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THE DISTRIBUTION OF STEROLS, ALKALOIDS AND FATTY ACIDS IN SENITA CACTUS, *LOPHOCEREUS SCHOTTII*, OVER ITS RANGE IN SONORA, MEXICO

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Abstract—Young and old stems of senita cactus have been examined for their sterol, alkaloid and fatty acid content. No systematic cline was observed when these phytochemical characteristics were compared with the location in Sonora where the plants were gathered. Instead, there were significant differences between young and mature stems of a single plant and between the epidermis and cortex of a single stem. These differences did not correlate with plant morphology or with a chromosomal inversion in *Drosophila pachea*, an insect that uses senita as its sole breeding site.

INTRODUCTION

SENITA, *Lophocereus schottii* (Engelmann) Britton and Rose, is a large columnar cactus abundant in the states of Sonora and Baja California, Mexico. Three varieties of senita have been described, two of which, *L. schottii* var. *schottii* and *L. schottii* var. *tenuis*, grow in Sonora.[1] Felger and Lowe reported a clinal variation in the surface–volume ratio and number of stem ribs in senita.[2] Larger, thicker (13–18 cm dia.) stems with fewer ribs are observed in northern Sonora (var. *schottii*). Shorter, thinner stems (7–12 cm dia.), with a larger surface–volume ratio (var. *tenuis*), occur in southern Sonora where there is more rainfall. In both areas, the green epidermis covers a pale green cortex in young stems, a color which changes to a golden yellow as the stems mature. On very old stems the epidermis turns grey and the cortex orange. A tubular woody skeleton filled with pith develops within the stems as they age.

Senita is an unusual plant. It contains at least five sterols, of which only two have been described, lophenol (4-α-methyl-Δ^7-cholesten-3β-ol) and schottenol (Δ^7-stigmasten-3β-ol).[3] Schottenol appears to be the main reason why a particular species of fruit fly, *Drosophila pachea*, uses senita as its sole breeding site.[4] The cactus is also rich in alkaloids. Djerassi and co-workers[5–7] isolated small quantities of lophocereine (1-isobutyl-2-methyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline) and larger amounts of two lophocereine trimers, pilocereine and piloceredine. The latter two are structurally the largest cactus alkaloids

[1] G. LINDSAY, *Cactus Succ. J.* **35**, 176 (1963).
[2] R. S. FELGER and C. H. LOWE, *Ecology* **48**, 530 (1967).
[3] C. DJERASSI, G. W. KRAKOWER, A. J. LEMIN, H. H. LIU, J. S. MILLS and R. VILLOTTI, *J. Am. Chem. Soc.* **80**, 6284 (1958).
[4] W. B. HEED and H. W. KIRCHER, *Science* **149**, 758 (1965).
[5] C. DJERASSI, N. FRICK and L. E. GELLER, *J. Am. Chem. Soc.* **75**, 3632 (1953).
[6] C. DJERASSI, T. NAKANO and J. M. BOBBITT, *Tetrahedron* **2**, 58 (1958).
[7] C. DJERASSI, H. W. BREWER, C. CLARK and L. J. DURHAM, *J. Am. Chem. Soc.* **84**, 3210 (1962).

known. These compounds are toxic to Sonoran species of *Drosophila* other than *D. pachea*, and prevent them from using rotting senita cactus as a breeding site.[8]

An added impetus for the initiation of this work was the discovery of an inversion in the salivary chromosomes of *D. pachea*.[9] Populations of this fly in northern Sonora had a much higher incidence of this genetic marker than those in the south. The purpose of this study was therefore threefold: (1) to correlate differences in the lipid content of senita with morphological differences, (2) to correlate differences in the lipid content with chromosomal variations in *D. pachea*, and (3) to determine which plants or parts of plants are the best sources of the three unknown sterols.

