New Hemisynthetic Manoyl Oxide Derivatives with Antimicrobial Activity

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The synthesis and antimicrobial activity of ten labdane-type diterpenes derived from *ent*-3-β-hydroxy-13-*epi*-manoyl oxide (ribenol) is reported. The chloroethyl carbamidic ester 9 showed the strongest antimicrobial activity against all the tested gram (+), gram (−) bacteria and pathogenic fungi. Moreover, the glycoside 11 exhibited an interesting activity against the three tested fungi.

Key words labdane-type diterpene; antimicrobial activity; manoyl oxide; *Cistus creticus*

Labdane type diterpenes possess a wide spectrum of biological activities, such as anti-mutagenic,[1] cell differentiation,[2] anti-(PAF),[3] and anti-inflamatory,[4] but the antibacterial and antifungal activities are more important.[5] Diterpenoids possessing an *ent*-manoyl oxide skeleton, such as *ent*-13-*epi*-manoyl oxide or its derivative hydroxylated in position C-3 (ribenol), have been proven to be powerful antimicrobial agents[6,7] and also have recently been cited to possess interesting activity against *Leismania donovani*.[8] On the other hand, ribenol (**1**) is one of the major compounds of the commercial resin "ladano" obtained from the aerial parts of *Cistus creticus*, and it constitutes 2% of the crude extract.[7] Therefore, the preparation of new analogues of this skeletal type could be carried out to obtain new leads in antimicrobial drug development.

As a part of our program regarding the discovery of new antimicrobial agents of natural origin, the antimicrobial activity of ten hemisynthetic compounds derived from *ent*-3-β-hydroxy-13-*epi*-manoyl oxide or ribenol (**1**) is reported here.

A total of ten *ent*-3-β-hydroxy-13-*epi*-manoyl oxides were prepared from ribenol (**1**): three carboxylates, **2**, **3**, **4**, five carbamates, **5**, **6**, **7**, **8**, **9**, the *ent*-13-*epi*-manoyl oxide-3-yl methanosulfonate (**10**), and the glycoside **11**. The carboxylates were synthesized by treatment of **1** with the corresponding anhydrides or acid chlorides. The carbamates were synthesized by treatment of **1** with the corresponding isocyanate derivatives in the presence of pyridine.[9] The lipophilic glycoside **11** was obtained by treatment of **1** with 4-*O*-acetyl-3-chloro-2,3,6-trideoxy-α-L-arabinosyl chloride, derived from α-L-rhamnal,[10] under modified Koenings–Knorr conditions (yellow mercury(II) oxide and mercury(II) bromide) in 40% yield, accompanied by 2′,3′-unsaturated compound **13**. It is interesting to point out that all the efforts for the nucleophilic substitution of the mesylate **10**, using NaN_3 or

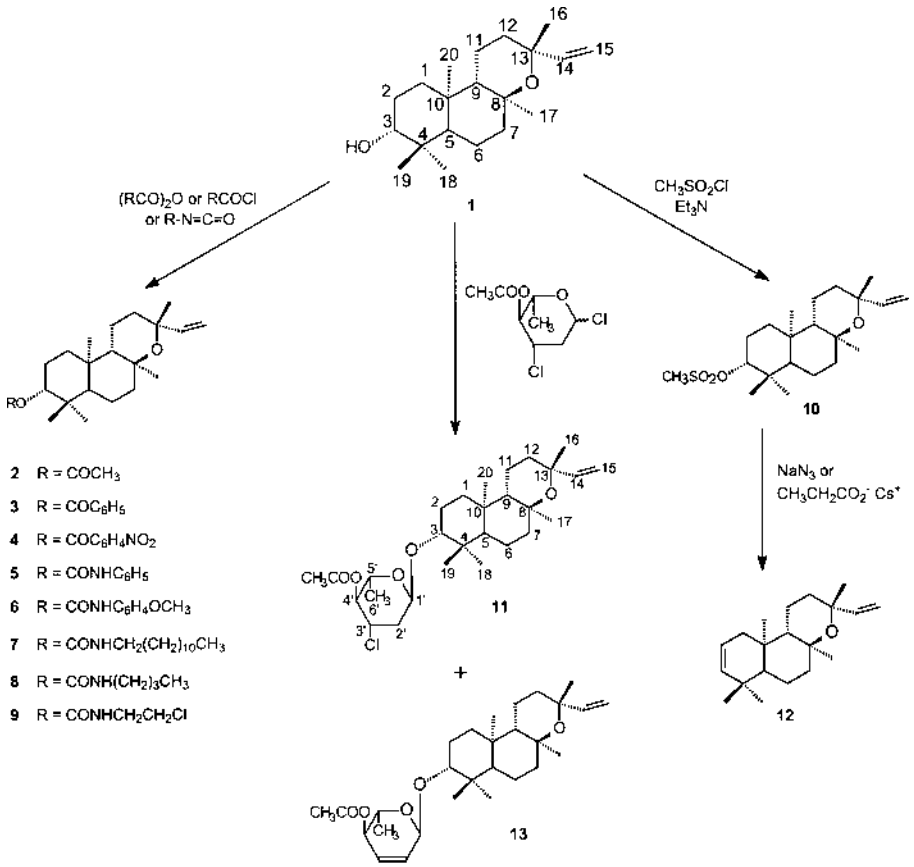

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Table 1. Antimicrobial Activitya of Compounds **1**—**12**

