemotherapeutic effects might be greater with plants selected by the local people for medicinal use than with plants selected at random. Whether this is so remains a moot point. No checks were carried out for possible potentiation corresponding to use of several plants in a given local remedy.

Each plant part from Western Samoa was powdered and extracted with methylene chloride and then 60% ethanol. In this manner, the extraction method gave two extracts, the methylene chloride extract containing the relatively nonpolar constituents and the aqueous ethanolic extracts containing the more polar constituents. All extracts were examined for antiviral, antitumor, and antibacterial activity as well as for systemic effects in the rat.

[1] They are deposited at the B. P. Bishop Museum, Honolulu, Hawaii; U. S. National Herbarium, Washington, D. C.; Gray Herbarium, Cambridge, England; Royal Botanic Gardens, Kew, England; and at several other herbaria.