Diterpenes from *Colophospermum mopane*: "Missing Links" in the Biogenesis of 9,13-Epoxylabdanes

Emily Reiter,[†] Edward Treadwell,[‡] Ericka Cederstrom,[§] Paul B. Reichardt,[†] and Thomas P. Clausen*[,†]

Department of Chemistry and Biochemistry, University of Alaska Fairbanks, Fairbanks, Alaska 99775-6160, Department of Chemistry, Eastern Illinois University, Charleston, Illinois 61920, and Department of Chemistry, University of Pittsburgh, Pennsylvania 15260

Received May 24, 2002

Moponeol A (**1**) and moponeol B (**2**) were isolated from *Colophospermum mopane* along with a mixture of their corresponding aldehydes (**3** and **4**). These substances are primitive diterpenes that we view as the "missing links" in the biosynthesis of the 9,13-epoxylabdanes. The structures of **1** and **2** were elucidated by a combination of spectra (NMR and MS) of the isolates and their mono-*p*-bromobenzoyl derivatives. The structures of **3** and **4** were confirmed by their ready reduction to **1** and **2**. The biosynthetic implications of the stereochemical assignments of these terpenoids are briefly discussed.

*Colophospermum mopan*e Kirk ex J. Leonard (Leguminosae), commonly called mopane, is a tree/shrub found throughout central and southern Africa (between the Tropic of Cancer and 10 degrees south latitude). Local uses of it as a medicinal agent for ailments from inflamed eyes to syphilis to temporary madness have been reported,[1] but no chemical studies investigating these claims have been reported. Its major anthropogenic importance arises from it being the host plant for the edible larvae of the Saturnid moth *Gonibrasia belina*,[1] for which the annual market runs into thousands of tons.[2]

We initially investigated the secondary chemistry of mopane to better understand the basis for elephants' selective use of mopane stands.[3] Early on, however, we discovered four novel primitive diterpenes which may considered to be the "missing links" in the biosynthesis of an important class of compounds, the 9,13-epoxylabdanes. We herein report the structures of these four compounds (**1**−**4**) and comment on their biosynthetic implications.

Results and Discussion

Flash chromatography of a hexanes extract of mopane leaves on silica gel yielded mopaneol A (**1**). The IR showed the presence of at least one alcohol (ν_{max} 3400 cm^{-1}), but no other functionalities (carbonyl or olefin) were apparent. The HREIMS of **1** indicated a molecular ion peak at *m*/*z* 324.2640, corresponding to the molecular formula $C_{20}H_{36}O_3$ (calcd 324.2666), implying a tricyclic structure. A cursory examination of the ^{13}C NMR and DEPT spectra (Table 1) showed five methyls, eight methylenes, three methines, and four quaternary carbons. Chemical shifts implied that four of the carbons (one methine, one methylene, and two quaternary) were oxygenated. Furthermore, a brief inspection of the ^{1}H NMR showed a methine triplet at δ 3.34 (O−CH−CH_2), diastereotopic methylenes at δ 3.71 (1H dt; J = 9, 2.5 Hz) and 3.96 (1H, td; J = 9, 3 Hz) (O−CH_2−CH_2), a methyl doublet (CH_3CH) at δ 0.90, and a downfield methyl (CH_3C−O) at δ 1.04. A 9,13-epoxylabdane with a hydroxy group at C-15 was the only known diterpene ring system[4] consistent with these observations. The placement of the second alcohol at C-3 was initially hypothesized on the basis of biosynthetic arguments and was subsequently confirmed by COSY, TOCSY, and HMBC experiments (Figure 1). Finally, an intense fragment peak in the EIMS with m/z = 169 was consistent with these assignments (Figure 1).[5]

A complete structure determination of **1** required assigning the stereochemistry of five centers relative to C-10 (which we assume to be *S*; Figure 2). The H-3 signal at δ 3.34 appeared as a triplet with a small coupling constant (J = 3 Hz) that implied an α-OH at C-3. This assignment of stereochemistry was consistent with subsequent NOESY experiments that showed strong NOE correlations of H-3 with both methyls at C-4 (Figure 2).