	Strain								
	Staphylococcus aureus	*Staphylococcus epidermidis*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Enterobacter cloacae*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*
Compound									
1	28	18	—	20	12	—	—	—	—
2	25	16	—	10	—	—	10	12	10
3	8	10	8	—	9	—	—	—	—
4	20	19	14	14	19	14	—	—	—
5	12	12	12	12	10	12	—	—	—
6	9	8	12	—	—	—	—	—	—
7	15	10	10	—	—	10	—	—	—
8	20	17	10	11	10	12	—	—	—
9	18	16	12	9	15	12	10	14	10
10	9	—	10	10	12	10	—	—	—
11	—	—	—	—	—	—	10	12	10
12	12	—	10	10	10	12	—	—	—
Netilmicin	26	20	30	24	22	20	—	—	—
Ceftriaxon	18	20	25	24	22	22	—	—	—
Ceftazidin	30	22	30	18	32	22	—	—	—
Amoxicillin	14	18	20	14	20	16	—	—	—
5-Flucytocine	—	—	—	—	—	—	30	34	33
Amphotericin B	—	—	—	—	—	—	20	25	20
Intraconazole	—	—	—	—	—	—	25	32	28

a) The results were reported as the diameter of the zone of inhibition around each disk (in mm).

$CH_3CH_2COO^-Cs^+$ at 90 °C, were unsuccessful and yielded a β elimination product, 8,13-epoxylabda-2,14-diene (**12**).[11] At lower temperatures (25 °C or 60 °C) we obtained only the starting material. The structure of the synthesized diterpenoids was determined by mass (CID), ^{1}H- and ^{13}C-NMR spectroscopic methods. DEPT 135° and 2D-NMR experiments were applied to assign unambiguously the ^{1}H and ^{13}C chemical shifts of all the signals of the above-mentioned diterpenoids.

The antimicrobial activity (Table 1) of the tested compounds (**1**—**12**) was determined by the standardized disk diffusion method of Bauer–Kirby[12] against two gram positive bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis*, four gram negative bacteria: *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and three pathogenic fungi: *Candida albicans*, *Candida tropicalis* and *Candida glabrata*. Netilmicin, ceftriaxon, ceftazidin, amoxicillin, 5-flucytocine, amphotericin B and intraconazole were used as standard antimicrobial agents for comparison for the tested bacteria as well as for the tested fungi. The results were reported as the diameter of the zone of inhibition around each disk (in mm).

As far as structure activity relationships are concerned, it is interesting to point out that the *ent*-3β-hydroxy-13-*epi*-manoyl oxide (**1**) showed good activity against *S. aureus*, *S. epidermidis*, *K. pneumoniae* and *P. aeruginosa* and so did the carboxylic acid esters **2**, **4**, and the carbamate esters **8** and **9**. Moreover, the lipophilic glycoside **11**, as well as the esters **2** and **9**, showed a good antimicrobial activity against the three pathogenic fungi. In addition compound **9** (possessing the chloroethyl group) showed the broadest spectrum of activity. In conclusion, in good accordance with previous findings,[13,14] antimicrobial activity increases with increasing lipophilicity. The evaluation of these three derivatives against clinically isolated resistant bacterial strains and the synthesis of new analogs are currently in progress in our laboratory.

Experimental

General Remarks Optical rotations were measured with a Perkin-Elmer 341 polarimeter. NMR spectra were recorded on a Bruker AC200 and a Bruker DRX400 spectrometer. Chemical shifts are given in δ values with TMS as an internal standard. The 2D experiments (COSY, HMQC, HMBC) were performed using standard Bruker microprograms. Mass spectra were recorded with a Nermag R 10-10C spectrometer using CID-MS (reagent gas, NH_3) techniques. Column chromatography was performed on silica gel [Merck, 0.04—0.06 mm (flash chromatography)]. Analytical thin layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck Silica gel F-254 plates.

General Method (A) for the Preparation of Carboxylates by the Treatment of the Anhydride or Acid Chloride with Ribenol Ribenol (**1**) was dissolved in dry pyridine (2.0 ml), and anhydride or acid chloride was added, then the mixture was stirred under Ar atmosphere. The pyridine was removed under reduced pressure and the residue was dissolved in CH_2Cl_2. The solution was washed with saturated aqueous solution $NaHCO_3$, the organic solvent was removed under reduced pressure, and the residue was chromatographed on silica gel.

General Method (B) for the Preparation of Carbamate Derivatives by the Treatment of the Corresponding Isocyanate Reagents with Ribenol Ribenol (**1**) was dissolved in dry pyridine (2.0 ml), isocyanate derivative was added, and the mixture was stirred under Ar atmosphere. The pyridine was removed under reduced pressure and the residue was chromatographed on silica gel.

