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HIV Inhibitory Natural Products. 26.[1] Quinoline Alkaloids from *Euodia roxburghiana*

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Bioassay-directed fractionation of the CH_2Cl_2−MeOH extract of *Euodia roxburghiana* resulted in the isolation of two known quinoline alkaloids, buchapine (**1**) and **2**, and three new furoquinoline alkaloids, roxiamines A, B, and C (**3**−**5**). Compounds **1** and **2** protected CEM-SS cells from the cytopathic effects of HIV-1 *in vitro* (EC_{50} 0.94 and 1.64 μM, respectively), but **3**−**5** were inactive against HIV-1.

Previous chemical studies of the genus *Euodia* were prompted by its use in folk medicines by indigenous peoples from Australia and Asia. A tree resin from *E. vitiflora* has been used by Queensland aborigines as an adhesive and for filling cavities in teeth.[2] A decoction of the leaves of *E. latifolia* has been used to treat fever and cramps.[3] Antifungal[4] and antibacterial[5] activities have been reported for *E. luna-ankenda* extracts. Compounds previously found in *Euodia* include terpenes,[6] coumarins,[7] and alkaloids.[4]

Observation of anti-HIV activity of an extract of *Euodia roxburghiana* Benth. (Rutaceae) in the NCI's anti-HIV *in vitro* primary screen[8] led to the present study. Two known quinolines and three new furoquinolines have been identified; in an XTT-tetrazolium assay,[9] the quinolines exhibited modest anti-HIV activity against HIV-1 in cultured human lymphoblastoid CEM-SS cells.

Results and Discussion

The CH_2Cl_2−MeOH (1:1) extract of *E. roxburghiana* was subjected to a solvent−solvent partition protocol, which concentrated anti-HIV activity in the hexane and CCl_4-soluble fractions. Gel permeation of the CCl_4-soluble fraction through Sephadex LH-20, followed by vacuum−liquid chromatography and, finally, HPLC purification on silica, afforded known quinolines **1** and **2** and three new compounds, designated roxiamines A (**3**), B (**4**), and C (**5**). Compounds **1** and **2** were also isolated from the hexane fraction in the same fashion. EIMS established that **1** and **2** had the same molecular formula, $C_{19}H_{23}NO_2$. Their ^{1}H-NMR, ^{13}C-NMR, IR, UV, and MS spectral data corresponded closely with those reported for the known compounds buchapine (**1**)[10] and 3-(3-methyl-2-butenyl)-4-[(3-methyl-2-butenyl)oxy]-2(1*H*)-quinolinone (**2**),[11] both originally isolated from *Haplophylum tuberculatum*. Buchapine (**1**) did not exhibit optical activity, consistent with the literature.[10]

The similar UV absorption maxima of **3**−**5** (332, 320, 309, and 244 nm) suggested that they shared a common chromophore. Their ^{1}H-NMR spectra revealed striking similarities in the aromatic region, also indicating that they were related structures.

High-resolution EIMS of roxiamine A (**3**) established a molecular formula of $C_{18}H_{19}NO_5$, with 10 sites of unsaturation. Its ^{13}C-NMR spectrum revealed only six carbons in the sp^3 region above 80 ppm, while the remaining 12 carbons resided in the downfield sp^2 area (below 100 ppm). The ^{1}H-NMR spectrum (see Table 1) was straightforward, with five aromatic protons comprising two spin systems. The resonance at δ 7.06 (dd, J = 9.5, 2.8 Hz) was coupled to the doublets at δ 8.13 (J = 9.5 Hz) and δ 7.35 (J = 2.8 Hz), typical of an 1,2,4-trisubstituted phenyl ring. The remaining two doublets at δ 7.05 (J = 3.0 Hz) and δ 7.56 (J = 3.0 Hz) were coupled to each other. These substitution patterns, combined with the fact that furoquinolines have been found in other Rutaceae,[12] strongly suggested the presence of a furoquinoline skeleton. Indeed, these aromatic proton resonances matched very well those of dictamnine.[13] Of the remaining protons, there were two methoxyl groups (δ 3.67, 4.43), one methyl doublet (δ 1.24), one methylene group (δ 4.14), and three other protons (all multiplets). Through application of ^{1}H−^{1}H COSY and 1D proton-decoupling techniques, the structure of the side chain was established. Finally,

