Bioorganic & Medicinal Chemistry Letters 18 (2008) 5178–5181

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and antiproliferative activity of unnatural enantiomers of 7-*epi*-goniofufurone and crassalactone C

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ARTICLE INFO

Article history:
Received 21 July 2008
Revised 24 August 2008
Accepted 26 August 2008
Available online 29 August 2008

Keywords:
Styryl-lactones
Stereocontrolled syntheses
D-Xylose
7-Epigoniofufurone
Crassalactone C
Goniofufurone
Unnatural enantiomers
Antitumor agents

ABSTRACT

A facile synthesis of 7-*epi*-(−)-goniofufurone as well as the first synthesis of (−)-crassalactone C was achieved starting from D-xylose. A comparison of their in vitro antitumour activities with those observed for the corresponding naturally occurring enantiomers was provided.

7-*epi*-(+)-Goniofufurone (**1**, Fig. 1) is a naturally occurring styryl lactone that was isolated from the stem bark of the tropical plant *Goniothalamus giganteus* (Annonaceae).[1] Its structure was elucidated by spectral methods and the relative configuration was established by X-ray crystal data.[1] The absolute stereochemistry of **1** was established from the total synthesis of 7-*epi*-(−)-goniofufurone (*ent*-**1**).[2] The combination of the unique structures and a promising biological activity[3] has prompted a number of syntheses of similar styryl lactones.[4,5] A new styryl lactone, (+)-crassalactone C (**2**), was recently isolated from the leaves and twigs of the Asian three *Polyalthia crassa*.[6] Its structure and the relative stereochemistry were assigned based on spectral data, and its absolute configuration was confirmed by semi-synthesis starting from the isolated (+)-goniofufurone. Recently, we have completed the total synthesis of the naturally occurring styryl lactones **1** and **2**,[7] and evaluated their antiproliferative activity against several tumour cell lines. As a consequence of the in vitro antitumour activity of these natural products, we were especially keen to prepare and evaluate the opposite enantiomers of **1** and **2**, since it is well known that enantiomers of certain bioactive compounds may exhibit improved potencies[8] or even different biological activities altogether.[9]

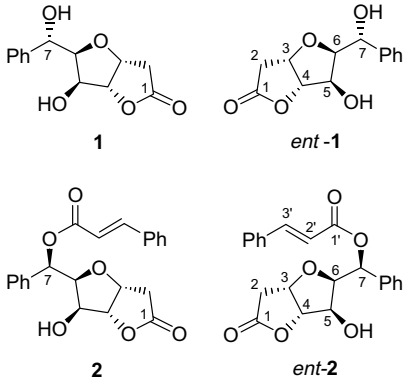

Figure 1. Structures of 7-*epi*-(+)-goniofufurone (1) and (+)-crassalactone C (**2**), and of the corresponding unnatural enantiomers *ent*-**1** and *ent*-**2**

Herein, we report the total synthesis of the unnatural enantiomers of 7-*epi*-goniofufurone (*ent*-**1**) and crassalactone C (*ent*-**2**) starting from D-xylose, and provide a comparison of their antiproliferative activities with those observed for the natural products **1** and **2**. Three syntheses of *ent*-**1** were reported,[2,10] but there was no

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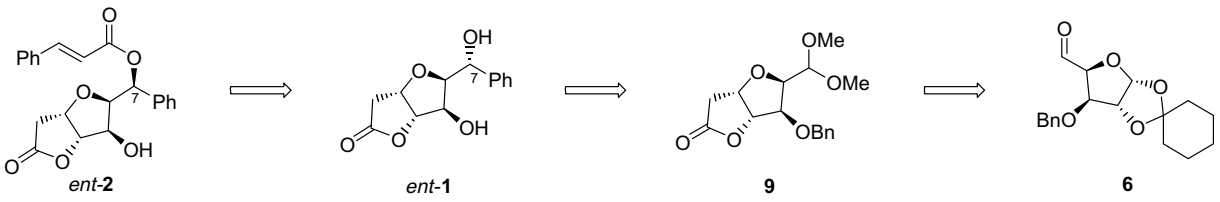
doi:10.1016/j.bmcl.2008.08.093