A *trans*-decalin system was consistent with biosynthetic arguments (vide infra) and literature precedent of other 9,13-epoxylabdanes. A NOESY experiment confirmed this hypothesis by the absence of a correlation between the methyl at C-10 and H-5, which would be expected for a *cis*-decalin system. Finally, the ^{13}C NMR chemical shift of the C-10 methyl (δ 18.3) is consistent only with a *trans*-decalin ring.[6]

With benzene-d_6 as the solvent, an unambiguous NOE correlation between the methyls at C-8 and C-10 was evident and thus confirmed a β-configuration for the methyl at C-8 (Figure 2). The proximity of these two methyls was established by molecular modeling using molecular mechanics (AM1) and semiempirical (PM3) standard algorithms available in HyperChem software. For instance, PM3 calculations predicted the chair-chair conformation shown in Figure 2 to be favored by 4.2 kcal over the chair-twist boat conformation that would place the C-8 and C-10 methyls far apart.

A review of the literature did not provide guidance on the relative stereochemistry at C-9 because of numerous examples of both α and β ether linkages of 9,13-epoxylabdanes. A strong NOE correlation between the methyl at C-10 and the pro-R H-11 (Figure 2), however, was observed and is consistent only with an α ether linkage.[7]

The stereochemical assignment at C-13 proved to be the most challenging. Use of models indicated that the C-13 methyl and 2-hydroxyethyl groups are too remote from the decalin system to allow strong NOE correlations. This proved true, as these substituents showed only correlations between themselves and the two H-12 hydrogens. These

* To whom correspondence should be addressed. Tel: (907) 474-5512. Fax: (907) 474-5640. E-mail: FFTPC@UAF.EDU.
[†] University of Alaska Fairbanks.
[‡] Eastern Illinois University, Charleston.
[§] University of Pittsburgh.

10.1021/np0202430 CCC: $25.00
Published on Web 12/02/2002

Table 1. NMR Chemical Shifts (relative to TMS in C_6D_6 and $CDCl_3$) for Mopaneol A (**1**) and Its Benzoate Derivative (**5**) in $CDCl_3$[a]

	mopaneol A (**1**); C_6D_6		mopaneol A (**1**); $CDCl_3$		*p*-bromobenzoyl ester (**5**)	
assign.	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR
1	26.7	**0.82**(eq)	26.3	1.05(eq)	26.2	1.05(eq)
		2.10(ax)		1.98(ax)		2.01(ax)
2	25.2	1.66(eq)	24.4	1.61(eq)	24.8	1.61(eq)
		1.80(ax)		1.93(ax)		1.97(ax)
3	76.0	3.34	76.1	3.30	76.2	3.38
4	38.0		37.7		40.9	
5	41.4	**1.95**	41.4	1.65	39.2	**1.87**
6	17.1	1.32 (ax and eq)	16.7	1.36 and 1.40	16.7	1.34
7	29.9	1.34(eq)	29.7	1.40(eq)	29.5	1.38(eq)
		2.07(ax)		1.98(ax)		2.02(ax)
8	40.5	1.75	40.4	1.88	40.8	**1.96**
9	93.8		94.0		92.5	
10	42.2		41.9		41.7	
11	29.2	**1.50** (ProS)	29.2	1.85 (ProS)	28.7	1.88
		1.68 (ProR)		2.02 (ProR)		1.88
12	39.6	**1.43** (ProR and S)	39.9	1.73/1.85	38.7	1.74/1.8
13	83.5		84.0		80.8	
14	42.9	**1.30**/1.91	42.2	1.61/2.08	40.6	**1.91**/2.0
15	59.8	3.71 and 3.96	60.1	3.75 and 4.02	62.9	**4.4**/**4.6**
C4-Me	22.4 (ax)	0.75	22.4	0.84	22.2	0.87
C4-Me	26.6 (eq)	1.03	28.5	1.00	28.5	0.99
C8-Me	18.11	0.90	18.0	1.05	18.2	1.06
C10-Me	18.3	**0.80**	18.1	0.98	18.2	0.97
C13-Me	28.9	**1.04**	26.3	1.26	27.5	1.31
aromatic (1′)					127.9	
(2′/6′)					131.2	
(3′/5′)					131.8	
(4′)					129.4	
C=O					166.0	

[a] Chemical shifts in bold represent major shifts from the corresponding ones of **1** in $CDCl_3$.