Antimicrobial Activity The antimicrobial activity of the tested compounds was determined by the disk diffusion method of Bauer–Kirby[12] as it has been described[15,16] against two gram positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228), and four gram negative bacteria: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883), as well as against three pathogenic fungi: *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 13801) and *Candida glabrata* (ATCC 28838). Netilmicin, ceftriaxon, ceftazidin, amoxicillin, 5-flucytocine, amphotericin B and intraconazole were used as standard antibiotics for comparison for the tested bacteria and the tested fungi. The results were reported as the diameter of the zone of inhibition around each disk (in mm). The isolated compounds were dissolved in CH_2Cl_2. For each experiment, control discs with pure solvent were used as a blind control. All the paper discs had a diameter of 6 mm and were deposited on the

surface of the seeded trypticase Muller–Hinton agar, while Sabouraud agar was used for growing the fungi. Petri dishes, which had been previously inoculated with the tested organisms, gave a final cell concentration of 10^7 cell/ml. 10 μl volumes of the above solutions were required to wet the test paper discs. The incubation conditions used were 24 h at 37 °C (for the bacteria) and 48 h at 28 °C (for the fungi). The experiments were repeated three times and the results were expressed as average values.

***ent*-3-β-Hydroxy-13-*epi*-manoyl Oxide (1)** This compound was isolated from the resin "ladano" of *Cistus creticus*, as described in ref. 7, and identified by comparison of its optical rotation and spectral data (CID-MS, ^{1}H- and ^{13}C-NMR) with published data.[17)]

***ent*-3β-Acetoxy-13-*epi*-manoyl Oxide (2)** This compound was synthesized according to the general method A by the treatment of **1** (15.8 mg, 0.052 mmol) with acetic anhydride (26.5 mg, 0.260 mmol), then identified by comparison of its optical rotation and spectral data (CID-MS, ^{1}H- and ^{13}C-NMR) with published data.[18,19)]

***ent*-13-*epi*-Manoyloxide-3-yl Benzoate (3)** This compound was synthesized according to the general method A by the treatment of **1** (15.8 mg, 0.052 mmol) with benzoic anhydride (58.8 mg, 0.260 mmol). The reaction mixture was stirred for 20 h at 60 °C. Compound **3** (13.9 mg, 0.034 mmol, 65.4%) was obtained by column chromatography on silica gel (cyclohexane : EtOAc 90 : 10): $[\alpha]_D^{20}$ −77.3° (c=0.09, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.78 (3H, s, H-20), 0.90 (3H, s, H-19), 0.97 (3H, s, H-18), 1.02—1.18 (6H, m, H-1ax, H-5, H-9, H-16), 1.21 (3H, s, H-17), 1.30—1.50 (5H, m, H-6ax, H-7ax, H-11ax, H-11eq, H-12ax), 1.63—1.88 (5H, m, H-1eq, H-2ax, H-2eq, H-6eq, H-7eq), 2.22 (1H, m, H-12ax), 4.71 (1H, dd, J=4.5, 11.3 Hz, H-3), 4.89 (1H, d, J=11.5 Hz, H-15a), 4.97 (1H, d, J=18.0 Hz, H-15b), 6.00 (1H, dd, J=11.5, 18.0 Hz, H-14), 7.37—7.60 (3H, m, H-3′, H-4′, H-5′), 8.02 (2H, d, J=8.2 Hz, H-2′, H-6′). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 16.0 (C-20), 16.0 (C-11), 16.6 (C-19), 19.4 (C-6), 23.6 (C-17), 23.8 (C-2), 28.1 (C-18), 32.6 (C-16), 34.6 (C-12), 36.5 (C-10), 37.2 (C-1), 38.2 (C-4), 42.8 (C-7), 55.3 (C-5), 58.1 (C-9), 73.5 (C-13), 75.8 (C-8), 81.3 (C-3), 109.6 (C-15), 128.3 (C-3′, C-5′), 129.5 (C-2′, C-6′), 130.8 (C-1′), 132.7 (C-4′), 147.5 (C-14), 166.2 (CO). CID-MS m/z: 411 $(M+H)^+$, 428 $(M+NH_4)^+$.