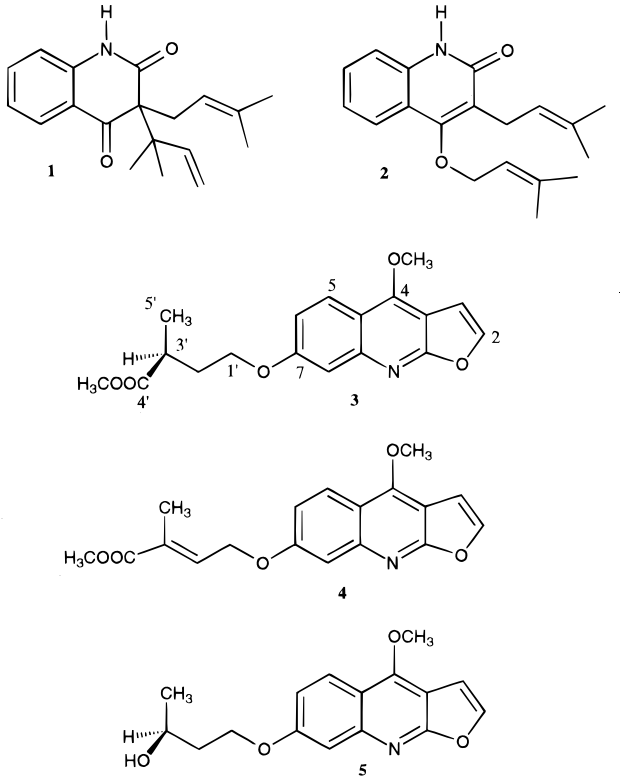

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Table 1. NMR assignments of Roxiamine A (**3**) in $CDCl_3$

C no.	^{13}C NMR (ppm)	^{1}H NMR [ppm (mult, *J* (Hz))]	HMBC corr to C no.
2	142.3	7.56 (d, 3.0)	3,4,9a
3	104.8	7.05 (d, 3.0)	2,4,9a
3a	101.8		
4	156.9		
4a	113.3		
5	123.5	8.13 (d, 9.5)	4,7,8a
6	116.7	7.06 (dd, 9.5, 2.8)	4a,8
7	160		
8	106.5	7.35 (d, 2.8)	4a,6,7,8a
8a	147.5		
9a	164.3		
1′	65.6	4.14 (br t, 6.3)	7,2′,3′
2′	32.8	2.25 (ddt, 14.1, 7.8, 6.3) 1.95 (ddt, 14.1, 6.4, 6.3)	1′,3′,4′,3′-CH_3
3′	36.3	2.76 (ddq, 7.8, 6.4, 7.3)	1′,2′,4′,3′-CH_3
4′	176.6		
5′	17.4	1.24 (d, 7.3)	2′,3′
4-OCH_3	58.8	4.43 (s)	4
4′-OCH_3	51.6	3.67 (s)	4′

HMBC correlations (Table 1) between the methoxyl protons (δ 4.43) and C-4 (δ 156.9), and between H-1′ (δ 4.14) and C-7 (δ 160.0) clarified the attachment sites of OMe and the side-chain at C-4 and C-7, respectively.

