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ALKALOIDS, LIMONOIDS AND FUROCOUMARINS FROM THREE MEXICAN *ESENBECKIA* SPECIES*

DAVID L. DREYER

Department of Chemistry, San Francisco State University, San Francisco, California, U.S.A.

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Key Word Index—*Esenbeckia litoralis*; *E. flava*; *E. berlandieri*; Rutaceae; limonoids; furoquinoline alkaloids; furocoumarins; 1-hydroxy-3-methoxy-*N*-methylacridone; 3,3-diisopentenyl-*N*-methyl-2,4-quinoldione; structure determination; chemotaxonomy.

Abstract—The chemical constituents of three Mexican *Esenbeckia* species have been determined. Rutaevin was the main limonoid present in the seeds of all three species, *E. litoralis*, *E. flava* and *E. berlandieri*. The husks, leaves, wood and bark contained a wide array of known furocoumarins and furoquinoline alkaloids. In addition, 1-hydroxy-3-methoxy-*N*-methylacridone was obtained from *E. litoralis* bark and a new natural 2-quinolone alkaloid, formulated as 3,3-diisopropyl-*N*-methyl-2,4-quinoldione, was obtained from *E. flava* wood. The structure was assigned from spectroscopic considerations and conversion to *N*-methylhaplofoline.

INTRODUCTION

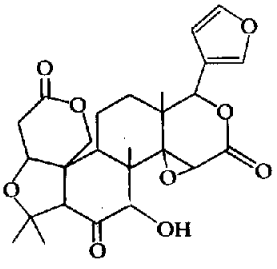
Limonoids have been found in some plants of all the major subfamilies of the Rutaceae at a relatively low frequency of occurrence [1, 2]. The evidence on limonoid distribution available [1] suggests that all species of limonoid-producing genera contain limonoids. Only a few limonoid-producing genera containing many species have been surveyed for limonoids. One moderately large genus which has been systematically investigated for its limonoid content is *Citrus*. Thus, seeds of all *Citrus* species and hybrids which have been examined have been found to contain limonoids [1, 3]. Similarly, although a much lower percentage of species of the large genus *Euodia* has been examined, limonoids have been reported in a number of cases [1, 4–7].

Esenbeckia is a new world genus of about 30 species [8]. Two species of *Esenbeckia* have been the subject of previous chemical studies and both contain limonoids [2, 9–10]. *Esenbeckia* thus appeared to be a promising genus to test the predictive idea that when limonoids occur in one species of a genus then all species of that genus will contain limonoids. The extractives of three Mexican *Esenbeckia* species [11], *E. litoralis*, *E. berlandieri* (Baill.) and *E. flava* (Don Smith) are the subject of this paper. The *Esenbeckia* species examined in this study contained a large variety of typical rutaceous furoquinoline alkaloids and furocoumarins. Limonoids were also present in the seeds of all three species.

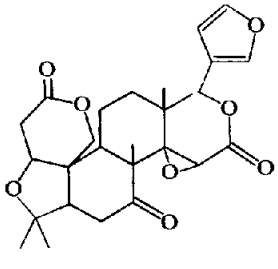
RESULTS

The availability of sufficient plant material permitted separate isolation work on different plant parts of *E. litoralis*. The seeds contained the expected limonoids, rutaevin (**1**) and limonin (**2**) itself. The seed husks contained bergapten, isopimpinellin and the furoquinoline alkaloid, kokusaginine. Only kokusaginine was isolated from the leaves and stems. It was possible to flake the brittle bark off the trunk wood, and this was studied separately to give imperatorin, isopimpinellin, 8-hydroxybergapten, phellopterin, alloimperatorin and 1-hydroxy-3-methoxy-*N*-methylacridone [12]. The trunk wood yielded the furocoumarins, bergapten, imperatorin, phellopterin and the furoquinoline alkaloids, dictamine, evolitrine, maculine, kokusaginine and skimmianine. All of these substances were identified by comparison with previously available, authentic samples except 1-hydroxy-3-methoxy-*N*-methylacridone (**17**). In order to provide a reference sample of **17** it was synthesized from *N*-methylanthranilic acid by adaption of the xanthone procedure of Patolia and Trivedi [13].