***ent*-13-*epi*-Manoyloxide-3-yl *p*-Nitrobenzoate (4)** This compound was synthesized according to the general method A by the treatment of **1** (32.0 mg, 0.104 mmol) with *p*-nitrobenzoyl chloride (38.7 mg, 0.208 mmol). The reaction mixture was stirred for 4 h at 70 °C. Compound **4** (29.0 mg, 0.064 mmol, 61.3%) was isolated by column chromatography on silica gel (cyclohexane : EtOAc 90 : 10): $[\alpha]_D^{20}$ −59.3° (c=0.19, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.76 (3H, s, H-20), 0.86 (3H, s, H-19), 0.94 (3H, s, H-18), 1.02—1.08 (2H, m, H-1ax, H-5), 1.12—1.18 (4H, m, H-9, H-16), 1.20 (3H, s, H-17), 1.30—1.50 (5H, m, H-6ax, H-7ax, H-11ax, H-11eq, H-12ax), 1.62—1.87 (5H, m, H-1eq, H-2ax, H-2eq, H-6eq, H-7eq), 2.21 (1H, m, H-12eq), 4.74 (1H, dd, J=4.8, 11.0 Hz, H-3), 4.88 (1H, d, J=11.4 Hz, H-15a), 4.96 (1H, d, J=18.3 Hz, H-15b), 5.98 (1H, dd, J=11.4, 18.3 Hz, H-14), 8.17 (2H, d, J=8.0 Hz, H-2′, H-6′), 8.26 (2H, d, J=8.0 Hz, H-3′/H-5′). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 16.0 (C-20), 16.1 (C-11), 16.6 (C-19), 19.4 (C-6), 23.6 (C-17), 23.8 (C-2), 28.2 (C-18), 32.6 (C-16), 34.7 (C-12), 36.5 (C-10), 37.3 (C-1), 38.2 (C-4), 42.8 (C-7), 55.3 (C-5), 58.1 (C-9), 73.5 (C-13), 75.7 (C-8), 82.7 (C-3), 109.7 (C-15), 123.5 (C-3′, C-5′), 130.6 (C-2′, C-6′), 136.2 (C-1′), 147.5 (C-14), 150.4 (C-4′), 164.3 (CO). CID-MS m/z: 456 $(M+H)^+$.

***ent*-13-*epi*-Manoyloxide-3-yl Methanosulfonate (10) and 8a,13-Epoxy-labda-2,14-diene (12)** To a solution of **1** (50.0 mg, 0.16 mmol) in dry Et_2O (4 ml) were added Et_3N (0.1 ml) and methanosulfonyl chloride (0.017 ml) at −10 °C. The mixture was stirred for 1 h at this temperature and extracted with an aqueous solution of 0.1 M HCl. The organic layer was washed with a saturated aqueous solution of $NaHCO_3$ and the organic solvent was removed under reduced pressure. Compound **10** (39.0 mg, 0.102 mmol, 63.5%) was isolated by column chromatography on silica gel (CH_2Cl_2). To a solution of **10** (35.0 mg, 0.091 mmol) in dimethylformamide (1.5 ml) was added NaN_3 (20 mg, 0.308 mmol) or $CH_3COO^-Cs^+$ (65 mg, 0.316 mmol), and the mixture was stirred for 14 h at 90 °C. The solvent was removed under reduced pressure, and compound **12** (21.0 mg, 0.073 mmol, 80.2%) was isolated by column chromatography on silica gel (cyclohexane : EtOAc 99 : 1).

Compound **10**: $[\alpha]_D^{20}$ −16.2° (c=0.52, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.75 (3H, s, H-20), 0.81 (3H, s, H-19), 0.99 (3H, s, H-18), 1.03—1.18 (6H, m, H-1ax, H-5, H-9, H-16), 1.21 (3H, s, H-17), 1.30—1.50 (5H, m, H-6ax, H-7ax, H-11ax, H-11eq, H-12ax), 1.63—2.00 (5H, m, H-1eq, H-2ax, H-2eq, H-6eq, H-7eq), 2.21 (1H, m, H-12eq), 3.00 (3H, s, $C\underline{H}_3SO_2$), 4.32 (1H, dd, J=5.0, 11.0 Hz, H-3), 4.89 (1H, d, J=11.5 Hz, H-15a), 4.96 (1H, d, J=18.2 Hz, H-15b), 5.98 (1H, dd, J=11.5, 18.2 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 15.8 (C-20), 15.8 (C-11), 15.9 (C-19), 19.4 (C-6), 23.6 (C-17), 25.1 (C-2), 28.1 (C-18), 32.5 (C-16), 34.4 (C-12), 36.2 (C-10), 37.2 (C-1), 38.4 (C-4), 38.6 ($\underline{C}H_3SO_2$), 42.6 (C-7), 55.2 (C-5), 59.2 (C-9), 73.4 (C-13), 75.4 (C-8), 90.0 (C-3), 109.6 (C-15), 147.2 (C-14).

Compound **12**: $[\alpha]_D^{20}$ −64.0° (c=0.17, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.72 (3H, s, H-20), 0.82 (3H, s, H-19), 0.91 (3H, s, H-18), 1.10 (3H, s, H-16), 1.15—1.30 (5H, m, H-5, H-9, H-17), 1.32—1.50 (5H, m, H-6ax, H-7eq, H-11ax, H-11eq, H-12ax), 1.53—1.67 (2H, m, H1ax, H-6eq), 1.73 (1H, m, H-1eq), 1.87 (1H, td, J=19.2, 5.7 Hz, H-7ax), 2.20 (1H, m, H-12eq), 4.89 (1H, d, J=11.5 Hz, H-15a), 4.94 (1H, d, J=18.2 Hz, H-15b), 5.33 (1H, dd, J=10.4, 2.6 Hz, H-2), 5.42 (1H, dd, J=10.4, 1.6 Hz, H-3), 5.99 (1H, dd, J=11.5, 18.2 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 16.0 (C-20), 16.1 (C-11), 20.7 (C-6), 22.4 (C-19), 23.1 (C-17), 31.6 (C-18), 32.7 (C-16), 34.6 (C-4), 34.8 (C-12), 35.9 (C-10), 40.2 (C-1), 42.2 (C-7), 52.0 (C-5), 57.1 (C-9), 73.5 (C-13), 75.9 (C-8), 109.5 (C-15), 121.2 (C-3), 138.2 (C-2), 147.6 (C-14). CID-MS m/z: 289 $(M+H)^+$, 306 $(M+NH_4)^+$.

