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SYNTHESIS AND ANTI-HIV ACTIVITY OF DIBENZYLBUTYROLACTONE LIGNANS

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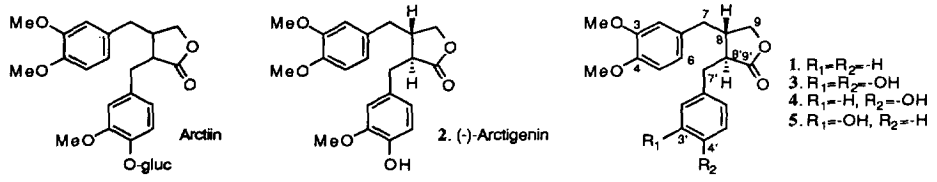
Abstract: Five optically active dibenzylbutyrolactone lignans were synthesized through a lipase-catalyzed transesterification route, and evaluated for their inhibitory activity against HIV-1 replication in acutely infected H9 cells. Compounds **1** and **2** demonstrated anti-HIV replication activity with an EC_{50} values of 2.2 and 0.16 μg/ml and a therapeutic index values of 9.1 and 5, respectively. Structure-antiviral activity relationships are discussed.

In our continuing search for new anti-HIV agents from natural products, we previously isolated three dibenzylbutyrolactone lignans, namely arctiin, trachelosiaside and matairesinoside, from *Trachelospermum gracilipes* (Apocynaceae).[1] When evaluated for their anti-HIV-1 activities, arctiin, the β-D-glucoside of (-)-arctigenin, demonstrated a potent inhibitory effect against HIV replication in H9 cells with an ED_{50} of 0.85 μg/ml and a therapeutic index of >118.[1]

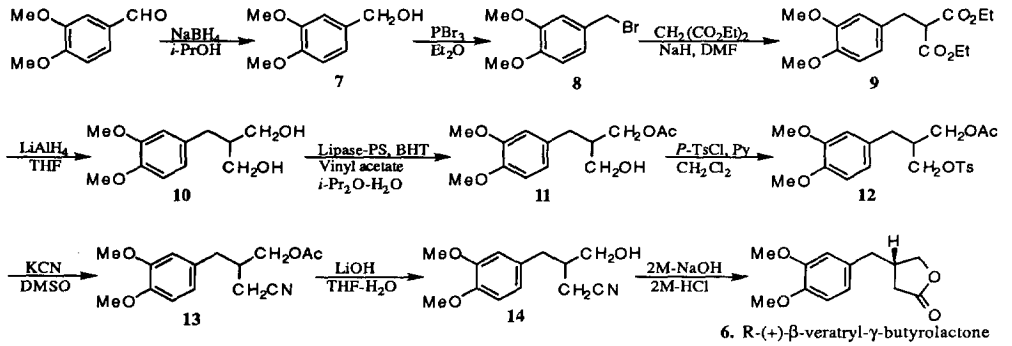
Other lignans of the podophyllotoxin and dibenzylbutyrolactone series have received considerable interest recently because of their wide range of biological activities,[2] including activity as antitumor[3] and antiviral agents,[4] function as platelet-activating-factor (PAF) antagonists[5] and use in folk medicine.[6] Several members of this family of natural products and their analogues have been shown to possess potent antiviral properties.[7] For example, (-)-arctigenin produces anti-HIV activity *in vitro*. This compound efficiently inhibits DNA topoisomerase II activity and strongly suppresses the integration of retroviral DNA into the cellular DNA genome.[8] Following this lead, we synthesized and evaluated (-)-arctigenin analogues with the aim of finding more potent and selective anti-HIV activity, and establishing the structure-antiviral activity relationships for the dibenzylbutyrolactone lignan derivatives.[9]

Several routes for the synthesis of racemic dibenzylbutyrolactone lignans have been reported.[10] We synthesized our compounds *via* an enzymatic transesterification[11] of the prochiral diol **10** (Scheme 1). This route leads to high yields of the dibenzylbutyrolactone lignan analogues in absolute enantiomeric optical purity. We used (-)-arctigenin, a natural dibenzylbutyrolactone lignan, as a template for the structural modifications on the benzyl moiety at position C-8′. No lignan has been isolated with an unsubstituted benzyl ring and monosubstituted examples are also rare.[12] Thus, in addition to the unsubstituted benzyl derivative **1**, we focused on chemical modifications at the 3′ and /or 4′ position of the C-8′ benzyl skeleton by introducing different chemical moieties such as 4′-hydroxy-3′-methoxybenzyl (**2**), 3′,4′-dihydroxybenzyl (**3**), 4′-hydroxybenzyl (**4**) and 3′-hydroxybenzyl (**5**) substituents. The synthesis and structure-antiviral relationships of these

dibenzylbutyrolactone lignan analogues are presented in this paper.



Scheme 1.