Less plant material of *E. berlandieri* was available and only rutaevin (**1**) was recovered from the seeds and bergapten from the fruit husks. Rutaevin (**1**) was also obtained from the seed extracts of *E. flava*. Wood



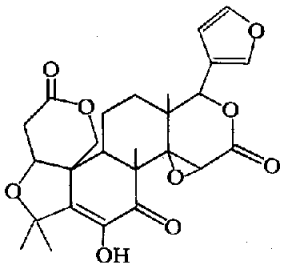
Rutaevin (**1**)



Limonin (**2**)

* Part XIV in the series "Chemotaxonomy of the Rutaceae". For Part XIII see Dreyer, D. L. and Brenner, R. C. (1980) *Phytochemistry* **19**, 935.

† Present address: U.S. Department of Agriculture, Science and Education Administration, 800 Buchanan Street, Berkeley, CA 94710, U.S.A.



Limonin diosphenol (**3**)

extracts of *E. flava* after chromatography yielded a nonpolar, noncrystalline quinolone alkaloid as well as flindersiamine, dictamine, evolitrine, macuosidine and skimmianine. The blue fluorescing, noncrystalline alkaloid appeared homogenous by TLC and its NMR spectrum. The pattern of signals in the aromatic region of the NMR spectrum represented four protons and was consistent with the presence of an anthranilic acid system [14, 15]. A three-proton singlet at 3.42 ppm indicated the presence of an *N*-methyl or *O*-methyl group. Finally, a vinyl triplet, a doublet at 2.69 ppm and two *C*-methyl resonances indicated the presence of an isopentenyl group. The chemical shift of the 2.69 ppm doublet indicated that the isopentenyl group must be attached to carbon rather than to oxygen [16]. Most striking, however, was the fact that all the NMR signals associated with the isopentenyl group had twice the area expected relative to the four aromatic protons. Thus, there must be two isopentenyl groups attached to the anthranilic acid system. Moreover, since there is only one set of signals for the isopentenyl system, the two isopentyl groups are most likely in identical environments.

The mass spectrum indicated a molecular weight of 311. This molecular weight and the NMR data were best reconciled in terms of structure **21**, 3,3-diisopentenyl-*N*-methyl-2,4-quinoldione. The carbon NMR spectrum supported the structural assignment. The chemical shift assignments were made by comparison of the chemical shifts with those of related *N*-methylanthranilic acid [14, 15] and isopentenyl systems [17–19] as well as a gated, decoupled spectrum. Moreover, the carbon spectrum indicated the presence of an *N*-methyl rather than a methoxy group.

The alkaloid (**21**) was recovered unchanged after heating with hydroxylamine hydrochloride and pyridine. Dealkylation and conversion to *N*-methylhaplofoline (**22**) was achieved by heating the alkaloid (**21**) for a short time with perchloric acid. *N*-Methylhaplofoline (**22**) has been previously synthesized [20–23], and the NMR spectrum compared well with data reported by Bowman and Grundon [23] for the linear isomer and was sufficient to distinguish the linear ring closed product (**22**) from the angular isomer (**23**).

Compound **21** has been synthesized by Mitscher *et al.* [24] but has not previously been reported as a natural product. The wide array of typical rutaceous furocoumarins and furoquinoline alkaloids found in the three *Esenbeckia* species reported here parallels the pattern of such compounds previously reported from *Esenbeckia*. *E. febrifuga* has been previously shown [9, 10] to contain skimmianine, flindersiamine, maculine and rutaevin (**1**). *E. hartmanii* has been previously shown [2] to contain the ubiquitous skimmianine as well as maculosidine and the limonoids, limonin (**2**), limonin diosphenol (**3**) and rutaevin (**1**).

Although only five out of about 30 *Esenbeckia* species have been examined chemically, the results support the generalization that if limonoids occur in one species of a genus of the Rutaceae, then all species of the genus will contain limonoids.

EXPERIMENTAL

Isolation from E. litoralis *seeds.* Ground seeds were defatted with petrol and then extracted with Me_2CO. Solvent was removed from the Me_2CO extracts and the residue chromatographed on acid-washed Al_2O_3. Fractions were combined, guided by an Ehrlich's test [25] on TLC and after removal of solvent crystallized from EtOH to give rutaevin [26]. IR spectrum identical with that of an authentic sample. Further work-up of the mother liquors gave limonin from *i*-PrOH–CH_2Cl_2. NMR and IR spectra were identical with that of an authentic sample.