***ent*-13-*epi*-Manoyloxide-3-yl Phenylcarbamate (5)** This compound was synthesized according to the general method B by the treatment of **1** (30.6 mg, 0.100 mmol) with phenyl isocyanate (60.0 mg, 0.500 mmol). The reaction mixture was stirred for 4 h at room temperature. Compound **5** (25.0 mg, 0.059 mmol, 59.0%) was isolated by column chromatography on silica gel (cyclohexane : EtOAc 95 : 5): $[\alpha]_D^{20}$ −49.3° (c=0.15, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.72 (3H, s, H-20), 0.81 (3H, s, H-19), 0.91 (3H, s, H-18), 0.97—1.07 (2H, m, H-1ax, H-5), 1.09—1.17 (4H, ∼s, H-9, H-16), 1.19 (3H, s, H-17), 1.30—1.50 (5H, m, H-6ax, H-7ax, H-11ax, H-11eq, H-12ax), 1.58—1.82 (5H, m, H-1eq, H-2ax, H-2eq, H-6eq, H-7eq), 2.20 (1H, m, H-12eq), 4.44 (1H, dd, J=4.5, 11.2 Hz, H-3), 4.88 (1H, d, J=11.4 Hz, H-15a), 4.94 (1H, d, J=18.3 Hz, H-15b), 5.98 (1H, dd, J=11.4, 18.3 Hz, H-14), 7.02 (1H, t, J=8.0 Hz, H-4′), 7.26—7.40 (4H, m, H-2′, H-3′, H-5′, H-6′). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 16.0 (C-20, C-11), 16.4 (C-19), 19.4 (C-6), 23.9 (C-17), 24.0 (C-2), 28.0 (C-18), 32.6 (C-16), 34.6 (C-12), 36.4 (C-10), 37.2 (C-1), 38.0 (C-4), 42.8 (C-7), 55.3 (C-5), 58.9 (C-9), 73.5 (C-13), 75.7 (C-8), 81.7 (C-3), 109.6 (C-15), 118.4 (C-2′, C-6′), 123.2 (C-4′),129.0 (C-3′, C-5′), 138.0 (C-1′), 147.5 (C-14), 153.6 (CO). CID-MS m/z: 426 $(M+H)^+$, 443 $(M+NH_4)^+$.

***ent*-13-*epi*-Manoyloxide-3-yl *p*-Methoxyphenylcarbamate (6)** This compound was synthesized according to the general method B by the treatment of **1** (30.6 mg, 0.100 mmol) with *p*-methoxyphenyl isocyanate (0.065 ml, 0.500 mmol). The reaction mixture was stirred for 20 h at room temperature. Compound **6** (36.7 mg, 0.081 mmol, 80.6%) was isolated by column chromatography on silica gel (cyclohexane : EtOAc 95 : 5): $[\alpha]_D^{20}$ −20.1° (c=0.19, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.72 (3H, s, H-20), 0.79 (3H, s, H-19), 0.90 (3H, s, H-18), 0.98—1.08 (2H, m, H-1ax, H-5), 1.09—1.16 (4H, m, H-9, H-16), 1.19 (3H, s, H-17), 1.32—1.50 (5H, m, H-6ax, H-7ax, H-11ax, H-11eq, H-12ax), 1.55—1.80 (5H, m, H-1eq, H-2ax, H-2eq, H-6eq, H-7eq), 2.20 (1H, m, H-12eq), 3.75 ($C\underline{H}_3O$), 4.43 (1H, dd, J=4.5, 11.6 Hz, H-3), 4.88 (1H, d, J=11.2 Hz, H-15a), 4.93 (1H, d, J=17.8 Hz, H-15b), 5.98 (1H, dd, J=11.2, 17.8 Hz, H-14), 6.80 (2H, d, J=8.5 Hz, H-3′, H-5′), 7.26 (2H, d, J=8.5 Hz, H-2′, H-6′). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 16.0 (C-20), 16.4 (C-19), 16.4 (C-11), 19.4 (C-6), 23.8 (C-17), 23.9 (C-2), 28.0 (C-18), 32.6 (C-16), 34.6 (C-12), 36.4 (C-10), 37.2 (C-1), 38.0 (C-4), 42.8 (C-7), 55.3 (C-5), 55.5 ($\underline{C}H_3O$), 58.1 (C-9), 73.5 (C-13), 75.7 (C-8), 77.5 (C-4′), 81.6 (C-3), 109.6 (C-15), 114.2 (C-3′, C-5′), 120.4 (C-2′, C-6′), 131.1 (C-1′), 147.5 (C-14), 153.9 (CO), 155.8 (C-4′). CID-MS m/z: 456 $(M+H)^+$, 473 $(M+NH_4)^+$.