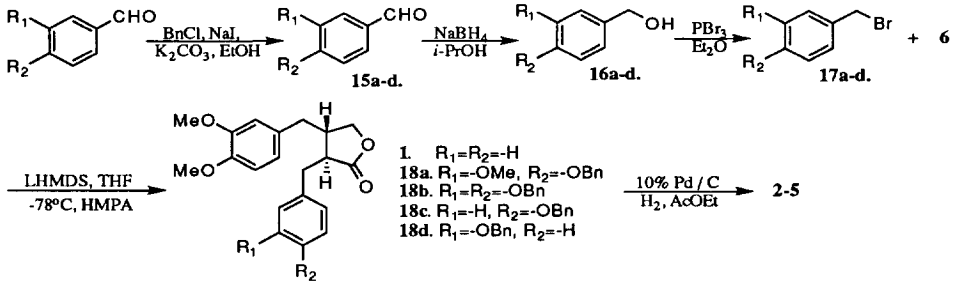
As shown in Scheme 1, the enantioselective synthesis of the key intermediate (R)-β-veratryl-γ-butyrolactone **6** was performed according to a literature procedure.[13] Veratraldehyde was reduced with sodium borohydride in *i*-PrOH to give 3,4-dimethoxybenzyl alcohol **7**, which was treated with PBr_3 in ether to give a quantitative yield of 3,4-dimethoxybenzyl bromide **8** according to the procedure of Charlton et al.[14] Deprotonation of diethyl malonate with sodium hydride in DMF followed by treatment with a THF solution of 3,4-dimethoxybenzyl bromide **8** gave diethyl 2-(3,4-dimethoxybenzyl)malonate **9** in 76% yield. The malonate **9** was reduced with $LiAlH_4$ in THF to give the substituted 1,3-propanediol **10** in 90% yield.

The desired enantiospecific monoacetylation[12] of 2-(3,4-dimethoxybenzyl)-1,3-propanediol **10** was accomplished by treatment with 50 wt% of lipase PS (from *Pseudomonas fluorescens*)[15] in the presence of vinyl acetate as an acyl donor in diisopropyl ether-water mixture. The optically pure monoacetate (R)-**11** was obtained in 96% yield. This monoacetate (R)-**11** was then converted to hydroxy nitrile (R)-**14** in high yield using a three-step procedure. Tosylation of the hydroxyl group of **11** with tosyl chloride in pyridine-dichloromethane gave acetoxy tosylate **12** in 94% yield. This was followed by a S_N2 displacement of the tosylate by treatment with potassium cyanide in dimethyl sulfoxide to afford acetoxy nitrile (R)-**13** in 87% yield. The acetoxy group of **13** was hydrolyzed by treatment with lithium hydroxide in THF-water to give hydroxy nitrile (R)-**14** in 74% overall yield from monoacetate (R)-**11**. Hydrolysis of the (R)-**14** in refluxing 2M NaOH followed by acidification with 2 M HCl gave optically pure (R)-β-veratryl-γ-butyrolactone **6** in 96% yield.

Scheme 2 summarizes the general synthetic procedures for the dibenzylbutyrolactone lignans **1-5**. The hydroxy substituted benzaldehyde were protected as benzyl ether derivatives **15a-d**, which were reduced to the alcohols **16a-d** with sodium borohydride in *i*-PrOH, followed by bromination with PBr_3 in ether to provide the

corresponding bromides **17a-d**.

Scheme 2.



(R)-β-Veratryl-γ-butyrolactone **6** was treated with lithium hexamethyldisilylamide (LHMDS) at -78 °C in THF. The resulting enolate anion was reacted with benzyl bromide or the aryl bromides **17a-d** in the presence of hexamethylphosphoric triamide (HMPA) to give **1** or compounds **18a-d**, respectively. The benzyl-protected dibenzylbutyrolactones **18a-d** were subsequently O-debenzylated by catalytic hydrogenolysis in the usual way to obtain the enantiomerically pure target compounds **2-5**.

The stereochemical structures of the dibenzylbutyrolactone lignans **1-5** were characterized by NMR chemical shifts analysis,[16] and the enantiomeric purity was verified by optical rotations.[2] The physical and spectral data of the synthetic lignan **2** were identical with those of an authentic sample of natural (-)-arctigenin.

Table 1. Anti-HIV activity of dibenzylbutyrolactone lignans **1-6**[a] in acutely infected H9 lymphocytes.

	EC_{50}(μg/ml)[b]	IC_{50}(μg/ml)[c]	T.I.[d]
1	2.2	20	9.1
2	0.16	0.8	5
3	1.5	6	4
4	0.25	0.8	3.2
5	1.8	4	2.2
6	34	100	2.9

[a] Data represent the averages for at least two experiments.
[b] Concentration which inhibited virus (HIV-1) replication by 50%.
[c] Concentration which inhibited H9 cell growth by 50%.
[d] Therapeutic Index.

The dibenzylbutyrolactone lignans **1-5** described in this paper were screened for their anti-HIV activity at Biotech Research Laboratories, Rockville, MD. They were evaluated for their inhibitory activity against HIV-1 replication in acutely infected H9 cells (human T-cell line, clone 9) according to a literature method.[16] The results obtained are shown in Table 1. Compound **1** demonstrated relatively potent anti-HIV activity with an EC_{50} value of 2.2 μg/ml. It also exhibited a good therapeutic index value of 9.1. Replacing the unsubstituted benzyl moiety at C-8′ in **1** with a 4′-hydroxy-3′-methoxybenzyl group gave (-)-arctigenin **2**, which showed increased anti-HIV activity with an EC_{50} value of 0.16 μg/ml but had a lower therapeutic index of 5. The C-4′-

monohydroxy derivative **4** was only slightly less active than **2**. Compounds **3** and **5**, the 3′,4′-dihydroxy and 3′-monohydroxy derivatives, respectively, were less active than **2** and **4**. Compound **6**, which lacks a C-8′ benzyl group, showed the lowest activity in this series. A comparison of the anti-HIV activities of **1-6** suggested that lignans without a hydroxy substituent in the C-8′ benzyl moiety such as **1** demonstrated more significant anti-HIV activity as reflected by therapeutic index values than the other compounds evaluated. Derivatives that were hydroxylated at the 3′ and/or 4′ positions of this benzyl group retained or had increased anti-HIV activity but were also more cytotoxic. Further studies to determine the structural features essential for potent anti-HIV activity and to elucidate a mechanisium(s) of action are warranted.

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