# Chapter 2

## Experimental

Melting points were determined in capillary tubes and are uncorrected : on a Fihser-Johns melting point apparatus. Optical rotation : on a Perkin Elmer digital polarimeter. UV spectra : on a Hewlett Packard 8452A. IR spectra : on a Perkin Elmer 1760X FT-IR Spectrophotometer. Low-resolution MS : 70 eV, on a Fisons Instruments Trio 2000 mass spectrometer. $^{1}H$ and $^{13}C$ NMR experiment were recorded on a Bruker 200 MHz, JEOL JNM-GSX400A (400 MHz), JEOL-GSX500A (500 MHz) spectrometer . Microanalyses were determined on a Perkin Elmer PE 2400 Series II.

Column chromatographic separations were performed on silica gel (Merck, Kieselgel 60, 0.063-0.2 mm and 0.04-0.063 mm) under pressure. THF was distilled from sodium-benzophenone. Benzene, n-hexane, toluene were distilled from sodium. Dichloromethane was distilled from calcium hydride ($CaH_2$).

### 2.1 Materials and Methods

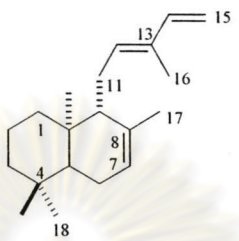
The stem bark of *C. oblongifolius* was collected from Ampur Pranburi, Prachuab KiriKhan Province, Thailand, in September 1996. Botanical identification was achieved through comparison with a voucher specimen No. BKF 084729 in the herbarium collection of the Royal Forest Department of Thailand.

### 2.2 Extraction and Isolations

The powdered, sun-dried stem bark (2.5 kg) of *C. oblongifolius* was extracted with hexane (5 x 5 L). The hexane extract was filtered, and evaporated under vacuum to obtain a yellowish green oil (90 g). The hexane extract (50 g) was fractionated by silica

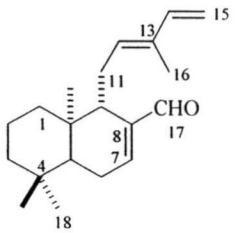
gel column chromatography and eluted with hexane, 5% ethyl acetate in hexane and 10 % ethyl acetate in hexane to give compounds **11** (4.25 g), **12** (1.6 g), **13** (4.29 g) and **14** (23.64 g) after purification by flash column chromatography.

**Labda-7,12(*E*),14-triene (11)**



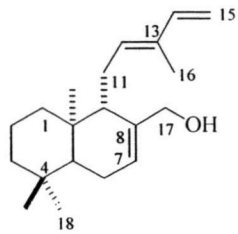

**Compound 11** is viscous transparent oil, ; $[\alpha]_D^{20}$ +3.8 (*c* 1.76, $CHCl_3$) Anal calcd. for $C_{22}H_{32}$ : C, 88.2 ; H, 11.8%. Found : C, 88.1 ; H, 11.6%. ; IR νmax (neat) 2924, 2867, 1672, 1645, 1453 and 1384 $cm^{-1}$. ; $^1H$ NMR ($CDCl_3$, 500 MHz) δ (ppm) : 6.35 (1H, dd, *J* = 10.7, 17.4 Hz, H-14), 5.51 (1H,t, *J* = 6.7 Hz, H-12), 5.42 (1H, ddd, *J* = 1.5, 1.5, 4.0 Hz, H-7), 5.04 (1H, d, *J* = 17.4 Hz, H-15a), 4.89 (1H, d, *J* = 10.7 Hz, H-15b), 2.29 (1H, ddd, *J* = 1.8, 4.0, 16.5 Hz, H-11a),2.11 (1H, ddd, *J* = 7.6, 7.6, 16.5 Hz, H-11b), 1.97 (1H, m, H-6a), 1.89 (1H, m, H-9), 1.87 (1H, m, H-6a), 1.85 (1H, m, H-1a), 1.74 (3H, s, Me-16), 1.60 (3H, s, Me-17), 1.53 (1H, m, H-2a), 1.45 (1H, m, H-2b), 1.41 (1H, m, H-3a), 1.19 (1H, dd, *J* = 4.9, 12.2 Hz, H-5), 1.16 (1H, ddd, *J* = 3.7, 3.7, 13.1), 1.00 (1H, ddd, *J* = 3.4, 3.4, 13.4 Hz, H-1b), 0.88 (3H, s, Me-19), 0.86 (3H, s, Me-18), 0.79 (3H, s, Me-20). ; $^{13}C$-NMR spectrum ($CDCl_3$, 125 MHz) δ (ppm) : 141.7 (C-14), 135.9 (C-12), 135.0(C-8), 132.4 (C-13), 122.6 (C-7), 109.8 (C-15), 55.2 (C-9), 50.2 (C-5), 42.3 (C-3), 39.7 (C-1), 36.8 (C-10), 33.3 (C-18), 33.0 (C-4), 26.3 (C-11), 23.8 (C-6), 22.5 (C-17), 22.0 (C-19), 18.9 (C-2), 14.0 (C-20), 11.9 (C-16). ; *m/z* (EI) (rel int.) : 272[$M^+$](4), 191(99), 135(19), 121(31), 109(100), 95(58), 81(29), 69(30), 55(35) and 41(43).

**Labda-7,12(*E*),14-triene-17-al (12)**



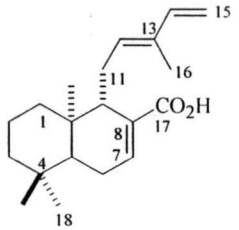

**Compound 12** is a white solid, $[\alpha]_D^{30}$ +37.5 ($CHCl_3$, *c* 1.51), mp 72-74 °C, Anal calcd. for $C_{20}H_{30}O$ ; C 83.92, H 10.49% Found C 83.86, H 10.66%. ; FT-IR spectrum (KBr) $\nu_{max}$ ($cm^{-1}$) : 2954, 2923, 2852, 2710, 1690, 1639, 1607, 1460, 1443, 1389, 1368. ; $^1$H-NMR spectrum ($CDCl_3$, 200MHz) δ (ppm) : 9.37 (1H, s, H-17), 6.83 (1H, ddd, *J* = 2.1, 2.1, 5.8 Hz, H-7), 6.29 (1H, dd, *J* = 10.9, 17.4 Hz, H-14), 5.44 (1H, t, *J* = 6.7 Hz, H-12), 5.00 (1H, d, *J* = 17.4 Hz, H-15a), 4.84 (1H, d, *J* = 10.9 Hz, H-15b), 2.62 (1H, ddd, *J* = 7.0, 7.0, 16.8 Hz, H-11a), 2.48 (1H, ddd, *J* = 2.1, 6.4, 16.8 Hz, H-11b), 2.34 (1H, m, H-6a), 2.30 (1H, m, H-9), 2.18 (1H, m, H-6b), 1.90 (1H, ddd, J = 3.0, 5.2, 13.1 Hz, H-1a), 1.70 (3H, s, Me-16), 1.50 (1H, m, H-2a), 1.43 (1H, m, H-3a), 1.40 (1H, m, H-2a), 1.16 (1H, m, H-3a), 1.13 (1H, dd, *J* = 4.3, 12.5 Hz, H-5), 1.00 (1H, ddd, *J* = 3.7, 3.7, 13.1 Hz, H-1b), 0.91 (3H, s, Me-19), 0.86 (3H, s, Me-18), 0.79 (3H, s, Me-20). ; $^{13}$C-NMR spectrum ($CDCl_3$, 50 MHz) δ (ppm) : 194.3 (C-17), 151.8 (C-7), 143.4(C-8), 141.8 (C-14), 133.8 (C-12), 132.7 (C-13), 109.8 (C-15), 49.4 (C-5), 49.4 (C-5), 41.9 (C-3), 39.9 (C-1), 36.7 (C-10), 33.2 (C-18), 32.9 (C-4), 24.9 (C-6), 24.8 (C-11), 22.1 (C-19), 18.5 (C-2), 14.7 (C-20), 11.8 (C-16). ; EIMS *m/z* (rel int.) : 286[$M^+$] (18), 271(15), 191(10), 163(14), 147(13), 124(24), 109(100), 91(38), 81(62), 55(22) and 41(25).

