# Bioactive Diterpenes from *Orthosiphon labiatus* and *Salvia africana-lutea*

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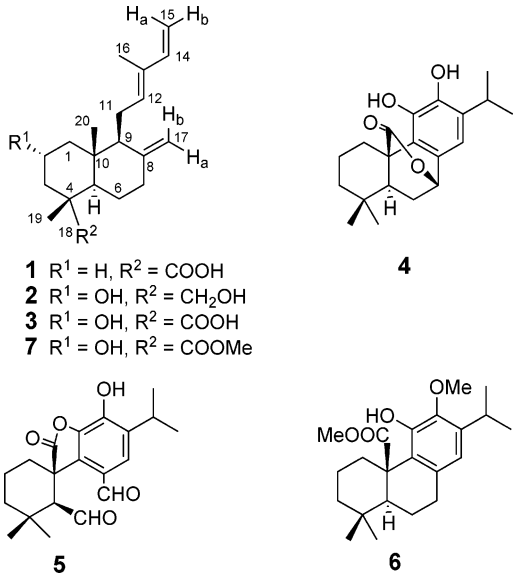
The known (+)-*trans*-ozic acid (**1**) and two new labdane diterpenoids (**2** and **3**) have been isolated from an ethanol extract of *Orthosiphon labiatus*. The structures of **2** and **3** were established mainly by 1D and 2D NMR spectroscopic means. The ethanolic extract of *Salvia africana-lutea* afforded the known abietane diterpenoids carnosol (**4**), rosmadial (**5**), and carnosic acid (characterized as its derivative **6**). Compounds **3** and **6** exhibited MICs of 157 and 28 $\mu$M, respectively, against *Mycobacterium tuberculosis*, while **2** and **6** showed cytotoxic activity with $IC_{50}$ 82 and 69 $\mu$M, respectively, against a breast (MCF-7) human cancer cell line.

Plants belonging to the *Orthosiphon* and *Salvia* genera (Labiatae) are rich sources of diterpenoids that display a vast array of interesting biological activities such as bactericidal, insecticidal, antiviral, and several others.[1] In particular, the isopimarane and staminane diterpenes isolated from *Orthosiphon* species growing in Southeast Asia have attracted interest in recent years on account of their nitric oxide inhibitory activity.[2,3] In continuation of our studies on biologically active diterpenoids from Labiatae plants of South Africa,[4] we have now investigated *Orthosiphon labiatus* N. E. Br. and *Salvia africana-lutea* L., two species that have not hitherto been studied chemically or pharmacologically, with the exception of the analgesic and antipyretic activities of the water extract of *S. africana-lutea*.[5] In this paper, we report on the isolation of three labdane diterpenoids, the known[6−8] (+)-*trans*-ozic acid (**1**) and two new analogues (**2** and **3**) from an EtOH extract of *O. labiatus*, and the abietane diterpenes [carnosol[9] (**4**), rosmadial[10] (**5**), and carnosic acid,[11] the latter identified as its derivative **6**] from *S. africana-lutea*. The structures of **2** and **3** were elucidated by 1D and 2D NMR spectroscopic means. In addition, we also report on the antimycobacterial and cytotoxic activities of these compounds.

Repeated chromatographic processes on the EtOH extract of fresh aerial parts of *O. labiatus* allowed the isolation of (+)-*trans*-ozic acid (**1**, also called 4-epicommunic acid[8]), previously found in Compositae, Umbelliferae, and Pinaceae species,[6−8] and the new labdane diterpenoids **2** and **3**, together with a mixture of ursolic and oleanolic acids.[12]

