a helical conformation.[7] Since the esterified radical is in intimate contact with the host polymer, the transition from helix to a random coil conformation is a reasonable explanation for the observed phenomenon. It is thus probable that the polymer molecules in the relaxation region I are in a random coil conformation. Even if the helical conformations still exist, their fluctuations are so rapid that they do not affect the spin rotational relaxation.

Because the nitroxyl radicals incorporated into PEG exhibit a transitional relaxation region (II) in the temperature range where the endothermic melting of polymer crystals occurs, it is evident that radicals are in close contact with the crystalline phase. The isotropic nature of the ESR spectra refers to the amorphous environment.[1] On the other hand, the bulky radicals would surely distort the tightly packed crystal lattice about them and exhibit an amorphous-like environment even though they were in the crystalline phase. The structural changes in the crystal phase are thus reflected in the rotating radicals as changes in the forces caused by crystalline phase in the amorphous environment of radicals. This hypothesis is supported by the observation that in the $\overline{M}_n$ region, where end group effects become negligible ($\overline{M}_n \geq 9500$), the degree of crystallinity and E_a^{II} decrease as a function of $\overline{M}_n$ (Fig. 1 in this report and Fig. 6 in Ref. 1).

Experimental. The PEG samples were of commercial origin (Fluka and Merck). Their M_n values were measured by a vapour pressure osmometer (Perkin-Elmer Modell 115) in chloroform at 32°C. The spin probe radical 3-methoxycarbonyl-2,2,5,5-tetramethyl-pyrroline-1-oxyl and the spin label radical 3-PEG-carbonyl - 2,2,5,5 - tetramethylpyrroline-1-oxyl were prepared and incorporated into PEG as reported earlier.[1] The history of the polymers was as follows: The PEG samples containing spin probes (set A) were dissolved in chloroform and the solvent was carefully evaporated in vacuum. The PEG samples containing spin labels (set B) were dissolved in chloroform precipitated with ether and dried in vacuum.[1] All these procedures were carried out at room temperature (24°C). DSC-curves were scanned at a rate of 8°C/min in an N_2-atmosphere using a Perkin Elmer DSC-1B calorimeter. T_m was measured from the maximum of the endothermic peak height.

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Isolation of D-Galactaric Acid and Isocitric Acid from *Ferocactus acanthodes* Br. et R.

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Phytochemical investigations during recent years have revealed that the predominating acids of several succulent plants are hydroxy acids, which are able to form lactones.[1-4] Some of these acids, like phorbic acid, were first isolated from a succulent.[5] The cumulation of hydroxy acids in succulent plants is undoubtedly connected with the unique form of metabolism that is so characteristic for this group of plants, and it is believed that

lactone forming hydroxy acids play an important part in the succulent metabolism, *e.g.* as pH regulators.[6,7]

In a current investigation on organic acids in succulents it was observed that *Ferocactus acanthodes* contains a rare organic acid which was isolated and identified as D-galactaric acid. This acid has been found in free state in plants only once, namely in the fruits of cylon olive,[8] which belongs to a quite different group of plants. During the investigation another lactone forming acid, namely isocitric acid, was isolated and identified.

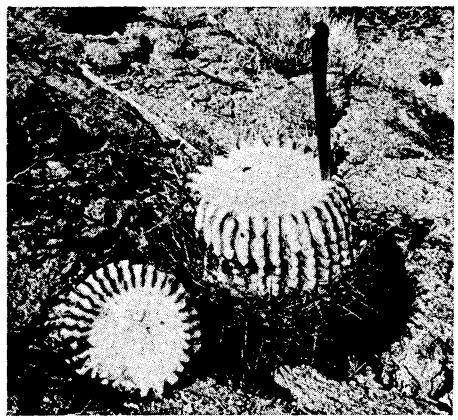

Fig. 1. Ferocactus acanthodes Br. et R.

Experimental. The plant used for the investigations (Fig. 1) was collected by one of the authors (A. N.) in the deserts of Baja California, Mexico, in 1967. The fresh plant was cut into 1 – 2 cm thick slices and dried at 60°C. The dried material, which was yellowish white, was kept in well closed containers till it was used for the following investigation.

30.0 g of freshly prepared powder was extracted for half an hour at room temperature with 400 ml of distilled water. The cations were removed from the filtrate with Dowex 50 W × 8 (H^+), and the acid extract was concentrated to about 250 ml. The extract was then treated with barium hydroxide solution till no more barium salt precipitated.

