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ALKALOIDS AND OTHER CONSTITUENTS OF *ZANTHOXYLUM WILLIAMSII, Z. MONOPHYLLUM* AND *Z. FAGARA**

FRANK R. STERMITZ, MARY A. CAOLO and JACQUELYN A. SWINEHART

Department of Chemistry, Colorado State University, Fort Collins, CO 80523, U.S.A.

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Key Word Index—*Zanthoxylum williamsii*; *Z. monophyllum*; *Z. fagara*; Rutaceae; alkaloids; chemotaxonomy.

Abstract—*Zanthoxylum williamsii* (Rutaceae) was found to contain (+)-asaranin, (+)-sesamin, esculetin dimethyl ether, nitidine, chelerythrine, magnoflorine, laurifoline, skimmianine and edulinine. The quaternary alkaloid fraction of *Z. monophyllum* contained berberine, magnoflorine, chelerythrine and a 1,2,9,10-substituted dihydroxydimethoxy-*N,N*-dimethylaporphinium salt. Leaves of *Z. fagara* were found to contain synephrine. Leaves of each species were examined for the presence of bishordeninyl terpene alkaloids, but none was found. Some chemotaxonomic relationships among *Zanthoxylum* species are discussed.

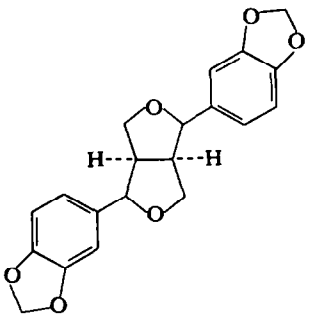
INTRODUCTION

The genera *Fagara* and *Zanthoxylum* of the Rutaceae were historically maintained separately because of the position [1] that perianth structures were fundamentally different in the two taxa. Brizicky, however, noted [2] that 3 species not described by Engler had perianth morphology which was transitional between those of the separated taxa and reduced *Fagara* to a subgenus under *Zanthoxylum*. Waterman has published [3] a summary of these and additional views on *Zanthoxylum* taxonomy. He gave *Zanthoxylum* names to the remaining *Fagara* species which had not previously been described under *Zanthoxylum*. Among key transitional species which influenced the taxonomic reduction were *Z. ferrugineum* Rdlk., *Z. maxatlanum* Sandw. and *Z. williamsii* Standl. & Steyerm., all of Central American origin. We were able to obtain *Z. williamsii* and report here the major constituents of this species. We have also completed a study of the quaternary alkaloids of *Z. monophyllum* and have analyzed the polar extract of the leaves of *Z. fagara*.

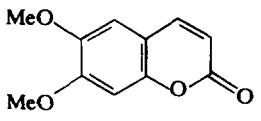
RESULTS

Z. williamsii. Roots yielded (+)-asaranin (**1**), (+)-sesamin (**2**), esculetin dimethyl ether (**3**), nitidine (**4**), chelerythrine (**5**) and magnoflorine (**6**). In the stems we found laurifoline (**7**) and (+)-asaranin, while the leaves yielded skimmianine (**8**) and edulinine (**9**). No bishordeninyl terpenes [4, 5] were present.

Z. monophyllum. The major constituents and tertiary alkaloids of this species were previously reported [6] but the quaternary fraction has not been investigated. Additional berberine was found along with magnoflorine and chelerythrine. A trace of an aporphine was isolated which could be assigned the partial structure **10**. It was nonidentical by MS and UV with a



1 Aryl ring *trans*
2 Aryl rings *cis*



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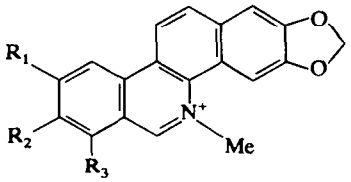
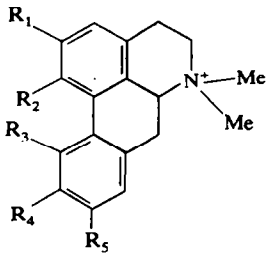
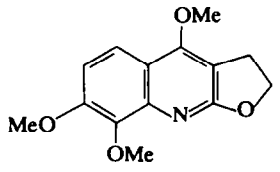


