CHEMICAL CONVERSION OF LABDANE-TYPE DITERPENOID ISOLATED FROM THE LIVERWORT *PORELLA PERROTTETIANA* INTO (-)-AMBROX[§]

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Abstract - As a part of our systematic investigation of biologically active substances of liverworts, the chemical conversion of labdane- type diterpenoid, labda-12, 14-dien-7α, 8α-diol (**1**) that the absolute configuration remained unidentified, isolated from the liverwort *Porella perrottetiana* into the animal perfume, (-)-ambrox (**2**) *via* 6 steps has been achieved. Consequently, the absolute configuration of **1** was determined.

Liverworts contain both terpenoids and aromatic compounds which constitute the oil bodies. We have reported the distribution of a number of new terpenoids and aromatic compounds possessing the interesting biological activities such as anti-microbial and cytotoxic activities in more than 200 species of the liverworts.[1,2] Previously, we reported the isolation and structure elucidation of a new labdane-type diterpenoid (**1**) from the liverwort *Porella perrottetiana* but the relative and absolute structures of **1** were not clear.[3] In the course of chemical conversion of a large amount of natural products isolated from liverwort into biologically active substances, **1** was converted into the most valuable animal perfume, (-)-ambrox (**2**) *via* 6 steps. Consequently, the absolute configuration of **1** was determined as labda-12, 14-dien-7α, 8α-diol (**1**).

[§]Dedicated to Dr. Bernhard Witkop

Isolation and Structure Elucidation of Labda-12, 14-dien-7α, 8α-diol

The ether extract (19.06 g) of dry material (1.13 kg) of *Porella perrottetiana* collected in Tokushima in 1994 was subjected repeatedly to column chromatography on silica gel with *n*-hexane-AcOEt, gradient to afford labda-12, 14-dien-7α, 8α-diol (**1**) (2.68 g).

The FT-IR and UV spectra of labda-12, 14-dien-7α, 8α-diol (**1**) ($C_{20}H_{34}O_2$) indicated the presence of a hydroxyl (3300 cm^{-1}) and a conjugated diene [λ_{max} 246 nm (log ε 4.05)] groups. The ^1H and ^{13}C NMR spectra of **1** showed the presence of four tertiary methyl [δ_H 1.04, 1.12, 1.15, 1.18 (each 3H, *s*)], a vinyl methyl [δ_H 1.75 (3H, *br s*)], a tri-substituted olefin [δ_H 4.99 (*br d*, J=9.9 Hz), 5.03 (*dd*, J=3.6, 9.6 Hz)], a di-substituted *trans*-olefine [δ_H 5.03 (*dd*, J=1.1, 16.2 Hz), 5.63 (*ddd*, J=6.9, 7.7, 16.2 Hz)], a tertiary hydroxyl group [δ_C 81.5 (*s*)] and a secondary hydroxyl group [δ_H 4.53 (*ddd*, J=5.5, 9.9, 9.9 Hz)], which was confirmed by the formation of a monoacetate (**3**) [δ 2.03 (3H, s)] on acetylation with Ac_2O and pyridine. The stereostructure of **1** was inferred by the careful analysis of HMBC and NOESY spectra as shown in Figure 1. The CD spectrum of *p*-bromobenzoate (**4**) of **1** showed a negative first Cotton effect at 247 nm (Δε-5.62) and a positive second Cotton effect at 220 nm (Δε+4.01) as shown in Figure 2. The absolute configuration of **1** was presumed to be *R* configuration by an exciton chirality method as applied to the conjugated diene.[4]

