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NOVEL NAPHTHOQUINONES FROM *CONOSPERMUM INCURVUM*

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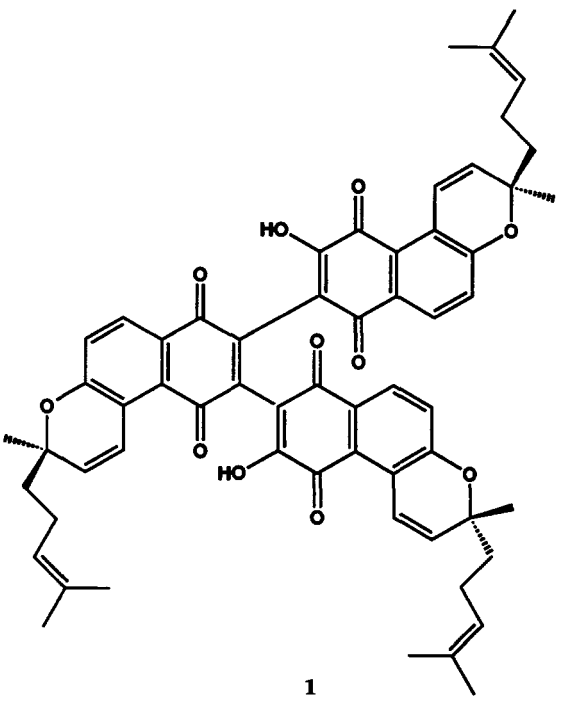
ABSTRACT.—During the reisolation of the trimeric naphthoquinone derivative conocurvone [**1**] from an extract of the Australian shrub *Conospermum incurvum*, six monomeric naphthoquinones were isolated. These include three novel 1,4-naphthoquinone derivatives: 3-methyl-14,15-dihydro-15-hydroxyteretifolione B [**3**], 3-methyl-14,15-dihydro-15-hydroxyteretifolione B methyl ether [**4**], and 2,3-dimethyl-6-hydroxy-7-methoxy-1,4-naphthoquinone [**5**]. In addition, the previously reported compounds 3-methylteretifolione B [**6**], 3-methylteretifolione B methyl ether [**7**], and 8-geranyl-2,7-dihydroxy-3-methyl-1,4-naphthoquinone [**8**] were isolated and identified. The structures of the novel 1,4-naphthoquinones were elucidated by spectral methods. While conocurvone [**1**] is a potent inhibitor of HIV-1-induced cell killing, all of the monomeric naphthoquinone derivatives were inactive against HIV-1.

A previous report from our laboratory detailed the isolation of conocurvone [**1**], a novel HIV-1 inhibitory trimeric naphthoquinone from an extract of the endemic Australian shrub *Conospermum incurvum* Lindley (Proteaceae) (1). The related monomeric quinone teretifolione B [**2**] was also obtained and its absolute stereochemistry estab-



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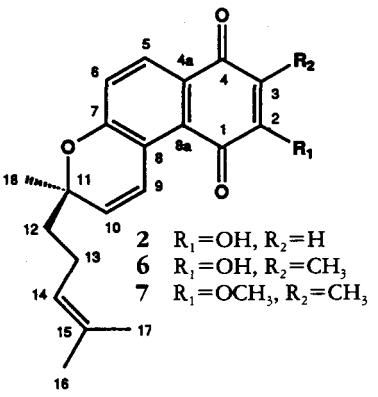
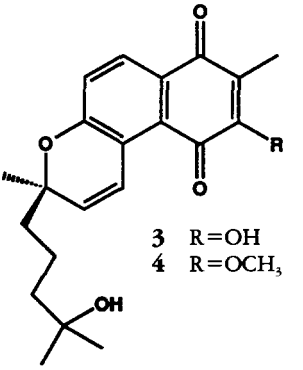
lished via an X-ray crystallographic analysis. We have been investigating the reisolation of conocurvone [**1**] on a larger scale (2) to support detailed biological evaluation of its anti-HIV-1 activity. The crude extract was initially fractionated on a Sanki centrifugal partition chromatograph (cpc) in the ascending mode with hexane-EtOAc-MeOH-H_2O (17:7:13:3). Fractions from the cpc were further purified by hplc or by reinjection on the Sanki cpc to afford conocurvone [**1**], teretifolione B [**2**], and six additional naphthoquinones. Three of these were novel 1,4-naphthoquinones, namely, 3-methyl-14,15-dihydro-15-hydroxyteretifolione B [**3**], the related methyl ether [**4**], and 2,3-dimethyl-6-hydroxy-7-methoxy-1,4-naphthoquinone [**5**].[3] The other three compounds, 3-methylteretifolione B [**6**], 3-methylteretifolione B methyl ether [**7**], and 8-geranyl-2,7-dihydroxy-3-methyl-1,4-naphthoquinone [**8**][3] have previously been reported from *C. teretifolium* (3,4). The structures of compounds **3**–**5** were elucidated by spectroscopic techniques, while the structures of **6**–**8** were confirmed by independent spectral analyses and comparison of their spectral data with published values.

