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Enantioselective synthesis and biological evaluation of α-hydroxylated lactone lignans*

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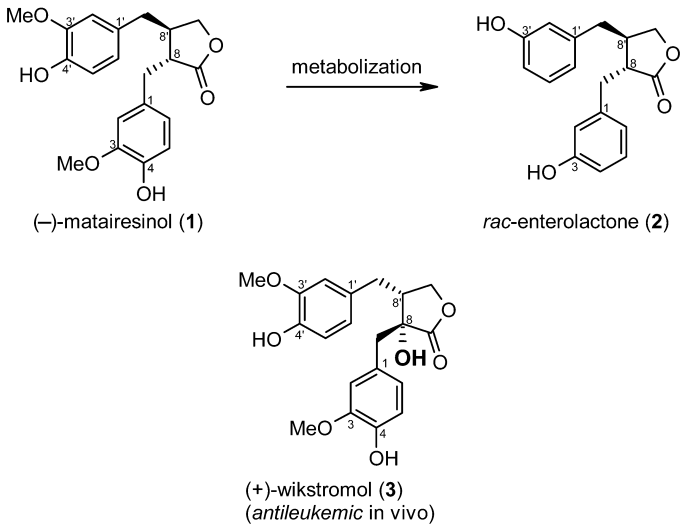
Abstract: A short and efficient synthesis of enantiomerically pure α-hydroxylated lactone lignans starting from commercially available diisopropyl malate is presented. Stereoselective alkylation with various benzyl bromides and saponification yielded the corresponding succinic acids. Acetalization afforded the dioxolanones, which were stereoselectively alkylated. Reduction (and deprotection, where required) yielded the lactone lignans in up to 30 % overall yield. The inhibition of the proliferation of HT29 colon cancer cells was investigated. One lignane, bis-2,4,6-trimethylbenzyllactone lignan, was active (IC_{50} = 35 μM), whereas all other tested lignans were inactive within the investigated concentration range.

INTRODUCTION

Lactone lignans are important secondary plant metabolites possessing a variety of biological activities [1,2]. A prominent derivative is (–)-matairesinol (**1**) [2]. It is widely accepted that **1** (Scheme 1) acts as phytoestrogen and competitive binder to the corresponding estrogen receptor [3]. Matairesinol (**1**) is metabolized in the gastrointestinal tract to *rac*-enterolactone (**2**) [4,5]. It is believed that lignans **1** and/or **2** have an inhibitory effect on hormone-dependent cancer [3].



Scheme 1

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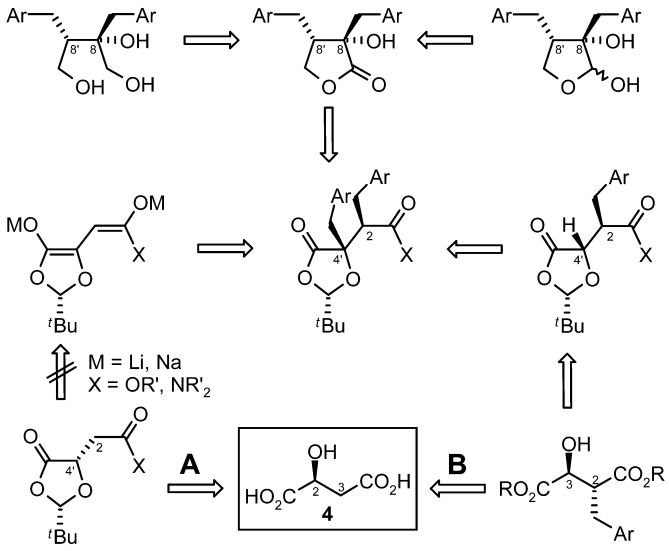
8- or α-Hydroxylated lactone lignans, such as (+)-wikstromol (**3**) [6], are also widespread in nature and exhibit interesting pharmacological properties [2]. For example, wikstromol (**3**) (Scheme 1), which differs from **1** only by the hydroxy group at C8 and the absolute configuration, was found to have antileukemic activity in vivo [7].

SYNTHESIS OF 8-HYDROXYLATED LACTONE LIGNANS

Despite their attractive properties, only few enantioselective syntheses of α-hydroxylated lactone lignans have been reported in the past. These were based on three general strategies: (a) α-hydroxylation of α,β-dibenzyl-γ-butyrolactone (8 steps/3–6 % overall yield) [8], (b) conversion of (+)-arabinose (20/0.5 %) [9], or (c) α-alkylation of protected α-hydroxy-β-benzyl-γ-butyrolactones (8/3 %) [10]. Major drawbacks of these routes were a multistep sequence (b) or a nonselective introduction of either the hydroxy group (a) or the benzyl moiety (c) in α-position to the carbonyl group.