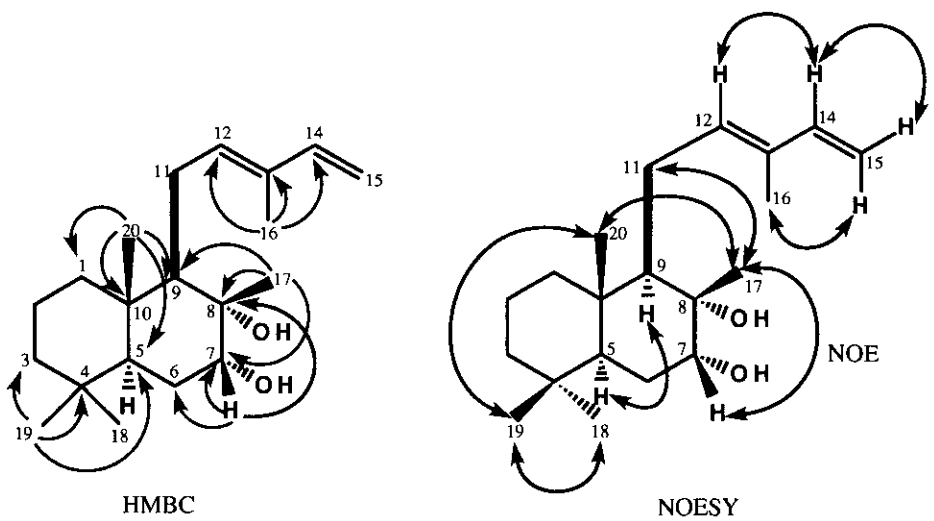


Figure 1. HMBC and NOESY Spectra of **1**

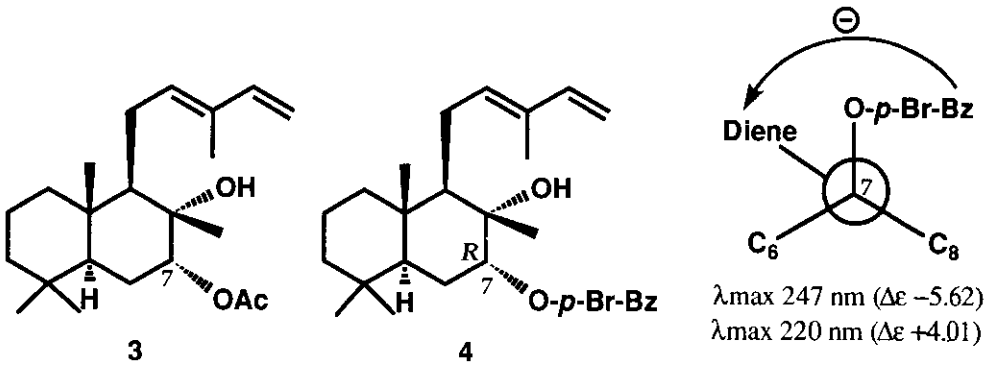


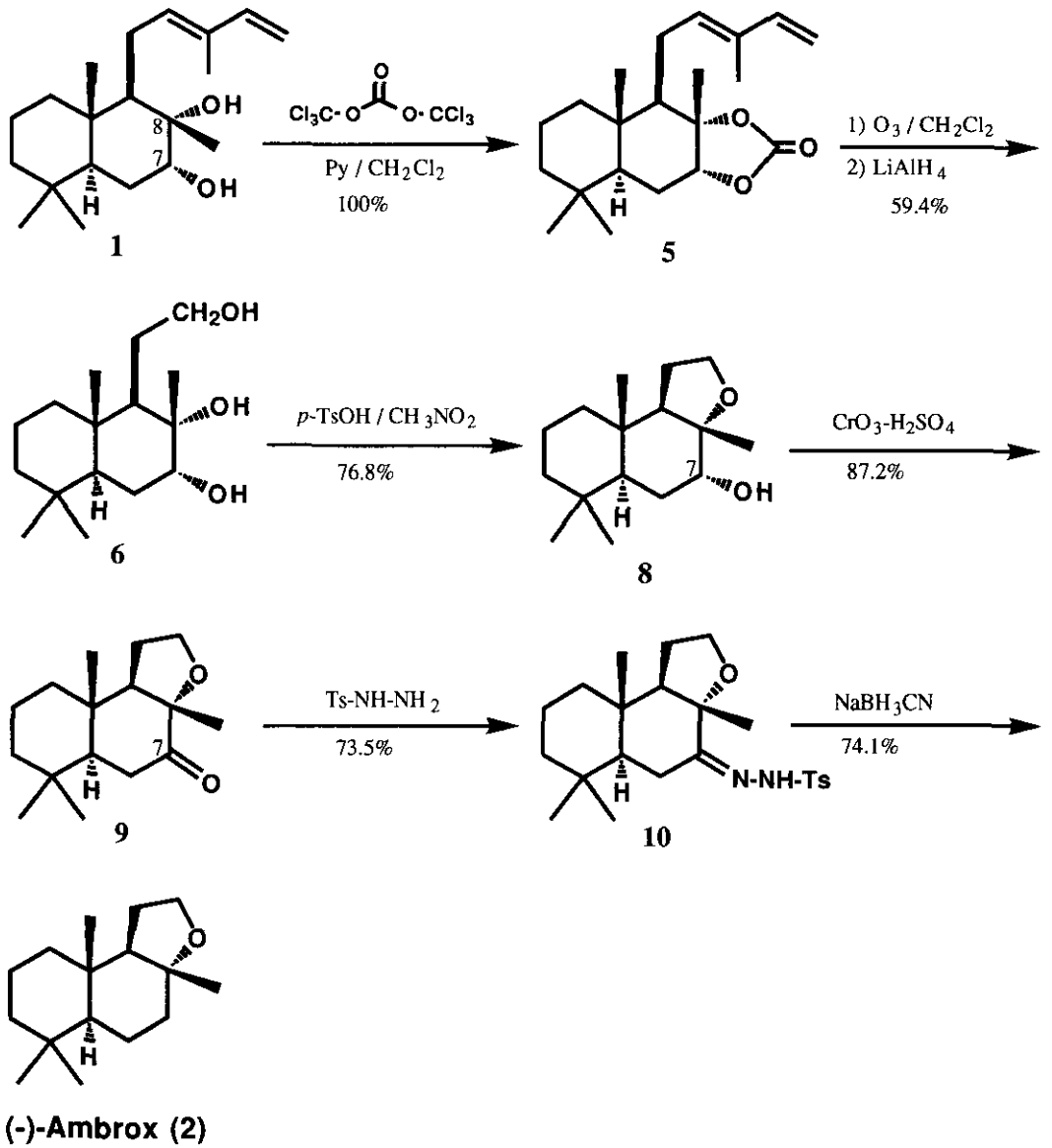
Figure 2. The CD spectrum of **4**

Chemical Conversion of Labda-12, 14-dien-7α, 8α-diol into (-)-Ambrox

Ambergris is a methabolic product found in the gut of some blue sperum whales. One of the constituents of the ambergris is the norlabdane oxide (-)-ambrox (**2**) which possesses a powerful amber-type aroma. Several syntheses of (-)-ambrox (**2**) have started from naturally occurring sesqui- or diterpenes such as (-)-drimenol,[5] (+)-manool,[6] manoyl oxide,[7] and (+)-*cis*-abienol[8]. In this paper, we report a new synthesis of (-)-ambrox (**2**) from labda-12, 14-dien-7α, 8α-diol (**1**), the main constituent of the liverwort *Porella perrottetiana via* 6 steps as shown in Scheme 1.

