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3,28-Di-*O*-(dimethylsuccinyl)-betulin Isomers as Anti-HIV Agents[1]

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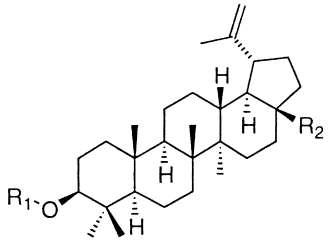
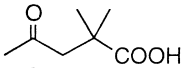
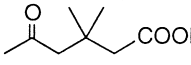
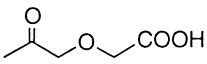
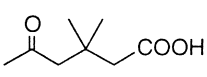
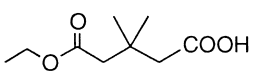
Abstract—Four isomers of 3,28-di-*O*-(dimethylsuccinyl)-betulin were prepared and evaluated for anti-HIV activity against HIV-1 replication in H9 lymphocyte cells. 3-*O*-(3′,3′-Dimethylsuccinyl)-28-*O*-(2″,2″-dimethylsuccinyl)-betulin (**11**) was the most potent anti-HIV compound with an EC_{50} value of 0.00087 μM and a TI value of 42,400.

Following isolation and identification of betulinic acid (**1**) as an anti-HIV agent from the leaves of *Syzygium claviflorum*,[2] modification of **1** was carried out to develop more potent anti-HIV derivatives. 3-*O*-(3′,3′-Dimethylsuccinyl)-betulinic acid (DSB, **2**) showed extremely potent anti-HIV activity with an EC_{50} value of $<0.00035\,\mu M$ and a therapeutic index (TI) value of >20,000. 3′,3′-Dimethylglutaryl (**3**) and diglycoryl (**4**) derivatives were also quite potent with EC_{50} values of 0.0023 and 0.01 μM and TI values of 1974 and 1172, respectively.[3,4] Based on the structural similarity of betulin and betulinic acid, various 3′-substituted glutaryl betulins were also prepared, and 3,28-di-*O*-(3′,3′-dimethylglutaryl)-betulin (**5**) was found to demonstrate potent anti-HIV activity with an EC_{50} value of $0.00066\,\mu M$ and a TI value of 21,515.[5] However, because reaction of betulin and 2,2-dimethylsuccinic anhydride yielded an isomeric mixture from which individual isomers could not be readily separated, substituted succinyl betulins, especially dimethylsuccinyl betulins, were not evaluated previously. In this study, we have now prepared four isomeric dimethylsuccinyl betulins and evaluated their anti-HIV activity.

Betulin and 2,2-dimethylsuccinic anhydride were reacted initially in pyridine at reflux to produce a mixture of 3,28-di-*O*-(dimethylsuccinyl)-betulins. HPLC examination showed the presence of four compounds in a ratio of 66:25:6:3. These isomers were successfully separated by semi-preparative scale HPLC to give four pure samples. However, their structural assignments were uncertain because the ^{1}H and ^{13}C NMR resonances for the two



