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ALFILERAMINE

A NEW ZANTHOXYLUM ALKALOID STRUCTURALLY RELATED TO TETRAHYDROCANNABINOL[1]

MARY A. CAOLO and FRANK R. STERMITZ*
Department of Chemistry, Colorado State University, Fort Collins, CO 80523, U.S.A.

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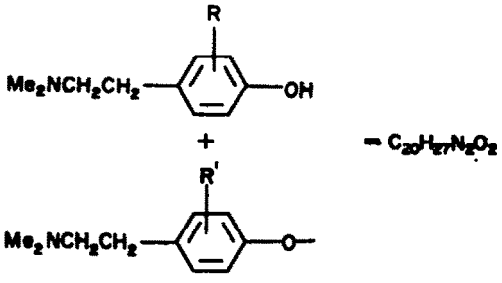
Abstract—A new alkaloid, named alfileramine, has been isolated from *Zanthoxylum punctatum*. It represents the first alkaloid which has a structure closely related to the tetrahydrocannabinols. The structure was assigned based upon ^1H and ^{13}C NMR, UV and mass spectroscopy and by relating alfileramine structure to that of the rearranged dihydrobromide salt. The structure of the dihydrobromide was known from X-ray diffraction. A possible biosynthetic pathway from a dehydrocitral equivalent and two molecules of hordenine is suggested.

Stems and branches of the Puerto Rican plant *Zanthoxylum punctatum* Vahl. (*Rutaceae* - the citrus family) were previously found[2] to contain the aporphine alkaloids N-methylisocorydine[3] and magnoflorine and the benzophenanthridinium alkaloid punctatine. We have now investigated the leaves of this species. Leaves contain none of the stem alkaloids, but instead a novel alkaloid whose structure is assigned below.

By the extraction and purification procedure given in the Experimental Section, a pure, powderey alkaloid (m.p. 185–187°), which we named alfileramine,[4] was isolated.

Combustion analysis and mass spectrometry established the molecular formula as $C_{30}H_{42}N_2O_2$. ^{13}C NMR (noise decoupled) showed only 24 of the expected 30 carbon resonances. The UV spectrum (λ_{max} 283) indicated a simple substituted benzene and a base shift to λ_{max} 290 showed the presence of a phenol. This was confirmed by the ^1H NMR which showed a one proton resonance at 9.30 ppm that disappeared upon addition of D_2O. Lack of a CO absorption in the IR indicated that the second oxygen was probably part of an ether structure. The base peak in the mass spectrum was *m/e* 58, which is attributable to $CH_2{=}\overset{+}{N}Me_2$. The presence of two different side chains which could yield this ion was indicated by two six proton singlets in the ^1H NMR at 2.28 and 2.33 ppm along with intense ^{13}C absorption at 45.6 ppm (a quartet in the off resonance decoupled spectrum). Six aromatic hydrogens were evident in the ^1H NMR and analysis of this region in the 220 MHz spectrum was consistent with the presence of two nearly identical 1,3,4-trisubstituted benzene rings. These data in summary suggested the following part structures for alfileramine:



$C_{30}H_{42}N_2O_2 - C_{20}H_{27}N_2O_2 = C_{10}H_{15}$

That the $C_{10}H_{15}$ remainder could represent a terpenoid structure was indicated by the presence of three singlet C–CH_3 absorptions in the ^1H NMR spectrum, one of which occurred at the highly shielded value of 0.56 ppm. A doublet at 5.53 ppm was probably attributable to one vinylic hydrogen. From the ^{13}C NMR only 12 sp^2 carbons could be observed, but ^{13}C of the easily prepared dimethiodide salt showed 14 sp^2 carbons, consistent with two aromatic rings and a C=C. Off resonance decoupled ^{13}C NMR was useful for the establishment of several additional structural units. A singlet was observed at 77.35 ppm which is typical for a quaternary carbon next to oxygen. Alfileramine showed doublets at 33.34 and 48.24 ppm for methine carbons, but in the dimethiodide the latter peak was resolved into two at 48.57 and 49.03 ppm. Thus alfileramine contains three methine carbons. Alfileramine showed four triplets for $-CH_2-$ groups at 33.11, 38.19, 61.59 and 61.71 ppm. The latter two were shifted to 67.39 and 67.47 ppm in the dimethiodide and could therefore be assigned to $-CH_2-$ groups next to nitrogen. It seemed likely that the two $-CH_2-$ groups next to the benzene rings (see above part structure) would be nearly identical and the 33.11 ppm triplet is best assignable as arising from both these groups. This leaves one $-CH_2-$ (38.19 ppm) to place in the presumed terpenoid portion of the molecule. ^1H NMR was not useful for distinguishing among these groups since all $-CH_2-$ absorptions were part of a broad absorption in the 2.4–2.8 ppm region.

