ent-12-Oxolabda-8,13(16)-dien-15-oic acid and ent-8β,12α-Epidioxy-12β-hydroxylabda-9(11),13-dien-15-oic Acid γ-Lactone: Two New Diterpenes from the Aerial Parts of *Premna oligotricha*.

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Two novel *ent*-labdane diterpenes have been isolated from the aerial parts of *Premna oligotricha* (Verbenaceae). Primarily through NMR studies one was identified as *ent*-12-oxolabda-8,13(16)-dien-15-oic acid. Similar analysis of the second diterpene was unable to distinguish between two possible structures, *ent*-8β,12α-epidioxy-12β-hydroxylabda-9(11),13-dien-15-oic acid γ-lactone and *ent*-8β,12α-epoxy-12β-hydroperoxylabda-9(11),13-dien-15-oic acid δ-lactone. The structure of the second diterpene was established as the γ-lactone by an X-ray diffraction study

In a recent paper[1] we reported the isolation of the novel clerodane diterpene **4** with antibacterial activity from the aerial parts of *Premna schimperi*. As a result of this we have undertaken the analysis of other *Premna* species and we now wish to record the presence of two novel labdane diterpenes in *Premna oligotricha* Baker. Neither of these compounds has strong antibacterial activity.

Fig. 1 X-Ray molecular structure of compound **2**

Results and Discussion

The more polar of the two compounds analysed (by electron-impact MS) for M[+] 318 ($C_{20}H_{30}O_3$). Major fragments were observed at m/z 191 ($C_{14}H_{23}$)[+], 190 ($C_{14}H_{22}$)[+] and 113 ($C_5H_5O_3$)[+], identical with those reported for compound **4**.[1] The presence of the same six-carbon side-chain found in compound **4** was confirmed from the ^{1}H NMR spectrum (Table 1), which revealed isolated methylene resonances for 11-H_2, 14-H_2 and 16-H_2. However, unlike compound **4** the ^{1}H NMR resonances for the decaline system showed four methyl singlets (one vinylic).

Further analysis and the assignment of structure **1** and ^{1}H and ^{13}C chemical-shift values (Table 1) are based on an HMBC (Heteronuclear Multiple Bond Coherence) NMR study.[2] In this experiment [2]J and [3]J heteronuclear interactions are employed to reveal connectivities. Key observations are shown in Table 2. A [3]J interaction between two methyls (δ_H 0.82, 0.89; δ_C 33.1, 21.6) permits them to be placed as the *geminal* C-4 substituents. A [2]J interaction from these methyl protons to a singlet at δ_C 33.2 identifies C-4, and [3]J couplings to a methylene (δ_C 41.4) and methine (δ_C 51.2) similarly identify C-3 and C-5, respectively. A further [3]J coupling to C-5 from another methyl resonance (δ_H 0.92, δ_C 19.8) allows assignment of the C-20 Me group, which also shows a [2]J coupling to identify C-10 and [3]J couplings to C-1 (methylene) and C-9 (quaternary olefinic). Thus the double bond in the decalin system must be placed at C-8/C-9 and this was substantiated by observations for the vinylic 8-methyl (δ_H 1.43, δ_C 20.0) which shows [3]J interactions to C-9 and the C-7 methylene, and a [2]J coupling to C-8.

This analysis of connectivities from HMBC can readily be extended into the side-chain. The 11-H_2 methylene protons reveal four interactions, [3]J to C-8 and C-10 and [2]J to C-9 and C-12. The C-12 carbonyl carbon is further linked to 14-H_2 and 16-H_2 by [3]J interactions, while [2]J couplings are observed for 16-H_2 to C-14, 14-H_2 and 16-H_2 to C-13, and 14-H_2 to the carboxylic acid carbon C-15. Thus, only C-2 and C-6 methylenes have not been directly assigned through this process.

