SOME ALKALOIDS AND OTHER CONSTITUENTS OF *ZANTHOXYLUM MICROCARPUM* AND *Z. PROCERUM*[1]

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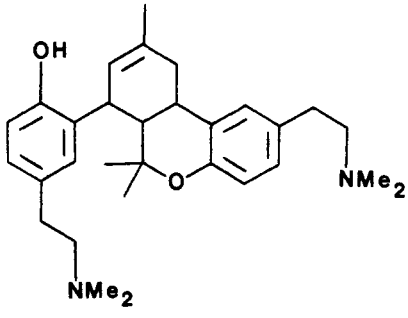
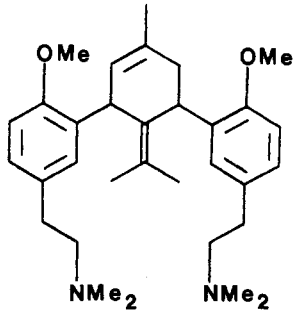
ABSTRACT.—Magnoflorine (**1**), haplopine (**2**) and hordenine (**3**) were isolated from the wood of *Zanthoxylum microcarpum* Griseb. (Rutaceae), while the bark yielded magnoflorine, decarine (**4**) and *N*-nornitidine (**5**). Small twigs and branches yielded *N*-methyltyramine (**6**) and 5,6-dihydro-6-hydroxymethylfagaridine (**8**). Hordenine was also found in the leaves. *N*-nornitidine was previously known as a synthetic, but not as a natural product. 5,6-Dihydro-6-hydroxymethylfagaridine is a new compound. Hordenine was isolated from the bark of *Z. procerum* Don., while the wood was found to contain 6,7,8-trimethoxycoumarin (**9**), 3,4,5-trimethoxycinnamaldehyde and methyl-3,5-dimethoxy-4-hydroxybenzoate. The leaves, of which only a small sample was was available, showed the presence of bishordeninyl terpene alkaloids, but not in sufficient quantity for separation and purification.

In a search for alkaloids of antitumor or other biological activity, we have continued the investigation of tropical American *Zanthoxylum* species. Since chemical investigation of species belonging to the Engler subsection Tobinia (1) yielded the novel bishordeninyl terpene alkaloids alfileramine (2) and culantraramine (3), a Costa Rican member of this subsection was studied: *Z. procerum*. Another Costa Rican species, *Z. microcarpum*, of the very diverse and non-homogeneous subsection Paniculatae (Neogaeae) was also available for study.



alfileramine



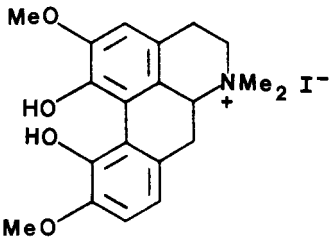
culantraramine

RESULTS

Zanthoxylum microcarpum. From the wood, we were able to isolate and characterize the known alkaloids magnoflorine (**1**), haplopine (**2**), and hordenine (**3**). From the bark, magnoflorine and the known decarine (**4**), were isolated along with an additional unknown benzophenanthridine alkaloid. Spectral data indicated that the unknown was *N*-nornitidine (**5**). This was proven by showing its identity with a synthesized sample. Small twigs and branches were difficult to debark and, hence, were analyzed *in toto*. We found two alkaloids, one the known *N*-methyltyramine (6).

[1]Paper 6 in the series "Constituents of Zanthoxylum." For paper 5 see F. R. Stermitz, M. A. Caolo and J. A. Swinehart, *Phytochemistry*, **19**, 1469 (1980).

The second alkaloid from the stems and twigs had a MW of 365 and exact mass measurement yielded the molecular formula $C_{21}H_{19}NO_5$. The uv spectrum was characteristic of a dihydrophenanthridine and ^1H nmr showed the presence of one N-Me, one OMe and a methylenedioxy group. The uv spectrum showed a strong base shift with added OH^-, so the presence of a phenolic group was indicated. The aromatic region was first order (150 MHz) and yielded two singlets and two AA'BB' patterns indicative of benzophenanthridines of the sanguinarine-chelerythrine, rather than nitidine-fagaronine types. Most instructive was a triplet at 3.10 and doublets of doublets at 3.47 and 4.53. These are indicative (4) of the $-CH-CH_2OH$ ABX system of 6-hydroxymethyldihydrobenzophenanthridines. In the mass spectrum, there was an *m/e* 344 peak which resulted from loss of $-CH_2OH$ from the molecular ion. The unknown gave a weak, but positive, Gibbs test for a phenol with a *para*-H. The positions and patterns of the aromatic



