

Further Observations on the Occurrence of Phorbic Acid in Plants

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Screening tests for phorbic acid were carried out with plant material originating from the following families and species: *Euphorbiaceae* (*Euphorbia palustris* L., *E. pilulifera* L., *E. pulcherrima* Willd., *E. splendens* Boj., *Croton eluteria* Benn., *Hevea brasiliensis* Müll. Arg., *Mallotus philippinensis* Müll. Arg., *Ricinus communis* Müll. Arg.); *Cactaceae* (*Cereus peruvianus* (L.) Mill., *Opuntia ficus-indica* Mill.); *Compositae* (*Kleinia repens* (L.) Haw.); *Portulacaceae* (*Portulaca oleracea* L.) The bark of *Croton eluteria* Benn. and the preserved latex of *Hevea brasiliensis* Müll. Arg. were used. In all other cases the leaves or chlorophyllcontaining stems served as starting materials. The acids were isolated over their lead salts and converted into ethyl esters, which in turn were investigated by means of gas chromatography in direct comparison with phorbic acid ethyl ester. In this way phorbic acid was detected in seven of the twelve investigated species. The acid was lacking or its presence found doubtful in the investigated samples of *Croton eluteria* Benn., *Hevea brasiliensis* Müll. Arg., *Ricinus communis* Müll. Arg., *Opuntia ficus-indica* Mill., and *Portulaca oleracea* L.

The object of this investigation was to extend our present knowledge on the distribution of phorbic acid within the vegetable kingdom. In a previous paper in this series,¹ phorbic acid was detected by means of the melting point and IR spectrum of its ethyl ester. During the present investigation comparative gas chromatography of phorbic acid ethyl ester and ethyl esters prepared from acid mixtures of the plants in question, served as basis for the identification of phorbic acid.

For the screening tests we used leaves and stems, except for *Croton eluteria* Benn., of which we used the bark (in pharmacy known as *Cortex cascarillae*), and for *Hevea brasiliensis* Müll. Arg., where we used the latex.

The non-volatile acids were isolated over their lead salts, and the mixtures of their ethyl esters investigated by means of gas chromatography in direct comparison with phorbic acid ethyl ester.² Altogether plant material from 12 different species, belonging to four different plant families was investigated.

Table 1. Main results of the screening tests. The acids are listed (from left to right) according to the relative retention times of their ethyl esters.

Plant family	Name of plant	Malonic acid	Succinic acid	Malic acid	Tartaric acid	Citric acid	Phorbic acid
Euphorbiaceae	<i>Euphorbia palustris</i> L.	+	+	+	+	+	+
	„ <i>pilulifera</i> L.	+ (?)	+	+	+ (?)	+	+
	„ <i>pulcherrima</i> Willd.	- (?)	+	+	+ (?)	+	+
	„ <i>splendens</i> Boj.	- (?)	+	+	+ (?)	+	+
	<i>Croton eluteria</i> Benn.	+	+	+ (?)	+	+	-
	<i>Hevea brasiliensis</i> Müll. Arg.	+ (?)	+	+	- (?)	+	-
	<i>Mallotus philippinensis</i> Müll. Arg.	+	+	+	+	+	+
	<i>Ricinius communis</i> L.	+	+	+	- (?)	+	-
Cactaceae	<i>Cereus peruvianus</i> (L.) Mill.	-	+	+	-	+	+
	<i>Opuntia ficus-indica</i> Mill.	+	+	+	-	+	-
Compositae	<i>Kleinia repens</i> (L.) Haw.	+ (?)	+	+	-	+	+
Portulacaceae	<i>Portulaca oleracea</i> L.	+	+	+	-	+	-

The screening tests, of which the main results are shown in Table 1, indicate that phorbic acid is widely distributed within the plant family *Euphorbiaceae*, and that the same acid is not confined to this plant family alone. From a biochemical and plant taxonomical point of view, the last mentioned finding is beyond doubt the most important result of the present investigation.

EXPERIMENTAL

Isolation of the non-volatile, organic acids. The materials used consisted of: fresh plants (*Euphorbia pulcherrima* Willd., *E. splendens* Boj., *Cereus peruvianus* (L.) Mill., *Opuntia ficus-indica* Mill.), air-dried plants (*Euphorbia palustris* L., *Mallotus philippinensis* Müll. Arg., *Ricinus communis* L.), Plants dried at 60°C (*Kleinia repens* (L.) Haw.), and in addition plant material obtained in dry state through Norsk Medisinaldepot, or preserved latex (*Hevea brasiliensis* Müll. Arg.). The amount of plant material processed varied between 100 g and 2 kg. Of the *Hevea* latex 5 l was used.

