Phytochemistry, Vol. 26, No. 3, pp. 727–730, 1987.
Printed in Great Britain

0031-9422/87 $3.00 + 0.00

THE CHARACTERIZATION OF ESULONE C AND CHEMOTAXONOMY OF JATROPHANE DITERPENES IN LEAFY SPURGE

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(*Revised received* 14 *August* 1986)

Key Word Index *Euphorbia* spp.; Euphorbiaceae; leafy spurge; chemotaxonomy; biological control; jatrophane diterpenes; esulone C.

Abstract A new jatrophane diterpene, esulone C, was isolated and characterized from the ether extract of the roots of a North Dakota accession of the noxious weed leafy spurge. The ether extracts of North Dakota, Oregon, Montana and Austrian leafy spurge accessions were chromatographically compared for the occurrence of jatrophane diterpenes relative to the taxonomy and biological control of leafy spurge.

INTRODUCTION

Leafy spurge is a toxic, perennial, noxious weed which poses a serious threat to livestock on open rangeland in the upper great plains of the United States [1]. While this weed can be controlled by herbicides, it cannot be eradicated by chemical means. European leafy spurge (*Euphorbia esula* L.) is controlled naturally in Europe by indigenous insect predators [2]. However, attempts to utilize these predators as biological control agents for North American leafy spurge have not been successful.

The North American population of leafy spurge is considered to include five taxa [*E. esula* L., *E.* × *pseudovirgata* (Schur) Soo', *E. uralensis* Fisch. ex Link., *E. agraria* M. Bieb., and *E. cyparissias* L.] based upon an assessment of the morphological characteristics of many accessions of the weed in the United States and Canada [3]. An analysis of leaf wax triterpenes of three North American leafy spurge accessions and a European *Euphorbia esula* accession showed distinct chemical differences on a geographical basis [4]. The extensive hybridization of leafy spurge suggests that insect biological control of this weed in North America will meet with limited success without a reliable means of correlating predator-susceptible European *Euphorbia esula* accessions with specific North American leafy spurge (*Euphorbia* spp.) accessions. The identification of easily detected unique chemical constituents in leafy spurge which are common to European and North American accessions of this weed could provide a chemical basis for the desired correlation.

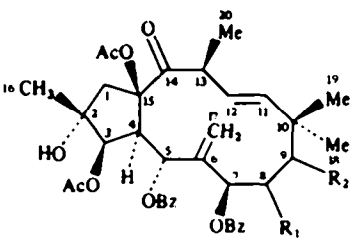
This investigation reports the identification of a new jatrophane diterpene in a North American leafy spurge accession, a chromatographic system for the separation and detection of this and other jatrophane diterpenes in *Euphorbia* spp. extracts and a qualitative evaluation of their occurrence in the extractives of a European *E. esula* and three North American accessions of leafy spurge.

RESULTS AND DISCUSSION

Characterization of esulone C

Esulone C (**3**) was obtained (0.0002 % of plant material) chromatographically (HPLC) from the ether extract of leafy spurge root material which previously yielded esulone A (**1**) and esulone B (**2**) [5]. The ^{1}H NMR spectrum of esulone C was similar to the previously reported spectrum of esulone A except for significantly higher field resonances for the C-15 acetate, C-2 methylene and C-17 vinyl protons and significantly lower field resonances for the C-9 carbinol proton and the C-5, and C-7 substituted benzoate protons.

Oxidation of esulone C gave a product chromatographically identical with an authentic sample of **4** obtained from the oxidation of esulone A. The attempted tautomerization of esulone A to esulone C with acid was unsuccessful. However, chromatography of the reaction mixture of a putative sample of esulone C treated with acid showed the presence of esulone A.



