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

Jinping L. McCormick, Tawnya C. McKee,* John H. Cardellina II, and Michael R. Boyd*

Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, Division of Cancer Treatment, Diagnosis, and Centers, National Cancer Institute, Frederick, Maryland 21702-1201

Received October 17, 1995[⊗]

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]

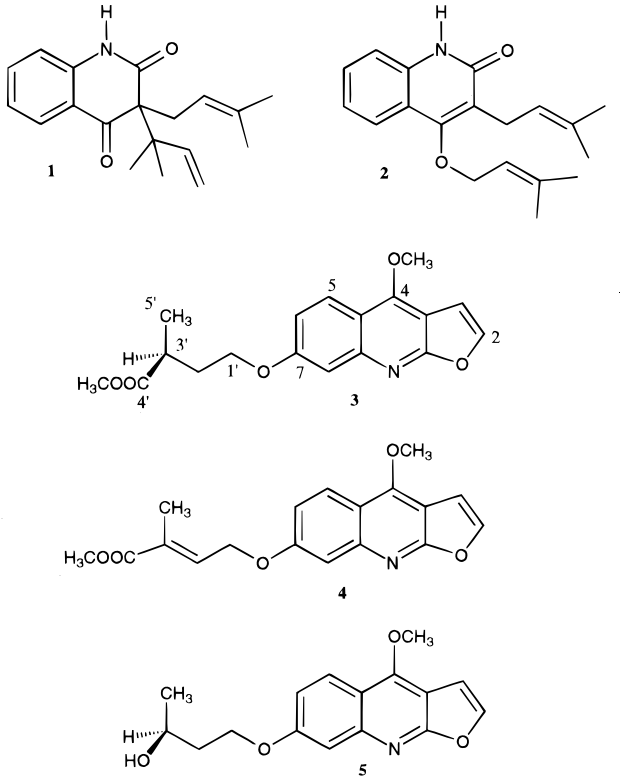
Observation of anti-HIV activity of an extract of *Euodia roxburghiana* Benth. (Rutaceae) in the NCI's anti-HIV *in vitro* primary screen[8] led to the present study. Two known quinolines and three new furoquinolines have been identified; in an XTT-tetrazolium assay,[9] the quinolines exhibited modest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



[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1996.

S0163-3864(96)00250-9

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

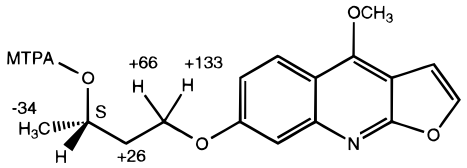
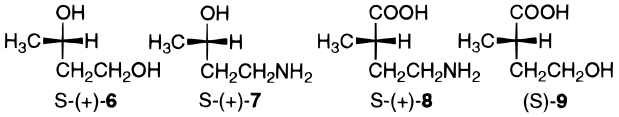


Figure 1. 500 MHz ^{1}H NMR $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$, Hz) for (*R*)- and (*S*)-MTPA esters of roxiamine C.

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.

Acknowledgment. The authors thank D. D. Soejarto, T. Smitinand, T. Santisuk, K. Taylor, and N. Nantasan for the contract plant collections, G. Cragg for coordinating collections, T. McCloud for extraction, J. B. McMahon, M. J. Currens, R. J. Gulakowski, B. Krepps, and D. Clanton for anti-HIV testing, G. Gray for mass spectral analyses, and M. A. Rashid for helpful discussions.

