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Alkaloids and Coumarins from *Ruta graveolens*

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Summary. The aerial parts of *Ruta graveolens* cultivated in Bulgaria afforded four new 2-alkyl-4-(1*H*)-quinolone alkaloids together with thirteen known components. The new alkaloids were obtained as a mixture of homologues. The structures of all compounds were determined by spectroscopic methods.

Keywords. *Ruta graveolens*; Rutaceae; Coumarins; 4-Quinolone alkaloids; Quinoline alkaloids.

Alkaloide und Cumarine aus *Ruta graveolens*

Zusammenfassung. Aus den oberirdischen Teilen von in Bulgarien gezogener *Ruta graveolens* konnten neben dreizehn bereits bekannten Substanzen vier neue 2-Alkyl-4(1*H*)-chinolonalkaloide als Gemisch homologer Verbindungen isoliert werden. Die Strukturen der neuen Komponenten wurden mit spektroskopischen Methoden aufgeklärt.

Introduction

Ruta graveolens is a native of the Mediterranean region but cultivated throughout Europe and many Asian countries, including China, India, and Japan [1]. It is known as medicinal plant since ancient times and currently used for treatment of various diseases [2]. The presence of many coumarins, alkaloids, terpenes, and flavonoids has already been reported [3, 4]. The components of *Ruta* species are of great interest in medicinal chemistry, as these compounds show a broad range of biological activity and a number of them are used in medicine [5, 6]. However, there are limited data on the chemical composition of *R. graveolens* of Bulgarian origin [7]. This motivated the present investigation on the aerial parts of *R. graveolens* cultivated in Bulgaria and currently sold as *Herba Rutae* in urban pharmacies. In this paper we describe the isolation and structure determination of four new 2-alkyl-4(1*H*)-quinolone alkaloids together with 13 known components. The new compounds were isolated as a mixture of homologues.

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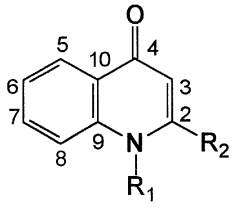
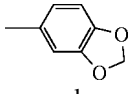
Results and Discussion

The crude ethanolic extract of the aerial parts of *R. graveolens* was worked up as described in the Experimental; 17 components were found. These included the coumarins rutamarin (**1**), bergapten (**2**), xanthotoxin (**3**), chalepin (**4**), and rutaretin (**5**), the furoquinoline alkaloids kokusagenin (**6**) and skimmianin (**7**), and the flavonoid glycoside rutin (**8**) [3, 4, 7]. The structures of the remaining compounds were established as the 2-aryl-4-(1*H*)-quinolone alkaloid graveoline (**9**) [4, 7] and its 2-*n*-alkyl analogues **10**–**17**. Compounds **1**–**10** and **17** have already been isolated from *R. graveolens* [3, 4, 8, 9]. Of the series of 4-quinolones **10**–**17** containing long alkyl chains in position 2, 2-heptyl-4(1*H*)-quinolone (**13**), 2-octyl-4(1*H*)-quinolone (**14**), and 2-decyl-4(1*H*)-quinolone (**16**) were isolated from plants for the first time, hitherto known only from synthesis [5, 10, 11]. The alkaloid 1-methyl-2-decyl-4(1*H*)-quinolone (**11**) proved to be a new compound. Compounds **12** and **15** have already been shown to be constituents of other rutaceous plants [11, 12], but their occurrence in *Ruta* species is now reported for the first time. The characterization of all compounds was accomplished by spectroscopic methods.

The 4-quinolone compounds **9**–**12** were isolated as a mixture. The ^{1}H NMR spectrum of the mixture revealed the presence of graveoline (**9**) and signals attributable to N-methylated pseudan derivatives with a saturated *n*-alkyl chain at C-2: $\delta = 8.48$ (1H, bd, $J = 9.0$ Hz, H-5), 6.42 (1H, s, H-3), 3.80 (3H, s, NMe), 2.76 (2H, t, $J = 8.0$ Hz, 2H-1′), and 0.87 (3H, t, $J = 8.0$ Hz, Me) ppm. The mass spectrum exhibited the characteristic fragmentation pattern of 1-methyl-2-alkyl-4(1*H*)-quinolone alkaloids ($m/z = 186$, 173 (base peak), and 144 [13]). The presence of **9** was supported by the peaks at $m/z = 279$ (M^+), 251, and 207 [8]. The peaks at $m/z = 285$, 299, and 313 corresponded to the molecular ions of 1-methyl-2-nonyl-4(1*H*)-quinolone (**10**), 1-methyl-2-decyl-4(1*H*)-quinolone (**11**), and 1-methyl-2-undecyl-4(1*H*)-quinolone (**12**). Reverse phase HPLC-MS (APCI) measurements



