TABLE I—RESULTS OF ASSAYS OF FERMENTATION SAMPLES

Fermentation Batch No.	Units of Penicillin Recovered from Chromatogram From 8 μl. of Broth: Pen. V	p-Hydroxy Pen. V	Total	10 mcg., Pen. V[a]	Control Assayed Directly, in Units	p-Hydroxy Pen. V, %
1	58.5	2.20	60.70	16.60	67.20	3.78
2	76.20	3.38	79.58	17.10	87.00	4.24
3	56.00	2.00	58.00	16.50	63.90	3.44
4	66.15	2.76	68.91	16.38	77.40	4.00
5	60.30	2.46	62.76	16.50	66.60	3.90

[a] 1 mcg. pen. V equals 1.695 unit.

After an hour's equilibration, start chromatography from outside by pushing to the bottom of the tank the rod on which the paper is suspended. Following development (up to 16 hr.), cut off the first strip containing 20 μl. of the extract, hold for a few sec. over a jet of steam, then hang for 10 min. in a closed jar containing concentrated ammonia. Spray the strip with starch-iodine solution, prepared by mixing 50 ml. of 1% soluble starch solution, 3 ml. glacial acetic acid, and 1 ml. 0.1 *N* iodine. The white spots on a blue background, which appear after a few min., indicate the position of the corresponding penicillins. A photograph of a chromatogram developed as a whole colorimetrically is shown as Fig. 1. The colored strip is used as a template for locating the penicillins on the remaining part of the chromatogram. Cut out the corresponding areas of the chromatogram (sample in duplicate and standard penicillin V), place in 1.9 cm. × 15.2 cm. test tubes and elute with a measured amount of 1% phosphate buffer pH 7.0. The amount of buffer used for elution per tube is 4 ml. for the *p*-hydroxyphenoxy methyl penicillin and 10 ml. for phenoxymethyl penicillin. Elution is achieved by shaking the stoppered test tubes for 0.5 hr. on a shaking machine. Transfer the same volume of original organic extract as was applied to the chromatogram (20 μl.) directly into a test tube and extract with 10 ml. of buffer to serve as a control. Assay biologically the penicillin content of the solutions as penicillin V and calculate the percentage of *p*-hydroxyphenoxy methyl penicillin.

RESULTS

The data for experiments performed on five samples taken from penicillin fermentation tanks are shown in Table I.

The completeness of extraction of penicillin V (99.82% recovery) under the described conditions was confirmed by biological assay.

The developed method permits the determination of the *p*-hydroxyphenoxy methyl penicillin in the fermented broth during all stages of the fermentation. It was also used in the consequent processes of penicillin extraction and purification and as a qualitative test in the final product.

REFERENCES

(1) de Flines, J., Waisvisz, J. M., Hoette, I., and Struyk, A. P., *Antibiot. Chemotherapy*, **7**, 497(1957).
(2) Vanderhaeghe, H., Parmentier, G., and Evrard, E., *Nature*, **200**, 891(1963).

Keyphrases

p-Hydroxyphenoxy methyl penicillin—analysis
Phenoxymethyl penicillin fermentations—*p*-hydroxyphenoxymethyl penicillin determination
Paper chromatography—separation
Biological analysis—penicillins

Antifertility Factors from Plants I. Preliminary Extraction and Screening

By CHARLES F. BARFKNECHT and HUNG CHIH PENG

Three plants were examined for possible antifertility effects. *Prunus emarginata* and *Lonicera ciliosa* were found to reduce litter production in mice, while *Lysichitum americanum* was not active. The initial extraction and screening procedures are described.

THE ETHNOBOTANICAL literature contains a report of the use of plant teas to affect the reproductive processes by Indians of the Northwest U. S. Specifically, the roots of *Lysichitum americanum*, the leaves of *Lonicera ciliosa*, and the wood of *Prunus emarginata* were used for antifertility and abortifacient effects (1). Generally the women drank the teas prepared from the ground plants, but some tribes made no distinction on the sex of the person. The report also failed to mention a dosage regimen, but apparently more than one dose was necessary to obtain the antifertility effect. It is the purpose of this study to verify the antifertility activity and determine the constituents responsible.

Cranston (2) studied the effect of *Lithospermum ruderale* extracts on mice. *Lithospermum* was used by Indians of the Southwest U. S. to affect reproduction. She prepared an ethanolic extract

Received August 25, 1967, from the Division of Medicinal Chemistry, College of Pharmacy, University of Iowa, Iowa City, IA 52240 and the College of Pharmacy, Idaho State University, Pocatello, ID 83201

Accepted for publication May 10, 1968.

This investigation was supported by a grant from the National Institute of Child Health and Human Development.

The authors wish to thank Dr. Robert B. Nelson, Idaho State University, for his assistance in designing the screening procedure and for his advice during the screening.

of the ground plant and fed a 20% extract diet to young adult males and females 10 days prior to and 10 days during cohabitation. The ethanolic extract, which was more potent than a simple aqueous extract, reduced litter production from 70% in the control group to 34% in the treated group. There was no effect on the number of young per litter or on the activity or physical appearance of the adult mice.