^1H NMR decoupling experiments were performed to gain information regarding the vinylic hydrogen environment. Irradiation of the 5.53 doublet caused the 4.36 resonance (which appeared as a broad absorption at 60 MHz) to change to a doublet. The latter resonance was probably originally a doublet of doublets with considerable overlap. Irradiation of the 4.36 resonance collapsed the 5.53 doublet to a singlet. This experiment showed that the vinylic 5.53 hydrogen was coupled to a methine hydrogen (4.36 ppm), which in turn was coupled to another methine hydrogen. In other words, alfileramine contained a C=CH–CH–CH– array (where missing valences are to R groups rather than to hydrogens).

A unique structure for alfileramine could not be assigned from these data so a suitable crystal for an X-ray diffraction study was sought. Neither alfileramine nor the dimethiodide provided such a crystal, but the dihy-

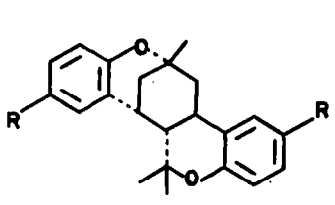
drobromide (formed from alfileramine in HBr/EtOH) did give a small useful crystal. Unfortunately, NMR and UV analysis of this salt showed that the vinylic hydrogen was no longer present nor was the phenolic group. The free base which was recovered from the dihydrobromide was not identical with alfileramine. The base, named isoalfileramine since it also showed a mass spectral molecular ion at *m/e* 462, lacked the vinylic hydrogen and the phenolic group and exhibited some other changes in the ^1H and ^{13}C NMR. Presence of C-CH_3 absorptions and other characteristics indicated that no deep-seated major rearrangement had occurred. A single crystal X-ray diffraction study was performed on isoalfileramine dihydrobromide, from which it was shown[5] that isolafileramine has structure 1 (absolute configuration not intended).

Examination of 1 in a retrosynthetic sense yields five phenols which might cyclize in acid to give alfileramine: 2, 3, 4, 5 and 6. Structures 4, 5 and 6 are immediately ruled out since their ^1H and ^{13}C NMR spectra would not be consistent with the interpretations given above. Alfileramine must therefore be assigned structure 2 or 3.

analysis of the reason for the abnormally high field C-methyl ^1H NMR resonance (0.50 ppm). This highly-shielded value arises because the C-9 Me is near the π-cloud of the C-5 aromatic ring. With the aromatic ring in that position, it cannot affect the vinylic proton at C-6. ^{13}C NMR methods[6] for distinguishing between 7 and 8 were not useful for the same comparison of 2 and 3.

Assignment of structure 2 to alfileramine is also consistent with its mass spectrum. Most of the ion current is carried by the molecular ion and the *m/e* 58 peak so attention must be focused on relatively minor fragmentations. These are depicted in Scheme 1. Loss of 58 from the molecular ion would yield the observed *m/e* 404 fragment, but a unique structure cannot be written for this moiety since $-CH_2NMe_2$ (58) can be lost from either of the two side chains. Essentially nothing appears between *m/e* 404 and a group of three peaks at *m/e* 230, 231 and 232. An explanation for this fragmentation is in exact accord with the reverse Diels–Alder cleavage which represents the main pathway for fragmentation of $\Delta^{1,6}$-THC (7).[9]

For 2, this would yield two *m/e* 231 fragments



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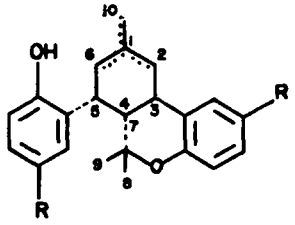
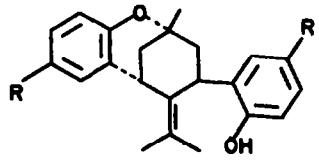


