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CERHEPTARIC ACID, A NEW LACTONE-FORMING ACID ISOLATED FROM *Cereus peruvianus* (L.) MILL.

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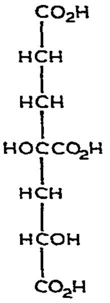
ABSTRACT

Cerheptaric acid, detected in *Cereus peruvianus* (a crassulacean, acid-metabolism plant), has been identified as 3-deoxy-D-*manno*-heptaric acid or its enantiomer. The acid occurred in high abundance in the cactus material investigated and was isolated as a crystalline monomethyl ester of the (7→4)-lactone.

INTRODUCTION

Cereus peruvianus has been included in two screening tests for phorbic acid (**1**), a lactone-forming acid first isolated from *Euphorbia resinifera*[1,2]. One investigation[3] indicated the presence of phorbic acid in *C. peruvianus*, whereas negative results were reported later[4]. T.l.c. data and a modified ferric hydroxamate test showed[5] that the cactus contained a lactone-forming acid having properties similar to those of phorbic acid. A reinvestigation of *C. peruvianus* is now reported.



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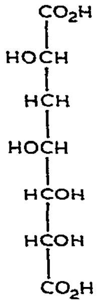

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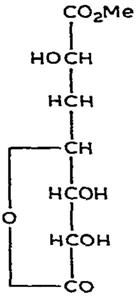

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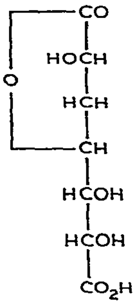

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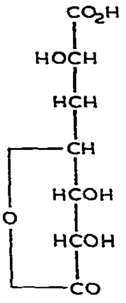

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EXPERIMENTAL

Plant material. — *Cereus peruvianus* (L.) Mill. (*Cactaceae*) was collected by Professor A. Nordal (Institute of Pharmacy, University of Oslo, Norway) in Tenerife during December 1971, immediately fixed with ethanol, and stored in the dark until

August 1975. The greyish precipitate which had then formed was dried and used in the present investigation. Fresh cactus material was collected in the neighbourhood of Agadir, Morocco, in February 1976, by Professor A. Nordal, and on the Canary Islands, during April 1976, by Professor E. Bernatek (Institute of Pharmacy, University of Oslo, Norway). Small plants, ~15 cm tall, were supplied by Löxa Nursery, Baerum, Norway. Professor A. Nordal was responsible for the identification of the plants.

Isolation of cerheptaric acid. — (*a*) *From the dried precipitate.* The precipitate (100 g) was extracted with 0.75M HCl (2 × 800 ml) for 2 h at 100°. The combined extracts were passed through columns of Dowex-50W X8 (H^+) resin (20–50 mesh, 300 ml) and Dowex-1 X8 (HO^-) resin (20–50 mesh, 900 ml). The anion-exchanger was eluted with 2M HCl (3 litres), the eluate was concentrated (~100 ml), treated with activated charcoal, and concentrated *in vacuo* over P_2O_5. The residue (~1 g) was a greyish, crystalline mass. Recrystallisation from dry methanol gave the lactone methyl ester 3 as white needles (~0.1 g), m.p. 159–160°, $[\alpha]_D^{20}$ +39° (*c* 1, methanol); ν_{max}^{KBr} 1770–1760 (γ-lactone) and 1745–1740 cm^{-1} (saturated ester); lit.[6] m.p. 160°.

Anal. Calc. for $C_8H_{12}O_7$: C, 43.64; H, 5.45. Found: C, 43.97; H, 5.87.

(*b*) *From fresh plant material.* Fresh stems (100 g) were cut into pieces and extracted with boiling ethanol for 30 min. The residue was extracted with water (3 × 500 ml). The combined extracts were concentrated and dialysed three times against water (1 litre), and passed through columns of Dowex-50W X8 (H^+) resin (20–50 mesh, 300 ml) and Dowex-1 X8 ($HCOO^-$) resin (20–50 mesh, 600 ml). Plant acids were eluted from the anion-exchanger with 2M HCl (3 litres), the eluate was concentrated, and the residues were subjected to g.l.c. and g.l.c.–m.s.

General methods. — Melting points were determined with a Mettler FPI instrument and are corrected. I.r. spectra were recorded with a Beckman IR 20 spectrophotometer. T.l.c. was carried out on Polygram cel 300 MN pretreated with, and developed with, pyridine–ethyl acetate–acetic acid–water (5:5:1:3); a modified ferric hydroxamate test, involving a hydroxylamine reagent adjusted to pH 6, was used for selective detection of unstable lactones[5].

G.l.c. of trimethylsilyl derivatives was performed[7] on a column of 10% OV-17 using a temperature programme rate of 2°/min from 120°. G.l.c.–m.s. was performed as described by Pettersen *et al.*[8].