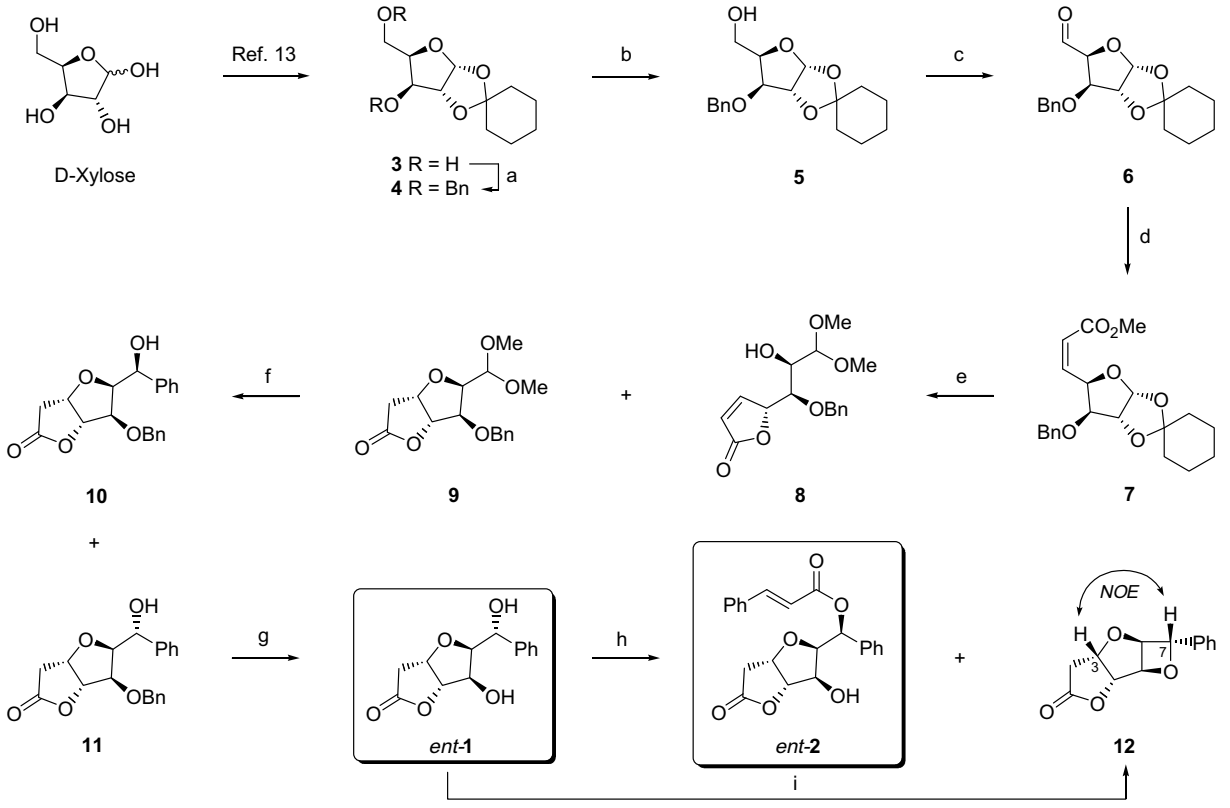


Scheme 1. Retrosynthetic analysis of target compounds *ent*-**1** and *ent*-**2**.

record in the literature concerning the biological activity of this molecule. Neither the synthesis nor biological activity of *ent*-**2** was reported in the literature.

Retrosynthetic analysis of *ent*-**1** and *ent*-**2** is outlined in Scheme 1. Both targets *ent*-**1** and *ent*-**2** contain five contiguous stereocenters but differ in stereochemistry and functionality at C-7. The presence of the cinnamoyl moiety in *ent*-**2** implies that this molecule could be obtained from *ent*-**1** through a regioselective Mitsunobu reaction with cinnamic acid. Further disconnection of *ent*-**1** shows that it can be derived from the protected aldehyde **9** by three successive transformations that involve a hydrolysis of the acetal functionality, stereo-selective addition of phenyl magnesium bromide to the liberated aldehyde group, followed by removal of benzyl protective group. The intermediate **9** should be accessible from the aldehyde **6**, through an adopted literature procedure.[11] Dialdose **6** is readily available from D-xylose in several synthetic steps.[12]