2: $\Delta^{1,6}$
3: $\Delta^{1,2}$
4: $\Delta^{1,10}$



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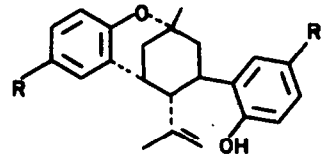

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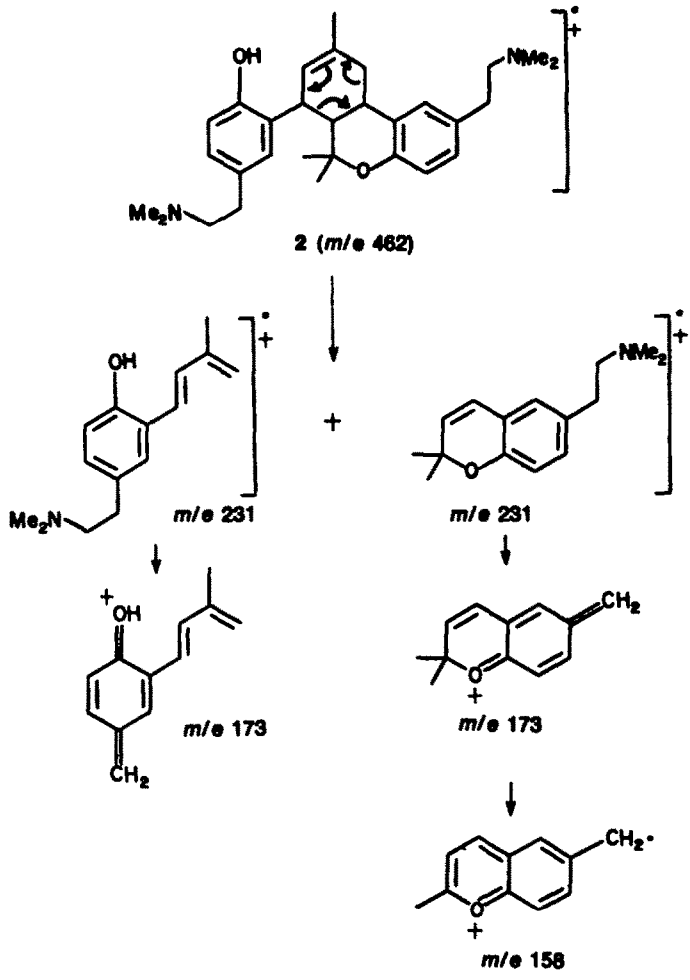
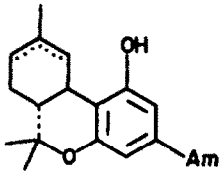
R = $CH_2CH_2NMe_2$

Structures 2 and 3 are closely related to $\Delta^{1,6}$- and Δ^1-*trans*-tetrahydrocannabinol (7 and 8) and a final assignment of structure 2 to alfileramine could be made based upon analogies with spectral data known for 7 and 8. One key to distinguishing between 7 and 8 lies in the position of the vinyl proton. That for Δ^1-THC (8) appears[6,7] as a broad singlet (60 MHz) at 6.42 ppm while that for $\Delta^{1,6}$-THC (7) is a broad singlet at 5.45 ppm. The large downfield shift for the vinylic proton at C-2 of 8 arises because it is in the plane of the aromatic ring and hence subjected to an induced diamagnetic field. The vinylic proton of alfileramine appears at 5.53 ppm and hence correlates very well with 7, but not with 8. The aromatic ring at C-5 could not have a major effect on a C-6 vinylic proton since the vinylic C–H bond is directed *perpendicular* to the C-5 aromatic ring. This is evident from a Dreiding model of 2 and also follows from (Scheme 1). Either could lose a benzylic hydrogen to produce the observed *m/e* 230 peak. Either of the 231 ions could also lose 58 to give an ion of *m/e* 173, which represents the third highest peak in the mass spectrum. Loss of a Me radical from *m/e* 173 would give the observed *m/e* 158 ion. Thus, the fragmentation pattern for alfileramine is, with the exception of the loss of 58, exactly analogous to that observed for $\Delta^{1,6}$-THC. The mass spectrum of Δ^1-THC (8) is not the same as that of 7 although it does undergo a minor pathway which yields some ions of the same *m/e* value as those attributed to the retro Diels–Alder of 7. It was originally suggested[9a] that Δ^1-THC might partially isomerize to $\Delta^{1,6}$-THC prior to decomposition, but this has been proven by deuterium labeling studies[10] not to be the case.

