Isolation, Structural Elucidation, and Chemical Synthesis of 2-Hydroxy-3-octadecyl-5-methoxy-1,4-benzoquinone (Irisoquin), a Cytotoxic Constituent of *Iris missouriensis*

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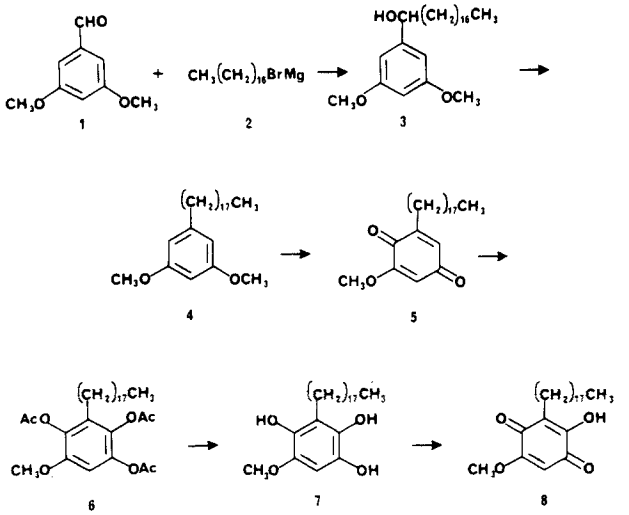
Abstract □ As part of a continuing effort to provide novel agents of potential value in cancer chemotherapy, 2-hydroxy-3-octadecyl-5-methoxy-1,4-benzoquinone (irisoquin) was identified as a component of *Iris missouriensis*. This novel species demonstrated cytotoxic activity with cultured KB and P-388 cells (ED_{50} = 1.8 and 0.03 μg/mL, respectively). The structure was assigned on the basis of spectral analyses and confirmed by chemical synthesis. The latter provides a facile method for the production of irisoquin and structural derivatives that may be of value for the examination of structure–activity relationships. A closely related compound, 3-octadecyl-5-methoxy-1,4-benzoquinone (deoxyirisoquin), was also isolated from *Iris missouriensis*, prepared synthetically, and found to be devoid of cytotoxic activity.

Iris missouriensis (Iridaceae) is a decorative household plant that commonly grows along the banks of the Missouri River and the highland of Northern California. Although a related species, *Iris pallasii* Fisch. var. *chinensis* Fisch., has been extensively investigated as a potential source of cancer chemotherapeutic agents,[1] the phytochemical constituents of *Iris missouriensis* Nutt., and biological activity thereof, have heretofore not been reported. Thus, the root of *Iris missouriensis*, collected in Nevada, was investigated for the presence of potential antitumor agents.[2] The roots of the plant were extracted with petroleum ether (b.p. 37.9–55.4°C), and the resulting material was found to demonstrate significant cytotoxicity toward cultured P-388 cells (ED_{50} = 0.19 μg/mL). Repeated column chromatography of this extract, using cultured P-388 cells for localizing the cytotoxic activity, afforded fractions containing a mixture of two compounds that were separated by an additional chromatographic step.



Scheme I

This article describes the isolation, structural eludication, and synthesis of these two compounds, irisoquin (8) and deoxyirisoquin (5).

Experimental Section

Melting points were recorded on a Thomas-Hoover melting point apparatus and are uncorrected. The ^1H NMR spectra were obtained on a Nicolet NT-1280 or Varian T-60A spectrometer using deuteriochloroform as the solvent. Chemical shifts are reported in parts per million, downfield from $(CH_3)_4$ Si. The broad band decoupled ^{13}C NMR spectra were obtained on a Nicolet NT-1280 spectrometer, while the Nuclear Overhauser experiments were performed on a Bruker and Nicolet NT-1280 spectrometer. Chemical shifts are reported in parts per million, downfield from $(CH_3)_4$ Si, with deuteriochloroform as both solvent and internal standard. Low-resolution mass, UV, and IR spectra were recorded with Varian MAT-112, Cary 118, and a Nicolet MX-1 FT-IR spectrometers, respectively.

