

several picrates are polymorphous, and in identifying amines by means of the picrates a careful examination of the literature is necessary before a decision is made.

Di-*sec*-butylamine picrate is a new compound: M.p. 105°. (Found: Equiv.wt. 359.9 (titration with perchloric acid). Calc. for  $C_{14}H_{22}N_2O_7$ ; Equiv.wt. 358.4).

The microanalyses have been performed by Mr. A. Bernhardt, Max Planck-Institut, Mülheim.

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## The Non-Volatile Acids of Succulent Plants Exhibiting a Marked Diurnal Oscillation in Their Acid Content

III. The Acids of *Kleinia repens* (L.) Haw., *Begonia tuberhybrida* (Hort) and *Mesembryanthemum criniflorum* L. fil.

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The two previous papers in this series<sup>1,2</sup> deal mainly with the detection of piscidic acid, and its occurrence in *Agave americana* L. and *Opuntia ficus-indica* L. In connection with the following, the authors would also like to draw attention to a third paper dealing with the occurrence of phorbic acid in plants.<sup>3</sup>

In this paper we submit the results of an investigation of the non-volatile acids of three more species, belonging to three different genera in which typical "crassulacean acid metabolism" has been observed.

For the investigation we used mainly the same methods as in the earlier papers in this series: Isolation of the non-volatile acids over their lead salts, esterification of the acid mixtures, distillation of the ester mixtures in high vacuum, and investigation of the ester fractions by means of gas chromatography, conversion of the acids into hydrazides etc. The gas chromatograph was a Wilkins Autoprep A-700. (Wilkins Instrument and Research, Inc.). The columns used was an aluminium column, 5' 1/4", with Chromosorb 45/60 coated with 2 % SE-30 as solid support, and a copper column, 6' 1/4", with Chromosorb 45/60 coated first with 0.1 % Carbowax 6000 and then with 5 % Apiezon-L as solid support. Helium served as carrier gas. All samples were tested on both columns and co-chromatography of known and unknown samples were carried out.

We also used thin layer chromatography for the detection of minor amounts of phorbic acid. Owing to its dilactone properties, phorbic acid is difficult to handle when subjected to paper or thin layer chromatography. During the investigation we therefore used a method worked out by Anne Kristine Krogh.\* This method, which will be published elsewhere, is based on comparative thin layer chromatography of the phorbic acid itself and its lactones under different conditions.

*Kleinia repens* (L.) Haw. (= *Senecio hawortii*, Sch. Bip.). 3.5 kg of the fresh plant (without roots) gave 40 g of non-volatile acids, of which 35 g were esterified with diazoethane and the ester mixture subjected to fractionation in high vacuum (about  $10^{-3}$  torr). The results of the fractionation and some of the investigations carried out with the different ester fractions and the corresponding acids, are shown in Table 1. The non-identified ester in fractions 1 and 2 gave on the 2 % SE 30 column a peak with a retention time that corresponded to succinic acid diethyl ester, but on the 2 % Apiezon L column the retention time of the peak was different from that of the above-mentioned compound. The non-identified ester made up an almost negligible part of the two fractions.

All the six non-identified esters that were found in fractions 3 and 4 had on both columns shown a longer retention time

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Table 1. Ester fractions and the corresponding acids from *Kleinia repens* (L.) Haw.

Number of fraction	B.p. interval in °C	Yield in g	Acids and non-identified esters detected by means of gas chromatography of the esters, and by other methods
1	40—80	7.0	Oxalic, oxalacetic and malic acid (dominant). One non-identified ester.
2	80—130	2.4	Malic acid (dominant), and citric acid. One non-identified ester.
3	130—160	1.4	Malic, pyruvic and citric acid (dominant). Six non-identified esters.
4	160—210	1.6	Malic, pyruvic and citric acid. Six non-identified esters.

Table 2. Ester fractions and the corresponding acids from *Begonia tuberhybrida* (Hort).

Number of fraction	B.p. interval in °C	Yield in g	Acids and non-identified esters detected by means of gas chromatography of the esters, and by other methods
1	20—80	5.2	Oxalic acid (dominant), succinic acid and malic acid.
2	80—120	4.0	Oxalic, succinic, malic (dominant) and citric acid.
3	120—140	1.5	Malic and citric acid (dominant).
4	140—180	0.5	Malic, citric (dominant) and phorbic acid.
5	180—240	0.2	Malic (traces), citric and phorbic acid.