RESULTS AND DISCUSSION

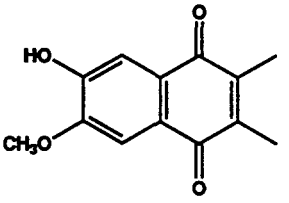
The presence of conocurvone [**1**] and teretifolione B [**2**] was confirmed by comparison with authentic samples. The spectral data obtained in this investigation for 3-methylteretifolione B [**6**], 3-methylteretifolione B methyl ether [**7**], and 8-geranyl-2,7-dihydroxy-3-methyl-1,4-naphthoquinone [**8**] were consistent with literature values



2 R_1=OH, R_2=H
6 R_1=OH, R_2=CH_3
7 R_1=OCH_3, R_2=CH_3



3 R=OH
4 R=OCH_3



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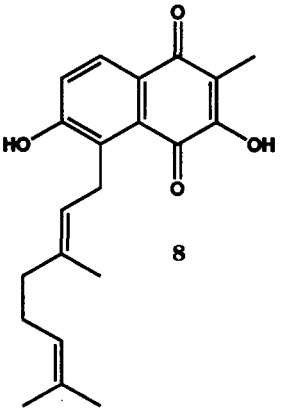

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[3]For clarity of discussion and comparison of spectral data, the numbering scheme for this compound has been assigned so that it is consistent with that of teretifolione B [**2**] and others in the series of monomeric naphthoquinones.

(3,4). However, only ^{1}H-nmr data have previously been reported for these compounds. We have independently confirmed the proposed structures and fully assigned the ^{1}H- and ^{13}C-nmr resonances of these compounds by a variety of nmr techniques, including HMQC and HMBC proton-detected heteronuclear correlation experiments. The ^{1}H-nmr data obtained for compounds **6** and **7** were in very close agreement with published values, but a slight discrepancy was noted for compound **8**. The C-10 olefin proton was observed at δ 5.13, while the previously reported value was δ 5.25 (3,4). All other proton resonances that were measured for **8** were similar to literature values. An nOe experiment showing significant nOe interactions between the C-10 proton and the C-12 allylic protons confirmed the olefin geometry as *E*. Therefore, we are unable to explain the minor difference between our data for **8** and the literature values (3,4).

Compound **3** was isolated as an optically active, $[\alpha]_D$ +38.8°, dark orange powder. Hreims revealed a molecular formula of $C_{21}H_{24}O_5$, with a molecular ion at *m/z* 356.1639. Absorptions in the ir (1644 cm^{-1}) and uv (219,292 nm) spectra of **3** indicated the presence of conjugated carbonyl groups. The ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) were closely related to those observed for 3-methylteretifolione B [**6**]. However, in the carbon spectrum of **3**, signals for the trisubstituted olefin previously assigned in **6** were replaced by an aliphatic methylene and a tertiary alcohol. Two exchangeable proton resonances at δ 7.44 and δ 4.07[4] indicated the presence of one phenol and one aliphatic