Compound **1** was assumed to be an *ent*-labdane in the light of its negative specific rotation. A perusal of the literature[3] revealed that *ent*-labdanes are laevorotatory while labdanes are dextrorotatory. The specific rotations of a number of labd-8-ene derivatives are recorded as dextrorotatory.[4]
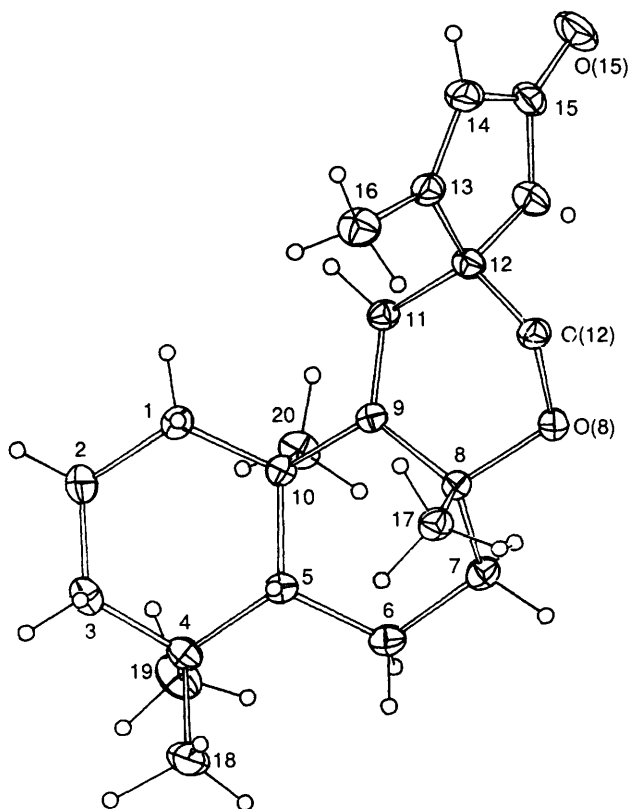
Table 1 ^{1}H and ^{13}C NMR chemical-shift data for compounds 1 and 2; all spectra run in CDCl$_3$

	1		2	
	^{1}H	^{13}C	^{1}H	^{13}C
C-1	1.03 and 1.43 m	36.1^a	1.26 ddd and 1.85 m	40.3
C-2	1.47 m	18.9^b	1.50–1.60 m	19.0
C-3	1.18 and 1.38 m	41.4	1.22 ddd and 1.50 m	41.8
C-4		33.2		33.5
C-5	1.27 dd	51.2	1.57 m	43.6
C-6	1.47 m	18.9^b	1.50–1.60 m	16.5
C-7	2.17 m	33.5	1.65 m	26.3
C-8		130.1		78.1
C-9		133.7		163.1
C-10		38.2		38.4
C-11	3.42/3.55 ABq (18.0)	36.1^a	5.23 s	111.2
C-12		198.8		107.1
C-13		141.7		162.4
C-14	3.35 s	37.5	5.98 q (2.0)	121.0
C-15		176.5		169.5
C-16	5.93 s, 6.25 s	126.4	2.01 d (2.0)	13.0
C-17	1.43 s	20.0	1.70 s	25.3
C-18	0.89 s	33.1	0.91 s	32.9
C-19	0.82 s	21.6	0.94 s	21.2
C-20	0.92 s	19.8	1.23 s	25.6

^{a,b} Signals with the same superscript were not identical and could not be unambiguously assigned.

Table 2 HMBC correlations for compound 1

	^{13}C	
^{1}H	^3J	^2J
18-H$_3$	21.6, 41.4, 51.2	33.2
19-H$_3$	33.1, 41.4, 51.2	33.2
20-H$_3$	36.1, 51.2, 133.7	38.2
17-H$_3$	33.5, 133.7	130.1
11-H$_2$	38.2, 130.1	133.7, 198.8
16-H$_2$	37.5, 198.8	141.7
14-H$_2$	198.8	141.7, 176.5

