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QUINOLINE ALKALOIDS AND CYTOTOXIC LIGNANS FROM *HAPLOPHYLLUM TUBERCULATUM*

GIUMA M. SHERIHA, KARIMA ABOUAMER, BAHLUL Z. ELSHTAIWI, AZIZA S. ASHOUR, FATMA A. ABED and HALA H. ALHALLAQ

Department of Chemistry, Al-Fateh University and Medicinal Plant Research Program, NASR, P. O. Box 13203, Tripoli, Libya

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Key Word Index—*Haplophyllum tuberculatum*; Rutaceae; alkaloids; lignans; quinoldione; quinolones; Polygamain; kusunokinin.

Abstract—Three quinoline alkaloids and two lignan lactones were isolated from *Haplophyllum tuberculatum*. Physicochemical and spectral evidence established the structures of two of the alkaloids as a new quinoldione, 3-(1′,1′-dimethylallyl)-3-(3″,3″-dimethylallyl)-1,2,3,4-tetrahydro-2,4-quinoldione and the known 4-(3′,3′-dimethylallyloxy)-3-(3″,3″-dimethylallyl)-2(1H)-quinolone. The former was shown to undergo facile [3,3]-sigmatotropic transformation into the latter. The remaining compounds were identified as the known Polygamain, kusunokinin and 1-methyl-2-n-nonyl-4(1H)-quinolone.

INTRODUCTION

In our earlier work [1, 2] when we isolated and characterized several 1-aryl-2,3-naphthalide lignans and the alkaloid (+)-tuberine, silica gel chromatography of the chloroform extract from the aerial parts of *Haplophyllum tuberculatum* plants yielded 164 fractions. Careful examination by analytical TLC and separation by preparative TLC of the residues from separately combined groups of these fractions resulted in the isolation of the lignan lactones **3** and **4** and the 4-quinolone alkaloid **5**.

Separation by preparative TLC of alkaloids of the residue from the petrol extract of the plant led to the isolation of the 2,4-quinoldione alkaloid **1** and the 2-quinolone alkaloid **2**. At the concentrations of 0.1–1.0 μg/ml the lignan lactone **3** showed strong antibacterial effects on *Staphylococcus aureus and Escherichia coli* when examined by the disk inhibition zone technique. This effect is presumably due to the well established antimitotic activity of podophyllotoxin lignan series [3, 4].

RESULTS AND DISCUSSION

The structure of compound **1** was assigned on the basis of the spectral and physicochemical data set out in the experimental section. These data were in close accord with those reported for similar 3,3-disubstituted 2,4-quinoldione type alkaloids [5–9]. The UV spectrum indicated the presence of a 2,4-quinoldione chromophore. The ^{13}C and ^{1}H NMR spectra contained nine carbon signals and five (1H, amide 4H, benzoid) one-proton resonances attributable to this moiety respectively. The benzoid region in the 400 MHz ^{1}H NMR spectrum of **1** revealed the C-5 proton doublet of doublets (δ7.87) and C-7 proton triplet of doublets (δ7.49) at lowerfield to the C-6 proton triplet of doublets (δ7.09) and C-8 proton doublet of doublets (δ6.92). This was attributed to H-5 and H-7 being subjected to proximate and/or conjugate deshielding effects (mesomeric effects) of the 4-carbonyl function. The IR spectrum exhibited absorption bands at 3200, 1655 (–HNCO–) and 1695 cm^{-1} (ArCO–) whereas the ^{13}C NMR spectrum displayed the 2- and 4-carbonyl signals at δ173.4 and 196.1 respectively. The ^{13}C and ^{1}H NMR spectra disclosed also the characteristic chemical-shift positions of carbon and proton absorptions ascribable to C-3 attached 1′,1′-dimethylallyl and 3″,3″-dimethylallyl chains. The former C-1″ signal appeared at δ43.9 and the latter C-3″ resonance occurred at δ135.0. The structure of the 1′,1′-dimethylallyl unit was defined by the up-field nonequivalent geminal dimethyl singlets (δ1.13 and 1.15) and the down-field three vinylic proton ABX system (δ_{AB} 4.91 and δ_X 5.90). While that of the 3″,3″-dimethylallyl unit was verified by the nonequivalent vinylic geminal dimethyl singlets (δ1.50 and 1.68) and the three proton ABX system comprising the nonequivalent AB methylene protons (δ_A 2.79 and δ_B 2.88) and X vinylic proton (δ_X 4.76) which was further coupled to the allylic geminal dimethyl protons. The geminal anisochronies of the C-1′ attached dimethyls and C-1′ AB protons were attributed to the chiral perturber centre at C-3 which is three and two bonds separated from the aforementioned sensors respectively [10]. The APT ^{13}C NMR spectra [11] differentiated the various carbon atom types to which the assignment of the respective chemical-shift positions were made by reference to the literature [5–7]. The high resolution mass spectral data corroborated the IR, UV, ^{1}H NMR and ^{13}C NMR findings and showed besides $[M]^+$ at m/z 297, the other diagnostic fragment ions which were consonant with structure **1**.

