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LACTONE-FORMING ACIDS IN SUCCULENT PLANTS

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Key Word Index—Angiosperms; succulent plants; isocitric acid; glucaric acid; phorbic acid; quinic acid; distribution.

Plants and sources. Families and species examined are listed in Table 1. Species of the Cactaceae, except for *Echinopsis triumphans*, *Opuntia ficus-indica* and *Pachycereus pringlei*, was supplied by Apotekare Jan G. Bruhn, Department of Pharmacognosy, Faculty of Pharmacy, University of Uppsala, Stockholm, Sweden. Identification was carried out by Dr. Helia Bravo and Dr. Hernando Sánchez-Mejorada, Departamento de Botánica, Instituto de Biologia, Universidad Nacional Autónoma de México, México D.F. *Pachycereus pringlei* was supplied by Dr. A. A. Benson, Scripps Institution of Oceanography, La Jolla, Calif., who was also responsible for the identification of this species. All the other species originate from the Botanical Garden, University of Oslo, Norway. They were identified and partly supplied by the director of the Garden, Dr. Per Sunding.

Previous work. Earlier investigations indicate that hydroxy acids, which can form lactones (lactone-forming acids), are widely distributed within succulent plants. [1–7].

Present work. The present investigation supports earlier findings, as all the plants investigated were found to contain one or more lactone-forming acids (Table 1). The following acids were used as reference compounds: dilactophorbic acid [3], erythronolactone, D-galactaric acid, D-glucaric acid (prepared from calcium D-saccharate by means of Dowex 50 [H^+]), hibiscus acid, homocitric acid lactone, DL-isocitric acid lactone, quinic acid, quinide [5] and shikimic acid.

Table 1. Lactone-forming acids in succulent plants

Plant family and species*	Lactone-forming acids identified	Criteria for identification
Liliaceae		
Aloe vera L.	Isocitric acid	TLC, GLC (OV-17, OV-225)
Amaryllidaceae		
Agave sisalana Perrine	Glucaric acid	TLC
	Isocitric acid	TLC, GLC (OV-17, OV-225)
Crassulaceae		
Echeveria elegans Rose	Isocitric acid	TLC, GLC (OV-17, OV-225), GC–MS
	Phorbic acid	TLC, GLC (OV-17), GC–MS
Kalanchoë longiflora Schltr.	Isocitric acid	TLC, GLC (OV-17, OV-225), GC–MS
Kalanchoë pinnata (Lamk.) Pers. (*Bryophyllum calycinum* Salisb.)	Isocitric acid [11]	TLC, GLC (OV-17, OV-225), GC–MS
Sedum acre L.	Isocitric acid [2]	TLC, GLC (OV-17, OV-225)

Table 1 (*cont.*)

Plant family and species*	Lactone-forming acids identified	Criteria for identification
Zygophyllaceae		
Zygophyllum fontanesii Webb et Berth.	Isocitric acid	TLC, GLC (OV-17, OV-225)
Euphorbiaceae		
Euphorbia canariensis L.	Glucaric acid [6]	TLC
Euphorbia resinifera Berg	Phorbic acid [3]	TLC, GLC (OV-17)
	Quinic acid [5]	TLC, GLC (OV-17, OV-225)
Cactaceae		
Aztekium ritteri (Boed.) Boed.	Glucaric acid	TLC
	Quinic acid	TLC, GLC (OV-17, OV-225), GC–MS
Carnegia gigantea (Eng.) Br. & R.	Glucaric acid	TLC
	Isocitric acid	TLC, GLC (OV-17, OV-225)
	Quinic acid	TLC, GLC (OV-17, OV-225)
Cephalocereus chrysocanthus Web.	Quinic acid	TLC, GLC (OV-17, OV-225), GC–MS
Coryphantha calipensis H. Bravo	Isocitric acid	TLC, GLC (OV-17, OV-225), GC–MS
Echinocereus cinerascens (DC.) Rümpl.	Glucaric acid	TLC
Echinopsis triumphans R. Mey.	Isocitric acid	TLC, GLC (OV-17, OV-225)
Lophophora diffusa (Croizat) Bravo	Glucaric acid	TLC
	Quinic acid	TLC, GLC (OV-17, OV-225), GC–MS
Lophophora williamsii (Lem.) Coult. (*Echinocactus williamsii* Coult.)	Glucaric acid	TLC
Obregonia denegrii Frič	Quinic acid	TLC, GLC (OV-17, OV-225)
Opuntia ficus-indica (L) Mill.	Unidentified	TLC
Pachycereus grandis Rose	Glucaric acid	TLC
	Isocitric acid	TLC, GLC (OV-17, OV-225)
Pachycereus marginatus (DC.) Br. & R. (*Marginatocereus marginatus, Stenocereus marginatus*)	Isocitric acid	TLC, GLC (OV-17, OV-225)
	Quinic acid	TLC, GLC (OV-17, OV-225)
Pachycereus pecten-aboriginum (Eng.) Br. & R.	Quinic acid	TLC, GLC (OV-17, OV-225)
Pachycereus pringlei Br. & R.	Glucaric acid	TLC
	Isocitric acid	TLC, GLC (OV-17, OV-225)
Pachycereus weberii (Coult.) Backbg. (*Stenocereus weberii*)	Glucaric acid	TLC
	Isocitric acid	TLC, GLC (OV-17, OV-225)
Pelecyphora aselliformis Ehrenberg	Quinic acid	TLC, GLC (OV-17, OV-225)
Strombocactus disciformis (DC.) Br. & R.	Isocitric acid	TLC, GLC (OV-17, OV-225)
Trichocereus pachanoi Br. & R.	Unidentified	TLC
Asclepidaceae		
Ceropegia fusca Bolle	Isocitric acid	TLC, GLC (OV-17, OV-225)
Staphelia nobilis N.E. Br.	Isocitric acid	TLC, GLC (OV-17, OV-225)
Labiatae		
Salvia canariensis L.	Isocitric acid	TLC, GLC (OV-17, OV-225)
Compositae		
Kleinia neriifloria Haw.	Glucaric acid	TLC
	Isocitric acid	TLC, GLC (OV-17, OV-225)