TABLE 1. ^{1}H-Nmr Spectral Data (in $CDCl_3$) for Compounds **3–8**.[a]

Position	Compound					
	3	**4**	**5**	**6**	**7**	**8**
H-5	7.95 d (8.5)	7.89 d (8.5)	7.53 s	7.93 d (8.5)	7.86 d (8.5)	7.99 d (8.5)
H-6	7.03 dd (8.5, 1.0)	6.99 dd (8.5, 1.0)		7.02 d (8.5)	6.96 dd (8.5, 1.0)	7.11 d (8.5)
H-8			7.49 s			
H-9	7.81 d (10.5)	7.71 d (10.5)		7.79 d (10.5)	7.69 d (10.5)	4.05 d (7.0, 2H)
H-10	5.92 d (10.5)	5.86 d (10.5)		5.90 d (10.5)	5.83 d (10.5)	5.13 m
H-12	1.70 m, 1.75 m	1.64 m, 1.74 m		1.66 m, 1.75 m	1.64 m, 1.72 m	2.05 m (2H)
H-13	1.47 m (2H)	1.50 m (2H)		2.07 m (2H)	2.04 m (2H)	2.05 m (2H)
H-14	1.44 m (2H)	1.43 m (2H)		5.04 m	5.02 m	5.01 m
CH_3-16	1.17 s	1.16 s		1.61 s	1.60 s	1.63 s
CH_3-17	1.18 s	1.17 s		1.52 s	1.51 s	1.56 s
CH_3-18	1.41 s	1.38 s		1.41 s	1.39 s	1.84 s
CH_3-2			2.11 s			
CH_3-3	2.04 s	2.02 s	2.11 s	2.02 s	1.99 s	2.04 s
OCH_3-2		4.00 s			3.97 s	
OCH_3-7			4.01 s			
OH	4.07[b] s 7.44 s	4.07[b] s	6.10 s	7.49 s		7.49 s

[a]Chemical shifts (δ) were referenced relative to the internal solvent signal. Coupling constants in parentheses are in Hz.

[b]Observed in DMSO-d_6.

[4]Exchangeable proton resonances were observed in DMSO-d_6 spectra.

TABLE 2. ^{13}C-Nmr Spectral Data (in $CDCl_3$) for Compounds **3–8**.[a]

Position	Compound					
	3	**4**	**5**	**6**	**7**	**8**
C-1	183.2	183.6	184.2	183.2	183.1	183.0
C-2	153.2	158.4	142.8[b]	153.3	158.3	153.5
C-3	118.6	130.4	142.9[b]	118.6	120.5	118.4
C-4	184.4	185.1	184.3	184.4	185.0	184.7
C-4a	126.8	126.3	127.7	126.9	126.2	127.8
C-5	128.7	128.3	112.0	128.7	128.2	127.6
C-6	121.7	121.0	150.1	121.7	120.8	121.0
C-7	157.8	158.5	150.4	158.0	158.5	159.4
C-8	121.2	120.6	107.6	121.2	130.3	130.3
C-8a	123.2	126.0	126.6	123.3	126.0	127.7
C-9	120.2	120.4		120.1	120.2	25.3
C-10	135.1	134.0		135.1	140.0	120.2
C-11	79.2	79.2		79.2	79.1	139.2
C-12	41.6	41.6		41.2	41.1	39.7
C-13	18.7	18.8		22.6	22.6	26.4
C-14	43.6	43.8		123.5	123.5	123.7
C-15	70.9	70.9		132.1	132.0	132.0
C-16	29.3[c]	29.3[c]		25.6	25.6	25.6
C-17	29.2[c]	29.2[c]		17.6	17.5	17.7
C-18	26.5	26.5		26.5	26.5	16.4
CH_3-2			12.8			
CH_3-3	8.5	9.1	12.8	8.5	9.0	8.5
OCH_3		60.8	56.5		60.7	

[a]Chemical shifts (δ) were referenced relative to the internal solvent signal.
[b,c]Assignments within a column may be reversed.