Synthesis of both *ent*-**1** and *ent*-**2** is shown in Scheme 2. The synthetic sequence commenced with the formation of the protected dialdose **6** from D-xylose derivative **3**[13] by a slight modification of a literature method.[12] A newly developed two-step sequence that included di-O-benzylation of **3**, followed by a regioselective C-5 deprotection gave the primary alcohol **5**. Oxidation of **5** under modified Moffat conditions[14] gave **6** in 71% overall yield (from three steps). The preceding preparation of **6** was accomplished in 62% overall yield over four linear steps.[12] The *Z*-selective Wittig olefination[15] of **6** with methyl (triphenylphosphoranylidene)acetate gave the *Z*-unsaturated ester **7** (83%) as a dominant reaction product, accompanied with a minor amount (12%) of the corresponding *E*-isomer (not shown in the reaction scheme). It was expected that the key intermediate **9** could be obtained by an acid-catalyzed methanolysis of **7**. The procedure followed here is analogous to that previously developed by Prakash and Rao[11] for the conversion of the isopropylidene analogue of **7** into lactone **9**. Accordingly, compound **7** was refluxed in dry methanol in the presence of a catalytic amount of sulphuric acid (2%). Although a complete conversion of starting compound was observed after 4.5 h, only traces of the desired lactone **9** were detected in the reaction mixture. The unsaturated lactone **8** was formed as a major reaction product under these reaction conditions. However, when



Scheme 2. Reagents and conditions: (a) BnBr, NaH, DMF, 0 °C for 0.5 h, then rt for 1 h; (b) H_2–Pd/C (0.05 equiv of Pd), EtOH, EtOAc, rt, 1.5 h, 80% from **3**; (c) DCC, anhyd H_3PO_4, Py, DMSO, rt, 3.5 h, 89%; (d) Ph_3P:$CHCO_2Me$, MeOH, 0 °C for 0.5 h, then rt for 1.5 h, 83%; (e) i—H_2SO_4, MeOH, reflux 4.5 h, ii—$NaHCO_3$, 35 °C, 1 h, 74% of **9**, 4% of **8**; (f) i—9:1 TFA/H_2O, 0 °C for 0.5 h, then rt for 0.5 h, ii—PhMgBr/THF, Et_2O, −7 °C, 3 h, 56% of **11** (from **9**), 3% of **10** (from **9**); (g) H_2–Pd/C, MeOH, rt, 72 h, 64%; (h) Cinnamic acid, Ph_3P, THF, DEAD, 0 °C for 1 h, then rt for 2.5 h, 53% of *ent*-**2**, 16% of **12**; (i) Ph_3P, DEAD, PhMe, reflux, 2.5 h, 40%.

the reaction solution was alkalized to pH 9 ($NaHCO_3$) and stirred for 1 h at 35 °C, the bicyclic lactone **9** was predominantly formed as a result of the stereospecific Michael ring closure in **8**. The bicyclic lactone **9** was isolated in a 74% yield, along with a minor amount of **8** (4%). These results are in accord with Prakash and Rao's assumption that lactonisation precedes Michael attack in this process, but dismisses their hypothesis that the tetrahydrofuran ring closure in **8** occurs under the acidic conditions.[11]

Hydrolytic removal of the dimethyl acetal protection in **9** afforded the corresponding aldehyde, which was subsequently treated with phenyl magnesium bromide to give two diastereomeric alcohols **10** and **11** in a 1:19 ratio and 59% combined yield (calculated to **9**). Major isomer **11** was converted to target *ent*-**1** after removal of the benzyl protecting group. The physical and spectral data[16] of thus obtained sample *ent*-**1** were identical to those previously reported.[10a] This new synthesis of *ent*-**1** proceeds in seven steps with 16% overall yield calculated to starting compound **3**. Along with the Gracza and Jäger approach, it appears to be one of the most efficient routes to this molecule yet disclosed.[17]

Compound *ent*-**1** was converted to (−)-crassalactone C (*ent*-**2**) upon treatment with cinnamic acid, under the standard Mitsunobu conditions.[18] The target *ent*-**2** that was isolated in 53% yield, displayed physical and spectral properties[19] in reasonable agreement with those previously reported for the natural (+)-crassalactone C.[6,7] A minor amount of 5,7-anhydro derivative **12** (16%) was also obtained from this reaction. The side-product **12** was presumably formed by a competitive intramolecular nucleophilic displacement process.[18] In order to verify this assumption, the Mitsunobu reaction was performed in absence of cinnamic acid (Ph_3P, DEAD, PhMe↑↓, 2.5 h), whereupon the oxetane **12**[20] was obtained as a major product. The stereochemistry at C-7 was resolved on the basis of a NOE interaction between H-3 and H-7 that is consistent with L-*glycero*-D-*ido* stereochemistry of **12**.

A side-by-side comparison of natural **1** and *ent*-**1**, as well as of **2** and *ent*-**2** in a range of cytotoxic assays[21] is presented in Table 1. Antitumour activity of **12** was also preliminary evaluated, since this compound formally represents a structurally constrained analogue of (−)-goniofufurone. The doxorubicin (DOX) was used as a reference compound.