Isolation—The isolation sequence was guided by in vitro cytotoxicity evaluations using the KB and P-388 cell lines. Approximately 1 kg of the roots of *Iris missouriensis* was extracted at room temperature with petroleum ether (b.p. 37.9–55.4°C) until the extract was colorless. The concentrated extract was found to demonstrate an ED_{50} of 0.19 μg/mL with cultured P-388 cells. Column chromatography of the extract (23.0 g) on silica gel (500 g) with gradient elution from petroleum ether to $CHCl_3$, and then from $CHCl_3$ to MeOH, afforded 15 fractions (2 L each). Fraction 12 (2.5 g), eluted with 20% petroleum-ether in $CHCl_3$, was found to demonstrate cytotoxic activity toward cultured P-388 cells. This material was again chromatographed over silica gel (150 g) with $CHCl_3$:ethyl acetate (98:2) to yield 20 fractions (30 mL each). Fractions 12–16 were then combined (130 mg) and chromatographed over silica gel (20 g) employing *n*-hexane:ethyl acetate:acetic acid (10:1:0.2) as the eluant. Fractions of 15 mL were collected, and a crystalline mixture (100 mg) containing compounds 5 and 8 was obtained from fraction 15. Further chromatography [silica gel (16 g), prepacked in benzene:acetic acid (50:1), and eluted with benzene:ethyl acetate (20:1)] afforded the inactive derivative, deoxyirisoquin (5, 18 mg), followed by the cytotoxic quinone, irisoquin (8, 42 mg).

Evaluation of Cytotoxic Activity—Cytotoxic activity was assessed utilizing cultured KB or P-388 cells, essentially by protocols developed by the National Cancer Institute,[3] as described previously.[4,5]

Irisoquin (2-Hydroxy-3-octadecyl-5-methoxy-1,4-benzoquinone) (8)—This compound was crystallized from a mixture of *n*-hexane and ethyl acetate as orange crystals, mp 98–101°C; UV: γ_{max}^{EtOH} nm (log ε) 290 (4.6), 218 (4.7) IR: ν_{max} (KBr) 3336, 2955, 2919, 2815, 1680, 1637, 1599, 1220, and 1207 cm^{-1}; ^1H NMR: δ 7.26 (br, 1), 5.84 (s, 1), 3.86 (s, 3), 2.44 (t, 2), 1.25 (br, 32), and 0.88 ppm (t, 3); ^{13}C NMR (broad band decoupling): 182.8, 181.7, 161.1, 151.5, 119.2, 102.4, 56.7, 31.9, 29.6, 29.4, 28.0, 22.7, and 14.1 ppm; EIMS (70 eV): *m/z* (relative intensity) 406 (M^+, 41), 169 (43), 168 (100), 167 (18), and 156 (15).

Anal.—Calc. for $C_{25}H_{42}O_4$: C, 73.89; H, 10.35. Found: C, 73.75; H, 10.41.

Deoxyirisoquin (3-Octadecyl-5-methoxy-1,4-benzoquinone) (5)—This compound was crystallized from a mixture of *n*-hexane and ethyl acetate as yellow crystals, mp 86–89°C, UV: λ_{max}^{EtOH} nm (log ε) 267 (4.21) and 364 (2.68); IR: ν_{max} (KBr) 2916, 2850, 1969, 1684, 1653, 1598, 1472, and 1234 cm^{-1}; ^1H NMR: δ 6.64 (m, 1), 5.85 (d, 1), 3.81 (s, 3), 2.40 (t, 2), 1.25 (br, 32), and 0.88 ppm (t, 3); EIMS (70 ev): *m/z* (relative intensity) 390 (M^+, 100) 154 (97), and 153 (70).

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Anal.—Calc. for $C_{25}H_{42}O_3$: C, 76.92; H, 10.77. Found: C, 76.99; H, 10.81.