Low-resolution mass spectrometry and combustion analysis indicated a molecular formula of $C_{20}H_{32}O_2$ for diterpenoid **2**. Its IR spectrum showed hydroxyl (3400 $cm^{-1}$) and olefinic (3080, 1643, 1606, 888 $cm^{-1}$) absorptions. The $^1H$ and $^{13}C$ NMR spectra displayed resonances for an exocyclic methylene group and a C-9 side chain (see Experimental Section) identical to those reported[6,7] for **1** and other labda-8(17),12*E*,14-triene derivatives.[13,14] The NMR data of **2** showed resonances indicating an equatorial secondary hydroxyl group in a cyclohexane ring, placed between two methylene groups ($H_{ax}$ at $\delta$ 3.96, 1H, tt, $J_{a,a'} = J_{a,a''} = 11.4$ Hz, $J_{a,e'} = J_{a,e''} = 4.2$ Hz, $\delta_C$ 65.5, CH), thus representing C-2 of the labdane hydrocarbon skeleton.[14,15] It also indicated an AB system at $\delta$ 3.42 and 3.14 (1H each, both d, $J_{gem} = 10.9$ Hz), reminiscent of a hydroxymethylene group attached to a quaternary $sp^3$ carbon. The location of the primary hydroxyl group at the C-18 equatorial position was suggested by the HMBC spectrum, which showed connectivities between the hydroxymethylene protons and the C-3, C-4, C-5, and C-19 carbons [$\delta$ 44.6 ($CH_2$), 39.4 (C), 47.4 (CH), and 18.6 ($CH_3$), respectively], and confirmed by the chemical shifts of the equatorial C-18 ($\delta$ 71.4, $CH_2$) and axial C-19 ($\delta$ 18.6, $CH_3$) carbons.[15,16]




NOE experiments on **2** corroborated its 12*E*-configuration, because irradiation at $\delta$ 5.39 (H-12) caused a noticeable NOE enhancement (+8.9%) of H-14 ($\delta$ 6.31) and a smaller NOE (less than +0.9%) of Me-16 ($\delta$ 1.74). NOE experiments also supported the relative configuration of the decalin part of **2**, as is depicted in the formula. Irradiation of H-2$\beta_{ax}$ ($\delta$ 3.96) led to NOE enhancements of H-1$\beta$ ($\delta$ 2.14, +6.7%), H-3$\beta$ ($\delta$ 1.67, +3.8%), and Me-19 and Me-20 ($\delta$ 0.78 and 0.79, respectively, +10.7% for both), but not of H-5$\alpha$ ($\delta$ 1.05). This established that the indicated hydrogens and methyl groups are cofacial relative to the plane defined by the decalin ring system and suggesting that the A/B-ring junction is *trans*. The absence of NOE associations between H-5$\alpha$ and Me-19 and Me-20 further supported such an allocation. Irradiation of H-7$\alpha_{ax}$ ($\delta$ 2.03) caused, among others, NOE enhancements of H-5$\alpha_{ax}$ and H-9$\alpha_{ax}$ ($\delta$ 1.47 and 1.87, +4.2% and +5.4%, respectively), thus confirming a *trans* A/B-ring junction and establishing that the C-9 side chain of **2** is in a $\beta$-orientation. NOE experiments allowed the unambiguous assignment of both C-17

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methylene protons, because irradiation at $\delta$ 4.85 (H-17a) caused enhancement (+4.1%) of H-7$\beta$ ($\delta$ 2.38); thus H-17a was the pro-*E* hydrogen.

Diterpenoid **3** ($C_{20}H_{30}O_3$) yielded a methyl ester derivative (**7**, $C_{21}H_{32}O_3$) by treatment with diazomethane. Compounds **3** and **7** showed $^1$H and $^{13}$C NMR spectra (see Experimental Section) very similar to those reported[6,7] for the methyl ester derivative of **1**. The C-5−C-9 and C-11−C-20 resonances were identical in **7** and (+)-*trans*-ozic acid methyl ester,[7] whereas the differences in the chemical shifts of C-1−C-4 and C-10 [$\Delta\delta = \delta$ (**7**) $- \delta$ (methyl ozate): +9.2, +46.5, +7.9, +1.0, and +1.4 ppm, respectively] were in agreement with the presence in **7**, and consequently in **3**, of an equatorial hydroxyl group at C-2, such as in **2**. This was supported by the $^1$H NMR spectra of **3** and **7**, which showed H-2$\beta$ at $\delta$ 3.94 and 3.91, respectively, as a triplet of triplets ($J_{a,a'} = J_{a,a''} = 11.4-11.5$ Hz, $J_{a,e'} = J_{a,e''} = 4.2$ Hz) like in **2**. 2D NMR (COSY, HSQC, and HMBC) and 1D NOESY experiments on **3** and **7** were in agreement with the proposed structures and allowed the unequivocal and complete assignment of their $^1$H and $^{13}$C NMR spectra.