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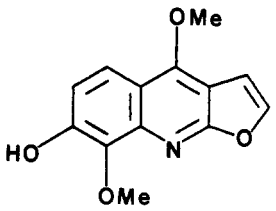

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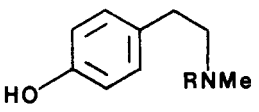
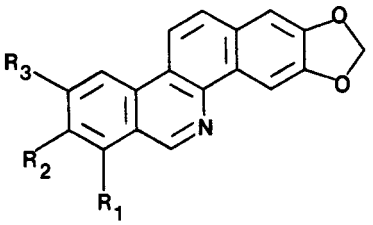


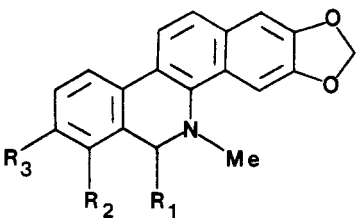
3: R = Me

6: R = H



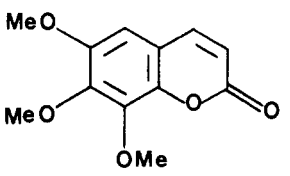
4: R_1 = OMe ; R_2 = OH; R_3 = H

5: R_1 = H; R_2 = R_3 = OMe



7: R_1 = CH_2OH; R_2 = R_3 = OMe

8: R_1 = CH_2OH; R_2 = OH; R_3 = OMe



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protons in the nmr were nearly identical with those reported (4) for bocconodine (**7**). The uv spectrum (except for the phenolic base shift) was identical to that reported for bocconoline. These data are all consistent with the structure of 5,6-dihydro-6-hydroxymethylfagaridine (**8**), for the unknown. Leaves were found to contain hordenine and trace alkaloids in amounts insufficient to characterize.

Zanthoxylum procerum Donn. Smith. The bark had one main alkaloid, hordenine, and traces of others in insufficient amounts to identify. A hexane extract of the wood contained no alkaloids, but yielded 6,7,8-trimethoxycoumarin (**9**), and 3,4,5-trimethoxycinnamaldehyde. The crude total basic fraction from a methanol extract of the wood was very small (80 mg from 450 g of wood). It showed trace amounts of polar alkaloids, but none could be isolated pure. A nonalkaloidal constituent was found to be methyl 3,5-dimethoxy-4-hydroxybenzoate.

The small amount of leaves available gave a $CHCl_3$ extract from which 60 mg of total bases was isolated. Tlc indicated the presence of four alkaloids (two major), but none could be isolated pure. The ^1H nmr of the crude mixture, however, showed resonances characteristic of C-Me, NMe_2, OMe, vinyl and aromatic protons. The mixture nmr was virtually superimposable with the 1:1 mixture of culantraramine and an unknown bishordeninyl terpene isolated from *Z. culantrillo*. The difficulty of working with these bishordeninyl terpene alkaloids has been pointed out (3) and, in the present case, we were unable to isolate a pure constituent.

DISCUSSION

We continue to find hordenine (**3**), as a common constituent of Central American and Carribean *Zanthoxylum* (Fagara) species, although it was not found in 61 species studied through 1976 (5). Its presence in *Z. microcarpum* did not lead to bishordeninyl terpene alkaloids, although these were encountered along with hordenine in *Z. procerum* and *Z. coriaceum* (6). Conversely, *Z. punctatum* contained bishordeninyl terpenes but did not accumulate hordenine.

With the exception of magnoflorine, the other alkaloids of *Z. microcarpum* are either new or rare in the *Zanthoxylum* family. Decarine was previously known from a Madagascar (7), Nigerian (8) and Asian (9) species. Haplopine, while common in *Haplophyllum* species, is only known from a single Asian *Zanthoxylum* (9). *N*-Nornitidine has not previously been identified as a natural product. 5,6-Dihydro-6-hydroxymethylfagaridine is the third known 6-hydroxymethyl benzophenanthridine, with the other (in addition to bocconoline) being corynolamine from *Corydalis incisa* (10). Fagaridine, but not the 6-hydroxymethyl derivative, has been reported (11) from *Fagara xanthoxyloides*. *N*-Methyltyramine is a common cactus alkaloid and has previously been reported in the Rutaceae from *Citrus aurantium* L. (12), but not from *Zanthoxylum*.