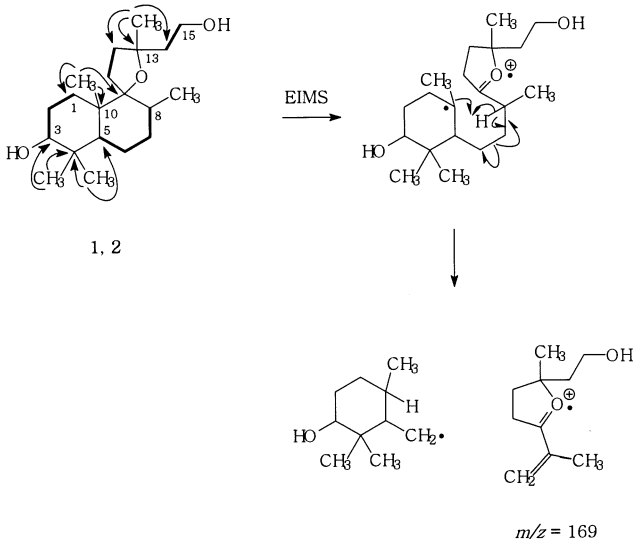

Figure 1. Skeletal structure of mopaneols A (**1**) and B (**2**). Solid lines indicate COSY and TOCSY connectivities, arrows indicate important HMBC correlations, and fragmentation scheme explains the base peak in the EIMS.

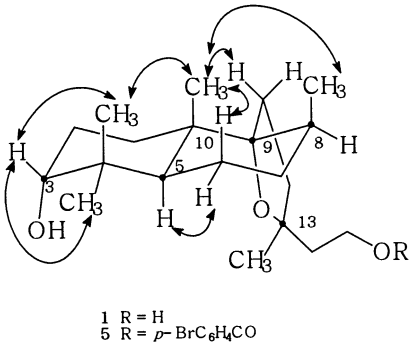

Figure 2. Mopaneol A (**1**) and its important NOESY correlations leading to stereochemical assignments at C-3, C-5, C-8, and C-9 relative to C-10.

latter hydrogens, while diastereotopic, were nonetheless magnetically equivalent in C_6D_6, making correlation of the stereochemistry at C-13 to C-10 indirectly through the two H-12 hydrogens impossible. A similar complication arose using $CDCl_3$. Under this solvent, the diastereotopic hydrogens at C-12 became differentiable (δ 1.73 and 1.85; Table 1), but the diastereotopic hydrogens at C-11 became magnetically equivalent.

We ultimately supplemented the NMR analysis with chemical methods to establish the stereochemistry at C-13. Specifically, we were able to selectively esterify the primary alcohol at C-15 with *p*-bromobenzoyl chloride in pyridine to yield **5** (Figure 2; R = *p*-BrC_6H_4CO). While no new useful NOE correlations were seen, a comparison of the ^{1}H NMR signals of the decalin system of **1** and **5** was informative (Table 1). In particular the ^{1}H NMR signals from the two decalin systems in $CDCl_3$ were essentially identical (Table 1) except for those arising from H-8 ($\Delta\delta$ = 0.12 ppm) and H-5 ($\Delta\delta$ = 0.27 ppm). The large change in the chemical shift of H-8 is consistent only with an *S*-configuration at C-13 in which the benzoyl group is placed on the same face as the saturated furan ring system as H-8. Interestingly, this observed shift of H-8 with benzoylation at C-13 independently confirms the stereochemistry at C-8 where the methine hydrogen is α, while the corresponding large shift of H-5 independently confirms the *trans*-decalin configuration and the stereochemistry of the ether linkage at C-9.