To substantiate that alkaloid **1** is a natural constituent of *H. tuberculatum* perennial herbs, carefully dried and powdered aerial parts of flowering plants were stirred for an hour with ether and the amount of **1** and **2** in the ether

extract determined by a combination of preparative TLC and UV spectroscopy. The results, which were repeatable, showed the occurrence of **1** and **2** in an almost 1:1 ratio in the fresh powdered plants. On storage, however, there was a gradual decrease in the amount of **1** with a concomitant increase in that of **2** until an approximately 1:4 equilibrium mixture had been established. Further confirmatory evidence for the aforementioned interconversion was provided by the finding that on application of **1** and **2** as pure compounds to separate preparative silica gel plates 24 hr before development resulted in the conversion of each alkaloid to an 1:4 equilibrium mixture of **1** and **2** respectively; indicative of the four fold faster rate of formation of **2** from **1** via the facile [3,3]-sigmatotropic transformation of the chiral centre of the 1',1'-dimethylallyl substituent to the C-4 oxygen, than that of **1** from **2** by the sluggish Claisen rearrangement of the ethereal 3',3'-dimethylallyl moiety to C-3. The observation that **1** shows no optical activity can readily be explained by examining its Drieding model which shows that the fused tetrahydropyridione ring of this alkaloid's presumably biosynthetic enantiomer is conformationally mobile and interconvertible between two half-chair pseudo-antipodal conformers which are most likely of equal and opposite molecular rotations. The finding thus are best reconciled in terms of structure **1** which has been encountered in nature for the first time. The individual spectral and physicochemical properties of the remaining four compounds isolated (2–5) were as described in the experimental section and correspond closely to those reported for 4-(3'.3'-dimethylallyloxy)-3-(3",3"-dimethylallyl)-2 (1H)-quinolone (**2**) [12], 1-methyl-2-*n*-nonyl-4 (1H)-quinolone (**5**) [13], (1*R*, 2*R*, 3*R*)-2,3-*trans*-6,7-methylenedioxy-1-(3',4'-methylenedioxyphenyl)-1,2,3,4-tetrahydro-3-hydroxymethylanaphthalene-2-carboxylic acid lactone (**3**) [14, 15] and (2*R*,3*R*)-(−)-2,3-*trans*-2-(3',4'-methylenedioxybenzyl)-3-(3",4"-dimethoxybenzyl) butyrolactone (**4**) [16].

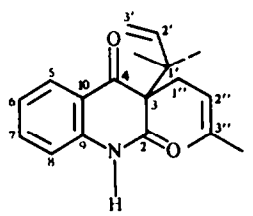
EXPERIMENTAL

Spectral measurements, microanalyses, optical rotations and Mps were carried out as described in a previous paper [1].