A relatively high concentration of hordenine was found in the bark of *Z. procerum*, but no alkaloids could be isolated from the wood. The leaves contained considerable amounts of the bishordeninyl terpene alkaloids, but our sample was not sufficiently great to provide separation. As in the cases of the same type of alkaloids from *Z. punctatum* (2), *Z. coiraceum* (6), and *Z. culantrillo* (3), they were found *only* in leaves. Of the known four occurrences of bishordeninyl terpenes, three have been in species of the Engler (1) section Tobinia. The exception is *Z. culantrillo*, which is from the Pterota section.

Whether or not these chemical data will help in distinguishing phylogenetic relationships in *Zanthoxylum* will probably have to await further work by botanists. As has been pointed out by Porter (13), "The American species of *Zanthoxylum* are badly in need of revision."

EXPERIMENTAL

GENERAL PROCEDURES.—Extractions were performed on a Soxhlet apparatus. A separation procedure referred to below as the "standard acid-base procedure" was accomplished as follows. A residue was dissolved in warm 1M H_2SO_4 and extracted three times with chloroform. The aqueous solution was brought to pH 8.6 by the addition of NH_4OH. A small portion of KI was added, and the solution was then extracted three times with chlorform and then once with 1-butanol.[2]

Tlc and plc solvents used are designated as follows: S_4=15:3:1 methanol-water-NH_4OH; S_3=15:9:1 ethanol-water-NH_4OH:R_1=6:2:2:1 hexane-ethyl acetate-chloroform-methanol; F=2:17:1 methanol-ethyl acetate-NH_4OH.

"Flash chromatography" refers to use of the low pressure system described by Still (14).

Z. microcarpum Griseb. was collected March 17, 1978, in uncut woods neighboring a banana-coffee plantation 5 km southeast of San Gabriel, Costa Rica, (Colorado State University herbarium accessium No. 58078). Foliage was sparse, having mostly dropped. A few dried fruits were present.

Debarked dried wood (1546 g) was ground to a powder and extracted with ethanol. The ethanol was evaporated to an oily residue and subjected to the standard acid-base procedure. The pH 8.6 chlorform extract yielded 1.6 g of crude alkaloid residue, which was chromatographed on Sephadex LH-20 (4:1 chloroform-methanol). Middle fractions were combined and purified by column chromatography on activity grade III neutral Al_2O_3 (1:1 methanol-acetone) and plc (solvent S_4) to yield magnoflorine iodide, **1**, (identified by nmr, uv, ms, tlc). Latter fractions from the Sephadex chromatography were combined and subjected to flash chromatography on Silica Gel (3:1 chloroform-methanol and 1:1 chloroform-methanol). Early fractions yielded (after residue trituration with 1:1 hexane-chloroform) a white solid identified as haplopine, **2**, (nmr; uv neutral, acid and base; ms) in comparison with literature (9) values. Later fractions yielded hordenine (**3**), (nmr, tlc, uv).

Dried, ground, and defoliated twigs (585 g) were extracted with chloroform, followed by methanol. The methanol was evaporated and the residue was dissolved in warm 1M H_2SO_4. The solution was extracted with chloroform at pH 1, 6, and 8.6 after successive additions of NH_4OH. The pH 6 residue (15 mg) was purified by plc (solvent R_1) and an R_f 0.35 band removed with warm chloroform. The solid residue showed the following characteristics. Ms (*m/e*, rel intensity): 365(10), 334(100), 319(80), 304(10), 284(10), 276(10), 245(15), 227(24); exact mass 365.1258, calc for $C_{21}H_{19}NO_5$: 365.1264. Uv λ max (EtOH) 228, 284, 324sh, 353sh; +HCl 223, 255, 268sh, 277, 307, 321, 339, 356; +OH^- 236, 268, 295, 336. ^1H nmr (NT150 MHz, $CDCl_3$): 2.72 (s, 3H, N-CH_3), 3.10 (t, 1H, *J* 11.8, CHCH_2), 3.47 (dd, 1H, *J* 11.3 and 5.07, CHCH_2), 3.90 (s, 3H, OCH_3), 4.53 (dd, 1H, 11.8 and 5.07, CHCH$_2$), 6.01 (s, 2H, OCH_2O), 6.93 (d, 1H, *J* 8.78), 7.05 (s 1H), 7.43 (d, 1H, *J* 8.78), 7.50 (d, 1H, *J* 8.55), 7.56 (s, 1H), 7.61 ppm (d, 1H, *J* 8.55). As discussed in the RESULTS section, these data (along with a positive Gibb's test) allow assignment of the 5,6-dihydro-6-hydroxymethylfagaridine structure, **7**, to the alkaloid. The pH 8.6 residue was purified by flash chromatography on silica gel with chloroform-methanol mixtures and finally 3% NH_4OH in methanol. Only the last fractions yielded a pure alkaloid which was identified as *N*-methyltyramine, **6**, (^1H nmr, uv, tlc compared with a standard sample (15)).