Methyl esters were formed by treating[9] the acid mixture with boiling, dry methanol for 4 h, using water-free Dowex-50W X8 (H^+) resin as catalyst[10].

RESULTS AND DISCUSSION

Each step in the procedure for isolation of the new acid was monitored by t.l.c. and the ferric hydroxamic acid reaction, and ion-exchange chromatography did not change the properties of the lactone-forming acid. A freshly prepared, aqueous solution of the isolated acid mixture gave two components (R_F 0.6, 0.7) which gave a positive reaction with the ferric hydroxamate test; after one week, an additional compo-

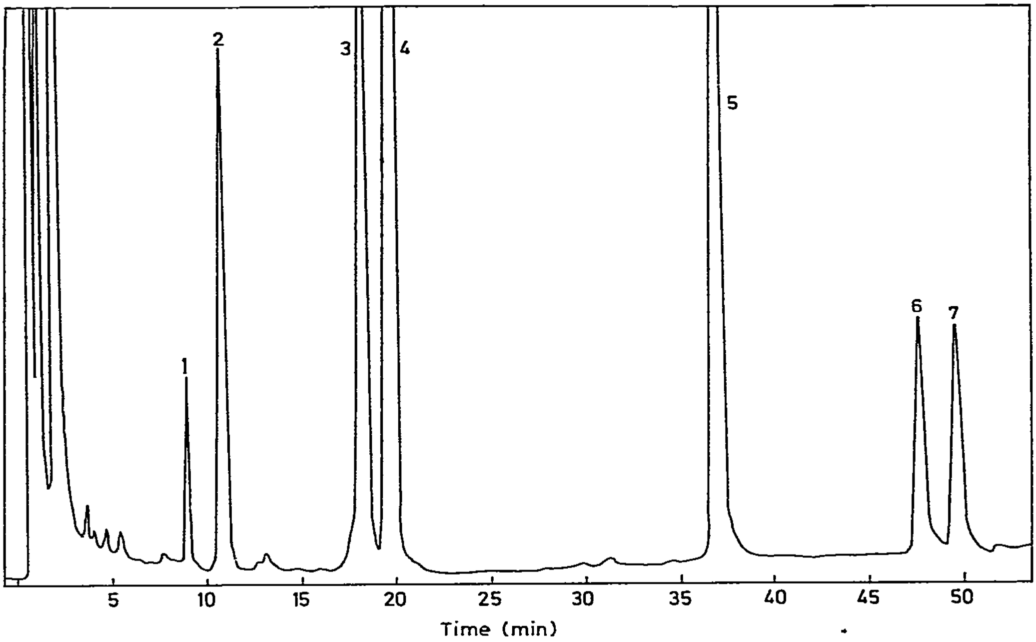

Fig. 1. Gas chromatogram of the trimethylsilylated acids isolated from *C. peruvianus*: 1, unknown; 2, phosphoric acid; 3, unknown; 4, malic acid; 5, citric acid; 6 and 7, cerheptaric acid monolactones.

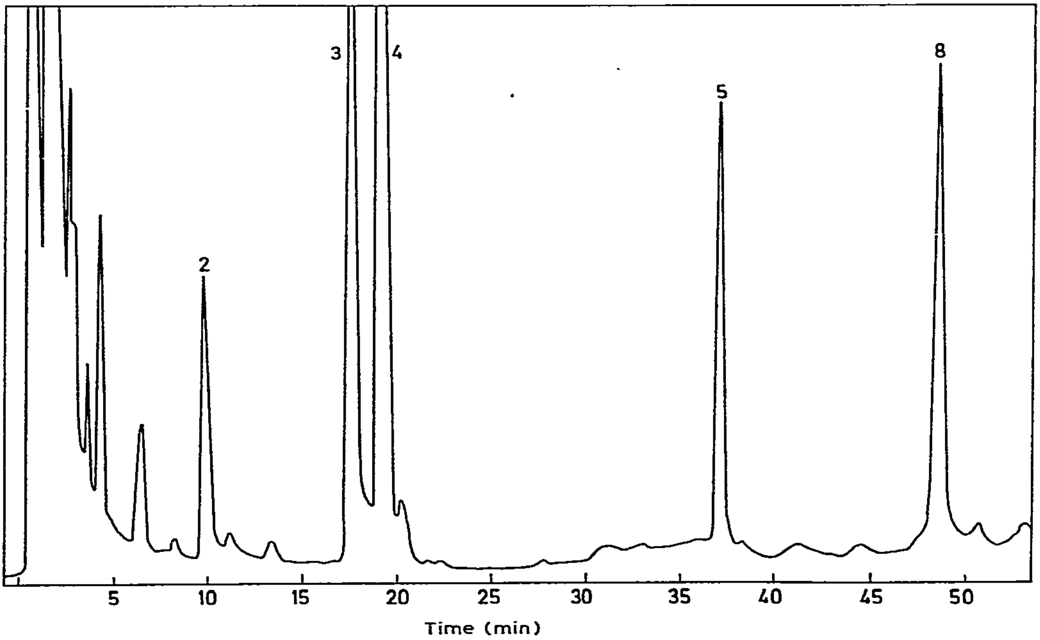

Fig. 2. Gas chromatogram of the trimethylsilylated sodium salts of acids isolated from *C. peruvianus*: 2, phosphoric acid; 3, unknown; 4, malic acid; 5, citric acid; 8, cerheptaric acid.

