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Note

Separation and identification of stereoisomers of sesquiterpene lactones by multiple development of thin-layer chromatograms

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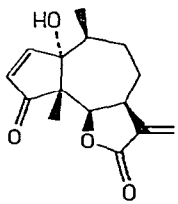
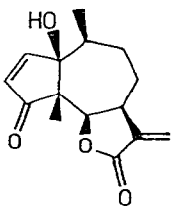
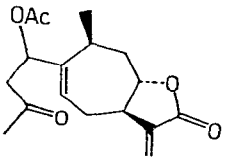
Because diastereoisomers have different physical properties, including different solubilities in a given solvent, they are often separated chromatographically[1]. However, thin-layer chromatography (TLC) techniques with various solvent systems which have been generally used for separation of sesquiterpene lactones[2] are inadequate for the separation of their stereoisomers. For example, two naturally occurring diastereoisomers, parthenin and hymenin (I, II), give identical R_F values on TLC using silica gel in various solvent systems even with heat-activated plates or plates impregnated with $AgNO_3$. In addition, they cannot be distinguished under UV light, using iodine vapours, $KMnO_4$ spray or vanillin or *p*-dimethylaminobenzaldehyde spray reagents[3].



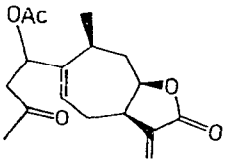
I, PARTHENIN



II, HYMENIN



III, XANTHININ



IV, XANTHUMIN

A better separation of similar compounds can be sometimes achieved through the multiple development of TLC plates in the same solvent system[4]. We report here the use of the multiple-development TLC technique for the separation of parthenin and hymenin. This method appears to be equally suitable for the separation of other isomeric sesquiterpene lactones.

EXPERIMENTAL

Methods

Silica gel plates, without gypsum and with fluorescent indicator (Polygram;

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Brinkmann, Westbury, NY, U.S.A.) were used. Strips of silica gel plate (12 cm) with parthenin and hymenin or xanthinin(III) and xanthumin(IV) applied to the same spot (approximately 1 μg of each compound) were developed 1–10 × in a standard chamber with heptane–diethyl ether–ethyl acetate (30:65:5), the plates being air dried in between runs. The compounds were visualized with the vanillin spray reagent[3]. Parthenin and hymenin give a bluish green color, xanthinin brownish red, and xanthumin reddish brown. Under these conditions parthenin moves ahead of hymenin and xanthinin ahead of xanthumin.

To determine which of the two stereoisomers, parthenin or hymenin, is present in crude extracts of *Parthenium hysterophorus*, two 20 × 20 cm plates were used. One plate was spotted (in one corner) with parthenin, the other one with hymenin, and to these spots the crude plant extract was added. The TLC plates were developed in chloroform–acetone (6:1) to separate parthenin (or hymenin) from other extract constituents, air dried, and then developed in a second direction in the above solvent system ten times. The presence of parthenin in an extract was indicated by two spots on a plate spotted with hymenin but only one spot on a plate spotted with parthenin. On the other hand, the presence of hymenin was indicated by two spots on a plate spotted with parthenin.

Materials

Parthenin and hymenin were isolated from *P. hysterophorus* collected in Texas (U.S.A.) and Argentina, respectively. The samples of xanthinin were provided by the late T. A. Geissman (University of California, Los Angeles, CA, U.S.A.) and T. J. Mabry (University of Texas, Austin, TX, U.S.A.). Xanthumin was a gift of E. Rodriguez (University of California, Irvine, CA, U.S.A.).

RESULTS AND DISCUSSION

The multiple development of TLC plates in the same solvent system facilitates the separation of stereoisomers which after a single development of plates move as one spot, the technique taking advantage of the slightly different solubilities of otherwise very similar compounds in a given solvent system. To achieve a separation the solvent system must be one in which the compounds move very slowly on the chromatographic plates.

In case of both mixtures (parthenin and hymenin, xanthinin and xanthumin) 1–6 developments of the TLC plates did not sufficiently separate the stereoisomers (Fig. 1). To separate parthenin and hymenin or xanthinin and xanthumin clearly it is necessary to develop the TLC plates 8 × or 10 ×, respectively (Fig. 1).

The method described proved to be useful in a comparative study of the sesquiterpene lactones of *P. hysterophorus* (*Compositae*) collected from various populations throughout its range of distribution. In general, North and Central American plants as well as Indian specimens were found to contain parthenin (I), while South American samples had hymenin (II) as the major sesquiterpene lactone[5]. Parthenin is responsible for an epidemic of allergic contact dermatitis in India[6] which is stereospecific[7]. Both stereoisomers were never found together in a single plant.

This technique, however, also makes it possible to identify stereoisomers of sesquiterpene lactones in cases where they are present in the same sample. "Xan-

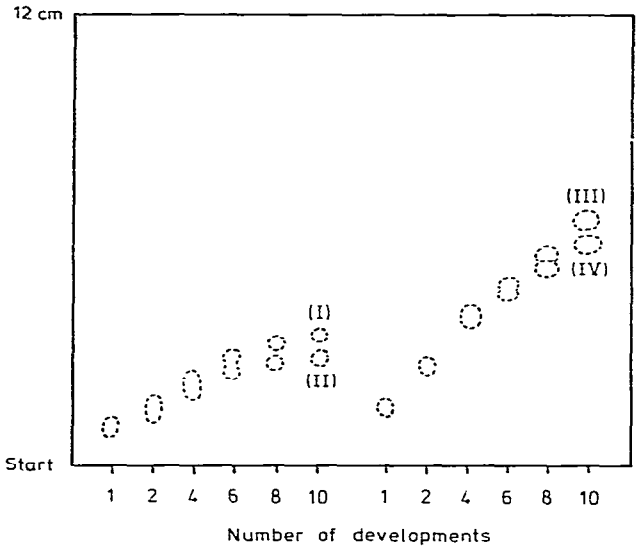

Fig. 1. Separation of parthenin (I) and hymenin (II), and xanthinin (III) and xanthumin (IV) by multiple-development technique.

thinin" which was available in our laboratory proved to be a mixture of authentic samples of stereoisomers, xanthinin(III) and xanthumin(IV). The shades of colors formed with the vanillin spray reagent are slightly different for these compounds. Both, xanthinin and xanthumin, co-occur in *Xanthium strumarium* (*Compositae*). The species is separated into the "italicum" morphological complex with the predominant sesquiterpene lactone being xanthinin and the "chinese" complex with the major compound being xanthumin. Some hybrids between these two complexes contain both stereoisomers[8]. Our TLC method could be successfully used in such comparative chemical studies. In addition, this technique is particularly useful when only small samples of plants are available for analyses and when other techniques such as nuclear magnetic resonance spectroscopy which may require the isolation and purification of a compound, cannot be successfully applied.

ACKNOWLEDGEMENTS

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