Table 3 HMBC correlations for compound 2

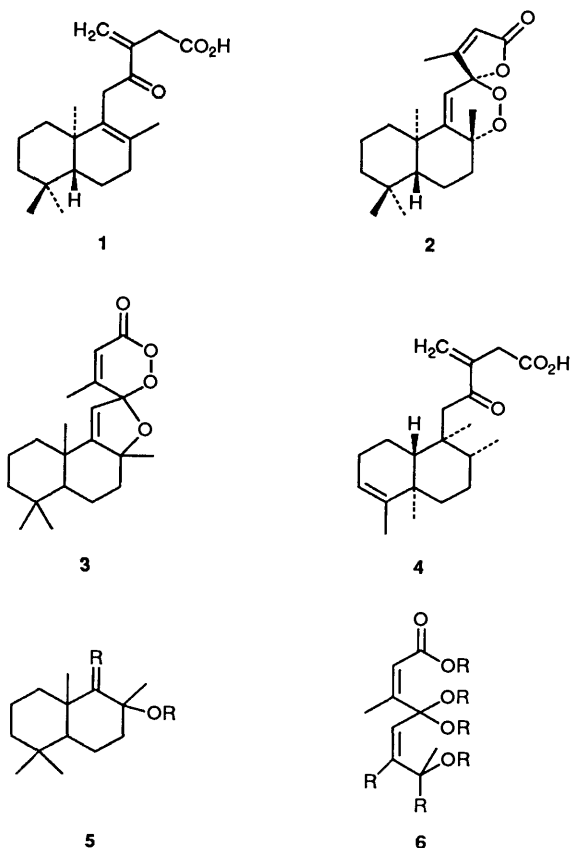|	^{13}C	
^{1}H	^3J	^2J
18-H$_3$	21.2, 41.8, 43.6	33.5
19-H$_3$	32.9, 41.8, 43.6	33.5
20-H$_3$	40.3, 43.6, 163.1	38.4
17-H$_3$	26.3, 163.1	78.1
11-H	38.4, 78.1	107.1
16-H$_3$	107.1, 121.0	162.4
14-H	107.1	169.5

The electron-impact MS of the less polar compound was ambiguous, giving a weak ion at m/z 332 $(C_{20}H_{28}O_4)^+$ and a much stronger ion at m/z 300 $(C_{20}H_{28}O_2)^+$. The validity of the 332 MU ion was confirmed by fast-atom bombardment MS, which revealed MH$^+$ at m/z 333. This suggested the presence of a peroxide, for which M$^+$ − 32 is a common fragment in electron-impact MS. The ^{1}H NMR spectrum (Table 1) showed the presence of five methyl singlets, of which one was vinylic and showed long-range coupling to an olefinic proton at δ_H 5.98. A second olefinic proton appeared as a sharp singlet at δ_H 5.23. All remaining signals occurred between δ_H 1.90 and 1.45, except for two ddds (1 H each) at δ_H 1.25 for axial methylene protons. The ^{13}C NMR spectrum revealed the presence of five methylenes, three methines and seven quaternary carbons (Table 1). ^{1}H and ^{13}C resonances were correlated using the HMQC (Heteronuclear Multiple Quantum Coherence) procedure.[5]

As with compound 1 ^{1}H–^{13}C connectivities were established through an HMBC experiment (Table 3). Assignments around the C-18 and C-19 methyl groups followed exactly the same arguments as for compound 1. For 20-H$_3$ and 17-H$_3$ ^3J correlations revealed that C-9 was again an olefinic carbon but in this case the ^2J coupling from 17-H$_3$ revealed that C-8 was an oxygen-bearing sp^3 carbon. This suggested that the second diterpene had partial structure 5.

The isolated olefinic proton had to be placed at C-11 as it showed ^3J interactions with C-8 and C-10, while a ^2J coupling

linked it to a singlet resonating at δ_C 107.1. This carbon must be assigned to C-12, which was confirmed by ^3J interactions with the second olefinic proton (14-H) and the vinylic methyl (16-H$_3$). The only other interaction of significance was a ^2J coupling linking 14-H to a carbonyl (C-15), which must form part of a lactone system (ν_{max} 1770 cm^{-1}).

In this diterpene only three carbons exist to which oxygen can be bonded, C-8, C-12 and C-15. The strongly deshielded nature of C-12 suggests it is bonded to two oxygen atoms (partial structure 6). Given that the compound contains four oxygens this requires that a peroxide be present. In theory this peroxide could be placed between C-8 and C-12 (structure 2) or between C-12 and C-15 (structure 3); none of the NMR experiments performed allowed for differentiation between these possibilities.