hydroxyl group. This suggested that compound **3** was the hydrate of **6** resulting from Markovnikov-type addition of H_2O to the C-14–C-15 side-chain olefin. Long-range heteronuclear correlations from the oxygenated C-15 carbon (δ 70.9) to the C-13 methylene protons and from C-14 (δ 43.6) to the C-16, C-17 methyl groups supported this assignment. The C-2 hydroxyl and C-3 methyl substitution pattern about the naphthoquinone moiety was confirmed by correlations from C-1 (δ 183.2) to the C-2 hydroxyl proton and from C-4 (δ 184.4) to H-5. All other heteronuclear correlations observed were fully consistent with the proposed structure of 3-methyl-14,15-dihydro-15-hydroxyteretifolione B for **3**, and permitted complete assignments of all ^{1}H- and ^{13}C-nmr signals to be made. The fact that both teretifolione B [**2**] and compound **3** have strong, positive optical rotations indicates that they have the same (*R*) absolute stereochemistry at C-11.

Compound **4**, $[\alpha]_D$ +32.1°, was also obtained as a dark orange powder. The hreims of **4** revealed a molecular formula of $C_{22}H_{26}O_5$ (M^+, *m/z* 370.1805) and the ir and uv spectra indicated the presence of conjugated carbonyl chromophores (1698, 1666 cm^{-1}; 222, 266 nm). The ^{1}H- and ^{13}C-nmr spectra of **4** (Tables 1 and 2) closely matched those of compound **3**. However, there was only one exchangeable proton signal (δ 4.07),[4] but there was an additional methoxyl group present (^{1}H δ 4.00 3H, s; ^{13}C δ 60.8). HMBC correlations from C-2 (δ 158.4) to the methoxyl protons and from C-4 (δ 185.1) to the C-3 methyl protons defined the regiochemical placement of these substituents. Complete ^{1}H- and ^{13}C-nmr assignments for compound **4** were made from heteronuclear correlation data. These data established that compound **4** is the C-2 methyl ether derivative of compound **3**.

Compound **5** was obtained as an optically inactive yellow powder. The hreims spectrum of **5** indicated a molecular formula of $C_{13}H_{12}O_4$ for the molecular ion at *m/z* 232.0729. The ir and uv spectra of **5** indicated the presence of conjugated carbonyl groups (ir 1644 cm^{-1}; uv 277 nm). The ^{1}H-nmr spectrum of **5** (Table 1) exhibited two singlet aromatic protons at δ 7.53 and 7.49, a six-proton singlet due to two methyl groups at δ 2.11, one methoxyl at δ 4.01 (s) and an exchangeable phenol resonance at δ 6.10. The ^{13}C-nmr spectrum (Table 2) displayed a two-carbon signal at δ 12.8 due to two methyls, one methoxyl group at δ 56.5 ppm, two quinone carbonyls at δ 184.2 and 184.3, and eight additional sp^2 carbons making up a naphthoquinone skeleton. This indicated that compound **5** was a 1,4-naphthoquinone substituted with two methyls, one methoxyl and one hydroxyl group.

The assignment of ^{1}H- and ^{13}C-nmr spectral data and the structural elucidation of compound **5** were supported by HMQC and HMBC experiments. Long-range coupling observed between the quinone carbonyl carbons and the methyl protons established the methyl substituents at C-2 and C -3. Correlations between H-5 (δ 7.53), C-4 (δ 184.3), C-7 (δ 150.4), and C-8a (δ 126.6), between H-8 (δ 7.49), C-6 (δ 150.1), and C-4a (δ 127.7), between the methoxy group (δ 4.01) and C-7 (δ 150.4), as well as between the phenolic OH (δ 6.10) and C-5 (δ 112.0) and C-7 (δ 150.4), firmly established the structure of compound **5** as 2,3-dimethyl-6-hydroxy-7-methoxy-1,4-naphthoquinone.