**Labda-7,12(*E*),14-triene-17-ol (13)**




**Compound 13** is a white solid, $[\alpha]_D^{30}$ +12.0 ($CHCl_3$, *c* 1.63), mp 90-92 °C, ; Anal calcd. for $C_{20}H_32O$ ; C 83.33, H 11.11% : Found C 83.19, H 11.28%. FT-IR spectrum (KBr) $\nu_{max}$ ($cm^{-1}$) : 3249(br), 2919, 2845, 1640, 1607, 1459, 1441, 1388, 1365, 1073, 1057. ; $^1$H-NMR spectrum ($CDCl_3$, 200 MHz) δ (ppm) : 6.34 (1H, dd, *J* = 10.7, 17.4 Hz, H-14), 5.75 (1H, ddd, *J* = 2.5, 2.5, 5.2 Hz, H-7), 5.55 (1H, t, *J* = 6.7 Hz, H-12), 5.06 (1H, d, *J* = 17.4 Hz, H-15a), 4.91 (1H, d, *J* = 10.7 Hz, H-15b), 4.05 (1H, ddd, *J* = 0.9, 1.8, 12.8 Hz, H-17a), 3.86 (1H, d, *J* = 12.8 Hz, H-17b), 2.33 (1H, d, *J* = 16.55 Hz, H-11a), 2.15 (1H, ddd, *J* = 8.2, 8.2, 16.5 Hz, H-11b), 2.07 (1H, m, H-9), 2.05 (1H, m, H-6a), 1.90 (1H, m, H-6b), 1.89 (1H, m, H-1a), 1.77 (3H, s, Me-16), 1.45 (1H, m, H-2a), 1.01 (1H, ddd, 3.7, 3.7, 13.1 Hz, H-1b), 0.87 (3H, s, Me-19), 0.85 (3H, s, Me-18), 0.77 (3H, s, Me-20). ; $^{13}$C-NMR spectrum ($CDCl_3$, 50 MHz) δ (ppm) : 141.3 (C-14), 138.6 (C-8), 134.7 (C-12), 133.5 (C-13), 125.4 (C-7), 110.7 (C-15), 66.0 (C-17), 52.2 (C-9), 49.9 (C-5), 42.2 (C-3), 39.6 (C-1), 36.7 (C-10), 33.2 (C-18), 33.0 (C-4), 25.9 (C-11), 23.6 (C-6), 22.0 (C-19), 18.9 (C-2), 14.0 (C-20),11.9 (C-16). ; EIMS m/z (rel int.) : 288 [$M^+$] (3), 270(19), 255(15), 202(12), 189(21), 176(20), 161(16), 147(25), 131(52), 109(100), 105(69), 91(84), 81(92), 55(76) and 41(96).

**Labda-7,12(*E*),14-triene-17-oic-acid (14).**

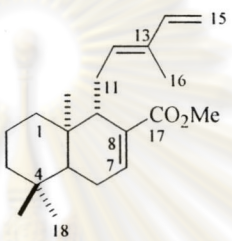



**Compound 14** is colourless crystals, $[\alpha]_D^{30}$ -15.9 ($CHCl_3$, *c* 1.67), mp 118-120 °C, Anal calcd. for $C_{20}H_{30}O_2$ : C 79.47, H 9.93% : Found C 79.49, H 9.66%. FT-IR spectrum $\nu_{max}$ ($cm^{-1}$) : 3421-2627(Br), 2946, 1707, 1652, 1604, 1459, 1430, 1383, 1345, 1208. ; $^1$H-NMR spectrum ($CDCl_3$, 200 MHz) δ (ppm) : 6.90 (1H, ddd, *J* = 2.1, 2.1, 6.1 Hz, H-7), 6.31 (1H, dd, *J* = 10.9, 17.4 Hz, H-14), 5.47 (1H, t, *J* = 6.4 Hz, H-12), 5.0 (1H, d, *J* = 17.4 Hz, H-15a), 4.84 (1H, d, *J* = 10.9 Hz, H -15b), 2.58 (1H, ddd, *J* = 7.3, 7.3, 16.8 Hz, H-11a), 2.37 (1H, m, H-9), 2.30 (1H, dd, *J* = 6.1, 16.8 Hz, H-11b), 2.19 (1H, dddd, *J* = 2.1, 4.3, 6.1, 19.2 Hz, H-6a), 1.87 (1H, ddd, *J* = 3.7, 5.2, 12.5 Hz, H-1a), 1.67 (3H, s, Me-16), 1.50 (1H, m, H-2a), 1.41 (1H, d, J = 2.5 Hz, H-2b), 1.18 (1H, dd, J = 4.3, 12.2 Hz, H-5), 0.89 (3H, s, Me-19), 0.86 (3H, s, Me-18), 0.82 (3H, s, Me-20). ; $^{13}$C-NMR spectrum ($CDCl_3$, 50 MHz) δ (ppm) : 174.1 (C-17), 141.8 (C-14), 140.5 (C-7), 133.6 (C-8), 133.4 (C-12), 133.1 (C-13), 109.9 (C-15), 50.0 (C-9), 49.3 (C-5), 41.9 (C-3), 40.1 (C-1), 36.9 (C-10), 33.3 (C-18), 32.8 (C-4), 26.0 (C-11), 24.0 (C-6), 22.2 (C-19), 18.6 (C-2),14.8 (C-20), 11.7 (C-16). ; EIMS m/z (rel int.) : 302 [$M^+$] (50), 284(14), 221(47), 203(44), 175(65), 151(67), 139(100), 125(80), 109(95), 81(94), 69(68), 55(64) and 41(67).

**2.3 Synthesizes**

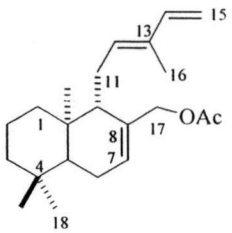
For this part, the labda-7,12(*E*),14-triene-17-oic acid (**14**) was the starting material for synthesis the modified labdanes. Compound **14** was modified by chemical reaction. The modified labdanes was confirmed the structure by the information from spectral data ($^1$H and $^{13}$C NMR, MS, IR).

**Preparation of Labda-7,12(*E*),14-triene-17-methyl ester (47).**




Compound **14** (100 mg, 0.33 mmol) was methylated with diazomethane in ether under the conditions described earlier [13], and gave compound **47** as a viscous oil (100.45 mg, 96%), $[\alpha]_D^{30}$ -12.0 ($CHCl_3$, *c* 0.97), ; FT-IR spectrum (neat) $\nu_{max}$ ($cm^{-1}$) : 2945, 2923, 1713, 1639, 1605, 1469, 1446, 1395, 1372, 1247, 1077. ; $^1$H-NMR spectrum ($CDCl_3$, 200 MHz) δ (ppm) : 6.62 (1H, dt), 5.42 (1H, t), 5.01 (1H, d), 4.85 (1H, d), 3.59 (3H, s), 2.50 (1H, m), 2.38 (1H, m), 2.22 (1H, m), 2.10 (1H, dt), 1.87 (1H, dd), 1.47 (1H, m), 1.43 (1H, m), 1.38 (1H, m), 1.27 (1H, d), 1.68 (3H, s), 1.14 (1H, d), 1.03 (1H, dd), 0.88 (3H, s), 0.85 (3H, s) and 0.81 (3H, s). ; $^{13}$C-NMR spectrum ($CDCl_3$, 50 MHz) δ (ppm) : 169.5 (C-17, 141.7 (C-14), 137.0 (C-7), 134.5 (C-8), 133.6 (C-12), 133.1 (C-13), 110.0 (C-15), 51.3 (C-21), 50.3 (C-9), 50.3 (C-5), 42.0 (C-3), 40.0 (C-1), 36.8 (C-10), 33.3 (C-4), 32.8 (C-18), 26.2 (C-11), 23.7 (C-6), 22.1 (C-19), 18.6 (C-2), 14.5 (C-20) and 11.7 (C-16). ; EIMS m/z (rel int.) : 316 [$M^+$] (46), 284(50), 235(42), 203(70), 175(96), 165(56), 153(80), 139(82), 133(55), 119(67), 190(100), 105(71), 93(53), 91(75), 81(79), 79(68), 69(59), 55(68) and 41(59).

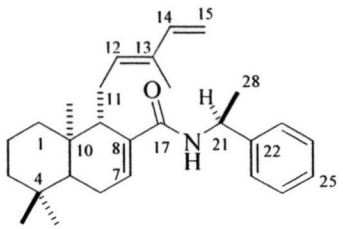
**Preparation of 17-Acetoxylabda-7,12(*E*),14-triene (48)**




Acetyl chloride ( 47.1 mg, 0.6 mmol ) was added to the solution of **13** (144 mg, 0.5 mmol) and triethylamine ( 60.7 mg, 0.6 mmol ) in $CH_2Cl_2$ ( 10 ml ). The reaction mixture was stirred for 3 h at room temperature and water was added to stop the reaction. The product was extracted with diethyl ether and with 5% $NaHCO_3$, water and then dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to give the product. The crude product was purified by silica-gel column chromatography with 60 % $CH_2Cl_2$ in hexane to obtain **48** ( 122 mg ) giving a 71 %yield.