Alfileramine and isoalfileramine each have three chiral centers, but are optically inactive. This was shown not



Scheme 1.

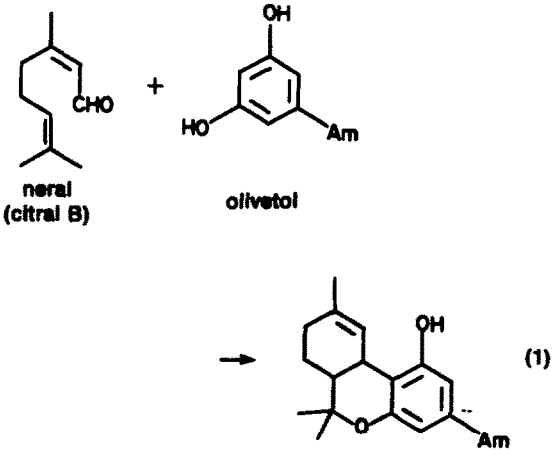


7: $\Delta^{1,6}$
8: $\Delta^{1,2}$

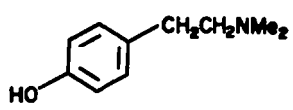
only at the sodium D line, but also at Hg wave lengths 578 and 546. The two molecules per unit cell found[3] in crystalline isoalfileramine dihydrobromide are also consistent with a *dl*-pair. Although the best procedure for isolation of alfileramine involved acid and base, tlc showed that the alkaloid was present in the crude MeOH extract prior to the acid-base treatment. It could be isolated partially pure by Sephadex LH-20 chromatography without an acid-base procedure. Alfileramine can be dissolved in 1M H_2SO_4 and recovered unchanged by $CHCl_3$ extraction after basification. It is to be noted that Δ^1-THC rearranges to $\Delta^{1,6}$-THC in the presence of acid. The lack of change in alfileramine in 1M H_2SO_4 is in keeping with the $\Delta^{1,6}$-assignment. Alfileramine does gradually decompose upon standing in $CHCl_3$ solution and tlc examination shows the formation of several lower R_f alkaloid-positive spots. This instability may be a function of the strongly basic alkylamine groupings which could react with $CHCl_3$.

DISCUSSION

As far as we are aware, alfileramine represents the first alkaloid with a tetrahydrocannabinol-type ring system which has been found in a plant species other than *Cannabis sativa*.[11] Structurally, just as THC is formally related to citral and olivetol (and can be synthesized *in vitro* from these components, Eqn 1), alfileramine can be



formally related to neral and two molecules of hordenine (9).

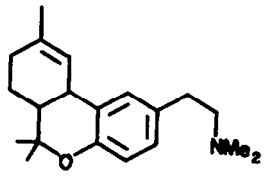


9
hordenine

There are, however, no cannabinoids which bear a substituent at C-5. The involvement of neral or an equivalent such as nerol pyrophosphate as alfileramine precursors is therefore unlikely although terpenoids are common constituents of species of the citrus family. An alternative precursor could be dehydrocitral or an equivalent. The outline of a mechanism for the conversion of dehydroneral, for example, and hordenine to alfileramine is given in Scheme 2.

Although we could find no hordenine in *Z. punctatum*, it does occur in the leaves of *Z. culantrillo* H.B.K., a species we have found[13] to contain at least two additional alkaloids of the alfileramine type. Neither is isomeric with alfileramine and both contain methoxy groups.[13] The co-occurrence of hordenine and alfileramine-type alkaloids in *Z. culantrillo* adds additional evidence to the suggested precursor role (Scheme 2) of hordenine.