References and Notes

(1) Part 25: ref 21.
(2) Briggs, M. J. *Australas. J. Pharm*. **1969**, *50*, 82−83.
(3) Perry, L. M.; Metzger, J. Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses; MIT Press: Cambridge, 1980; pp 335−369.
(4) Kumar, V.; Karunaratne, V.; Sanath, M. R.; Meegalle, K.; MacLeod, J. K. *Phytochemistry* **1990**, *29*, 243−245.
(5) Manandhar, M. D.; Hussaini, F. A.; Kapil, R. S.; Shoeb, A. *Phytochemistry* **1985**, *24*, 199−200.
(6) Goh, S. H.; Chung, V. C.; Sha, C. K.; Mak, T. C. W. *Phytochemistry* **1990**, *29*, 1704−1706.
(7) Lassak, E. V.; Southwell, I. A. *Aust. J. Chem*. **1972**, *25*, 2491−2496.
(8) Boyd, M. R. In *AIDS, Etiology, Diagnosis, Treatment and Prevention*; DeVita, V. T., Hellman, S., Rosenberg, S. A., Eds.; Lippincott, J. B: Philadelphia, 1988; pp 305−319.
(9) Gulakowski, R. J.; McMahon, J. B.; Staley, P. G.; Moran, R. A.; Boyd, M. R. *J. Virol. Methods* **1991**, *33*, 87−100.
(10) (a) Nesmelova, E. F.; Bessonova, I. A.; Yunusov, S. *Khim. Prir. Soedin*. **1982**, 532−533; *Chem. Abstr*. **1983**, *98*, 14361d. (b) Sheriha, G. M.; Abouamer, K.; Elshtaiwi, B. Z.; Ashour, A. S.; Abed, F. A.; Alhallaq, H. H. *Phytochemistry* **1987**, *26*, 3339−3341.
(11) Lavie, D.; Danieli, N.; Weitman, R.; Gletier, E. *Tetrahedron* **1968**, *24*, 3011−3018.
(12) Mohan, P. S.; Ramesh, M.; Shanmugan, P. *J. Nat. Prod*. **1985**, *48*, 501.
(13) Fauvel, M. T.; Gleye, J.; Moulis, C.; Blasco, F.; Stanislas, E. *Phytochemistry* **1981**, *20*, 2059−2060.
(14) (a) Ohtani, I.; Kusumi, T.; Ishitsuka, M. O.; Kakisawa, H. *Tetrahedron Lett*. **1989**, *30*, 3147−3150. (b) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. J. *J. Org. Chem*. **1991**, *56*, 1296−1297.
(15) Fredga, A.; Jennings, J. P.; Klyne, W.; Scopes, P. M.; Sjöberg, B.; Sjöberg, S. *J. Chem. Soc*. **1965**, 3928−3933.
(16) (a) Levene, P. A.; Haller, H. L. *J. Biol. Chem*. **1926**, *69*, 165−173; (b) **1926**, *69*, 569−574; (c) **1928**, *77*, 555−562. (d) Lemieux, R. U.; Giguere, J. *Can. J. Chem*. **1951**, *29*, 678−690.
(17) Adams, R.; Fles, D. *J. Am. Chem. Soc*. **1959**, *81*, 4946−4951.
(18) (a) Dreyer, D. L. *J. Org. Chem*. **1970**, *35*, 2420−2422. (b) Grundon, M. F.; Harrison, D. M.; Sypropoulos, C. G. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2181−2184. (c) Eastwood, F. W.; Hughes, G. K.; Ritchie, E. *Aust. J. Chem*. **1954**, *7*, 87−98.
(19) (a) Clark, P. K.; Ferris, A. L.; Miller, D. A.; Hizi, A.; Kim, K. W.; Deringer, B. S.; Mellini, M. L.; Clark, A. J.; Arnold, G. F.; Lebherz, W. *AIDS Res. Hum. Retrovirus* **1990**, *6*, 753−764. (b) Hizi, A.; McGill, C.; Hughes, S. H. *Proc. Natl. Acad. Sci. U.S.A*. **1988**, *85*, 1218−1222. (c) Hizi, A.; Tal, R.; Shaharabany, M.; Loya, S. *J. Biol. Chem*. **1990**, *266*, 6230−6239. (d) Boyer, P. L.; Currens, M. J., McMahon, J. B.; Boyd, M. R.; Hughes, S. H. *J. Virol*. **1993**, *67*, 2412−2420.
(20) Loya, S.; Rudi, A.; Tal, R.; Kashman, Y.; Loya, Y.; Hizi, A. *Arch. Biochem. Biophys*. **1994**, *309*, 315−322.
(21) Cardellina, J. H., II; Bokesch, H. R.; McKee, T. C.; Boyd, M. R. *BioMed. Chem. Lett*. **1995**, *5*, 101l-1014.

NP960250M