***ent*-13-*epi*-Manoyloxide-3-yl *n*-Dodecylcarbamate (7)** This compound was synthesized according to the general method B by the treatment of **1** (30.6 mg, 0.100 mmol) with *n*-dodecyl-isocyanate (105.7 mg, 0.500 mmol). The reaction mixture was stirred for 12 h at 60 °C. Compound **7** (31.0 mg, 0.060 mmol, 60.0%) was isolated by column chromatography on silica gel (cyclohexane : EtOAc 90 : 10): $[\alpha]_D^{20}$ −36.5° (c=0.20, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.72 (3H, s, H-20), 0.76 (3H, s, H-19), 0.87 (6H, m, H-12′, H-18), 0.96-1.06 (2H, m, H-1ax, H-5), 1.10—1.15 (4H, m, H-9, H-16), 1.18 (3H, s, H-17), 1.20—1.50 (35H, m, H-6ax, H-7ax, H-11ax, H-11eq, H-12ax, H-2′, H-3′, H-4′, H-5′, H-6′, H-7′, H-8′, H-9′, H-10′, H-11′), 1.58—1.80 (5H, m, H-1eq, H-2ax, H-2eq, H-6eq, H-7eq), 2.19 (1H, m, H-12eq), 3.16 (1H, ∼q, J=5.6 Hz, H-1′), 4.37 (1H, dd, J=4.2, 11.0 Hz, H-3), 4.89 (1H, d, J=11.5 Hz, H-15a), 4.93 (1H, d, J=18.0 Hz, H-15b), 5.98 (1H, dd, J=11.5, 18.0 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 14.1 (C-4′), 15.9 (C-20), 16.0 (C-11), 16.3 (C-19), 19.4 (C-6), 22.7 (C-11′), 23.8 (C-17), 24.0 (C-2), 26.7 (C-10′), 27.9 (C-18), 29.3-30.0 (C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-9′), 31.9 (C-2′), 32.6 (C-16), 34.6 (C-12), 36.4 (C-10), 37.2 (C-1), 38.0 (C-4), 41.0 (C-1′), 42.8 (C-7), 55.3 (C-5), 58.1 (C-9), 73.4 (C-13), 75.7 (C-8), 80.8 (C-3), 109.6 (C-15), 147.5 (C-14), 156.7 (CO). CID-MS m/z: 518 $(M+H)^+$, 535 $(M+NH_4)^+$.

***ent*-13-*epi*-Manoyloxide-3-yl *n*-Amylcarbamate (8)** This compound

was synthesized according to the general method B by the treatment of **1** (34.0 mg, 0.110 mmol) with *n*-amylisocyanate (0.075 ml, 0.55 mmol). The reaction mixture was stirred for 12 h at 60 °C. Compound **8** (29.0 mg, 0.069 mmol, 62.9%) was isolated by column chromatography on silica gel (CH_2Cl_2: MeOH 99.8 : 0.2): $[\alpha]_D^{20}$ −37.0° (c=0.27, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.72 (3H, s, H-20), 0.76 (3H, s, H-19), 0.86 (3H, s, H-18), 0.87 (3H, t, J=6.6 Hz, H-5′), 0.93—1.06 (2H, m, H-1ax, H-5), 1.09—1.15 (4H, m, H-9, H-16), 1.18 (3H, s, H-17), 1.21—1.80 (19H, m, H-1eq, H-2ax, H-2eq, H-6ax, H-6eq, H-7ax, H-7eq, H-11ax, H-11eq, H-12ax, H-2′, H-3′, H-4′), 2.18 (1H, m, H-12eq), 3.12 (1H, m, J=6.6 Hz, H-1′), 4.32 (1H, dd, J=4.2, 11.0 Hz, H-3), 4.88 (1H, d, J=11.5 Hz, H-15a), 4.93 (1H, d, J=18.0 Hz, H-15b), 5.97 (1H, dd, J=11.5, 18.0 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 14.1 (C-5′), 16.0 (C-20), 16.0 (C-11), 16.4 (C-19), 19.5 (C-6), 22.4 (C-4′), 23.9 (C-17), 24.1 (C-2), 28.0 (C-18), 29.0 (C-3′), 29.8 (C-2′), 32.7 (C-16), 34.7 (C-12), 36.5 (C-10), 37.3 (C-1), 38.1 (C-4), 41.0 (C-1′), 42.9 (C-7), 55.4 (C-5), 58.2 (C-9), 73.6 (C-13), 75.8 (C-8), 80.9 (C-3), 109.7 (C-15), 147.6 (C-14), 156.8 (CO). CID-MS m/z: 420 $(M+H)^+$, 437 $(M+NH_4)^+$.

