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LINEAR FUROCOUMARINS AND OTHER CONSTITUENTS FROM *THAMNOSMA TEXANA**

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Key Word Index—*Thamnosma texana*; Rutaceae; furocoumarins; psoralen; bergapten; xanthotoxin; isopimpinellin; imperatorin; alloimperatorin methyl ether epoxide; heraclenin; oxypeucedanin; photosensitization.

Abstract—Eight linear furocoumarins and three coumarins were isolated and identified from *Thamnosma texana*. They were xanthotoxin, imperatorin, bergapten, alloimperatorin methyl ether epoxide, heraclenin, isopimpinellin, psoralen, oxypeucedanin, and the coumarins herniarin, osthol and thamnosmin. The linear furocoumarins appear to be agents that account for the known photosensitizing properties of *Thamnosma texana*, and consequently its colloquial name, 'blisterweed.' This is the first report on the occurrence of imperatorin, heraclenin, oxypeucedanin, herniarin or osthol in any *Thamnosma* species.

INTRODUCTION

Linear furocoumarins are potent photosensitizers that occur in a number of plant species from several families [1, 2]. They also have medical applications [3] and are phototoxic to both domestic animals [2] and man [1]. *Thamnosma texana* (Gray) Torr. (Dutchman's breeches, Texas desert rue) is an odorous perennial plant of the Rutaceae that grows in limestone soils from central Texas westward. The plant has been recognized as having photosensitizing properties [4] and is often locally referred to as 'blisterweed.' We have previously reported that *T. texana* is a potential photosensitizing plant for livestock [5], and report here the results of investigations into the linear furocoumarin chemistry of the plant. *T. texana* contains at least eight linear furocoumarins (Fig. 1), and the total content appears to be at least 0.5% (dry wt basis) of the above ground parts of the plant. There remains little doubt that these compounds account for the phototoxicity associated with *T. texana*.

RESULTS AND DISCUSSION

T. texana plants were collected during October, 1980, in Tom Green County, Texas. A specimen is on deposit at the Texas A&M University herbarium, College Station, Texas. Although both root (including root crown) and aerial portions were examined, identification and quantification studies were directed primarily at the above ground portions of the plant because of interest in the phototoxic properties of this species to grazing livestock. All compounds (**1**–**11**) reported here were found in the aerial portion of *T. texana* (Fig. 1), but the roots also contained each of these except herniarin (**9**).

Quantification of various compounds in the plant revealed that xanthotoxin (**8**) exceeded the next highest concentration of a furocoumarin by a ratio of 3:1 (Table 1). Samples spiked with xanthotoxin were utilized to evaluate the extraction and analytical techniques, establishing an overall 83% recovery.

Reverse phase HPLC was used for final separation of components from bands isolated on TLC plates from sample extracts. Quantification was performed on a normal phase, 5μ silica column (15 cm) with 0.1% formic acid and 0.1% ethyl acetate in chloroform at a flow rate of 2.5 ml/min with detection at 250 nm [6]. Whenever authentic standards were available, their chromatographic and spectral data were compared with the isolated compounds from *T. texana*. Authentic samples of alloimperatorin methyl ether epoxide (**1**) and osthol (**10**) were not available; therefore, these two compounds were identified by comparison of experimental data with reported spectral data [7, 8]. Alloimperatorin methyl ether epoxide (**1**), bergapten (**2**), isopimpinellin (**5**), psoralen (**7**) and xanthotoxin (**8**) have previously been isolated

Table 1. Levels of linear furocoumarins in the aerial portions of *Thamnosma texana*

Compound*	ppm†
Psoralen (**7**)	840
Xanthotoxin (**8**)	2725
Bergapten (**2**)	950
Isopimpinellin (**5**)	575

*These were the only compounds quantitated. On the basis of xanthotoxin, all other linear furocoumarins not recorded here appeared in quantities < 575 ppm by HPLC at 250 nm.

†Dry weight basis.

*Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

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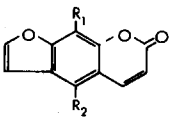
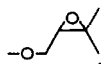
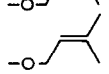
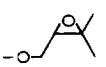
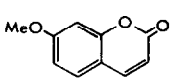
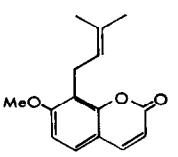


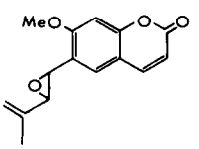
COMPOUND (MW)	R_1	R_2
1 Alloimperatorin Methyl Ether Epoxide (300)	-OMe	
2 Bergapten (216)	H	-OMe
3 Heraclenin (286)		H
4 Imperatorin (270)		H
5 Isopimpinellin (246)	-OMe	-OMe
6 Oxypeucedanin (286)	H	
7 Psoralen (186)	H	H
8 Xanthotoxin (216)	-OMe	H



9 Herniarian (176)



10 Osthol (244)



11 Thamnosmin (258)

from *T. montana* [7], but this is the first reported occurrence of heraclenin (**3**), imperatorin (**4**), oxypeucedanin (**6**), herniarin (**9**), or osthol (**10**) from any *Thamnosma* species. An additional compound, thamnosmin (**11**), also found in *T. montana* [7], was identified from *T. texana* by comparison of mass spectral data.*

EXPERIMENTAL

IR spectra were recorded from KBr discs. ^{1}H NMR spectra ($CDCl_3$ or CD_2Cl_2) were measured at 90 MHz, and the δ-values are given in ppm downfield from TMS as an internal standard. HPLC was performed on a Waters Model M-6000 pump with UV detection on a Tracor Model 970A Variable Wavelength Detector. Sample peaks were recorded and integrated with a Hewlett Packard Model 3390A Integrator.

