Investigation of Anthocyanins and Betalains in Mammillaria Vivapara Var. Vivapara

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ABSTRACT

A characterization of betalain and anthocyanin pigments from Christmas Cactus (Schlumbergera buckleyi) and Beehive cactus (Mammillaria vivapara var. vivapara) flower petals by LC/MS-MS/ESI was conducted. The analysis of the Christmas Cactus flower petals by mass spectrometry was compared to previous work. The molecular weights and retention times obtained for betalain and its many isomers allowed the assignment of several previously identified molecules. Further analysis of the data yielded several previously unknown betalain isomers. As reported in the research literature anthocyanins were not found in the mass spectrometry analysis of the Christmas Cactus flower petals. The analysis of the mass spectrometry data for the Beehive cactus petals was carried out and several betalain isomers common to the Christmas cactus were identified. In addition to the expected betalains two anthocyanins were identified in the flower petals. The identification of these anthocyanins in Mammillaria Vivapara var. vivapara is a novel discovery in the species.

INTRODUCTION

Anthocyanins and betalains are water soluble vacuolar pigments. Anthocyanins appear red to blue depending on pH. Betalains appear as red-violet betacyanins or as yellow betaxanthins. They are synthesized exclusively by organisms of the plant kingdom, and have been observed to occur in all tissues of higher plants, providing color in leaves, stems, roots, flowers, and fruits. In flowers, anthocyanin and betalain pigments function as pollinator attractants, and in fruits, the colorful skins attract animals which will eat the fruits and disperse the seeds. In photosynthetic tissues (such as plant leaves or the stems of cacti), anthocyanins and betalains have been shown to act as a "sunscreen", protecting cells from photodamage by absorbing UV and blue-green light, thereby protecting the tissues from photoinhibition, or high light stress. The chemical structure of anthocyanidins is based upon the flavonoid family of molecules and this in turn is based on the C6-C3-C6 configuration in the flavan nucleus. There are six common naturally occurring anthocyanidins. Figure 1 shows the structure of these anthocyanidins. Anthocyanins are anthocyanidins linked with one or more sugar moieties. The most common sugars are glucose, galactose, rhamnose, and arabinose. These sugars can also be acylated by acetic acid, malonic acid, and coumaric acid. Not all land plants contain anthocyanins and in the *Caryophyllales*, *Cactus* and *Galium mollugo* they are replaced by betacyanins which are based on betalamic acid (FIGURE 2) and vary depending on the components bonded to the main structure. Betacyanins (FIGURE 3) are formed when the group is 3,4-dihydroxyphenylalanine (DOPA), which may or may not be glycosylated. Betaxanthins are formed if the conjugation partners are amino acids or derived amines (FIGURE 4).

FIGURE 2.
Betalamic acid

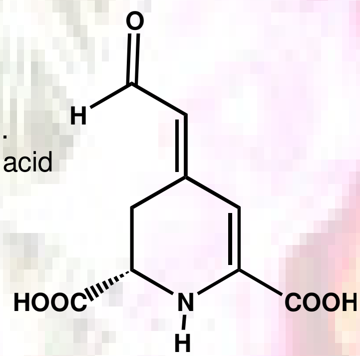
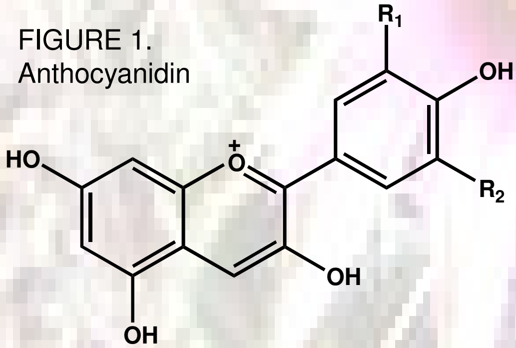
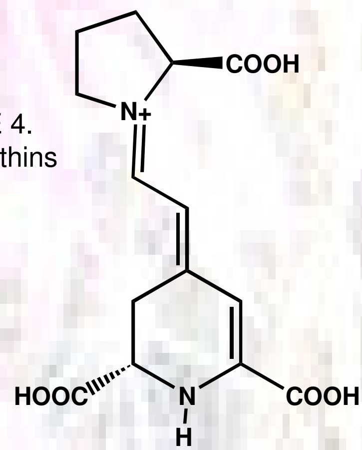


FIGURE 1.
Anthocyanidin



Anthocyanidin	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Peonidin	OCH_3	H
Delphinidin	OH	OH
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