The identity of the new compound as compound 2 was finally confirmed by means of an X-ray diffraction study (Fig. 1). This revealed that the A-ring is in a normal chair conformation but ring B is severely distorted due, primarily, to the 9(11) double bond. The peroxide ring is almost planar between C(9)–C(11)–C(12)–O(12), while O(8) is displaced below the plane of the ring. The absolute stereochemistry of compound 2 has not been unambiguously assigned but it seems probable that, like compound 1, it is based on the ent-labdane skeleton.

It is possible to envisage the formation of compound 2 from

Table 4 Non-hydrogen atom fractional co-ordinates for compound 2

Atom	x	y	z
C(1)	0.4765(2)	0.3217(3)	0.5928(3)
C(2)	0.5686(3)	0.2942(4)	0.5140(3)
C(3)	0.6561(3)	0.2597(3)	0.5923(3)
C(4)	0.6877(2)	0.3427(2)	0.6884(3)
C(5)	0.5927(2)	0.3739(2)	0.7644(3)
C(6)	0.6141(2)	0.4509(3)	0.8721(3)
C(7)	0.5205(2)	0.4899(3)	0.9402(3)
C(8)	0.4301(2)	0.4171(2)	0.9183(3)
O(8)	0.3492(1)	0.4765(2)	0.9761(2)
C(9)	0.4090(2)	0.4137(2)	0.7805(2)
C(10)	0.4978(2)	0.4077(2)	0.6900(2)
C(11)	0.3148(2)	0.4260(2)	0.7434(3)
C(12)	0.2283(2)	0.4274(2)	0.8306(3)
O(12)	0.2564(1)	0.4148(2)	0.9545(2)
C(13)	0.1522(2)	0.3399(2)	0.8064(3)
C(14)	0.0647(2)	0.3848(3)	0.7819(3)
C(15)	0.0752(2)	0.5013(3)	0.7873(3)
O(15)	0.0139(2)	0.5703(2)	0.7711(2)
O	0.1724(2)	0.5257(2)	0.8161(2)
C(16)	0.1823(3)	0.2252(3)	0.8118(5)
C(17)	0.4401(3)	0.3084(3)	0.9830(3)
C(18)	0.7637(3)	0.2886(4)	0.7762(4)
C(19)	0.7422(3)	0.4364(3)	0.6264(5)
C(20)	0.5068(3)	0.5190(3)	0.6284(4)

Table 5 Non-hydrogen bond lengths (Å) for compound 2

Atom	Distance
C(1)–C(2)	1.532(5)
C(1)–C(10)	1.529(4)
C(2)–C(3)	1.503(5)
C(3)–C(4)	1.526(5)
C(4)–C(5)	1.558(4)
C(4)–C(18)	1.542(5)
C(4)–C(19)	1.530(5)
C(5)–C(6)	1.536(4)
C(5)–C(10)	1.555(4)
C(5)–C(7)	1.527(4)
C(7)–C(8)	1.526(4)
C(8)–O(8)	1.448(3)
C(8)–C(9)	1.517(4)
C(8)–C(17)	1.531(4)
O(8)–O(12)	1.473(3)
C(9)–C(10)	1.536(4)
C(9)–C(11)	1.325(4)
C(10)–C(20)	1.543(4)
C(11)–C(12)	1.488(4)
C(12)–O(12)	1.400(3)
C(12)–C(13)	1.511(4)
C(12)–O	1.442(3)
C(13)–C(14)	1.318(4)
C(13)–C(16)	1.486(5)
C(14)–C(15)	1.461(5)
C(15)–O(15)	1.199(4)
C(15)–O	1.364(4)

