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CONSTITUENTS OF *ERYSIMUM INCONSPICUUM*. TWO SULFUR-CONTAINING LACTONE COMPOUNDS

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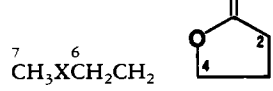
ABSTRACT.—The alcohol extract of *Erysimum inconspicuum* fruits, which exhibited cytotoxic activity against the KB cell line and some activity against the P-388 lymphocytic leukemia in vivo, was studied. Strophanthidin, uzarigenin, and two sulfur-containing lactones, erysulfone[6-methylsulfonyl-4-hydroxyhexanoic acid lactone] and erysulfoxide[6-methylsulfinyl-4-hydroxyhexanoic acid lactone], were isolated and characterized by spectral data.

Erysimum inconspicuum (S. Wats.) MacM. (Cruciferae), a pale yellow-flowered perennial, is a non-studied native species. The genus has cardiotonic properties that undoubtedly result from its significant cardiac glycoside content (1). *Erysimum repandum*, a well-studied relative, is known to contain periplogenin, strophanthidin, uzarigenin, and various glycosides of these three cardenolides (2-8). Other compounds of interest found in this genus include a variety of isothiocyanates in which a sulfur atom is present as a methylsulfonyl, methylsulfinyl, or methylthio group; a chain of 3-6 carbons links the sulfur and isothiocyanate groups; and an alcohol group is present on the carbon chain (9-13). These isothiocyanates apparently arise from hydrolysis of glucosinolates, like glucoerypestrin and glucoerysolin, which are also found in this genus (14, 15).

Our interest in this plant stems from our observation that alcohol extracts of the fruits, leaves and stems, and roots give cell growth inhibitory activity against the NCI KB cell line (16) with ED_{50} values below 12 μg/ml. The extract from the fruits was particularly promising with a confirmatory sample ED_{50} of 0.53 and T/C values of 119% at 200 mg/kg and 132% at 400 mg/kg in the P-388 lymphocytic leukemia in vivo screen (16), so all further isolation work centered on this fraction. The air-dried fruits were extracted, separated, and chromatographed as described in the Experimental section to yield strophanthidin and uzarigenin, which are already known (2-8) from this genus, and two sulfur-containing lactones, which we have termed erysulfone (**1a**) and erysulfoxide (**1b**). Evaluation of the lactones in the KB assay indicated an ED_{50} of 2.1 for **1a** and 2.3 for **1b**, while an ED_{50} of 0.44 was secured for strophanthidin. Values less than 4 μg/ml for pure compounds are considered active (16).



1a X=SO_2
1b X=SO

It was initially established that the two lactones were closely related in structure since much of the spectral data, in particular ^{13}C-nmr spectra, were similar. Cims indicated that the two differed only by a mass of 15.9955, which could be attributed to an oxygen atom (15.9949). For erysulfone (**1a**), an ir spectrum had a peak at 1775 indicative of a γ-lactone, which was supported by a broad ^1H-nmr signal at δ4.68 equivalent to 1H for the proton on the γ carbon of the lactone. The ^1H nmr also had a sharp singlet at δ2.94, suggestive of a methyl substituted on a heteroatom, an apparent triplet at δ3.24 possibly for a methylene group on the same heteroatom, and a group of indistinguishable overlapping signals at δ1.8-2.8. Single-frequency decoupling studies in

which the signal at δ4.68 was irradiated caused collapse of an apparent doublet at δ2.55, but any further nmr information could not be extracted because of the close proximity of the remaining signals.

An off-resonance decoupled ^{13}C-nmr spectrum of **1a** had seven signals. A carbonyl carbon signal at δ176 taken with one at δ78.24 further supported a γ-lactone system. Two other clearly discernible peaks were visible at δ50.96 and 41.03 and could be assigned to carbons attached to heteroatoms. Determination of the multiplicity of the protons on each carbon was facilitated by an off-resonance spectrum. Amongst others, a doublet at δ78 supported a -CH- on a heteroatom, a quartet at δ41 corroborated a methyl group on a heteroatom, and a distinct triplet at δ51 established a methylene moiety on a heteroatom.