FIGURE 4.
Betaxanthins



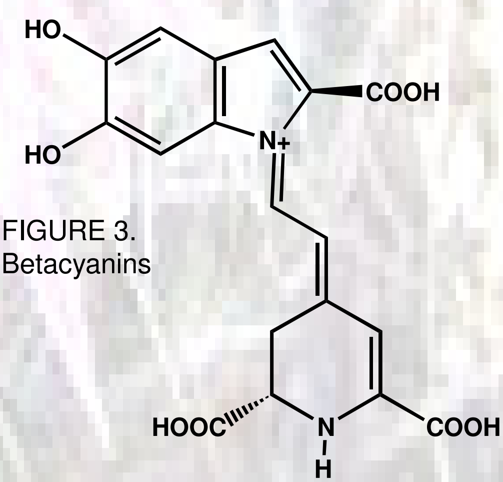


FIGURE 3.
Betacyanins

EXTRACTION AND ISOLATION OF PIGMENTS

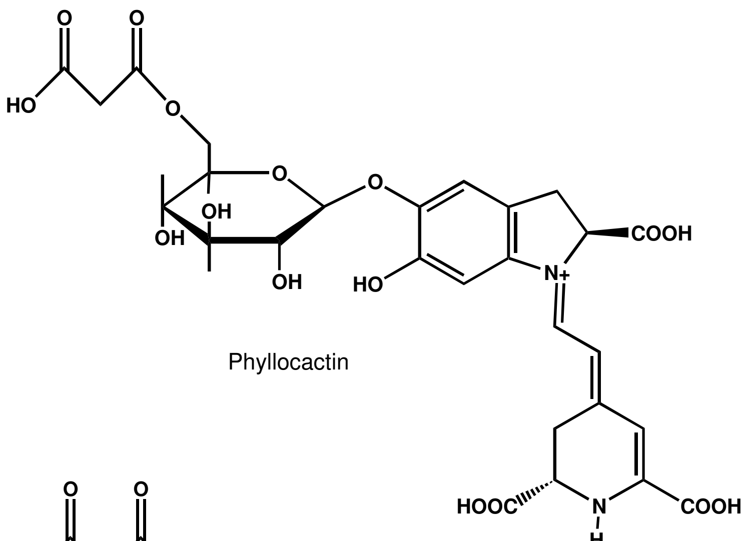
Fresh blooms from both the Christmas Cactus and Beehive Cactus were extracted by means of a procedure which treated the blooms with 50/50 methanol/ water with 0.1% formic acid, ground with a glass stirring rod and placed in sonicator for one hour to extract the anthocyanins and betalains. The mixture was placed in a glass-sintered funnel and the filtrate was washed three times with an equal volume of 50/50 ethyl acetate/cyclohexane, to remove flavonoid compounds. A small aliquot of the aqueous fraction was filtered through a 0.2 µm nylon syringe filter (Whatman Inc., Clifton, NJ) prior to introduction into the LC.

INSTRUMENTATION

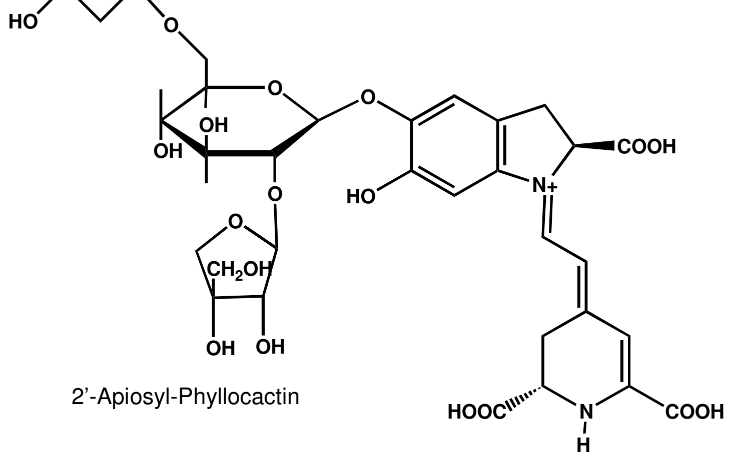
HPLC/DAD/ESI-MS/MS ANALYSES. LC/ESI-MS/MS experiments were performed on an Agilent MSD XCT ion trap mass spectrometer (Palo Alto, CA) equipped with an electrospray ionization (ESI) interface, 1100 HPLC, a DAD detector, and Chemstation software. The column used was a 150 x .5 mm i.d., Zorbax SB- C18 5 µm (Agilent, Palo Alto, CA). Solvents were (A) 0.1% formic acid/ 99.9% water (v/v) and (B) 0.1% formic acid/ 99.9% acetonitrile (v/v). Solvent gradient was 0-20 min, 10-50% B; 20-31 min, 50-10% B; and 31-35 min, 10% B. Flow rate was 6.000 µL/min, injection volume was 0.5µL, and column temperature was 25 °C. The ESI parameters were as follows: nebulizer, 13 psi; dry gas (N2), 4.00 L/min; dry temperature, 325 °C; trap drive, 76.5; skim 1, 40 V; lens 1, -5.00 V; octopole RF amplitude, 150 Vpp; capillary exit, 158.5 V. The ion trap mass spectrometer was operated in positive ion mode scanning from m/z 100 to m/z 2200 at a scan resolution of 13000 amu/s. Trap ICC was 30000 units and maximal accumulation time was 300000 µs. MS-MS was operated at a fragmentation amplitude of 1.2 V and threshold ABS was 3,000,000 units.