1 R^1 = OH, R^2 = =O
2 R^1 = OAc, R^2 = =O
3 R^1 = =O, R^2 = OH
4 R^1 = =O, R^2 = =O

Mass spectral data for the esulone A, B, and C could only be obtained utilizing ammonia chemical ionization mass spectral techniques. These data revealed unusual and distinctive fragmentation patterns related to the loss of the C-8, C-9 carbonyl and hydroxyl, C-3 and C-15 acetates and C-5 and C-7 benzoates (as benzoic acid and ammonium benzoate) from the parent ion [Haddon, W. F. and Manners, G. D., unpublished results]. The prominent loss of two acetic acid fragments and a benzoic acid fragment in the ammonia chemical ionization spectrum of **3** and **4**, while ammonium benzoate and benzoic acid fragments are lost in the spectrum of **1**, was consistent with carbonyl functionality at C-8 for esulone C. The oxidation results, mass spectral and ^{1}H NMR data structurally designated esulone C as **3**.

Attempts to obtain a single crystal X-ray analysis of esulone C were unsuccessful thereby preventing the determination of the absolute stereochemistry of the compound. However, application of the excitation chirality method [6, 7] to esulone C showed the interaction of the benzoate groups at C-5 and C-7 to produce a positive first (longer wavelength) Cotton effect ($\Delta\varepsilon_{236} = +19.6$) indicating a positive chirality for the compound. The negative chirality observed for compound **1**, which established the (5*R*, 7*S*)-configuration in esulone A [5], is opposite to the observed positive chirality of compound **3**. The absolute configuration of compound **1**, established by X-ray crystallography, revealed that the exocyclic vinyl methylene group was directed into the 12-membered macrocyclic ring. Although the absolute configuration of esulone C has not been determined, molecular models indicate that a conformation of esulone C in which the exocyclic vinyl methylene group is oriented away from the macrocyclic ring would produce a positive chirality and retain the (5*R*, 7*S*)-configuration. The absolute stereochemistry of esulone C awaits confirmation by X-ray crystallography.

The characterization of esulone C from the roots of the North Dakota accession of leafy spurge substantiates the existence of the stabilized keto–enol tautomers **1** and **3** in this plant as proposed earlier [5]. Although esulone C can be converted to esulone A in acidic media, the reverse is not observed indicating esulone A to be the more thermodynamically stable tautomer. The tautomeric equilibrium of the jatrophanes is reflected in the comparative occurrence of the compounds in the ether extracts (esulone A 2% vs esulone C 0.03%).

Chromatographic detection of jatrophane diterpenes in leafy spurge accessions

Ether extracts of four accessions of leafy spurge roots were chromatographically examined for the occurrence of jatrophane diterpenes. Chromatographically separated compounds which exhibited UV absorbance at 230 nm were considered potential jatrophane diterpenes based upon previously reported UV absorption data for jatrophane diterpenes [5, 7]. Simultaneous measurements of UV absorption at 254 and 280 nm were also conducted. The small quantities (124–184 mg) of the ether extracts (acetone soluble) of some of the accessions did not permit complete characterization of detected components, although UV spectra were obtained for some of the chromatographically separated compounds.

The chromatographic separation of the acetone soluble portions of the ether extracts of the four leafy spurge accessions are compared in Figs 1 and 2. Figure 1 demonstrates the distinct difference between the North Dakota accession and the Oregon, Montana and Austrian accessions. Quantitation of esulones A, B and C in ether extracts of roots of flowering North Dakota leafy spurge showed yields of 2, 0.1 and 0.03%, respectively. Significantly smaller amounts of esulone A were detected in the roots of a non-flowering sample (0.1%). These three diterpenes were not present in the three other accessions examined.

Figure 1 indicates that all potential jatrophane diterpenes of the Oregon, Montana and Austrian leafy spurge ether extracts were less polar than the esulones of the North Dakota accession (R_t < 4.0 min). Analysis of these accessions in a less polar chromatographic system (Fig. 2) revealed one prominent potential jatrophane diterpene (B1, R_t = 4.43) in the Oregon sample and two possible jatrophanes (A1, A2, R_t = 4.20, 5.08) in the Austrian sample. The Montana sample showed the least polar components with potential diterpenes eluting in less than 3 min (Fig. 2). Chromatographic analysis of the North Dakota sample in the less polar solvent produced greatly increased retention times for esulones A, B and C (R_t > 18 min). Analysis of this sample is not included in Fig. 2.

