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Chemical and Enzymatic Modifications of Integric Acid and HIV-1 Integrase Inhibitory Activity

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Abstract—Integric acid (**1**), an acyl eremophilane sesquiterpenoid, was identified as an inhibitor of HIV-1 integrase, the enzyme responsible for provirus entry into the host cell nucleus and integration in to the host genome. Chemical and enzymatic modification of integric acid led to the preparation of several selective chemical derivatives of integric acid. Preparation, HIV-1 inhibitory activity, and the structure–activity relationship against coupled and strand transfer assays are described. It appears that most of the groups present in the natural product are required for inhibition of HIV-1 integrase strand transfer activity. In contrast, inhibition of 3′ processing activity is less stringent suggesting distinct SAR for the two integrase reactions. © 2000 Elsevier Science Ltd. All rights reserved.

HIV-1 integrase is an enzyme responsible for a series of important reactions essential for viral replication.[1–4] These include cleavage of a dinucleotide from each end of the viral DNA (3′-end processing) and insertion of the viral DNA into the host cellular DNA (strand transfer).[1–4] This enzyme appears to be absent in the mammalian host and therefore represents a potential target for the development of non-toxic antiviral therapeutic agents to be used either as monotherapy or in combination with other anti-HIV agents.

We recently reported the isolation of equisetin and phomasetin[5,6] and integric acid (**1**)[6,7] as inhibitors of HIV-1 integrase. Integric acid, an acylated eremophilane sesquiterpenoid, is defined by two distinct structural units; a bicyclic terpenoid unit consisting of a free carboxylic acid, a vinyl aldehyde, and a conjugated ketone and a 2,4-dimethyloctenoic acyl chain. In order to assess the minimum pharmacophore required for HIV-1 integrase inhibitory activity a systematic modification of the functional groups of integric acid (**1**) was carried out. Selective chemical and enzymatic modifications of the aldehyde, carboxylic acid, vinyl, keto groups and the acyl chain and HIV-1 integrase activity of resulting derivatives are described herein.

Catalytic Hydrogenation

Hydrogenation of integric acid with 5% Pd/C in THF selectively reduced the vinyl group and gave a ~1:1 mixture of two stereoisomeric dihydro products, **2** and **3**, in 39% yield along with ~10% of 2,4-dimethyloctenoic acid (**4**). Hydrogenation of **1** with Wilkinson's catalyst in toluene at 40 psi produced the same two dihydro products **2** and **3** in 64% combined yield. These compounds were purified by reverse phase HPLC at neutral pH. However, chromatography at acidic pH (TFA at pH~2.5) led to hydration of the aldehyde group and production of compound **5** [δ: 202.0 (C-8); 101.5 (C-13)], which exists in equilibrium with cyclic compound **6** [δ: 101.5 (C-8); 101.2 (C-13)]. Hydrogenation of integric acid with PtO_2 in THF gave two major products **7** and **8** in a combined yield of 40% along with small amounts of 2,4-dimethyloctenoic acid (**4**). The tricyclic products are formed as a result of the hydrogenation of the vinyl group, $\Delta^{9,10}$, and the aldehyde group followed by cyclization. The stereochemistry of the new stereogenic centers of compound **8** was assigned by NOESY correlations between H-7 and H-11, H13α and H-12, H_3-12 and H6α, as well as H-7 and H_3-14. In addition, the comparison of the ^1H NMR spectra of compounds **7** and **8** showed that H-11 of compound **7** experienced a 1 ppm upfield shift due to a 1,3-*syn* relationship with the α-hydroxy group at C-8 and appeared at δ 1.90, whereas H-11 of compound **8** appeared at δ 2.90. The methyl protons at C-12 of compound **7** likewise experienced an

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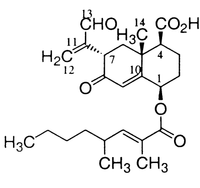
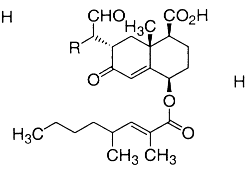
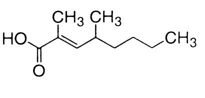


1: Integric acid



2: R = α-CH_3
3: R = β-CH_3



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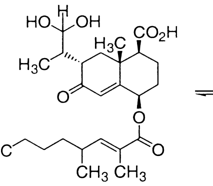

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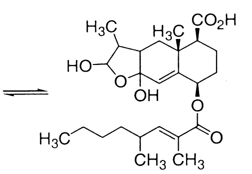

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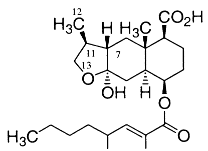
upfield shift of δ 0.15 ppm compared to those in compound **8** (δ 1.02 vs δ 1.17). When the hydrogenation reactions were conducted in polar solvents such as methanol the formation of acid **4** predominated.