Malic acid (**4**) was selected as starting material, owing to its availability in both enantiomeric forms, and attendant potential for developing enantioselective syntheses of both (+)- and (–)-α-hydroxylated lactone lignans. Additionally, the two carboxy groups can easily be distinguished, which is necessary for the synthesis of the target molecules. A further advantage is that these targets can also serve as precursors for the synthesis of other naturally occurring structural variants, such as lactol and triol lignans.

Our initial strategy was a diastereo- and enantioselective double alkylation of a malic acid derivative in one step via a chiral 1,3-dien-1,4-diolate (Scheme 2, Path **A**). Therefore, a dioxolanone amide (X = NMe_2) and a dioxolanone ester (X = O^{t}Bu) were prepared, though the preparation of the chiral dienolate was not achieved due to rapid β-elimination, even at –105 °C [11].

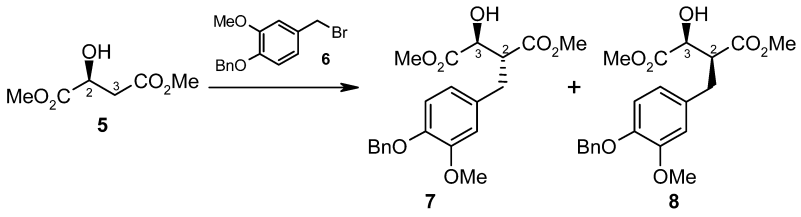


Scheme 2

The second strategy was the consecutive stereoselective alkylation of the 2- and the 3-position of malic acid (**4**) (Scheme 2, Path **B**). The advantage of this approach was that both alkylation reactions were known in the literature [12,13]. However, the stereoselectivity of the second alkylation was uncertain because dioxolanones having a stereogenic center in the side chain have never been used before. Herein, we present the enantioselective synthesis of wikstromol (**3**) [14] and analogs [15], and a pre-

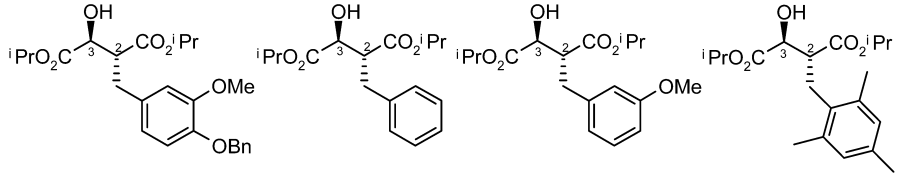
liminary evaluation of their biological activity against HT29 cancer cells. The compounds were obtained in 5–6 steps from dialkyl malates **4** and in overall yields of up to 30 %.

The initial step of the synthesis was the diastereoselective alkylation of dialkyl malates, a reaction that has been used frequently in the past [16]. However, according to the standard procedure [12b] (base, then dialkyl malate, then –78 → –20 °C for 2 h, then –78 °C, then electrophile, then –78 → 0 °C, 15–20 h), the alkylation of dimethyl malate (**5**) with benzyl bromide **6** proceeded in only 20 % to afford **8** and **9** in 89:11 diastereoselectivity (Scheme 3). A survey of the literature revealed that this procedure was never changed significantly. Furthermore, average yields of about 50–60 % with diastereoselectivities in the range of 9:1 *anti*/*syn* have been reported [16]. A major problem encountered in this procedure is the complete enolization of the malate before addition of the electrophile because the second carboxy group contained in dialkyl malates can act as an electrophile, affording ester condensation products [17]. We found that the alkylation can be carried out with improved yield (75 %) when ester **5**, benzyl bromide **6**, and lithium hexamethyldisilazide (LHMDS) are mixed together at –78 °C, and the reaction mixture is warmed to 10 °C [14,15]. This procedure for the α-alkylation of esters (addition of the base in the presence of the electrophile) is uncommon with metal bases, but is used frequently when highly reactive neutral P-bases are employed [18].



Scheme 3 Standard procedure: LDA, then dialkyl malate, –75 °C, THF, then –75 → –20 °C, 2h then –75 °C, then electrophile, then –75 → –5 °C, 15–20 h. **Modified procedure:** dialkyl malate + electrophile, –75 °C, THF then LHMDS, –75 → 10 °C, 14–16 h.