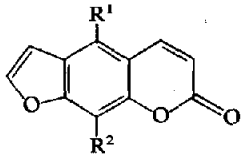
Isolation from E. litoralis *husks. E. litoralis* was collected 7 miles west of the Tehuantepec–Salina Cruz junction along Mexican Highway #190 at Puente Las Tejas, Oaxaca. Ground seed husks were extracted with Me_2CO, and after removal of solvent, residue was chromatographed on silicic acid. The first fractions showing fluorescence on TLC were worked up to give **4**. Fractions which followed yielded **5**. Mother liquors from these operations were combined and extracted with 10% HCl. The HCl extracts were made basic and the product collected with Et_2O. After removal of the Et_2O, the residue was crystallized from EtOAc–hexane to give **14**.

Isolation from E. litoralis *wood.* Wood shavings were extracted with Me_2CO and solvent removed from the extracts. Residue was chromatographed on silicic acid. Phellopterin (**8**) was recovered from the first fractions to show fluorescence on TLC. TLC indicated that the more polar fractions were a complex mixture. These polar fractions were recombined and partitioned between Et_2O and 10% HCl. The HCl phase was made basic and the alkaloids recovered with Et_2O. Chromatography of this alkaloid mixture on silicic acid gave **10**, **11**, **15**, and **14** by elution with successively more polar solvents. Rechromatography of the neutral fraction gave **4**.

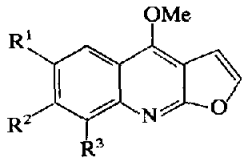
Isolation from E. litoralis *bark.* Ground bark was extracted with Me_2CO. Residue, after removal of solvent, was chromatographed on silicic acid. The initial chromatogram gave **5**, 1-hydroxy-3-methoxy-*N*-methylacridone (**17**) and **6**, eluted in that order. The mother liquors from the above operations were combined and rechromatographed to give **7**, **8**, **17**, **9** and more **6**. All of the coumarins were identified by comparison of IR and NMR spectra with those of authentic samples previously isolated from rutaceous species in this laboratory.

1-*Hydroxy*-3-*methoxy*-N-*methylacridone* (**17**). Orange crystals; mp 163–164° (EtOAc–petrol); green $FeCl_3$; λ_{max}^{EtOH} nm: 222, 249, 263, 271, 295, 324, 392 ^{1}H NMR 8.18(8, 1H, H-8), 7.58(*t*, *d*, 1H, H-6), ~7.3(*m*, 2H, H-5 and H-7), 6.03(*s*, 2H, H-2 and H-4), 3.80 (*s*, 3H, methoxy), 3.60(*s*, 3H, *N*-methyl) ppm ($CDCl_3$–DMSO); MS *m/e* (rel. int.): 255(100), 226(42). Metastable peak at *m/e* 200. The ^{13}C NMR spectrum compared well with that previously reported [27].

1-*Acetoxy*-3-*methoxy*-N-*methylacridone* (**18**). This substance was prepared from **17** with Ac_2O–Py; mp 219–220° (EtOAc–petrol); ^{1}H NMR 8.42(*q*, 1H, $J = 7,2$ Hz, H-8), 7.57



Bergapten (**4**)	$R^1 = OMe$; $R^2 = H$
Isopimpinellin (**5**)	$R^1 = R^2 = OMe$
8-Hydroxybergapten (**6**)	$R^1 = OMe$; $R^2 = OH$
Imperatorin (**7**)	$R^1 = H$; $R^2 = OCH_2CH{=}CMe_2$
Phellopterin (**8**)	$R^1 = OMe$; $R^2 = OCH_2CH{=}CMe_2$
Alloimperatorin (**9**)	$R^1 = CH_2CH{=}CMe_2$; $R^2 = OH$

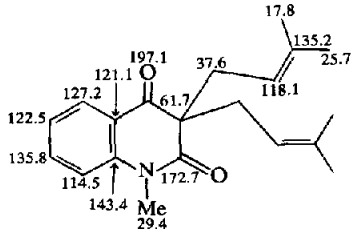


Dictamine (**10**)	$R^1 = R^2 = R^3 = H$
Evolitrine (**11**)	$R^1 = R^3 = H$; $R^2 = OMe$
Skimmianine (**12**)	$R^1 = H$; $R^2 = R^3 = OMe$
Maculosidine (**13**)	$R^1 = R^2 = OMe$; $R^2 = H$
Kokusaginine (**14**)	$R^1 = R^2 = OMe$; $R^3 = H$
Maculine (**15**)	R^1, $R^2 = OCH_2O$; $R^3 = H$
Flindersiamine (**16**)	R^1, $R^2 = OCH_2O$; $R^3 = OMe$