Table 3. Ester fractions and the corresponding acids from *Mesembryanthemum criniflorum* L. fil.

Number of fraction	B.p. interval in °C	Yield in g	Acids and non-identified esters detected by means of gas chromatography of the esters, and by other methods
1	20—80	3.2	Oxalic, malonic, succinic and malic acid. The two last mentioned acids in nearly equal amounts. One non-identified ester.
2	80—110	3.7	Oxalic, malonic (negligible amounts), succinic, malic (dominant), and citric acid. One non-identified ester.
3	110—130	6.1	Malic and citric acid (dominant).
4	130—160	1.2	Malic (small amounts) and citric acid (dominant).
5	160—210	0.3	Malic (negligible amounts) and citric acid (dominant).

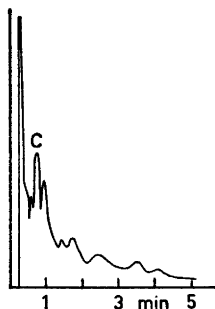


Fig. 1. Gas chromatogram of fraction 4 from *Kleinia repens* (L.) Haw. Column: Apiezon-L. Temperature: 190°C. Peak C corresponds to citric acid triethyl ester.

than citric acid triethyl ester (Fig. 1). It is in these regions of the gas chromatograms that the monoethyl ester of dilactophorbic acid and the diethyl ester of piscidic acid are found.

In a screening test for phorbic acid in plants, carried out with a small sample of *Kleinia repens* (L.) Haw. in 1965 (cf. Ref. 3), it was found that one of these non-identified peaks corresponded to that of dilactophorbic acid monoethyl ester. This finding could not be verified in the present investigation, neither on the gas chromatograph nor in the thin layer chromatographic test carried out with the acids isolated from the fractions 3 and 4. From fraction 4 a crystalline compound (or a mixture) was obtained by adding a few drops of ethanol to the fraction and leaving it in the refrigerator for some days; but the yield — a fraction of a milligram — was too small for a closer investigation.

Our investigations so far seem to indicate that the acids corresponding to the ester peaks shown in Fig. 1 are different from all the organic acids that have so far been isolated from succulent plants in their free (uncoupled) state.

*Begonia tuberhybrida* (Hort). 6.5 kg of the fresh flowering plant (without roots) was used. The yield of crude acids was 67 g of which 60 g were converted into the ethyl esters by boiling for 3 h with 1 l of anhydrous ethanol using 30 g of conc. sulphuric acid as a catalyst. For the results of the fractionation of the ester mixture and some of the investigations carried out with the fractions, see Table 2.

According to our investigation, oxalic acid is the dominating acid in this plant. Then comes malic, and finally citric acid. Phorbic acid, which is present in small amounts was identified by means of gas chromatography and also by means of thin layer chromatography. The gas chromatographic investigations were carried out in direct comparison with pure dilactophorbic acid diethyl ester (m.p. 91–92°C). For thin layer chromatography we used pure lactophorbic acid (m.p. 154°C). All the comparative tests corresponded completely.

*Mesembryanthemum criniflorum* L. fil. 27.5 kg of the fresh flowering plant (without roots) was used. The yield of crude acids was 75 g of which 70 g was esterified as described under *Begonia*. For the results of the fractionation and the examination of the fractions, see Table 3. The results show that citric acid is the dominant acid in *Mesembryanthemum criniflorum* L. fil. Then follows malic acid. The non-identified ester, which had a shorter retention time than malic acid diethyl ester, was present only in small quantities.

If the ester fractions of *Mesembryanthemum* were kept in the refrigerator for a couple of weeks, small amounts of colourless crystals separated from fractions 2, 3, and 5. The crystals from fractions 2 and 3 were identical. They melted at about 110°C and were insoluble in water, ethanol and ethyl acetate. The amount of the substance, a fraction of a milligram, was too small for a closer investigation. The crystalline substance isolated from fraction 5 melted at about 135°C. The yield of the crystalline substances was only a fraction of a milligram, and was not investigated further.