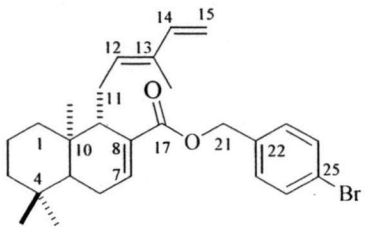
**Compound 48** ; $[\alpha]_D^{20}$ –10.7 (*c* 1.4, $CHCl_3$) Anal calcd. for $C_{22}H_{34}O_2$ : C, 79.95 ; H, 10.37%. Found : C, 79.90 ; H, 10.34%. ; IR νmax (neat) 2924, 2868, 1736, 1460,1245, 1388, 1024 and 758 $cm^{-1}$ ; $^1H$ NMR ($CDCl_3$, 200 MHz)δ 0.90-2.30 (13H, m, H-1~H-6 and H-9~H-11), 6.29(1H, dd, *J* = 10.7,17.4 Hz, H-14), 5.80(1H, m, H-7), 5.41(1H, t, *J* = 7.4 Hz, H-12), 5.04(1H, d, *J* = 17.40 Hz, H-15a), 4.84(1H, d, *J* = 10.7 Hz, H-15b), 4.48(1H, d, *J* = 12.1 Hz, H-17a), 4.26(1H, d, *J* = 12.1 Hz, H-17b), 1.99(3H, s, Me-22), 1.72(3H, s, Me-16), 0.88(3H, s, Me-19), 0.85(3H, s, Me-18), 0.77(3H, s, Me-20). ; $^{13}C$ NMR (CDCl3, 50 MHz)δ 170.6 (C-21), 141.3 (C-14), 134.4 (C-12), 133.8 (C-13), 133.1 (C-8), 129.0 (C-7), 110.4 (C-15), 67.9 (C-17), 52.3 (C-9), 49.6 (C-5), 42.1 (C-3), 39.5 (C-1), 36.6 (C-10), 33.1 (C-4), 33.1 (C-18), 25.8 (C-11), 23.7 (C-6), 21.9 (C-19), 21.0 (C-22), 18.8 (C-2), 13.9 (C-20) and 11.9 (C-16) ; EIMS *m/z* (rel. int.) : 271(10), 270(35), 255(20), 202(17), 189(44), 176(30), 161(18), 147(37), 133(61), 131(64), 119(75), 109(92), 105(100), 95(60), 93(60), 91(78), 81(65), 69(38) and 43(41).

**Preparation of N-[(*S*)-1-Phenylethyl]-labda-7,12(*E*),14-triene-17-amide (49).**



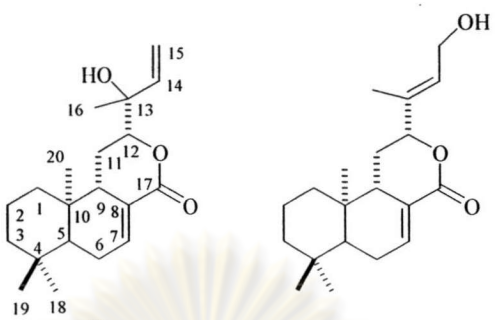

Methylbenzylamine ( 60.5 mg, 0.5 mmol ) was added to a solution of **14** ( 302.45 mg, 1 mmol ) in $CH_2Cl_2$ ( 10 ml ). The reaction mixture was stirred in ice-bath, then DCC solution ( 206.3, 1 mmol ) was added slowly drop wise and the reaction mixture was stirred for 12 hr. at room temperature in the presence of DMAP as a catalyst. The reaction mixture was filtered and the solvent was removed by evaporation under reduce pressure to give the residue product. The product was purified by silica-gel column chromatography eluted with 15 % ethyl acetate in hexane to yield **49** ( 318 mg, in 79 %yield).; $[\alpha]_D^{20}$ +0.9 (*c* 1.2, $CHCl_3$) m .p 146-148 °C ; Anal calcd. for $C_{28}H_{39}ON$ : C, 82.96 ; H, 9.63 ; N, 3.46 %. Found : C, 82.91 ; H, 9.69 ; N, 3.45 % ; IR νmax (KBr) 3267, 2924, 2858, 2842, 1659, 1618 and 1541 $cm^{-1}$ ; $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.3 (5H, s, aromatic), 6.29 (1H, dd, *J* = 10.7, 17.3 Hz, H-14), 6.09 (1H, m, C-7), 5.75 (1H, d, *J* = 8.2 Hz, -NH), 5.5 (1H, t, *J* = 7.3 Hz, H-12), 5.06 (1H, m, H-21), 5.01 (1H, d, *J* = 17.3 Hz, H-15a), 4.86 (1H, d, *J* = 10.7 Hz, H-15b), 1.70 (3H, s, Me-16), 1.40 (3H, d, *J* = 6.8 Hz, Me-28), 0.89 (3H, s, Me-20), 0.87 (3H, s, Me-18,19) and 0.90-2.60 (13H, m, H-1~H-6 and H-9~H-11). $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 170.0 (C-17), 143.6 (C-22), 141.8 (C-14), 139.7 (C-25), 133.9 (C-12), 133.0 (C-13), 129.7 (C-7), 128.9 (C-23,27), 127.2 (C-8), 126.2 (C-24,26), 109.9 (C-15), 50.5 (C-9), 48.3 (C-21), 41.9 (C-3), 39.8 (C-1), 36.6 (C-10), 33.2 (C-18), 33.2 (C-4), 25.9 (C-11), 23.1 (C-6), 22.1 (C-19), 21.5 (C-28), 18.6 (C-2), 14.7 (C-20) and 11.8 (C-16). ; EIMS *m/z* (rel. int.) : 405 $[M^+]$ (100), 390(20), 336(40), 301(87), 300(62), 286(50), 281(35), 176(52), 160(57) and 105(95).

**Preparation of 4-Bromobenzyl labda-7,12(*E*),14-triene-17-oate (50)**




4-Bromobenzyl alcohol ( 187 mg, 1 mmol ) was added to a solution of **14** ( 302 mg, 1 mmol ) in $CH_2Cl_2$ ( 20 ml ). The mixture was stirred in an ice-bath and a solution of DCC ( 206.3, 1 mmol ) was added slowly drop wise in the presence of DMAP as a catalyst. The reaction mixture was stirred for 12 hr at room temperature and the reaction product was obtained. This crude product was purified by silica-gel column chromatography eluted with 5 % diethyl ether in hexane to give **50** ( 504 mg ) with a 86 %yield. $[\alpha]_D^{20}$ –13.4 (*c* 1.16, $CHCl_3$) Anal calcd. for $C_{27}H_{35}O_2Br$ : C, 68.78 ; H, 7.48 ; Br, 16.95%. Found : C, 68.71 ; H, 7.49 ; Br, 17.02%. ; IR νmax (neat) 2924, 2863, 2847, 1716, 1644, 1490, 1362, 1239 and 1070 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 200 MHz)δ 7.45 (2H, d, *J* = 8.4 Hz, H-24,25), 7.18 (2H, d, *J* = 8.4 Hz, H-23,27), 6.70 (1H, m, H-7), 6.25 (1H, dd, *J* = 11.1, 17.4 Hz, H-14), 5.40 (1H, t, *J* = 6.8 Hz, H-12), 5.02 (2H, d, *J* = 11.6 Hz, H-21), 5.01 (1H, d, *J* = 17.4 Hz, H-15a), 4.85 (1H, d, *J* = 11.1 Hz, H-15b), 1.65 (3H, s, Me-16), 0.88 (3H, s, Me-19), 0.85 (3H, s, Me-18), 0.81 (3H, s, Me-20) and 0.90-2.60 (13H, m, H-1~H-6 and H-9~H-11). ; $^{13}C$ NMR ($CDCl_3$, 50 MHz)δ 168.5 (C-17), 141.6 (C-14), 138.1 (C-7), 135.2 (C-22), 133.4 (C-12,13), 133.2 (C-8), 131.6 (C-24,26), 130.0 (C-23,27), 128.5 (C-25), 110.1 (C-15), 65.2 (C-21), 50.1 (C-9), 49.3 (C-5), 41.9 (C-3), 39.9 (C-1), 36.8 (C-10), 33.3 (C-18), 33.2 (C-4), 26.0 (C-11), 23.8 (C-6), 22.2 (C-19), 18.6 (C-2), 14.7 (C-20) and 11.8 (C16). ; EIMS *m/z* (rel. int.) : 471 [$M^+$] (7), 470(12), 315(10), 301(92), 283(43), 255(24), 219(24), 203(37), 177(89), 169(100) and 159(46).