Although the Montana extract contained no polar constituents with jatrophane absorbtion characteristics, detection at 254 nm (hexane–IPA, 95:5) revealed two

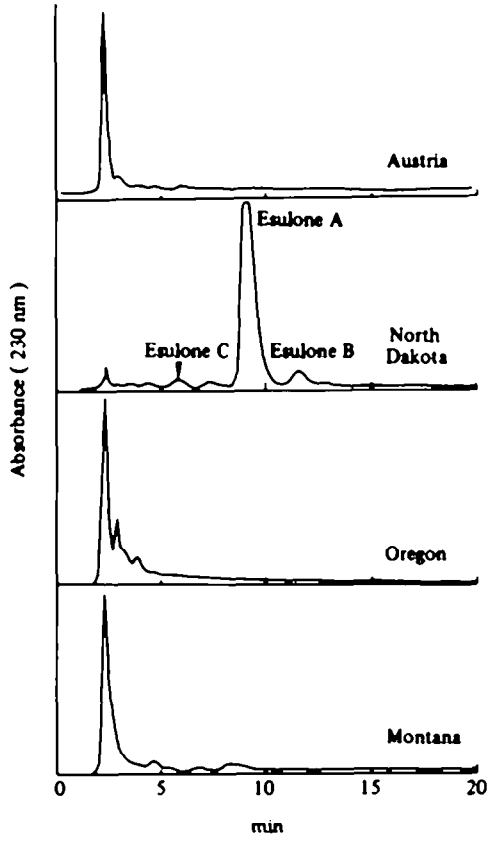


Fig. 1. The chromatographic separation of the ether extract (acetone soluble) of four accessions of leafy spurge (silica gel, hexane isopropyl alcohol, 95:5).

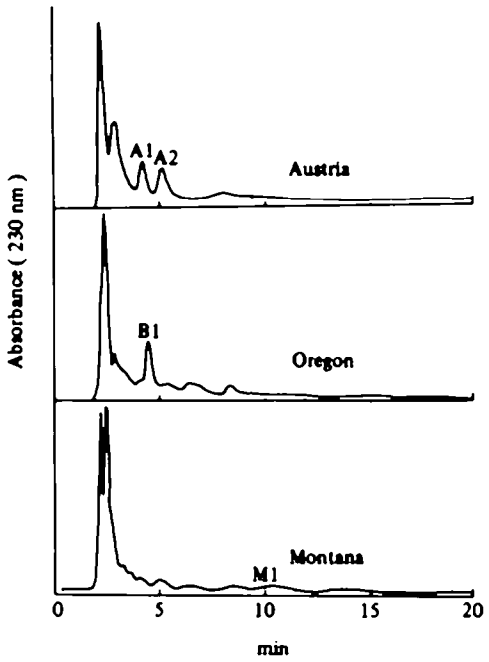


Fig. 2. The chromatographic separation of the ether extract (acetone soluble) of the Austrian, Oregon and Montana accessions of leafy spurge (silica gel, hexane isopropyl alcohol 98:2).

prominent peaks (M1, M2; R_f = 4.64, 8.21) with on-line UV spectral maxima at 262 (M1) and 235, 350 nm (M2). Compound A2 of the Austrian sample showed greater UV absorption at 280 nm than at 230 nm and its on-line spectrum maxima at 228, 287 and 312 nm. Examination of the 280 nm scan of all four accessions showed the occurrence of A2 in all samples. This compound appears to be the only co-occurring constituent among the four accessions.

The broad morphological diversity in the numerous species of *Euphorbia* complicates their taxonomic classification by classical means. Attempts to taxonomically classify the genus on the basis of secondary metabolites have led to reclassification proposals based upon the observed distribution of diterpenoid [8] and triterpenoid [9 11] constituents occurring in the latex of numerous *Euphorbia* species. The limited distribution and distinct chemical character of the diverse diterpenes occurring in Euphorbiaceae [12] suggest these compounds may be the most important taxonomic markers in this family. In particular, the jatrophane diterpenes are unusual secondary metabolites with restricted occurrence among *Euphorbia* species [5, 13] which appear to be of taxonomic importance at the species and sub-species level in this genus.