The absolute configuration of **2** and **3** was not ascertained by direct methods. However, on biogenetic grounds, we assume that these compounds belong to the *normal* series, like **1**,[6−8] a labdane diterpenoid co-occurring in the same species. From a biogenetic point of view, it is of interest that *O. labiatus* growing in South Africa contains labdane diterpenoids, whereas other *Orthosiphon* species belonging to the flora of Southeast Asia possess staminane and isopimarane derivatives.[2,3]

Chromatography of an EtOH extract of *S. africana-lutea* (see Experimental Section) yielded only three known abietanes: carnosol[9] (**4**), rosmadial[10] (**5**), and carnosic acid,[11] together with ursolic acid.[12] Carnosic acid was identified through its 12-*O*-methyl-20-methyl ester derivative **6**. Compounds **4**−**6** have also been found in other Labiatae species.[1,9−11]

Compounds **2**−**4** and **6** were tested against *Mycobacterium tuberculosis* (strain no. H37Rv ATCC27294). Compounds **3** and **6** exhibited inhibitory activity (MIC) of 157 and 28 $\mu$M, respectively, while the other two compounds were not active at the highest concentration tested (650 $\mu$M). Compounds **2** and **6** showed moderate cytotoxicity, with $IC_{50}$ values of 82 and 69 $\mu$M, respectively, against the breast (MCF-7) human cancer cell line, and the other two compounds showed no activity at 650 $\mu$M. It has been reported that carnosic acid showed higher antibacterial, antioxidant, and antimutagenic activities than carnosol[17−19] (**4**). An interesting point of note is that the 12-*O*-methyl-20-methyl ester of carnosic acid (**6**) showed higher antimycobacterium and cytotoxic activities than carnosol (**4**).[17−19]

## Experimental Section

**General Experimental Procedures.** Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. UV spectra were recorded on a Perkin-Elmer Lambda 2 UV/vis spectrophotometer. IR spectra were obtained on a Perkin-Elmer Spectrum One spectrophotometer. $^1$H and $^{13}$C NMR spectra were recorded in $CDCl_3$ solution on a Varian INOVA 400 spectrometer at 400 and 100 MHz, respectively. Chemical shifts are reported relative to the residual $CHCl_3$ signal ($\delta$ 7.25) for protons and $\delta_{CDCl3}$ 77.00 for carbons. All the assignments for protons and carbons were in agreement with 2D COSY, gHSQC, gHMBC, and 1D NOESY spectra. Mass spectra were registered in the positive EI mode on a Hewlett-Packard 5973 instrument (70 eV). Elemental analyses were conducted on a Leco CHNS-932 apparatus. Merck Si gel (70−230 mesh and 230−400 mesh, for gravity flow and flash chromatography, respectively) was used for column chromatography. Merck 5554 Kieselgel 60 F254 sheets were used for TLC analysis. Petroleum ether (bp 50−70 °C) was used for column chromatography.

**Plant Materials.** Aerial parts of *O. labiatus* N. E. Br. and *S. africana-lutea* L. were collected in September 2004, at the Botanic Garden of the Department of Botany, University of Pretoria, South Africa, and were identified by HGWJ Schweickerdt Herbarium of the University of Pretoria, where voucher specimens (registry numbers: *O. labiatus*, A. H. 095055; *S. africana-lutea*, A. H. 095053) are preserved.

**Extraction and Isolation.** Fresh aerial parts of *O. labiatus* (900 g) were crushed and extracted with EtOH (5 L) at room temperature for 1 day. Filtration and evaporation of the solvent at 160 mbar and 40 °C yielded a residue (24.9 g). The extract was subjected to column chromatography (Si gel 70−230 mesh, 1.3 kg) eluting with petroleum ether (1 L), then with petroleum ether−EtOAc (9:1, 3:1, 1:1, 1:2; 3 L each) mixtures, and finally with EtOAc (3 L), collecting fractions of 250 mL.