**Preparation of 13-Hydroxylabda-7,14-diene-17,12-olide (51) and 15-Hydroxylabda-7,13(*E*)-diene-17,12-olide (52).**




51 52

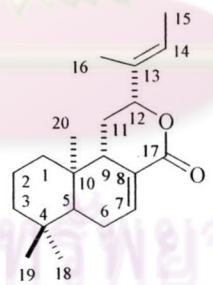
To a reaction mixture of **14** ( 302 mg, 1 mmol ) and $NaHCO_3$ ( 84.01 mg, 1.0 mmol ) in $CH_2Cl_2$ ( 10 ml ) was added *m*-CPBA ( 295.71 mg, 1.2 mmol ) in $CH_2Cl_2$ ( 10 ml ) was stirred for 3.5 hr at 0°C. The solvent was removed and the residue was extracted with diethyl ether, washed with 10% $Na_2SO_3$ , 5 % $NaHCO_3$, water, brine and dried over anhydrous $MgSO_4$. The solvent was evaporated to give the crude product. The crude product was separated by silica-gel column chromatography eluted with ethyl acetate-hexane gradient in a stepwise fashion to obtain **51** and **52** with a 60 % ( 191.0 mg ) and 29 %yields ( 92.4 mg ), respectively.

**Compound 51**: $[\alpha]_D^{20}$ +19.8 (*c* 1.1, $CHCl_3$) m.p. 105-107 °C : Anal calcd. for $C_{20}H_{30}O_3$ : C, 75.43 ; H, 9.50 %. Found : C, 75.44 ; H, 9.64 %. ; IR νmax (KBr) ; 3346 (br.), 2929, 2868, 2847, 1710, 1639, 1460, 1362, 1255, 1122 and 1075 $cm^{-1}$. ; $^1H$ NMR ($CDCl_3$, 200 MHz)δ 7.3 (1H, m, H-7), 5.86 (1H, dd, *J* = 10.7, 17.3 Hz, H-14), 5.35 (1H, d, *J* = 17.3 Hz, H-15a), 5.18 (1H, d, *J* = 10.7 Hz, H-15b), 4.06 (1H, d, *J* = 11.53 Hz, H-12), 1.35 (3H, s, Me-16), 0.89 (3H, s, Me-19), 0.87 (3H, s, Me-18), 0.72 (3H, s, Me-20) and 1.00-2.45 (13H, m, H-1~H-6 and H-9~H-11). ; $^{13}C$ NMR ($CDCl_3$, 50 MHz)δ 165.6 (C-17), 143.2 (C-7), 139.9 (C-14), 125.8 (C-8), 114.6 (C-15), 84.6 (C-12), 73.9 (C-13), 48.4 (C-9, 5), 41.8 (C-3), 38.5 (C-1), 34.7 (C-10), 32.8 (C-18), 32.7 (C-4), 25.0 (C-11), 24.0 (C-6), 22.5 (C-16), 21.3 (C-19), 18.4 (C-2) and 13.3 (C-20). ; EIMS

*m/z* (rel. int.) : 318 [$M^+$] (7), 300(50), 285(44), 261(32), 247(100), 217(58), 187(50), 177(40), 145(41) and 109(74).

**Compound 52** : $[\alpha]_D^{20}$ –3.4 (*c* 1.31, $CHCl_3$) m.p.128-130 °C ; Anal calcd. for $C_{20}H_{30}O_3$ : C, 75.43 ; H, 9.50 9%. Found : C, 75.42 ; H, 9.54 %. ; IR νmax (KBr) : 3450 (br), 2929, 2858, 1716, 1634, 1465, 1424, 1367, 1255, 1127, 1086 and 1014 $cm^{-1}$. ; $^1H$ NMR ($CDCl_3$, 200 MHz)δ 7.31(1H, m, H-7), 5.70 (1H, t, *J* = 6.5 Hz, H-14), 4.60 (1H, d, *J* = 9.8 Hz, H-12), 4.20 (2H, d, *J* = 6.5 Hz, H-15), 1.70 (3H, s, Me-16), 0.88 (3H, s, Me-19), 0.85 (3H, s, Me-18), 0.71 (3H, s, Me-20), 1.00-2.65 (13H, m, H-1~H-6 and H-9~H-11). ; $^{13}C$ NMR ($CDCl_3$, 50 MHz)δ 165.9 (C-17), 143.3 (C-7), 136.1 (C-13), 127.3 (C-14), 125.7 (C-8), 83.8 (C-12), 58.9 (C-15), 49.1 (C-9), 48.9 (C-5), 41.9 (C-3), 38.6 (C-1), 34.7 (C-10), 32.9 (C-18, 4), 27.2 (C-11), 25.2 (C-6), 21.5 (C-19), 18.5 (C-2), 13.5 (C-20) and 12.2 (C-16). ; EIMS *m/z* (rel. int.) : 318 [$M^+$] (8), 300(12), 287(87), 247(22), 217(45), 195(52), 177(50), 131(53), 124(58) and 109(100).

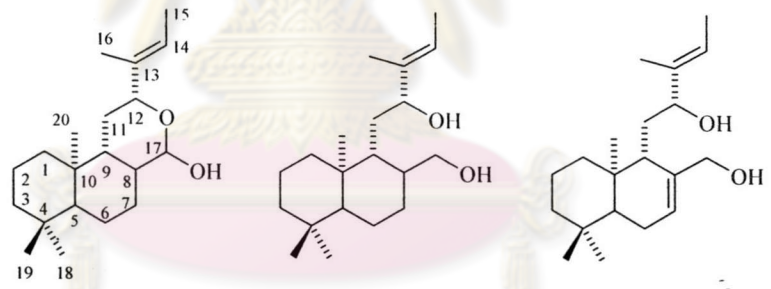
**Preparation of Labda-7,13(E)-diene-olide (53).**




TsOH ( 19 mg, 0.1 mmol) was added to the solution of **14** ( 302 mg, 1 mmol ) in anhydrous toluene ( 20 ml ). The reaction mixture was refluxed for 7 hr. and after the reaction had taken placed the reaction product was examined following the usual procedure. The product was then purified by silica-gel column chromatography and eluted with 10% EtOAc in hexane to obtain **53** ( 297 mg ) with a 98% yield. $[\alpha]_D^{20}$ –9.8 (*c* 1.2, $CHCl_3$) m.p. 104-106 °C ; Anal calcd. for $C_{20}H_{30}O_2$ : C, 79.42 ; H, 10.00 %.

Found : C, 79.40 ; H, 9.90 %. ; IR νmax (KBr) : 2924, 2863, 1716, 1644, 1460, 1367, 1255 and 1076 $cm^{-1}$. ; $^1H$ NMR ($CDCl_3$, 200 MHz)δ 7.33 (1H, m, H-7), 5.59 (1H, d, $J$= 6.4 Hz , H-14), 4.60 (1H, d, $J$ = 11.3 Hz, H-12), 1.60 (6H, s, Me-15,16), 0.92 (3H, s, Me-19), 0.90 (3H, s, Me-18), 0.76 (3H, s, Me-20) and 0.90-2.40 (13H, m, H-1~H-6 and H-9~H-11). ; $^{13}C$ NMR ($CDCl_3$, 50 MHz)δ 166.0 (C-17), 142.7 (C-7), 134.0 (C-13), 126.0 (C-8), 123.2 (C-14), 84.7 (C-12), 49.2 (C-9), 48.9 (C-5), 41.9 (C-3), 38.6 (C-1), 34.7 (C-10), 32.9 (C-18), 32.8 (C-4), 27.3 (C-11), 25.1 (C-6), 21.5 (C-19), 18.6 (C-2), 13.4 (C-20), 13.1 (C-15) and 11.4 (C-16). ; EIMS m/z (rel.int.) : 302 [$M^+$] (70), 287(25), 217(50), 203(50), 179(50), 161(53), 133(40), 124(65) and 109(100).