Testing of **3–8** in an in vitro XTT-based anti-HIV-1 assay (5,6), revealed that none of these "monomeric" naphthoquinone derivatives were active. This is in sharp contrast to the reported potent anti-HIV activity of the "trimeric" conocurvone [**1**], but consistent with the reported inactivity of the monomer teretifolione B [**2**] and a related 1,4-naphthoquinone "dimer" (1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Centrifugal partition chromatography (cpc) was performed at 24° with a cartridge Sanki model NMF instrument. An ISCO V absorbance detector (280 nm) and a Foxy fraction collector were connected to the main Sanki cpc unit. Hplc was performed on a Rainin system equipped with Rabbit-HP HPX pumps and a Dynamax UV-1 detector. Optical rotations were measured on a Perkin-Elmer 241 polarimeter; uv spectra were recorded on a Beckman DU-64 spectrophotometer; ir spectra were recorded on a Perkin-Elmer 1600 series Ftir; nmr spectra were recorded on a Varian VXR 500 spectrometer at 25°. Hreims spectra were recorded with a Finnigan MAT 90 spectrometer.

PLANT MATERIAL.—The roots of *Conospermum incurvum* Lindley (collection number Q73H128) were collected in February 1991, on the Northern Sand Plains, Western Australia. Ground plant material (11.416 kg) was percolated at room temperature in CH_2Cl_2, and then in CH_2Cl_2-MeOH (1:1), and finally washed with 100% MeOH. Solvent was removed *in vacuo* to produce CH_2Cl_2, CH_2Cl_2-MeOH (1:1), and MeOH extracts (69.81 g, 145.73 g, and 23.81 g, respectively).

EXTRACTION AND ISOLATION.—A 1.5-g portion of the CH_2Cl_2 extract was fractionated by cpc with hexane-EtOAc-MeOH-H_2O (17:7:13:3), using the ascending mode, a spin rate of 500 rpm, a flow rate of 2.7 ml/min, and uv detection at 280 nm. A total of 12 fractions were obtained. Conocurvone [**1**] (9 mg) and compound **6** (30 mg) were obtained from cpc fraction 3 by two different hplc methods. Pure conocurvone [**1**] was obtained by phenyl-bonded phase hplc using CH_3CN-H_2O (17:3, 0.1% HOAc by volume), while compound **6** was purified by Si gel hplc eluting with CH_2Cl_2-MeOH (19:1). Teretifolione B [**2**] (30 mg) was isolated from cpc fraction 5, compound **4** (20 mg) was isolated from cpc fraction 7 (68 mg), and compound **8** (5 mg) was isolated from cpc fraction 6 (24 mg) using the same hplc conditions as those used for **6**. Hplc on a phenyl-bonded phase, eluting with CH_2CN-H_2O (3:1, 0.1% HOAc by volume), of 23 mg of cpc fraction 9 and 40 mg of cpc fraction 8, provided compound **3** (12 mg) and compound **5** (7 mg), respectively. A 100-mg portion of cpc fraction 2 was reinjected on the Sanki cpc using the exact same conditions as the original cpc run to give 60 mg of compound **7**.

3-Methyl-14,15-dihydro-15-hydroxyteretifolione B [**3**].—$[\alpha]_D$ +38.8° (c=0.56, $CHCl_3$); uv λ max (MeOH) (log ε) 219 (5.33), 292 (5.12), 392 (4.66) nm; ir (film) ν max 3373, 2970, 1644, 1561, 1391, 1351, 847 cm^{-1}; for ^{1}H and ^{13}C nmr, see Tables 1 and 2, respectively; eims *m/z* 356 (M^+, 4), 341 $[M-CH_3]^+$ (2), 338 $[M-H_2O]^+$ (1), 270 $[M-C_5H_{10}O]^+$ (1), 255 $[M-C_5H_{10}O-CH_3]^+$ (100), 227

$[M-C_5H_{10}O-CH_3-CO]^+$ (19), 199 (1), 149 (4), 85 (1), 69 (1); hreims *m/z* 356.1639 (M^+, $C_{21}H_{24}O_5$, calcd 356.1624).