The qualitative chromatographic comparison of the four leafy spurge accessions examined in this investigation reveals distinct differences in the occurrence of identified and unidentified jatrophane diterpenes. The characterization of these unique diterpenes in predator-susceptible European leafy spurge accessions could provide an important chemical correlation basis to aid in determining which North American leafy spurge accessions are most likely to be susceptible to biological control by insect predation.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR (200 MHz) spectra were obtained for $CDCl_3$ solns. Analytical and semi-preparative HPLC were performed on a Waters Model 840 chromatography system with a Model 490 multi-wavelength UV detector. On-line UV spectra of chromatographic samples were obtained from a scan mode of the Model 490 detector under non-flow conditions. Preparative silica chromatography was performed on a Waters Prep 500 preparative chromatography system.

Extraction of Euphorbia *spp.* Root material of a morphologically diverse stand of flowering *E.* spp. was collected near Fargo, North Dakota in June 1983. The material from this accession was air dried (4.3 kg), hammermilled to pass a 1/8" screen and sequentially extracted with *n*-hexane, Et_2O, Me_2CO and MeOH for 1-week periods in a Soxhlet extractor. The resulting extracts were concentrated to dryness under red. press. and stored at 0°.

Et_2O extracts were similarly obtained from root material of *Euphorbia* spp. accessions maintained in greenhouse facilities in Fargo, North Dakota [Baker, Oregon accession (15.4 g) and Krems, Austria *E. esula* accession (13.6 g)] and Albany, California [Reedpoint, Montana accession (14.2 g)].

Isolation of esulone C. A portion (22.3 g) of the North Dakota accession Et_2O extract (28.1 g) was mixed with Me_2CO (300 ml) and filtered. The Me_2CO soluble portion (14.9 g) was evaporated *in vacuo* to dryness, dissolved in $CHCl_3$ (25 ml) and applied to a preparative silica column ($CHCl_3$). Ten fractions (500 ml) were collected and concentrated to dryness *in vacuo*. Fractions 2 8 deposited crystalline material upon standing. Fractions 2 and 3 (1.1 g) were triturated with MeOH, filtered and the filtrate was concentrated to dryness (0.7 g), dissolved in $CHCl_3$ (10 ml) and applied to a Sephadex LH-20 chromatography column (4 × 50 cm) eluted with $CHCl_3$. Five fractions (250 ml) were collected and evaporated to dryness *in vacuo*. Fraction 2 (352 mg) was dissolved in $CHCl_3$ (1 ml) and semi-preparatively chromatographed, as 60 μl aliquots, on a Waters microporosil column (7.8 mm × 30 cm, hexane isopropyl alcohol, 95:5). Twenty fractions (2 ml) were collected and multiple chromatographic runs were combined. Fractions 12–15 and 17 crystallized upon standing. Fraction 12 (28 mg) was rechromatographed (hexane–isopropyl alcohol, 95:5) and 20 fractions were collected.