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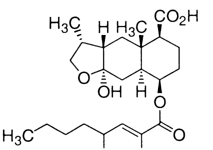

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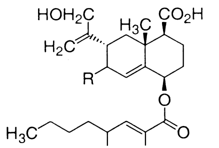


9: R = β-OH
10: R = α-OH

Borohydride Reduction

Sodium borohydride reduction of **1** in a mixture of THF and methanol under carefully controlled conditions at −78 °C gave a mixture of two isomeric diols (**9** and **10**) in a ratio of 9:1 in a 50% overall yield along with 30% yield of the acid **4**.

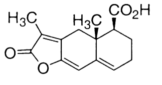
Hydrolytic Reactions

Chemical hydrolysis

Both basic and acidic hydrolysis of **1** proved to be extremely challenging and produced acid **4** and an intractable mixture of products originating from the terpenoid part of the molecule.

Enzymatic hydrolysis

Lipase catalyzed hydrolysis of integric acid in ammonium phosphate buffer (pH 7.4) at 40 °C either in monophasic conditions using Triton-100^{TM} as a solubilizing agent or in biphasic conditions using *tert*-butylmethyl ether produced the expected acid **4** and an unexpected tricyclic compound **11** in 40% isolated yield. The biphasic reaction conditions were superior (rate, yield and ease of isolation) and in all cases reactions were complete in less than 16 h. All three common lipases — Amano PS (*Pseudomonas* lipase P 30), lipase from *Candida antarctica*, and lipase from *Mucor meihei* — used for this study produced the same reaction profile. Analytical HPLC indicated exclusive formation of these two products. Since the putative hydrolysis product (**12**) could not be isolated, the question arose whether **4** and **11** were formed as a result of enzyme catalysis or by chemical reaction. Therefore, the reaction was also conducted without enzyme. In enzyme free reactions only very small amounts of both products **4** and **11** were formed but $>$80% of integric acid was left unchanged indicating that the enzyme may be accelerating the reaction.



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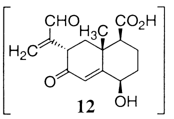

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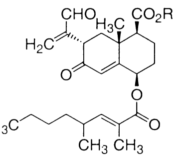


13: R = (S)-Leu-OMe
14: R = (*S*)-Phenylglycine-OMe
15: R = (*R*)-Phenylglycine-OMe

The tricyclic compound **11** is probably formed after the ester hydrolysis and concomitant elimination of water from alcohol **12** after cyclization originating from enolization involving H-7 followed by olefin rearrangements. Alternatively, enolization lead cyclization followed by olefin rearrangements followed by direct elimination of the acyl chain could lead to compound **11** (Fig. 1). Molecular modelling suggests that the acyl chain at C-1 forms a 175° dihedral angle with H-2α and is ideally disposed for a direct β-elimination.

Preparation of amides

Reaction of integric acid with amino acid methyl ester hydrochlorides in THF using diethylphosphoro cyanidate (DEPC) as a coupling reagent produced the corresponding peptides **13–15** in $\sim$50–60% isolated yield. In a typical experiment $\sim$40 μM of **1** was reacted with 1.2

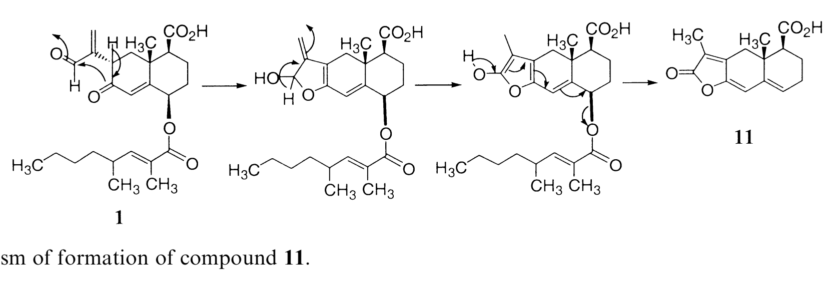
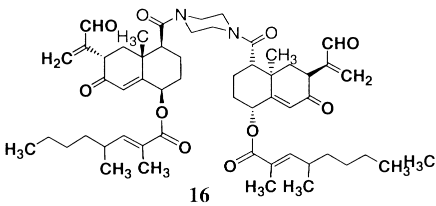


Figure 1. Probable mechanism of formation of compound **11**.