The residue (1.75 g) of the fractions eluted with 3:1 petroleum ether−EtOAc was rechromatographed (Si gel 230−400 mesh column, 350 g, eluted with a petroleum ether−EtOAc gradient from 4:1 to 2:1). The fractions eluted with 3:1 petroleum ether−EtOAc yielded (+)-*trans*-ozic acid[6−8] (**1**, 770 mg, 0.086% of plant material).

The residue (83 mg) of the fractions eluted with 1:1 petroleum ether−EtOAc from the initial chromatography was crystallized from MeOH, giving white crystals (62 mg, 0.0068%) of a mixture of ursolic and oleanolic acids.[12]

The fractions from the initial chromatography eluted with 1:2 petroleum ether−EtOAc gave a residue (692 mg), which, after crystallization from $Et_2O$−*n*-pentane, yielded pure **2** (502 mg, 0.0558%).

The residue (1.16 g) of the fractions eluted with EtOAc from the initial chromatography was rechromatographed (Si gel 230−400 mesh column, 100 g, eluted with 97:3 $Et_2O$−MeOH), giving pure **3** (720 mg), after crystallization from $CHCl_3$ (0.08% yield).

Fresh aerial parts of *S. africana-lutea* (1 kg) were crushed and extracted with EtOH (6.5 L) at room temperature for 1 day. Evaporation of the solvent as described above yielded a residue (19.7 g), which was dissolved in EtOAc (300 mL) and then washed with water (4 × 200 mL). The EtOAc solution was dried ($Na_2SO_4$), filtered, and evaporated in vacuo (230 mbar) at 40 °C, giving a residue (8.73 g). This residue was subjected to column chromatography (Si gel 230−400 mesh, 400 g) eluting with a petroleum ether−EtOAc gradient from 10% to 100% and collecting fractions of 150 mL. The fractions eluted with 3:1 petroleum ether−EtOAc gave a residue (877 mg), from which pure carnosol[9] (**4**, 609 mg, 0.06% yield) was obtained after crystallization from $Me_2CO$−*n*-pentane. Rechromatography (Si gel 230−400 mesh column, 30 g, 3:1 petroleum ether−EtOAc as eluent) of the residue (680 mg) of the fractions eluted with 1:1 petroleum ether−EtOAc subsequently yielded rosmadial[10] (**5**, 6 mg, 0.0006%) and ursolic acid[12] (240 mg, 0.024%).

The residue (1.30 g) of the fractions eluted with 1:2 petroleum ether−EtOAc from the initial chromatography showed in TLC a major constituent together with a complex mixture of other compounds. This residue was treated with an excess of an ethereal solution of $CH_2N_2$ at room temperature for 3 h and then subjected to column chromatography (Si gel 230−400 mesh, 70 g, eluted with 4:1 petroleum ether−EtOAc) to yield 12-*O*-methylcarnosic acid methyl ester[11] (**6**, 760 mg, 0.076%).

The known compounds (+)-*trans*-ozic acid[6−8] (**1**), carnosol[9] (**4**), rosmadial[10] (**5**), ursolic acid,[12] and methyl 12-*O*-methylcarnosoate[11] (**6**) were identified by their mp, $[\alpha]_D$, $^1$H and $^{13}$C NMR, and mass spectra, whereas the mixture of ursolic and oleanolic acids was characterized from $^1$H NMR data[12] and by comparison (TLC) with authentic samples.