In general, the unnatural enantiomer of crassalactone C (*ent*-**2**) exhibited antiproliferative activities towards the all tested cell lines while compound *ent*-**1** was active against K562, Jurkat and HeLa cell lines but was completely inactive against Raji cells. Remarkably, all styryl lactones were devoid of any significant toxicity against the normal foetal lung fibroblasts (MRC-5). A comparison of IC_{50} values revealed not only that the unnatural 7-*epi*-(−)-goniofufurone (*ent*-**1**) was slightly more potent than the corresponding natural enantiomer **1** against HL-60 cells, but also that this molecule was approximately 345-fold more potent than the natural enantiomer **1** against Jurkat cell line. On the other hand, *ent*-**1** showed a similar potency as the reference compound (DOX) in the same cell line. The unnatural enantiomer of crassalactone C (*ent*-**2**) showed a potent antiproliferative activity towards HL-60 cells, while the corresponding natural enantiomer **2** was completely inactive against this cell line. Moreover, compound *ent*-**2** demonstrated a 27- and 10-fold greater cytotoxicity in Raji and HeLa cells, respectively, when compared to the natural product **2**. At the same time, this molecule demonstrated a 5-fold greater cytotoxicity than DOX towards the Raji cells. Remarkably, tricyclic derivative **12** showed sub-micromolar antiproliferative activities against HL-60, Raji and HeLa malignant cells. In fact, it was the most active compound towards these cell lines. Finally, molecule **12** demonstrated a 37- and 7-fold stronger cytotoxicity in HL-60 and Raji cell lines when compared to doxorubicin, respectively.

Table 1
In vitro cytotoxicity of **1**, *ent*-**1**, **2**, *ent*-**2** and **12**

Compound	IC_{50}[a] (μM)				
	HL 60	Jurkat	Raji	HeLa	MRC-5
1	22.02	18.64	1.25	0.89	>100
ent-**1**	13.93	0.05	>100	4.38	>100
2	>100	25.45	15.46	11.25	>100
ent-**2**	1.87	15.67	0.57	1.18	>100
12	0.025	28.45	0.45	0.97	>100
DOX	0.92	0.03	2.98	0.07	0.10

[a] IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control.

It is notable that natural styryl lactones and analogues present promising and diverse biological activities. Some important mechanisms of action of this class of bioactive compounds have been recently reviewed.[3] The remarkable activity of the lactone **12** towards the all malignant cell lines tested deserves some additional comments. It is possible that this analogue acts as a specific alkylating agent, and that the observed cytotoxicities originated from an irreversible covalent binding to cellular serine or thiol proteases. However, further studies will be needed to verify this hypothesis.

In summary, the unnatural styryl lactones (−)-7-*epi*-goniofufurone (*ent*-**1**), (−)-crassalactone C (*ent*-**2**), as well as a new conformationally constrained analogue of (−)-goniofufurone (**12**) have been synthesized and evaluated for their in vitro antitumour activities against a number of human neoplastic cell lines. Compound *ent*-**1** demonstrated a sub-micromolar antiproliferative activity against Jurkat cells, comparable to that recorded for the reference compound (DOX). The highest cytotoxicity of *ent*-**2** was observed in Raji cell line, with approximately 5-fold higher potency then doxorubicin. Compound **12** showed the most potent antiproliferative activity towards HL-60 cells being ca. 37-fold more active than the reference compound (DOX). Based upon the potent antitumour activities of *ent*-**1**, *ent*-**2** and **12**, as well as upon their non-toxicity against normal MRC-5 cells, we believe that these compounds may serve as convenient leads in the synthesis of more potent and selective antitumour agents. Finally, from a synthetic perspective, the fact that both enantiomers efficiently inhibit tumour cells growth also means that both are equally valuable synthetic targets. In this sense, the synthesis of *ent*-**1** and *ent*-**2**, along with our preceding approach to **1** and **2**,[7] represents a new enantiodivergent route that provided the opportunity to access the cytotoxic properties of not only the natural products, but also their unnatural enantiomers.

Acknowledgment

Financial support from the Ministry of Science of the Republic of Serbia (Project No. 142005) is gratefully acknowledged.

