New Quinoline Alkaloids from *Ruta chalepensis*

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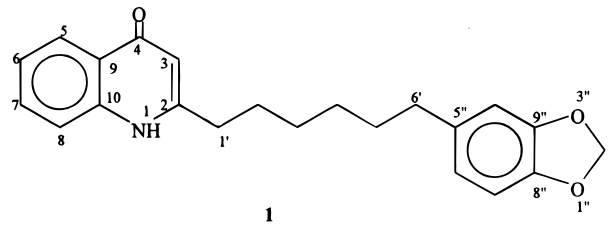
The roots of *Ruta chalepensis*, collected from the northern Saudi desert, yielded two new quinoline alkaloids, namely, 2-{6′-(2H-benzo[*d*]1″,3″-dioxolen-5″-yl)hexyl}-hydroquinolin-4-one (**1**) and 2-{6′-(2H-benzo[*d*]1″,3″-dioxolen-5″-yl)hexyl}-4-methoxy-quinoline (**2**). Nine previously reported alkaloids, dictamnine, pteleine, skimmianine, rutacridone, isogravacridonechlorine, maculosidine, graveoline, graveolinine, and 4-methoxy-1-methyl-2(1H)-quinolinone, and coumarins, chalepensin, and umbelliferone were also isolated. Structure elucidations were based primarily on 1D and 2D NMR analyses and chemical transformations. Antimicrobial activity of these compounds is discussed.

Ruta chalepensis L. (Rutaceae) is a perennial herb widely used in folk medicine as an antirheumatic, an antispasmodic, an aphrodisiac, and a treatment for snakebites, headaches, and wounds.[1] The plant is a rich source of several acridone and quinoline alkaloids, as well as coumarins.[2,3] A few of the coumarins from *R. chalepensis* exhibited antifertility effects,[4] and some quinoline alkaloids isolated from *Ruta* species displayed mutagenic,[5,6] ganglionic-blocking,[7] curare-like,[7] and spasmolytic activities.[8] The present study deals with the isolation, characterization, and evaluation of the antimicrobial activity of a number of new and known metabolites from *R. chalepensis*.

The $CHCl_3$ fraction obtained from an EtOH extract of *R. chalepensis* roots was subjected to flash chromatography followed by centrifugal preparative TLC to give the alkaloids **1** and **2**, as well as several known alkaloids and coumarins (see Experimental Section). The HRFTMS of **1** displayed a molecular ion peak $[M + H]^+$ at *m/z* 350.1723, suggesting the molecular formula $C_{22}H_{23}O_3N$ and 12 degrees of unsaturation. The IR spectrum of **1** showed a broad absorption band at 3105−3125 cm^{-1}, suggesting the presence of an NH group. It also showed a strong absorption band at 1655 cm^{-1}, consistent with the presence of an α,β-unsaturated carbonyl functionality. The ^{13}C and ^1H NMR spectra of **1** were in agreement with a quinolone skeleton.[2,9,10] The ^{13}C NMR spectra of **1** demonstrated the presence of a trisubstituted double bond, a vinylogous amino ketone group, and a disubstituted aromatic ring system. The H-3 olefinic proton and the aromatic protons H-6 and H-8 displayed ^3J-HMBC correlations with the quaternary carbon at δ 125.0 (C-9). Similarly, the aromatic protons H-5 and H-7 each displayed a ^3J-HMBC correlation with the quaternary carbon at δ 140.5 (C-10), thus confirming the quinolone system. The H-3 proton displayed a ^3J-HMBC correlation with the methylene carbon at δ 33.6 (C-1′). The COSY and HMBC data of **1** supported the NMR assignments of the hexyl side chain. Also, the benzylic methylene proton signal (H_2-6′) displayed ^3J-HMBC correlations to the aromatic methine carbon signals at δ 109.0 and 121.3 (C-4″ and C-6″, respectively) and a ^2J-HMBC correlation with the quaternary carbon at δ 136.5 (C-5″). Both aromatic protons, H-4″ and H-6″, were HMBC-coupled to the same quaternary carbon at δ 145.4 (C-8″). Both H-6″ and H-7″ displayed COSY correlation to each other due to ortho coupling. The H-2″ methylenedioxy group resonated as a two-proton singlet at δ 5.94, which correlated with the methylene carbon at δ 100.9. On the other hand, the H_2-2″ protons displayed ^3J-HMBC correlations with C-8″ and C-9″ to confirm the proposed structure **1**. Thus, compound **1** was found to be a new quinolone alkaloid, 2-{6′-(2H-benzo[*d*]1″,3″-dioxolen-5″-yl)hexyl}-hydroquinolin-4-one. A related compound {2-[4-(3,4-methylenedioxyphenyl)butyl]-4-(1H)-quinolinone} was reported to occur in *R. graveolens*,[9] but with a butyl rather than a hexyl side chain.