	R_1	R_2
1	H	COOH
2		COOH
3		COOH
4		COOH
5		

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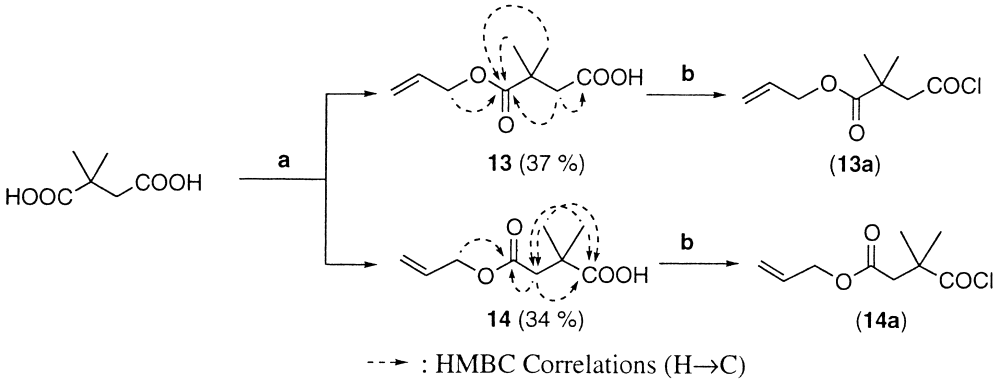
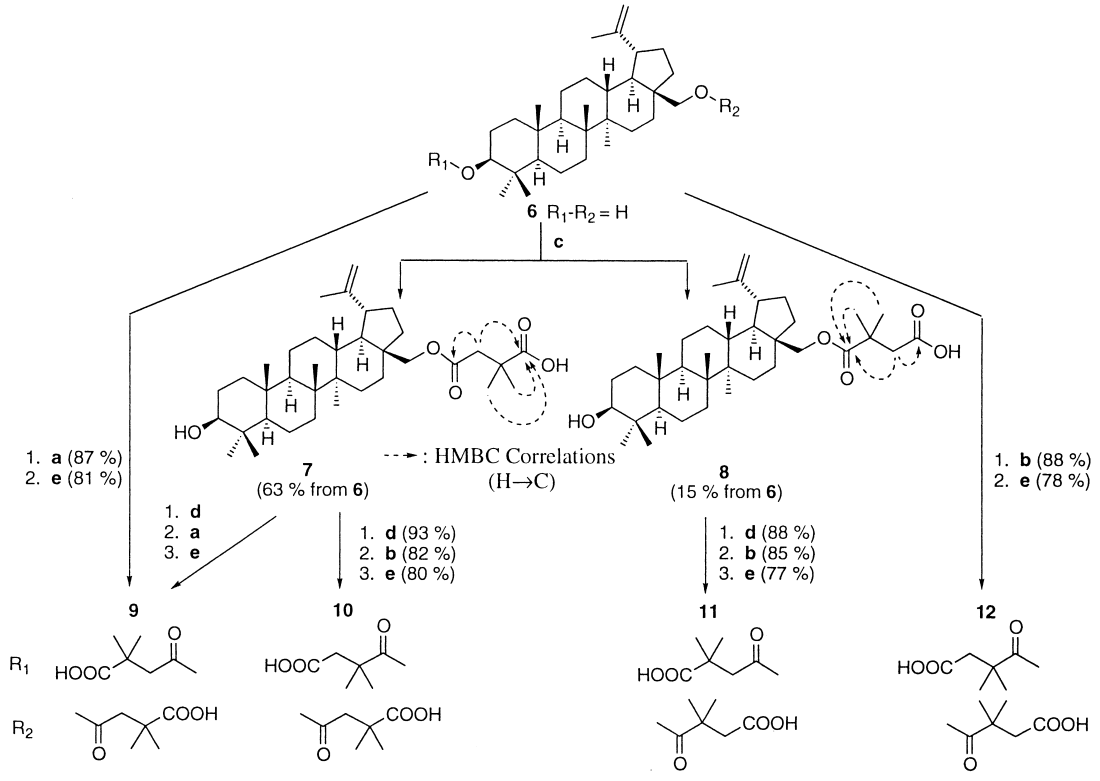
dimethylsuccinyl moieties were quite complex. Therefore, these four isomers (**9**–**12**) were prepared by a different procedure to establish their structures unequivocally.[7]

First, 2,2-dimethylsuccinic acid was treated with a molar equivalent of allyl alcohol in dry pyridine at reflux to give an isomeric mixture of 1-allyl-2,2-dimethyl- and 1-allyl-3,3-dimethylsuccinate, which was separated by HPLC. The structures of 1-allyl-2,2-dimethyl- (**13**) and 1-allyl-3,3-dimethylsuccinate (**14**) were assigned by HMBC examination. (Scheme 1). Treatment of betulin with the acid chloride of **13** or **14** (**13a** and **14a**, respectively) gave 3,28-*O*-(3′,3′-dimethylsuccinyl)- and 3,28-*O*-(2′,2′-dimethylsuccinyl)-betulin diallyl esters, respectively. These allyl groups were deprotected by $(Ph_3P)_4Pd$/morpholine to furnish **9** and **12**, respectively.

Second, reaction of a molar equivalent of betulin and 2,2-dimethylsuccinic acid in dry pyridine at reflux gave two products which were successfully separated by HPLC to yield **7** and **8**. The structural assignment of **7** was performed by HMBC examination; the important HMBC correlations are shown in Scheme 2. This structure was further supported by the fact that the diallyl ester of **9** was produced by introducing an allyl ester group into **7**, followed by reaction with **13a**. In the case of **8**, the observed HMBC correlations shown in Scheme 2 established the positions of the dimethyl groups at C-2′. The



Scheme 1. (a) Allyl alcohol, pyridine, reflux; (b) $(COCl)_2$, benzene, reflux.