**Preparation of 17-Hydroxylabda-13(E)-ene-12,17-epoxide (55), 12,17-Dihydroxylabda-13(E)-ene (56) and 12,17-Dihydroxylabda-7,13(E)-diene (54).**




**55** **56** **54**

$NaBH_4$ (79.44 mg, 2.1 mmol) was added to a solution of **53** (90.6 mg, 0.3 mmol) in MeOH. The reaction was stirred at room temperature for 1 hr, quenched with water, extracted with diethyl ether and dried over anhydrous $MgSO_4$. The solvent was then removed by evaporation to give a mixture of reaction product. The mixture was separated by silica-gel column chromatography and eluted with EtOAc – hexane gradient in a stepwise fashion to give **55,56** and **54** with 20% (18.4 mg) . 36% (33.3 mg) and 26% ( 27.6 mg ), respectively.

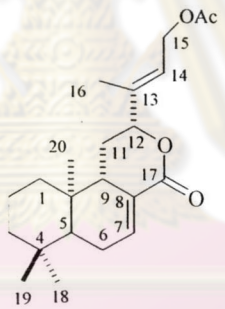
**Compound 55** as a solid ; $[\alpha]_D^{20}$ -8.5 (*c* 1.08, $CHCl_3$) m.p. 150-152 °C ; Anal calcd. for $C_{20}H_{34}O_2$ : C, 78.38 ; H, 11.18 %. Found : C, 78.39 ; H, 11.21 %. ; IR νmax (KBr) : 3385 (br), 2924, 2863, 2842, 1449, 1388, 1132 and 1029 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 200 MHz)δ 5.5 (1H, q, *J* = 6.1, 12.7 Hz, H-14), 4.33 (1H, d, *J* = 7.4 Hz, H-17), 3.75 (1H, d, *J* = 10.0 Hz, H-12), 1.63 (3H, s, Me-16), 1.62 (3H, s, Me-15), 1.50-1.55 (1H, m, H-8), 1.10-1.30 (2H, m, H-7), 0.83 (3H, s, Me-19), 0.82 (3H, s, Me-18), 0.80 (3H, s, Me-20) and 0.90-2.10 (15H, m, H-1~H-6 and H-9~H-11). ; $^{13}C$ NMR ($CDCl_3$, 50 MHz)δ 136.0 (C-13), 120.6 (C-14), 100.9 (C-17), 80.8 (C-12), 55.0 (C-9), 51.9 (C-5), 42.1 (C-3), 41.7 (C-8), 38.8 (C-1), 36.3 (C-10), 33.5 (C-18), 33.2 (C-4), 28.7 (C-6), 28.5 (C-7), 21.7 (C-19), 20.7 (C-11), 18.1 (C-2), 14.2 (C-20), 13.0 (C-15) and 12.4 (C-16). ; EIMS m/z (rel.int.) : 306 [$M^+$] (7), 288(60), 273(12), 177(25), 163(47), 149(68), 135(100), 123(70), 109(78) and 107(69).

**Compound 56** as a solid ; $[\alpha]_D^{20}$ –15.4 (*c* 1.18, $CHCl_3$) ; m.p.161-163 °C ; Anal calcd. for $C_{20}H_{36}O_2$ : C, 77.87 ; H, 11.76 %. Found : C, 77.82 ; H, 11.79 %. ; IR νmax (KBr) : 3252(br), 2924, 2868, 1460, 1444, 1383 and 1045 $cm^{-1}$. ; $^1H$ NMR ($CDCl_3$, 200 MHz) δ 5.45 (1H, q, *J* = 6.5, 12.5 Hz, H-14), 3.95 (1H, d, *J* = 11.9 Hz, H-17a), 3.95 (1H, d, *J* = 5.9 Hz, H-12), 3.30 (1H, d, *J* = 11.9 Hz, H-17b), 1.59 (6H, s, Me-15,16), 1.40-1.43 (1H, m, H-8), 1.10-1.13 (2H, m, H-7), 0.83 (3H, s, Me-19), 0.79 (3H, s, Me-18), 0.75 (3H, s, H-20) and 0.90-1.50 (15H, m, H-1~H-6 and H-9~H-11). ; $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 138.9 (C-13), 119.9 (C-14), 80.4 (C-12), 64.5 (C-17), 54.7 (C-9), 47.8 (C-5), 42.2 (C-3), 40.4 (C-8), 39.2 (C-1), 38.5 (C-10), 33.4 (C-7), 33.2 (C-4), 32.3 (C-18), 30.3 (C-6), 21.8 (C-11), 21.5 (C-19), 18.7 (C-2), 13.9 (C-20), 12.9 (C-15) and 11.3 (C-16). ; EIMS *m/z* (rel.int.) : 308 [$M^+$] (6), 290(100), 275(77), 261(18), 224(22), 191(21), 123)36), 109(41) and 105(45).

**Compound 54** as a solid ; $[\alpha]_D^{20}$ –7.6 (*c* 1.06, $CHCl_3$) ; m.p. 109-111 °C ; Anal calcd. for $C_{20}H_{34}O_2$ : C, 78.38 ; H, 11.18 %. Found : C, 78.56 ; H, 11.21%. ; IR νmax (KBr) :

3262(br), 2929, 2863, 1460, 1383, 1050 and 999 $cm^{-1}$. ; $^1H$ NMR ($CDCl_3$, 200 MHz) δ 5.68 (1H, t, *J* = 2.6 Hz, H-7), 5.45 (1H, q, *J* = 5.7, 13.0 Hz, H-14), 4.21 (1H, d, *J* = 12.1 Hz, H-17a), 3.95 (1H,dd, *J* = 4.8, 8.7 Hz, H-12), 3.75 (1H, d, *J* = 12.1 Hz, H-17b), 1.59 (6H, s, Me-15,16), 0.85 (3H, s, Me-19), 0.83 (3H, s, Me-18), 0.71 (3H, s, Me-20) and 1.90-2.70 (13H, m, H-1~H-6 and H-9~H-11). ; $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 138.7 (C-13), 126.4 (C-7,8), 119.9 (C-14), 80.1 (C-12), 66.3 (C-17), 49.9 (C-9), 48.6 (C-5), 42.2 (C-3), 39.2 (C-1), 37.0 (C-10), 32.9 (C-18), 30.8 (C-4), 23.8 (C-6, 11), 21.7 (C-19), 18.7 (C-2), 13.6 (C-20), 13.0 (C15) and 11.3 (C-16). ; EIMS *m/z* (rel.int.) : 306 [$M^+$] (98), 288(72), 270(40), 213(38), 203(32), 189(42), 164(55), 133(68), 119(79), 109(100) and 105(71).

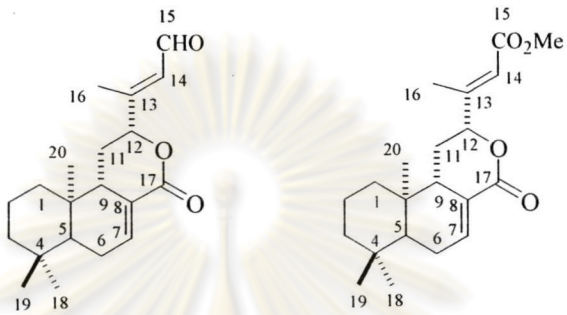
**Preparation of 15-Acetoxylabda-7,13(E)-diene-17,12-olide (60)**




To a solution of **51** ( 300 mg, 0.94 mmol) in $CH_2Cl_2$ (15 ml) was added with $BF_3$.2AcOH (0.78 ml, 2.83 mmol) in ice-bath and stirred for 3.5 h. The solution was added by water (7 ml), stirred 5 min and diluted with EtOAc (20 ml). The organic layer was washed with 5% $NaHCO_3$ (20 x 4), washed with water (20 ml), washed with brine and dried with MgSO4. The crude product was purified by column chromatography (20%, 50% EtOAc in hexane) to give **60** in 60% yield as colorless oil. $[\alpha]_D^{20}$ +4.6 (*c* 0.54, $CHCl_3$), IR νmax (neat) : 2926, 2850, 1739, 1718, 1639, 1368, 1235, 1124 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.35 (1H, ddd, *J* = 2.8, 2.8, 7.6 Hz, H-7), 5.69 (1H, t, *J* = 6.8 Hz, 14-H), 4.65 (2H, dd, *J* = 2.8, 6.8 Hz, 15-H), 4.62 (1H, d, *J* = 11.2 Hz, 12-H),

2.07 (3H, s, -$COCH_3$), 1.76 (3H, s, $CH_3$), 0.93 (3H, s, $CH_3$), 0.90 (3H, s, $CH_3$), 0.77 (3H, s, $CH_3$).