Preparation of Carbinol (3)—Heptadecyl bromide (3.8 g), dissolved in 20 mL of ether, was added into a stirring mixture containing 0.5 g of Mg and 15 mL of dry ether over a 30 min period in a dropwise manner. The mixture was then allowed to reflux for 3 h, cooled, and the 3,5-dimethoxybenzaldehyde (1) (1.5 g dissolved in 10 ml of ether) was added in a dropwise manner. This mixture was then refluxed for an additional 5 h, cooled, and diluted with 300 mL of ice water. Work-up of the separated organic layer yielded carbinol (3) (3.0 g, 82% yield): mp 65–68°C; ^{1}H NMR: δ 6.58 (d, 2), 6.40 (d, 1), 4.65 (t, 1); 3.80 (s, 6), 1.85 (br, 1, D_2O exchangeable), 1.28 (s, 32), and 0.88 ppm (t, 3).

Anal.—Calc. for $C_{26}H_{46}O_3$: C, 76.85; H, 11.33. Found: C, 76.78; H, 11.37.

Preparation of 3,5-Dimethoxyphenyloctadecane (4)—Three grams of the carbinol (3), dissolved in 75 mL of ethyl acetate, was hydrogenated over 10% Pd/C (5 atm pressure) at room temperature for 4 h and filtered. Work-up of the filtrate gave 4 as needles (2.3 g, 80% yield): mp 44–45°C; ^{1}H NMR: δ 6.25 (br, 3), 3.80 (s, 6), 2.5 (t, 2), 1.28 (br, 32), and 0.88 ppm (t, 3).

Anal.—Calc. for $C_{26}H_{46}O_2$: C, 80.00; H, 11.79. Found: C, 79.88; H, 11.94.

Preparation of Deoxyirisoquin (5)—A sample of 4 (2 g) dissolved in 15 mL of warm acetic acid was added in a dropwise manner to a stirring solution of chromium trioxide (2.5 g) in water (1.0 mL) and acetic acid (25 mL). The mixture was heated on a steam bath for 2 h, diluted with 250 mL of ice water, and extracted with two 250-mL portions of ether. The material remaining after removal of the ether crystallized from acetonitrile as yellow needles (1.06 g, 48% yield): mp 86–89°C; UV: λ_{max}^{EtOH} nm (log ϵ) 267 (4.21) and 364 (2.68); IR: ν_{max} (KBr) 2916, 1850, 1969, 1684, 1653, 1598, 1472, and 1234 cm^{-1}; ^{1}H NMR: δ 6.64 (m, 1,6-H), 5.85 (d, 1, J = 2.7 Hz, 2-H), 3.81 (t, 3, OCH_3), 2.40 (t, 2, J = 7.2 Hz, —CH_2—), 1.25 (br, 32, —$(CH_2)_{16}$—) and 0.88 ppm (t, 3, J = 7.2 H_z, —CH_3); EIMS (70 eV): *m/z* (relative intensity) 390 (32), 153 (40), and 152 (100).

Anal.—Calc. for $C_{25}H_{42}O_3$: C, 76.92; H, 10.77. Found: C, 76.81; H, 10.89.

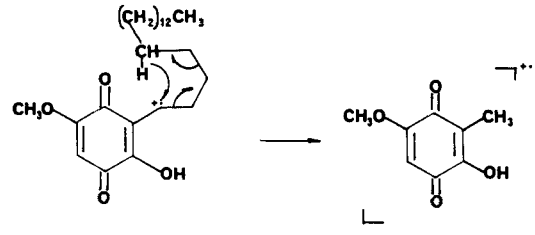
Preparation of 2,5,6-Triacetoxy-3-methoxy-phenyloctadecane—Deoxyirisoquin (5) (1.0 g) in acetic anhydride and 3 mL of concentrated sulfuric acid was kept overnight. After diluting with 300 mL of ice water, the mixture was extracted twice with 300-mL portions of ether. The ether extract was then washed thoroughly with water. The brown oil (1.1 g) obtained by evaporation of the ether showed a single spot on TLC chromatograms developed with two solvent systems [chloroform and petroleum ether:ethyl acetate (9:1)]. ^{1}H NMR: δ 6.69 (s, 1), 3.78 (s, 3), 2.30 (s, 3), 2.29 (s, 3), 2.25 (s, 3), 2.5 (m, 2), 1.26 (br, 32), and 0.88 ppm (t, 3).