Isolation of quinoline alkaloids and lignan lactones. Repeated prep. TLC (silica gel) of the crude alkaloidal constituents of the residue of the petrol extract [1] gave a blue fluorescing alkaloid (**1**, R_f 0.83 on florisil developed with Et_2O–hexanel, 1:1; detection by UV fluorescence) and a violet fluorescing alkaloid (**2**, (R_f 0.49). Recrystallization of the former from EtOAc–petrol and the latter from Me_2CO yielded crystalline **1** (150 mg) and pure **2** (155 mg) respectively.

Because they gave similar patterns on TLC (silica gel 60) fractions 35F–80F and 85F–135F [1, 2] were combined separately and evapd. From the residue of the former, the lignan lactones **3** and **4** and from that of the latter the 4-quinolone alkaloid **5** were separated by prep. TLC (0.5 mm silica gel 60 GF_{254}, EtOAc–hexane, 3:1, visualized with Dragendorff's reagent). Recrystallization of **3** (R_f 0.64) from MeOH and of **5** (R_f 0.36) from EtOAc–petrol afforded polygamain (20 mg) and 1-methyl-2-*n*-nonyl-4-(1H)quinolone (35 mg) respectively. Whereas **4** (R_f 0.54), after removal of the solvent furnished kusunokinin (110 mg).

3-(1',1'-*Dimethylallyl*)-3-(3",3"-*dimethylallyl*)-1,2,3,4-*tetrahydro*-2,4-*quinoldione* (**1**). Colourless needles (150 mg) from EtOAc–petrol; mp 121–123°; MS *m/z* (rel. int.): 297.1728 (11) $[M]^+$ (Found: C, 76.67, H, 7.89, N, 4.71. Calc. for $C_{19}H_{23}NO_2$:C, 76.77, H, 7.74, N, 4.71%, *M*, 297.1729), 282 (3) $C_{18}H_{20}NO_2$, 254 (2) $C_{16}H_{16}NO_2$, 229 (32) $C_{14}H_{15}NO_2$, 228 (100) $C_{14}H_{14}NO_2$, 227 (3) $C_{14}H_{13}NO_2$, 214 (9) $C_{13}H_{12}NO_2$, 212 (11) $C_{13}H_{10}NO_2$, 200 (9) $C_{12}H_{10}NO_2$, 186 (52) $C_{11}H_8NO_2$, 174 (29) $C_{10}H_8NO_2$, 161 (1) $C_9H_7NO_2$, 69 (28) C_5H_9; ^{1}H NMR($CDCl_3$)2,4-quinoldione nucleus: δ9.56 (1H, *br*, *s*, N-H), 7.87 (1H, *dd*, *J* = 7.8, 1.3Hz, H-5), 7.09(1H, *dt*, *J* = 7.8, 1.3 Hz, H-6), 7.49(1H, *dt*, *J* = 7.8, 1.3 Hz, H-7), 6.92 (1H, *dd*, *J* = 7.8, 1.3 Hz, H-8); 1',1'-dimethylallyl unit: δ1.13 (3H, *s*, Me-1'), 1.15 (3H, *s*, Me-1'), 5.90 (1H, *dd*, *J* = 17.4, 10.6 Hz, H-2'), 4.91 (1H, *dd*, *J* = 10.6, 1.0 Hz, H_{cis}-3'), 4.91 (1H, *dd*, *J* = 17.4, 1.0 Hz, H_{trans}-3');



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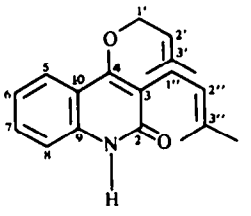

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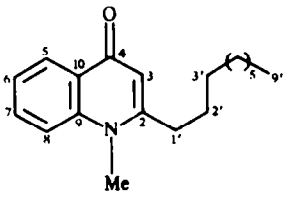