Roxiamine B (**4**) had a molecular formula of $C_{18}H_{17}NO_5$, determined by HREIMS. Its ^{1}H- and ^{13}C-NMR spectra revealed that it also contained the 4-methoxyfuro[2,3-*b*]quinoline skeleton. Compared to the NMR spectra of **3**, roxiamine B had two fewer protons in the aliphatic region and two new sp^2 carbons (δ 136.4, 129.9), indicating the presence of an additional olefinic bond. In the ^{1}H-NMR spectra, the downfield shift of H-1′ and H-2′ from δ 4.14 and δ 2.25/1.95 in **3** to δ 4.86 and δ 6.99 in **4**, respectively, confirmed the side-chain assignment. As was the case for **3**, attachment positions were established by HMQC and HMBC experiments. The (*E*)-configuration of the double bond was derived from NOE experiments; when H-5′ (CH_3) was irradiated, the only enhancement was observed at δ 4.86 (H-1′), whereas when δ 3.76 ($COOCH_3$) was irradiated, a weak enhancement was seen at δ 6.99 (H-2′). The (*E*)-configuration was further supported by the downfield chemical shift (δ 6.99) of the olefinic proton (H-2′), which was in the deshielding region of the carbonyl group, and the upfield chemical shift (δ 13.1) of C-5′, arising from steric compression between C-5′ and C-1′.

Roxiamine C (**5**) was a white solid with a molecular formula of $C_{16}H_{17}NO_4$. Because of the number of sites of unsaturation inherent in the furoquinoline skeleton, the rest of the molecule had to be saturated. From comparison of its mass and ^{1}H-NMR spectra with those of **3**, it was obvious that the carbomethoxy group was missing. In addition, the signal for H-3′ occurred at δ 4.14 in **5**, in contrast to δ 2.76 in **3**, suggesting an oxygen substituent at C-3′. A deuterium-exchangeable proton resonance at δ 2.31 confirmed a hydroxyl group, thereby completing the side-chain composition. The connecting point of the side chain to the quinoline was again confirmed by HMBC.

The absolute stereochemistry at C-3′ in **5** was determined to be *S* by a modified Mosher's method.[14] Both (*R*)- and (*S*)-MTPA esters of **5** were prepared, and $\Delta\delta$ values from their ^{1}H-NMR spectra were calculated ($\Delta\delta = \delta_S - \delta_R$, see Figure 1). The stereochemistry at C-3′ in **3** was deduced as follows. Both **3** and **5** have positive

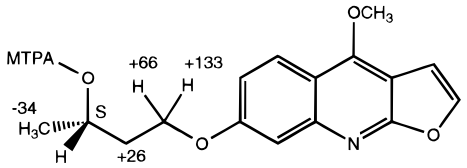
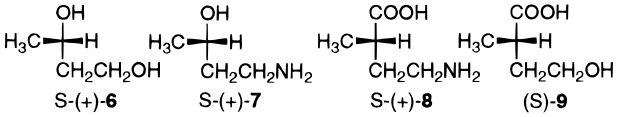


Figure 1. 500 MHz ^{1}H NMR $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$, Hz) for (*R*)- and (*S*)-MTPA esters of roxiamine C.

optical rotations at 589, 578, and 546 nm. The (*S*)-(+) configuration determined for roxiamine C (**5**) correlates with model compound (*S*)-(+)-**6**. The quinoline group in **5** is well-removed from the chiral center; previous studies have indicated that a phenyl group at such a distal position should not alter the sign of the ORD curve.[15] (*S*)-(+)-**6** has been correlated with (*S*)-(+)-**7**,[16] which, in turn, has been correlated with (*S*)-(+)-**8**.[17] It follows, then, that (*S*)-**9** should be dextrorotatory. Since the aryloxy substituent should have no effect on the ORD curve,[15] the 3′S configuration was deduced for roxiamine A (**3**).