* Plant families are listed in the order given by Englers [12]. The species in the same family are arranged alphabetically.

EXPERIMENTAL

Acids were isolated from fresh or dried leaves or stems. Material supplied by Dr. Bruhn consisted of conc. ethanolic extracts prepared from fr. plants in Mexico, Peru and Arizona.

Isolation of the acid mixtures. Starting material was extracted with boiling H_2O for 5 min. Extracts were treated first with Dowex 50 W × 8 [H^+], 20–50 mesh, and then with Dowex 1 × 8 [OH^-], 20–50 mesh. From the latter anion exchanger, the organic acids were eluted with 2 N HCl which was removed from the extracts by repeated evaporation after addition of H_2O. Residues were freeze-dried and stored in desiccators in a cold place.

TLC of the acid mixtures was carried out on Polygram cel 300 MN (0·1 mm, 20 × 20 cm), pretreated with the solvent system [C_6H_5N–EtOAc–HOAc–H_2O (5:5:1:3)]. Methods of detection: (a) UV light; (b) hydroxylamine–ferric chloride [8]; and (c) bromophenol blue 0·1% in MeOH. Before application of (a) and (b), plates were heated for 1 hr at 105°. Inspection of the plates in UV light was found especially useful for the detection of glucaric acid, which shows up as 3–4 bright yellow, fluorescent spots.

Preparation of the methyl esters. Acid mixtures (50–100 mg) were esterified with CH_2N_2 [9].

GLC of the methyl esters. GLC was on a glass column (3 m × 6 mm), filled with 7·5% OV-17 on Gas Chrom Q, 80–

100 mesh, or 3·5% OV-225 on Varaport 30, 100–120 mesh. Standard injector temp. was 250° and detector temp. 300°. The column temp. was kept isothermal at 170° (OV-17 and OV-225) and 190° (OV-17).

GC–MS of the methyl esters. Apparatus and conditions used have been described by Pettersen and Stokke [10]. For our purpose a column containing OV-17 (10%) was selected.

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TRIGLOCHININ IN *ARUM MACULATUM**

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Key Word Index—*Arum maculatum*; Araceae; cyanogenic glycoside; triglochinin.

Arum maculatum ist schon lange als Blausäure-produzierende Pflanze bekannt. 1884 zum ersten Mal von Jorisslen erwähnt [1] vermutete Greshoff in der Cyanogenese eine Schutzfunktion gegen Insekten in der Spatha nach erfolgter Befruchtung [2]. 1921 berichtete Brunswik [3] über die Cyanogenese einer Reihe von Arten aus der Unterfamilie der Aroideae; zuletzt beschäftigte sich Dillemann mit der cyanogenen Substanz dieser Pflanze und fand, daß ein Glykosid vorliegen muß [1].

Unser Untersuchungsmaterial stammt aus den Auwäldern der Rheinebene (Vergleichsexemplar im Bot. Garten der Universität). Die oberirdischen, jungen Teile wurden in flüssigem Stickstoff gesammelt, gefriergetrocknet, pulverisiert und mit siedendem Wasser extrahiert. Die Aufreinigung erfolgte im wesentlichen nach [4]. Kontrolle der einzelnen Schritte auf CN-Gehalt und pc-Überprüfung zeigten, daß nur eine cyanogene Verbindung vorlag. Das nach Lyophilisation erhaltene, gelbliche, nicht kristalline hygroskopische Pulver besitzt gleiches Laufverhalten wie Triglochinin [4,9] mit einer Reihe von Fließmitteln an Kieselgel, Cellulose und Papier [4,5] und schließt Triglochininmonomethylester [5] aus. Das UV-Spektrum in Wasser weist ein λ_{max} bei 275 nm auf, das IR-Spektrum ist identisch mit dem von Triglochinin [4,6]. Säurehydrolyse in 0·5 N HCl liefert nach GC-Untersuchung [7] als einzigen Zuckerpartner Glucose. β-Glucosidase aus *Alocasia macrorrhiza* [8], die für Triglochinin eine hohe Spezifität aufweist, hydrolysiert das vorliegende Glucosid ebenso schnell wie Triglochinin. Das 60-MHz-NMR Spektrum des trimethylsilylierten Glucosides ist mit dem von TMS-Triglochinin identisch [4,6]; es zeigt, daß auch hier geringe Mengen an Isotriglochinin [9,6] vorliegen, wobei ungeklärt ist, ob Isotriglochinin genuin vorliegt.

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* 2. Mittlg. über die Cyanogenese der Araceen.