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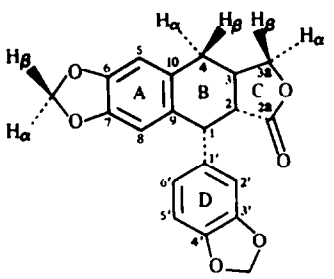

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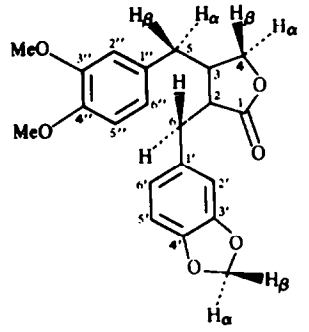

4

3″,3″-dimethylallyl unit: δ2.79 (1H, *dd*, *J* = 13.4, 6.9 Hz, H_A-1″), 2.88 (1H, *dd*, *J* = 13.4, 6.9 Hz, H_B-1″), 4.76 (1H, *t. sept.*, *J* = 6.9, 1.4 Hz, H_X-2″), 1.50 (3H, *s*, 3″-Me), 1.68 (3H, *s*, 3″-Me); ^{13}C NMR ($CDCl_3$)2,4-quinoldione nucleus: δ173.4(C-2), 67.6 (C-3), 196.1 (C-4), 126.9 (C-5), 123.2 (C-6), 135.5 (C-7), 115.7 (C-8), 140.8 (C-9), 121.7 (C-10); 1′,1′-dimethylallyl unit: δ43.9 (C-1′), 23.2 (1′-Me), 23.4 (1′-Me), 142.7 (C-2′), 113.1 (C-3′); 3″,3″-dimethylallyl unit: δ29.6 (C-1″), 119.4 (C-2″), 135.0 (C-3″), 18.2 (3″-Me), 25.9 (3″-Me); UV λ_{max}^{EtOH} nm: (log ε): 232 (4.57), 236 (4.56), 257 (3.85), 263 (3.82), 346 (3.53); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200, 1695, 1655.

4-(3′,3′-*Dimethylallyloxy*)-3-(3″,3″-*dimethylallyl*)-2(1H)-*quinolone* (**2**). Colourless needles (155 mg) from Me_2CO, mp 112–114°; MS *m/z* (rel. int.): 297.1728 (6) $[M]^+$ (Found: C, 76.65, H, 7.88, N, 4.77. Calc. for $C_{19}H_{23}NO_2$: C, 76.77, H, 7.74, N, 4.71%, M_r 297.1729), 229 (33), 228 (100), 227 (6), 70 (48), 69 (8), 68 (13); ^{1}H NMR ($CDCl_3$)2-quinolone nucleus: δ12.26 (1H, *br*, *s*, N-H), 7.78 (1H, *dd*, *J* = 7.5, 1.1 Hz, H-5), 7.20 (1H, *td*, *J* = 7.5, 1.1 Hz, H-6), 7.46 (1H, *td*, *J* = 7.5, 1.1 Hz, H-7), 7.42 (1H, *dd*, *J* = 7.5, 1.1 Hz, H-8); 3′,3′-dimethylallyloxy unit: δ4.56 (2H, *d*, 7.2 Hz, H-1′), 5.64 (1H, *t. sept.*, *J* = 7.2, 1.3 Hz, H-2′), 1.82 (3H, *s*, Me-3′), 1.85 (3H, *s*, Me-3′); 3″,3″-dimethylallyl unit: δ3.47 (2H, *d*, *J* = 6.8 Hz, H-1″), 5.32 (1H, *t. sept.*, *J* = 6.8, 1.3 Hz, H-2″), 1.71 (3H, *s*, Me-3″), 1.72 (3H, *s*, Me-3″); APT [11] ^{13}C NMR ($CDCl_3$) [6] 2-quinolone nucleus: δ161.2 (C-2), 117.7 (C-3), 165.8 (C-4), 123.1 (C-5), 122.1 (C-6),129.8 (C-7), 115.9 (C-8), 139.0 (C-9), 122.8 (C-10); 3′,3′-dimethylallyloxy unit: δ71.2 (C-1′), 119.7 (C-2′), 137.4 (C-3′), 18.1 (3′-Me), 25.9 (3′-Me); 3″,3″-dimethylallyl unit: δ23.8 (C-1″), 121.7 (C-2″), 132.5 (C-3″), 18.0 (3″-Me); 25.8 (3″-Me); UV λ_{max}^{EtOH} nm (log ε) [8]: 226 (4.61), 263 (3.89), 272 (3.95), 279 (3.87), 313 (3.83), 324 (3.93), 337 (3.80); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 1640, 1600.