Assignment of the nmr signals and a partial structural sequence were enhanced through the use of the shift reagent Eu(fod)$_3$, which would be expected to complex with the heteroatom and/or lactone ring oxygen and cause protons on carbons attached to these moieties to shift the most. Although the exact coupling patterns were not fully discernible, single-frequency decoupling after addition of shift reagent permitted correlation of signals from adjacent protons. As noted in Table 1, the ^{1}H-nmr spectrum was eventually discerned into six proton signal groups consisting of a singlet, an apparent triplet, three multiplets, and a broad unresolved multiplet. Irradiation at A, which spilled over into B, gave a doublet for the multiplet at D and some restructuring of the multiplet at F. When the multiplet at D was irradiated, the triplet at B collapsed to a singlet, and the broad multiplet at A sharpened. Therefore, a tentative arrangement of B-D-A-F was assumed. If this combination is taken together with the γ-lactone moiety and the ^{13}C-nmr data, then all seven carbon atoms can be accounted for as depicted in **1**, and only the nature of the heteroatom need be established.

TABLE 1. ^{1}H-nmr Chemical Shifts for **1a** and **1b** upon Addition of Eu(fod)$_3$

Signal Group[a]	Carbon	Multiplicity Discerned	δ at different Eu(fod)$_3$ concentrations (mg)[b]					
			1a			**1b**		
			10	15	25	15	25	51.6
A	4	br m	5.6(F)	6.0	6.27(D,F)[d]	5.55(F)	6.2(D,F)	7.0(D,F)[d,e]
B	6	t	4.91(D)	5.53(D)	6.17(D,F)[d]	5.1(D)	6.0	9.2(D)[d]
						5.85	8.6	18.26
C	7	s	4.25	4.72	5.27	4.28	5.1	7.20
D	5	m	3.51[c]	4.14(A,B)	4.65(A,B)	4.05(A,B)	4.6	6.15(A,B_1)
E	2	m	3.51[c]	3.86	4.25(F)	2.74	2.84	3.28(F)
F	3	m	2.75	2.92	3.08	2.5	2.7	2.92(A)

[a]Signal groups assigned by increasing magnetic field.
[b]Letters in parentheses represent signal group affected when single frequency irradiation is applied at the δ noted.
[c]These signals are not resolved yet.
[d]Simultaneous irradiation occurred at both signals owing to close proximity.
[e]Total decoupling of D results from irradiation at A and B_1.

Initially, a positive Dragendorff's test implicated a nitrogen for the heteroatom; however, an exact mass of 193.0538 for the MH^+ ion from a cims did not support its introduction into the structure. Computation of an exact mass using an -SO_2- group as the heteroatom did furnish an appropriate match with a value of 193.0534 as did a comparison of the relative intensities of the $MH^+ + 1$ and $MH^+ + 2$ peaks, the latter of which is known to be quite characteristic for the presence of a sulfur atom. Reexamination of the ir spectrum also supported this moiety with characteristic (17) sharp absorptions at 1278, 1138, and 1125 cm^{-1}, and the nmr chemical shift data correlated well with values expected for adjacent carbons and protons (18-20). The cims fragmentation patterns and appropriate exact masses for the fragments further substantiated the struc-

ture (see Experimental section for data). The positive response to Dragendorff's reagent is apparently due to the lactone ring (21).

For erysulfoxide (**1b**), both similarities and differences with the spectral data for **1a** could be noted. For example, the presence of a γ-lactone ring was evident from an ir peak at 1760 cm^{-1} and a ^{1}H-nmr signal at δ4.68. The methyl singlet, however, was found at δ2.62, implying a less deshielded environment and, perhaps, a different heteroatom. In an off-resonance decoupled ^{13}C-nmr spectrum, again seven carbon signals were in evidence with the carbonyl carbon at δ176, the lactone ring oxygen carbon at δ80, and the carbons attached to the heteroatom at δ51.35 and 39.19. An off-resonance spectrum provided the same proton substitution pattern as in **1a**. When Eu(fod)$_3$ was added to shift the ^{1}H-nmr spectrum (Table 1), the signals did not seem to shift rapidly, but eventually the same coupling patterns and the same single-frequency irradiation effects as for **1a** emerged. Therefore, the same carbon arrangement as in **1a** was applicable to **1b**.