***ent*-13-*epi*-Manoyloxide-3-yl 2-Chloroethylcarbamate (9)** This compound was synthesized according to the general method B by the treatment of **1** (30.6 mg, 0.100 mmol) with 2-chloroethyl-isocyanate (0.043 ml, 0.500 mmol). The reaction mixture was stirred for 20 h at room temperature. Compound **9** (28.0 mg, 0.068 mmol, 68.0%) was isolated by column chromatography on silica gel (CH_2Cl_2: cyclohexane 70 : 30): $[\alpha]_D^{20}$ −13.6° (c=1.07, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.72 (3H, s, H-20), 0.77 (3H, s, H-19), 0.86 (3H, s, H-18), 0.95—1.05 (2H, m, H-1ax, H-5), 1.10—1.17 (4H, m, H-9, H-16), 1.19 (3H, s, H-17), 1.30—1.50 (5H, m, H-6ax, H-7ax, H-11ax, H-11eq, H-12ax), 1.58—1.82 (5H, m, H-1eq, H-2ax, H-2eq, H-6eq, H-7eq), 2.19 (1H, m, H-12eq), 3.50 (1H, t, J=4.1 Hz, H-2′), 3.61 (1H, t, J=4.1 Hz, H-1′), 4.38 (1H, dd, J=4.5, 11.2 Hz, H-3), 4.89 (1H, d, J=11.6 Hz, H-15a), 4.94 (1H, d, J=18.1 Hz, H-15b), 5.97 (1H, dd, J=11.6, 18.1 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 16.0 (C-20), 16.2 (C-11), 16.5 (C-19), 19.7 (C-6), 24.2 (C-17), 24.2 (C-2), 28.3 (C-18), 32.8 (C-16), 34.7 (C-12), 36.4 (C-10), 37.3 (C-1), 38.0 (C-4), 42.4 (C-2′), 42.8 (C-7), 44.8 (C-1′), 55.3 (C-5), 58.3 (C-9), 73.5 (C-13), 75.7 (C-8), 82.5 (C-3), 109.8 (C-15), 147.5 (C-14), 155.6 (CO). CID-MS m/z: 412 $(M+H)^+$, 429 $(M+NH_4)^+$.

1-*O*-(*ent*-13-*epi*-Manoyloxide-3-yl) 4-*O*-Acetyl-3-chloro-2,3,6-trideoxy-α-L-arabinoside (11) Dry hydrogen chloride was purged for 15 min into a solution of methyl 4-*O*-acetyl-3-chloro-2,3,6-trideoxy-α-L-arabinohexopyranoside (50.0 mg, 0.200 mmol) in dry benzene (5 ml) at 0 °C. The solvent was then vacuum evaporated, and the last traces of HCl were azeotropically removed with toluene. The residue was dissolved in dry dichloromethane (3 ml) and yellow mercury(II) oxide (65.0 mg, 0.299 mmol), mercury (II) bromide (55.0 mg, 0.154) and a solution of **1** (34.0 mg, 0.111 mmol) in dry dichloromethane (2 ml) were added and the mixture was stirred at room temperature for 24 h. The insoluble material was then filtered off, washed with dichloromethane, and the filtrate was washed with a saturated aqueous $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4), the solvent was evaporated to dryness, and the residue was purified by column chromatography using a mixture of CH_2Cl_2: MeOH 99.8 : 0.2 as the eluent. Compound **11** (22.0 mg, 0.044 mmol, 39.9%) together with compound **13** (7.0 mg, 0.015 mmol, 13.5%) was obtained.

Compound **11**: $[\alpha]_D^{20}$ −93.9° (c=0.10, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.70 (3H, s, H-20), 0.74 (3H, s, H-19), 0.85—0.95 (5H, m, H-1ax, H-5, H-18), 1.10—1.20 (10H, m, H-9, H-16, H-17, H-6′), 1.25—1.50 (5H, m, H-6ax, H-7ax, H-11ax, H-11eq, H-12ax), 1.55—1.82 (5H, m, H-1eq, H-2ax, H-2eq, H-6eq, H-7eq), 2.00—2.40 (6H, m, H-2′eq, H-2′ax, H-12eq, CH_3COO-4′), 3.14 (1H, dd, J=4.5, 11.5 Hz, H-3), 3.87 (1H, m, H-5′), 4.23 (1H, m, H-3′), 4.76 (1H, t, J=11.2 Hz, H-4′), 4.89 (1H, d, J=11.4 Hz, H-15a), 4.92 (1H, d, J=17.8 Hz, H-15b), 4.99 (1H, d, J=1.3 Hz, H-1′), 5.98 (1H, dd, J=11.4, 17.8 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 15.8 (C-20), 16.0 (C-11), 16.2 (C-19), 17.6 (C-6′), 19.5 (C-6), 20.8 ($\underline{C}H_3$COO-4′), 21.8 (C-2), 23.2 (C-17), 28.4 (C-18), 32.6 (C-16), 34.7 (C-12), 36.4 (C-10), 37.1 (C-1), 38.4 (C-4), 40.8 (C-2′), 43.0 (C-7), 55.5 (C-5), 55.7 (C-3′), 58.2 (C-9), 67.3 (C-5′), 73.4 (C-13), 75.7 (C-8), 77.5 (C-4′), 82.0 (C-3), 92.6 (C-1′), 109.6 (C-15), 147.5 (C-14), 170.0 ($CH_3\underline{C}$OO-4′). CID-MS m/z: 514 $(M+NH_4)^+$.

