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FIELD TEST FOR SCREENING MILKWEED LATEX FOR CARDENOLIDES

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ABSTRACT.—A simple test was devised to screen for cardenolides in latex of milkweed. The test is based on a documented spectrophotometric assay for cardenolides in plants. During field collections for a chemotaxonomic study of milkweeds endemic to the intermountain U.S. region, the test qualitatively facilitated on-site screening of potential specimens.

Many genera of the milkweed family Asclepiadaceae, comprising some 2500 species, examined thus far appear to contain cardioactive chemicals known as cardenolides (1). The cardenolide content of species in the genus *Asclepias* has been relatively well characterized (2–5). Most *Asclepias* species examined contain a wide variety of cardenolides which vary in concentration and type between species (6) and between leaves, stems, roots, seeds, and latex within a given species (7). Latices of different *Asclepias* species can vary in cardenolide concentration from quite low in *Asclepias californica* Greene (<0.06%) to quite high (>40%) in *Asclepias curassavica* L. (4). Within the visual detection limits of the new colorimetric test described in this study, the latex of *Asclepias speciosa* Torr., in spite of having one of the lowest reported values for latex cardenolide content (4), consistently tested positive on numerous field samples over a broad geographic distribution of the intermountain region. The test may have potential application in the field for the phytochemist, range manager, and pharmacognosist interested in screening the relative potency of milkweeds and other cardenolide-containing plant families like the Apocynaceae (e.g., *Nerium oleander* L.).

The field test was performed as a modificaiton of the spectroassay formulated on the base-catalyzed reaction of cardenolide with 2,2′,4,4′-tetranitrodiphenyl (TNDP) that produces a charge transfer complex with an anion on the butenolide portion of the cardenolide (8). A strip of Whatman No. 1 filter paper impregnated with the TNDP was used to test a drop of fresh latex on a plant specimen in the field. After a drop of 10% aqueous NaOH solution was added to the strip the appearance of a blue color was the determining factor for a positive reaction. Table 1 shows the field testing of various

TABLE 1. Results on the Use of Field Test for Detecting Cardenolides in Various Latices, with the *Asclepias* Species Listed as Enumerated by A. Cronquist *et al.* (11).

Specimen	Results[a]
1 *Asclepias incarnata* L.	+++[b]
2 *Asclepias fascicularis* Decne.	−
6 *Asclepias speciosa* Torr.	+
7 *Asclepias macrosperma* Eastw.	−
11 *Asclepias labriformis* M.E. Jones	+
12 *Asclepias ruthiae* Maguire	−
13 *Asclepias eastwoodiana* Barneby	+15+
16 *Asclepias cordifolia* (Benth.) Jepson	+++
Asclepias cryptoceras S. Wats.	+
Nerium oleander L.	+++
Tragopogon dubius Scop.	−
Taraxacum officinale Weber	−
Euphorbia pulcherrima Willd. ex Klotzsch	−
calotropin	+++[c]
digitoxin	+++[c]

[a]Relative intensity of color +++>++>+. No color indicated by −. Each test was performed on a minimum of 3 different plants within a species.

[b]The latex of *A. incarnata* darkened rapidly when collected and gave an intense red color with the field test.

[c]From solutions in $CHCl_3$, these standard cardenolides were tested at a concentration of ca. 1 mg/ml.

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latices of *Asclepias* species and other genera from different plant families.

The results in Table 1 compare favorably with published tlc data from this laboratory (4) for relative cardenolide abundance in the latex: *Asclepias cordifolia* (Benth.) Jepson ⩾*A. speciosa*> *Asclepias fascicularis* Decne. In that study, which employed TNDP in a laboratory spectroassay, quantitative analysis of the total cardenolide content demonstrated that neither the latex nor the leaf of *A. fascicularis* had cardenolide above the detection limit of 0.57 mg of digitoxin equivalents per gram of dry material. By contrast, a mean value of 4.68 mg/g was reported for whole latex of *A. cordifolia* (4), a species which tested strongly positive in the field. Specificity is demonstrated by a strongly positive test with calotropin and digitoxin standards, the former commonly found in the latex of numerous milkweed species.