Because the molecular ions of the two compounds differed only by one oxygen atom and there were no changes in the carbon substitution patterns, it was concluded that the difference between the two was the oxidation state of the sulfur. A high resolution cims supported this assumption, because the experimental value for the MH^+ ion at *m/z* 177.0583 coincided with the calculated value of 177.0585, and the relative intensities of the $MH^+ + 1$ and $MH^+ + 2$ peaks were in agreement with calculated values. Ir peaks at 1030 and 1000 cm^{-1} indicative of a sulfoxide group (17) and the cims fragmentation assignments corroborated the structure. The oxidation of **1b** with m-CPBA to **1a**, which was identical to authentic **1a** by tlc and ir, fully confirmed the relationship.

Interestingly, during the ^{1}H-nmr studies with sulfoxide **1b** and shift reagent, the signal for the two protons on the methylene group attached to the sulfur gave rise to an apparent triplet and an unresolved multiplet at δ9.2 and 18.26 after addition of 51.6 mg of Eu(fod)$_3$. This separation can probably be explained by a magnification of the nonequivalent character of sulfoxide methylene protons as has been observed (22) with uranyl-β-diketone-sulfoxide adducts.

EXPERIMENTAL

GENERAL.—Melting points were measured on a Fisher-Johns apparatus and are uncorrected. Ir spectra were done as KBr pellets on a Sargent-Welch 3-200 instrument. ^{1}H- and ^{13}C-nmr spectra were recorded with an IBM-NR 80 spectrometer on $CDCl_3$ solutions containing TMS as an internal standard. Uv spectra were performed on EtOH solutions with a Cary 14 spectrophotometer, and optical rotatory dispersion spectra, on $CHCl_3$ solutions with a JASCO Model ORD-UV 5.

Precoated tlc sheets of aluminum oxide F_{254} neutral type T and silica gel 60 F_{254} from EM reagents were used for qualitative tlc and silica gel 60 (less than 230 mesh) for medium pressure liquid column chromatography (mplc).

KB assays were performed at the University of Miami under the auspices of NCI (16). Cims (CH_4) were done at the University of Nebraska.

PLANT MATERIAL.—The whole plants were collected in the northwest section of DeKalb County, Illinois; separated into fruits, stems and leaves, and roots; and air dried. A voucher specimen is deposited in the Northern Illinois University Herbarium located in the Department of Biological Sciences.

EXTRACTION OF *E. INCONSPICUUM* FRUITS.—The dried fruits (5.97 kg) of *E. inconspicuum* fruits were first extracted for 8 h with hexane to remove the fats and oils (100 kg). The air-dried material was then extracted with EtOH for 16 h. The EtOH was removed in vacuo, H_2O added to the residue to provide a slurry, and the slurry was extracted successively with hexane (108 g; ED_{50} 2.8), Et_2O (7.6 g; ED_{50} 3.0), $CHCl_3$ (13.5 g; ED_{50} 0.24), 10% MeOH-$CHCl_3$ (4.9; ED_{50} 6.8×10^{-2}), 20% MeOH-$CHCl_3$ (8.0 g; ED_{50} 0.29), and 30% MeOH-$CHCl_3$ (25.2 g; ED_{50} 0.28) to provide the fractions indicated as well as a H_2O soluble fraction (269.8 g; ED_{50} 0.30).

A portion (6.0 g) of the $CHCl_3$ soluble material was dissolved in $CHCl_3$, silica gel (40 g) was added, and the slurry was evaporated until a free-flowing powder resulted. The dry material was packed into a mplc column and connected to a second silica gel column (300 g) packed with hexane. The columns were

eluted with solvents of increasing polarity (hexane-$CHCl_3$, $CHCl_3$, MeOH-$CHCl_3$) to yield 25-30 ml fractions, which were combined on the basis of tlc profiles to provide 27 fractions.

