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TERPENOIDS AND ALKALOIDS FROM *ESENBECKIA BELIZENCIS*. SPONTANEOUS OXIDATION OF FUROQUINOLINE ALKALOIDS[1]

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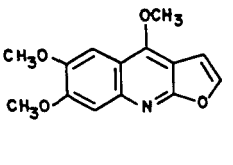
ABSTRACT.—Several terpenoids and the alkaloids kokusaginine [**1**] and flindersiamine [**2**] were isolated from the aerial parts of *Esenbeckia belizencis* (Rutaceae). Kokusaginine showed activity in the brine shrimp lethality test. The structures of the main autoxidation products derived from the natural alkaloids were established.

Previous chemical work on species of *Esenbeckia* (Rutaceae, Rutoideae) revealed the presence of coumarins, alkaloids, and limonoids (1–3), and recently, from *Esenbeckia leiocarpa*, two quinolinone alkaloids considered as biological poisons were isolated (4). Chemical investigation of the organic extracts obtained from the leaves of *Esenbeckia belizencis* Lundell, a tree that grows in the neotropical Mesoamerican forest, allowed us to isolate decaprenol (5), β-sitosterol, cariophyllene β-oxide (6), spathulenol (7), lupenone, friedelin, friedelanol, and two alkaloids, kokusaginine [**1**] and flindersiamine [**2**] (8,9), whose structures were established by comparison of physical and spectroscopic data with those published in the literature. The structures of the alkaloids have been further confirmed by synthesis (10,11).



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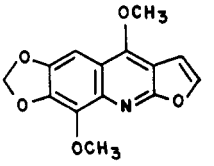

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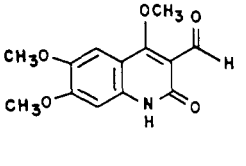

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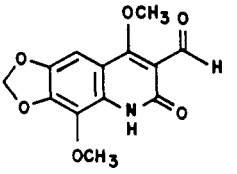

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Compounds **1** and **2** were tested for biological activity in the brine shrimp toxicity assay (12). Compound **1** was found to be mildly toxic (LC_{50} 367 ppm), while **2** did not display toxicity (LC_{50}>1000 ppm).

During the course of this study, it was observed that solutions of alkaloids **1** and **2** in $CHCl_3$ undergo partial transformation upon standing. The transformations also proceed in other solvents such as EtOAc, *n*-hexane–EtOAc (3:2), and Me_2CO, at room temperature, in the presence or absence of Si gel, and with the necessary presence of air and light.

[1]Contribution No. 1127 from the Instituto de Química, UNAM.

Preparative cc allowed isolation of the main transformation products derived from **1** and **2**. The product derived from **1** had the molecular formula $C_{13}H_{13}O_5N$ (ms and elemental analysis) corresponding to the addition of a molecule of oxygen and loss of the elements of carbon monoxide. The presence of a 2-quinolone system in the molecule was indicated by the uv absorptions at 233, 260, and 315 nm (13) and by an ir band at 1647 cm^{-1}. A band at 1683 cm^{-1} suggested the presence of an α,β-unsaturated aldehyde, which was confirmed by a signal at δ 10.35 in the ^1H nmr. The ^1H-nmr spectrum also contained signals at δ 7.25 (s, 1H, H-5) and 6.79 (s, 1H, H-8) for the isolated protons on the benzenoid ring. Three singlet Me resonances at δ 4.14, 3.96, and 3.85 indicated the 4,6,7- substitution pattern present in the starting material, **1**. Therefore, this substance was established as 3-formyl-4,6,7-trimethoxy-2-quinolone [**3**]. The structure of the oxidation product of **2** was analogous to the oxidation product of **1**, and was established as 3-formyl-4,8-dimethoxy-6,7-methylenedioxy-2-quinolone [**4**].

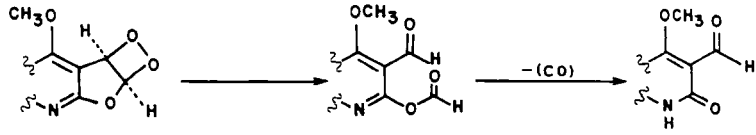
A plausible mechanism for the oxidative degradation of **1** and **2** involves the addition of oxygen to the furan ring to form the dioxetan **5**. This intermediate could not be isolated but was detected by nmr in some chromatographic fractions obtained during the isolation of **3**. ^1H-nmr analysis of these fractions showed signals for an AB system at δ 6.18 and 5.58 (1H each, $J = 6$ Hz), confirmed by decoupling experiments, corresponding to the acetalic and benzylic hydrogens of **5**, respectively, in addition to the signals due to the product **3**. Analogous intermediates have been proposed in the transformation of 2,3-dimethylbenzo [*b*]furane to yield 2-acetoxyacetophenone (14) and in the cycloaddition of acetophenone to furan to yield the corresponding oxetane derivative. The acetalic proton in this oxetane is located at δ 6.10 (15). Dioxetan ring opening of **5** with C-C fission (14) would lead to the dicarbonyl intermediate **6**, which upon hydrolysis afforded the final products (Scheme 1).