4 $R_1 = R_2 = OMe$; $R_3 = H$
5 $R_1 = H$; $R_2 = R_3 = OMe$

* Part 5 in the series "Chemistry of *Zanthoxylum*". For Part 4 see Swinehart, J. A. and Stermitz, F. R. (1980) *Phytochemistry* **19**, 1219.



6 $R_1 = R_4 = OMe$; $R_2 = R_3 = OH$; $R_5 = H$
7 $R_1 = R_4 = OMe$; $R_2 = R_5 = OH$; $R_3 = H$
10 R_1, R_2, R_4, R_5 = 1OH,3OMe; $R_3 = H$
11 $R_1 = R_4 = R_5 = OMe$; $R_2 = OH$; $R_3 = H$
12 $R_1 = R_2 = R_5 = OMe$; $R_4 = OH$; $R_3 = H$
13 $R_1 = R_2 = R_4 = OMe$; $R_2 = OH$; $R_3 = H$



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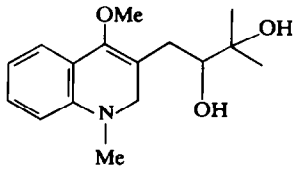

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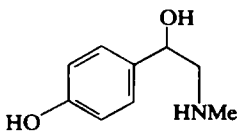
standard sample of '*Fagara* base', **11**, from *Z. tingoassuiba* [7]. Its neutral and base-shifted UV spectra were similar to those reported [8] for cocsarmine, **12**, and only slightly shifted from the literature values [9] for xanthoplanine, **13**. Standard samples were not available and insufficient **10** was available for definitive structure proof.

Z. fagara. Engler [1] and Waterman (private communication) consider *Z. fagara* L. and *F. pterota* L. to be conspecific. This species has previously been studied, but leaf analysis for bishordeninyl terpenes was not accomplished. It was first investigated [10] under the name *F. pterota* L. and yielded candicine, tembetarine, magnoflorine, *N*-methylisocorydine, laurifoline, chelerythrine and a trace of nitidine. It was investigated [11] under the name *Z. fagara* L. and again found to contain magnoflorine and laurifoline. Recently, the leaves have been specifically studied and found [12] to contain skimmianine and scopoletin, but only the hexane extract was analysed. Since bishordeninyl terpenes have so far only been found in more polar extracts and occur in the leaves of the supposedly related species *Z. culantrillo* [5], we analysed for alkaloids in the MeOH leaf extract. Only synephrine (**14**) and no bishordeninyl terpenes or other alkaloids were found.



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DISCUSSION

In Engler [1], only 13 species were originally assigned to *Zanthoxylum* and the remainder to *Fagara*. Of the 13, 12 were from the China and Japan areas, while the last, *Z. americanum* is native to North America. The taxon *Z. clava-herculis*, also from North America, was considered [1] synonymous with *Z. americanum*, but most later workers have considered these separate species. The two American species contain 6 identical alkaloids, although berberine occurs only in *Z. clava-herculis*, while *Z. americanum* has skimmianine and γ-fagarine in the leaves. Waterman has pointed out [13] that the major alkaloid content of these two species (candicine, chelerythrine, laurifoline, magnoflorine, nitidine and tembetarine) is typical of the small Central and South American section *Pterota* of the subgenus *Fagara*. Of the Asian original *Zanthoxylum* species, constituents of *Z. alatum*, *piperitum* and *arnottianum* have been reported. Nitidine is absent from all 3, chelerythrine from two and each contains at least two of the 3 furoquinoline alkaloids dictamnine, skimmianine and γ-fagarine. Two also contain the lignans asaranin and/or sesamin. Thus, these 3 Asian species form a reasonably close group chemically, but one which is somewhat different from the American members of the original *Zanthoxylum*. Our results with *Z. williamsii* are interesting in that it contains asaranin, sesamin, and two furocoumarin alkaloids (typical of the Asian species), but also nitidine, chelerythrine and the coumarin esculetin dimethyl ether (more typical of the American species). The isolation of edulinine from the leaves of *Z. williamsii* may be of importance since its only previous reported [14] occurrence was in the leaves of the South American species *Z. mayu* (*Fagara mayu*).