Compound **13**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.72 (3H, s, H-20), 0.75 (3H, s, H-19), 0.90—1.00 (5H, m, H-1ax, H-5, H-18), 1.10—1.20 (10H, m, H-9, H-16, H-17, H-6′), 1.25—1.55 (6H, m, H-6ax, H-2ax, H-7ax, H-11ax, H-11eq, H-12ax), 1.60—1.80 (4H, m, H-1eq, H-2eq, H-6eq, H-7eq), 2.08 (3H, s, $C\underline{H}_3$COO-4′), 2.20 (1H, m, H-12eq), 3.26 (1H, dd, J=3.9, 11.8 Hz, H-3), 3.98 (1H, m, H-5′), 4.90 (1H, d, J=11.4 Hz, H-15a), 4.94 (1H, d, J=17.8 Hz, H-15b), 5.03 (1H, dd, J=11.2, 2.1 Hz, H-4′), 5.05 (1H, d, J=1.0 Hz, H-1′), 5.21 (1H, d, J=10.8, 2.1 Hz, H-1′), 5.32 (1H, dd, J=10.8, 1.0 Hz, H-3′), 5.98 (1H, dd, J=11.4, 17.8 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 15.9 (C-20), 16.0 (C-11), 16.1 (C-19), 17.6 (C-6′), 19.6 (C-6), 21.1 ($\underline{C}H_3$COO-4′), 22.8 (C-2), 23.8 (C-17), 27.8 (C-18), 32.7 (C-16), 34.7 (C-12), 36.5 (C-10), 37.4 (C-1), 38.3 (C-4), 43.0 (C-7), 55.7 (C-5), 58.3 (C-9), 65.0 (C-5′), 70.9 (C-4′), 73.4 (C-13), 75.8 (C-8), 83.3 (C-3), 91.3 (C-1′), 109.6 (C-15), 128.6 (C-2′), 129.3 (C-3′), 147.6 (C-14), 170.6 ($CH_3\underline{C}$OO-4′).

References

1) Miyazawa M., Shimamura H., Nakamura S., Kameoka H., *J. Agric. Food Chem*., **43**, 3012—3015 (1995).
2) Matsuda T., Kurounagi M., Sugiyama S., Umehara K., Ueno A., Nishi K., *Chem. Pharm. Bull.*, **42**, 1216—1225 (1994).
3) Han B.H., Yang H. O., Kang Y. H., Suh D. Y., Go H. J., Song W. J., Kim Y. C., Park M. K., *J. Med. Chem*., **41**, 2626—2630 (1998).
4) Alcarez M. J., Ochoa S. G., Jimenez M. J., Valverde S., Villar A., *Phytochemistry*, **28**, 1267—1268 (1989).
5) Singh M., Pal M., Sharma R. P., *Planta Med*., **65**, 2—8 (1999).
6) Chinou I., Demetzos C., Harvala C., Roussakis C., Verbist J. F., *Planta Med*., **60**, 34—36 (1994).
7) Kalpoutzakis E., Chinou I., Mitaku S., Skaltsounis A. L., Harvala C., *Natl. Prod. Lett*., **11**, 173—179 (1998).
8) Granados A. G., Linan E., Martinez A., Rivas F., Mesa-Vale C. M., Castilla-Calvente J. J., Osuma, A., *J. Nat. Prod.*, **60**, 13—16 (1997).
9) Antonini I., Claudi F., Cristalli G., Franchetti P., Grifantini M., Martelli S., *J. Med. Chem.*, **31**, 260—264 (1988).
10) Mitakou S., Skaltsounis A. L., Tillequin F., Koch M., *Synthesis*, **1992**, 1068—1090.
11) Cambie R. C., Leong S. H., Palmer B. D., Preston A. F., *Aust. J. Chem*., **33**, 155—168 (1980).
12) Bauer A.W., Kirby W. M. M., Sherris J. C., Turck M., *Am. J. Clin. Pathol*., **45**, 493—496 (1966).
13) Roth B., Baccanari D. P., Sigel C. W., Hubbell J. P., Eaddy J., Kao J. C., Grace M. E., Rauckman B. S., *J. Med. Chem.*, **31**, 122—129 (1988).
14) T'Ang A., Lien E. J., *J. Clin. Hosp. Pharm*., **6**, 245—249 (1981).
15) Cruickshank R., Duguid I. P., Marmion B. P., Swain R. H. A., "Medical Microbiology," 12nd edn. Churchill Livingstone, Edinburgh, London, New York, Vol. III, 1975.
16) Magiatis O., Melliou E., Skaltsounis A. L., Chinou I., Mitakou S., *Planta Med*., **65**, 749—752 (1999).
17) Konishi T., Azuma M., Itoga R., Kiyosawa S., Fujiwara Y., Shimada Y., *Chem. Pharm. Bull.*, **44**, 229—231 (1996).
18) Gonzalez A. G., Fraga B. M., Hernadez M. G., Luis J. G., *Phytochemistry*, **12**, 1113—1116 (1973).
19) Fraga B. M., Guillermo R., Hernadez M. G., Mestres T., Arteaga J. M., *Phytochemistry*, **30**, 3361—3364 (1991).