Protection of **1** with triphosgene afforded the cyclic acetal (**5**) [IR (KBr)cm^{-1}: 1776 (C=O)] in 100% yield indicating that the relative configuration of C_7, C_8-diol was represented as *cis*. Oxidation of **5** with ozone in CH_2Cl_2 and subsequent reduction of the resulting ozonide with $LiAlH_4$ afforded the triol (**6**) [IR (KBr)cm^{-1}: 3341 (OH)] in 59.4% yield. The relative configuration of the triol (**6**) was determined by the NOESY spectrum of di-*p*-bromobenzoate (**7**) of **6** as shown in Figure 3. Thus, the NOEs between (i) H-11 and H-17 (ii) H-11 and H-20, (iii) H-7 and H-17, and (iv) H-17 and H-20 were observed. Cyclization of **6** in CH_3NO_2 at rt, using *p*-TsOH afforded a single product (**8**) in 76.8% yield. The structure of **8** was assigned as by careful analysis of the NOESY spectrum [NOEs between (i) H-11 and H-17 and (ii) H-7 and H-17] as shown in Figure 3. Oxidation of **8** with Jones reagent (CrO_3-H_2SO_4) in acetone gave the corresponding ketone (**9**) in 87.2% yield. The absolute configuration of **9** was presumed by a negative single Cotton effect [290 nm (Δε-2.50)] in the CD spectrum. Reaction of **9** with *p*-toluenesulfonylhydrazine in absolute EtOH under reflux gave *p*-toluenesulfonylhydrazone (**10**) in 73.5% yield. The stereostructure of

10 was established by X-Ray crystallographic analysis as shown in Figure 4. Finally, reduction of **10** with $NaBH_3CN$ afforded (-)-ambrox (**2**). The spectral data (^1H and ^{13}C NMR, IR, and MS) of 2 were identical with those of the natural product.[7] The melting point and specific rotation of the synthetic product were mp 72-74° and $[\alpha]_D^{20}$ -29.8° (*c* 0.92, $CHCl_3$), while the literature values were mp 73-74° and $[\alpha]_D^{20}$ -28.8° (*c* 2.35, $CHCl_3$). Consequently, the absolute configuration of labda-12, 14-dien-7α, 8α-diol was established as formulated by **1**.



Scheme 1. Chemical conversion of **1** into (-)-ambrox (**2**)