RESULTS

Senita was collected from October 1967 to February 1968 in the places indicated on Fig. 1, dried *in vacuo*, and extracted with 2:1 $CHCl_3$–MeOH. The extracts of young stems varied from 9 to 25 per cent and of mature stems from 15 to 21 per cent of the dry plant material. Except for a few cases, between 45 and 65 per cent of the extracts were recovered in three

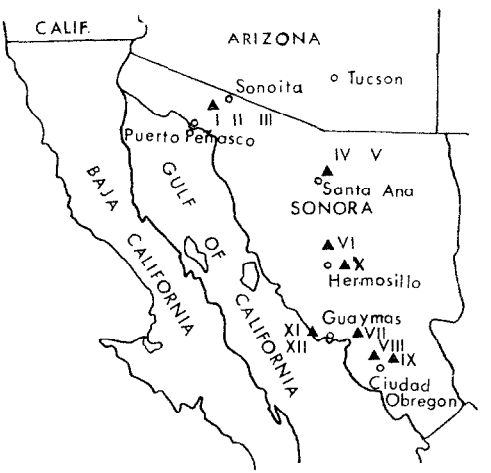


FIG. 1. MAP OF SONORA, MEXICO, SHOWING SENITA COLLECTION SITES.
Triangles and Roman numerals represent individual cacti that were sampled and that are represented in subsequent figures.

crude fractions: non-saponifiables, fatty acids (plus chlorophytin), and alkaloids; the remainder of the $CHCl_3$–MeOH extracts was water-soluble material. The contents of the three fractions in whole stems of nine plants collected from Sonoita to Ciudad Obregon is shown in Fig. 2. The contents of the epidermis and cortex (and of the wood and pith in one case) of young and mature stems of three plants collected near Hermosillo and Guaymas is shown in Fig. 3. The crude extracts of the epidermi (15–44 per cent) varied more than those of the cortexes (8·5–23 per cent of the dry plant material).

The fatty acid fractions from all plants had as their main constituents palmitic, oleic, linoleic, and linolenic acids (Fig. 4*a–h*). Greatest differences were observed between young and mature stems of a single plant (4*a,b*) and between mature stems in northern plants vs.

[8] H. W. KIRCHER, W. B. HEED, J. S. RUSSELL and J. GROVE, *J. Insect Physiol.* **13**, 1869 (1967).
[9] B. L. WARD, W. B. HEED and J. S. RUSSELL, *Genetics* **60**, 235 (1968).

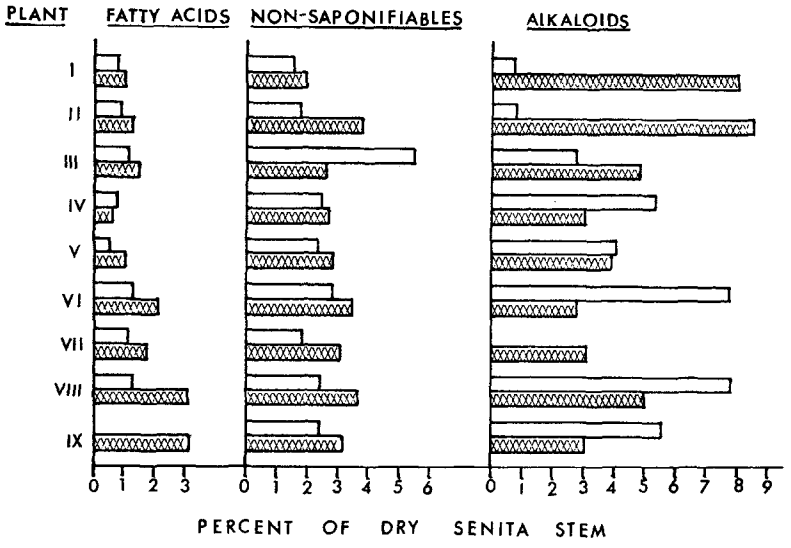


FIG. 2. FATTY ACID (INCLUDING CHLOROPHYTIN), NON-SAPONIFIABLE, AND ALKALOID CONTENTS OF YOUNG (OPEN BARS) AND MATURE STEMS (SHADED BARS) FROM NINE SENITA CACTI.

