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

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Senita cactus, *Lophocereus schottii* (Englem.) Br. and R., lacks typical Δ^5 phytosterols and instead contains 4α-methyl, Δ^7 and $\Delta^{8,14}$ sterols as well as several unusual alkaloids (lophocereine, its dimer and trimer) (1–5). 4α-Methyl, Δ^8 and $\Delta^{8,14}$ 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. opuntiae, 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 $AgNO_3$-tlc of the steryl acetates showed the sterols present to be principally ergosterol with traces of zymosterol. No 4α-methyl, Δ^7 or $\Delta^{8,14}$ 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).

Yeast Species	U. of Ariz. Strain No.	Dry Wt. (g) From 25 Plates		Ergosterol Conc. (mg/g dry wt.)	
		With Alkaloids	Without Alkaloids	With Alkaloids	Without Alkaloids
*P. cactophila**	80-328.1	1.71	0.69	4.4	4.3
*P. opuntiae**	76-211	1.43	1.20	7.6	3.4
P. mexicana	80-124.1	2.41	1.98	8.1	7.0
*C. sonorensis**	80-230.4	1.51	1.47	5.5	5.2
*C. ingens**	81-208.3	2.23	1.92	3.8	5.7
*Cr. cereanus**	79-259.1	1.74	1.60	9.7	8.2
Average		1.84	1.48	6.5	5.6

*Have been isolated from senita rots in nature.

briefly washed with 1.0 N HCl before saponification. Thus, the observation that senita alkaloids appear to be taken up by cactophilic yeasts but do not inhibit or interrupt ergosterol biosynthesis by the cells does not support the hypothesis that these alkaloids are responsible for the accumulation of phytosterol intermediates in senita cactus (5).

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 *Backerbergia* 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 NH_3 and extracted with CH_2Cl_2. The crude alkaloids were purified through another ether-HCl-NH_3-ether sequence followed by solution in petroleum ether, filtration, and evaporation; 57 g (4% of dry matter, 0.7% of fresh tissue) of purified senita alkaloids (4) was obtained. The alkaloids were diluted with ethanol to 1 g/3 ml for addition to the yeast media.

PREPARATION OF MEDIA.—a) Yeast Extract-Malt Extract (Difco YM) agar (4.1 g/90 ml H_2O) and 10 ml 0.3 N HCl were separately autoclaved; 1.8 ml of alkaloid solution was added to the latter, and the two solutions were combined. This mixture, when poured into sterile Petri plates, gave a test medium of pH 4.0 containing 0.6% senita alkaloids. Control plates without alkaloids were similarly prepared.

b) Difco YM broth (2.1 g/90 ml) and 10 ml 0.2 N HCl were separately autoclaved and 1.2 ml of alkaloid solution was added to the latter; mixing the two solutions, gave a broth of pH 4.5 containing 0.4% alkaloids.

c) Difco yeast carbon base-agar medium was supplemented with senita alkaloids (0.2 and 0.4 g/100 ml) to test the ability of the alkaloids to serve as a nitrogen source for yeast growth.

GROWTH TESTS.—Strains of each of the cactophilic yeasts were streaked on YM agar (without alkaloids) and grown at 37° for 48 hr. Yeast suspensions were made by placing a loopful of each 48 hr yeast culture in a small amount of sterile water. A mechanical replica plater was then used to transfer a small drop of the suspension of each yeast species to the test media. Plate order in the replica series was YM agar+alkaloids, YCB agar+alkaloids, YM agar (control).

ANALYSIS.—Yeasts grown for 5 days on YM agar±alkaloids were washed from the plates with a stream of water, centrifuged, washed twice with water, dried in vacuo, and weighed. They were hydrolyzed on the steam bath overnight in loosely stoppered flasks under N_2 with NaOH and pyrogallol (9) in aqueous alcohol. The resulting mixtures were cooled and diluted with water, and ether extracted. The ether solutions were extracted with 1 N HCl to remove alkaloids, washed with water