UZARIGENIN.—Fraction 8 was purified by preparative tlc on silica gel PF_{254} (1 mm) plates with 4% MeOH-$CHCl_3$. The uv active band gave crystalline uzarigenin (80.5 mg) which was a single spot by qualitative tlc. Recrystallization from $CHCl_3$ gave pure material with mp 226°/232-234° [reported (23) 234-238°]. Ir, ^{1}H-nmr, and ms data agreed with literature values (24).

Anal. calcd for $C_{23}H_{40}O_4$: 374.2458. Found (ms): 374.2458.

STROPHANTHIDIN.—A portion (27 mg) of fraction 12 was recrystallized from $CHCl_3$ to afford colorless crystals of strophanthidin, mp 138-141°/222-228° [lit. (25) 128-142°/228-241°]. Ir and ^{1}H-nmr spectra were identical to those of an authentic sample. Tlc on silica gel with $CHCl_3$-MeOH-H_2O (70:26:4) EtOAc-cyclohexane (1:1), and MeOH-$CHCl_3$ (15:75) and on alumina with MeOH-$CHCl_3$ (15:75) and $CHCl_3$-MeOH-H_2O (70:26:4) gave Rf values identical with those of an authentic sample. A pure sample had a KB ED_{50} of 0.44 μg/ml.

ERYSULFONE (**1a**).—This compound crystallized from fraction 7 as colorless needles (80 mg) using $CHCl_3$. Recrystalization from $CHCl_3$ gave 58 mg of colorless prisms, mp 116.5-117.5°; ir ν max 1775, 1320, 1278, 1190, 1138, 1124 cm^{-1}; ^{1}H nmr δ4.68(br m, 1H), 3.24(t, 2H, J=8.5 Hz), 2.94(s, 3H), 2.8-1.8(m); ^{13}C nmr δ176, 78.24(d), 50.96(t), 41.03(q), 28.35(t), 28.17(t), 27.71(t); ms *m/z* (rel. int.) 195 (1.71, MH^++2), 194 (2.77, MH^++1), 193 (30.66, MH^+), 177 (4.07, M-CH_3), 175 (84.61, M-OH), 147 (96.6, M-COOH), 113 (15.11, M-CH_3SO_2), 95 (25.87, M-CH_3SO_2-H_2O), 85 (61.6, M-CH_3SO_2-C_2H_2), 67 (100, M-CH_3SO_2-CO-H_2O); ord (c, 2.534 mg/5ml) $[\phi]_{600}$-75.79, $[\phi]_{589}$-75.79, $[\phi]_{500}$-113.68, $[\phi]_{400}$-189.47, $[\phi]_{300}$-530.51.

Anal. calcd for $C_7H_{12}SO_4$+H: 193.0534. Found (ms): 193.0538.

ERYSULFOXIDE (**1b**).—Preparative tlc of Fraction 9 with 8% MeOH-$CHCl_3$ yielded 285 mg of crude material from which 18 mg of colorless crystals were obtained. Recrystallization from $CHCl_3$ gave colorless prisms (13.5 mg), mp 86-88°; ir ν max 1760, 1180, 1030, 1000 cm^{-1}; ^{1}H nmr δ4.68(br m, 1H), 2.62(s, 3H), 3.2-1.7(m); ^{13}C nmr δ176(s), 80.0(d), 51.35(t), 39.19(s), 29.46(t), 28.11(t), 27.43(t); ms *m/z* (rel. int.) 179 (5.54, MH^++2), 178(8.54, MH^++1), 177 (100, MH^+), 159 (19.7, M-OH), 113 (43.1, M-CH_3SO), 112 (21.3, M-CH_3SO-H), 95(23.1 M-CH_3SO-H_2), 85 (15.16, M-CH_3SO-C_2H_2], 67 (14.15, M-CH_3SO-CO-H_2O); ord (c, 2.538 mg/5 ml) $[\phi]_{600}$ 86.71, $[\phi]_{589}$ 86.71, $[\phi]_{500}$ 121.39, $[\phi]_{400}$ 190.76, $[\phi]_{300}$ 346.82.

Anal. calcd for $C_7H_{12}SO_3$+H: 177.0585. Found (ms): 177.0583.

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