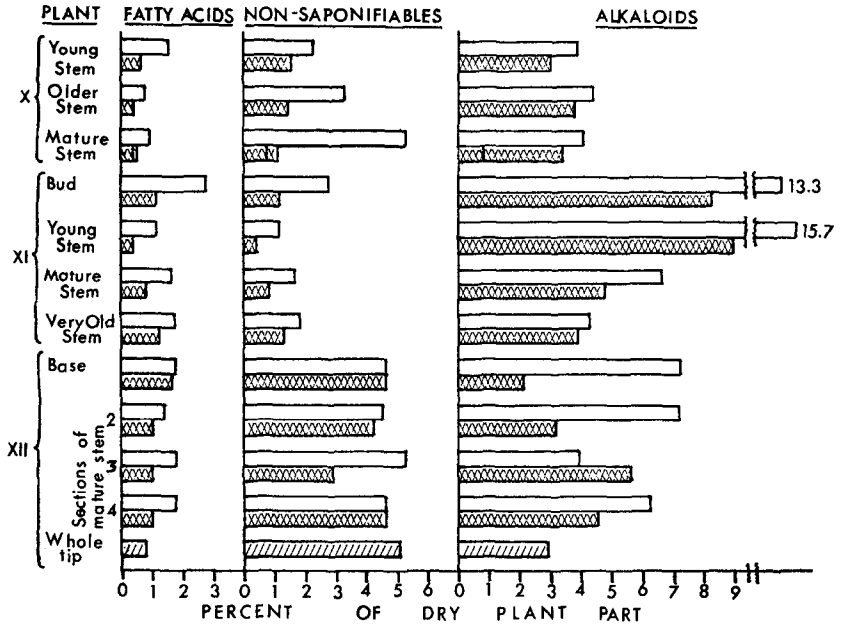


FIG. 3. FATTY ACID (INCLUDING CHLOROPHYTIN), NON-SAPONIFIABLE, AND ALKALOID CONTENTS OF THE EPIDERMIS (OPEN BARS) AND CORTEX OF THREE SENITA CACTI.

A mature stem from plant XII was cut from its base to its tip into five 25-cm sections. The dotted lines in the shaded bar of plant X, mature stem, represent the constituents in the woody skeleton and pith of this stem.

southern plants (4*c*, *d*). In general, younger stems contained more linolenic acid and relatively smaller amounts of acids of carbon number less than 16 than mature stems. Mature stems in southern Sonora were very rich in these shorter chain fatty acids. The epidermis was richer in linolenic acid than the cortex of a single stem (4*e*, *f*), a difference which was reduced as the stem matured (4*g*, *h*). There are very little difference in the fatty acid pattern in the epidermis and cortex of different sections along the length of a single long stem.

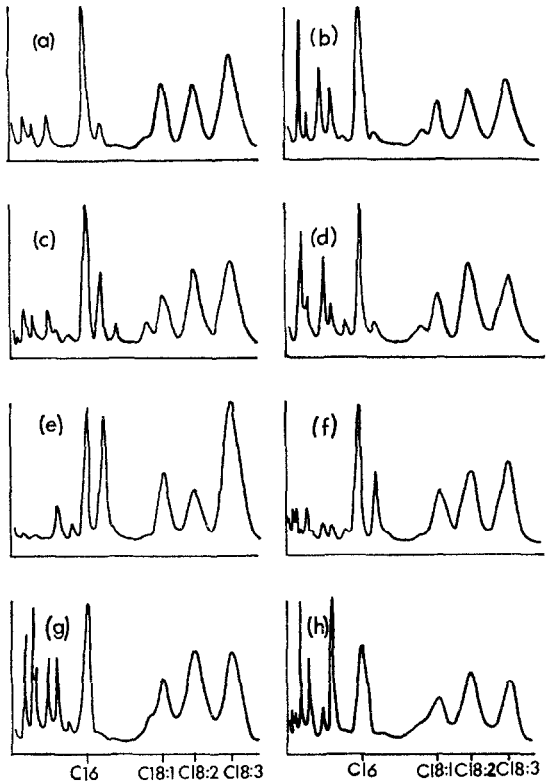


FIG. 4. GLC SEPARATION DIAGRAMS OF FATTY ACID METHYL ESTERS.