Esulone C (3). [(*E*)-(+)-3,15-Diacetoxy-5,7-dibenzoyloxy-2,9-dihydroxyjatropha-6(17),11-diene-8,14-dione]. Fraction 13 crystallized upon standing to yield esulone C (3) as needles (1.4 mg), mp 210–213°. (Found: MS $M^+ + NH_4$, 708.3033; $C_{38}H_{42}O_{12} \cdot NH_4$ requires 708.3019.) CIMS (ammonia-probe) 70 eV, *m/z* (rel. int.): 708 $[M]^+$ (100), 650 [M − C(9)OH, C(10) CO]$^+$ (7), 588 [M − 2AcOH]$^+$ (17), 586 [M − BzCOOH]$^+$ (52), 528 [M − C(9), C(10) CO − BzCOOH]$^+$ (17), 466 [M − 2BzCOOH]$^+$ (7), 406 (5), 378 (8), 348 (13), 139 (49), 105 (49), 77 (32), 58 (32). CD (*c* 0.025, MeOH) $\Delta\varepsilon_{236}$ = +19.6 (64 600), $\Delta\varepsilon_{216}$ = −25.3 (84 200). λ_{max}^{MeOH} 204 (ε_{max} 30 600), 232 (35 500), 276 (2500). ^{1}H NMR: δ 1.28 (3H, *s*, H_3-18 or H_3-19), 1.36 (3H, *s*, H_3-18 or H_3-19), 1.40 (3H, *s*, H_3-16), 1.42 (3H, *d*, *J* = 6.5 Hz, H_3-20), 1.57 (3H, *s*, OAc at C-15), 1.98 (1H, *br s*, OH at C-2), 2.13 (3H, *s*, OAc at C-13), 2.26 (1H, *dd*, *J* = 16.0, 1.5 Hz, H-1a), 2.50 (1H, *d*, *J* = 16.0 Hz, H-1e), 3.65 (1H, *dd*, *J* = 10.0, 5.0 Hz, H-4), 4.32 (1H, *s*, H-9), 4.43 (1H, *dddd*, *J* = 10.0, 6.5 (3 ×) Hz, H-13), 5.42 (1H, *br s*, H-17), 5.50 (1H, *s*, H-17), 5.57 (1H, *d*, *J* = 5.0 Hz, H-3), 5.59 (1H, *br s*, OH at C-9), 5.62 (1H, *br m*, H-5), 5.86 (1H, *dd*, *J* = 10.0, 16.0 Hz, H-12), 6.12 (1H, *d*, *J* = 16.0 Hz, H-(11), 6.24 (1H, *s*, H-7), 7.36–7.62 (6H, *m*, OBz), 7.97–8.12 (4H, *m*, OBz).

Oxidation product, 4, *of esulone A.* [(*E*)-(−)-3,15-Diacetoxy-

5,7-di-benzoyloxy-2-hydroxyjatropha-6(17),11-diene-8,9,14-trione]. Esulone A (20 mg) was dissolved in $CHCl_3$ (1 ml) and Jones reagent (2.5 molar eq.) was added. After warming (40°, 30 min), H_2O (5 ml) was added and the mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was dried ($MgSO_4$), concentrated and subjected to multiple semi-preparative chromatography (silica, hexane–isopropyl alcohol, 95:5) to obtain **4**, needles (MeOH) (9 mg), mp 214–215°. (Found: MS $M^+ + NH_4$, 706.2878, $C_{38}H_{40}O_{12}$. NH_4 requires 706.2859.) CIMS (ammonia, probe) *m/z* (rel. int.): 706 $[M^+]$ (54), 586 $[M - 2AcOH]^+$ (32), 569 (5), 530 (5), 466 $[M - AcOH - BzCOOH]^+$ (41), 406 (6), 279 (16), 139 (100), 77 (71), 74 (53); $[\alpha]_D^{20}$ −0.14° (*c* 2.5 in Me_2CO); CD (*c* 0.033, MeOH) $\Delta\varepsilon_{235}$ = −31.4 (104, 300), $\Delta\varepsilon_{210}$ = +16.4 (54, 300); UV λ_{max}^{MeOH} nm: 208, 232, 276; ^1H NMR (50°): δ1.10 (3H, *s*, H_3-18 or H_3-19), 1.29 (3H, *d*, *J* = 6.5 Hz, H_3-13), 1.37 (3H, *s*, H_3-16), 1.40 (3H, *s*, H_3-20), 1.77 (3H, *s*, OAc at C-15), 1.81 (1H, *d*, *J* = 1.5 Hz, OH at C-2), 2.09 (3H, *s*, OAc at C-3), 2.26 (1H, *dd*, *J* = 16.5, 1.5 Hz, H-1a), 2.98 (1H, *dd*, *J* = 4.0, 2.0 Hz, H-4), 3.04 (1H, *d*, *J* = 16.5 Hz, H-1e), 4.32 (1H, *dddd*, *J* = 9.5, 6.5 (3 ×) Hz, H-13), 5.44 (1H, *s*, H-17), 5.61 (1H, *s*, H-17), 5.63 (1H, *d*, *J* = 4.0 Hz, H-3), 5.75 (1H, *dd*, *J* = 9.5, 16.5 Hz, H-12), 5.89 (1H, *d*, *J* = 16.5 Hz, H-11), 5.96 (1H, *br d*, *J* = 2.0 Hz, H-5), 6.60 (1H, *s*, H-7), 7.27 7.62 (6H, *m*, OBz), 7.92 8.10 (4H, *m*, OBz).