The fresh latex of oleander (*N. oleander*), which has a reported mean cardenolide content for leaves and stems of 3.85 mg/g (9), tested as strongly positive in color intensity as that of *A. cordifolia* for its latex (see Table 1). Specimens like the common dandelion (*Taraxacum officinale* Weber) and yellow salsify (*Tragopogon dubius* Scop.), which are both lactiferous plants from the family Asteraceae that overlap in habitat with most milkweeds, tested negative for cardenolides. Poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) also tested negatively for cardenolides in its latex. Though the negative results were predicted for these latter three plants, it should be emphasized that the test appears to only screen latex for cardenolide at the concentration (>0.057%) limits of detection cited above for *A. fascicularis*.

There is a fair amount of variation in cardenolide content between the intermountain species of *Asclepias* tested in Table 1. As part of a chemotaxonomic study we are particularly interested in comparing any differences in the cardenolide content between two morphologically similar, but taxonomically contested, species of *Asclepias*: *Asclepias eastwoodiana* Barneby and *Asclepias ruthiae* Maguire. At least one author considers these two to be one and the same, *A. ruthiae* (10). Others consider them to constitute distinct taxa (11). In our laboratory we have found that TNDP can cross-react with some non-cardenolide ketones that give blue- to red-colored residues on the test strips. The deep red color noted when testing the latex of *Asclepias incarnata* L. (Table 1) may be the result of pregnane steroids, according to an observation made by other *Asclepias* investigators (12). Whether the apparent lack of cardenolide or other TNDP-positive compounds in the latex of *A. ruthiae* truly indicates a chemotaxonomic difference when compared with the central Great Basin endemic *A. eastwoodiana* or is but a manifestation of phytogeographic or edaphic factors is a question under current study and requires more sampling and selective analytical methods to measure chemical differences precisely.

EXPERIMENTAL

Because it is not commercially available, TNDP was prepared by a modification of a procedure based on the nitration of 2,2′-dinitrodiphenyl (8) and stored under refrigeration until use. A saturated solution of the TNDP was made by mixing ca. 100 mg of TNDP in 50 ml of 95% EtOH. The saturated solution of TNDP should be prepared fresh each day of use. Strips of Whatman No. 1 filter paper were cut from a larger piece about 1.5 cm wide and 10 cm long. The bottom 1 cm of a strip was soaked for ca. 3–5 sec in the above TNDP/EtOH solution and allowed to dry. The strips were stored at room temperature in a plastic vial or bag.

To conduct the test a drop (ca. 5 μl) of latex is placed on the strip end containing the TNDP, followed by a drop of 10% aqueous NaOH solution. The appearance in 1–2 min of a blue color indicates a positive test. The color varies in intensity with the total cardenolide concentration at the time of the test. In the field a drop of fresh latex can be placed on the end of the test strip, allowed to dry, and stored for up to 6 h before adding the 10% aqueous NaOH solution with no apparent loss in color intensity. Neither the temper-

ature of the reaction conditions nor the presence or absence of sunlight appears to have any effects on the color intensity in various tests recorded in the field. TNDP is notoriously unstable in solution for any length of time and normally has to be prepared fresh when performing spectroassay work. But the apparent stability for 2 months on dry impregnated strips suggests that the Whatman filter paper, and perhaps other stationary media of similar chemistry, may stabilize the TNDP for longer periods. This latter attribute has been exploited here to make this a test that can be conducted on specimens in the field over long periods of time during the summer months when collections take place.

The latex was obtained in the field by breaking a leaf away from the stem of a plant, which caused the flow of sufficient material to test directly or to collect and store at $-20°$ for further study. *Asclepias* species used in this study were tested and collected in June and July 1990, from various locations in Nevada and Utah. Identifications of some of the specimens were made by June McCaskill based on vouchers DAV 111646 (*A. speciosa*), DAV 111640, 111642, 111643 (*A. cordifolia*), DAV 111644 (*A. fascicularis*), DAV 111641 (*Asclepias cryptoceras* S. Wats.) and DAV 111645 (*A. eastwoodiana*) deposited at the Botany Department Herbarium, University of California, Davis. The remaining specimens were identified by Leila M. Shultz based on vouchers UTC 206555 (*Asclepias macrosperma* Eastw.), UTC 206512 (*A. ruthiae*), UTC 206510 (*Asclepias labriformis* M.E. Jones), UTC 206511 (*A. incarnata*), and UTC 206513 (*A. eastwoodiana*) deposited at the Intermountain Herbarium, Utah State University, Logan.

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