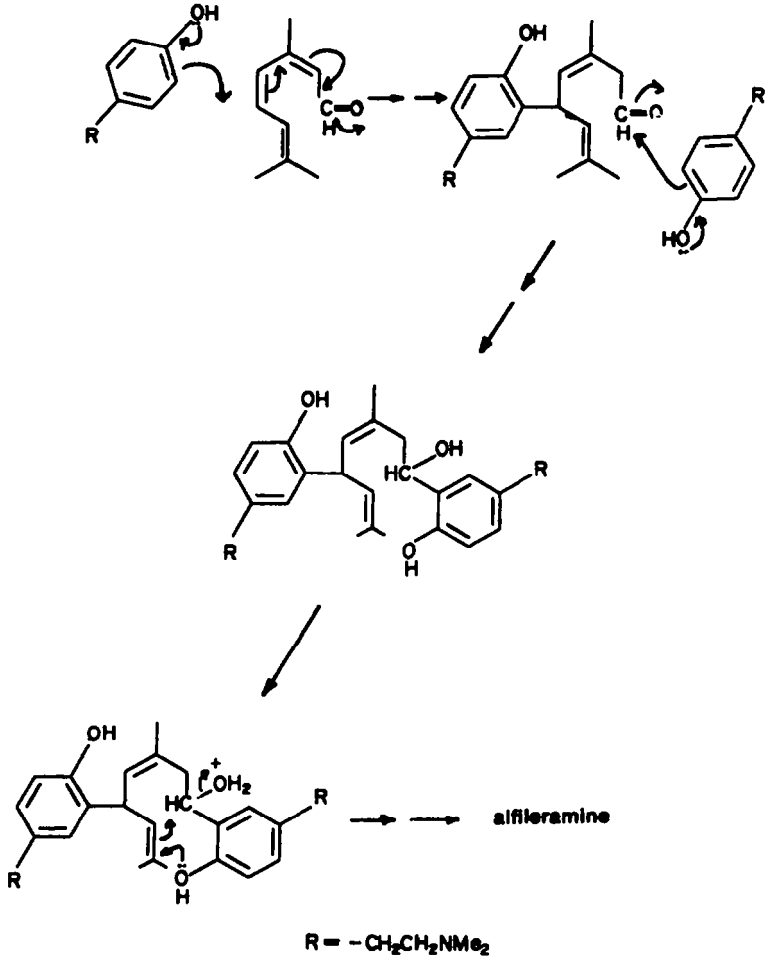
It has been reported[14] that hordenine occurs in *Cannabis sativa*. It would seem quite possible then that hordenine could react similarly to olivetol with citral of *C. sativa* (Eqn 1) and produce an alkaloid such as **10**.



10

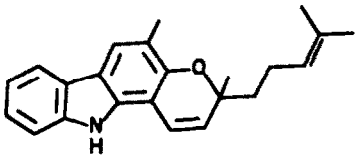
The isolation of four alkaloids from leaves (but not roots or stems) of *Cannabis* was indeed reported[15] some time ago. Only mass spectral evidence for the structure of these alkaloids was available, but one alkaloid (cannabamine B) had a molecular weight of 299, which is exactly that of **10**. Fragmentation patterns indicated[15] that the unknown alkaloids could be phenethylamine derivatives and hence **10** may represent the structure of cannabamine B.

The isolation of a compound with three chiral centers, but in an inactive form, is unusual although not without precedent. It does raise questions about artifactual synthesis, transformations or racemizations, none of which



Scheme 2.

can be simply answered at this time. Analogies occur in both cannabinoid and *Murraya koenigii* chemistry. The alkaloid mahanimbine (11) was isolated[16] from *M. koenigii* in a chiral form, but mahanimbidine (12) and cyclomahanimbine (13), were isolated[17] inactive even though each has three chiral centers. Compound 12 was isolated simultaneously by another group[18] and named curryangin. They showed that it could be formed from (+)-mahanimbine (11) by treatment with acetic anhydride or *p*-toluenesulfonic acid and that the product was optically inactive.



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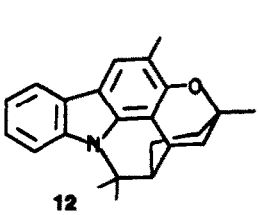

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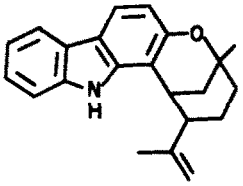

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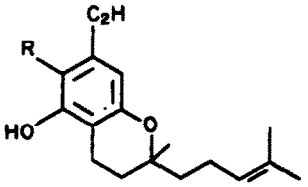
On the other hand, cannabichromene (14) and cannabichromenic acid (15) are optically inactive as isolated[19] and a biogenetic scheme which involves a symmetrical intermediate has been proposed[20] for these compounds in *C. sativa*.