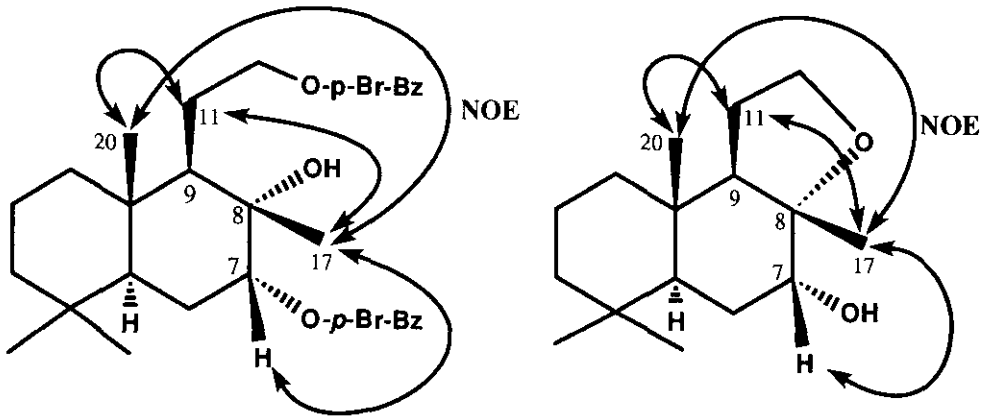


Figure 3. The NOESY spectra of 7 and 8

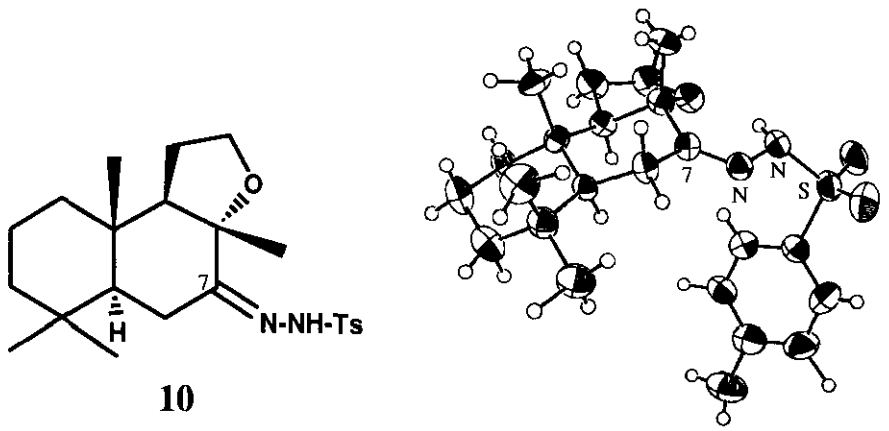


Figure 4. ORTEP dawing of **10**

In conclusion, we accomplished the chemical conversion of (-)-labda-12, 14-dien-7α, 8α-diol (**1**), isolated from the Japanese liverwort *P. perrottetiana*, into (-)-ambrox (**2**) *via* 6 steps in overall yield 21.7%. Our synthetic method includes good advantage to obtain (-)-ambrox and C_7-substituted ambrox from naturally abundant diterpene diol with short step and satisfactory yield.

EXPERIMENTAL

IR spectra were measured on a Jasco FT-IR 500 spectrophotometer. ^1H and ^{13}C NMR were recorded on a Varian untiy 600 (^1H; 600 MHz, ^{13}C; 150 MHz) or a Varian Unity 200 (^1H; 200 MHz, ^{13}C; 50 MHz) spectrometer. The solvent used for NMR spectra was $CDCl_3$ unless otherwise

stated. MS spectra were measured on a JEOL JMS HX-100 or a JEOL AX-500 spectrometer. The specific rotation and the CD spectra were taken on a JASCO DIP-140 POLARIMETER and a JASCO J-500 spectrometer, respectively. Slica gel 60 for column chromatography was purchased from Merk.

Isolation of Labda-12, 14-dien-7α, 8α-diol (1) Dried powders (1.13 kg) of *Porella perrottetiana* collected in Kito-son, Tokushima, Japan, in July 1994, was extracted with Et_2O (10 L) for 1 week at rt. The Et_2O extract (19.06 g) was chromatographed on silica gel (500 g) with a gradient solvent system of *n*-Hexane-AcOEt increasing the amount of 5% portions AcOEt stepwise to give a number of fractions. 40% AcOEt-n-Hex. eluate (Fr. 31-39) was evaporated in *vacuo* to afford the crude crystal (2.679 g), which was recrystallized from Et_2O and *n*-hexane to give labda-12, 14-dien-7α, 8α-diol (**1**) (2.096 g). Abienol (146 mg) from 20% AcOEt-n-Hex. eluate (fr. 17-18) and perrottetial A (1.009 g) from 25% AcOEt-n-Hex. eluate (fr. 20-22) were isolated, respectively.

Labda-12, 14-dien-7α, 8α-diol (1) colorless needles, mp 125-127°, $[\alpha]_D^{25}$ -9.4° (*c* 1.55, $CHCl_3$); EI-MS: *m/z* 306 (M^+), 288, 270, 177, 150 (100), 123; HR-MS: *m/z* 306.2549 (M^+), $C_{20}H_{32}O_2$ requires 306.2559; FT-IR (KBr)cm^{-1}: 3383 (OH), 3090, 2926, 1640, 1127, 1055; UV (EtOH) λ_{max}nm (logε); 232.5 (4.38); ^{1}H NMR($CDCl_3$): δ 0.80 (3H, s, H-19), 0.84 (3H, s, H-20), 0.88 (3H, *s*, H-18), 1.19 (3H, *s*, H-17), 1.79 (3H, *br s*, H-16), 3.65 (1H, *br t*, J=2.0 Hz, H-7), 4.93 (1H, *d*, J=10.7 Hz, H-15), 5.07 (1H, *d*, J= 17.6 Hz, H-15), 5.58 (1H, *t*, J= 7.3 Hz, H-12), 6.35 (1H, *dd*, J= 10.7, 17.6 Hz, H-14), ^{13}C NMR ($CDCl_3$): δ 11.9 (*q*, C-16), 14.9 (*q*, C-20), 18.5 (*t*, C-2), 21.5 (*q*, C-19), 23.4 (*q*, C-17), 23.5 (*t*, C-11), 25.9 (*t*, C-6), 32.7 (*s*, C-4), 33.1 (*q*, C-18), 38.5 (*s*, C-10), 39.7 (*t*, C-1), 41.8 (*t*, C-3), 46.3 (*d*, C-5), 55.5 (*d*, C-9), 74.3 (*d*, C-7), 75.0 (*s*, C-8), 110.7 (*t*, C-15), 133.0 (*s*, C-13), 135.6 (*d*, C-12), 141.4 (*d*, C-14).

