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2-Phenyl-4-quinolinone Alkaloids from *Casimiroa edulis* Llave et Lex (Rutaceae)

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Summary. Three new 4-quinolinone alkaloids (5,6-dimethoxy-2-(3-methoxyphenyl)-1*H*-quinolin-4-one, 5,6-dimethoxy-2-(3,4-dimethoxyphenyl)-1*H*-quinolin-4-one, 5,6-dimethoxy-2-(2,5,6-trimethoxyphenyl)-1*H*-quinolin-4-one) were isolated from the leaves of *Casimiroa edulis* Llave et Lex (Rutaceae) cultivated in Egypt. Their structures were determined by UV/Vis, IR, ^{1}H and ^{13}C NMR, and EI mass spectroscopy. The alkaloids were also detected in the kernels of the seeds.

Keywords. Quinolinone alkaloids; Natural products; Spectroscopy.

Introduction

Casimiroa edulis Llave et Lex (Rutaceae) is a tree distributed in the temperate zones of Mexico and central America (popularly called *Zapote blanco*, or white sapote). It is known for its interesting sedative-like effect and as sleep inducer [1–3]. The tree is cultivated in Egypt for its edible fruits. In folk medicine, a decoction of the leaves and, less frequently, of the seeds is administered [4]. The aqueous extract of the leaves has shown anticonvulsant and sedative activities [5]. Also, the leaf is most frequently used to treat ailments related to hypertension [4]. The seed, root, and bark of *C. edulis* have extensively been worked up to yield histamine derivatives, such as N^{α}, N^{α}-dimethylhistamine [6], casmidine, and casimiroedin [7–9], compounds of marked hypotensive activity. Zapotidine alkaloid [10], yet another hypotensive constituent, has been isolated from the seeds. Furoquinoline alkaloids together with 2-quinolones and 4-quinolones, such as edulein, edulitin, edulinine, casmiroin, and others constitute the major part of the total bases [11, 12]. Coumarins, flavonoids, and limonoids have been also reported as constituents [13]. Only two reports about the constituents of the leaves were found [4, 14]. Imidazole alkaloid derivatives have also been isolated from leaves of *C. edulis* [4]. In addition, isopimpinellin, casmiroin, skimmianine, 1-methyl-2-phenyl-4-quinolone, edulein, and scopoletin methyl ether have been isolated from the leaves [14]. Thus, the leaves were thought to be in need of further investigations, aiming to the isolation of new compounds from the plant.

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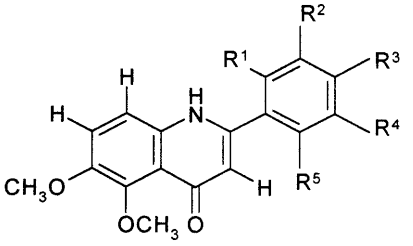
Results and Discussion

All isolated compounds **1**–**3** have a yellow fluorescence in UV light and give an orange colour on TLC plates when sprayed with *Dragendorff's* reagent. They give a positive test for the presence of nitrogen [15].



	R^1	R^2	R^3	R^4	R^5
1	H	OCH_3	H	H	H
2	H	OCH_3	OCH_3	H	H
3	OCH_3	H	H	OCH_3	OCH_3

The presence of the γ-pyridone moiety and, therefore, the 4-quinolinone structure of **1**–**3** was indicated by their IR (ν_{max} (KBr) = 3450–3500 (NH), 1650 (C=O), and 1590 (aryl NH) cm^{-1} [16, 17]) and UV/Vis (λ_{max} = 250–270 and 302–332 nm [18]) spectra as well as by the signal of the carbonyl group at 180–182 ppm in their ^{13}C NMR spectra.