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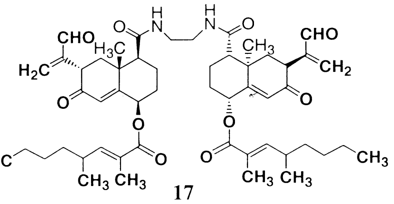

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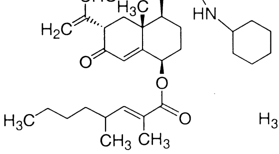
molar equivalents of the respective amino acid methyl ester hydrochloride (*S*-Leu-OMe.HCl, or *S* and *R*-phenylglycine-OMe.HCl) and 1.5 molar equivalents of DEPC, and 2.4 molar equivalents of TEA. Similarly, the reaction of integric acid with 0.5 molar equivalents of either piperazine or ethylene diamine hydrochlorides gave ~30% yield of dimeric compounds **16** and **17**, respectively. The dimeric compounds were prepared to examine the effect of dimerization on HIV-1 integrase activity. The coupling reactions were successful only when reacting amines were used as the hydrochloride salt. Aside from a tendency to Schiff base formation, free primary and secondary amines lead to elimination of the side chain and serious decomposition. Formation of compound **11** was not detected in these coupling reactions except in the case of oxalyl chloride activated coupling. The activation of the carboxyl group with oxalyl chloride and the subsequent reaction with primary amines such as *p*-xylenediamine produced side chain acid **4** and the tricyclic lactone **11**; the same two products obtained during enzymatic hydrolysis reactions. Tertiary bases such as TEA, diisopropylethyl amine, and DMAP had no effect either on the starting compound or on the products.

Replacement of DEPC with other coupling reagents such as DCC or isobutyl chloroformate resulted in stable adducts such as **18** which did not undergo the coupling reaction. In fact, the DCC adduct **18** was easily isolated and purified by silica gel chromatography. When less than one molar equivalent of amine was used the DEPC adduct **19** was readily isolated. Stability and lack of reactivity of **18** could not be easily explained other than by invoking the steric hindrance caused by the cyclohexyl rings.



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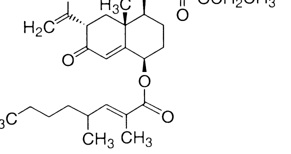

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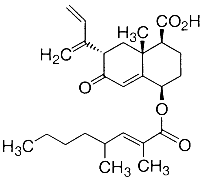
Wittig reaction

Reaction of unprotected integric acid with methyltriphenylphosphorane produced a 2:1 ratio of the expected product **20** and the isomeric product **21**, respectively, in 30% overall yield.



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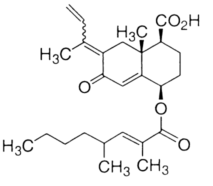

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Structure–activity relationship

All compounds were evaluated in the coupled and strand transfer versions of HIV-1 integrase assays.[6] As described previously the coupled assay requires both the 3′ processing and strand transfer activities of the enzyme. In the coupled assay these compounds exhibited the following IC_{50}s; **1**: 3 μM; **2/3**: 11 μM; **4**: 12 μM; **5/6**: 40 μM; **7**: 100 μM; **8**: 45 μM; **9**: 73 μM; **11**: >100 μM; **13**: 3 μM; **14**: 5 μM; **15**: 5 μM; **16**: 24 μM; **17**: 10 μM; **18**: 88 μM; **19**: 81 μM; **20**: 30 μM; and **21**: 75 μM. Compounds **1**, **2/3**, **4**, **13**, **14**, **15**, **16** and **17** were all active in the 5–20 μM range, comparable to integric acid. In contrast, none of these compounds retained strand transfer activity at 100 μM except for the two dihydro-compounds **2** and **3**, which exhibited weak inhibition with IC_{50} values of 50 μM. Integric acid exhibited IC_{50} value of 10 μM in strand transfer assay. The formation of carboxy amides (**13**–**17** did not have any significant effect on the inhibitory activity in the coupled assay. Reduction of the vinyl group (**2** and **3**) led to only a minor reduction in potency, however the reduction of both the aldehyde and ketone groups (**9**), removal of the aldehyde group either by cyclization (**5/6**, **7**, and **8**) or by homologation (**20** and **21**) caused significant decrease in the inhibitory activity. Elimination of acyl side chain considerably diminished the activity (**8** vs **11**) but surprisingly, the side chain acid **4** by itself retained most of the inhibitory activity in the coupled assay. These structure function relationships indicate that certain modifications are tolerated for the coupled assay activity but the acid groups at C-4, vinyl aldehyde at C-11 and the ester group at C-1 may be required for strand transfer activity. The observation that the coupled assay, requiring both catalytic activities, is less affected by these modifications suggests different structure activity relationships for the 3′ processing and stand transfer reactions of the enzyme.

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