Acetylation of 1 A solution of **1** (100 mg) in pyridine (3 mL) was treated with acetic anhydride (3 mL). The mixture was stirred overnight at rt . Water was added and the mixture was extracted with $CHCl_3$. The organic phase was washed with 1N HCl, 5% $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated to give a residue. The residue was purified by a silica gel column chromatography with hexane-AcOEt gradient to afford 7-acetyllabda-12, 14-dien-7α, 8α-diol (**3**)(127 mg, 100%) as colorless needles; mp 94-95°, $[\alpha]_D^{20}$ -50.7° (*c* 0.67, $CHCl_3$); CI-MS (CH_4): *m/z* 349 (M^++1),

331, 289, 270 (100), 255; 192, 150; FT-IR (KBr)cm^{-1}: 3530 (OH), 3088, 2928, 1640, 1242, 1128; UV (EtOH) λ_{max}nm (logε); 231 (4.40); ^{1}H NMR($CDCl_3$): δ 0.78 (6H, s, H-19, H-20), 0.86 (3H, s, H-18), 1.20 (3H, *s*, H-17), 1.77 (3H, *br s*, H-16), 2.12 (3H, s, -OAc), 4.80 (1H, *br t*, J=2.3 Hz, H-7), 4.90 (1H, *d*, J=10.7 Hz, H-15), 5.06 (1H, *d*, J= 17.5 Hz, H-15), 5.57 (1H, *t*, J= 7.2 Hz, H-12), 6.36 (1H, *dd*, J= 10.7, 17.5 Hz, H-14); ^{13}C NMR ($CDCl_3$): δ 11.8 (*q*, C-16), 14.9 (*q*, C-20), 18.4 (*t*, C-2), 21.3 (*q*, -OAc), 21.4 (*q*, C-19), 22.5 (*q*, C-17), 23.5 (*t*, C-11), 24.3 (*t*, C-6), 32.6 (*s*, C-4), 33.1 (*q*, C-18), 38.7 (*s*, C-10), 39.8 (*t*, C-1), 41.7 (*t*, C-3), 47.8 (*d*, C-5), 57.9 (*d*, C-9), 73.1 (*s*, C-8), 77.8 (*d*, C-7), 110.1 (*t*, C-15), 132.1 (*s*, C-13), 136.4 (*d*, C-12), 141.8 (*d*, C-14), 170.4 (*s*, -OAc).

***p*-Bromobenzoylation of 1** A solution of **1** (80 mg) in pyridine (4 mL) was treated with *p*-bromobenzoyl chloride (347 mg) and DMPA (100 mg). The mixture was stirred overnight at rt. Water was added and the mixture was extracted with $CHCl_3$. The organic phase was washed with 1N HCl, 5% $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated to give a residue. The residue was purified by a silica gel column chromatography with hexane-AcOEt gradient to afford 7-*p*-bromobenzoyllabda-12, 14-dien-7α, 8α-diol (**4**)(127 mg, 100%) as colorless oil; $[\alpha]_D^{20}$ -61.3° (*c* 0.55, $CHCl_3$); EI-MS: *m/z* 472 (M^++2-H_2O), 470 (M^+-H_2O), 332, 270 (100), 185, 183; CI-MS (CH_4): *m/z* 489 (M^++1), 435, 248, 183 (100); FT-IR (KBr) cm^{-1}: 3534 (OH), 3038, 2926, 1719 (C=O), 1589, 1275, 1119; UV (EtOH) λ_{max}nm (logε); 203 (4.48), 235 (4.60); ^{1}H NMR ($CDCl_3$): δ 0.74 (3H, s, H-18), 0.80 (3H, s, H-19), 0.91 (3H, s, H-20), 1.28 (3H, *s*, H-17), 1.77 (3H, *br s*, H-16), 4.90 (1H, *d*, J=10.7 Hz, H-15), 5.06 (1H, *d*, J= 17.1 Hz, H-15), 5.10 (1H, *br t*, J=2.3 Hz, H-7), 5.57 (1H, *t*, J= 7.3 Hz, H-12), 6.35 (1H, *dd*, J= 10.7, 17.1 Hz, H-14), 7.61 (2H, d, J=8.3 Hz, H-3' and H-5'), 7.89 (2H, d, J=8.3 Hz, H-2' and H-6'); ^{13}C NMR ($CDCl_3$): δ 11.9 (*q*, C-16), 15.0 (*q*, C-20), 18.4 (*t*, C-2), 21.2 (*q*, C-19), 22.9 (*q*, C-17), 23.5 (*t*, C-11), 24.8 (*t*, C-6), 32.6 (*s*, C-4), 33.2 (*q*, C-18), 38.8 (*s*, C-10), 40.0 (*t*, C-1), 41.8 (*t*, C-3), 48.5 (*d*, C-5), 58.2 (*d*, C-9), 73.6 (*s*, C-8), 78.3 (*d*, C-7), 110.2 (*t*, C-15), 128.1 (*s*, C-4'), 129.4 (*s*, C-1'), 131.0 (*d*, C-2' and C-6'), 131.9 (*d*, C-3' and C-5'), 132.3 (*s*, C-13), 136.2 (*d*, C-12), 141.6 (*d*, C-14), 165.3 (*s*, C-7').