14: R = H
15: R = COOH

Since alfileramine was shown to be present in the $CHCl_3$ and methanol plant extracts prior to purification attempts and was partially purified by Sephadex chromatography with inert solvents, it seems likely that it is not an artifact of the isolation procedure. Whether it could have formed during the plant drying process or is simply formed in the plant cells by a nonenzymatic process is difficult to determine without access to fresh plant material. The eventual structure elucidation of the additional alfileramine-type alkaloids present in *Z. culantrillo*[13] may assist in solving this problem.

EXPERIMENTAL

Dried and ground leaves of *Z. punctatum*[2] were extracted successively in a Soxhlet with petroleum ether (b.p. 40–60°), $CHCl_3$ and MeOH. Tlc on Si gel (EtOH/H_2O/NH_4OH, 15:9:1) showed alkaloid spots at R_f 0.51 and 0.63 in the $CHCl_3$ residue, but the amount of total residue precluded work on this fraction. The MeOH residue showed mainly the R_f 0.51 alkaloid with trace spots at 0.25, 0.30, 0.63 and 0.74. From 412 g of leaf material, 28 g of crude residue was obtained from the MeOH extract by evaporation *in vacuo* at 40°. The major alkaloid could be obtained nearly pure in relatively low yield by column chromatography of the MeOH residue on Sephadex LH 20 ($CHCl_3$/MeOH 1:1). Mass spectrometry and ^1H NMR indicated that it was the same as the alkaloid purified much more successfully by the following procedure. The MeOH residue was dissolved in 200 ml 1M H_2SO_4 and the soln was extracted with $CHCl_3$. The aqueous was made basic to pH 9 with NaOH and extracted again with $CHCl_3$. The $CHCl_3$ was dried ($NaSO_4$) and evaporated *in vacuo* to yield 0.9 g of the R_f 0.51 alkaloid with only faint traces of the other alkaloidal components. Trituration of the crude alkaloid with hot acetone and recrystallization from acetone/EtOH yielded alfileramine, m.p. 185–187°. IR (KBr): 3250, 2970, 1620, 1500, 1280, 1260, 850, 820 cm^{-1}; UV λ_{max}^{EtOH} nm (log ε), 283 (5.5), 290 sh (5.3), 212 (26.8) with OH^- shift to 290 and 306 nm; ^1H NMR ($CDCl_3$, 60 MHz) ppm: 0.56 (s, 3H), 1.75 (s, 3H), 1.84 (s, 3H), 2.32 (s, 6H), 2.38 (s, 6H), 2.48–2.96 (br s, 11H), 2.96–3.66 (br m, 2H), 4.44 (br s or t, 1H), 5.53 (br d, 1H), 6.48–7.23 (m, 6H), 9.30 (br s, 1H, disappears with D_2O); ^1H NMR ($CDCl_3$, 220 MHz) ppm: similar to 60 MHz except 3.29 (m, 1H), 6.56 (d, 1H, J = 8 Hz), 6.64 (d, 1H, J = 9 Hz), 6.83 (dd, 1H, J = 8 and 2 Hz), 6.88 (dd, 1H, J = 9 and 2 Hz), 7.04 (s, 1H), 7.14 (s, 1H); ^{13}C NMR ($CDCl_3$) ppm: 20.04 q, 23.36 q, 27.88 q, 33.11 t, 33.34 d, 38.19 t, 45.05 q, 45.09 q, 48.24 d, 61.59 t, 61.71 t, 77.35 s, 115.20 d, 116.95 d, 125.82 d, 126.62 s, 127.01 d, 128.74 s, 129.69 s, 130.84 s, 131.32 s, 132.20 d, 150.86 s, 152.98 s; MS: *m/e* 462 (43), 418 (4), 404 (5), 232 (7), 231 (4), 230 (9), 187 (14), 173 (13), 158 (20), 58 (100); $[\alpha]_D^{25°} = 0°$ at 589, 578 and 546 nm (c, 1.9 $CHCl_3$). *Anal.* Found: C, 77.21; H, 8.55; N, 5.95. $C_{30}H_{42}N_2O_2$ requires: C, 77.88; H, 9.15; N, 6.05%.