Dried and ground bark (600 g) was extracted with hexane, followed by methanol. The methanol residue was dissolved in warm 1M H_2SO_4 and extracted with chloroform. The pH was adjusted to 6 (NH_4OH), at which point a black, insoluble gum formed. This was taken up in pH 11 aqueous solution adjusted to pH 9 and extracted with butanol. The butanol was evaporated, and the residue was dissolved in methanol and separated from a nonalkaloidal ppt. The methanol residue was purified by plc (silica gel, solvent S_4). An R_f 0.30 band proved to be magnoflorine iodide (^1H nmr, uv, tlc). A set of bands at R_f 0.75–0.90 were removed together and separated by plc (Si gel, solvent R_1). A 0.53 band (yellow long wave length uv fluorescence) was shown to be *N*-nornitidine, **5**, by the data given below. An R_f 0.39 band was not pure, but was purified by a second plc (silica gel, 20:3:1 ethanol/2-propanol/NH_4OH, R_f 0.87, green-brown fluorescence). The spectroscopic data below allowed assigning the structure of decarine, **4**, to this unknown.

N-Nornitidine showed the following. Ms: 334(2), 333(10), 236(17), 223(33), 205(37), 186(33), 177(20), 149(100). Uv λ max (EtOH): 227, 274, 280, 315sh, 334sh, 352sh, 369sh nm; +HCl: 229, 268, 286, 297, 325, 381 nm. ^1H nmr (NT150 MHz, d_6-acetone): 4.02 ppm (s, 3H,

[2]We have found that addition of KI to a basic solution allows extraction of quaternary alkaloids directly as the iodide salts.

OCH_3), 4.13 (s, 3H, OCH_3), 6.15 (s, 2H, OCH_2O), 7.63 (s, 1H), 7.90 (d, 1H, *J* 10.0), 8.13 (s, 1H), 8.50 (d, 1H, *J* 10.0), 8.65 (s, 1H), 9.25 (s, 1H). A standard sample of nitidine chloride was heated at 200° and 0.05 mm pressure to yield solid *N*-nornitidine, mp 279–281°, lit. (16) mp 278–280°. The prepared and isolated samples of *N*-nornitidine had identical uv and 150 MHz ^{1}H nmr spectra.

Decarine showed the following. Ms: 320(38), 319(100), 305(23), 304(69), 290(8), 287(10), 286(92), 276(92), 247(6), 218(15). Uv λ max (EtOH): 248, 257, 269sh, 276, 285sh, 326 nm; +HCl: 213, 242sh, 266, 275sh, 313, 335, 385; $+OH^-$: 253, 297, 330sh. ^{1}H nmr (NT150 MHz d_6-acetone): 4.11 (s, 3H, NCH_3), 6.21 (s, 2H, OCH_2O), 7.40 (s, 1H), 7.63 (d, 1H, *J* 9.0), 7.97 (d, 1H, *J* 9.0), 8.48 (d, 1H, 9.0), 8.52 (d, 1H, *J* 9.0), 8.68 (s, 1H), 9.66 (s, 1H). These data conform to the literature (7, 8) values for decarine with one minor exception. The mw of decarine is 319 so there should be no 320 peak. Our ms sample, however, was the same sample whose nmr spectrum had been taken in d_6-acetone. This solvent contained a little D_2O and it is quite likely that the phenolic OH had exchanged to give an OD group.