A hexane extract of seed husks (90 g) was flash chromatographed on silica gel to yield mopaneol B (**2**) as an oil (17 mg). The HREIMS, EIMS, IR, COSY, ^{1}H NMR, and ^{13}C NMR spectra were nearly identical with those of **1** (see Tables 1 and 2 for NMR comparisons), suggesting **1** and **2** are stereoisomers. We concluded that **2** is a C-13 epimer

Table 2. NMR Chemical Shifts (δ relative to TMS in C_6D_6) for Mopaneol B (**2**)

assignment	^{13}C NMR	^{1}H NMR
1	26.4	0.88(eq) 2.05(ax)
2	25.4	1.59(eq) 1.76(ax)
3	76.0	3.24
4	37.9	
5	42.1	1.99
6	17.2	1.31 (ax and eq)
7	30.0	1.39(eq) 2.14(ax)
8	38.4	1.86
9	93.8	
10	42.0	
11	27.7	1.54(ProS) 1.62(ProR)
12	39.0	1.36 and 1.51
13	83.5	
14	44.6	1.33 and 1.74
15	60.6	3.68 and 3.90
C4-Me(ax)	22.4	0.71
C4-Me(eq)	28.8	0.89
C8-Me	18.6	0.91
C10-Me	18.4	0.78
C13-Me	25.7	1.13

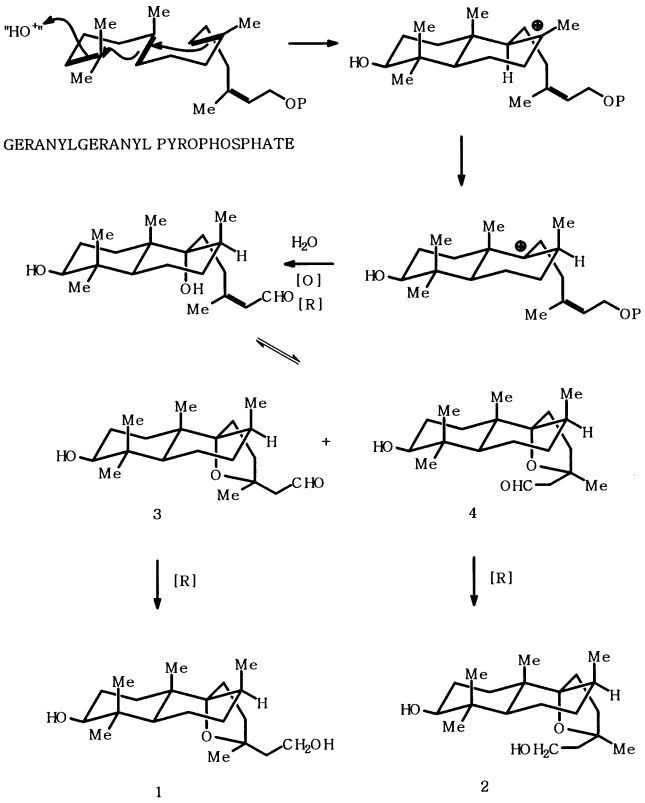

Figure 3. Proposed biogenesis of **1**−**4** from geranylgeraniol pyrophosphate.

of **1** (Figure 3). Any other epimeric structure would be expected to have significant differences in the chemical shifts of the decalin system. This was subsequently verified by a NOESY experiment, which demonstrated identical spatial relations for the decalin system described above for these experiments in **1**.

A mixture of two compounds that resisted further purification (other than GC and TLC) was obtained by flash chromatography (silica gel) of a hexane extract of mopane roots. Aldehyde functionalities were seen from ^{13}C NMR (δ 202.3), ^{1}H NMR (δ 9.8; triplet), and EIMS (strong M − 1 peak at $m/z = 321$). A comparison of the combined spectra of the aldehydes (see Experimental Section) and those of moponeols A and B strongly suggested structures **3** and **4**, which could exist in equilibrium through a reversible Michael addition mechanism (Figure 3). This conclusion was subsequently confirmed by their reduction ($NaBH_4$/alcohol) to a mixture of **1** and **2**, as confirmed by GC/MS comparisons with authentic material.