Reaction of 1 with Triphosgene To a solution of **1** (710 mg) and pyridine (1.69 mL) in dry CH_2Cl_2 (45 mL) at -78°C was added triphosgene (1.032 g). The mixture was stirred at 0°C for 4

hr, diluted with saturated NH_4Cl sol. (50 mL) and extracted with CH_2Cl_2 (50 mLx2). The organic layer was washed with brine, dried ($MgSO_4$) and evaporated under reduced pressure. The crude product (1.155 g) was recrystallized from Et_2O to give labda-12, 14-dien-7α, 8α-cyclocarbonate (**5**) (770 mg; 100%) as colorless needles; mp 130-133°, $[\alpha]_D^{19}$ -47.1° (*c* 0.52, $CHCl_3$); EI-MS: *m/z* 332 (M^+), 288, 270, 255, 190, 175, 146, 133, 119, 81 (100); HR-MS: *m/z* 332.2360 (M^+), $C_{21}H_{32}O_3$ requires 332.2352; FT-IR (KBr)cm^{-1}: 3069, 2957, 1776 (C=O), 1609, 1053; UV (EtOH)λ_{max}nm (logε); 231 (4.33); ^1H NMR($CDCl_3$): δ 0.83 (3H, s, H-19), 0.85 (3H, s, H-20), 0.89 (3H, s, H-18), 1.44 (3H, *s*, H-17), 1.76 (3H, *br s*, H-16), 4.45 (1H, *br t*, J=2.6 Hz, H-7), 4.92 (1H, *d*, J=10.7 Hz, H-15), 5.07 (1H, *d*, J= 17.2 Hz, H-15), 5.55 (1H, *t*, J= 7.2 Hz, H-12), 6.36 (1H, *dd*, J= 10.7, 17.2 Hz, H-14), ^{13}C NMR ($CDCl_3$): δ 11.7 (*q*, C-16), 14.2 (*q*, C-20), 18.2 (*t*, C-2), 21.2 (*q*, C-19), 22.5 (*q*, C-17), 23.8 (*t*, C-11), 23.8 (*t*, C-6), 32.7 (*s*, C-4), 32.7 (*q*, C-18), 37.1 (*s*, C-10), 39.1 (*t*, C-1), 41.4 (*t*, C-3), 46.8 (*d*, C-5), 56.3 (*d*, C-9), 83.0 (*d*, C-7), 86.1 (*s*, C-8), 110.5 (*t*, C-15), 132.9 (*d*, C-12), 133.2 (*s*, C-13), 141.4 (*d*, C-14). 154.5 (*s*, -O-CO-O-)

Synthesis of 6 Ozone gas was blown through the solution of **5** (815 mg) in dry CH_2Cl_2 (25 mL) at -78 °C for 1 h. The reaction mixture was evaporated in *vacuo* to give a colorless residue (823 mg). To a suspension of $LiAlH_4$ (1.86 g) in dry Et_2O (20 mL) was added dropwise a solution of the residue in dry Et_2O (15 mL) and dry THF (5 mL) at 0°C. The reaction mixture was stirred at 0°C for 1 h. AcOEt (20 mL) and 1N HCl (20 mL) was added successively to decompose excess $LiAlH_4$. The organic layer was washed with brine, dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by a silica gel column chromatography with $CHCl_3$-AcOEt gradient to afford a triol (**6**)[5] (127 mg, 100%) as colorless needles; mp 154-156°, $[\alpha]_D^{25}$ -30.2° (*c* 0.53, EtOH); EI-MS: *m/z* 270 (M^+, 100), 252, 165, 151, 137, 123, 109, 95, 69; HR-MS: *m/z* 270.2204 (M^+), $C_{21}H_{32}O_3$ requires 270.2195; FT-IR (KBr)cm^{-1}: 3341 (OH), 2949, 1238, 1161, 1057; UV (EtOH) λ_{max}nm (logε); 231 (4.33); ^1H NMR ($CDCl_3$): δ 0.80 (6H, *s*, H-19, H-20), 0.89 (3H, *s*, H-18), 1.18 (3H, *s*, H-17), 3.46 (1H, *m*, H-12), 3.70 (1H, *br s*, H-7), 3.83 (1H, *m*, H-12); ^{13}C NMR ($CDCl_3$): δ 14.8 (*q*, C-20), 18.3 (*t*, C-2), 21.4 (*q*, C-19), 23.5 (*q*, C-17), 25.6 (*q*, C-6), 27.0 (*t*, C-11), 32.6 (*s*, C-4), 32.9 (*q*, C-18), 38.5 (*s*, C-10), 39.0 (*t*, C-1), 41.8 (*t*, C-3), 46.1 (*t*, C-5), 52.8 (*d*, C-9), 63.6 (*t*, C-12), 73.5 (*s*, C-8), 73.8 (*d*, C-7).