**Labda-8(17),12*E*,14-triene-2α,18-diol (2):** colorless needles ($Et_2O$−*n*-pentane), mp 134−136 °C; $[\alpha]_D^{22}$ +24.1 (*c* 0.851, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 231 (4.31) nm; IR (KBr) $\nu_{max}$ 3400, 3080, 2935, 1643, 1606, 1433, 1388, 1036, 888 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 6.31 (1H, ddd, $J_{14,15a} = 17.4$ Hz, $J_{14,15b} = 10.7$ Hz, $J_{14,12} = 0.7$ Hz, H-14), 5.39 (1H, br t, $J_{12,11a} = J_{12,11b} = 6.5$ Hz, H-12), 5.04 (1H, ddd, $J_{15a,15b} = 1.2$ Hz, $J_{15a,14} = 17.4$ Hz, $J_{15a,12} = 0.6$ Hz, pro-Z H-15a), 4.87 (1H, ddd, $J_{15b,15a} = 1.2$ Hz, $J_{15b,14} = 10.7$ Hz, $J_{15b,12} = 1.0$ Hz, pro-*E* H-15b), 4.85 (1H, q, $J_{17a,17b} = J_{17a,7\alpha} = J_{17a,9\alpha} = 1.6$ Hz, pro-*E* H-17a), 4.49 (1H, q, $J_{17b,17a} = J_{17b,7\alpha} = J_{17b,9\alpha} = 1.6$ Hz, pro-*Z* H-17b), 3.96 (1H, tt, $J_{2\beta,1\alpha} = J_{2\beta,3\alpha} = 11.4$ Hz, $J_{2\beta,1\beta} = J_{2\beta,3\beta} = 4.2$ Hz, H-2$\beta$), 3.42 (1H, d, $J_{18a,18b} = 10.9$ Hz, H-18a), 3.14 (1H, d, $J_{18b,18a} = 10.9$ Hz, H-18b), 2.39 (1H, m,* H-11a), 2.38 (1H, ddd, $J_{7\beta,7\alpha} = 13.0$ Hz, $J_{7\beta,6\alpha} = 2.4$ Hz, $J_{7\beta,6\beta} = 4.3$ Hz, H-7$\beta$), 2.16 (1H, ddd, $J_{11b,11a} = 17.6$ Hz, $J_{11b,9\alpha} = 10.9$ Hz, $J_{11b,12} = 6.5$ Hz, H-11b), 2.14 (1H, ddd, $J_{1\beta,1\alpha} = 11.9$ Hz, $J_{1\beta,2\beta} = 4.2$ Hz, $J_{1\beta,3\beta} = 2.2$ Hz, H-1$\beta$), 2.03 (1H, br td, $J_{7\alpha,7\beta} = J_{7\alpha,6\beta} = 13.0$ Hz, $J_{7\alpha,6\alpha} = 5.3$ Hz, H-7α), 1.87 (1H, br dd, $J_{9\alpha,11a} = 2.2$ Hz, $J_{9\alpha,11b} = 10.9$ Hz, H-9α), 1.74 (3H, d, $J_{16,12} = 1.1$ Hz, Me-16), 1.67 (1H, ddd, $J_{3\beta,3\alpha} = 12.1$ Hz, $J_{3\beta,2\beta} = 4.2$ Hz, $J_{3\beta,1\beta} = 2.2$ Hz, H-3$\beta$), 1.62 (1H, dddd, $J_{6\alpha,6\beta} = 12.7$ Hz, $J_{6\alpha,5\alpha} = 2.7$ Hz, $J_{6\alpha,7\alpha} = 5.3$ Hz,