	R^1	R^2
9	Me	
10	Me	*n*-nonyl
11	Me	*n*-decyl
12	Me	*n*-undecyl
13	H	*n*-heptyl
14	H	*n*-octyl
15	H	*n*-nonyl
16	H	*n*-decyl
17	H	*n*-undecyl

showed that the fractions with retention times of 19.33, 20.36, and 21.35 min corresponded to compounds **10**, **11**, and **12** with molecular ions at $m/z = 286$, 300, and 314. This result strongly supported the presence of these N-methyl-2-alkyl-4(1*H*)-quinolones.

The N-demethylated quinolone **13** was isolated as a mixture with its homologue **15**. The amorphous powder showed characteristic UV maxima and IR bands. The ^{1}H NMR spectrum revealed signals assignable to NH ($\delta = 10.80$ ppm, bs), a conjugated olefinic proton at $\delta = 6.22$ ppm (s, H-3), four adjacent aromatic protons, and an *n*-alkyl group. The resonance at $\delta = 8.35$ ppm (bd, $J = 10.0$ Hz) is indicative for the aromatic proton at C-5 of 4-quinolone alkaloids. These data provided evidence for the existence of a 4-quinolone moiety substituted at C-2 with *n*-alkyl group. The presence of two methyl signals at $\delta = 0.84$ and 0.85 ppm as well as the integral of the methylene signal at $\delta = 1.30$–1.15 ppm suggested the existence of the two homologuous compounds 2-heptyl-4(1*H*)-quinolone (**13**) and 2-nonyl-4(1*H*)-quinolone (**15**). The mass spectrum showed the M^+ peaks of **13** and **15** at $m/z = 243$ and 271, the respective $[M\text{-}Me]^+$ ions at $m/z = 228$ and 256, and peaks at $m/z = 172$, 159 (base peak), and 130 which are diagnostic for the N-demethyl quinolone skeleton. The fragment ions at $m/z = 214$, 200, and 186 were related to the cleavage of the side chains [14]. RP-HPLC separation confirmed the presence of two compounds. The APCI mass spectra in further HPLC-MS experiments gave the expected molecular ions at $m/z = 244$ and 272 for compounds **13** and **15**; their daughter ions contained fragments characteristic for 2-alkyl-4(1*H*)-quinolones at $m/z = 130$, 159 (base peak), and 172, thus confirming the structures of **13** and **15**.

Compound **15** was also obtained as an amorphous powder. Its UV, IR, and NMR spectroscopic data were similar to those of **13**. Both the ^{1}H and ^{13}C NMR spectra exhibited signals due to the 4-quinolone nuclei with an aliphatic *n*-nonyl chain at position 2. The mass spectrum revealed the $[M]^+$ ion at $m/z = 271$ and the expected fragment ions at $m/z = 172$, 159 (base peak), and 130. The peak at $m/z = 256$ ($[M\text{-}Me]^+$) and fragment ions at $m/z = 242$, 228, 214, 200, and 186 with differences of 14 mass units confirmed the existence of 2-*n*-nonyl side chain. The mass spectrum of **15** also showed peaks of lower intensity at $m/z = 257$, 285, and 299 which were attributed to the molecular ions of the minor components 2-octyl-4(1*H*)-quinolone (**14**), 2-decyl-4(1*H*)-quinolone (**16**), and 2-undecyl-4(1*H*)-quinolone (**17**). The structures of **14**, **16**, and **17** were further supported by the presence of the $[M\text{-}Me]^+$ ions at $m/z = 242$, 270, and 284. Lack of material prevented detailed HPLC-MS measurements.

Experimental

General

^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX 250 Spectrometer using *TMS* as internal standard. Mass spectra: 70 eV: Varian MAT 311A, APCI: Finnigan TSQ 700; RP-HPLC: C_{18} column 125×4 mm, flow rate 0.8 cm^3/min, detection at 235 nm; TLC, prep. TLC: aluminium sheets, silica gel 60 F_{254} (Merck), bands detected under UV light, by exposure to I_2 vapour, or by spraying with H_2SO_4 and heating; liquid vacuum chromatography (LVC): silica gel 60 (Merck).

