

## A NEW BISHORDENINYL TERPENE ALKALOID FROM *ZANTHOXYLUM CHIRIQUINUM*

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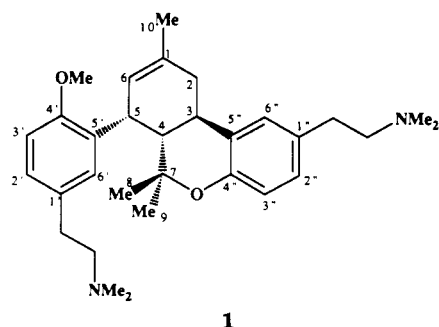
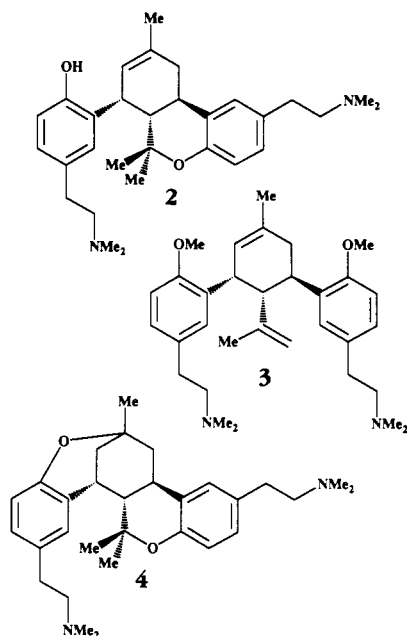
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**ABSTRACT.**—A new bishordeninyl terpene alkaloid, *O*-methylalfileramine [**1**], was isolated from the MeOH extract of the leaves of *Zanthoxylum chiriquinum*, along with the already known alfileramine [**2**], culantramine [**3**], and isoalfileramine [**4**]. This is the first report of the isolation of the two skeleton types of bishordeninyl terpene alkaloids from the same species.

*Zanthoxylum chiriquinum* Standl. (Rutaceae) is a very rare species, but our finding of a few trees in the cloud forest south of San Jose, Costa Rica, has both provided a new location and made material available for this work. At the time of our collection, "carboneros" were in the area cutting trees and burning them to manufacture charcoal, so this new location may not survive for long. In this note we describe the isolation of the new alkaloid *O*-methylalfileramine [**1**].

Dry leaves of *Z. chiriquinum* were extracted, and the alkaloidal fraction was submitted first to Si gel chromatography and then to hplc (normal phase), affording compounds **1–4**. Compounds **2–4** were shown by spectroscopic studies and direct comparison with authentic samples to be the known alkaloids alfileramine [**2**], culantramine [**3**], and isoalfileramine [**4**] (1,2).

This is the first report of the isolation of the two skeleton types of bishordeninyl terpene alkaloids from the same species. The small quantity of



isoalfileramine [**4**] isolated might suggest the possibility that this compound is an artifact derived from alfileramine during the acid-base isolation procedure.

The new compound, *O*-methylalfileramine [**1**], was isolated as an oil that solidified upon standing. It has  $[\alpha]_D^{25}$  as with all bishordeninyl terpene alkaloids isolated so far. The uv spectrum [ $\lambda_{max}$  (EtOH) = 283] is coincident with that of a substituted  $C_6H_6$ , and the lack of displacement with base shows that the molecule does not have a phenolic group.

The fabms spectrum showed an  $[MH]^+$  signal at  $m/z$  477, indicating that the mol wt is 476. The molecular formula was established as  $C_{31}H_{44}N_2O_2$  by hreims (found 476.3400, calcd 476.3405), and the fragmentation pattern of this compound was found to be very similar to that of alfileramine [2], the most remarkable signals being  $m/z$  476, 474, 418, 246, 244, 230, 229, 185, 173, 159, and 58.

The  $^1H$ -nmr spectrum of this alkaloid is also very similar to that of 2, but it does not present the duplicity of signals which characterized 2 as a result of the hindered rotation around the  $sp^2$ - $sp^3$  bond 5-5' (3). Also, it shows the presence of a methyl singlet at 3.82  $\delta$  for an aromatic methoxyl group; this was corroborated by its  $^{13}C$ -nmr spectrum, which shows an additional signal at 51.43  $\delta$ . These observations allowed us to identify compound 1 as the new alkaloid *O*-methylalfileramine. The assignment of the  $^1H$  nmr is supported by a 2D COSY experiment.

The relative stereochemistry of the three chiral centers was deduced by transformation of 1 (heating in acidic media) to isoalfileramine [4]; the stereochemistry of this compound has been established by X-ray crystallography (4).