The three compounds have related ^1H NMR spectra. The number of protons and the deshielded methoxy groups suggested that all compounds contain the phenyl quinolinone structure. Because of lack of literature on the suggested alkaloid structure, interpretation of the obtained data was carried out in the light of available data on this type of alkaloids (some of them isolated from the same plant) as well as of methoxyflavones (especially those isolated from the same plant). The ^1H NMR spectra of **1**–**3** indicated 5,6-dimethoxy substitution of ring A by the presence of two doublets with an *ortho*-coupling constant of 9.2 Hz at δ = 7.37–7.49 and 7.47–7.56 ppm (H-7 and H-8; cf. 5,6-dimethoxyflavones [19, 20]). Also, all show the presence of a sharp singlet in the range of 6.20–6.74 ppm, which could be assigned to a proton in position 3. This evidence was supported by the mass fragmentation pattern.

Compound **1**

The ^1H NMR spectrum of **1** indicated monosubstitution in ring B. This was postulated from a complex absorption pattern in the aromatic region, a multiplet at δ = 7.13 ppm, integrating for one proton assignable to H-4′ and a multiplet at δ = 7.44–7.57 ppm integrating for three protons at C-2′, C-5′, and C-6′. The presence of one methoxy group in ring B was confirmed from the mass spectrum of the compound which showed a fragment at m/z = 132. The protons of the B-ring moiety could be clearly assigned by comparison with those of

3′-methoxyflavones [12]. Compound **1** is thus 5,6-dimethoxy-2-(3-methoxyphenyl)-1*H*-quinolin-4-one.

Compound **2**

The ^{1}H NMR spectrum of **2** showed the characteristic pattern of ring A in addition to the presence of four deshielded methoxy groups and 30 mass units more in the mass spectrum than **1**. The fragment ion peak at $m/z = 162$ confirmed the presence of two methoxy groups in ring B. The 3′,4′-dimethoxy substitution in ring B was indicated by the presence of an ABX system in the ^{1}H NMR spectrum as a doublet at $\delta = 7.18$ ppm ($J_m = 1.8$ Hz) for H-2′, a doublet at $\delta = 7.20$ ppm ($J_o = 7.6$ Hz) for H-5′, and a doublet of doublets at $\delta = 7.33$ ppm ($J_m = 1.8$ Hz, $J_o = 7.6$ Hz) for H-6′. From these data, **2** could be assigned as 5,6-dimethoxy-2-(3,4-dimethoxyphenyl)-1*H*-quinolin-4-one.

Compound **3**

The ^{1}H NMR spectrum of **3** revealed the presence of five methoxy groups and two *ortho*-coupled doublets at $\delta = 6.81$ and 7.14 ppm belonging to ring B. The signal of H-3 integrated for one proton and appeared as a singlet at $\delta = 6.20$ ppm. The upfield shift of this singlet with respect to its position in **1** and **2** suggested a different substitution pattern in ring B. As the resonance of H-3 in the corresponding 2′-methoxyflavones is shifted downfield [12], the expected substitution pattern is that of a 2′,6′-dimethoxyquinolone (cf. shift of H-3 in the zapotin flavonoid isolated from the seed of the same plant [12]). From the mass spectrum it was concluded that there are 3 methoxy groups in ring B. Thus, **3** could be assigned as 5,6-dimethoxy-2-(2,5,6-trimethoxyphenyl)-1*H*-quinolin-4-one.

The ^{13}C NMR data of the isolated alkaloids were in accordance with the suggested structures and are given in the experimental part. The assignments of chemical shifts was substantiated using a HETCOR experiment for **3**.

Experimental

General

UV/Vis spectra were measured on a Shimadzu 265 spectrophotometer. IR spectra were run in KBr discs using a Shimadzu IR-435 spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured on a Varian instrument operating at 400 (^{1}H) and 100 (^{13}C) MHz. Compounds were analyzed in CD_3OD with *TMS* as internal standard. EI-MS were obtained using a Varian Mat CH5 instrument at 70 eV.

