Journal of Chromatography, 258 (1983) 297–301
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,546

Note

Determination of grandiflorenic acid in organic and aqueous extracts of *Montanoa tomentosa* (zoapatle) by reversed-phase high-performance liquid chromatography*

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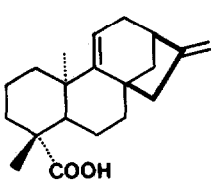
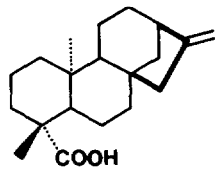
(First received September 6th, 1982; revised manuscript received November 20th, 1982)

Montanoa tomentosa Cerv. (popularly known as "zoapatle") is a shrub widely distributed in Mexico that has been known since prehispanic times for its reputation in facilitating labour and its potential as a terminator of pregnancy[1–4].

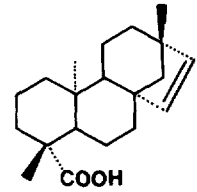
A chemical–biological examination of a hexane extract of zoapatle leaves was recently undertaken as it possesses uterotonic effect *in vivo* and *in vitro* animal studies. Fractionation of this extract by column chromatography and *in vitro* pharmacological assay allowed a correlation to be shown between those fractions containing grandiflorenic acid (GA) (I) and an uteroconstrictive biological effect[5]. As with a hexane extract of the roots[6], GA is found to be associated in the corresponding extract of leaves with other closely related diterpenic acids, *e.g.*, kaurenoic (KA) (II) and monoginoic (MA) (III). The latter are under pharmacological investigation since they also possess uterotropic effect[7].



I



II



III

A pharmacological assay with pure GA made it apparent that this compound was responsible for at least a part of the biological activity observed with the crude extract. In popular practice, zoapatle is used as an aqueous extract (decoction) of leaves, which is administered orally. On the other hand, gastric absorption of GA has already been demonstrated[8]. Therefore, it became necessary to determine the contents of GA in the aqueous extract of leaves and a reversed-phase high-performance liquid chromatographic (HPLC) method was developed for this purpose.

* Taken in part from the MSc Theses of L.I.E. and M.L.R.

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EXPERIMENTAL

The HPLC instrument was a Varian Model 5000 equipped with a Rheodyne Model 7125 injector (20-μl sample loop) and a Model UV-50 variable-wavelength UV detector operated at 220 nm connected to a Vista 401 CDS printer. A stainless-steel column (30 × 0.4 cm I.D.) packed with octadecylsilane bonded to 10 μm silica (Micropak MCH-10 from Varian) was used with a mobile phase of acetonitrile–water (80:20) at a flow-rate of 1.0 ml/min, giving a pressure drop of 78 atm.

All solvents were of HPLC grade from Merck (Darmstadt, G.F.R.), filtered through Millipore membranes (1.0 μm) before use. For determinations, extracts of roots, stems and leaves were prepared by exhaustic maceration in diethyl ether at room temperature of 2 g of the dried, ground material. Pure GA was obtained from a separate hexane extract of leaves.

A 10-g amount was chromatographed on a column (60 × 4 cm I.D.) of deactivated silica gel (5% water), isocratically eluted with hexane–ethyl acetate (95:5). Elution of GA, which occurred simultaneously with both KA and MA, was monitored by thin-layer chromatography (TLC) on silica gel plates impregnated with 10% silver nitrate solution[9,10]. From the collected GA-enriched fractions, the pure compound crystallized out from hexane on standing at 0°C. Recrystallization yielded 225 mg of I, which was further characterized by its m.p. (157°C), ^{1}H NMR spectrum[11] and mass spectrum[6], which were identical with previously reported spectroscopic data.

The aqueous extract was prepared by boiling 3 g of dried, powdered leaves in 30 ml of distilled water for 15 min, then filtering the solution through Whatman No. 2 filter-paper. To 10 ml of the filtrate was added an equal volume of methanol. The insoluble material formed was filtered off and washed with methanol. The organic solvent was removed *in vacuo* from this aqueous–methanolic solution, followed by extracion (three times) with 50-ml portions of diethyl ether. The organic layers were dried over anhydrous sodium sulphate and evaporated to dryness *in vacuo* and the residue was used for sample preparation. The organic extracts of leaves, roots and stems were prepared for determinations by dissolving an appropriate amount of the corresponding residue in acetonitrile and filtering through a 1.0-μm Millipore mem-

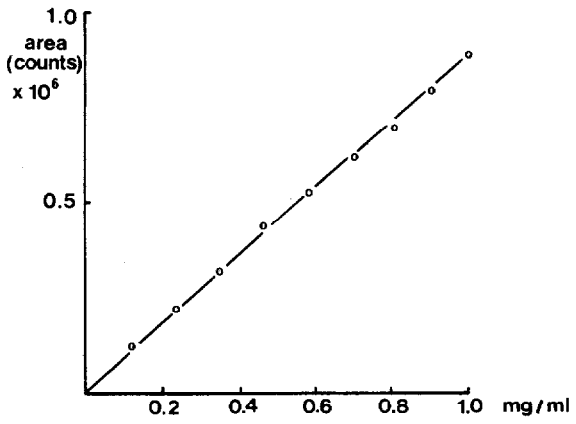

Fig. 1. Calibration graph for grandiflorenic acid.