1-*Methyl*-2-n-*nonyl*-4(1H)-*quinolone* (**5**). Colourless plates (35 mg) from EtOAc–petrol; mp 73–75°; MS *m/z* (rel. int.): 285.2094 (14) $[M]^+$ (Found: C, 79.80, H, 9.57, N, 4.82. Calc. for $C_{19}H_{27}NO$: C, 80.00, H, 9.47, N, 4.91%, M_r 285.2093), 270 (2) $C_{18}H_{24}NO$, 256 (3) $C_{17}H_{22}NO$, 242 (3) $C_{16}H_{20}NO$, 228 (3) $C_{15}H_{18}NO$, 214 (1) $C_{14}H_{16}NO$, 200 (5) $C_{13}H_{14}NO$, 186 (50) $C_{12}H_{12}NO$, 173 (100) $C_{11}H_{11}NO$; ^{1}H NMR ($CDCl_3$) 4-quinolone nucleus: δ3.73 (3H, *s*, N-Me), 6.23 (1H, *s*, H-3), 8.45 (1H, *dd*, *J* = 7.8, 1.4 Hz, H-5), 7.36 (1H, *td*, *J* = 7.8, 1.4 Hz, H-6), 7.65 (1H, *td*, *J* = 7.8, 1.4 Hz, H-7), 7.50 (1H, *dd*, *J* = 7.8, 1.4 Hz, H-8); *n*-nonyl chain: δ 2.70 (2H, *t*, *J* = 7.7 Hz, H-1′), 1.68 (2H, *pent.*, *J* = 7.7 Hz, H-2′), 1.46 (2H, *pent.*, *J* = 7.7 Hz, H-3′) 1.31 (10H, *br s*, H-4′- to 8′), 0.91 (3H, *t*, *J* = 7.7 Hz, H-9); UV λ_{max}^{MeOH} nm (log ε): 217 (4.32), 239 (4.47), 321 (4.18), 333 (4.19); $\lambda_{max}^{1N\,HCl}$ in MeOH 235 (4.52), 302 (4.16); IR $\nu_{max}^{CHCl_3}$ cm^{-1}; 1635, 1610.