Furoquinolines **3**−**5** differed from most known 7-*O*-"prenylated" furoquinolines[18] in that they lacked an 8-methoxy group; they provided essentially no protection against HIV-1 in the NCI primary screen. In contrast, buchapine (**1**) and quinolone **2** were active against infectious HIV-1, as confirmed in an XTT-tetrazolium assay[9] using human lymphoblastoid (CEM-SS) host cells ($EC_{50} = 0.94\ \mu M$, $IC_{50} = 29.0\ \mu M$ and $EC_{50} = 1.64\ \mu M$, $IC_{50} = 26.9\ \mu M$ for **1** and **2**, respectively). Both **1** and **2** also showed inhibitory activity (IC_{50} 12 and 8 μM, respectively) in an HIV-1 reverse transcriptase (RT) assay.[19] HIV-1 RT-inhibitory activity has been reported previously for simple quinolones from marine sponges.[20] Taken together, these results suggest that quinolones might be candidates for further study (medicinal or combinatorial chemistry) as potential anti-HIV agents.

Experimental Section

General. All NMR experiments were performed on a Varian VXR-500 spectrometer; ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ and referenced to residual solvent peaks at δ 7.24 and δ 77.00, respectively. UV and IR spectra were obtained on Beckman DU-64 and Perkin-Elmer 1600 spectrometers, respectively. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Mass spectra were obtained on a Finnigan MAT95 spectrometer. HPLC separations were performed on a Waters 600E system equipped with a Waters 990 diode array detector and employing Rainin Dynamax columns (2.1 × 25 cm).

Plant Material. Flowers, leaves, and twigs of *E. roxburghiana* were collected under contract from the National Cancer Institute in Surat Thani, Thailand, in April 1987. The plant was identified by J. S. Burley; a voucher specimen (Soejarto et al. 5877) was deposited at the Smithsonian Institution.

Isolation. The crude organic extract (5.13 g) was partitioned between 90% aqueous MeOH and hexane (1.850 g). The MeOH solution was adjusted to 80%

MeOH and extracted with CCl_4 to yield 0.692 g. The bulk of the activity was concentrated in the CCl_4 fraction. The CCl_4 fraction was subjected to gel permeation on Sephadex LH-20 (hexane−CH_2Cl_2−MeOH, 2:5:1) to yield two active fractions which were further purified by vacuum−liquid chromatography on silica (7−100% EtOAc−CH_2Cl_2), followed by HPLC purification (silica, 20% EtOAc−CH_2Cl_2), to afford pure buchapine (**1**, 27.0 mg), **2** (40.0 mg), and roxiamines A (**3**, 52.1 mg), B (**4**, 7.4 mg), and C (**5**, 13.5 mg). Compounds **1** (7.4 mg) and **2** (20.1 mg) were also isolated from the hexane fraction (738 mg) in the same fashion.

Buchapine (1): white solid; HREIMS *m/z* 297.1728 (calcd for $C_{19}H_{23}NO_2$, 297.1729). IR, UV, ^{13}C-NMR, and ^{1}H NMR ($CDCl_3$) data were consistent with the literature.[10]

3-(3-Methyl-2-butenyl)-4-[(3-methyl-2-butenyl)-oxy]-2(1*H*)-quinolinone (2): white solid; HREIMS *m/z* 297.1735 (calcd for $C_{19}H_{23}NO_2$, 297.1729). All spectral data including IR, UV, ^{13}C-NMR, and ^{1}H NMR ($CDCl_3$) correspond closely with literature reports.[10,11]

Roxiamine A (3): yellow oil; $[\alpha]_D$ +2.0° (*c* 1.0, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 244 (4.41), 309 (3.73), 320 (3.73), 332 (3.65) nm; IR (film) υ_{max} 3156, 2949, 1732, 1621, 1584, 1453, 1423, 1367, 1209 cm^{-1}; LREIMS *m/z* 329 (30), 215 (20), 115 (100); HREIMS *m/z* 329.1285 (calcd for $C_{18}H_{19}NO_5$, 329.1263); ^{1}H-NMR and ^{13}C-NMR, see Table 1.