$J_{6\alpha,7\beta}$ = 2.4 Hz, H-6α), 1.47 (1H, dd, $J_{5\alpha,6\alpha}$ = 2.7 Hz, $J_{5\alpha,6\beta}$ = 12.7 Hz, H-5α), 1.41 (1H, dd, $J_{3\alpha,3\beta}$ = 12.1 Hz, $J_{3\alpha,2\beta}$ = 11.4 Hz, H-3α), 1.31 (1H, br qd, $J_{6\beta,6\alpha} = J_{6\beta,5\alpha}$ = 12.7 Hz, $J_{6\beta,7\alpha}$ = 13.0 Hz, $J_{6\beta,7\beta}$ = 4.3 Hz, H-6β), 1.05 (1H, dd, $J_{1\alpha,1\beta}$ = 11.9 Hz, $J_{1\alpha,2\beta}$ = 11.4 Hz, H-1α), 0.79 (3H, s, Me-20), 0.78 (3H, s, Me-19);[20] $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 147.4 (C, C-8), 141.5 (CH, C-14), 133.6 (C, C-13), 133.4 (CH, C-12), 110.0 ($CH_2$, C-15), 108.5 ($CH_2$, C-17), 71.4 ($CH_2$, C-18), 65.5 (CH, C-2), 56.8 (CH, C-9), 47.8 ($CH_2$, C-1), 47.4 (CH, C-5), 44.6 ($CH_2$, C-3), 40.7 (C, C-10), 39.4 (C, C-4), 37.5 ($CH_2$, C-7), 23.5 ($CH_2$, C-6), 23.3 ($CH_2$, C-11), 18.6 ($CH_3$, C-19), 15.9 ($CH_3$, C-20), 11.9 ($CH_3$, C-16); EIMS *m/z* 304 $[M]^+$ (0.5), 286 (5), 271 (8), 255 (22), 241 (11), 199 (17), 187 (25), 173 (31), 161 (29), 159 (41), 147 (42), 145 (54), 131 (50), 107 (68), 105 (93), 95 (50), 91 (79), 81 (100), 55 (84), 53 (38); *anal*. C 78.71%, H 10.68%, calcd for $C_{20}H_{32}O_2$, C 78.89%, H 10.59%.

**2α-Hydroxylabda-8(17),12E,14-trien-18-oic acid (3):** colorless prisms ($CHCl_3$), mp 130−134 °C; $[\alpha]_D^{22}$ +32.5 (*c* 0.283, 1:1 $CHCl_3$−MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 231 (4.46) nm; IR (KBr) $\nu_{max}$ 3412, 3087, 2926, 1678, 1642, 1389, 1224, 1169, 1033, 893 $cm^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) δ 6.31 (1H, ddd, $J_{14,15a}$ = 17.3 Hz, $J_{14,15b}$ = 10.7 Hz, $J_{14,12}$ = 0.8 Hz, H-14), 5.39 (1H, br t, $J_{12,11a} = J_{12,11b}$ = 6.4 Hz, H-12), 5.05 (1H, ddd, $J_{15a,15b}$ = 1.2 Hz, $J_{15a,14}$ = 17.3 Hz, $J_{15a,12}$ = 0.7 Hz, pro-*Z* H-15a), 4.88 (1H, ddd, $J_{15b,15a}$ = 