Dried and ground leaves (187 g) were extracted with hexane, followed by ethanol. The extract was put through the standard acid-base procedure and screened by tlc. Although several iodoplatinic acid reactive spots were observed, only hordenine was present in sufficient quantity to allow isolation and identification (^{1}H and ^{13}C nmr, tlc).

Z. procerum Donn. Sm. was collected March 17, 1978, off a nature trail at the Instituto Internacional Agricola, Turrialba, Costa Rica, (Colorado State University Herbarium accession No. 58077). It should be noted that on the nature trail itself there is a tree labeled *Z. procerum*, which is clearly not that species.

Dried and ground bark (457 g) was extracted with hexane and then methanol. The residue was treated by the standard acid-base procedure, which yielded 50 mg of crude alkaloid mixture (three iodoplatinic acid positive spots) from the chloroform pH 8.6 extract. No pure alkaloids could be isolated. Extraction at pH 8.6 with 1-butanol and evaporation yielded 110 mg of crude material which was purified by plc (silica gel, 100:100:1 chloroform-methanol-conc. HCl). Hordenine was isolated as the hydrochloride (^{1}H nmr, uv, tlc).

Dried and ground leaves (180 g) were extracted with hexane, chloroform and then methanol. The chloroform residue (2.6 g) was subjected to the standard acid-base procedure to yield 60 mg of red-orange glass. Tlc showed four alkaloids (R_f 0.16, 0.44, 0.60 and 0.73 in solvent S_3). The last two were major. The ^{1}H nmr of the crude showed 1.27, 1.37 and 1.77 ppm singlets (CCH_3), a large 2.33 ppm peak (NMe_2), a CH_2 envelope at 2.66 and an aromatic multiplet from 6.6 to 7.4. This crude nmr had the main peaks superimposable with those of a 1:1 mixture of culantraramine and an unknown bishordeninyl terpene isolated (3) from *Z. culantrillo*. In our previous work with bishordeninylterpene alkaloids from *Z. culantrillo*, *Z. punctatum* and *Z. coriaceum*, we have found the presence of the distinctive C-Me and N Me_2 ^{1}H nmr resonances to be diagnostic for such alkaloids. Attempted isolation of pure alkaloids resulted in loss of the material without separation.

Dried and ground wood (452 g) was extracted with hexane, followed by methanol. The hexane extract yielded 1.59 g of viscous yellow oil. This was triturated with hexane and an insoluble residue discarded. The hexane was evaporated, and the methanol soluble portion of the residue was separated by plc (silica gel, 1:1 hexane/ethyl acetate). An R_f 0.79 band yielded 20 mg of a white solid, mp 100–103°. The solid was identified as 6,7,8-trimethoxycoumarin (**9**) (nmr, ir), lit. (17) mp 104°. A band at R_f 0.24 was removed to yield 8 mg of *trans*-3,4,5-trimethoxycinnamaldehyde. Ms: 222(100), 207(25), 179(99), 151(62); ir ($CHCl_3$) 1674 cm^{-1}; ^{1}H nmr (60 MHz, d_6-acetone): 3.82 (s, 3H, OMe), 3.92 (s, 6H, 2 OMe), 9.73 ppm (d, 1H, *J* 8.0); 360 MHz: 6.76, 6.79 (dd, 1H, *J* 8.0, 14.3), 7.60 (d, 1H, *J* 14.3), 7.12 (s, 2H); uv (EtOH) 234, 320 nm.

The methanol extract was subjected to the standard acid-base procedure to yield 80 mg of red-orange residue which showed two polar alkaloid spots (Rf 0.0 and 0.05 in solvent S_4). Neither could be isolated pure by flash chromatography or plc. Plc (silica gel, solvent R_1) did allow isolation of a nonalkaloidal R_f 0.44 compound which was identified as methyl 3,5-dimethoxy-4-hydroxybenzoate from the following data. Ms: 212(100), 181(98), 149(59), 91(43); ir ($CHCl_3$) 3400, 1716 cm^{-1}; uv λ max (EtOH) 228, 279, 332 nm; $+OH^-$ 239, 328 nm. ^{1}H nmr (60 MHz, $CDCl_3$) 3.87 (s, 3H, COOMe), 3.97 (s, 6H, 2 OMe), 7.30 ppm (s, 2H).

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