Polygamain (**3**). Colourless needles (250 mg) from MeOH; mp 187–188°; $[\alpha]_D^{22}$–140.0° (*c* 0.178; $CHCl_3$; MS *m/z* (rel. int): 352.095 (100) $[M]^+$, (Found: C, 68.16, H, 4.58, Calc. for $C_{20}H_{16}O_6$: C, 68.12, H, 4.56%, M_r 352.095), 307 (14) $C_{19}H_{15}O_4$, 267 (12) $C_{16}H_{11}O_4$, 185 (17) $C_{12}H_9O_2$, 135 (17) $C_8H_7O_2$; ^{1}H NMR ($CDCl_3$): δ2.70 (1H, *dd*, *J* = 14.5, 4.6 Hz, H-2), 2.74 (1H, *m*, H-3), 2.76 (1H, *dd*, J = 10.6, 3.0 Hz, Hα-4), 3.05 (1H, *t*, *J* = 10.6 Hz, Hβ-4), 3.91 (1H, *t*, *J* = 8.6 Hz, Hβ-3a), 4.45 (1H, *dd*, *J* = 8.6, 5.9 Hz, Hα-3a), 4.57 (1H, *d*, *J* = 4.6 Hz, H-1), 5.89, 5.90 (1Hα, 1Hβ, 2 × *d*, *J* = 1. 5 Hz, 6,7–OCH_2O–), 5.92 (2H, *s*, 3′,4′–OCH_2O–), 6.47 (1H, *s*, H-8), 6.60 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.61 (1H, *dd*, *J* = 7.2, 1.8 Hz, H-6′)), 6.65 (1H, *s*, H-5), 6.68 (1H, *d*, *J* = 7.2 Hz, H-5′); APT [11] ^{13}C NMR ($CDCl_3$) [15]: δ43.2 (C-1), 47.2 (C-2), 174.7 (C-2a), 32.5 (C-3), 72.0 (C-3a), 33.0 (C-4), 108.4 (C-5), 147.0 (C-6), 146.9 (C-7), 110.3 (C-8), 131.0 (C-9), 128.1 (C-10), 134.4 (C-1′), 111.1 (C-2′), 147.2 (C-3′), 146.7 (C-4′), 107.6 (C-5′), 124.1 (C-6′), 101.0; (6,7–OCH_2O–), 101.1 (3′,4′–OCH_2O–); UV λ_{max}^{EtOH} nm (log ε): 237 (3.95), 287 (3.98); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 243 (3.91), 288 (3.97); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1790, 1485, 936.

Kusunokinin (**4**). A viscous liquid (110 mg): $[\alpha]_D^{22}$–31.1° ($CHCl_3$; *c* 0.679); MS [17,18] *m/z* (rel. int.): 370.141 (71) $[M]^+$, (Found: C, 68.08, H, 5.96, Calc. for $C_{21}H_{22}O_6$: C, 68.11, H, 5.95%, M_r 370.142), 235 (4) $C_{13}H_{15}O_4$, 219 (3) $C_{12}H_{11}O_4$, 192 (3) $C_{10}H_8O_4$, 178 (12) $C_{11}H_{14}O_2$, 151 (84) $C_9H_{11}O_2$, 135 (100) $C_8H_7O_2$; ^{1}H NMR ($CDCl_3$) [19]: δ2.49 (1H, *dd*, *J* = 11.7, 8.2 Hz, Hβ-5), 2.50 (1H, *m*, H-2), 2.55 (1H, *m*, H-3), 2.62 (1H, *dd*, *J* = 11.7, 4.6 Hz, Hα-5), 2.85 (1H, *dd*, *J* = 14.1, 7.0 Hz, Hα-6), 2.97 (1H, *dd*, *J* = 14.1, 5.1 Hz, Hβ-6), 3.83 (3H, *s*, 3″-Me), 3.86 (3H, *s*, 4″-OMe), 3.88 (1H, *dd*, *J* = 9.3, 7.4 Hz, Hβ-4), 4.15 (1H, *dd*, *J* = 9.3, 6.9 Hz, Hα-4), 5.93, 4.94 (1Hα, Hβ, 2 × *d*, *J* = 1.4 Hz, 3′,4′-OCH_2O), 6.48 (1H, *d*, *J* = 2.0 Hz, H-2″), 6.57 (1H, *dd*, *J* = 8.1, 2.0 Hz, H-6″), 6.58 (1H, *dd*, *J* = 7.7, 1.9 Hz, H-6′), 6.60 (1H, *d*, *J* = 1.9 Hz, H-2′) 6.71 (1H, *d*, *J* = 7.7 Hz, H-5′), 6.77 (1H, *d*, *J* = 8.1 Hz, H-5″); UV λ_{max}^{EtOH} nm (log ε): 230 (3.94), 281 (3.69), 285 (3.68); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 242 (3.75), 282 (3.71), 286 (3.71): IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1510, 930.

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