The quaternary alkaloid analysis of *Z. monophyllum* has not greatly changed its chemical position [6]. It was unique in having berberine and the pyranoquinoline zanthophylline as major components. The additional finding of chelerythrine and aporphine alkaloids simply reinforces its place as a prolific synthesizer of both 1-benzyltetrahydroisoquinoline and anthranilic acid derived alkaloids. This has been suggested [15] to be a mark of evolutionarily advanced species.

The well-worked species *Z. fagara* represented an interesting case in regard to our newly-discovered bishordeninyl terpene alkaloids. *Z. fagara* is in the *Pterota* section of the subgenus *Fagara* and its main alkaloid content is very similar to that of *Z. americanum* and *Z. clava-herculis*. The main exception is the occurrence of *N*-methylisocorydine as a major alkaloid, which is absent in the other two species. Morphologically, *Z. fagara* is apparently closely related to *Z. culantrillo* and the alkaloid content of roots and stems of these two species is indeed very similar [5]. We found [5] bishordeninyl terpene alkaloids in *Z. culantrillo* leaves, and they are absent in *Z. fagara*. There had been some hope that these alkaloids would provide a good taxonomic marker since the first investigations of species from the section *Tobinia*, *Z. punctatum* [4] and *Z. coriaceum* [5], both

yielded bishordeninyl terpenes. They also occur in a third member of the section, *Z. procerum* (Boulware, R., unpublished results). Their presence in *Z. culantrillo*, but absence in the closely-related *Z. fagara* may however, indicate a sporadic or random rather than specific occurrence. Certainly additional work (chemical and botanical) in both the *Pterota* and *Tobinia* sections of the genus would be valuable.

EXPERIMENTAL

Z. williamsii Standl. & Steyerm. Plants were collected in April 1977 near Zamorano, Honduras by J. R. Stermitz and verified by A. Molina (Voucher Accession No. 2754, CSU Herbarium).

Dried and ground leaves (53 g) were extracted in a Soxhlet with hexane and then MeOH. The hexane residue (2.3 g) was chromatographed through a 2.8 × 11 cm Si gel column (Et_2O–$CHCl_3$, 1:1). From fractions 57–70 were obtained (after trituration with MeOH) 10 mg of a gummy solid identified as skimmianine (UV, ^1H NMR comparison with an authentic sample). The MeOH residue (6.8 g) was triturated with MeOH and the soln separated from a nonalkaloidal solid. The MeOH was evapd and the residue triturated with hexane to leave 2.5 g of gummy solid. This was dissolved in M H_2SO_4, extracted with $CHCl_3$, made basic to pH 8.6 and extracted with $CHCl_3$. The $CHCl_3$ soln (after drying and evapn) left 17 mg of a semi-solid alkaloid identified as (−)-edulinine (UV, ^1H NMR and OR data compared with lit. [14] values).

Dried and ground roots (300 g) were extracted successively with hexane and then MeOH. The hexane residue (8 g) was triturated with hot hexane to leave 4 g of insoluble residue. This was dissolved in $CHCl_3$–EtOAc (9:1) and chromatographed by prep. LC (Si gel). Fractions 21–26 yielded 0.22 g of crystalline (+)-asarinin (mp, OR and ^1H NMR comparison with lit. [16, 17] values). Fractions 48–60 yielded 0.099 g of crystalline (+)-sesamin (mp, OR, UV, ^1H NMR comparison with lit. [16] values). Fractions 137–188 yielded a gummy solid which was recrystallized from Me_2CO–MeOH to give 0.9 mg of crystalline esculetin diMe ether (scoparone) (mp, UV, IR, ^1H NMR comparison with lit. [18] values).