**Preparation of 15-Aldehydelabda-7,13(E)-diene-17,12-olide (64) and 15-Methyl ester labda-7,13(E)-diene-17,12-olide (65)**




**64** **65**

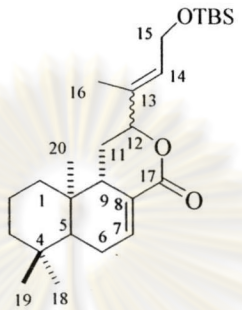
To a solution of **52** ( 107.4 mg, 0.34 mmol) in benzene (5.3 ml) was added by $MnO_2$ (586.32 mg, 6.74 mmol). The solution was refluxed for 1.5 h after the solution was cooled to rt. The mixture was dilutued with PhH (20 ml) and filtrated with celite. The crude product was detected by $^1$H NMR. The aldehyde crude product (**64**) which was dissolved in MeOH (3.3 ml) was treated with NaCN (82.63 mg, 1.69 mmol), AcOH (0.03 ml, 0.51 mmol) and stirred at rt for 5 min. The mixture was added with $MnO_2$ (586.32 mg, 6.74 mmol) and stirred at rt for 1 h. The mixture was diluted with EtOAc (10 ml) and filtrated with celite. The organic layer was washed with water (10 mL x 2), brine (10 ml) and dried with $MgSO_4$. The crude product was purified by column chromatography (15% EtOAc in hexane) to produce compound **65** (58.16 mg) in 50% yield.

**Compound 64** : $^1$H NMR ($CDCl_3$, 400 MHz) δ 10.08 (1H, s, -CHO), 7.36 (1H, s, H-7), 6.13 (1H, d, $J$ = 4.8 Hz, H-14), 4.74 (1H, d, $J$ = 7.2 Hz, H-12), 2.22 (3H, s, $CH_3$), 0.93(3H, s, $CH_3$), 0.91 (3H, s, $CH_3$), 0.77 (3H, s, $CH_3$)

**Compound 65** : $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.38 (1H, ddd, $J$= 2.8, 2.8, 5.2 Hz, H-7),

6.03 (1H, t, $J$ = 0.8 Hz, H-14), 4.66 (1H, d, $J$ = 10.8 Hz, H-12), 3.72 (3H, s, -COOMe), 2.18 (3H, s, $CH_3$), 0.93 (3H, s, $CH_3$), 0.91 (3H, s, $CH_3$), 0.76 (3H, s, $CH_3$).

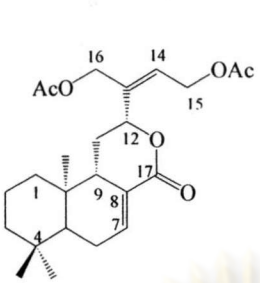
**Preparation of 15-t-Butyldimethy dimethyloxylabda-7,13(E)-diene-17,12-olide (62)**



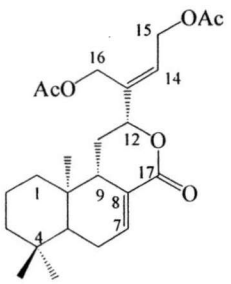

**62**

To a solution of **52** (50 mg, 0.16 mmol) in DMF (0.5 ml) was added by immidazole (26.75 g, 0.393 mmol) and added TBSC1 (28.43 mg, 0.19 mmol) and stirred at rt for 15 min. The solution was diluted with EtOAc (10 mL), washed with sat. $NaHCO_3$ (10 mL x 2), washed with water (10 mL), washed with brine (10 mL) and dried with $MgSO_4$. Crude product was purified by column chromatography (7% EtOAc in hexane) to obtain compound **62** (62.5 mg) in 92% yield as colorless solid. IR νmax (neat) : 2927, 2855, 1719, 1643, 1459, 1246, 1075, 835 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.29 (1H, s, br, H-7), 5.60 (1H, t, $J$ = 6.0 Hz, H-14), 4.58 (1H, d, $J$ = 11.2 Hz, H-12), 4.21 (2H, d, $J$ = 6.0 Hz, H-15), 1.64 (3H, s, $CH_3$), 0.88 (3H, s, $CH_3$), 0.87 (3H, s, $CH_3$), 0.72 (3H, s, $CH_3$), 0.10 (6H, s, -$SiCH_3$).

**Preparation of 15,16-Diacetoxylabda-7,13(Z)-diene-17,12-olide (66b) and 15,16-Diacetoxylabda-7,13(E)-diene-17,12-olide (66a)**



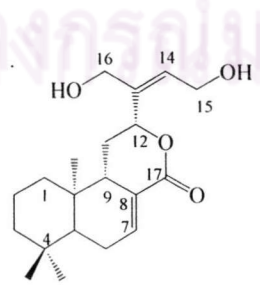

**66b**



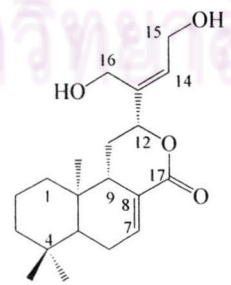

**66a**

To a solution of **60** (60 mg, 0.17 mmol) in $Ac_2O$ (1.7 ml) was added with $SeO_2$ (27.69 mg, 0.25 mmol) and was refluxed at 140°C for 20.5 h. The reaction was cooled to room temperature and diluted with EtOAc (10 mL). The organic layer was washed with 5% $NaHCO_3$ (10 mL x 3), washed with water (10 mL), washed with brine (10 mL) and dried with $MgSO_4$. The crude product was purified by column chromatography (20% EtOAc in hexane) to afford the mixture of **66a** and **66b** in 28% yield (19.3 mg) as colorless oil. $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.37 (1H, ddd, $J$ = 2.4, 2.4 , 5.2 Hz, H-7), 5.97 (0.7H/1H, t, $J$ = 6.4 Hz, H-14 of **66a**), 5.80 (1H, t, $J$ = 6.8 Hz, H-14), 5.20 (1H, dd, $J$ = 2.8, 11.6 Hz, H-12), 4.70 (4H, m, H-15,16), 2.09 (3H, s, -$COCH_3$), 2.08 (3H, s, -$COCH_3$), 0.93 (3H, s, $CH_3$), 0.91 (3H, s, $CH_3$), 0.78 (3H, s, $CH_3$).

**Preparation of 15,16-Dihydroxylabda-7,13(Z)-diene-17,12-olide (67b) and 15,16-Dihydroxylabda-7,13(E)-diene-17,12-olide (67a)**



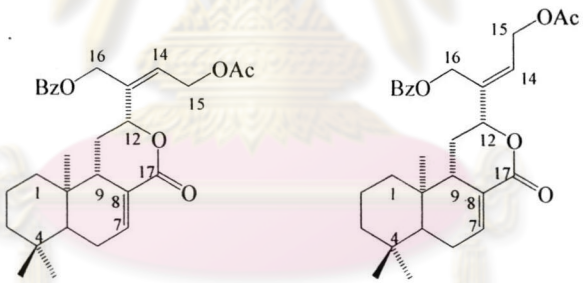

**67b**




**67a**

To a solution of mixture of **66a** and **66b** (19.8 mg, 0.05 mmol) in EtOH (0.5 ml) were added with 5% NaOH (0.16 ml, 0.20 mmol) in ice-bath. The reaction mixture was stirred for 30 min and added 5% HCl (2 mL). The suspension was diluted with EtOAc (10 ml). Organic layer was washed with water (10 mL x 2), washed with brine (10 mL) and dried with $MgSO_4$. The crude product was purified by PTLC (6% EtOH in CHCl3) to result the mixture of **67a** and **67b** in 66% yield as colorless. $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.36 (1H, s, br, H-7), 5.97 (0.15H/1H, t, $J$ = 6.4 Hz, H-14 of **67a**), 5.89 (1H, t, $J$ = 6.4 Hz, H-14), 5.24 (1H, d, $J$ = 11.2 Hz, H-12), 4.82 (0.15H/1H, d, $J$ = 11.2 Hz, H-12 of **53**), 4.23 (4H, m, H-15,16), 0.93 (3H, s, $CH_3$), 0.91(3H, s, $CH_3$), 0.77 (3H, s, $CH_3$).