Plant material

A commercial sample of *R. graveolens* aerial parts cultivated in the region of Gorna Orjahovitza, collected in 1996 and purchased from *Bilkocoop Ltd*., Sofia, was investigated.

Extraction and isolation

The dried and powdered aerial parts (0.5 kg) of *R. graveolens* were extracted with 6 l 95% EtOH (3×24 h) at room temperature. Concentration of the combined EtOH solutions yielded 34.0 g crude extract to which 300 cm^3 of 95% of EtOH were added; the deposited rutin (**8**, 3.4 g) was removed by filtration. The mother liquor (after treatment with charcoal) was concentrated and subjected to solvent-solvent partition to give the petrol (1.3 g), EtOAc (2.5 g), and water-methanol (10.7 g) extracts. LVC of the EtOAc extract using $CHCl_3$ and $CHCl_3$-EtOH with increasing polarity afforded 9 fractions (F1–F9). F3 was further separated by CC over silica gel with hexane-EtOAc (increasing polarity) and preparative TLC ($CHCl_3$, multiple development) to give **1** (14.0 mg), **2** (3.0 mg), **3** (3.0 mg), **4** (3.7 mg), **6** (5.4 mg), and **7** (2.9 mg). Analogous treatment of F6 afforded S1 (1.8 mg, mixture of **9**–**12**), graveoline (**9**, 5.3 mg), S2 (5.2 mg, **15** and minor amounts of **14**, **16**, and **17**), S3 (2.3 mg, **13** and **15**), and **5** (5.0 mg).

1-Methyl-2-nonyl-4(1H)-quinolone (**10**) *as a mixture with graveoline* (**9**) *and 1-methyl-2-alkyl-4(1H)-quinolones* (**11**) *and* (**12**)

Amorphous powder; EIMS, 70 eV: *m/z* (rel. int.) = 313 (3.0), 299 (1.8), 298 (2.0), 285 (7.1), 284 (3.9), 279 (1.0), 270 (4.3), 256 (3.6), 251 (1.0), 242 (4.4), 228 (4.3), 214 (1.8), 207 (1.0), 200 (6.8), 186 (58.7), 173 (100.0), 144 (21.6); ^{1}H NMR (250 MHz, δ, $CDCl_3$): 8.48 (1H, bd, $J = 9.0$ Hz, H-5), 7.80–7.40 (3H, H-8, H-7, H-6 overlapping with the signals of graveoline), 6.42 (1H, s, H-3), 3.80 (3H, s, N-Me), 2.76 (2H, t, $J = 8.0$ Hz, 2H-1′), 1.50–2.00 (2H-2′ to 2H-8′, overlapping with the signal of H_2O in $CDCl_3$), 0.87 (3H, t, $J = 8.0$ Hz, Me) ppm; Analytical reverse phase HPLC separation (methanol-water gradient starting with 25% MeOH) and further RP-HPLC-MS (APCI) measurements revealed that the peaks with retention times 19.33, 20.00, and 21.15 min corresponded to compounds with molecular ions at *m/z* = 286, 300, and 314. On the basis of the HPLC-MS experiments, a ratio of approximately 46:47:4:3 was estimated for components **9**–**12**.

2-Heptyl-4(1H)-quinolone (**13**) *as a mixture with 2-nonyl-4(1H)-quinolone* (**15**)