3-Methyl-14,15-dihydro-15-hydroxyteretifolione B methyl ether [**4**].—$[\alpha]_D$ +32.1° (*c*=0.80, $CHCl_3$); uv λ max (MeOH) (log ε) 222 (5.23), 266 (5.12), 370 (4.41) nm; ir (film) ν max 3478, 2970, 1698, 1666, 1564, 1372, 1279, 983 cm^{-1}; for ^1H and ^{13}C nmr, see Tables 1 and 2, respectively; eims *m/z* 370 (M^+, 6), 355 $[M-CH_3]^+$ (5), 352 $[M-H_2O]^+$ (4), 297 (2), 285 (11), 284 $[M-C_5H_{10}O]^+$ (1), 270 (33), 269 $[M-C_5H_{10}O-CH_3]^+$ (100), 241 $[M-C_5H_{10}O-CH_3-CO]^+$ (1), 226 (12), 170 (4), 149 (5), 115 (2), 83 (1), 59 (2); hreims *m/z* 370.1805 (M^+, $C_{22}H_{26}O_5$, calcd 370.1780).

2,3-Dimethyl-6-hydroxy-7-methoxy-1,4-naphthoquinone [**5**].—Uv λ max (MeOH) (log ε) 277 (5.45), 352 (4.28) nm; ir (film) ν max 3300–3400, 1644, 1580, 1524, 1356, 1313, 1200, 889 cm^{-1}; for ^1H and ^{13}C nmr, see Tables 1 and 2, respectively; eims *m/z* 232 (M^+, 100), 217 $[M-CH_3]^+$ (6), 215 (4), 214 $[M-H_2O]^+$ (1), 204 $[M-CO]^+$ (42), 189 $[M-CO-CH_3]^+$ (57), 161 (17), 150 (11), 133 (5), 122 (13), 115 (5), 83 (3), 63 (2), 51 (6); hreims *m/z* 232.0729 (M^+, $C_{13}H_{12}O_4$, calcd 232.0736).

3-Methylteretifolione B [**6**].—$[\alpha]_D$ +29.7° (*c*=0.33, $CHCl_3$); for ^1H and ^{13}C nmr, see Tables 1 and 2, respectively; eims *m/z* 338 (M^+, 14), 323 $[M-CH_3]^+$ (4), 295 $[M-CH_3-CO]^+$ (1), 270 (1), 269 $[M-C_5H_9]^+$ (2), 255 $[M-C_6H_{11}]^+$ (100), 227 $[M-C_6H_{11}-CO]^+$ (29), 199 (1), 115 (3), 69 (5).

3-Methylteretifolione B methyl ether [**7**].—$[\alpha]_D$ +37.3° (*c*=0.73, $CHCl_3$); for ^1H and ^{13}C nmr, see Tables 1 and 2, respectively; eims *m/z* 352 (M^+, 49), 337 $[M-CH_3]^+$ (16), 309 $[M-CH_3-CO]^+$ (4), 284 (1), 283 $[M-C_5H_9]^+$ (3), 269 $[M-C_6H_{11}]^+$ (100), 255 (7), 241 $[M-C_6H_{11}-CO]^+$ (4), 226 (24), 213 (2), 182 (13), 144 (6), 115 (6), 77 (2), 69 (15).

8-Geranyl-2,7-dihydroxy-3-methyl-1,4-naphthoquinone [**8**].—For ^1H and ^{13}C nmr, see Tables 1 and 2, respectively; eims *m/z* 340 (M^+, 71), 322 $[M-H_2O]^+$ (4), 297 $[M-C_3H_7]^+$ (54), 285 $[M-C_4H_7]^+$ (2), 271 $[M-C_5H_9]^+$ (62), 257 $[M-C_6H_{11}]^+$ (22), 253 (68), 229 $[M-C_8H_{15}]^+$ (100), 218 (66), 187 (44), 149 (37), 123 (35), 115 (18).

ANTI-HIV EVALUATIONS.—DMSO solutions of the purified compounds were tested in the XTT-based in vitro anti-HIV assay as described elsewhere (5,6).

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