*Discussion.* The non-volatile acids from 15 succulents or semi-succulents belonging to 9 different genera in which a typical crassulacean acid metabolism has been observed, have so far been investigated in this laboratory. In all these plants the acids from the Krebs cycle, especially malic and citric acid, make up the major part of the acid content. In a few cases it was found that certain acids from the Krebs cycle, which usually do not accumulate, make up a major part of the acid mixture. (Cf. the accumulation of isocitric acid in *Sedum*, *Sempervivum*, *Bryophyllum*, and other genera of the plant family Crassulaceae<sup>4</sup>). Phorbic acid, which was first found in the succulent *Euphorbia resinifera*<sup>5,6</sup> (Euphorbiaceae), is an example

of an "extra-cyclic" acid that tends to accumulate in certain succulents, and so is piscidic acid, which has lately been found to make up a major part of the acids in *Agave americana* L. (Agavaceae)<sup>1</sup> and *Opuntia ficus-indica* L. (Cactaceae).<sup>2</sup> Succulents from the plant family Compositae, too, seem to contain interesting acids that might serve to throw light on the biology of succulent plants.

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## X-Ray Crystallographic and IR Data on Some Divalent Selenium and Tellurium Compounds

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The unit cells, space groups and IR data of the following derivatives of divalent selenium and tellurium have been determined in the course of crystal structure work on analogous compounds.<sup>1-3</sup> The X-ray data were derived from single-crystal oscillation and Weissenberg photographs using CuK radiation with  $\lambda = 1.5418$  Å. The calculated cell parameters are believed to be accurate within 0.5 %.

*Selenium bis(diethylthioselenophosphate),*  $\text{Se}[\text{Et}_2\text{P}(\text{S})\text{Se}]_2$  (I). Yellow monoclinic prisms from benzene, elongated along  $c$ , with  $a = 12.94$  Å,  $b = 12.30$  Å,  $c = 13.31$  Å,  $\beta = 126.0^\circ$ . There are four molecules per unit cell; density calc. and found  $1.85$  g/cm<sup>3</sup>. The space group, from systematic extinctions, is  $C_{2h}^6-C2/c$  or  $C_s^4-Cc$ . However, three of the compounds reported here, (I), (II), and (III), show the same morphology and X-ray extinctions. Further similarities are found in unit cell data and X-ray reflection patterns. The three compounds are thus isomorphous. Structure work on II and III is in progress in this laboratory. Patterson and Fourier maps of the  $b$  and  $c$  axis projections indicate that the molecules possess twofold symmetry, with the central atoms on twofold symmetry axes parallel with  $b$ . The two structures seem to be similar to that of tellurium bis(dimethylthiophosphate).<sup>3</sup> Accordingly space groups without twofold symmetry axes are not likely in any of the three isomorphous compounds I, II, and III. Thus  $C_{2h}^6-C2/c$  is the probable space group for I, as the other possible space group does not possess such an axis.

*Tellurium bis(diethylthioselenophosphate),*  $\text{Te}[\text{Et}_2\text{P}(\text{S})\text{Se}]_2$  (II). Orange, monoclinic prisms from a mixture of ethanol and benzene. The crystals are elongated along  $c$ , with  $a = 13.07$  Å,  $b = 12.37$  Å,  $c = 13.37$  Å,  $\beta = 126.5^\circ$  and four molecules in the unit cell. The density is, calc.  $2.01$ , found  $1.99$  g/cm<sup>3</sup>. The space group, from systematic extinctions and structure work in progress, is  $C_{2h}^6-C2/c$ .

*Selenium bis(diethyldiselenophosphate),*<sup>4,5</sup>  $\text{Se}[\text{Et}_2\text{P}(\text{Se})\text{Se}]_2$  (III). Yellow and orange, monoclinic prisms from a mixture of ethanol and benzene, elongated along  $c$ , with  $a = 12.92$  Å,  $b = 12.49$  Å,  $c = 13.43$  Å,  $\beta = 125.0^\circ$ . There are four molecules in the unit cell, with density, calc. and found  $2.14$  g/cm<sup>3</sup>. The space group from systematic extinctions and structure work in progress, is  $C_{2h}^6-C2/c$ .

*Tellurium bis(diethyldiselenophosphate),*  $\text{Te}[\text{Et}_2\text{P}(\text{Se})\text{Se}]_2$  (IV). Orange-red, monoclinic prisms from mixtures of ethanol and benzene. The crystals are elongated along  $b$ , with  $a = 21.40$  Å,  $b = 11.03$  Å,  $c = 16.99$  Å and  $\beta = 117.3^\circ$ . There are eight molecules in the unit cell, with density, calc.  $2.31$ , found  $2.33$  g/cm<sup>3</sup>. The space group from systematic extinctions, is either  $C_{2h}^6-C2/c$  or  $C_s^4-Cc$ .

The isolation of selenium bis(diethylthioselenophosphate) has been reported