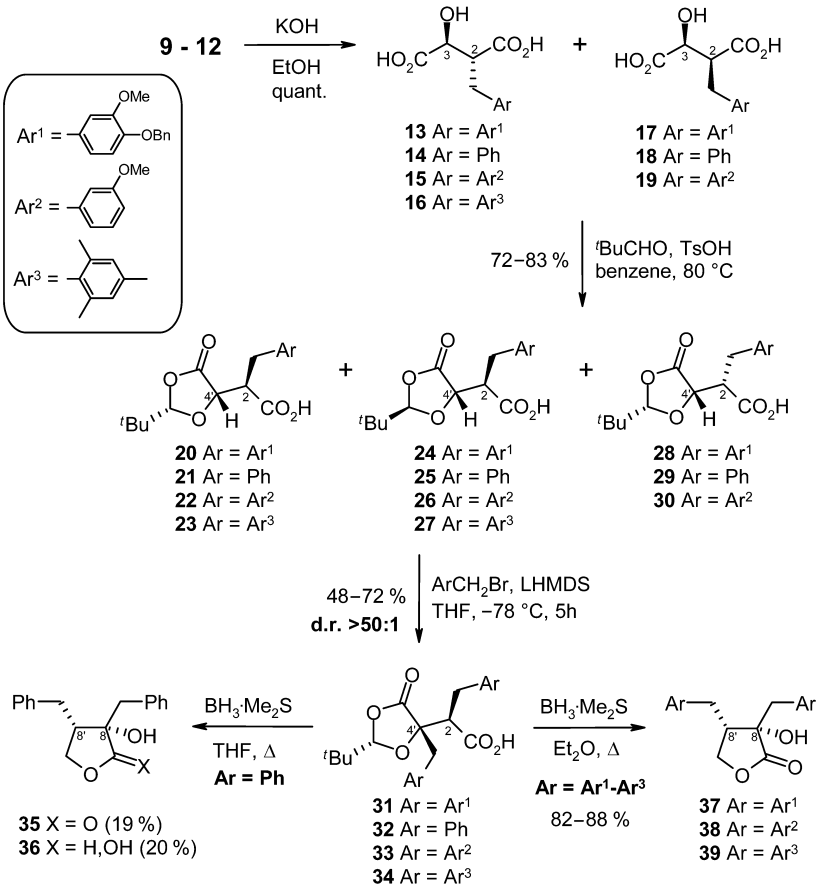
Yield and diastereoselectivity of the α-alkylation of dialkyl malates are not only dependent on the procedure followed, but also on the two alkylester groups involved [19]. We found that the highest yields were obtained with a ^{t}Bu group at C4 (90–94 %), although the diastereoselectivity decreased from 89:11 to 82:18–84:16. On the other hand, the maximum diastereoselectivity was obtained with an ^{i}Pr group at C4 and a ^{t}Bu group at C1 > 97:3, but the product was attained in only 78 % yield. The best compromise with respect to reactivity *and* availability of the starting material was the use of diisopropyl malate [19]. This malic acid ester is easy to prepare, and its alkylation with various benzyl bromides was achieved with good yields and high stereoselectivities (~95:5, **9**–**11**). An exception to these results was the 2,4,6-trimethylbenzyl substituted malate **12**, which was obtained in a d.r. of 83:17 (Scheme 4). The yields of **10**–**12** were obtained under nonoptimized conditions.



9 (80 %, d.r. 95:5) **10** (53 %, d.r. 94:6) **11** (65 %, d.r. 97:3) **12** (56 %, d.r. 83:17)