(*a*) Plant IV, young stem
(*b*) Plant IV, mature stem
(*c*) Plant I, mature stem
(*d*) Plant IX, mature stem
(*e*) Plant XI, young stem epidermis
(*f*) Plant XI, young stem cortex
(*g*) Plant XI, mature stem epidermis
(*h*) Plant XI, mature stem cortex

TLC of the non-saponifiable fractions from the various plants gave five to seven spots in the sterol–triterpene area of the plates (Fig. 5). Three of the spots corresponded in R_f to available reference materials, lupeol, lophenol, and schottenol, all of which have been identified in senita by Djerassi.[3]

GLC separation diagrams of the silyl ethers of the constituents of the non-saponifiable fractions of young and mature stems from plants collected along the length of Sonora are shown in Fig. 6. No consistent changes in the composition of these fractions were apparent. The younger stems were richer in unknown sterols, schottenol, and lupeol, the older stems had proportionately more lophenol.

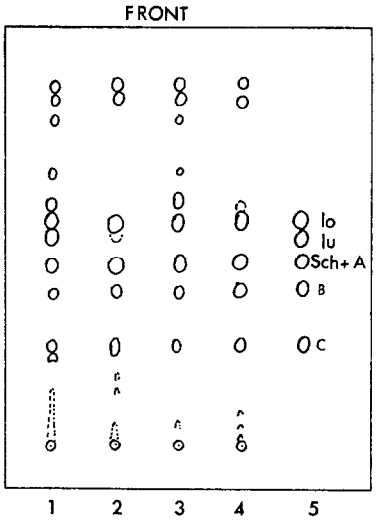


FIG. 5. TLC OF THE NON-SAPONIFIABLE FRACTIONS FROM PLANT XI.
(1) Young stem epidermis, (2) young stem cortex, (3) mature stem epidermis, (4) mature stem cortex, (5) reference compounds—lophenol (lo), lupeol (lu), schottenol (sch), unknown sterol (A), unknown sterol (B), unknown sterol (C).

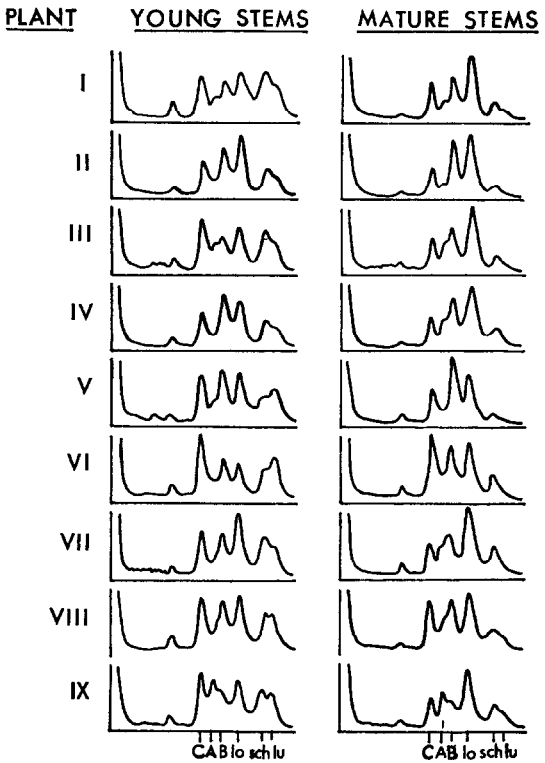


FIG. 6. GLC SEPARATION DIAGRAMS OF THE SILYLATED NON-SAPONIFIABLE FRACTIONS FROM YOUNG AND MATURE STEMS OF SENITA PLANTS I TO IX.
Retention times—lophenol (lo), lupeol (lu), schottenol (sch), unknown sterols A, B, and C.