(*t*, *d*, 1H, $J = 7{,}2$ Hz, H-6), 7.33–7.07 (*m*, 2H, H-7 and H-5), 6.60 and 6.44 (*d*, 1H each, $J = 2$ Hz, H-2 and H-4); 3.83 (*s*, 3H, methoxy), 3.62 (*s*, 3H, *N*-methyl), 2.56 (*s*, 3H, acetoxy) ppm ($CDCl_3$).

1,3-*Dimethoxy*-N-*methylacridone* (**19**). This material was obtained by methylation of **17** with Me_2SO/aq. NaOH; mp 160° (EtOAc); λ_{max}^{EtOH} nm: 225, 261, 269, 281, 313, 381; ^{1}H NMR 8.47 (*q*, $J = 2$, $J = 8$ Hz, 1H, H-8), 7.6–6.98 (*m*, 3H, aromatic), 6.20 (*s*, 2H, H-2 and H-4), 3.97, 3.83 (*s*, methoxy), 3.52 (*s*, *N*-methyl) ppm ($CDCl_3$).

Synthesis of 1-*hydroxy*-3-*methoxy*-N-*methylacridone* (**17**). Equal molar amounts of phloroglucinol and *N*-methylanthranilic acid were heated in diphenyl ether for 30 min near the bp. The cooled mixture was taken up in EtOAc and washed with 5% $NaHCO_3$. The organic phase was then extracted with 5% NaOH. The base extracts were acidified and extracted with CH_2Cl_2. The dried CH_2Cl_2 extracts were chromatographed on SiO_2 and those fractions which showed a green $FeCl_3$ test were combined and solvent removed.



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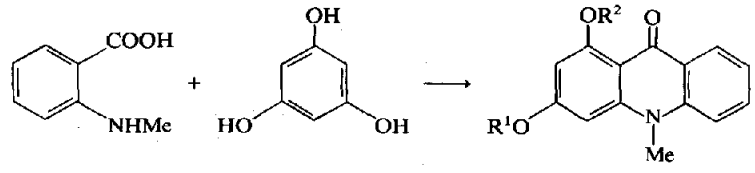
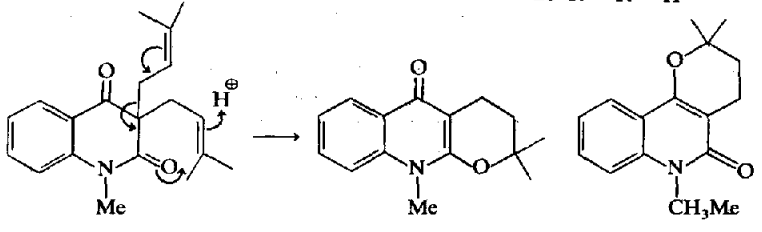
Residue (**20**) was crystallized from EtOAc–C_6H_6. Crude **20** was methylated with Me_2SO_4 and aq. NaOH on a steam bath. The reaction mixture was extracted with CH_2Cl_2 and after removal of solvent chromatographed on SiO_2. Those fractions showing a green $FeCl_3$ test were combined, solvent removed and the residue crystallized from EtOAc to give **17**, mp 164–165°. The IR spectrum was identical with that of the natural sample.

Isolation from E. flava *seeds. E. flava* was collected 6 miles south of the junction with Mexican Highway #1 on the road to Todos Santos in Baja California, Mexico. Ground seeds were defatted with petrol and then extracted with Me_2CO. After removal of solvent, the residue was chromatographed on Al_2O_3. Fractions which gave a positive Ehrlich's test [25] were worked up to give rutaevin.