Preparation of Irisoquin (8) Via 2,5,6-Trihydroxy-3-methoxy-phenyloctadecane—Compound 6 (1.0 g), in methanol (40 mL) and concentrated hydrochloric acid was (2 mL), was refluxed over a steam bath under nitrogen for 30 min, and treated with 30% aqueous ferric chloride (45 mL). The solution was diluted with water (2°C) and extracted with ether. Chromatography of the residue from the organic extract on silica gel prepacked in benzene:acetic acid (50:1) and eluted with benzene:ethyl acetate (25:1) afforded 8 (160 mg, 15.2% yield from 5). This substance crystallized from hexane as orange crystals: mp 98–101°C; UV: λ_{max}^{EtOH} nm (log ϵ) 290 (4.6), 218 (4.7); IR: ν_{max} (KBr) 3336, 2955, 2919, 2815, 1680, 1637, 1599, and 1219 cm^{-1}; ^{1}H NMR: δ 7.26 (br, 1) 5.84, (s, 1), 3.86 (s, 3), 2.44 (t, 2), 1.25 (br, 32), and 0.88 ppm (t, 3); EIMS (70 eV): *m/z* (relative intensity) 406 (41), 169 (43), 168 (100), 167 (18), and 156 (15).

Anal.—Calc. for $C_{25}H_{42}O_4$: C, 73.89; H, 10.35. Found: C, 73.71; H 10.46.

Results and Discussion

Elemental and MS analyses [EIMS: *m/z* 406 (M^+) and CIMS *m/z* 407 $(M+H)^+$] of 8 (given the trivial name of irisoquin) indicated a molecular formula of $C_{25}H_{42}O_4$. Absorption bands characteristic of conjugated carbonyls and C=C bonds of a trisubstituted benzoquinone (1680, 1637, and 1599 cm^{-1})[6] were found in the IR spectrum (KBr), as were strong bands (3336 and 1220 cm^{-1}) indicative of hydroxy and methoxy groups. The UV spectrum was consistent with the structure of a 2-hydroxy-5-methoxy benzoquinone chromophore [λ_{max}^{EtOH} 218, 290 nm (log ϵ 4.7, 4.6)].[7,8] In the ^{1}H NMR spectrum (360 MHz, $CDCl_3$), a singlet at 3.86 ppm (3 protons), and a broad D_2O exchangeable signal at 7.26 ppm (1 proton), also supported the attachment of a methoxy and a hydroxy group to a benzoquinone ring. Further, a triplet signal for the methylene protons alpha to C=C at 2.44 ppm, a broad singlet at 1.25 ppm (representing 32 protons), and a triplet for methyl protons at 0.88 ppm, revealed an octadecyl side chain attached to the quinone moiety. A trisubstituted 1,4-benzoquinone was also supported by a strong peak at *m/z* 168 (base); this correlates with the hydroquinone fragment that is formed by the loss of a heptadecyl side chain of irisoquin (Scheme II).[7]



Scheme II

To establish the relative positions of the substituents, the methoxy proton signal at 3.86 ppm was irradiated and found to cause a Nuclear Overhauser enhancement of the C_6 proton signal at 5.84 ppm (26% increase in intensity). This procedure caused no change in the intensity of signals at 7.26 (hydroxy proton) and 2.44 ppm (methylene hydrogen of the alkyl side chain). Hence, the substitution of a methoxy group at position-5 of the 1,4-benzoquinone, and absence of substitution at position-6, were confirmed.