1.2 Hz, $J_{15b,14}$ = 10.7 Hz, $J_{15b,12}$ = 1.0 Hz, pro-*E* H-15b), 4.86 (1H, q, $J_{17a,17b} = J_{17a,7\alpha} = J_{17a,9\alpha}$ = 1.4 Hz, pro-*E* H-17a), 4.51 (1H, q, $J_{17b,17a} = J_{17b,7\alpha} = J_{17b,9\alpha}$ = 1.4 Hz, pro-*Z* H-17b), 3.94 (1H, tt, $J_{2\beta,1\alpha} = J_{2\beta,3\alpha}$ = 11.4 Hz, $J_{2\beta,1\beta} = J_{2\beta,3\beta}$ = 4.2 Hz, H-2β), 2.37 (1H, m,* H-11a), 2.36 (1H, ddd, $J_{7\beta,7\alpha}$ = 12.8 Hz, $J_{7\beta,6\alpha}$ = 2.8 Hz, $J_{7\beta,6\beta}$ = 3.6 Hz, H-7β), 2.18 (1H, m,* H-11b), 2.17 (1H, m,* H-1β), 2.07 (1H, br td, $J_{7\alpha,7\beta} = J_{7\alpha,6\beta}$ = 12.8 Hz, $J_{7\alpha,6\alpha}$ = 5.0 Hz, H-7α), 1.98 (2H, m,* H-3β and H-5α), 1.90 (1H, br dd, $J_{9\alpha,11a}$ = 2.1 Hz, $J_{9\alpha,11b}$ = 11.4 Hz, H-9α), 1.75 (1H, dd, $J_{3\alpha,3\beta}$ = 12.6 Hz, $J_{3\alpha,2\beta}$ = 11.4 Hz, H-3α), 1.75 (3H, d, $J_{16,12}$ = 0.8 Hz, Me-16), 1.42 (1H, qd, $J_{6\beta,6\alpha} = J_{6\beta,5\alpha} = J_{6\beta,7\alpha}$ = 12.7 Hz, $J_{6\beta,7\beta}$ = 3.6 Hz, H-6β), 1.28 (1H, m,* H-6α), 1.19 (3H, s, Me-19), 1.18 (1H, dd, $J_{1\alpha,1\beta}$ = 12.0 Hz, $J_{1\alpha,2\beta}$ = 11.4 Hz, H-1α), 0.80 (3H, s, Me-20);[20] $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 182.9 (C, C-18), 146.6 (C, C-8), 141.4 (CH, C-14), 133.8 (C, C-13), 133.0 (CH, C-12), 110.2 ($CH_2$, C-15), 109.1 ($CH_2$, C-17), 65.0 (CH, C-2), 56.9 (CH, C-9), 49.2 (CH, C-5), 48.4 (C, C-4), 47.4 ($CH_2$, C-1), 45.4 ($CH_2$, C-3), 40.3 (C, C-10), 37.3 ($CH_2$, C-7), 25.7 ($CH_2$, C-6), 23.1 ($CH_2$, C-11), 17.4 ($CH_3$, C-19), 15.6 ($CH_3$, C-20), 11.9 ($CH_3$, C-16); EIMS *m/z* 318 $[M]^+$ (3), 300 (15), 285 (17), 272 (10), 255 (21), 239 (22), 199 (28), 173 (90), 159 (73), 131 (59), 119 (84), 105 (100), 91 (79), 79 (89), 55 (74); *anal*. C 75.31%, H 9.39%, calcd for $C_{20}H_{30}O_3$, C 75.43%, H 9.50%.