The precipitate, which mainly consisted of barium sulphate, was removed, and the filtrate (pH near to 1) was adjusted with barium hydroxide solution to pH 6. The extract was concentrated to 20 ml and put in a refrigerator for two days, during which time a white precipitate had formed. The precipitate (Substance A) was removed by centrifugation and the supernatant (in the following referred to as Solution I) was put aside for further investigation.

Substance A, which was a mixture of barium salts, was suspended in 30 ml of distilled water and deionized with Dowex 50 W × 8 (H^+). On evaporation to about 5 ml, a white microcrystalline substance (Substance B) separated. It was washed with distilled water and ethanol, and dried. Yield 12.4 mg. The mother liquor from this substance will in the following be referred to as Solution II.

Investigation of Substance B. The substance melted at about 227°C, when inserted into the micro melting point apparatus at 205°C. When mixed with D-galactaric acid no melting point depression was observed.

The melting point of the substance varied a great deal according to the conditions,[9,10] but corresponded always to the values observed with authentic D-galactaric acid under the same conditions. For the melting point determination, a Reichert micro melting point apparatus No. 250282 was used. (Found: C 34.07; H 4.99. Calc. for $C_6H_{10}O_8$: C 34.29; H 4.80.)

The IR-spectrum was identical with that of the authentic substance. The isatine test[11] and the *p*-dimethyl-aminobenzaldehyde test[12] were positive.

Thin-layer chromatography (TLC) was run on Polygram cel 300 MN. Solvent system: pyridine – ethyl acetate – acetic acid – water (5 : 5 : 1 : 3). Detecting reagents: a. Bromophenol Blue 0.1 % in methanol. b. Hydroxylamine – ferric chloride.[13]

The chromatographic behaviour of the substance was identical with that of authentic D-galactaric acid. Both acids gave two spots.

Investigation of Solution I and Solution II. Solution I was deionized with Dowex 50 W × 8 (H^+), and the acid extract passed through a column of Dowex 1 × 8 (OH^-). After washing of the column with distilled water, the acids were eluted with hydrochloric acid (2 N), the eluate was evaporated and the residue dried. TLC of the acid residue, performed under the same conditions as above, gave two purple-brown spots – one weak and one stronger – when using detecting reagent b. The spots corresponded to the two spots given by isocitric acid under the same conditions. As reference was used DL-isocitric acid lactone (No. 1 – 1377 Sigma).

To confirm the presence of isocitric acid, the acid residue was converted into the ethyl esters, and the ester mixture subjected to gas chromatography, using the ethyl ester of DL-

isocitric acid and an isocitric acid ethyl ester isolated from *Sedum acre* [14] as references.

Esterification of the acid from Solutions I and II, and of the reference sample, was performed by boiling one part of the acids with about 50 parts of anhydrous ethanol for 10 h, using 2–3 g of Dowex 50 W × 8 (H^+) as a catalyst.

The gas chromatograph used was an Aerograph Hy-Fi 600 B with a hydrogen flame ionization detector. Nitrogen served as a carrier gas. The column used was of stainless steel, 2 m × 3 mm with Gas Chrome Q, coated with 3 % DEGS. The temperature of the column chamber was kept constant at 180°C during the runs.

Under the conditions described above, isocitric acid ethyl ester gives two well separated peaks on the gas chromatogram.[15] The first peak often shows a shoulder indicating a double peak. Sometimes this double peak is fairly clear.

Both solutions were found to contain isocitric acid ethyl ester. The main part was found in Solution I.

Discussion. D-Galactaric acid might easily be overlooked during an investigation on organic acids in plants, as it in its physical and chemical properties differs much from other organic acids commonly found in plant tissues. In our case we came across the acid more or less incidentally as it was noticed that a white substance separated when a deionized solution of a calcium salt isolated from *Ferocactus acanthodes* was left for some days in the cold store. A preliminary investigation of the white precipitate pointed towards D-galactaric acid, for which reason a more thorough investigation of the problem was initiated.

In the method for isolation of D-galactaric acid described above, sulphuric acid is precipitated as barium salt under conditions where the barium salt of galactaric acid remains in solution (pH 1). After removal of the barium salts which separate at pH 1, the pH of the filtrate is adjusted to pH 6, and the solution concentrated. Under these conditions the barium salt of D-galactaric acid will separate (admixed, *e.g.*, with the barium salts of isocitric and malic acids). On deionization of the barium salt mixture and concentration of the acid solution, D-galactaric acid will first separate from the acid solution owing to its slight solubility in water.

We assume that D-galactaric acid is much more common in plants, especially in succulents, than hitherto believed, and that this might be shown when suitable methods for its detection are applied.

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