Another report of a natural feeding assay of antifertility activity was made by Dewald and co-workers (3). They studied the effectiveness of a synthetic compound in mice by combining it with the food and allowing the pregnancies to go full term. Activity was again expressed as a ratio of the litter production in the treated group *versus* litter production in the control group.

DISCUSSION

For the plants in this study a 50% aqueous ethanolic solution was found to be a more efficient and more effective extraction system than a hot aqueous decoction. The natural feeding method of assay outlined previously was used with the exception that the extract food combination was reformed into pieces resembling the normal food. Since mice are gnawing creatures, it was felt that this technique would encourage them to feed normally. No difference in average food intake was found for any group, thus endorsing the technique.

In the initial screening to determine the validity of the ethnobotanical report, the discovery of antifertility activity was more important than establishing either which sex was affected or what was the mechanism of action. Thus the extracts were fed to both sexes prior to and during cohabitation. The results of the screening are summarized in Table I. Two consecutive runs were made, separated by 1 month, using two different concentrations. The consecutive runs permitted the evaluation of the extracts' effects on virgin and nonvirgin mice, the determination of the relative effectiveness of the dosage levels, and the gathering of the maximum data from the mouse colony. The extracts did not appear to affect the behavior or activity of the mice. Those extracts which caused reduction of litter production did so without decreasing the number of young per litter or without causing visible birth defects.

Present studies are directed toward the isolation of the active antifertility factors from *Lonicera ciliosa* and *Prunus emarginata*. Preliminary work on *Lonicera* leaves indicates the presence of alkaloid and flavonoid components. References to the antifertility effect of both components are found in the literature (4, 5). Two alkaloids have been separated and detected by thin-layer chromatography. One of them has been isolated in sufficient quantities to begin structure elucidation studies. The results of these studies will be the subject of a future communication.

Table I—Antifertility Screening of Plant Extracts

	Run	No. Females	No. Litters	% Litters	Av. Dose Extract/ Day/Mouse, mg.
Control	1st	15	9	60	—
	2nd	18	11	60	—
Lysichitum, roots	1st	15	7	46	10
	2nd	15	8	56	20
Prunus, wood	1st	12	4	33	10
	2nd	12	4	33	20
Lonicera, leaves	1st	15	5	33	10
	2nd	15	4	27	20

EXPERIMENTAL

Collection—The plants were gathered in the vicinity of Corvallis, Ore., in late summer, 1966 and authenticated under the direction of LaRea D. Johnston, Oregon State University Herbarium, Corvallis, to whom the authors are extremely grateful. Voucher specimens are on deposit in the Oregon State University Herbarium and in the College of Pharmacy, University of Iowa.

Extraction—The air-dried plant parts, mentioned in the ethnobotanical report were ground in a Waring blender with 50% v/v aqueous ethanol (20 ml./g. of plant material). The mixture was macerated 3 days with occasional stirring, filtered through paper, and evaporated to dryness under reduced pressure at temperatures not exceeding 50°. By this procedure extracts of 13.0, 1.7, and 17.0% were obtained for *Lysichitum*, *Prunus*, and *Lonicera*, respectively. Preliminary experiments had shown this extraction procedure to be the best method.

Food-Extract Combination—Purina dog chow, the normal food, was ground in a mill. The dried extract was dissolved in a minimum of 50% ethanol, combined with the proper amount of ground dog chow to prepare a 1 or 2% extract diet, and mixed into a mass. The mass was cut into pieces and allowed to air-dry at temperatures not exceeding 40°.

Screening—White mice, 8–10 weeks old (Berkeley Pacific Laboratories, Berkeley, Calif.), were maintained on a diet of Purina dog chow and water for 3 weeks prior to screening. To begin the screening, separated males and females (four to a cage) were fed the dog chow extract diet for 2 weeks. The only exceptions were the mice which would constitute the control group, they received only dog chow throughout the entire screening period. The mice were cohabitated, one male and three females per cage, and fed the test diet an additional 3 weeks. After this period, the males were removed from the cages and the cages examined for litters daily during the next 4 weeks. After the last litter was born, a period of 4 weeks elapsed before the second run. The second run was conducted in an analogous manner. No attempt was made to maintain the mice in the same test group during both runs.

REFERENCES

(1) Gunther, E., *Univ. Wash. Publ. Anthropol.*, **10**, 1(1945).
(2) Cranston, E. M., *J. Pharmacol. Exptl. Therap.*, **83**, 130(1945).
(3) Dewald, H. A., Bird, O. D., Rodney, G., Kaump, D. H., and Black, M. L., *Nature*, **211**, 538(1966).
(4) deLaszlo, H., and Henshaw, P. S., *Science*, **119**, 626 (1954).
(5) Moersch, G. W., Morrow, D. F., and Neuklis, W. A., *J. Med. Chem.*, **10**, 154(1967).

Keyphrases

Antifertility factors—plant extracts
Prunus emarginata extract—mouse litter reduction
Lonicera ciliosa extract—mouse litter reduction