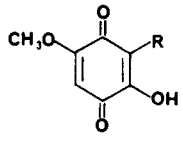
As the UV spectrum suggested the presence of a 2-hydroxy-3-alkyl-1,4-benzoquinone, when compared with the published data of 9 and the study of Ogawa et al,[7] it appeared most likely that the octadecyl side chain was situated at the 3-position. Data from the ^{13}C NMR were also indicative of a 2-hydroxy-3-alkyl-5-methoxy-1,4-benzoquinone.[6]

For final confirmation, a synthetic method of producing the proposed substances was devised. The procedure was based on the method reported by Ridley et al. for the synthesis of 3-tridecyl-5-methoxy-1,4-benzoquinone,[9] and is summarized in Scheme I. Reaction of 3,5-dimethoxybenzaldehyde (1) with the Grignard reagent 2 afforded the carbinol 3, which, on hydrogenation over 10% palladium-charcoal and hydrogen, gave 4. Oxidation of compound 4 with chromium trioxide in acetic acid afforded 5 which, by means of the Thiele–Winter acetoxylation,[10] yielded a product, 6, that appeared from TLC chromatograms (chloroform and a mixture of petroleum ether and ethyl acetate as developing solvents) and ^{1}H NMR spectroscopy to be a single substance with the proposed structure. This structure was confirmed by irradiation of the methoxy proton signal at 3.78 ppm that resulted in an enhancement of 26% in the aromatic proton signal at 6.99 ppm.

Finally, hydrolysis of 6 with methanolic HCl furnished a crude phenol, 7, which was immediately oxidized by ferric chloride to 8. Compound 8 (2-hydroxy-3-octadecyl-5-methoxy-1,4-benzoquinone) was found to be identical with the natural product, irisoquin, isolated from *Iris missouriensis*, by mixing melting point, co-TIC and direct comparison of NMR and MS spectral data.

Elemental and EIMS analyses [*m/z* 390 ($M^{+\cdot}$)] of the second compound isolated from *Iris missouriensis* indicated a molecular formula of $C_{25}H_{42}O_3$. The 1,4-benzoquinone moiety was revealed by its IR[11,12] (1684, 1653, 1623, and 1598 cm^{-1}) and

UV spectra [λ_{max}^{EtOH} 267, 364 nm (log 4.21, 2.68)]. The presence of a methoxy group was indicated by a strong absorption band at 1234 cm^{-1} and a ^1H NMR signal at 3.8 ppm (3 protons). The ^1H NMR spectrum was similar to that of 8, except in the low field region where a doublet at 5.85 (1 proton, J = 2.7) and a multiplet at 6.46 ppm (1 proton) (arising from the ring protons) were observed, and the absence of a hydroxy proton signal at 7.26 ppm. The signals of the ring protons were obviously coupled, indicating that they occupy the 2,6-positions. Thus, it was speculated that this substance may be a 2,6-disubstituted quinone[1] structurally similar compound 5, obtained during the synthesis of irisoquin (Scheme I). A comparison of these substances, including spectral data, mixture melting point and co-TLC, indicated that they were identical. By analogy with compound 8, the natural product, 2-methoxy-6-octadecyl-1,4-benzoquinone, was assigned the trivial name of deoxyirisoquin 5.



9 R = $(CH_2)_9CH{=}CH(CH_2)_7CH_3$
10 R = $(CH_2)_9CH{=}CH(CH_2)_3CH_3$
11 R = $(CH_2)_{14}CH_3$

The cytotoxic potential of these substances was investigated. Irisoquin 8 was found to be active against the KB (ED_{50} = 1.8 μg/mL) and the P-388 (ED_{50} = 0.03 μg/mL) cell lines. Conversely, deoxyirisoquin 5 was found to be devoid of activity (ED_{50} for the KB and P-388 cells were both greater than 100 μg/mL). Since these two substances differ structurally only by the presence or absence of a hydroxyl group at position-2, this substituent appears to play a critical role in the mediation of cytotoxicity. The potential of irisoquin to prolong the survival time of P-388-infected mice was also examined. No effect was observed when the drug was administered intraperitoneally for 9 d with daily dosages ranging from 11 to 90 mg/kg body weight. In contrast, benzoquinones 9 and 11 have been reported as active antitumor compounds.[1,13] Thus, it should be of interest to synthetically prepare a series of 2-hydroxy and 2-deoxy derivatives of irisoquin 8 for the establishment of antitumor efficacy and structure–activity relationships, as well as for the evaluation of bactericidal activity[13] and the mediation of host defense responses, such as those facilitated by compound 10.[6]

Acknowledgments

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