The MeOH extract was concd to 50 ml and $\frac{1}{2}$ was added to H_2O. The sol. portion was evapd to dryness, the residue dissolved in MeOH and pptd with Me_2CO. The ppt. was added to H_2O, filtered and the soln divided in two parts. One part was concd to a small vol. and pptd with KI soln. The alkaloid ppt. was separated by prep. LC to yield nitidine and chelerythrine (UV, UV acid and base shifts, TLC in two solvent systems, fluorescence compared with standard samples). The second aq. portion was evapd to dryness, redissolved in MeOH and pptd with Me_2CO. The ppt. was identified as magnoflorine (UV, UV base shift, TLC in two solvent systems, fluorescence compared with standard sample). The second portion of the original MeOH extract was distributed between $CHCl_3$ and M HCl. The acidic layer was made basic to pH 8.6, KI was added and the soln was continuously extracted with $CHCl_3$. The $CHCl_3$ residue (0.5 g) was chromatographed on neutral Al_2O_3 (activity I, 10–50% MeOH–Me_2CO), fractions 71–79 combined and rechromatographed on acid-washed Al_2O_3 (same solvents). Fractions 50–70 yielded a few mg of residue shown to be laurifoline (UV, UV base shift, TLC and MS when compared with standard sample).

Z. monophyllum. To 100 g of syrupy MeOH extract of 1 kg of *Z. monophyllum* stems [6] was added 450 ml 2N HCl. A brown ppt. (mostly berberine HCl) was filtered off, the soln made basic to pH 8.6 and extracted with $CHCl_3$. Evapn left 5 g of brown–yellow solid. By PLC (Si gel, C_6H_6–MeOH, 3:2), chelerythrine was isolated (UV, UV acid and base shifts, TLC in two solvents, compared with standard sample). Chelerythrine and berberine represented the major alkaloids. PLC (Si gel, MeOH–$CHCl_3$–formamide–H_2O, 7:7:4:1) allowed isolation of two minor alkaloids. An R_f 0.6 alkaloid was shown to be magnoflorine (UV, UV base shift, MS, TLC in two solvent systems, compared with standard sample). A trace of an R_f 0.3 alkaloid was found: MS (*m/e*, rel. int.): 356 (28), 355 (80), 341 (20), 297 (20), 192 (19), 58 (100); λ_{max}^{EtOH} nm: 274 sh, 282, 308, 313 sh, λ_{min} nm: 260, 290; OH^- λ_{max} nm: 352, λ_{min} nm: 308. The *A* of the 308 nm max was 10% higher than that of the 282 max and is typical of 1,2,9,10-tetrasubstituted aporphines (boldine-type substituent pattern). The MS and UV base shift were consistent with a dihydroxydimethoxy-*N,N*-dimethylaporphinium salt. TLC was identical with standard sample of 1-hydroxy-2,9,10-trimethoxy-*N,N*-dimethylaporphimium chloride, but significant differences were apparent in the MS and UV spectra. There was insufficient material for further investigation.

Z. fagara. A collection was made by J. M. Poole near Galveston Island, Texas (CSU Herbarium Accession No. 35044). Dried and ground leaves (600 g) were extracted with hexane EtOH and then MeOH. The EtOH and MeOH residues showed mainly one alkaloid by TLC and this was identified as synephrine after isolation and characterization as described previously [5]. Extensive TLC work on extracts and column fractions showed no alkaloids or residues indicative of the bishordeninyl terpene alkaloids nor was hordenine present.

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