Screening Tests for Phorbic and Piscidic Acids in Plants by Thin-Layer Chromatography

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The following investigation is based upon an earlier described method.[1] According to the method the acids were isolated by means of ionic exchangers, and chromatographed on cellulose plates (Macherey-Nagel MN 300).

Isolation of the acids. For the present investigation the acids were isolated in the following way, which is a simpler and less time-consuming method than the one described earlier. 5 g of fresh plant material were crushed or pulverized. Concentrated hydrochloric acid was added (pH 1), and the mixture shaken with diethyl ether. The ether layer was filtered off, evaporated, and the residue dissolved in acetone (1 ml), and chromatographed.

Chromatograms of the acid mixtures isolated according to the two methods showed sometimes minor differences. This is probably due to a destruction of labile acids (*i.e.* L-ascorbic acid) by the basic solutions used in the first method.

Chromatography of the acids. As shown in the previous paper,[1] the *piscidic acid* could easily be detected in the extracts, using 1-pentanol-formic acid (98 %)-water (50:50:2.5) as a solvent. In the cases where one acid made up the major part of the acid mixture, a multiple development [2] gave a satisfactory result (*cf.* Fig. 1).

Because of the special problems connected with the *phorbic acid dilactone*, a two-dimensional chromatographic method was developed, using SRS-technique.[3] The chromatograms were first developed in the above mentioned solvent. Then the plates were dried for 1 h at 110°C to lactonize the possible openchained acids, and after cooling, chromatographed in the second direction in the same solvent (*cf.* Fig. 2). As a second solvent, also

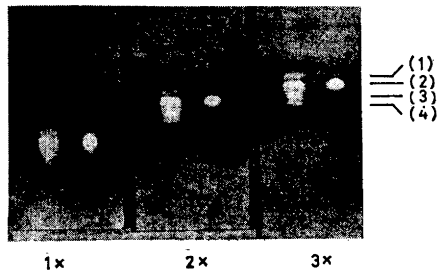

Fig. 1. Detection of piscidic acid in *Agave americana* L. by multiple development. 1× shows a chromatogram which was developed one time and then sprayed for acids. 2× shows a corresponding chromatogram which was heated for 10 min at 110°C and rechromatographed. 3× shows a chromatogram corresponding to 2× heated for 10 min at 110°C and chromatographed for a third time. Solvent: 1-pentanol-formic acid-water (50:50:2.5). Length of run 10 cm. Sorbent layer: Cellulose Macherey-Nagel MN 300. Spray reagent: Bromophenol blue, 0.1 % in methanol. Acids: (1) Malic acid, (2) piscidic acid, (3) citric acid,[4] (4) ascorbic acid.[5]

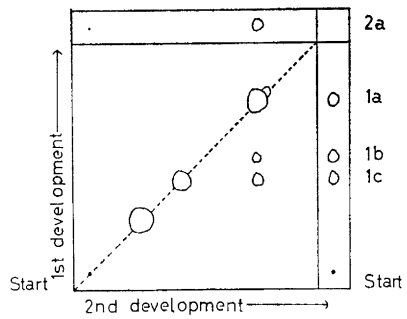

Fig. 2. Two-dimensional thin-layer chromatogram of acids from *Euphorbium*.

After development in the first direction, the plate was heated 1 h at 110°C, cooled, and developed in the second direction using the same solvent.
1a, 2a: Phorbic acid dilactone. 1b: Phorbic acid monolactone. 1c: Phorbic acid (open chain). Solvent: 1-Pentanol-formic acid (98 %)-water (50:50:2.5). Sorbent layer: Cellulose Macherey-Nagel MN 300. Spray reagent: Bromophenol blue, 0.1 % in methanol.

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Table 1. The distribution of phorbic and piscidic acid in the listed plants.

Plant family	Name of plant	Part	Phorbic acid	Piscidic acid
Liliaceae	*Aloe zèbrina* Bater Engl.		−	+
	Agave americana L.		−	+
	Narcissus poeticus L.	bulbs	−	+
	Galanthus nivalis L.	bulbs	−	+
Portulacaceae	*Portulaca oleracea* L.		−	−
Mesembryanthemaceae	*Glottiphyllum linguieforme* L.		−	−
Cactaceae	*Cereus peruvianus* Mill.		−	+
Crassulaceae	*Crassula arborescens* Mill.	−	−	−
	Crassula falcata Wendl.		−	−
Compositae	*Kleinia repens* Haw.		−	−
Euphorbiaceae	*Euphorbium*	latex	+	−
Aristolochiaceae	*Aristolochia clematitis* L.	leaves	−	−
	Aristolochia elegans Mast.	leaves	−	−
	Aristolochia brasiliensis Mart. et Zucc.	leaves	−	−
	Asarum canadense L.	roots	−	−
	Asarum europaeum L.	leaves	−	−
		roots	−	−

toluene-ethyl formate-formic acid (95 %) (50:40:10) was used. This gives a better separation of the non-polar substances than the pentanol-formic acid-water solvent.

From Fig. 2 it will be noticed, that phorbic acid monolactone and phorbic acid gave the same R_F-values as the phorbic acid dilactone. This is due to the heating process, which converts the open chain compound and the monolactone into the dilactone.

Detection of the acids. As formerly, bromophenol blue 0.1 % in methanol was used for the detection of the acids and in addition the hydroxamic acid reaction was used for detection of the lactones. Fast blue salt B was used for detection of piscidic acid, which also is visible as a dark spot in short-wave UV-light. The plates had to be dried 1 h at 110°C before the applying of the hydroxamate test.

The result of the distribution of piscidic and phorbic acids in the plants is listed in Table 1. The piscidic acid was mainly restricted to the family Liliaceae, while the phorbic acid was found only in the family of Euphorbiaceae.

Acknowledgement. I am grateful to Professor Dr. Egon Stahl, Institute of Pharmacognosy and Analytical Phytochemistry, University of the Saar, who supplied the reagents and equipment for this investigation. I also wish to thank Professor Dr. Arnold Nordal, Institute of Pharmacy, University of Oslo, for his interest and encouragement extended to me in connection with this work.

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Received August 14, 1969.