Table 6 Non-hydrogen bond angles (°) for compound 2

Atoms	Angle
C(2)–C(1)–C(10)	113.0(3)
C(1)–C(2)–C(3)	111.7(3)
C(2)–C(3)–C(4)	113.8(3)
C(3)–C(4)–C(5)	107.8(2)
C(3)–C(4)–C(18)	107.7(3)
C(3)–C(4)–C(19)	110.5(3)
C(5)–C(4)–C(18)	108.4(3)
C(5)–C(4)–C(19)	115.1(3)
C(18)–C(4)–C(19)	107.1(3)
C(4)–C(5)–C(6)	114.0(2)
C(4)–C(5)–C(10)	117.0(2)
C(6)–C(5)–C(10)	112.0(2)
C(5)–C(6)–C(7)	114.4(2)
C(6)–C(7)–C(8)	112.2(3)
C(7)–C(8)–O(8)	102.4(2)
C(7)–C(8)–C(9)	108.4(2)
C(7)–C(8)–C(17)	112.8(2)
O(8)–C(8)–C(9)	107.5(2)
O(8)–C(8)–C(17)	108.7(2)
C(9)–C(8)–C(17)	116.1(2)
C(8)–O(8)–O(12)	106.6(2)
C(8)–C(9)–C(10)	119.0(2)
C(8)–C(9)–C(11)	118.0(2)
C(10)–C(9)–C(11)	122.6(2)
C(1)–C(10)–C(5)	108.4(2)
C(1)–C(10)–C(9)	109.3(2)
C(1)–C(10)–C(20)	110.4(2)
C(5)–C(10)–C(9)	107.9(2)
C(5)–C(10)–C(20)	113.8(2)
C(9)–C(10)–C(20)	107.0(2)
C(9)–C(11)–C(12)	122.7(3)
C(11)–C(12)–O(12)	113.5(2)
C(11)–C(12)–C(13)	113.5(2)
C(11)–C(12)–O	109.9(2)
O(12)–C(12)–C(13)	105.3(2)
O(12)–C(12)–O	109.7(2)
C(13)–C(12)–O	104.5(2)
O(8)–O(12)–C(12)	108.5(2)
C(12)–C(13)–C(14)	108.6(3)
C(12)–C(13)–C(16)	120.6(3)
C(14)–C(13)–C(16)	130.8(3)
C(13)–C(14)–C(15)	109.3(3)
C(14)–C(15)–O(15)	130.1(3)
C(14)–C(15)–O	108.8(3)
O(15)–C(15)–O	121.2(3)
C(12)–O–C(15)	108.9(2)

HMBC and HMQC experiments where a shift of 60° was applied.

Plant Material.—Collected from beside the Yabello–Mega road at *ca.* 1600 m altitude, Sidamo Province, southern Ethiopia, in September 1989. A voucher (SHM-12) has been deposited at the National Herbarium of Ethiopia, University of Addis Ababa.

Extraction and Isolation.—Ground aerial parts (1 kg) were extracted with cold ethanol for 5 days. Evaporation of the solvent yielded a gum (150 g), which was subjected to vacuum chromatography over silica gel and elution with light petroleum (boiling range 40–60 °C)–ethyl acetate mixtures of increasing polarity. The 7:3 eluent was collected and chlorophyll was removed by elution through a short column of Sephadex LH-20. The resulting yellow gum was then subjected to column chromatography over silica gel, again with light petroleum–ethyl acetate mixtures of increasing polarity as eluent. Compound 2 (250 mg) was obtained by elution with a 95:5 mixture. Impure compound 1 (127 mg) was obtained by elution with an 8:2 mixture and was subsequently purified by preparative TLC [silica gel; solvent light petroleum–chloroform–ethyl acetate (4:6:4)].

compound 1 and hence to speculate that compound 2 is an artefact. While not ruling out this possibility an attempt to convert compound 1 into compound 2 by bubbling oxygen through a solution of the former failed to lead to the production of detectable amounts of the latter.

Experimental

NMR experiments were conducted on a Bruker AC300 instrument which was modified in order to allow detection in the reverse mode. For the homonuclear experiments matrixes were 256 × 2K data points. Sine bell multiplication was applied in both dimensions before Fourier transformation, except for

Table 7 Ring torsion angles (°). Atoms are denoted by number only, O-italicized.[a]

Atoms	Angle
10–1–2–3	56.2(4)
1–2–3–4	−57.3(4)
2–3–4–5	52.8(4)
3–4–5–10	−51.2(3)
4–5–10–1	51.0(3)
2–1–10–5	−51.3(3)
10–5–6–7	39.3(4)
5–6–7–8	19.9(4)
6–7–8–9	−59.1(3)
7–8–9–10	40.5(3)
8–9–10–5	15.9(3)
6–5–10–9	−56.6(3)
8–8–9–11	−23.5(3)
8–9–11–12	−8.9(4)
9–11–12–12	0.1(4)
11–12–12–8	39.9(3)
8–8–12–12	−74.1(3)
9–8–8–12	63.2(2)