TABLE I

GRANDIFLORENIC ACID CONTENTS OF EXTRACTS OF *MONTANOA TOMENTOSA*

Plant material	*Solvent*	*GA content**
Roots	Diethyl ether	71.9 ± 3.81 mg/g
Stems	Diethyl ether	52.1 ± 2.76 mg/g
Leaves	Diethyl ether	43.6 ± 2.31 mg/g
Leaves	Water	0.19 ± 0.01 mg/ml

* Given as $\bar{x} \pm \frac{ts}{\sqrt{n}}$ ($n = 4$, $\alpha = 5\%$).

brane filter. For quantification, a single standard solution of GA (2.34 mg/ml) was used to construct a calibration graph of area *versus* concentration using a maximum injection volume of 10 μl (Fig. 1).

Vegetable specimens were cultivated in the Experimental Field Station of the Instituto Nacional de Investigaciones Agrícolas (SARH)[12].

RESULTS AND DISCUSSION

The method described here allowed a rapid and reproducible determination of GA in both the organic and aqueous extracts studied, the latter being of importance as many plant extracts in popular use are administered in the form of infusions. GA contents are summarized in Table I.

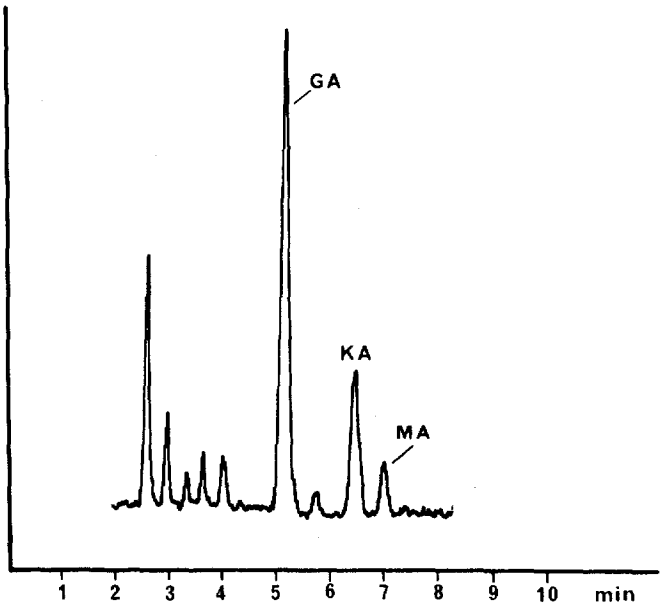

Fig. 2. HPLC profile of GA-enriched fraction from column chromatography of a hexane extract of leaves. Conditions as in Fig. 3.

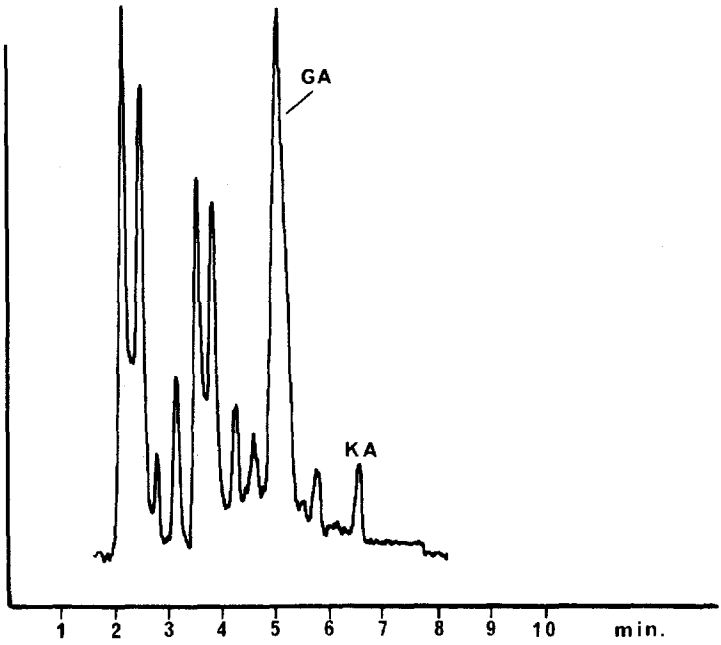

Fig. 3. Reversed-phase separation of diethyl ether soluble substances from an aqueous extract of leaves. Column 30 × 0.4 cm I.D., MCH-10; mobile phase 80% acetonitrile–water; temperature, 50°C; flow-rate, 1 ml/min; detection UV (220 nm); attenuation was changed from 1024 to 64 at 3.3 min; 0.02 a.u.f.s.

After crystallization, the residue containing the mixture of I, II and III showed very poor resolution in silica gel TLC with various solvent systems, although it was resolved by using silica gel TLC plates pre-coated with silver nitrate, which affected mainly the R_F value of MA (0.5–0.2 in hexane–ethyl acetate, 90:10). Additionally, in the reversed-phase HPLC examination of the residue a good separation of these compounds was achieved (Fig. 2), although under the conditions used MA was not detected in the chromatogram of the compounds obtained from the aqueous extract (Fig. 3). The method had a detection limit of 60 ng with a signal-to-noise ratio of 4 and the extraction efficiency ranged from 94 to 102%. In addition, this procedure may be adequate for the study of the diterpenic acids content in the plant during its growth cycle.

ACKNOWLEDGEMENTS

We are grateful to Professor F. Walls of the Instituto de Química, UNAM, who kindly provided us with a sample of monoginoic acid, and to Professor F. Bohlmann for providing us with a sample of kaurenoic acid. Partial financial support from PSPA (UNAM) to L.I.E. and M.L.R. is also acknowledged. Botanic assessment throughout this work was provided by A. Estrada and A. Aguilar.

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