Roxiamine B (4): white solid; UV (EtOH) λ_{max} (log ϵ) 242 (4.64), 309 (3.93), 320 (3.93), 332 (3.86) nm; IR (film) υ_{max} 2950, 1714, 1621, 1585, 1451, 1367, 1238 cm^{-1}; LREIMS *m/z* 327 (56), 268 (40), 240 (35), 215 (100), 200 (40), 156 (25), 113 (40); HREIMS *m/z* 327.1124 (calcd for $C_{18}H_{17}NO_5$, 327.1141); ^{1}H NMR δ 8.17 (d, J = 9.3 Hz, 1H, H-5), 7.57 (d, 2.7, 1H, H-2), 7.28 (d, 2.7, 1H, H-8), 7.10 (dd, 9.3, 2.7, 1H, H-6), 7.05 (d, 2.7, H-3), 6.99 (tq, 5.6, 1.2, 1H, H-2′), 4.86 (dq, 5.6, 1.2, 2H, H-1′), 4.43 (s, 3H, 4-OCH_3), 3.76 (s, 3H, 4′-OCH_3), 1.97 (q, 1.2, 3H, H-5′); ^{13}C-NMR δ 167.6 (C-4′), 164.5 (C-9a), 159.6 (C-7), 157.0 (C-4), 147.5 (C-8a), 142.6 (C-2), 136.4 (C-2′), 129.9 (C-3′), 123.9 (C-5), 116.8 (C-6), 113.6 (C-4a), 106.4 (C-8), 104.8 (C-3), 102.1 (C-3a), 65.0 (C-1′), 58.9 (4-OCH_3), 52.0 (4′-OCH_3), 13.1 (C-5′).

Roxiamine C (5): white solid; $[\alpha]_D$ + 4.0° (*c* 1.0, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 239 (4.67), 309 (3.86), 321 (3.86), 334 (3.79) nm; IR (CH_2Cl_2) υ_{max} 3608, 2962, 1622, 1585, 1453, 1368, 1091, 1013 cm^{-1}; LREIMS *m/z* 287 (55), 215 (100), 200 (40)169 (30); HREIMS *m/z* 287.1147 (calcd for $C_{16}H_{17}NO_4$, 287.1157); ^{1}H-NMR δ 8.10 (d, J = 9.3 Hz, 1H, H-5), 7.54 (d, 2.9, 1H, H-2), 7.29 (d, 2.7, 1H, H-8), 7.03 (dd, 9.3, 2.7, 1H, H-6), 7.01 (d, 2.9, 1H, H-3), 4.40 (s, 3H, 4-OCH_3), 4.29 (ddd, 9.7, 6.4, 5.6, 1H, H-1′), 4.22 (ddd, 9.7, 6.3, 5.4, 1H, H-1′), 4.14 (m, 1H, H-3′), 2.31 (bs, 1H, OH), 1.98 (m, 2H, H-2′), 1.28 (d, 6.4, 3H, H-4′); ^{13}C-NMR δ 164.4 (C-9a), 160.0 (C-7), 156.9 (C-4), 147.5 (C-8a), 142.5 (C-2), 123.6 (C-5), 116.7 (C-6), 113.4 (C-4a), 106.6 (C-8), 104.8 (C-3), 101.9 (C-3a), 66.0 (C-3′), 65.8 (C-1′), 58.9 (4-OCH_3), 38.0 (C-2′), 23.7 (C-4′).

Mosher's Esters of 5. To a dry round-bottom flask containing **5** (2.5 mg) were added sequentially dry pyridine (0.5 mL), DMAP (1.0 mg), and (*R*)-MTPA-Cl (10 μL). The reaction was allowed to stir for 4 h under Ar. Solvent was evaporated under a stream of N_2, and the residue was purified on a short column of silica to afford the (*S*)-MTPA ester (4.7 mg). The (*R*)-MTPA ester was prepared similarly using (*S*)-MTPA-Cl.

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