Thin-layer chromatography was performed on Kieselgel 60 GF_{254} precoated plates (Merck) and developed by hexane : ethyl acetate = 2 : 1.5 (system A) and $CHCl_3$: MeOH = 97 : 3 (system B); the compounds were visualized in by UV light and *Dragendorff's* reagent. Silica gel H type 60 for vacuum liquid chromatography (VLC), silica gel (Merck 60 A, 70–230 mesh ASTM), and sephadex LH-20 were used for column chromatography.

Plant material

The leaves as well as the seeds of *C. edulis* Llave et Lex were obtained from Dakahlia governorate, Nile delta, Egypt in the fruiting stage identified by the author and verified kindly by Dr. *N. El Hadidi*, Faculty of Science, Cairo University.

Extraction and isolation

One kg of powdered leaves of *C. edulis* was extracted with 10 dm^3 EtOH to give 50 g residue. The alcoholic extract was suspended in 100 cm^3 distilled H_2O and extracted successively with *n*-hexane, $CHCl_3$, and *n*-BuOH. The $CHCl_3$ extract (8 g) was fractionated on a VLC column (90 g, 30 × 5 cm, column 1). The column was eluted with *n*-hexane : ethyl acetate = 2 : 1.5. Fractions of 75 cm^3 were collected. Fractions 13–28 (4 spots, $R_f = 0.42$, 0.32, 0.26, 0.17 in system A, orange colour with *Dragendorff's* reagent) were pooled (1.5 g) and subjected to a silica gel G column chromatography (60 g, 25 × 3 cm, column 2). The column was eluted with *n*-hexane : ethyl acetate = 2 : 1.5, and fractions of 20 cm^3 were collected. Fractions 11–15 of column 2 (64 mg) were further purified by chromatography on silica gel G using the same solvent system, filtering through sephadex LH-20 using MeOH as the eluent, and washing the obtained residue with a low amount of cold ethyl acetate to give compound **1** (15 mg). Fractions 25–30 of column 2 (200 mg) were collected and purified by the same method as employed for **1** to give **2** (20 mg). Fractions 41–44 (80 mg) were treated similarly and further purified by crystallization from hexane : ethyl acetate = 2 : 1.5 and washing the crystals with a low amount of cold ethyl acetate to give 15 mg of pure **3**.

The isolated compounds were also detected in the kernels of the seeds using TLC, the solvent system A, and *Dragendorff's* reagent as the visualizing agent.

5,6-Dimethoxy-2-(3-methoxyphenyl)-1H-quinolin-4-one (**1**; $C_{18}H_{17}NO_4$)

White amorphous powder (15 mg); m.p.: 111–114°C; $R_f = 0.42$ (A), 0.72 (B); UV/Vis (MeOH): λ_{max} (ε) = 270 (14297), 302 (8136), 324 (6593) nm; IR (KBr): $\nu = 3450$, 2950, 1650, 1590 cm^{-1}; ^{1}H NMR (400 MHz, δ, CD_3OD): 7.56 (d, $J = 9.2$ Hz, H-8), 7.49 (d, $J = 9.2$ Hz, H-7), 7.57–7.44 (m, H-2′, 5′, 6′), 7.13 (m, H-4′), 6.74 (s, H-3), 3.93 (s, OCH_3), 3.88 (s, $2OCH_3$) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 180.1 (C-4), 164.0 (C-2), 161.8 (C-3′), 151.8 (C-5), 133.0 (C-1′), 131.2 (C-4′), 120.9 (C-7), 119.6 (C-6′), 118.4 (C-2′), 115.0 (C-8), 112.7 (C-3), 108.2 (C-5′), 62.0 (OCH_3), 57.3 (OCH_3), 55.9 (OCH_3) ppm; EI-MS (70 ev): *m/z* (%) = 313 (23, $M^+ + 2$), 312 (78, $M^+ + 1$), 298 (19), 297 (100, $M^+ + 1 - CH_3$), 296 (4, $M^+ - CH_3$), 283 (12, $M^+ - CO$), 281 (10, $M^+ - 2CH_3$), 266 (6, $M^+ - 3CH_3$), 165 (6), 149 (13), 137 (17), 132 (6).

