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Note

High-performance liquid chromatographic separation and quantitation of maytansinoids in *Maytenus ilicifolia*

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Maytenus ilicifolia Mart. *ex.* Reiss. (Celastraceae) is a large shrub found in Southern Brazil, Paraguay, Uruguay and Argentina[1–4]. It has been used in Argentinian folk medicine as a sialogogue, antiasthmatic, antiseptic and vulnerary[5], and as an indigenous antitumor remedy in Brazil[6]. The plant, known as Cangorosa, is used by Indian tribes and rural populations in Paraguay as a fertility regulating agent[7–11].

Confirmation of the use of this plant as a menses inducer by the indigenous population in Paraguay was obtained by interviews with a number of individuals in Ascuncion, Paraguay during a field visit in 1979. Among the people interviewed were "Yuyos" (herb women), waitresses, and housewives. The method of use is usually by boiling or steeping the plant material in water and drinking the decoction/infusion at the time of anticipated menses, or shortly after the absence of it is noted. The dose was determined to be approximately nine grams of plant material per day until menstruation begins.

Because of the known presence of maytansine and other antitumor and cytotoxic ansamacrolides in related *Maytenus* species[12–15], the present study was initiated to determine the maytansinoid content in an ethnomedical dose of *M. ilicifolia* tea in order to ascertain its potential adverse effects on the Paraguayan women who continue to use this plant as a fertility regulator.

EXPERIMENTAL

Plant material

Maytenus ilicifolia Mart. *ex.* Reiss was procured in Asuncion, Paraguay in April, 1980 and identified by one of the authors (D.D.S.). Voucher specimens are deposited at the John G. Searle Herbarium of the Field Museum of Natural History, Chicago, IL, U.S.A.

Apparatus

Liquid chromatographic separations were conducted with a Waters Assoc. (Milford, MA, U.S.A.) Model 6000A liquid chromatograph equipped with a

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Rheodyne (Berkeley, CA, U.S.A.) Model 7120 syringe-loading sample injector and 100-μl sample loop, a Waters Assoc. Model 450 variable-wavelength UV spectrophotometer, and a Beckman (Lincolnwood, IL, U.S.A.) 10-in. strip chart recorder. Separations were carried out with a Waters Assoc. 30 × 0.2 cm I.D. μPorasil column.

Chemicals

All chemicals and solvents used in this investigation were reagent grade. Solvents for high-performance liquid chromatography (HPLC) were redistilled in glass.

Reference maytansine, maytanprine, and maytanbutine were generously provided by Dr. D. E. Nettleton, Jr. (Bristol Labs, Syracuse, NY, U.S.A.).

Extraction and fractionation of M. ilicifolia

Decoctions (teas) were prepared from three samples (9.0 g each) of the leaf + twig and three samples (9.0 g each) of the stem + root parts by boiling each sample in 750 ml of distilled water for 5 min in a glass container. The decoctions were cooled, filtered and separately processed for total maytansinoids as illustrated in Fig. 1.