Alfileramine was treated with $CH_3\bar{I}$ in benzene to yield a precipitate which was recrystallized from acetone/MeOH to yield a white powder, m.p. 253–255°, of alfileramine dimethiodide: λ_{max}^{EtOH} 230, 283, 288 sh, OH^- shift to 290, 305 nm; ^1H NMR (d_6-acetone/D_2O) ppm: 0.31 (s, 3H), 1.52 (s, 3H), 1.72 (s, 3H), 2.66–3.83 (m, 14H), 3.26 (s, 18H), 5.31 (d, 1H), 6.50–7.10 (m, 4H), 7.25 (br s, 1H), 7.53 (br s, 1H); ^{13}C NMR (d_6-acetone/D_2O) ppm: 20.00 q, 23.26 q, 27.81 (q or t, partially obscured by solvent peak), 28.68 (t or q, partially obscured by solvent peak), 33.18 d, 39.30 t, 48.57 d, 49.04 d, 53.49 q, 53.58 q, 67.40 t, 67.47 t, 77.88 s, 115.99 d, 117.16 d, 125.04 d, 126.28 s, 127.27 s or d, 127.80 s, 127.88 s or d, 128.32 s or d, 128.76 s, 129.19 d, 132.87 two resonances s and d, 151.52 s, 153.47 s; MS: *m/e* (rel. intensity) 462 (2), 417 (2), 372 (7), 187 (7), 171 (7), 91 (7), 75 (7), 58 (100).

Alfileramine (0.1 g) was dissolved in 10 ml of abs EtOH and 2.5 ml of 48% HBr in 2.5 ml of EtOH was added. The solution was stirred at 25° for 10 min and the solvent was evaporated. The tan solid was recrystallized from EtOAc/MeOH to yield crystals of isoalfileramine dihydrobromide, m.p. 284–287°: ^1H NMR ($CDCl_3$) ppm: 0.80 (s, 3H), 1.47 (s, 3H), 1.67 (s, 3H), 1.93–2.17 (m, 7H), 2.97 (s, 12H), 3.27 (m, 8H), 6.57–7.13 (m, 6H); ^{13}C NMR ($CDCl_3$/CD_3OH) ppm: 20.49, 24.63, 28.08, 28.54, 28.88, 29.40, 30.09, 30.27, 33.71, 37.84, 42.73, 44.74, 45.60, 50.60, 51.57, 58.52, 74.32, 76.28, 114.42, 115.86, 121.26, 123.79, 124.88, 125.05, 125.74, 126.60, 128.61, 149.46, 152.91. The structure was determined by a single crystal X-ray diffraction study.[3]

Isoalfileramine dihydrobromide was dissolved in aq MeOH and a few drops of 5% NaOH was added until precipitation ceased. The mixture was extracted with $CHCl_3$ and the $CHCl_3$ layer was dried, filtered and the solvent evaporated *in vacuo* to leave a tan solid, m.p. 284–287°, of isoalfileramine: ^1H NMR ($CDCl_3$) ppm: 0.87 (s, 3H), 1.47 (s, 3H), 1.67 (s, 3H), 1.93 (m, 4H), 2.33 (s, 12H), 2.5–3.0 (m, 10H), 3.30 (m, 1H), 6.57–7.10 (m, 6H); ^{13}C NMR ($CDCl_3$) ppm: 19.16 q, 27.11 q, 27.53 t, 27.75 q, 32.34 t, 32.63 d, 37.03 t, 44.31 q, 50.94 d, 60.70 t, 73.56 s, 75.60 s, 114.34 d, 115.85 d, 120.97 s, 123.67 s, 125.01 d, 126.20 s, 127.08 d, 128.95 d, 130.11 s, 149.69 s, 153.12 s; MS *m/e* (rel. intensity): 462 (22), 443 (3), 429 (11), 405 (19), 369 (13), 355 (11), 295 (21), 281 (7), 221 (28), 207 (6), 187 (17), 173 (3), 159 (3); $[\alpha]_D^{25°} = 0°$ at 589, 578 and 546 nm (c, 5.0 $CHCl_3$).

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[1]This work was supported in part by research grant CA 19243 from the National Cancer Institute.

[2]F. R. Stermitz and I. Sharifi, *Phytochemistry* 16, 2003 (1977).

[3]This alkaloid was originally reported[2] as N-methylcorydine, but further work (F. R. Stermitz, unpublished results) established it instead as N-methylisocorydine. Details regarding this assignment revision will be published subsequently.

[4]*Z. punctatum* is known in Puerto Rico as "*the toothache tree*" (because chewing the bark causes a numbness in the mouth) and "alfiler" because of the sharp spines on the branches (alfiler = pin in Spanish).

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