**Methyl 2α-hydroxylabda-8(17),12*E*,14-trien-18-oate (7).** A solution of **3** (200 mg, 0.629 mmol) in $Et_2O$ (60 mL) was treated with an excess of an ethereal solution of $CH_2N_2$ at room temperature for 5 h. After evaporation of the solvent a residue (209 mg) remained. This residue was chromatographed (Si gel 230−400 mesh column, 2 g, 1:1 petroleum ether−EtOAc as eluent), yielding **7** (205 mg, 0.617 mmol, 98.1%): amorphous, white solid; $[\alpha]_D^{22}$ +30.4 (*c* 0.293, $CHCl_3$); IR (KBr) $\nu_{max}$ 3433, 3080, 2937, 1729, 1643, 1606, 1435, 1390, 1237, 1122, 1040, 891, 757 $cm^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) δ 6.30 (1H, ddd, $J_{14,15a}$ = 17.4 Hz, $J_{14,15b}$ = 10.7 Hz, $J_{14,12}$ = 0.8 Hz, H-14), 5.37 (1H, br t, $J_{12,11a} = J_{12,11b}$ = 6.6 Hz, H-12), 5.03 (1H, ddd, $J_{15a,15b}$ = 1.2 Hz, $J_{15a,14}$ = 17.3 Hz, $J_{15a,12}$ = 0.6 Hz, pro-*Z* H-15a), 4.87 (1H, ddd, $J_{15b,15a}$ = 1.2 Hz, $J_{15b,14}$ = 10.7 Hz, $J_{15b,12}$ = 1.0 Hz, pro-*E* H-15b), 4.84 (1H, q, $J_{17a,17b} = J_{17a,7\alpha} = J_{17a,9\alpha}$ = 1.4 Hz, pro-*E* H-17a), 4.48 (1H, q, $J_{17b,17a} = J_{17b,7\alpha} = J_{17b,9\alpha}$ = 1.4 Hz, pro-*Z* H-17b), 3.91 (1H, tt, $J_{2\beta,1\alpha} = J_{2\beta,3\alpha}$ = 11.5 Hz, $J_{2\beta,1\beta} = J_{2\beta,3\beta}$ = 4.2 Hz, H-2β), 3.66 (3H, s, COO*Me*), 2.36 (1H, m,* H-11a), 2.32 (1H, ddd, $J_{7\beta,7\alpha}$ = 13.0 Hz, $J_{7\beta,6\alpha}$ = 2.3 Hz, $J_{7\beta,6\beta}$ = 4.4 Hz, H-7β), 2.16 (1H, m,* H-11b), 2.14 (1H, ddd, $J_{1\beta,1\alpha}$ = 12.0 Hz, $J_{1\beta,2\beta}$ = 4.2 Hz, $J_{1\beta,3\beta}$ = 2.1 Hz, H-1β), 2.03 (1H, br td, $J_{7\alpha,7\beta} = J_{7\alpha,6\beta}$ = 13.0 Hz, $J_{7\alpha,6\alpha}$ = 5.1 Hz, H-7α), 1.95 (1H, dd, $J_{5\alpha,6\alpha}$ = 2.8 Hz, $J_{5\alpha,6\beta}$ = 12.5 Hz, H-5α), 1.90 (1H, ddd, $J_{3\beta,3\alpha}$ = 12.7 Hz, $J_{3\beta,2\beta}$ = 4.2 Hz, $J_{3\beta,1\beta}$ = 2.1 Hz, H-3β), 1.89 (1H, m,* H-9α), 1.73 (3H, d, $J_{16,12}$ = 1.0 Hz, Me-16), 1.70 (1H, dd, $J_{3\alpha,3\beta}$ = 12.7 Hz, $J_{3\alpha,2\beta}$ = 11.4 Hz, H-3α), 1.40 (1H, dddd, $J_{6\beta,6\alpha}$ = 12.9 Hz, $J_{6\beta,5\alpha}$ = 12.5 Hz, $J_{6\beta,7\alpha}$ = 13.0 Hz, $J_{6\beta,7\beta}$ = 4.4 Hz, H-6β), 1.24 (1H, dddd, $J_{6\alpha,6\beta}$ = 12.9 Hz, $J_{6\alpha,5\alpha}$ = 2.8 Hz, $J_{6\alpha,7\alpha}$ = 5.1 Hz, $J_{6\alpha,7\beta}$ = 2.3 Hz, H-6α), 1.17 (3H, s, Me-19), 1.16 (1H, dd, $J_{1\alpha,1\beta}$ = 12.0 Hz, $J_{1\alpha,2\beta}$ = 11.5 Hz, H-1α), 0.78 (3H, s, Me-20);[20] $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 178.0 (C, C-18), 146.7 (C, C-8), 141.4 (CH, C-14), 133.7 (C, C-13), 133.0 (CH, C-12), 110.2 ($CH_2$, C-15), 108.9 ($CH_2$, C-17), 64.9 (CH, C-2), 56.8 (CH, C-9), 52.2 ($CH_3$, $COOCH_3$), 49.4 (CH, C-5), 48.7 (C, C-4), 47.4 ($CH_2$, C-1), 45.5 ($CH_2$, C-3), 40.3 (C, C-10), 37.3 ($CH_2$, C-7), 25.7 ($CH_2$, C-6), 23.0 ($CH_2$, C-11), 17.6 ($CH_3$, C-19), 15.6 ($CH_3$, C-20), 11.9 ($CH_3$, C-16); EIMS *m/z* 332 $[M]^+$ (1), 314 (6), 299 (5), 278 (4), 255 (18), 239 (23), 199 (25), 173 (84), 159 (67), 131 (54), 119 (100), 105 (75), 91 (64), 79 (70), 55 (65); *anal*. C 75.91%, H 9.58%, calcd for $C_{21}H_{32}O_3$, C 75.86%, H 9.70%.

**Biological Assays.** The antimycobacterial activity of **2**−**4** and **6** was evaluated against a drug-sensitive strain of *M. tuberculosis* (strain no. H37Rv ATCC27294) using a rapid radiometric method.[21] Susceptibility testing of *M. tuberculosis* was also performed for the two primary tuberculosis bacterium drugs, streptomycin and ethambutol, at concentrations of 0.004 and 0.006 mg/mL, respectively, against the H37Rv strain. The cytotoxic activity was determined against the breast (MCF-7) human cancer cell line according to the method given by Monks et al.[22]

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