5,6-Dimethoxy-2-(3,4-dimethoxyphenyl)-1H-quinolin-4-one (**2**; $C_{19}H_{19}NO_5$)

White amorphous powder (20 mg); decomposition above 100°C; $R_f = 0.32$ (A), 0.63 (B); UV/Vis (MeOH): λ_{max} (ε) = 266 (22960), 302 (12215), 332 (9582) nm; IR (KBr): $\nu = 3450$, 2950, 1650, 1590 cm^{-1}; ^{1}H NMR (400 MHz, δ, CD_3OD): 7.47 (d, $J = 9.2$ Hz, H-8), 7.37 (d, $J = 9.2$ Hz, H-7), 7.33 (dd, $J = 7.6$, 1.8 Hz, H-6′), 7.20 (d, $J = 7.6$ Hz, H-5′), 7.18 (d, $J = 1.8$ Hz, H-2′), 6.65 (s, H-3), 3.93 (s, OCH_3), 3.91 (s, OCH_3), 3.85 (s, $2OCH_3$) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 182.0 (C-4), 163.0 (C-2), 155.0 (C-4′), 153 (C-3′), 151.8 (C-5), 129.9 (C-1′), 125.6 (C-6′), 121.6, (C-5′), 121.0 (C-7), 119.0 (C-10), 116.9 (C-2′), 115.0 (C-8), 112.6 (C-3), 62.0 (OCH_3), 61.4 (OCH_3), 57.4 (OCH_3), 56.6 (OCH_3) ppm; EI-MS (70 ev): *m/z* (%) = 343 (100, $M^+ + 2$), 342 (62, $M^+ + 1$), 327 (64, $M^+ + 1 - CH_3$), 313 (28, $M^+ - CO$), 311 (10, $M^+ - 2CH_3$), 297 (13), 296 (2, $M^+ - 3CH_3$), 281 (2, $M^+ - 4CH_3$), 165 (10), 162 (2), 149 (3), 137 (23).

5,6-Dimethoxy-2-(2,5,6-trimethoxyphenyl)-1H-quinolin-4-one (**3**; $C_{20}H_{21}NO_6$)

White needle crystals (15 mg); m.p.: 155°C; $R_f = 0.26$ (A), 0.57 (B); UV/Vis (MeOH): $\lambda_{max}(\varepsilon) = 250$ (11163), 256 (11145), 328 (5472) nm; IR (KBr): $\nu = 3450$, 3000, 1650, 1590 cm^{-1}; ^{1}H NMR (400 MHz, δ, CD_3OD): 7.54 (d, $J = 9.2$ Hz, H-8), 7.39 (d, $J = 9.2$ Hz, H-7), 7.14 (d, $J = 9.2$ Hz, H-4′), 6.81 (d, $J = 9.2$ Hz, H-3′), 6.20 (s, H-3), 3.92 (s, OCH_3), 3.88 (s, OCH_3), 3.85 (s, OCH_3), 3.81

(s, OCH_3), 3.75 (s, OCH_3) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 180.2 (C-4), 161.6 (C-2), 153.4 (C-6′), 153.0 (C-2′), 151.4 (C-5), 149 (C-6), 148.3 (C-5′), 120.9 (C-7), 119.0 (C-1′), 118.0 (C-10), 117 (C-4′), 115.1 (C-3′), 115.0 (C-8), 107.7 (C-3), 62.0 (OCH_3), 61.8 (OCH_3), 57.4 (OCH_3), 57.0 (OCH_3), 56.7 (OCH_3) ppm; EI-MS (70 ev): *m/z* (%) = 373 (89, $M^+ + 2$), 372 (100, $M^+ + 1$), 358 (24), 357 (86, $M^+ + 1–CH_3$), 343 (7, $M^+ –CO$), 341 (9, $M^+ –2CH_3$), 192 (6), 179 (2), 165 (12), 149 (9), 137 (19).

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