The plant material was chopped or powdered and extracted with 10–20 parts of water (50–60°C), filtered, and the acids precipitated, first with a saturated solution of lead acetate, and then, before filtering, with basic lead acetate solution, until no more precipitate had formed. The precipitated compounds were filtered off, washed thoroughly with water, suspended in 50% ethanol and decomposed with H₂S. After removal of the PbS, the filtrates were evaporated *in vacuo*, whereupon the remaining thick acid syrup was treated with 5–10 volumes of anhydrous ethanol, filtered, and the

ethanol removed by distillation. The last mentioned operation was repeated with anhydrous ethanol, and then with acetone. The acid syrups that finally remained, will in the following be referred to as "the acid-mixtures".

To isolate the non-volatile acids from the latex, the latter was diluted with two volumes of water, the mixture heated to about 50°C, whereupon the caoutchouc was precipitated with concentrated acetic acid under stirring. The filtrate was evaporated to a thick syrup under reduced pressure and diluted with 10 parts of water. From this solution the acids were precipitated with lead acetate and basic lead acetate and isolated in the same way as the acid-mixtures referred to above.

Esterification of the acid-mixtures and of the pure acids used for comparison. The esterification was performed in the usual way with diazoethane, and the acid-mixtures and the pure acids were treated as nearly as possible in the same way. The ethyl esters of the following pure acids were prepared and used in gas chromatography for comparison: acetylacetic, fumaric, malic malonic, citric, isocitric, tartaric, succinic and phorbic acid.

Comparative gas chromatography of the reference esters and the ester-mixtures. The gas chromatograph used was a Wilkins Autoprep A-700 (Wilkins Instruments and Research, Inc.) and the samples were tested on two different columns, A and B. Column A was an aluminium column, 10 ft long and 3/8 in i.d. Chromosorb W 45/60, coated with 3 % SE-30 served as solid support.

Column B was a copper column, 6 ft long and 1/4 in i.d. Chromosorb W 45/60, coated, first with 0.1 % of Carbowax 6000, and then with 5 % of Apiezon L, served as solid support. Helium served as carrier gas and the flow-rate was 60–85 ml per min. Usually 2–5 μ l of the liquid esters were injected at a time or a corresponding amount of the solid esters dissolved in ethanol.

The actual identification of the components of the ester mixtures was carried out in direct comparison with the known esters. All the samples were tested on both the above mentioned columns. The relative retention times of the known esters were compared with those of the ester mixtures, and co-chromatography of known and unknown samples was carried out.

As the number of components in the ester mixtures was very large, and as the respective quantities and boiling points of the components varied over a wide range, it was found impossible to perform a complete analysis of the mixtures in one working operation – even if programmed gas chromatography was applied. We therefore decided to perform the comparative analyses at different temperature levels, in most cases at 120, 160, 200, and 220°C.

A gas chromatogram of an ester mixture, containing phorbic acid ethyl ester mixed with the ethyl esters of five of the acids listed in Table 1, is shown in Fig. 1.

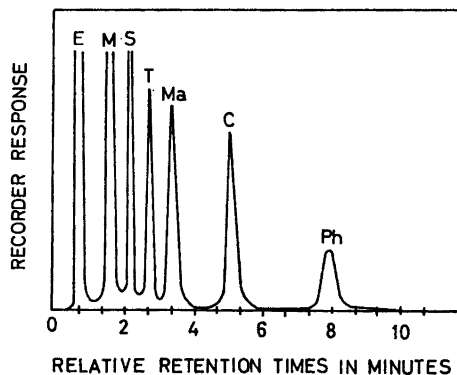


Fig. 1. Gas chromatogram (temperature programmed, 140–230°C in 10 min) of ethanol (E = solvent) and ethyl esters of malonic (M), succinic (S), tartaric (T), malic (Ma), citric (C), and phorbic (Ph) acid. SE-30, 3 %.

RESULTS AND DISCUSSION

During the process of gas chromatographing the ester mixtures, several unidentified components were recorded, but as the main scope of this investigation was to study the distribution of phorbic acid within the vegetable kingdom, a more thorough investigation of these compounds was put aside for the time being. While phorbic acid was found in varying amounts in all the euphorbiaceous plants of which the assimilating tissue (leaves and chlorophyll-containing stems) was used for the investigation, the acid could not be demonstrated in the bark of *Croton eluteria* Benn. nor in the latex of *Hevea brasiliensis* Müll. Arg. On a later occasion we hope to be able to test the leaves of these plants for phorbic acid too.

The above-mentioned findings indicate that phorbic acid is one of the most commonly occurring organic acids within the plant family *Euphorbiaceae*, and the fact that the same acid was found in two of three succulent species selected more or less at random from three different plant families, makes it reasonable to assume that phorbic acid by no means represents a unique or rare structure within the vegetable kingdom.

The basis for such a conclusion is of course that the method used for the detection of phorbic acid is reliable, and here there may be room for doubt. It is therefore our intention to try to isolate phorbic acid in the form of its ethyl ester from *Kleinia repens* (L.) Haw. and *Cereus peruvianus* (L.) Mill. as soon as sufficient amounts of these plants are made available.

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