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TRITERPENE GLYCOSIDES AND QUERETAROIC ACID IN ORGAN PIPE CACTUS*

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Key Word Index—*Lemairocereus thurberi*; Cactaceae; organ pipe cactus; triterpene glycosides; queretaroic acid; *Drosophila* breeding; chemical ecology.

Abstract—The concentration of triterpene glycosides in mature stems of organ pipe cactus was shown to decrease from the surface to the inner portions of the plant. In addition to the previously described oleanolic acid and thurberogenin, queretaroic acid was also shown to be present as an aglycone, and glucose and rhamnose were two of the constituent sugars. Addition of the glycosides to the medium inhibited the maturation of *Drosophila nigrospiracula*, a fly which cannot breed in organic pipe cactus.

INTRODUCTION

Organ pipe cactus (*Lemairocereus thurberi*) grows in Sonora and Baja California, Mexico and in Organ Pipe National Monument, Arizona. The decaying tissues of the plant serve as a breeding site for *Drosophila mojavensis* but cannot be used by *D. nigrospiracula* [1]. The cactus has been reported to lack alkaloids and contain oleanolic acid (1) and thurberogenin (2) as glycosides [2, 3]; betulin and calenduladiol were later isolated from a neutral fraction [4, 5]. Our interest in organ pipe cactus was initiated by the *Drosophila* ecology [6] and the lack of information about other lipids in the cactus.

RESULTS AND DISCUSSION

The cross section of a mature stem of organ pipe cactus is shown in Fig 1. The skin refers to the tough, wax-coated outer layer of translucent material covering the plant, the photosynthetic layer is a gelatinous, bright green tissue under the skin, the transition zone is where green gradually changes to the yellow of the cortex, a dry-appearing, soft, partly fibrous tissue extending inward to the woody, cylindrical rib, whose center is filled with a soft pith. The composition of each

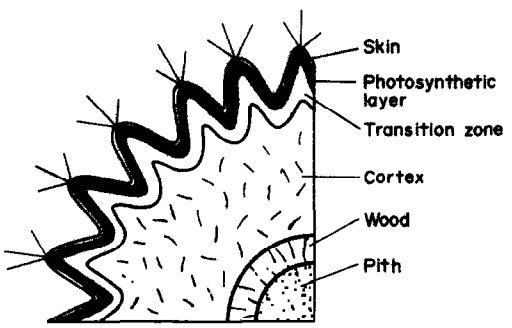

Fig. 1. Cross section of organ pipe cactus; 1/4 of mature stem.

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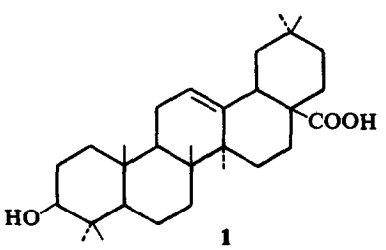

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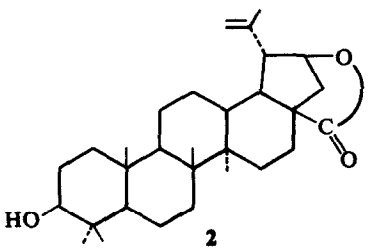

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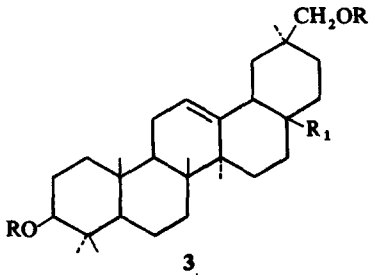

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	R	R_1
3a	H	COOH
3b	Ac	COOH
3c	Ac	COOMe
3d	H	COOMe
3e	H	CH_2OH
3f	Ac	CH_2OAc

of these components is shown in Table 1. The glycoside and lipid contents of the tissues decrease from just under the skin of the cactus to the inner portions of the stem. The relatively large quantities of triterpene glycosides in the outer portion of the stem may reflect their use against animal predation and/or for osmotic protection against freezing.

A third triterpene was encountered in the hydrolyzate of the glycoside fractions. It was shown to be queretaroic acid (**3a**) on the basis of the physical and spectroscopic properties of the compound and its derivatives. Queretaroic acid has been described in three other *Lemairocereus* species [7] as well in two plants from India [8, 9].

The photosynthetic layer, transition zone, cortex, wood and pith all had approximately the same ratio of **1**, **2**, and **3a** in the hydrolyzates of their glycoside fractions; that of the skin contained principally oleanolic acid. No betulin or calenduladiol were detected in the hydrolyzates of these fractions. From weight loss and PC, 2 glucose and 2 rhamnose anhydro-units made up the carbohydrate moiety of the saponins.

When a mixture of the triterpene glycosides was added to a regular laboratory diet for Drosophila, the number of adult *D. nigrospiracula* reared per test dropped from 30 to 2–3. These preliminary results show that the presence of high concentrations of saponins in organ pipe cactus may be the reason that *D. nigrospiracula* is unable to use the plant as a breeding site. More extensive tests with this species and with *D. mojavensis* are in progress and will be reported elsewhere.

