SENITA ALKALOIDS: NO INHIBITION OF STEROL BIOSYNTHESIS IN YEASTS OR CACTI

JAMES C. FOGLEMAN
Department of Ecology and Evolutionary Biology

HENRY W. KIRCHER
Department of Nutrition and Food Science, The University of Arizona, Tucson, AZ 85721

Senita cactus, Lophocereus schottii (Englem.) Br. and R., lacks typical Δ⁷ phytosterols and instead contains 4α-methyl, Δ⁷ and Δ⁸,14 sterols as well as several unusual alkaloids (lophocereine, its dimer and trimer) (1-5). 4α-Methyl, Δ⁷ and Δ⁸,14 sterols have been shown to accumulate when nitrogenous inhibitors of several steps in the normal biosynthetic pathway were added to rat liver homogenates (6) or chlorella (7,8), yeast (9,10) and bramble cell (11,12) cultures. Based on these observations, it was hypothesized that senita alkaloids may inhibit normal phytosterol biosynthetic reactions in the cactus and cause a buildup of the observed intermediate sterols (5). In this paper we present the sterol composition of several yeasts grown with or without senita alkaloids in their medium and of Backebergia militaris (Andot.) Bravo ex Sánchez Mejorada, a cactus related to senita (13).

Thirteen cactophilic yeast species were tested for their ability to grow on a complete medium supplemented with senita alkaloids (0.6 g crude alkaloids per 100 ml medium) at 25°. Included were Pichia cactophila, P. amethionina amethionina, P. amethionina pachycereana, P. mexicana, P. mexicana, P. heedii, Candida ingens, C. sonorensis, C. mucilagina, C. species “A”, C. species “K”, Cryptococcus cereanus, Clavispora opuntiae, and Kluyveromyces marxianus. These species were all isolated from necroses of columnar cacti in the Sonoran Desert (14). All species grew on this medium as they did on control plates without alkaloids. Since the average concentration of alkaloids in senita cactus is about 0.7 g per 100 g of fresh tissue (4), the supplemented medium contained a comparable concentration. Although senita alkaloids did not inhibit the absolute growth of the yeasts, their effect on the growth rate of yeasts remains undetermined. None of the yeast species could use the alkaloids as a nitrogen source.

Six of the thirteen species listed above were chosen for the test of the effect of alkaloids on sterol biosynthesis. After extraction and saponification of the lipids in the yeasts, gc of the free sterols and gc and AgNO₃-tlc of the steryl acetates showed the sterols present to be principally ergosterol with traces of zymosterol. No 4α-methyl, Δ⁷ or Δ⁸,14 sterols comparable to those in senita were detected. The ergosterol contents of the six yeast species grown on YM-agar with and without alkaloids (0.4 g per 100 ml) are shown in table 1. Although both the average dry weight of yeast harvested and the average ergosterol concentration were slightly greater on the medium containing alkaloids, these increases are not statistically significant.

One of the six species, C. ingens, was grown in YM-broth plus alkaloids (0.4 g/100 ml) in order to determine if this condition represents a qualitative difference from growth on agar plates. Broth culture more closely resembles the natural situation in a necrotic cactus. Again, only the typical yeast sterols were detected.

Alkaloids were observed on tlc plates in the extracts of all six yeasts. While this could be due to either the presence of alkaloids inside the yeast cells or the adherence of alkaloids to the outer surface of the cells, they were still detectable after cells were
Table 1. Ergosterol in yeasts grown on a medium with and without senita alkaloids (0.4 g/100 ml).

<table>
<thead>
<tr>
<th>Yeast Species</th>
<th>U. of Ariz. Strain No.</th>
<th>Dry Wt. (g) From 25 Plates</th>
<th>Ergosterol Conc. (mg/g dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With Alkaloids</td>
<td>Without Alkaloids</td>
<td>With Alkaloids</td>
</tr>
<tr>
<td><strong>P. capitophila</strong>*</td>
<td>80-328.1</td>
<td>1.71</td>
<td>0.69</td>
</tr>
<tr>
<td><strong>P. opuntiae</strong>*</td>
<td>76-211</td>
<td>1.43</td>
<td>1.20</td>
</tr>
<tr>
<td><strong>P. mexicana</strong>*</td>
<td>80-124.1</td>
<td>2.41</td>
<td>1.98</td>
</tr>
<tr>
<td><strong>C. sonorensis</strong>*</td>
<td>80-230.4</td>
<td>1.51</td>
<td>1.47</td>
</tr>
<tr>
<td><strong>C. ingens</strong>*</td>
<td>81-208.3</td>
<td>2.23</td>
<td>1.92</td>
</tr>
<tr>
<td><strong>Cr. cereusus</strong>*</td>
<td>79-259.1</td>
<td>1.74</td>
<td>1.60</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>1.84</td>
<td>1.48</td>
<td></td>
</tr>
</tbody>
</table>

*Have been isolated from senita rots in nature.

THE CACTUS *Backebergia militaris* is phylogenetically related to senita in that they both belong to the subtribe Pachycereinae (13). However, unlike senita, it contains only a small amount of less complex alkaloids (0.75% of 5,6-dimethoxyisoquinoline and 0.04% 3,4-dimethoxyphenylethylamine) (15). The non-saponifiable portion of the lipids from *Backebergia* were compared to those from senita by tlc and gc (4,5). In both instances (fig. 1) the *Backebergia* sterols resembled those from senita, again supporting the view that senita alkaloids have no effect on sterol biosynthesis in this cactus. The factors which do cause the interruption of the normal sequence of sterol biosynthesis in *Backebergia militaris* and senita remain to be determined.

**EXPERIMENTAL**

**ALKALOIDS.**—Outer tissues of two fresh senita cactus arms were extracted with methanol followed by chloroform, and the combined evaporated extracts were partitioned between ether and dilute HCl. The aqueous phase was made alkaline with conc. aq NH₃ and extracted with CH₃Cl₂. The crude alkaloids were purified through an-
and evaporated to dryness in vacuo. The residues were dissolved in 3 ml benzene for quantitative gc (5% OV-101, 250). and these solutions were acetylated (acetic anhydride-pyridine) for AgNO₃-tlc (chloroform) and gc. The HCl extracts were made basic (NaOH), and other extracted for alkaloid tlc (4).

Candida ingens grown in broth culture containing alkaloids (0.4 g/100 ml) was harvested after two weeks by centrifugation, washed briefly with 1 N HCl and then water, and analyzed for sterols and alkaloids as above.

Backebergia Militaris.—A small arm of the cactus was cut into pieces, air dried and extracted twice with 2:1 chloroform-methanol. The combined extracts were evaporated and partitioned between ether and water; 5.31 g (75% of dry wt) of ether soluble lipids were obtained. The ether extract was saponified, and the non-saponifiable fraction compared to that of senita cactus by gc and by tlc on AgNO₃ plates (4).

ACKNOWLEDGMENTS
This work was supported by NSF grant DEB 7912924 to H. W. Kircher and W. B.
Heed. We thank Dr. Arthur Gibson for the sample of Backebergia militaris.

Received 13 July 1988

LITERATURE CITED

8. L. G. Dickson and G. W. Patterson, Lipids, 7, 635 (1972).