Compound **2** ($C_{23}H_{25}O_3N$) exhibited ^{13}C and ^1H NMR data that were closely similar to **1**. Compound **2** lacked the carbonyl group at C-4 (quinolin-4-one), which was methoxylated instead to become a 4-methoxyquinoline moiety. The proton methyl singlet resonating at δ 4.05, which correlated with the methyl carbon resonating at δ 55.9, was assigned to the 4-methoxy group. The latter displayed a ^3J-HMBC correlation with the quaternary carbon at δ 162.8 (C-4). Both H_2-1′ and H_2-2′ displayed ^2J- and ^3J-HMBC correlations, respectively, with the downfield quaternary carbon resonating at δ 164.6 (C-2). Methylation of **1** using CH_3I/Na_2CO_3 in acetone afforded compound **2**, as indicated by TLC and MS analysis. Therefore, **2** was identified as 2-{6′-(2H-benzo[*d*]1″,3″-dioxolen-5″-yl)hexyl}-4-methoxy-quinoline, a new quinoline alkaloid.



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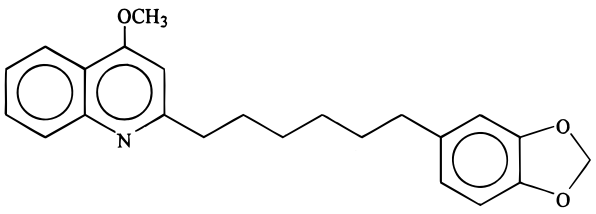

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The leaves of the same plant afforded the known aromatic compound, moskachan C,[11] along with the known coumarins, rutamarin, chalepin, isopimpinellin, and bergapten.[9,11]

All isolated new and known compounds were tested for antimicrobial activity against a wide range of microorganisms, using a modified microtiter-plate assay.[12] The known furanoquinoline alkaloid, pteleine showed moderate antimicrobial activity by inhibiting the growth of *Mycobacterium smegmatis*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*, with an MIC range of 50−100 μg/mL, while moskachan C, dictamnine, skimmianine, and isogravacridonechlorine were less active (MIC 100 μg/mL) against *M. smegmatis* and *B. subtilis* and inactive against the other microorganisms.

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were recorded on an Electrothermal 9100 instrument. UV spectra were obtained in MeOH, using a Shimadzu UV-1601PC spectrophotometer, and IR spectra were obtained as KBr disks or in $CHCl_3$ on a Perkin-Elmer 5808 spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, using TMS as internal standard, on a Bruker AMX NMR spectrometer operating at 500 MHz for ^1H NMR and at 125 MHz for ^{13}C NMR. The HRMS were measured using a Bioapex FT-ICR mass spectrometer with electrospray ionization. The EIMS were measured using an E. I. Finnigan model 3200 (70 eV ionization potential) with INCOS data system, an E. I. Finnigan model 4600 quadruple system or a Shimadzu QP500 GC/mass spectrometer. TLC analyses were carried out on precoated Si gel G_{254} 500−1000 μm, with the following developing system: $(CH_3)_2CO-CCl_4$ (10:90). For flash column chromatography, Si gel 60 40 μm was used, and a mixture of $CHCl_3$−MeOH was used as eluent. Centrifugal preparative TLC was performed using a Chromatotron (Harrison Research Inc. model 7924), 1- or 4-mm Si gel PF_{254} disk, and a flow rate of 3 mL/min. The isolated compounds were visualized using UV light (λ_{max} 254 nm) and by spraying with 1% vanillin−H_2SO_4 spray reagent. The IUPAC nomenclature of the isolated compounds was determined using a Chemistry 4-D Draw Pro 3.0 program (ChemInnovation Software, Inc.).