**Preparation of 15-Acetoxy-16-benzoate labda-7,13(*Z*)-diene-17,12-olide (68b) and 15-Acetoxy-16-benzoate labda-7,13(*E*)-diene-17,12-olide (68a)**

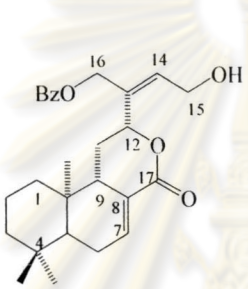



**68b** **68a**

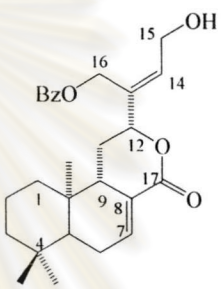
To a compound **60** (63 mg, 0.17 mmol) and Benzoic anhydried (1.00g, 4.42 mmol) was heated at 50 °C for 10 min to generate the solution. The mixture was added by $SeO_2$ (29.09mg, 0.26 mmol) and heated to 140 °C for 22.5 h. After cooling to roomtemperature the mixture was diluted with EtOAc (20 ml). Organic layer was washed with 5% $NaHCO_3$ (20 ml x 2), water (20 ml), brine (20 ml) and dried with $MgSO_4$. The crude product was purified by column chromatography (20% EtOAc in hexane) to give the mixture of **68a** and **68b** (34.9mg) in 42% yield. $^1$H NMR ($CDCl_3$,

400 MHz) δ 8.02 (2H, dd, $J$ = 7.6, 9.2 Hz, Aromatic), 7.50 (1H, t, $J$ = 7.6 Hz, Aromatic), 7.44 (2H, t, $J$ = 7.6 Hz, Aromatic), 7.37 (1H, s, H-7), 6.03 (0.7/1H, t, $J$ = 6.4 Hz, H-14 of **55**), 5.25 (1H, d, $J$ = 10.4 Hz, H-12), 5.04-4.70 (4H, m, H-15, 16), 2.05 (3H, s, $-COCH_3$), 0.92 (3H, s, $CH_3$), 0.90 (3H, s, $CH_3$), 0.74 (3H, s, $CH_3$),

**Preparation of 15-Hydroxy-16-benzoate labda-7,13(*Z*)-diene-17,12-olide (69b) and 15-Hydroxy-16-benzoate labda-7,13(*E*)-diene-17,12-olide (69a)**



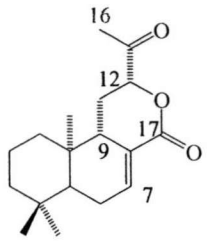

**69a**




**69b**

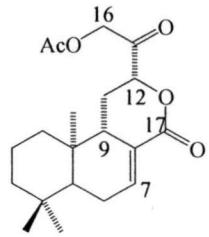
To a solution of mixture of **68a** and **68b** (14.7 mg, 0.03 mmol) in EtOH (0.4 ml) was added 5% $Na_2CO_3$ (0.14 ml, 0.06 mmol) at rt. The suspension was stirred at rt for 1 d and 5% HCl (2 mL) was added. The solution was diluted with EtOAc (10 ml) and washed with water (10 ml x 2), brine (10 ml) and dried with $MgSO_4$. The crude product was purified by column chromatography (50% EtOAc in hexane) to afford the mixture of **69a** and **69b** in 50% yield (6.7 mg). $^1$H NMR ($CDCl_3$, 200 MHz) δ 8.02 (2H, dd, $J$ = 6.8, 8 Hz, Aromatic), 7.57 (1H, m, Aromctic), 7.44 (2H, t, J = 8.0 Hz, Aromatic), 7.36 (1H, s, .H-7), 6.42 (0.8/2H, t, $J$ = 4.8 Hz, H-15 of **69a**), 6.10 (0.39/1H, t, $J$ = 6.4 Hz, H-14 of **69a**), 6.00 (1H, t, $J$ = 6.8 Hz, H-14), 5.22 (1H, dd, $J$ = 2.0, 11.6 Hz, H-12), 4.93 (2H, m, H-16), 4.30 (2H, t, $J$ = 6.8 Hz, H-15), 0.92 (3H, s, $CH_3$), 0.89 (3H, s, $CH_3$), 0.73 (3H, s, $CH_3$).

**Preparation of 13-Oxo-14,15-dinor-7-labda-17,12-olide (72)**



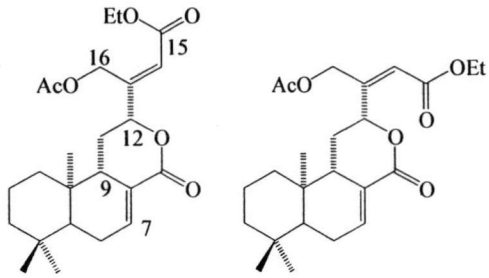

To a solution mixture of **53** (2000 mg, 6.61 mmol) which dissolved in DCM (70 mL) was added *m*-CPBA (1956.26 mg, 7.94 mmol) and the solution was stirred at room temperature for 4 h. The reaction mixture was diluted with $Et_2O$ (50 mL), washed with 10% $NaHSO_3$ (50 mL), washed with 5% $NaHCO_3$ (50 mL x 2), water (50 mL x 2) and brine (50 mL). The organic solvent was dried with $Na_2SO4$. The solvent was evaporated in vacuum to give epoxide crude product (**71**). The epoxide crude product (**71**) was dissolved in THF (82 mL) and added $H_5IO_6$ (2863.2 mg, 12.56 mmol). The reaction mixture was stirred at room temperature for 4 h and diluted with $Et_2O$ (50 mL), washed with water (50 mL), washed with 10% $NaHSO_3$ (50 mL), washed with water again (50 mL x 2) and washed with brine (50 mL). The organic solvent was dried with $Na_2SO_4$. The crude product was purified by column chromatography (16 % EtOAc in hexane) to obtain **72** in 65% yield in 2 steps as solid. as a solid ; $[\alpha]_D^{20}$ -37.5 (*c* 0.2, $CHCl_3$) ; m.p. = 97-99 °C ; Anal calcd. for $C_{18}H_{26}O_3$ : C, 74.45 ; H, 9.02 %. Found : C, 74.41 ; H, 8.98 %. ; IR νmax (KBr) : 2959, 2921, 1720, 1640, 1461, 1376, 1258, 1197, 1127 and 1084 $cm^{-1}$. ; $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.36 (1H, s, H-7), 4.54 (1H, dd, *J* = 2.0, 12 Hz, H-12), 2.32 (3H, s, $CH_3CO$), 0.88 (3H, s, $CH_3$), 0.86 (3H, s, $CH_3$) and 0.72 (3H, s, $CH_3$) ; $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 206.8 (C-13), 164.28 (C-17), 144.7 (C-7), 125.1 (C-8), 82.8 (C-12), 48.7 (C-9), 41.8 (C-3), 38.6 (C-1), 34.8 (C-10), 32.8 (C-4), 32.8 (C-18), 25.9 (C-16), 25.3 (C-6), 25.0 (C-11), 21.4 (C-19), 18.5 (C-2) and 13.3 (C-20) ; EIMS *m/z* (rel.int.) : 290[$M^+$](14), 247(100), 187(10), 167(20), 109(65), 95(29), 91(33), 83(35), 69(34) and 55(26)

**Preparation of 16-Acetoxy-13-oxo-14,15-dinor-7-labda-17,12-olide (73)**




To a solution of **72** (1068.7 mg, 3.68 mmol) which was dissolved in benzene (53 mL) was added by $PbOAc_4$ (8168.1 mg, 18.40 mmol) and added drop wise at room temperature of $BF_3.Et_2O$ (9.3 mL, 73.6 mmol). The reaction mixture was heated at 50 $^0C$ for 8 h and cooled to room temperature, diluted with $Et_2O$ (50 mL), washed with water (50 mL x 3), washed with brine (50 mL) and dried with $Na_2SO_4$. After evaporation of solvent the crude product was purified by column chromatography (20% EtOAc in hexane) to obtain 60 in 69 % yield as solid. $[\alpha]_D^{20}$ -38.9 (*c* 0.193, $CHCl_3$) ; m.p. = 106-108 $^oC$ ; Anal calcd. for $C_{20}H_{28}O_5$ : C, 68.94 ; H, 8.10 %. Found : C, 68.91 ; H, 8.14 %. ; IR νmax (KBr) : 2955, 2919, 2858, 1736, 1644, 1460, 1372, 1260, 1091, 1019 and 804 $cm^{-1}$. ; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.39 (1H, s, H-7), 5.13 and 4.99 (2H, d, *J* = 12.8 Hz, $CH_2OAc$), 4.77 (1H, d, *J* = 11.8 Hz, H-12), 2.17 (3H, s, OAc), 0.92 (3H, s, Me), 0.89 (3H, s, Me) and 0.76 (3H, s, Me) ; $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ ; EIMS *m/z* (rel.int.) : 348[$M^+$](4), 247(80), 225(18), 164(20), 124(22), 109(50), 105(20), 101(48), 91(45), 79(27), 73(100), 69(32) and 55(35).