Amorphous powder; UV: $\lambda_{max}^{MeOH} = 213$, 236, 317, 329 nm; IR: $\nu_{max}^{KBr} = 3060$, 2952, 2920, 2850, 1636, 1590, 1530, 1500, 1470, 1440, 770 cm^{-1}; EIMS, 70 eV: *m/z* (rel. int.) = 271 (5.7), 256 (1.5), 243 (7.2), 242 (3.0), 228 (3.0), 214 (3.8), 200 (3.0), 186 (7.5), 172 (48.3), 159 (100.0), 130 (15.0); ^{1}H NMR (250 MHz, δ, $CDCl_3$): 10.80 (bs, NH), 8.35 (bd, $J = 10.0$ Hz, H-5), 7.7–7.5 (m, H-8 and H-7), 7.32 (t, $J = 8.0$ Hz, H-6), 6.22 (s, H-3), 2.66 (t, $J = 8.0$ Hz, 2H-1′), 1.72 (qn, $J = 8.0$ Hz, 2H-2′), 1.30–1.15 (m, $(CH_2)_4$ (**13**) and $(CH_2)_6$ (**15**)), 0.85 (t, $J = 8.0$ Hz, Me(**13**)), 0.84 (t, $J = 8.0$ Hz, Me(**15**)) ppm. Analytical RP-HPLC separation (methanol-water gradient starting with 25% MeOH) showed two peaks with retention times 16.49 and 19.17 min. The APCI mass spectra of the corresponding fractions in further HPLC-MS measurements gave the expected peaks at *m/z* = 244 and 272, respectively. Daughter ion APCI spectrum of M^+ of **13** (relative intensities in parentheses): *m/z* = 244 (parent ion, 6.3%), 200 (0.1), 186 (1.0), 172 (24.0), 159 (100.0), 130 (8.0); daughter ion APCI spectrum of M^+ of **15** (relative intensities in parentheses): *m/z* = 272 (parent ion, 14.4%), 228 (0.1), 214 (1.0), 186 (4.0), 172 (100.0), 159 (100.0), 130 (3.0). The ^{1}H NMR and HPLC data suggested the presence of **13** and **15** in the mixture in a ratio of approximately 7:3.

2-Nonyl-4(1H)-quinolone (**15**)

Amorphous powder; UV: $\lambda_{max}^{MeOH} = 213, 236, 316, 330$ nm; IR: $\nu_{max}^{KBr} = 3060, 2952, 2925, 2850, 1634, 1580, 1530, 1505, 1440, 1350, 770\,cm^{-1}$; EIMS, 70 eV: *m/z* (rel. int.) = 299 (0.4), 285 (0.5), 284 (0.5), 271 (9.0), 270 (3.5), 257 (2.3), 256 (1.0), 242 (3.1), 228 (5.5), 214 (5.5), 200 (2.1), 186 (10.2), 172 (75.6), 159 (100.0), 130 (14.4); ^{1}H NMR (250 MHz, δ, $CDCl_3$): 11.70 (1H, bs, NH), 8.36 (1H, dd, $J = 8.0$ and 1.2 Hz, H-5), 7.71 (1H, bd, $J = 8.0$ Hz, H-8), 7.58 (1H, ddd, $J = 8.0$ and 1.5 Hz, H-7), 7.33 (1H, ddd, $J = 8.0$ and 1.0 Hz, H-6), 6.24 (1H, s, H-3), 2.68 (2H, t, $J = 8.0$ Hz, 2H-1′), 1.73 (2H, qn, $J = 8.0$ Hz, 2H-2′), 1.23 (12H, m, 2H-3′ to 2H-8′), 0.84 (3H, t, $J = 8.0$ Hz, Me) ppm; ^{13}C NMR (62.89 MHz, δ, $CDCl_3$): 178.9 (C-4), 154.9 (C-2), 140.5 (C-9), 131.8 (C-7), 125.4 (C-5), 125.0 (C-10), 123.6 (C-6), 118.3 (C-8), 108.3 (C-3), 34.4, 31.8, 29.5, 29.3, 29.3, 29.2, 29.0, 22.6 (C-1′ to C-8′), 14.1 (Me) ppm. The ^{1}H NMR spectrum suggested a total amount of the minor components of less than 5–6%.

Rutaretin (**5**)

^{1}H NMR (250 MHz, δ, $CDCl_3$): 7.52 (1H, d, $J = 9.5$ Hz, H-4), 6.74 (1H, s, H-5), 6.15 (1H, d, $J = 9.5$ Hz, H-3), 4.79 (1H, t, $J = 9.0$ Hz, H-2′), 3.25 (2H, m, ABX, 2H-3′), 1.25 (6H, s, 2Me) ppm; ^{13}C NMR (62.89 MHz, δ, $CDCl_3$): 160.5 (C-2), 149.9 (C-7), 144.2 (C-4), 142.8 (C-9), 127.9 (C-8), 125.5 (C-6), 113.9 (C-5), 112.8 (C-10), 111.5 (C-3), 91.3 (C-2′), 71.9 (C-4′), 30.1 (C-3′), 26.0 (Me), 24.3 (Me) ppm.

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