EXPERIMENTAL

Mps in air or *vacuo* are corrected, TLC on Si gel CCl_4–$CHCl_3$–MeOH–HOAc, (45:45:10:1) R_f **1**, **2**, **3a** 0.67, 0.78, 0.38 resp. MS and NMR spectra of **3a–f** are in accord with the structures and are available on request. PC of sugars on EtOAc–Py–H_2O (8:2:1) and EtOAc–HOAc–H_2O (9:2:2).

Plant material. A mature stem (16 cm dia, 85% H_2O) of the cactus was collected 30 km south of the Arizona border near Sonoyta, Sonora in NW Mexico. The tip and base were removed and the remainder sectioned into the components shown in Fig 1. Each of these was homogenized with MeOH several times and the residue air dried. The aq. mixture left after removal of MeOH and the dry residue were each contin-

Table 1. Composition (g/100 g dry wt) of various tissues of organ pipe cactus (Fig. 1)

Tissue	MeOH sol.*	Et_2O sol.†	Insol. residue‡
Skin	5.0	2.8	92.2
Photosynthetic layer	50.5	12.2	37.3
Transition zone	34.2	10.0	55.8
Cortex	15.0	8.1	76.9
Wood	3.5	3.6	92.9
Pith	12.6	7.3	80.1

* Triterpene glycosides † Lipids ‡ Carbohydrates

uously extracted several days with Et_2O to yield the 3 fractions listed in Table 1.

Hydrolysis of saponins. The saponins were hydrolyzed with 3N H_2SO_4 in H_2O–PrOH (2:1) on a steam bath for 24 hr. Hydrolysis with HCl in MeOH [2] failed to liberate much **3a** from its glycosides. The mixtures were cooled, diluted with H_2O and filtered to remove the aglycones. Portions of the filtrates were neutralized and analyzed (PC) for sugars.

Isolation of **1**, **2** *and* **3b**. Saponins (410 g) from the outer tissues of the cactus were hydrolyzed to yield 170 g of a *ca* 2:2:1 mixture of **1**, **2** and **3a**. The sapogenins were fractionated with Me_2CO, EtOH Et_2O plus aq. NaOH [2] and C_6H_6 to yield fractions rich in **1**, **2** and **3a** (25 g). **1** mp 300–4°, lit.[2] 308–10° and **2** mp 296–300°, lit. [2] 293–5° from $CHCl_3$–EtOH. The **3a** rich fraction was acetylated and chromatographed on Si gel with C_6H_6–$CHCl_3$ (4:1) and (2:1) and crystallized from $CHCl_3$–MeOH to give chromatographically pure **3b**, mp 297–300° (air) 302° (vac), $[\alpha]_D$ + 79.7° (c3, $CHCl_3$); lit. [9] mp 295–9°, [10] mp 292–5°, $[\alpha]_D$ + 82°.

Methyl queretaroate diacetate (**3c**). Diacetate **3b** was converted to the acid chloride ($SOCl_2$), treated with MeOH and reacetylated. **3c** was crystallized ×2 from C_6H_6–MeOH, mp 234–5° (air and vac), $[\alpha]_D$ + 79.6° (c3, $CHCl_3$); lit.[9] mp 210°, [8] mp 204–6°, $[\alpha]_D$ + 71.9°, [10] mp 211–12°, $[\alpha]_D$ + 68.5°. (Found: C, 73.7; H, 9.46. Calc for $C_{35}H_{54}O_6$: C, 73.7; H, 9.46%).

Methyl queretaroate (**3d**). Ester diacetate **3c** was hydrolyzed with NaOMe in MeOH, **3d** crystallized from C_6H_6–MeOH, mp 228–8.5° (air) 230.5–31° (vac), $[\alpha]_D$ + 69.6° (c3, $CHCl_3$); lit.[8] mp 226–8°, $[\alpha]_D$ + 77.8°, [10] mp 223–4°, $[\alpha]_D$ + 67°. (Found: C, 76.6; H, 10.32. Calc. for $C_{31}H_{50}O_4$: C, 76.5; H, 10.35%).

Queretaroic acid (**3a**). Diacetate **3b** hydrolyzed (KOH, EtOH) and **3a** filtered from solution after acidification, mp 345–8° (air) 345° (vac), $[\alpha]_D$ + 81.1° (c3, py); lit.[9] mp 318–20°, [10] mp 318–23°, [8] mp > 310°, $[\alpha]_D$ + 77.9° (Py).

Queretarol (**3e**). Ester diacetate **3c** was reduced ($LiAlH_4$, refl. THF), **3e** crystallized from Et_2O–Me_2CO–EtOH, mp 283–5° (air) 285–6° (vac), $[\alpha]_D$ +86.7° (c3, Py), +75.7 (c3, MeOH); lit. [8] mp 275–6°, $[\alpha]_D$ +85.5° (Py), [11] mp 269–72° [12] mp 280–1°, $[\alpha]_D$ +71.9° (MeOH).

Queretarol triacetate (**3f**). Recrystallized from petrol, mp 135–6° (air), $[\alpha]_D$ + 73.0° (c3, $CHCl_3$); lit.[12] mp 135–7°.

Drosophila *tests.* The saponin mixture was added to a *Drosophila* medium (bananas, yeast, corn syrup, malt extract, agar, propionic acid) to give a concentration equivalent to 30% of the dry wt of the medium. Bottles of media (5 each of control and test) were charged with *D. nigrospiracula* adults, and after the appearance of about 50 eggs, the adults were removed and the media observed for the next 3 weeks.

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