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16. *Selected data for ent-**1**:* Mp 205–206 °C, $[\alpha]_D^{20}$ −98.3 (*c* 0.47, Me_2CO); lit.[2] mp 208–209 °C, $[\alpha]_D^{20}$ −92.5 (*c* 1.1, Me_2CO). ^{1}H NMR (250 MHz, DMSO-d_6 + D_2O): δ 2.53 (d, 1H, $J_{2a,2b}$ = 18.7 Hz, H-2a), 2.85 (dd, 1H, $J_{2a,2b}$ = 18.7, $J_{2b,3}$ = 6.5 Hz, H-2b), 3.60 (d, 1H, $J_{5,6}$ = 2.8 Hz, H-5), 3.84 (dd, 1H, $J_{5,6}$ = 2.8, $J_{6,7}$ = 8.3 Hz, H-6), 4.73 (d, 1H, $J_{6,7}$ = 8.3 Hz, H-7), 4.78 (d, 1H, $J_{3,4}$ = 4.7 Hz, H-4), 4. 94 (dd, 1H, $J_{3,4}$ = 4.7, $J_{2b,3}$ = 6.5 Hz, H-3), 7.21–7.37 (m, 5H, Ph). ^{13}C NMR (62.9 MHz, DMSO-d_6): δ 36.42 (C-2), 72.16 (C-7), 73.48 (C-5), 77.35 (C-3), 85.35 (C-6), 88.48 (C-4), 127.54, 128.35, 128.84 and 142.40 (Ph), 177.44 (C-1). LR-MS (ESI): *m/z* 289 (M^++K), 273 (M^++Na).
17. Gracza and Jäger[10b] synthesis: 16% yield over seven linear steps; Zhao et al.[10a] synthesis: 11% yield over nine steps Shing et al.[2] synthesis: 10 steps and 12% overall yield.
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19. *Selected data for ent-**2**:* Mp 147–150 °C, $[\alpha]_D^{20}$ −110.1 (*c* 0.5, EtOH). ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.54 (d, 1H, $J_{2a,2b}$ = 18.6 Hz, H-2a), 2.69 (dd, 1H, $J_{2a,2b}$ = 18.6, $J_{2b,3}$ = 5.9 Hz, H-2b), 4.25 (br s, 1H, OH), 4.27 (dd, 1H, $J_{6,7}$ = 9.3, $J_{5,6}$ = 2.4 Hz, H-6), 4.43 (br s, 1H, H-5), 5.01 (m, 2H, H-3 and H-4), 6.00 (d, 1H, $J_{6,7}$ = 9.3 Hz, H-7), 6.46 (d, 1H, $J_{2',3'}$ = 15.8 Hz, H-2′), 7.35–7.60 (m, 10H, 2× Ph), 7.78 (d, 1H, $J_{2',3'}$ = 15.8 Hz, H-3′). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 35.79 (C-2), 72.80 (C-7), 73.10 (C-5), 77.09 (C-3), 82.43 (C-6), 87.01 (C-4), 116.59 (C-2′), 127.70, 128.35, 128.65, 128.95, 128.98, 130.95, 133.71 and 136.71 (2× Ph), 147.29 (C-3′), 167.46 (C-1′), 175.40 (C-1). HR MS (ESI): *m/z* 403.1133 (M^++Na). Calcd for $C_{22}H_{20}NaO_6$: 403.1152.
20. *Selected data for **12**:* Mp 146°C (CH_2Cl_2/hexane), $[\alpha]_D^{20}$ −48.1 (*c* 0.5, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.83 (d, 1H, $J_{2a,2b}$ = 18 Hz, H-2a), 2.93 (dd, 1H, $J_{2a,2b}$ = 18, $J_{2b,3}$ = 4.1 Hz, H-2b), 2.84 (dd, 1H, $J_{5,6}$ = 4.2, $J_{6,7}$ = 2.6 Hz, H-6), 5.09 (d, 1H, $J_{3,4}$ = 3.5 Hz, H-4), 5.37 (dd, $J_{2b,3}$ = 4.1, $J_{3,4}$=3.5 Hz, H-3), 5.50 (d, 1H, $J_{5,6}$ = 4.2 Hz, H-5), 5.52 (d, 1H, $J_{6,7}$ = 2.6 Hz, H-7), 7.50–7.32 (m, 5H, Ph); NOE contact: H-3 and H-7. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 36.04 (C-2), 79.24 (C-3), 84.92 (C-4), 85.08 (C-6), 85.31 (C-5), 89.11 (C-7), 125.0, 128.51, 128.85 and 138.56 (Ph), 174.16 (C-1). HR MS (ESI): *m/z* 233.0805 (M^++H). Calcd for $C_{13}H_{13}O_4$: 233.0808.
21. Cytotoxic activities were evaluated by using standard MTT assay after exposure of cells to the tested compounds for 72 h. Results are presented as mean values of three independent experiments done in quadriplicates. Coefficients of variation were <10%.