Scheme 2. (a) **13a**, DMAP, CH_2Cl_2; (b) **14a**, DMAP, CH_2Cl_2; (c) 2,2-dimethylsuccinic acid, pyridine, reflux; (d) allyl bromide, K_2CO_3, acetone; (e) $(Ph_3P)_4Pd$, morpholine.

Table 1. Anti-HIV activities[6] for 28-*O*- (**7** and **8**), and 3,28-di-*O*-dimethylsuccinyl-betulin isomers (**9**–**12**)

	IC_{50} (μM)[a]	EC_{50} (μM)[b]	TI[c]
7	37.4	< 0.18	>207
8	28.6	0.18	159
9	33.6	0.02	1680
10	38.6	0.40	96.5
11	36.9	0.00087	42,400
12		—[d]	—
AZT	1873	0.045	41,622

[a]Concentration which is toxic to 50% of mock-infected H9 cells.
[b]Concentration (μM) which inhibits HIV-1 replication by 50%.
[c]TI (therapeutic index) is defined by IC_{50}/EC_{50}.
[d]Toxic at all concentrations.

allyl ester derivatives of **7** and **8** were treated successfully with **14a** and **13a**, respectively, then with $(Ph_3P)_4Pd$/morpholine to furnish **10** and **11**, respectively.

Anti-HIV data for 3,28-di-*O*-dimethylsuccinyl-betulin isomers (**9**–**12**) and 28-*O*-dimethylsuccinyl isomers (**7** and **8**) are summarized in Table 1. Among these derivatives, **11** demonstrated the highest anti-HIV activity in acutely infected H9 cells with an EC_{50} value of 0.00087 μM and inhibited uninfected H9 cell growth with an IC_{50} value of 36.9 μM. Its calculated TI value (42,400) was comparable to that of AZT. Compound **9** was also extremely potent with an EC_{50} value of 0.02 μM and a TI value of 1680. Compound **10** displayed fairly potent anti-HIV activity with an EC_{50} value of 0.40 μM and a TI value of 96.5, whereas **12** was toxic. Compounds **10** and **12**, which contain the 3-*O*-(2′,2′-dimethylsuccinyl)-moiety, were less active or more toxic than the derivatives with the 3-*O*-(3′,3′-dimethylsuccinyl)-moiety (**9** and **11**). Notably, the latter substitutent pattern is also present in DSB (**2**).[3,4] The monoesterified 28-*O*-dimethylsuccinyl isomers (**7** and **8**) also showed moderately potent anti-HIV activity with EC_{50} values of < 0.18 and 0.18 μM and TI values of >207 and 159, respectively. The results for the diesters suggest that the 3-*O*-(3′,3′-dimethylsuccinyl)-moiety is essential for potent anti-HIV activity and its combination with a 28-*O*-(2′,2′-dimethylsuccinyl) group is optimal for exhibiting extremely potent anti-HIV activity. In contrast, although the 28-*O*-dimethylsuccinyl moiety enhances anti-HIV activity, the position of the dimethyl groups does not seem to be closely correlated with the potency of anti-HIV activity in the monoesters **7** and **8**.

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6. The HIV-1 (IIIB isolate) growth inhibition assay in the H9 cells was performed by the procedure described in refs 1–5.
7. All new compounds gave satisfactory analytical and spectroscopic data. Selected spectroscopic data for 3-*O*-(3′,3′-dimethylsuccinyl)-28-*O*-(2″,2″-dimethylsuccinyl)-betulin (**11**): A white amorphous powder; $[\alpha]_D$ +7.7° (*c* 1.01, $CHCl_3$); ^1H NMR (400 MHz, pyridine-d_5) δ: 0.75 (3H, s, CH_3-25), 0.93 (6H, s, CH_3-24 and -27), 0.96 (3H, s, CH_3-26), 0.97 (3H, s, CH_3-23), 1.48, 1.50 (each 3H, s, dimethylsuccinyl-CH_3), 1.55 (6H, s, dimethylsuccinyl-CH_3), 1.73 (3H, s, CH_3-29), 2.89, 2.97 (each 1H, d, J = 15.0 Hz, dimethylsuccinyl-CH_2), 2.93, 2.99 (each 1H, d, J = 16.0 Hz, dimethylsuccinyl-CH_2), 4.12, 4.60 (each 1H, d, J = 11.0 Hz, H_2-28), 474 (1H, dd, J = 4.5, 10.0 Hz, H-3), 4.74, 4.87 (each 1H, br s, H-30); Positive FABHRMS *m*/*z* 721.4656 ($[M + Na]^+$), $C_{42}H_{66}O_8Na$ requires 721.4655.