A. CHRISTMAS CACTUS DATA

Compound	M+H
1. Vulgaxanthin I	341
2. Betanin	551
3. Phyllocactin	637
4. 2'-Apiosyl-Phyllocactin	769
5. 5''-O-E-feruloyl-2'-apiosyl-betanin	859
6. 5''-O-E-feruloyl-2'-apiosyl-phyllocactin	945

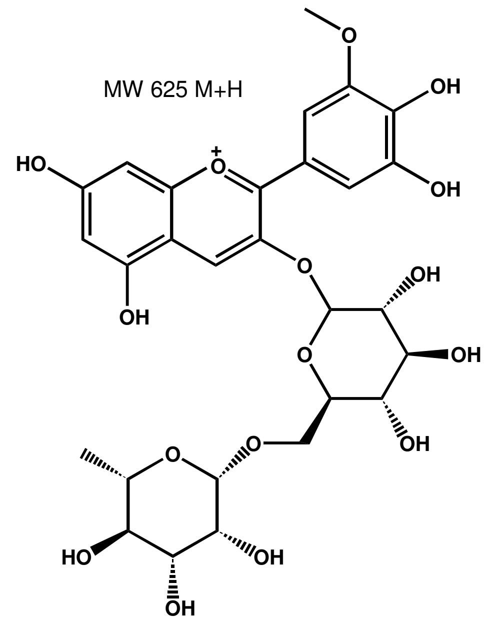


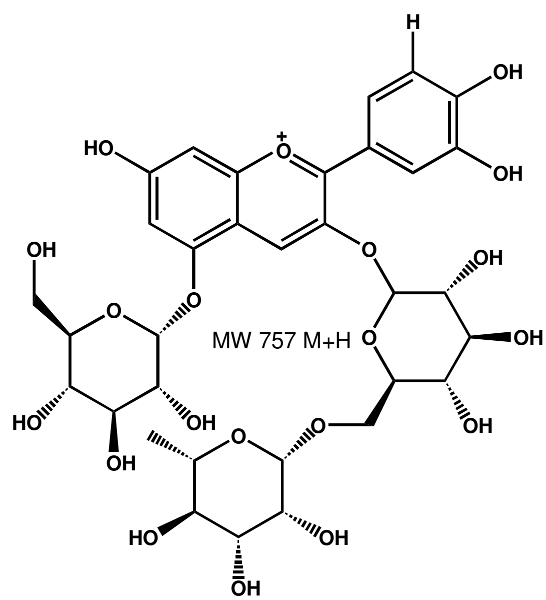
Phyllocactin



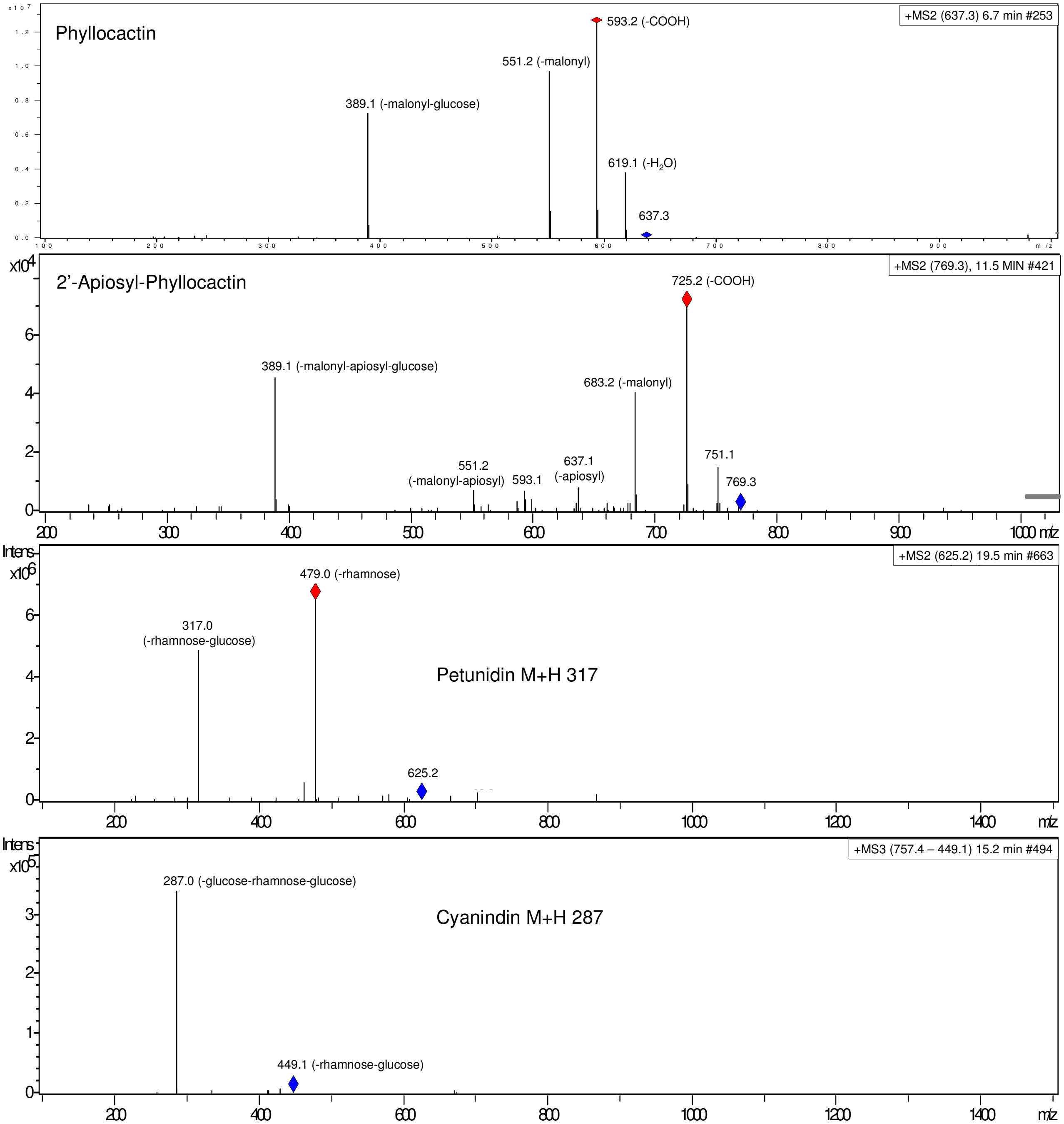
2'-Apiosyl-Phyllocactin

B. BEEHIVE CACTUS DATA





MASS SPECTRAL DATA



CONCLUSION

According to recent reviews in betalain research, the betalains replace the anthocyanins in flowers and fruits of plants of most families of the order Caryophyllales with the exception of the suborder Caryophyllineae and its Caryophyllaceae and Molluginaceae families.[1] Previous work and our work on Chrismas Cactus seems to support this observation that the families of the Caryophyllales produce only betalains.[2] Our work on the intact bloom (sepals and petals) did find phyllocactin and 2'-apiosyl phyllocactin in the extract; however, the discovery of the two anthocyanin pigments based on cyanindin and petunidin in the petals of the Beehive Cactus seem to contradict the conventional wisdom and classification of this cactus (Mammillaria vivapara var. vivapara) as an anthocyanin-free plant. The cacti diverged with respect to evolutionary development rather recently from other plants that have maintained their anthocyanin-producing abilities and even members of the same order as the cacti produce anthocyanins. We believe that the identification of these anthocyanin pigments in the petals of the Beehive Cactus represents a significant discovery and may require the reclassification of the Mammillaria family.

FUTURE WORK

We plan to extract and analyze pigments from the Beehive Cactus and other members of the Mammillaria family in order to ascertain whether or not there are anthocyanin pigments present in all members of the family. Additionally, we are curious as to whether or not there are similar anthocyanin pigments in other closely related families of cacti.

REFERENCES

1. Dieter Strack, Thomas Vogt, and Willibald Schliemann. Recent advances in betalain research. Phytochemistry 62 (2003) 247-269.
2. Naoko Kobayashi, Jurgen Schmidt, Manfred Nimtz, Victor Wray, and Willibald Schliemann. Betalains from Christmas cactus. Phytochemistry 54 (2000) 419-426.