***p*-Bromobenzoylation of 6** A solution of **6** (50 mg) in pyridine (4 mL) was treated with *p*-bromobenzoyl chloride (347 mg) and DMPA (100 mg). The mixture was stirred overnight at rt. Usual work-up and purification by silica gel column chromatography with $CHCl_3$-AcOEt gradient afforded di-*p*-bromobenzoate (**7**)(107 mg, 90.8%) as a colorless oil; $[\alpha]_D^{19}$ -54.1° (*c* 2.20, $CHCl_3$); EI-MS: *m/z* 638 (M^++4), 636 (M^++4), 634 (M^+), 436, 434, 185, 183 (100); HR-MS: *m/z* 634.0926 (M^+), $C_{30}H_{36}O_5Br_2$ requires 634.0930; FT-IR (KBr)cm^{-1}: 3231 (OH), 1743 (C=O), 1236, 1055; UV (EtOH) λ_{max}nm (logε); 203 (4.62), 244 (4.60); ^{1}H NMR($CDCl_3$): δ0.74 (3H, s, H-18), 0.80 (3H, s, H-19), 0.89 (3H, s, H-20), 1.30 (3H, *s*, H-17), 4.36, 4.48 (2H, m, H-12), 5.13 (1H, *dd*, J=2.2, 3.6 Hz, H-7), ; ^{13}C NMR ($CDCl_3$): δ14.9 (*q*, C-20), 18.3 (*t*, C-2), 21.2 (*q*, C-19), 22.9 (*q*, C-17), 24.0 (*t*, C-6), 24.7 (*t*, C-11), 32.7 (*s*, C-4), 33.1 (*q*, C-18), 38.5 (*s*, C-10), 39.7 (*t*, C-1), 41.8 (*t*, C-3), 48.5 (*d*, C-5), 52.9 (*d*, C-9), 73.5 (*s*, C-8), 78.2 (*d*, C-7), 128.0, 128.2 (*s*, C-4' and C-4"), 129.2 (*s*, C-1' and C-1"), 131.0 (*d*, C-2', C-2", C-6' and C-6"), 131.7, 131.9 (*d*, C-3', C-3", C-5' and C-5"), 165.3, 166.1 (*s*, C-7' and C-7").

Reaction of 6 with *p*-TsOH A solution of **6** (400 mg) in nitromethane (50 mL) was treated with *p*-TsOH (468 mg). The reaction mixture was stirred overnight at rt and evaporated in *vacuo* to give a colorless oil (456 mg). The crude product was purified by a silica gel column chromatography with $CHCl_3$-AcOEt gradient to afford 7α-hydroxyambrox (**8**)[5] (287 mg, 76.8%) as colorless needles, mp 61-63°, $[\alpha]_D^{20}$ -64.6° (*c* 0.65, $CHCl_3$); EI-MS: *m/z* 252 (M^+, 100), 237, 151, 137, 124, 109; HR-MS: *m/z* 252.2090 (M^+), $C_{16}H_{28}O_2$ requires 252.2090; FT-IR (KBr)cm^{-1}: 3429 (OH),2928, 1148, 1069; ^{1}H NMR($CDCl_3$): δ0.83 (3H, s, H-19), 0.84 (3H, *s*, H-20), 0.88 (3H, s, H-18), 1.09 (3H, *s*, H-17), 3.88 (1H, *m*, H-7), 3.86 (2H, *m*, H-12), ^{13}C NMR ($CDCl_3$): δ 14.7 (*q*, C-20), 18.3 (*t*, C-2), 21.1 (*q*, C-17 or C-19), 21.3 (*q*, C-17 or C-19), 22.1 (*t*, C-11), 32.5 (*s*, C-4), 33.2 (*q*, C-18), 36.0 (*s*, C-10), 39.6 (*t*, C-1), 42.3 (*t*, C-3), 48.7 (*d*, C-5), 51.7 (*d*, C-9), 65.4 (*t*, C-12), 71.2 (*d*, C-7), 81.5 (*s*, C-8).

Jones Oxidation of 8 To a solution of **8** (216 mg) in acetone (3 mL) was added Jones reagent (0.4 mL) at 0°C. The reaction mixture was stirred at 0°C for 10 min. Isopropanol (8 mL) was added to decompose excess Jones reagent. Usual work-up and purification by silica gel column chromatography with CH_2Cl_2-AcOEt gradient afforded 7–oxo-ambrox (**9**)[4](25 mg, 87.2%) as

colorless needles, mp 132-134°; $[\alpha]_D^{20}$ -147.7° (*c* 1.01, $CHCl_3$); EI-MS: *m/z* 250 (M^+), 222, 207, 137 (100), 124, 111; HR-MS: *m/z* 250.1908 (M^+), $C_{16}H_{26}O_2$ requires 250.1933; FT-IR (KBr)cm^{-1}: 2930, 1723 (C=O), 1150, 1063; 1011; CD (EtOH): 290 nm (Δε-2.50); ^{1}H NMR($CDCl_3$): δ 0.88 (6H, s, H-19 and H-20), 1.06 (3H, *s*, H-18),1.33 (3H, *s*, H-17), 2.40 (1H, *dd*, J=3.7, 13.7 Hz, H-6), 2.53 (1H, *dd*, J=13.8, 13.8 Hz, H-6), 3.99 (2H, *m*, H-12), ^{13}C NMR ($CDCl_3$): δ 14.5 (*q*, C-20), 18.1 (*t*, C-2), 20.0 (*q*, C-17 or C-19), 20.6 (*q*, C-17 or C-19), 21.9 (*t*, C-11), 33.0 (*q*, C-18), 33.7 (*s*, C-4), 36.0 (*s*, C-10), 37.0 (*t*, C-6), 39.4 (*t*, C-1), 41.7 (*t*, C-3), 59.1 (*d*, C-5), 60.6 (*d*, C-9), 65.2 (*t*, C-12), 86.0 (*s*, C-8), 209.2 (*s*, C-7).