Plant Material. Fresh whole plants of *R. chalepensis* were collected in March 1989, near Al Baha, in the Southern Region of Saudi Arabia. The plant was identified at the College of Pharmacy, King Saud University. A voucher specimen has been deposited at the Herbarium of Medicinal, Aromatic and Poisonous Plants Research Center, King Saud University (MAPPRC 12539, 1989).

Extraction and Isolation. The powdered, air-dried roots (1.3 kg) and leaves (1.25 kg) were separately extracted with EtOH (95%, 3 × 2 L), and the combined extracts from each organ were evaporated under reduced pressure to give 160 g (roots) and 65 g (leaves) of dry extracts. A portion of the antimicrobially active root EtOH extract was subjected to bioautography[13] on Si gel plates (5 × 10 cm), using $CHCl_3$ as a solvent system, against *B. subtilis* (NCTC 10400) as a test organism. Four clear, elongated inhibition zones with R_f values of 0.45, 0.27, 0.13, and 0.07 were observed after 24 h of incubation. Hence, the EtOH root extract was dissolved in $CHCl_3$ (1L × 2), and the $CHCl_3$-soluble fraction (10 g) was flash-chromatographed on Si gel 60 (300 g) using petroleum ether−$CHCl_3$ (9:1), with increasing concentrations of MeOH, as eluent. The crude fractions obtained were further subjected to repeated column chromatography followed by centrifugal preparative TLC using the systems: $(CH_3)_2CO-CCl_4$ (10:90) to afford **1** (48 mg, R_f 0.06), **2** (51 mg, R_f 0.44), dictamnine (70 mg, R_f 0.29), pteleine (57 mg, R_f 0.25), skimmianine (40 mg, R_f 0.12), rutacridone (18 mg, R_f 0.43), isogravacridonechlorine (9 mg, R_f 0.10), maculosidine (20 mg, R_f 0.11), graveoline (25 mg, R_f 0.30, system: $[CH_3]_2CO-CH_2Cl_2$ 30:70), graveolinine (63 mg, R_f 0.55), 4-methoxy-1-methyl-2(1H)-quinolinone (50 mg, R_f 0.09), chalepensin (150 mg, R_f 0.28, system: EtOAc−*n*-hexane 10:90), and umbelliferone (7.5 mg, R_f 0.45, system: EtOAc−*n*-hexane 40:60). The dry leaf EtOH extract (65 g) was flash-chromatographed on Si gel 60 (900 g) using petroleum ether−$CHCl_3$−MeOH gradient as a solvent system to give several crude fractions which were further subjected to repeated column chromatography followed by centrifugal preparative TLC using the system: $(CH_3)_2CO-CCl_4$ (10:90) to afford moskachan C (19 mg, R_f 0.68), rutamarin (37 mg, R_f 0.91), chalepin (30 mg, R_f 0.49), isopimpinellin (10.5 mg, R_f 0.32), and bergapten (78 mg, R_f 0.34). The known compounds were all identified by comparing their physical and spectroscopic data with literature values.[9−11]