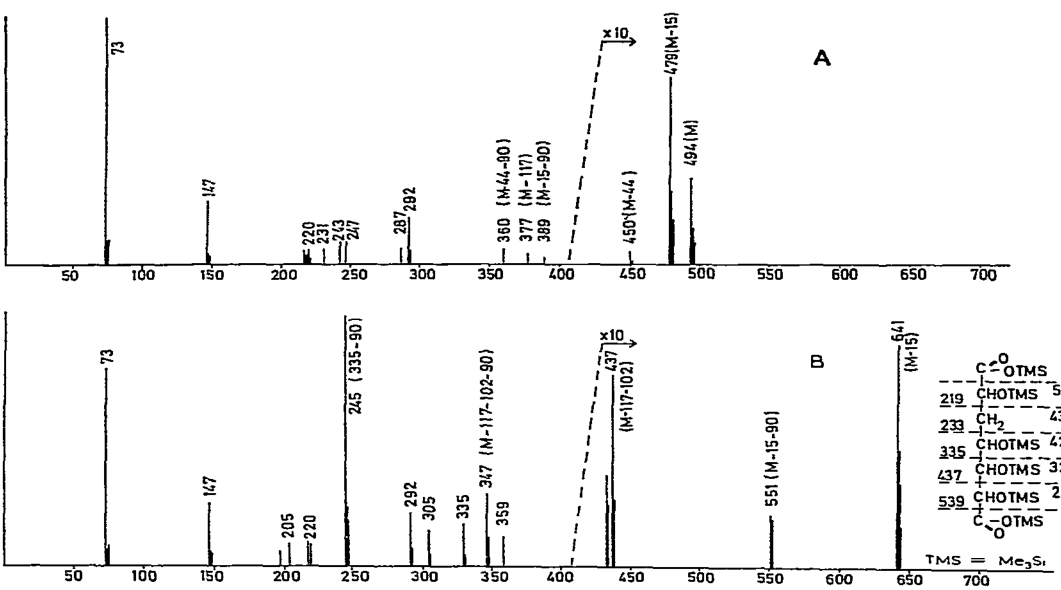

Fig. 3. Mass spectra of: A peak 6 in Fig. 1; B peak 8 in Fig. 2.

nent appeared (R_F 0.2), indicating that the acid is able to form more than one lactone. The component with R_F 0.2 probably corresponds to the acyclic acid **2**. Similar behavior has been suggested[11] for glucaric acid and shown[12] for phorbic acid.

Recrystallisation of the acid gave a new compound (R_F 0.9), which has been identified[13] as the (7→4)-lactone methyl ester **3**; no (1→4)-lactone methyl ester was detected. The absolute configuration has not been determined; the D form is depicted solely for convenience. Similar, easy esterification has been reported for other acids, *e.g.*, piscidic acid[14]. The ready formation of **3** is now utilised for the isolation of the acid from plant material[6].

The gas chromatograms of the trimethylsilyl derivatives of the acid mixture (Fig. 1) and of the sodium salts of the acid mixture (Fig. 2) extracted from small cacti indicate the amount of cerheptaric acid relative to those of other acids in *C. peruvianus*. The peaks 6 and 7, in Fig. 1, due to different lactones (**4** and **5**) of cerheptaric acid, gave nearly identical electron-impact mass spectra (70 eV). Spectrum A in Fig. 3 corresponds to peak 6 in Fig. 1. The mass spectra of trimethylsilylated, carbohydrate acyclic acids usually lack a peak for the molecular ion[15]. This was also true for the acyclic form of cerheptaric acid (Fig. 3, B).

The (1→4)- and (1→5)-lactones of aldonic acids are easily distinguished[16] by mass spectrometry of the trimethylsilyl derivatives. The mass spectra for the material in peaks 6 and 7 in Fig. 1 each correspond to a γ-lactone (**4** or **5**). The available data do not permit unequivocal assignment. The assignments in Fig. 3 are based on literature data[15,16].

Under the appropriate g.l.c. conditions, cerheptaric acid can be distinguished from the common plant-acids, such as malic and citric acid (Figs. 1 and 2), and appears to contain equal amounts of two γ-lactones. T.l.c. gave the same result.

Screening for cerheptaric acid in *Cereus* and *Trichocereus* species revealed its wide distribution in these genera of the cactacean family[6]. Its physiological function is unknown, as is that of phorbic acid (**1**).

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