Reaction of 9 with *p*-Toluenesulfonylhydrazine A solution of **9** (72.7 mg) in absolute EtOH (1.5 mL) was treated with *p*-toluenesulfonylhydrazine (109.8 mg). The reaction mixture was refluxed for 30 min and evaporated in *vacuo* to give a colorless oil (222 mg). The crude product was purified by a silica gel column chromatography with hexane-AcOEt gradient to afford 7–oxo-ambrox tosylhydrazone (**10**)(106 mg; 87.1%) as colorless needles. mp 196-198°, $[\alpha]_D^{25}$ -75.4° (*c* 0.63, $CHCl_3$); EI-MS: *m/z* 418 (M^+), 403, 234 (100), 219, 111; HR-MS: *m/z* 418.2286 (M^+), $C_{23}H_{34}O_3N_2S$ requires 418.2290; FT-IR (KBr)cm^{-1}: 3180 (NH), 3067, 2926, 1597, 1167; UV (EtOH) λ_{max}nm (logε); 206 (4.12), 224 (4.06); ^{1}H NMR($CDCl_3$): δ0.81 (3H, *s*, H-19), 0.87 (3H, *s*, H-18), 0.85 (3H, *s*, H-20), 1.17 (3H, *s*, H-17), 2.43 (3H, *s*, H-7'), 3.88 (2H, *m*, H-12), 7.29 (2H, *d*, J=8.0 Hz, H-3' and H-5'), 7.80 (2H, *d*, J=8.0, Hz, H-2' and H-6'), 10.38 (1H, *s*, -NH); ^{13}C NMR ($CDCl_3$): δ 14.4 (*q*, C-20), 18.2 (*t*, C-2), 20.0 (*q*, C-7'), 20.7 (*q*, C-19), 21.0 (*t*, C-11), 21.6 (*q*, C-17), 30.9 (*t*, C-6), 33.2 (*s*, C-4), 33.2 (*q*, C-18), 35.5 (*s*, C-10), 39.3 (*t*, C-1), 42.0 (*t*, C-3), 57.4 (*d*, C-5), 65.9 (*t*, C-12), 85.6 (*s*, C-8), 127.8 (*d*, C-2' and C-6'), 129.3 (*d*, C-3' and C-5'), 136.4 (*s*, C-4'), 143.2 (*s*, C-1'), 157.9 (*s*, C-7).

The crystal data for 10 Orthorhombic, space group $P2_12_12_1$, a= 16.435 (3)Å, b = 18.626 (3)Å, c = 7.344 (3)Å, V =2248 (1)Å^3, Z = 4, D_x= 1.234 Mg m^{-3}, D_m= 1.230 Mg m^{-3}, μ (Cu Kα) =14.404 mm^{-1}, Final R and R_w were 0.051 and 0.064 for 1496 reflections with $I > 3\sigma(I)$. The structure was solved by direct method (Monte-Carlo Multan) and refined by full-matrix least-squares techniques. Diffraction data were obtained using a Mac Science MXC18 diffractiotometer at rt. All diagrams and calculations were performed using CRYSTAN (Mac Science, Japan).

Reduction of 10 with $NaBH_3CN$ A solution of **10** (56.2 mg) in DMF (1 mL) and sulfolane (1 mL) was treated with *p*-TsOH (40 mg) and $NaBH_3CN$ (90.8 mg). The reaction mixture was stirred at 110°C for 3 h. Reaction mixture was diluted with water and extracted with Et_2O. The organic layer was washed with brine, dried ($MgSO_4$) and evaporated in *vacuo* to give a colorless oil (57 mg). The crude product was purified by a silica gel column chromatography with hexane-AcOEt gradient to afford (-)-ambrox (**2**)(23.5 mg; 74.1%) as colorless needles. Synthetic compound (**2**); mp 72-74°, $[\alpha]_D^{20}$ -29.8° (*c* 0.92, $CHCl_3$); FT-IR (KBr)cm^{-1}: 2930, 1460, 1377, 1128, 1003; EI-MS: *m/z* 236 (M^+), 221 (100), 203, 137, 97; HR-MS: *m/z* 236.2151 (M^+), $C_{16}H_{28}O$ requires 236.2140; ^1H NMR($CDCl_3$): δ 0.83 (6H, s, H-19, H-20), 0.88 (3H, s, H-18), 1.09 (3H, *s*, H-17), 3.82 (1H, *dd*, J=8.5, 16.8 Hz, H-12), 3.90 (1H, *ddd*, J=3.2, 3.2, 16.8 Hz, H-12), ^{13}C NMR ($CDCl_3$): δ 15.1 (*q*, C-20), 18.4 (*t*, C-2), 21.2 (*q*, C-17), 21.2 (*q*, C-19), 22.7 (*t*, C-11), 33.1 (*s*, C-4), 33.6 (*q*, C-18), 36.2 (*s*, C-10), 40.0 (*t*, C-1), 42.5 (*t*, C-3), 57.3 (*d*, C-5), 60.2 (*d*, C-9), 65.0 (*t*, C-12), 80.0 (*s*, C-8); Natural product (**2**)[7] ; mp 73-74°, $[\alpha]_D^{20}$ -28.8° (*c* 2.35, $CHCl_3$). The spectral data of synthetic (-)-ambrox were identical with those of natural (-)-ambrox.

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