[a] The lactone ring is planar.

ent-12-*Oxolabda*-8,13(16)-*dien*-15-*oic* Acid **1**.—Gum, $[\alpha]_D$ −71° (c 0.1, CHCl$_3$); λ_{max}(EtOH)/nm 230; ν_{max}(film)/cm^{-1} 3500, 1701 and 1680; ^{1}H and ^{13}C NMR—see Table 1; m/z (EI–MS) 318 (M$^+$, C$_{20}$H$_{30}$O$_3$, 49%), 191 (C$_{14}$H$_{23}$, 59), 190 (C$_{14}$H$_{22}$, 100), 175 (C$_{13}$H$_{19}$, 71) and 113 (C$_5$H$_5$O$_3$, 43).

ent-8β,12α-*Epidioxy*-12β-*hydroxylabda*-9(11),13-*dien*-15-*oic* Acid γ-*Lactone* **2**.—Needles, m.p. 216 °C (from MeOH); $[\alpha]_D$ −128° (c 0.1, CHCl$_3$); λ_{max}(EtOH)/nm 227; ν_{max}(film)/cm^{-1} 1770 and 1655; ^{1}H and ^{13}C NMR—see Table 1; m/z(EI–MS) 332 (M$^+$, C$_{20}$H$_{28}$O$_4$, 4%), 300 (C$_{20}$H$_{28}$O$_2$, 100) and 177 (C$_{13}$H$_{21}$, 15); (FAB–MS, glycerol matrix) 333 (M + H)$^+$.

Crystal Data.—C$_{20}$H$_{28}$O$_4$, M 332.4; Orthorhombic, space group P2$_1$2$_1$2$_1$, (D$_2^4$, No. 19), a = 13.301(3), b = 12.471(4), c =

* *Supplementary data.* (see section 5.6.3 of Instructions for Authors, January issue); thermal and hydrogen-atom parameters have been lodged at the Cambridge Crystallographic Data Centre.

10.818(3) Å. V 1795 Å^3. $D_c(Z$ = 4) = 1.23 g cm^{-3}, F(000) = 720. μ_{Mo} = 0.5 cm^{-1}; specimen: 0.48 × 0.35 × 0.30 mm.

Structural Determination and Analysis.—A unique data set was measured at ≈295K to 2θ$_{max}$ 50° [Enraf–Nonius CAD-4 diffractometer, monochromatic Mo-Kα radiation (λ 0.7107$_3$ Å), 2θ/θ scan mode]. 1808 Independent reflections were obtained, 1478 with I > 3σ(I) being considered 'observed' and used in the full-matrix least-squares refinement without absorption correction, after solution of the structure by direct methods. Anisotropic thermal parameters were refined with U_{iso} Conventional residuals R, R' on $|F|$ were 0.033, 0.034 [statistical weights derivative of σ^2(I) = σ^2(I_{diff}) + 0.0004 σ^4(I_{diff})]. Neutral atom complex scattering factors were employed,[6] the chirality being assumed from the chemistry; computation used the XTAL 3.0 program system implemented by Hall.[7] Pertinent results are given in Fig. 1 and Tables 4–7.*

References

1 S. Habtemariam, A. I. Gray, G. W. Halbert and P. G. Waterman, *Planta Med.*, 1990, **56**, 187.
2 Ad. Bax and M. F. Summers, *J. Am. Chem. Soc.*, 1986, **108**, 2093.
3 *Dictionary of Chemical Compounds*, ed. J. Buckingham, 5th edn. and supplements, Chapman and Hall, London 1982–1987.
4 R. Caputo, L. Mangoni, P. Monaco and L. Previtera, *Phytochemistry*, 1974, **13**, 471.
5 Ad. Bax and S. Subramaniam, *J. Magn. Reson.*, 1986, **67**, 565.
6 *International Tables for X-Ray Crystallography*, ed., J. A. Ibers and W. C. Hamilton, Kynoch Press, Birmingham, 1974, vol. 4.
7 The XTAL User's Manual, Version 3.0. S. R. Hall and J. M. Stewart for Universities of Western Australia and Maryland, 1990.