The structures of **1**−**4** are supportive of a classic biosynthesis of 9,13-epoxylabdanes from geranylgeraniol pyrophosphate (Figure 3). The *trans*-decalin system, for instance, is a logical consequence of a concerted electrophilic initiated cyclization as the first step. The resulting 3-β-hydroxy substituent is easily epimerized at a later stage by standard biochemical processes such as oxidation followed by reduction. Similarly, the hydride shift shown in the second step of Figure 3 clearly forces the C-8 methyl to occupy a β configuration. The capture of H_2O by the resulting C-9 carbocation faces extreme steric interactions on the β-face from the three axial methyls at C-4, C-8, and C-10. Consequently, formation of an α alcohol at C-9 is energetically favored over the corresponding β alcohol and sets the stage for the α ether linkage at C-9 for most known 9,13-epoxylabdanes. Finally, formation of the ether linkage probably involves a reversible Michael addition to the α,β-unsaturated aldehyde, leading directly to an equilibrating mixture of **3** and **4**, which upon reduction provides **1** and **2**.

Our biogenesis scheme clearly explains why all reported 9,13-epoxylabdanes have a *trans*-decalin ring systems with highly variable stereochemistries at C-9 and C-13. Until recently, however, all reported 9,13-epoxylabdanes have had a C-8-α methyl. Recent examples of C-8-β methyls, including a 3-deoxy analogue of **3** and other "biogenetically primitive" 9,13-epoxylabdanes from mopane,[8] along with our results suggest that C-8-α methyls of 9,13-epoxylabdanes are the result of epimerization of initially formed C-8-β methyls.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a 300 MHz Varian Mercury spectrometer using standard pulse sequences. Peak assignments were made using a combination of standard gHSQC, gHMBC, gCOSY, gNOESY, and TOCSY experiments. For **2**, limited material required ^{13}C assignments to be made from indirect ^{1}H NMR techniques (gHSQC and gHMBC). Chemical shifts were determined from residual solvent peaks and are reported as δ values. Mass spectrometry measurements were performed on a HP 5972 spectrometer interfaced with a HP 5890 gas chromatograph containing a 5% phenyl methyl siloxane capillary column (30m × 0.53 mm; Alltech).

Plant Material. *C. mopane* was collected in the Sengwa Wildlife Research Area located within the Sebungwe region of Zimbabwe (between 18°01′ and 18°13′ S, 28°03′ and 28°20′ E. Further details of this collection have been reported earlier.[3] A voucher specimen (#V136702) for this collection has been deposited at the University of Alaska Fairbanks Museum.

Isolations. Mopaneols A (**1**) and B (**2**) along with the corresponding aldehydes (**3** and **4**) were detected in the hexane extracts of most plant parts (leaves, stems, roots, and seeds) by TLC (silica gel). The only exception was **2**, which was absent in the seed samples. In a typical isolation, seed husks (86.5 g) were pulverized in a Waring blender and exhaustively extracted with hexanes. After removal of solvent under reduced pressure, the crude extract was chromatographed (flash chromatography) on silica gel using 10% EtOAc in $CHCl_3$ to yield **1** (71 mg) and **2** (34 mg) in a second eluting fraction. These were further purified by chromatography using a $CHCl_3/Et_2O$ solvent gradient from 10%, 30%, and 50% ether in $CHCl_3$.

Compound 1: pale yellow oil; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 324 $[M]^+$ (16), 207 (12), 183 (32), 170 (23), 169 (100), 156 (14), 151 (32), 139 (35), 137 (10), 134 (15), 125 (10), 123 (24), 121 (16), 119 (12), 111 (11), 109 (30), 107 (21), 105 (12); HREIMS *m*/*z* 324.2640 (calcd for $C_{20}H_{36}O_3$, 324.2666).

Compound 2: pale yellow oil; ^{1}H and ^{13}C NMR data, see Table 2; EIMS *m*/*z* 324 $[M]^+$ (12), 183 (26), 170 (22), 169 (100), 156 (13), 151 (28), 139 (27), 135 (10), 123 (14), 121 (14), 109 (23), 107 (16); HREIMS *m*/*z* 324.2657 (calcd for $C_{20}H_{36}O_3$, 324.2666).