2-{6′-(2H-Benzo[*d*]1″,3″-dioxolen-5″-yl)hexyl}hydroquinolin-4-one (1): colorless needles from $CHCl_3$, mp 163 °C ; UV λ_{max} (log ϵ) (MeOH) 235 (2.61), 290 (1.97), 315 (2.14), 328 (2.12) nm; IR ν_{max} (KBr): 3105−3125 (NH), 3040−2820, 1655 (C═O), 1605 (C═C), 1510, 1450, 1270 (C−O) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 11.50 (1H, s, NH-1), 8.05 (1H, d, J = 8.0 Hz, H-5), 7.61 (1H, dd, J = 8.1, 7.1, H-7), 7.53 (1H, d, J = 8.3, H-8), 7.28 (1H, dd, J = 7.7, 7.3, H-6), 6.77 (1H, d, J = 7.9, H-7″), 6.75 (1H, br s, H-4″), 6.61 (1H, d, J = 7.8, H-6″), 5.94 (2H, s, H_2-2″), 5.93 (1H, s, H-3), 2.57 (2H, dd, J = 7.7, 7.6, H_2-1′), 2.47 (2H, dd, J = 7.6, 7.6, H_2-6′), 1.66 (2H, m, H_2-2′), 1.52 (2H, m, H_2-5′), 1.32 (4H, m, H_2-3′ and H_2-4′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.3 (s, C-4), 153.9 (s, C-2), 147.5 (s, C-9″), 145.4 (s, C-8″), 140.5 (s, C-10), 136.5 (s, C-5″), 131.8 (d, C-7), 125.1 (d, C-5), 125.0 (s, C-9), 123.0 (d, C-6), 121.3 (d, C-6″), 118.2 (d, C-8), 109.0 (d, C-4″), 108.3 (d, C-7″), 108.0 (d, C-3), 100.9 (t, C-2″), 35.1 (t, C-6′), 33.6 (t, C-1′), 31.4 (t, C-5′), 28.7 (t, C-4′), 28.6 (t, C-2′), 28.5 (t, C-3′); EIMS [*m*/*z*] (% rel int) 349 $[M]^+$ (14); HRFTMS [*m*/*z*] (% rel int) 350.1723 $[M + H]^+$ (37) (calcd for $C_{22}H_{24}O_3N$, 350.1756).

2-{6′-(2H-Benzo[*d*]1″,3″-dioxolen-5″-yl)hexyl}-4-methoxyquinoline (2): colorless oil; UV λ_{max} (log ϵ) (MeOH) 227 (3.49), 285 (3.43), 311 (2.64) nm; IR ν_{max} (KBr) 3000−2860, 1620 (C═C), 1580, 1470, 1260 (C−O), 1070 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.16 (1H, dd, J = 7.5, 0.8 Hz, H-5), 8.00 (1H, d, J = 7.4, H-8), 7.68 (1H, ddd, J = 8.3, 7.0, 1.4, H-7), 7.46 (1H, ddd, J = 7.9, 7.2, 0.8, H-6), 6.73 (1H, d, J = 7.9, H-7″), 6.68 (1H, d, J = 1.4, H-4″), 6.64 (1H, s, H-3), 6.62 (1H, dd, J = 8.0, 1.3, H-6″), 5.93 (2H, s, H_2-2″), 4.05 (3H, s, 4-OMe), 2.93 (2H, dd, J = 8.0, 7.9, H_2-1′), 2.54 (2H, dd, J = 7.7, 7.6, H_2-6′), 1.84 (2H, m, H_2-2′), 1.61 (2H, m, H_2-5′), 1.48 (2H, m, H_2-3′), 1.41 (2H, m, H_2-4′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.6 (s, C-2), 162.8 (s, C-4), 149.2 (s, C-10), 147.8 (s, C-9″), 145.8 (s, C-8″), 137.1 (s, C-5″), 130.1 (d, C-7), 128.6 (d, C-8), 125.2 (d, C-6), 122.0 (d, C-5), 121.4 (d, C-6″), 120.4 (s, C-9), 109.2 (d, C-4″), 108.4 (d, C-7″), 101.1 (t, C-2″), 100.2 (d, C-3), 55.9 (q, 4-OMe), 40.3 (t, C-1′), 36.0 (t, C-6′), 32.0 (t, C-5′), 30.4 (t, C-2′), 29.8 (t, C-3′), 29.4 (t, C-4′); EIMS [*m*/*z*] (% rel int) 363 $[M]^+$ (12); HRFTMS [*m*/*z*] (% rel int) 364.1632 $[M + H]^+$ (51) (calcd for $C_{23}H_{26}O_3N$, 364.1913).

Methylation of 1. To a solution of 2 mg of **1** in 5 mL of anhydrous acetone, 200 mg of anhydrous Na_2CO_3 and 5 mL of CH_3I were added. The reaction mixture was refluxed for 5 h, then filtered over a short bed of Si gel 60. The residue, after evaporating the filtrate under vacuum, was dissolved in 0.5 mL of $CHCl_3$ and chromatographed over 5 g of Si gel 60 using $CHCl_3$ as eluent. The fraction containing the product was further subjected to preparative TLC on Si gel G_{254}, using $(CH_3)_2CO-CCl_4$ (10:90), to afford a product (0.7 mg, R_f 0.44), which was identical to compound **2** as suggested by co-chromatography and EIMS analysis.

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