Isolation from E. flava *wood.* Ground wood including the bark was extracted with Me_2CO. Solvent was removed from the extracts and the residue chromatographed on Al_2O_3. Elution with hexane gave a large amount of noncrystalline oil. Elution with petrol–C_6H_6 mixtures and C_6H_6 alone gave fractions from which **16** was recovered; NMR 7.55(*d*, $J = 2$ Hz, 1H, H-2), 7.33(*s*, 1H, H-5), 6.99(*d*, $J = 2$ Hz, 1H, H-3), 6.02(*s*, 2H, methylenedioxy); 4.36, 4.23(s, methoxys) ppm ($CDCl_3$). Later fractions from the column yielded **12**. The mother liquors from the more polar fractions of the initial column above were combined, taken up in EtOAc and extracted with 10% HCl. The HCl extracts were neutralized and extracted with $CHCl_3$. After drying and removal of solvent, the residue was chromatographed on alumina to give **10** and **11** eluted with C_6H_6, **13** followed by additional amounts of **12** eluted with C_6H_6–$CHCl_3$ mixtures. The mother liquors from these operations were combined and their composition checked by HPLC using a Partisil PXS10/25 PAC column and a 2:1 $CHCl_3$–hexane solvent



17 $R^1 = Me$; $R^2 = H$
18 $R^1 = Me$; $R^2 = Ac$
19 $R^1 = R^2 = Me$
20 $R^1 = R^2 = H$



21 **22** **23**

system. By this means, the mother liquors largely contained only further amounts of the alkaloids isolated above. The nonpolar oil was rechromatographed on Al_2O_3 with petrol. The initial fractions were unsaturated fatty acid esters (IR and NMR) followed by fractions showing complex IR adsorption. The content of the fractions was monitored by observing the carbonyl region in the IR of each fraction. The initial fractions containing largely fatty acid esters showed an IR band at 1750 cm^{-1}. The IR spectra of the later fractions (**21**) were free of the component causing the 1750 cm^{-1} band. Solvent was removed from these fractions to give a heavy oil (**21**); ν cm^{-1}: 1682, 1650, 1595; λ_{max}^{EtOH} nm: 235(3400), 258(450), 342(250); ^1H NMR 7.94(*q*, $J = 7$, 1 Hz, 1H, H-5), 7.58 (*t*, *d*, $J = 7$, 1Hz, 1H, H-7), ~7.14 (*m*, 2H, H-6 and H-8), 4.82(*t*, $J = 8$ Hz, 2H, vinyl), 3.42(*s*, 3H, *N*-methyl), 2.69(*d*, $J = 8$ Hz, 4H, methylene), 1.54, 1.48(*s*, 12H, *C*-methyls) ppm ($CDCl_3$); MS *m/e* (rel. int.): 311(2), 242(100), 200(20), 188(11), 69(13), 43(10), 41(25); M^+ 311.1889 (calc. for $C_{20}H_{25}NO_2$, 311.1885).

N-Methylhaplofoline (**22**). A soln of **21** in 20% $HClO_4$ in HOAc was warmed on a steam bath for 1 hr. The mixture was diluted with a large vol. of H_2O and extracted with $CHCl_3$. The dried extracts were filtered through a short column of Al_2O_3, solvent removed from the filtrates to give **22**, mp 130–131° (EtOAc–cyclohexane); λ_{max}^{EtOH} nm: 213, 233, ~285, ~292, 314, 326 nm; NMR 8.33 (*q*, $J = 7$, 2 Hz, 1H, H-5); 7.60–7.10(*m*, 3H, H-6, 7 and 8); 3.58 (*s*, 3H) N-methyl; 2.62(*t*, $J = 7$ Hz, 2H allyl methylene); 1.78(*t*, $J = 7$ Hz, 2H) methylene; 1.40 (*s*, 6H) C-methyls ppm ($CDCl_3$).

Isolation from E. flava *husks.* Ground husks of *E. flava* were extracted as described above for *E. litoralis.* Extracts were washed with 10% HCl, and after recovery of the acid-soluble fraction and chromatography over Al_2O_3, fractions were obtained which yielded **12**.

Isolation from E. berlandieri *seeds.* Fruit of *E. berlandieri* was collected from trees growing from limestone ledges along a branch of the Rio Dan Marcos *ca* 3 miles south of Ciudad Victoria on Highway #101. Seeds were removed from the dried fruit, ground and defatted with petrol. The marc was then extracted with Me_2CO. Solvent was removed to give a residue which was crystallized from EtOH–$CHCl_3$ to give rutaevin. The IR and NMR spectra were identical with those of an authentic sample.

Isolation from E. berlandieri *husks.* Ground husks were extracted with Me_2CO. After removal of solvent the residue was chromatographed on silicic acid. Work-up of fractions showing fluorescence on TLC gave only bergapten, identical by spectroscopic criteria with an authentic sample.

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