Tautomerization of esulone C. Aq. H_2SO_4 (20%, 0.1 ml) was added to a soln of esulone C (< 0.1 mg in 0.25 ml MeOH) and the mixture was heated (80°, 2 min). The mixture was diluted with H_2O (2 ml), $CHCl_3$ (2 ml) was added and the mixture extracted. The $CHCl_3$ layer was dried ($MgSO_4$), concentrated and chromatographically compared to esulone A. The chromatographic comparison confirmed the formation of esulone A from esulone C.

Qualitative chromatographic analysis of leafy spurge extracts. The Et_2O extracts of the Oregon, Montana, and Austrian accessions were concentrated to dryness *in vacuo*, redissolved in Me_2O (2 ml), filtered and concentrated to give samples for the qualitative chromatographic analysis (Oregon, 151 mg; Montana, 128 mg; Austria, 124 mg). The samples were dissolved in $CHCl_3$ (0.5 ml) and chromatographic aliquots (10–20 μl) were injected onto a silica column (Waters microporosil Z column, hexane–isopropyl alcohol, 95:5, 98:2, 2 ml/min). Ultraviolet detection of chromatographic runs was measured simultaneously at 230, 254 and 280 nm; the results are summarized in Figs 1 and 2.

Quantitative analysis of esulone A, B and C. A portion (1.0 g) of the Et_2O extract of the North Dakota accession of leafy spurge was dissolved in Me_2CO (50 ml). The soln was allowed to stand and a 1% aliquot (0.5 ml) of the Me_2CO soln was withdrawn, evaporated to dryness, redissolved in $CHCl_3$ (0.1 ml) and applied to a Waters Silica Sep Pak. The Sep Pak was eluted with $CHCl_3$ (10 ml) and the eluant was evaporated to dryness under N_2 and the residue redissolved in $CHCl_3$ (0.1 ml) and applied (10 μl) to an analytical silica gel HPLC column (Waters microporosil Z column, hexane–isopropyl alcohol, 95:5, 230 nm detection). The amounts of esulone A, B and C in the sample were determined by comparison with a standard soln of these diterpenes analysed on the same system under identical conditions. Esulone A, R_t = 9.06; yield 2.12% in the Et_2O extract; 0.01% on the dry plant basis. Esulone B, R_t = 11.56; yield 0.12% in the Et_2O extract; 0.0008% of plant dry wt. Esulone C, R_t = 5.88; yield 0.03% in the Et_2O extract; 0.0002% of plant dry wt.

The Et_2O extract (16.7 g) of root material (1.4 kg) obtained from a non-flowering plant (19.4 g) was analysed according to the above procedure. The analysis showed: esulone A, yield 0.14% of the Et_2O extract; 0.002% of plant dry wt.

Acknowledgements—The assistance of Mrs. M. Benson for NMR spectra, Dr. William Haddon for mass spectra, and Dr. William Gaffield for CD measurements and discussions of stereochemistry is gratefully acknowledged. The supply of Montana root materials by Dr. Robert Pemberton is greatly appreciated. Reference to a company and/or a product is only for the purposes of information and does not imply approval or recommendation of that product by the Department to the exclusion of others which may also be suitable.

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