It could be expected that electron donor substituents on the heteroaromatic nucleus would enhance the rate (and yield) of the transformation. The spontaneous oxidation of natural furoquinoline alkaloids has not been previously reported. Interestingly, the products of reserpine autoxidation have recently been discussed (16).

EXPERIMENTAL

PLANT MATERIAL AND ISOLATION.—*E. belizencis* was collected in Tuxtepec, Oaxaca, Mexico. Voucher specimens are preserved at the National Herbarium, Instituto de Biología, UNAM (MEXU CHR-89). Dried and ground aerial parts (2.5 kg) were extracted with 12 liters of *n*-hexane for 2 days at room temperature to afford 53.5 g of residue. The defatted plant material was then extracted with 12 liters of MeOH, yielding 131 g residue. The hexane extract was adsorbed on Si gel and chromatographed using vlc (17) (100→0% *n*-hexane/EtOAc) and subsequent cc, to give friedelin (132 mg), lupenone (19 mg), cariophyllene β-oxide (6) (93 mg), a mixture of polyprenols (600 mg) from which was isolated decaprenol (5) (purified by repeated reversed-phase hplc, using *n*-PrOH as eluent, ORV column, 40 mg), spathulenol (7) (68 mg), friedelanol (85 mg), and β-sitosterol (215 mg).

The MeOH extract was also chromatographed by vlc (100→0% *n*-hexane/EtOAc) and subsequent cc to give a mixture of kokusaginine [**1**]



5 **6** **3–4**

SCHEME 1

and flindersiamine [**2**] (ca. 2 g). Part of this mixture was separated by careful fractional crystallization from MeOH-EtOAc (7:3), yielding an amorphous solid, flindersiamine [**2**], and a crystalline solid, kokusaginine [**1**]. Physical data of **1** and **2** corresponded to those reported (8,9).

AUTOXIDATION OF KOKUSAGININE [**1**] AND FLINDERSIAMINE [**2**].—The alkaloid (200 mg of each) was placed in $CHCl_3$ (20 ml) and allowed to stand in the presence of light and air at room temperature for 2 days. After this period the transformation was slower (tlc control). The reaction also proceeded in EtOAc, *n*-hexane–EtOAc (3:2), and Me_2CO. The solvent was removed under reduced pressure, and the residue was adsorbed in Si gel (500 mg) and chromatographed by cc with *n*-hexane–EtOAc (9:1), eluting with increasing amounts of EtOAc. This procedure resulted in isolation of 12 mg of **3** from kokusaginine [**1**] and 15 mg of **4** from **2**. Compound **3**: yellow needles from EtOH; mp 190–192°, uv λ max (MeOH) nm (ε) 217 (22815), 233 (23640), 260 (10194), 315 (6743); ir ($CHCl_3$) 1683, 1647, 1515, 1462, 1265, 1009 cm^{-1}; ^1H nmr ($CDCl_3$ + DMSO, 80 MHz) δ 10.35 (1H, s, -C*H*=O), 7.25 (1H, s, H-5), 6.79 (1H, s, H-8), 4.14 (3H, s, -OCH_3 at C-4), 3.96 (3H, s, -OCH_3 at C-6), 3.85 (3H, s, -OCH_3 at C-7); eims *m/z* (rel. int.) $[M]^+$ 263 (47), 235 (51), 234 (100), 220 (74), 189 (23). *Anal.* found C 59.63, H 4.93, N 5.52; calcd for $C_{13}H_{13}NO_5$, C 59.31, H 4.98, N 5.32.

In order to obtain additional information about the intermediates of the transformation, some chromatographic fractions obtained during isolation of **3** were analyzed by ^1H nmr. ^1H-nmr and tlc analysis of these fractions showed the presence of **3** in addition to **5** in a ca. 5:1 ratio. The ^1H-nmr data obtained for 4,6,7-trimethoxy-1′,2′-dihydro-1′,2′-peroxy[2,3*b*]furoquinoline [**5**] were the following ($CDCl_3$, 300 MHz): δ 7.15 and 7.39 (singlets for H-5 and H-8), 6.18 (d, J = 6 Hz, H-1′), 5.58 (d, J = 6 Hz, H-2′), 3.92–3.90 (signals for OCH_3 groups).

The transformation of **2** gave 15 mg of **4**: yellow needles from EtOH; mp 276–277°; ir ($CHCl_3$) 1682, 1640, 1457, 1090, 1045 cm^{-1}; ^1H nmr ($CDCl_3$, 300 MHz) 10.41 (1H, s, -C*H*=O), 8.95 (1H, br s, -N*H*), 7.06 (1H, s, H-5), 6.04 (2H, s, -OCH_2O-), 4.18 (3H, s, -OCH_3 at C-4), 4.16 (3H, s, -OCH_3 at C-8).

Artemia salina bioassays for the alkaloids were performed as described in the literature (12).

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