Compounds **3** and **4** were isolated as an epimeric mixture from a hexanes extract of roots using similar chromatographic systems. In one isolation, 14 mg of **3**/**4** was isolated from 1.5 g of root as a pale yellow oil. The NMR spectra of the mixture showed strong similarities to **1** and **2** with significant differences only at C-13, C-14, and C-15: ^{13}C NMR δ 26.0 (C-1), 24.6 (C-2), 76.1 (C-3), 37.6 (C-4), 41.1 (C-5), 29.3 (C-6), 16.6 (C-7), 40.9 (C-8), 93.2 (C-9), 41.6 (C-10), 28.8 (C-11), 38.2 and 38.4 (C-12 of **3** and **4**), 80.6 (C-13), 55.3 and 55.9 (C-14 of **3** and **4**), 203.2 (C-15), 26.1 (C-13 methyl), 18.0 (C-8 methyl), 28.6 (C-4 ax methyl), 22.1 (C-4 eq methyl), 18.0 (C-10 methyl); ^{1}H NMR (partial) δ 9.8 (t, J = 2.5 Hz; H-15), 3.3 (t, J = 3 Hz; H-3), 2.6 (m, H-14), 1.3 (s, C-13 methyl), 0.90 (br s, C-4 methyls), and 0.80 (s, C-10 methyl); EIMS (both isomers) *m*/*z* 322 $[M]^+$ (13), 209 (6), 197 (13), 184 (14), 183 (100), 170 (11), 165 (10), 124 (5), 123 (16), 109 (10), 107 (5); HREIMS *m*/*z* 322.2492 and 322.2496 (calcd for $C_{20}H_{34}O_3$, 322.2509).

Esterification of Mopaneol A (1). In a typical procedure, **1** (16 mg) was dissolved in dry pyridine (1.0 mL) and 24 mg of *p*-bromobenzoyl chloride was added. The reaction was allowed to proceed under reflux for 1 h, and then the reaction mixture was partitioned between Et_2O (10 mL) and two portions of 10% HCl (10 mL). After neutralization ($NaHCO_3$) and drying ($MgSO_4$), the ether was removed under reduced pressure to yield a crude product, which after flash chromatography (silica gel; 20% Et_2O in $CHCl_3$) yielded **5** (12.0 mg, 62%).

Compound 5: pale yellow oil; ^{1}H and ^{13}C NMR data, see Table 1.

Reduction of 3/4 to Mopaneols A and B. A mixture of **3** and **4** (7 mg) was dissolved in dry MeOH (2.0 mL). A stock solution containing $NaBH_4$ (100 mg) and NaOMe (50 mg) in dry MeOH (5 mL) was added in 0.5 mL aliquots. After each addition, the solution was stirred at room temperature for 30 min and was then analyzed by TLC (silica gel; 10% Et_2O in $CHCl_3$). After 1.5 mL of the above stock solution had been added, TLC showed the formation of two spots consistent with **1** and **2** and no starting material. GC/MS comparisons with authentic samples of **1** and **2** confirmed this result.

Acknowledgment. We wish to thank the National Science Foundation for their support (DUE-9850731) in the purchase of the 300 MHz NMR that made this study possible. We would also like to thank Elliot Rachlin of the Department of Chemistry at the University of Utah for running the HRMS of our samples.

References and Notes

(1) Timberlake, J. R. *Zimbabwe Bull. For. Res.* **1995**, *11*, 1−49.
(2) Bartlett, E. *Electron. Mail Guardian* **1997**, *May 23*.
(3) Dudley, J. P. Foraging Ecology and Conservation Biology of African Elephants: Ecological and Evolutionary Perspectives on Elephant-Woody Plant Interactions in African Landscapes. Ph.D. Thesis, University of Alaska Fairbanks, Fairbanks, AK, May 1999, pp 1−188.
(4) Devon, T. K.; Scott, A. I. *Handbook of Naturally Occurring Compounds, Vol. II Terpenes*; Academic Press: New York, 1972; pp 185−279.
(5) Rivett, D. E. A. *J. S. Afr. Chem. Inst.* **1975**, *28*, 305−311.
(6) Capon, R. J.; Faulkner, D. J. *J. Chem. Soc.* **1984**, *106*, 1819−1822.
(7) Chan, J. A.; Freyer, A. J.; Carté, B. K.; Hemling, M. E.; Hofmann, G. A.; Mattern, M. R.; Mentzer, M. A.; Westley, J. W. *J. Nat. Prod.* **1994**, *57*, 1543−1548.
(8) Mebe, P. *Phytochemistry* **2001**, *57*, 537−541.

NP0202430