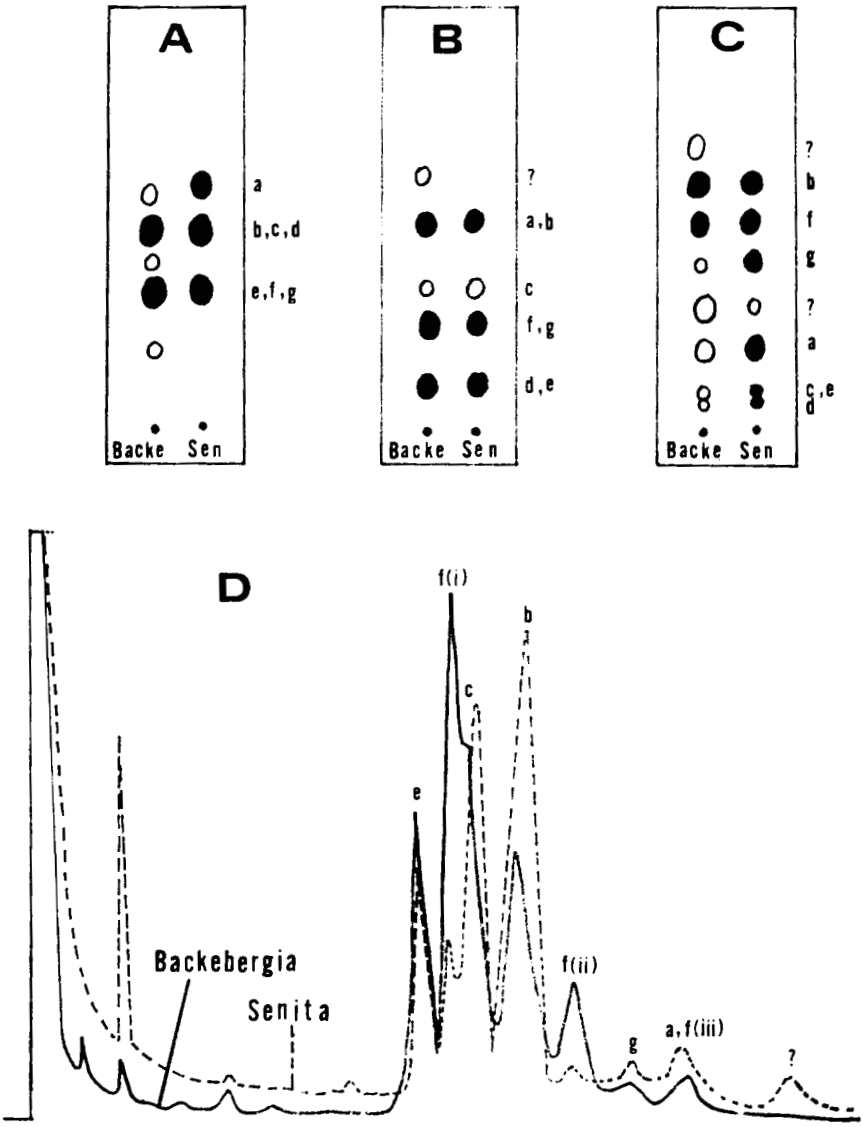

FIG. 1. Chromatographic comparisons of *Backebergia militaris* and senita cactus non-saponifiable fractions. TLC: **A**. 60/40 hexane-EtOAc, Sigel plate. **B**. 95/5 $CHCl_3$-Me_2CO, $AgNO_3$ Plate, 2 X. **C**. CH_2Cl_2, $AgNO_3$ plate, 2 X, acetates. GC: **D**. 5% OV-101, 270°.
a) lup-20(22)-en-3β-ol, b) 4α-methylcholest-7-en-3β-ol, c) 4α-methylcholesta-8,14-dien-3β-ol, d) 4α-methylergosta-7,24(28)-dien-3β-ol, e) cholesta-8,14-dien-3β-ol, f) (i) cholest-7-en-3β-ol, (ii) campest-7-en-3β-ol, (iii) stigmast-7-en-3β-ol, g) stigmasta-7,22-dien-3β-ol.

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 $AgNO_3$-tlc (chloroform) and gc. The HCl extracts were made basic (NaOH), and ether 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 (7% 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 $AgNO_3$ plates (4).

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