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—Uv spectra were recorded in EtOH solutions on a Pye-Unicam 1700 and ir spectra on a Perkin-Elmer 180. Mass spectral analyses were carried out with a Kratos MS-25 and MS-50. Nmr spectra were recorded in  $CDCl_3$  with a Bruker WM 250. Chemical shifts are given in ppm from TMS standard. Optical rotations were determined on a Perkin-Elmer 141. Hplc separations of the compounds were performed on a Whatman Partisil column using a Waters Associates Model 590 chromatograph, equipped with a uv detector operating at 254 nm and an R401 differential refractometer.

**PLANT MATERIAL.**—*Z. chiriquinum* was collected by F. R. S. in the deep woods east of the Pan American Highway at km 68 from San Jose, Costa Rica. It was identified by Luis Poveda,

Museo Nacional, San Jose, Costa Rica, and a voucher deposited in the Colorado State Herbarium, FRS Collection Number 192.

**EXTRACTION AND ISOLATION PROCEDURE.**—The finely powdered plant material (248 g) was extracted in a Soxhlet extractor with MeOH. Concentration of the extract under reduced pressure gave a dark green residue (54.2 g), that was treated with 1 N HCl, filtered, and extracted with  $CH_2Cl_2$  to yield 32.4 g after concentration. The acidic aqueous phase was neutralized with  $NH_4OH$  and extracted with  $CH_2Cl_2$  to provide 9.2 g of free bases after low pressure distillation at approximately 30°. The bases were submitted to chromatography on Si gel packed in  $CH_2Cl_2$  and using as eluent the lower layer of a 9:1  $CH_2Cl_2$ /concentrated  $NH_3$  (aqueous) mixture polarized with MeOH (from 5 to 20%). Rechromatography of the fractions by hplc (Si gel, normal phase, using the same eluent as before but with 3-4% of MeOH) yielded 75 mg of *O*-methylalfileramine [1], 174 mg of alfileramine [2] (white powder mp 190-193°), 62 mg of culantramine [3], and 10 mg of isoalfileramine [4].

***O*-Methylalfileramine [1].**—Ir (film) 3250, 2960, 1620, 1490, 1290, 1250  $cm^{-1}$ ; uv (EtOH) 283 (5.5), 290 (5.3) nm;  $^1H$  nmr (250 MHz,  $CDCl_3$ )  $\delta$  7.10 (br s, 2H, Ph), 6.99 (dd,  $J=2.2$  and 8.3 Hz, 1H, Ph), 6.90 (dd,  $J=2.2$  and 8.3 Hz, 1H, Ph), 6.78 (d,  $J=8.3$  Hz, 1H, Ph), 6.65 (d,  $J=8.3$  Hz, 1H, Ph), 5.41 (br d,  $J=5.3$  Hz, 1H, H-6), 4.32 (br t, 1H, H-5), 3.82 (s, 3H, OMe), 3.32 (m, 1H, H-3), 2.83 (dd,  $J=6.2$  and 17.0 Hz, 1H, H-2), 2.69 (m, 4H,  $Ph-CH_2$ ), 2.52 (m, 4H,  $N-CH_2$ ), 2.31 (s, 6H, NMe), 2.25 (s, 6H, NMe), 2.20 (dd,  $J=5.4$  and 12.6 Hz, 1H, H-4), 2.10 (m, 1H, H-2), 1.79 (s, 3H, Me-10), 1.54 (s, 3H, Me-8), 0.40 (s, 3H, Me-9);  $^{13}C$  nmr  $\delta$  153.39 and 151.33 (C-4' and C-4"), 132.66 (C-6' or C-6"), 132.39, 131.59, 130.72, 129.34, and 129.12 (C-1, C-1', C-1", C-5' and C-5"), 127.43, 127.19, 126.93, and 126.00 (C-2', C-2", C-6, and C-6' or C-6"), 117.23 and 115.48 (C-3' and C-3"), 77.42 (C-7), 53.16 and 52.96 ( $CH_2-N$ ), 51.43 (OMe), 42.15 (C-4), 38.06 (C-2), 35.80 and 35.70 (Me-N), 34.91 and 34.81 ( $CH_2-Ph$ ), 34.71 and 32.96 (C-3 and C-5), 27.85 (C-8), 23.23 (C-10), 19.80 (C-9); hreims  $m/z$  calcd for  $C_{31}H_{44}N_2O_2$   $[M]^+$  476.3405, found  $[M]^+$  476.3400; eims  $m/z$  (rel. int.)  $[M]^+$  476 (0.1), 475 (0.3), 474 (0.67), 418 (0.11), 246 (0.16), 244 (0.2), 230 (0.13), 229 (0.42), 185 (0.15), 173 (0.68), 159 (0.42), 58 (100).

## ACKNOWLEDGMENTS

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