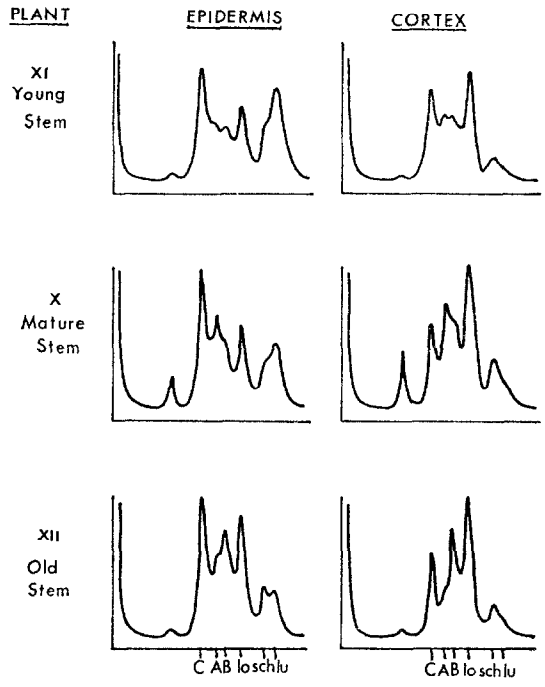


FIG. 7. GLC SEPARATION DIAGRAMS OF THE SILYLATED NON-SAPONIFIABLE FRACTIONS FROM THE EPIDERMIS AND CORTEX OF A YOUNG, MATURE, AND OLD SENITA STEM.
Retention times—lophenol (lo), lupeol (lu), schottenol (sch), unknown sterols A, B, and C.

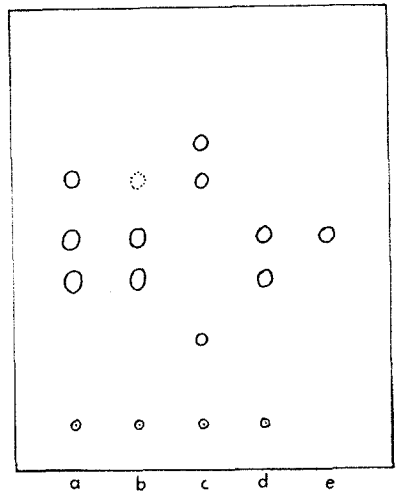


FIG. 8. TLC OF THE CRUDE ALKALOID FRACTIONS FROM A YOUNG (a) AND MATURE (b) STEM OF SENITA, THE PHENOLIC ALKALOID FRACTION (c), THE NON-PHENOLIC ALKALOID FRACTION (d), AND PILOCEREINE (e).

Comparisons of the non-saponifiable fractions of the epidermis and cortex of single stems are shown in Fig. 7. The epidermis were richer in two of the unknown sterols and lupeol, the cortexes were richer in lophenol.

TLC of the alkaloid fractions revealed no north–south changes in alkaloid composition. Young stems from two plants contained a higher proportion of phenolic alkaloids in relation to total alkaloids (0·4, 0·5 per cent phenolic vs. 1·1, 1·2 per cent total) than older stems from the same two plants (0·7, 0·6 per cent phenolic vs. 8·7, 9·1 per cent total). The distribution of alkaloids by type did not appear to vary between the epidermis and cortex of a single stem as did the fatty acid and non-saponifiable fractions. Typical results are shown in Fig. 8. The large spot below pilocereine may be piloceredine; it is not known at present which of the phenolic alkaloid spots correspond to lophocereine.

DISCUSSION

There is no apparent cline in either the content or composition of the fatty acid, non-saponifiable or alkaloid fractions of senita that correlate with the morphological changes in the cactus[1,2] or with the chromosomal inversion frequencies in *Drosophila pachea*.[9] The only consistent difference noted is the presence of a higher proportion of short-chain (less than C16) fatty acids in older stems in central and southern Sonora.