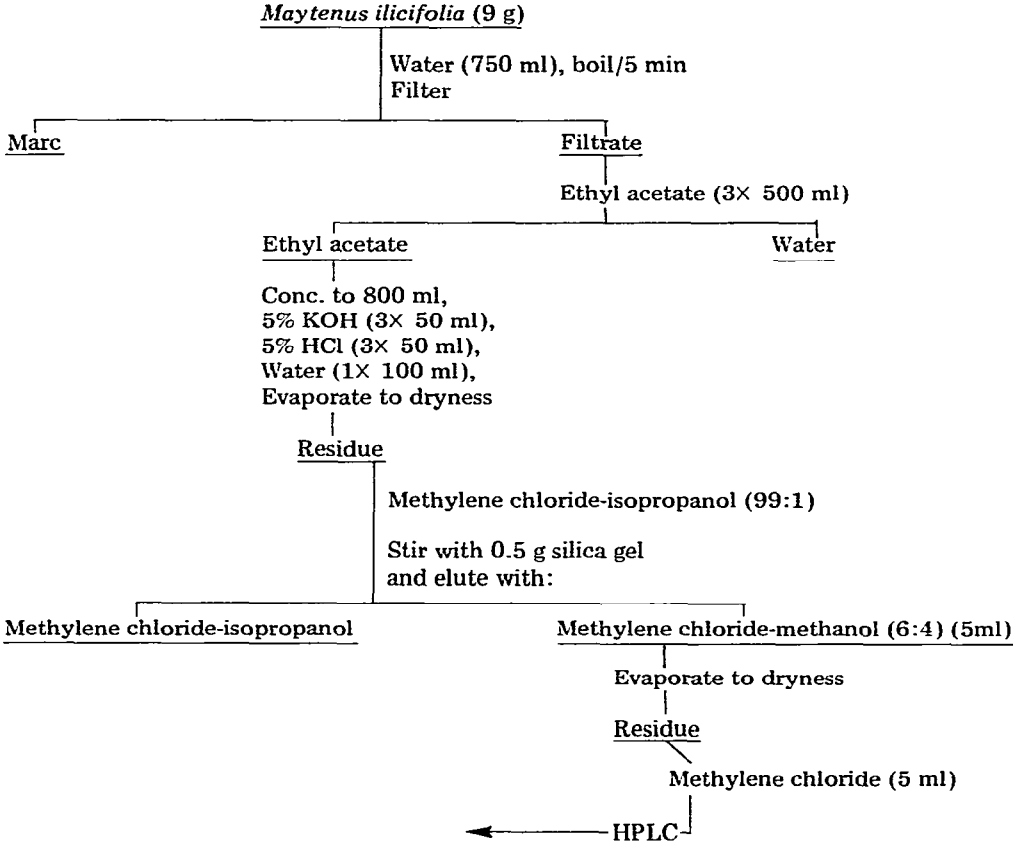
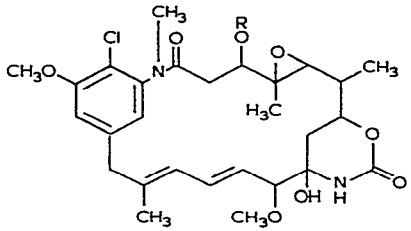


Fig. 1. Extration and purification of *Maytenus ilicifolia*.

RESULTS AND DISCUSSION

The variety and complexity of constituents present in the initial extracts of *M. ilicifolia* leaf + twig and stem + root precluded HPLC detection and quantitation of maytansinoids. Therefore, a purification procedure was employed prior to liquid chromatography. Separation of maytansine (I), maytanprine (II), and maytanbutine (III) was then achieved with methylene chloride–isopropanol–water (96:4:0.5) at a flow-rate of 1.0 ml/min and UV detection at 254 nm. The solvent system is that reported by Nettleton *et al.*[14]. Beer's law curves for I, II, and III showed a linear detection response for concentrations of 12.5 ng to 1 μg. The slopes, *y* axis (peak height) intercepts, and correlation coefficients were calculated by linear regression analyses and are shown in Table I, along with other maytansinoid-HPLC characteristics.



Maytansine (I) R=COCHCH$_3$NCH$_3$COCH$_3$
Maytanprine (II) R=COCHCH$_3$NCH$_3$CHCH$_2$CH$_3$
Maytanbutine (III) R=COCHCH$_3$NCH$_3$COCH(CH$_3$)2

TABLE I

HPLC CHARACTERISTICS OF MAYTANSINOIDS

Compound	*Retention time (min)*	*Slope*	*y axis intercept*	*Correlation coefficient*	*Minimum detectable concentration (ng)**
Maytansine	24.8	0.14	−1.89	0.998	5
Maytanprine	15.6	0.12	+0.91	0.999	5
Maytanbutine	12.0	0.21	−1.09	0.999	5

* UV detector, 254 nm; 0.01 a.u.f.s.