**Preparation of Ethyl 16-acetoxy labda-17,12-olide-7,13(*E*)-diene (74) and Ethyl 16-acetoxy labda-17,12-olide-7,13(*Z*)-diene (75)**



74 75

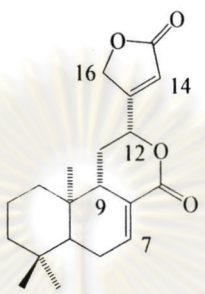
To a solution of **73** (341.7 mg, 0.98 mmol) which was dissolved in benzene (14 mL) was added by $Ph_3PCHCO_2Et$ (683.30 mg, 1.96 mmol). The reaction mixture was heated to reflux for 2 h, cooled to room temperature, diluted with $Et_2O$ (15 mL), washed with water (20 mL x 2), washed brine (20 mL) and dried with $Na_2SO_4$. The crude product was purified by column chromatography (14 % EtOAc in hexane) to give **74** and **75** in 54 and 43 % yield respectively.

**Compound 74** : $[\alpha]_D^{20}$ -17.1 (*c* 0.187, $CHCl_3$) ; Anal calcd. for $C_{24}H_{34}O_6$ : C, 68.87 ; H, 8.19 %. Found : C, 68.86 ; H, 8.15 %. ; IR νmax (KBr) : 2960, 2919, 2863, 1721, 1639, 1460, 1372, 1260, 1081, 1024 and 799 $cm^{-1}$. ; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.38 (1H, s, H-7), 6.25 (1H, s, H-14), 5.39 and 5.10 (2H, d, *J* = 14.0 Hz, $CH_2OAc$), 4.87 (1H, d, *J* = 8.6 Hz, H-12), 4.19 (2H, d, *J* = 7.0 Hz, $OCH_2CH_3$), 2.08 (3H, s, OAc), 1.28 (3H, s, $OCH_2CH_3$), 0.92 (3H, s, Me), 0.90 (3H, s, Me) and 0.72 (3H, s, Me) ; $^{13}C$ NMR ($CDCl_3$, 50 MHz)δ ; EIMS *m/z* (rel.int.) : 418 [$M^+$] (2), 358(9), 331(10), 312(20), 295(30), 235(11), 207(9), 184(12), 124(22), 109(100), 105(27), 91(42), 81(26), 69(45), 55(34).

**Compound 75** : $[\alpha]_D^{20}$ -46.1 (*c* 0.23, $CHCl_3$) ; Anal calcd. for $C_{24}H_{34}O_6$ : C, 68.87 ; H, 8.19 %. Found : C, 68.84 ; H, 8.17 %. ; IR νmax (KBr) : 2960, 2929, 2852, 1751, 1721, 1644, 1470, 1372, 1265, 1229, 1152, 1075, 1034 and 804 $cm^{-1}$. ; $^1H$ NMR ($CDCl_3$, 400 MHz)δ 7.37 (1H, s, H-7), 6.06 (1H, d, *J* = 11.3 Hz, H-12), 5.87 (1H, s, H-14), 4.92 and 4.82 (2H, d, *J* = 16.2 Hz, $CH_2OAc$), 4.17 (2H, d, *J* = 7 Hz, $OCH_2CH_3$), 2.13 (3H, s, OAc), 1.28 (3H, s, $OCH_2CH_3$), 0.91 (3H, s, Me), 0.89 (3H, s, Me) and 0.76 (3H, s, Me) ;

$^{13}C$ NMR ($CDCl_3$, 50 MHz)δ ; EIMS *m/z* (rel.int.) : 418 [$M^+$] (3), 372(7), 358(29), 331(14), 312(70), 295(42), 235(19), 207(20), 188(32), 184(29), 160(18), 124(34), 109(100), 105(13), 91(18), 81(17) and 69(22).

**Preparation of (+)-Limonidilactone (33)**




**(+)-limonidilactone (33)**

To a solution of **74** (77.2 mg, 0.18 mmol) which was dissolved in MeOH (3 mL) was added TsOH (280.7 mg, 1.46 mmol). The reaction mixture was stirred at 50 $^0C$ for 1.5 h and cooled to room temperature, diluted with $Et_2O$ (10 mL), washed with 5 % $NaHCO_3$ (10 mL), washed with water (10 mL), brine (10 mL) and dried with $Na_2SO_4$. After removal of solvent, the reaction gave compound **33** as white solid in 92 % yield. $[\alpha]_D^{20}$ +14.0 (*c* 0.193, $CHCl_3$) ; m.p. = 227-229 °C ; Anal calcd. for $C_{20}H_{26}O_4$ : C, 72.70 ; H, 7.93 %. Found : C, 72.72 ; H, 7.90 %. ; IR νmax (~~KBr~~) : 2960, 2924, 2847, 1782, 1751, 1721, 1644, 1255, 1142, 1086, 1024 and 804 $cm^{-1}$. ; $^1H$ NMR ($CDCl_3$, 200 MHz) δ 7.40 (1H, s, H-7), 6.07 (1H, d, *J* = 1.7 Hz, H-14), 5.21 (1H, d, *J* = 11.2 Hz, H-12), 4.95 (2H, s, H-16), 0.92 (3H, s, Me), 0.89 (3H, s, Me) and 0.76 (3H, s, Me) ; $^{13}C$ NMR ($CDCl_3$, 200 MHz) δ ; EIMS *m/z* (rel.int.) : 330 [$M^+$] (4), 312(9), 253(5), 239(4), 207(32), 124(100), 109(95), 105(8), 95(9), 91(12), 81(14), 75(19), 69(13) and 55(9).

**2.4 The cytotoxicity test**

The bioassay of cytotoxicity against human cell cultures *in vitro* was performed by the MTT[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method [41]. Doxorubicin hydrochloride was used as a positive control substance.

**2.5 The $Na^+$, $K^+$-ATpase test**

All procedures of enzyme purification were carried out in an ice bath. Rat were stunned by means of blow on the neck under an anesthetic with CO2, decapitated, and exsanguinated. The brain (cerebrum) were quickly removed and the gray matter was minced with a pair of scissors after eliminating white matter and capillaries. The homogenizing medium (HM), consisting of 0.32 M sucrose, 5 mM Tris-HCl (pH 7.5), and 2 mM ethylenediamine tetraacetic acid (EDTA), was added at a ratio of 1 g of tissue to 9 mL of HM, and the tissue was homogenized with 10 strokes of a Potter-Elverhjem homogenizer, using a Teflon pestle. The homogenate was centrifuged at 7,700 x g for 10 min in a refrigerated centrifuge, and the supernatant was recentrifuged at 25,000 x g for 40 min. The pellet was suspended in 5 mM Tris-HCl (pH 7.5) containing 1 mM EDTA-Tris, washed twice with the same buffer.

**2.6 Preparation of Kidney Microsomes**

Rat kidneys from which the pelvis had been removed were chopped with a pair of scissors, and homogenized in 9 volumes of HM with 15 strokes of a Teflon homogenizer. The homogenate was centrifuged at 2,450 x g for 15 min in a refrigerated centrifuge and the supernatant was decanted off and saved. The pellet was suspended in one-half of the original volume of HM and washed. The resulting supernatant was combined with the previous one and the pooled fraction was centrifuged at 32,800 x g

for 35 min. The pellet was suspended in 5 mM Tris-HCl (pH 7.5) containing 1 mM EDTA. The washing procedure was repeated twice with the same buffer.

**2.7 Assay of $Na^+$,$K^+$-ATPase activity**

The standard assay mixture containing, in a final volume of 100 µl, 3 mM ATP-Tris, 5 mM MgCl2, 0.5 mM EDTA, 140 mM NaCl, 14 mM KCl and 50 mM Imidazole (pH 7.2). 5 µg of the microsomes enzyme was added and incubation was carried out for 15 to 30 min at 37 $^0$C. The reaction was stopped by using 50 % trichloroacetic acid (TCA). Liberated orthophosphate (Pi) was determined by the method of Fiske and Subbarow. One unit of specific activity is defined as the liberation of 1 µmol of inorganic phosphate per mg protein per min.