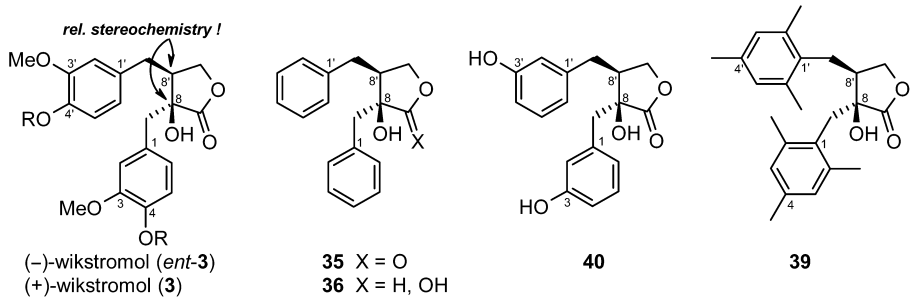
Scheme 4

Saponification of diastereoisomerically pure diesters **9**–**12** afforded the diacids **13**–**16** in quantitative yields. However, isomerization took place during saponification of the esters **9**–**11**, and the *anti*-diacids **13**–**15** were contaminated with ca. 10 % of the corresponding *syn*-diacids **17**–**19**. Only diacid **16** was obtained as an isomerically pure compound. The crude diacids were used for the subsequent acetalization of pivaldehyde. This reaction was best achieved in benzene, affording the *cis*-dioxolanones **20**–**23** and the *trans*-dioxolanones **24**–**27** in a 5:1 ratio [20]. When diacids **13**–**15** were contaminated with the *syn*-isomers **17**–**19**, dioxolanones **28**–**30** were obtained as a further by-product (ca. 10 %), whereas the fourth possible isomer having (2*S*,2′*R*,4′*S*)-configuration was not detected in any case. Isomerically pure dioxolanones **20**–**23** could be obtained from the crude reaction mixture after recrystallization. However, this process was not reliably reproducible and the yield for stereoisomerically pure **20** was low (50–70 %). Therefore, a mixture of the isomers was reacted with the benzyl bromides, in the presence of LHMDS at –78 °C. Surprisingly, only dioxolanones **20**–**23** were alkylated under these reaction conditions, providing isomerically pure dioxolanones **31**–**34** according to the ^{1}H NMR spectra of the crude reaction mixtures [19]. Dioxolanones **24**–**30** were recovered unchanged. The alkylation products **31**–**34** were isolated in 48–72 % yield. Selective reduction of the carboxy group with borane-dimethylsulfide followed by acidic workup, produced the phenol-protected α-hydroxylated butyrolactone lignans. The solvent was critical in this reaction. When tetrahydrofuran (THF) was employed, over-reduction occurred. Thus, the reduction of **32** in THF furnished a mixture of lactone **35** (19 %) and lactol **36** (20 %). The reduction of **31**, **33**, and **34** was performed in ether. Yields of the corresponding lignans **37**–**39** were generally over 80 % in ether (Scheme 5).



Scheme 5

Hydrogenolysis of lactone lignan **37** afforded (–)-wikstromol (*ent*-**3**), and BBr_3-mediated demethylation of **38** yielded lignan **40** (Scheme 6). (+)-Wikstromol (**3**) was obtained by the same method as described above, starting with *R*-diisopropyl malate.



Scheme 6

BIOLOGICAL ACTIVITY OF THE SYNTHESIZED LACTONE LIGNANS

The inhibition of proliferation of HT29 colon cancer cells was investigated with the compounds shown in Scheme 6 in concentration ranges from 0.001 to 100 μM. In addition to both enantiomers of wikstromol, the "parent" lignans **35** and **36** were tested to quantify the effect of the carbonyl group. Lignan **39** was chosen because it was demonstrated in a previous study that its analog lacking the hydroxy group at C8 was highly active relative to other lignans, including enterolactone (**2**). Lignan **40**, the hydroxylated analog of entereolactone (**2**) [21], might be an important metabolite of wikstromol, in a relationship similar to that of matairesinol (**1**) and enterolactone (**2**). Two HT29 cell lines were used (strain and clone 19a). Both cell lines were similarly inhibited by the lignans. The results of HT29 clone 19a are shown in Fig. 1. As expected, the only active compound was lignan **39**, having an IC_{50} of ca. 35 μM.

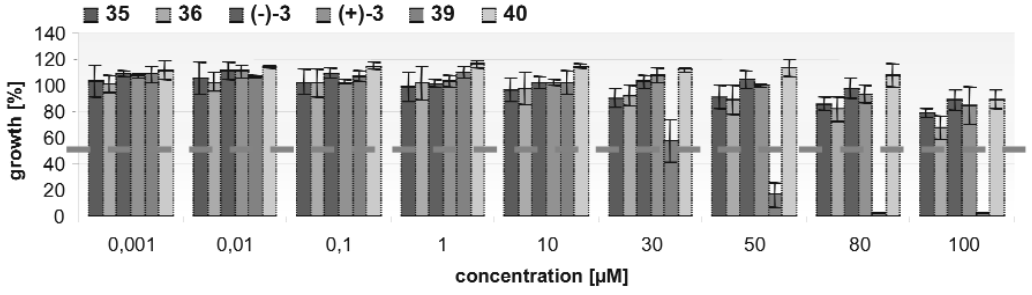


Fig. 1 Proliferation of HT29 clone 19a after 72 h incubation with 8-hydroxy-butyrolactone lignans **35**, **36**, (+)-**3**, (–)-**3**, **39**, **40** (n = 3).

In conclusion, the first efficient and enantioselective synthesis of α-hydroxylated lactone lignans has been demonstrated. Evaluation of the biological properties of these lignans revealed a non-natural derivative with relatively high cytotoxic activity. The synthesis of unsymmetrically substituted lactone lignans, as well as their biological activity with respect to other tumor cell lines, is currently underway.

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