Extracts of leaf + twig, as well as of stem + root, showed satisfactory resolution of I, II and III from other plant constituents. Their presence in both extracts was confirmed by spiking with reference standards. Representative separations of standards, extracts and spiked extracts are shown in Fig. 2. Quantitation of the maytansinoid content of the three leaf + twig samples showed an average total maytansinoid concentration of 5.331 μg (2.162 μg maytansine + 2.377 μg maytanprine + 0.792 μg maytanbutine) per dose of 9.0 g plant material. The average maytansinoid content in the stem + root decoctions was found to be 6.981 μg (2.360 μg maytansine + 2.343 μg maytanprine + 2.278 μg maytanbutine) per dose. In view

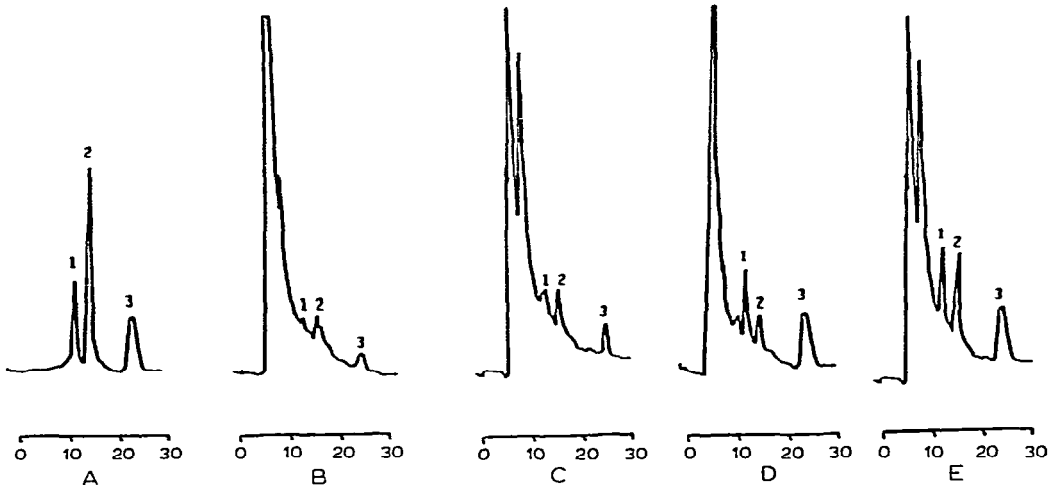


Fig. 2. Chromatograms of (A) mixture of maytansinoids, (B) *Maytenus ilicifolia* leaf + twig extract, (C) *Maytenus ilicifolia* stem + root extract, (D) *Maytenus ilicifolia* leaf + twig extract spiked with maytansinoids, (E) *Maytenus ilicifolia* stem + root extract spiked with maytansinoids. Peaks: 1 = maytanbutine; 2 = maytanprine; 3 = maytansine. Conditions: column, μPorasil (30 × 0.2 cm); solvent, methylene chloride–isopropanol–water (96:4:0.5); flow-rate, 1 ml/min; detector, UV at 254 nm; chart speed, 4 in./60 min.

of the fact that no adverse effects were seen in normal mice given dailly oral doses of 0.5 to 128 μg/kg/9 days in a National Cancer Institute study[16], the low concentration of maytansinoids in the daily dose of Cangorosa tea would probably not cause any adverse effect in the Paraguayan women taking it as a fertility regulator.

Due to the low concentrations of maytansine (0.000001 to 0.00025%)[15] in *Maytenus* species, quantitative analysis of ansamacrolides in less than 1 kg of plant material has not been attempted prior to this study. Thus, the present detection and quantitation of maytansinoids from 9.0-g samples represents the first effort in the quantitative analysis of ansamacrolides from very small samples of plant material.

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