Greater compositional differences were observed between parts of a single plant than between plants 350 miles (580 km) apart. In general, lophenol concentrations are higher in older stems and in the cortex rather than the epidermis. The three unknown sterols and triterpenes are most abundant in the epidermis of younger stems. Preliminary evidence (i.r. spectra and TLC mobility) suggests that two of the sterols may be the elusive $\Delta^{8,14}$ dienes recently postulated[10,11] to be intermediates in the decarboxylative removal of the 14-α-methyl group of the sterol nucleus.[12]

A more general conclusion relates to the phytochemical variability that is found in a single species of a long-lived plant. One should be chary of assigning numerical data to the types and amounts of compounds in a particular plant with the idea that these figures are representative for all plants of that species. One can assume *a priori* that the biochemical composition of tissues reflects their function, yet when the functions of compounds such as sterols, fatty acids and alkaloids in plants are still largely unknown, the correlation of phytochemical content with biological function remains obscure.

EXPERIMENTAL

Extraction and Separation

Portions of senita collected in the field were sliced into strips and dried *in vacuo* at 50–60°. A fresh stem contains 80–90 per cent water. The dry material was ground to a free-flowing powder in a blendor. From 20 to 40 g of the dry plant material was stirred overnight with 200 ml 2:1 $CHCl_3$–MeOH and filtered. The filter cake was washed with fresh solvent and the combined filtrates evaporated in a tared flask. After saponification with 10 vols. of 7% KOH in 95% ethanol under N_2 at room temperature for 24 hr, water was added and the non-acid material (sterols, waxes, carotenoids, triterpenes, non-phenolic alkaloids) was extracted with ether. The ether layer was extracted with 10% H_2SO_4 to remove the alkaloids, washed with water, and dried in a tared flask (non-saponifiable fraction). The acid solution was made basic with conc.

[10] L. Canonica, A. Fiecchi, M. Galli Kienle, A. Scala, G. Galli, E. Grossi Paoletti and R. Paoletti, *J. Am. Chem. Soc.* **90**, 6532 (1968).

[11] M. Akhtar, I. A. Watkinson, A. D. Rahimtula, D. C. Wilton and K. A. Munday, *Chem. Commun.* 1406 (1968).

[12] Dr T. J. Scallen, Univ. of New Mexico, personal communication.

NH_4OH and extracted with $CHCl_3$ to yield the crude alkaloids. Finally, the aqueous alkaline solution from the saponification was acidified and extracted with ether to give the fatty acids plus chlorophytin (from saponification of chlorophyll). It was then made alkaline in a few cases with conc. NH_4OH and the phenolic alkaloids extracted with ether.

Chromatography

(*a*) *Non-saponifiables.* These fractions were analyzed by TLC and GLC. The TLC system used 10% $AgNO_3$ on silica gel plates, activated at 120° for 20–30 min just prior to use, with 95:5 $CHCl_3$–acetone as the solvent and 30% H_2SO_4 as the spray. The fractions were also silylated with bis(trimethylsilyl)acetamide[13] for GLC on a 1·8 × 4 mm stainless-steel column, 3% QF-1 on 80/90 Anachrom SD, ^{90}Sr detector, at 215° and 40 psig argon; Research Specialties Co. instrument.

(*b*) *Acid fractions.* These were methylated at room temperature overnight with 7% BF_3 in methanol and extracted from the mixture with petroleum ether after dilution with water. The methyl esters were analyzed by GLC on a 20% DEGS on Chromosorb P column on the same machine as above, 215°, 20 psig argon.

(*c*) *Alkaloid fractions.* These were analyzed by TLC on silica gel G plates with benzene–EtOH–5% aqu NH_4OH, 150:25:7·5 v/v; the spray was a modified Dragendorff reagent.[14] Most reproducible results were obtained with plates that had not been heat activated.

Acknowledgements—The best conditions for TLC of the alkaloid fractions were worked out by James Chafoulas. This work was supported by NSF Grant GB-6996 to W. B. Heed and H. W. Kircher.

[13] J. F. KLEBE, H. FINKBEINER and D. M. WHITE, *J. Am. Chem. Soc.* **88**, 3390 (1966).
[14] J. ZARNAK and S. PFEIFER, *Die Pharmazie* **19**, 216 (1964).

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