Isolation. The green plant was separated into aerial and root (including crown) portions, each was dried with forced warm air (35°), ground and frozen. Samples (5 g) were extracted with EtOAc (5 × 15 ml) utilizing Polytron homogenization. The combined extracts were dried (Na_2SO_4) and the solvent removed by rotary evaporation. The residues were then dissolved with $CHCl_3$ or Me_2CO and aliquots were spotted on silica gel TLC plates (20 × 20 cm, 0.25 mm thick, Brinkmann Silplate F-22) and developed with CH_2Cl_2 (× 3) for aerial extracts, or with C_6H_6–Et_2O (20:1; × 3) for root extracts. Linear furocoumarin fractions were visualized on the developed plates as yellow or golden bands under long-wave UV light. These bands were scraped and the components eluted with Et_2O. The eluted components were further separated by reverse phase HPLC on a 5μ, C-18 column using a mobile phase of 37% MeCN in H_2O at a flow rate of 1 ml/min with UV detection.

Identification. Xanthotoxin (**8**) (mp 144–145°), bergapten (**2**) (mp 184–185°), and imperatorin (**4**) (mp 96–97°) were isolated from aerial portions of *T. texana*, and crystallized from Et_2O–C_6H_{12}. These three compounds exhibited spectral data identical to those of the authentic compounds [9]. A fourth product was isolated from the aerial portion of *T. texana* and crystallized from Et_2O–C_6H_{12} (mp 118–119°). ^{1}H NMR ($CDCl_3$): δ8.12 (1H, *d*, *J* = 11 Hz, H-4), 7.72 (1H, *d*, *J* = 2 Hz, H-7), 6.93 (1H, *d*, *J* = 2 Hz, H-6), 6.42 (1H, *d*, *J* = 11 Hz, H-3), 4.29 (3H, *s*, OMe), 3.53–2.81 (3H, *m*, CH_2–CH), 1.53 (3H, *s*, Me), 1.35 (3H, *s*, Me). EIMS (probe) 45 eV, *m/z* (rel. int.): 300 $[M]^+$ (43), 285 $[M-Me]^+$ (9), 271 $[M-CHO]^+$ (2), 257 $[M-C_3H_7]^+$ (21), 230 (80), 229 $[M-C_4H_7O]^+$ (100), 215 (46), 214 $[M-C_5H_{10}O]^+$ (50), 202 (35), 201 $[M-C_6H_{11}O]^+$ (53), 186 (82). IR ν cm^{-1}: 1718 (C–O), 1588 (C–C). NMR and mass spectral data agree with the product containing both aromatic methoxyl and 3-methyl-2,3-epoxybutyl substituents [8]. Since alloimperatorin methyl ether epoxide (**1**) is known to occur in the related species *T. montana* [7], we concluded that this compound may be **1**. Comparison of the IR, NMR and mass spectral data from the *T. texana* product with published spectral data for alloimpera-

*Two other compounds, thought to be byakangelicol and its 5,8 positional isomer (isobyakangelicol) by mass spectroscopy, were obtained in very minute quantities.

torin methyl ether epoxide (**1**) [7], established that the products were, in fact, the same. Heraclenin (**3**), isopimpinellin (**5**), oxypeucedanin (**6**) and psoralen (**7**) were identified by mass spectral data of samples obtained by preparative reverse phase HPLC. Confirmative identification was accomplished by co-injection on HPLC with the authentic compounds [9]. Herniarin (**9**) was identified by its MS and NMR data and by co-injection on HPLC with a commercially obtained sample. Osthol (**10**) was confirmed by NMR and MS. ^{1}H NMR (CD_2Cl_2): δ7.63 (1H, *d*, H-4), 7.35 (1H, *d*, H-5), 6.86 (1H, *d*, H-6), 6.18 (1H, *d*, H-3), 5.22 (3H, *m*, CH_2–CH), 3.52 (1H, *d*, CH), 1.84 (3H, *s* Me), 1.67 (3H, *s*, Me). EIMS (probe) 70 eV, *m/z* (rel. int.): 244 $[M]^+$ (100), 229 $[M-Me]^+$ (91), 213 $[M-MeO]^+$ (54), 201 $[M-C_2H_3O]^+$ (60), 189 $[M-C_3H_3O]^+$ (50). Thamnosmin (**11**) was also identified on the basis of mass spectral data: EIMS (probe) 70 eV, *m/z* (rel. int.): 258 $[M]^+$ (50), 229 $[M-CHO]^+$ (100), 203 (10), 201 (12), 199 (19), 198 (12), fragmentation explaining additional ions at *m/z* 214 (32), 189 (69) and 159 (41) is proposed by Kutney *et al.* [7].

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