Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3295–3298

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A CONCISE SYNTHESIS AND *IN VITRO* CYTOTOXICITY OF NEW LABDANE DITERPENES+

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Received 24 August 1998; accepted 6 October 1998

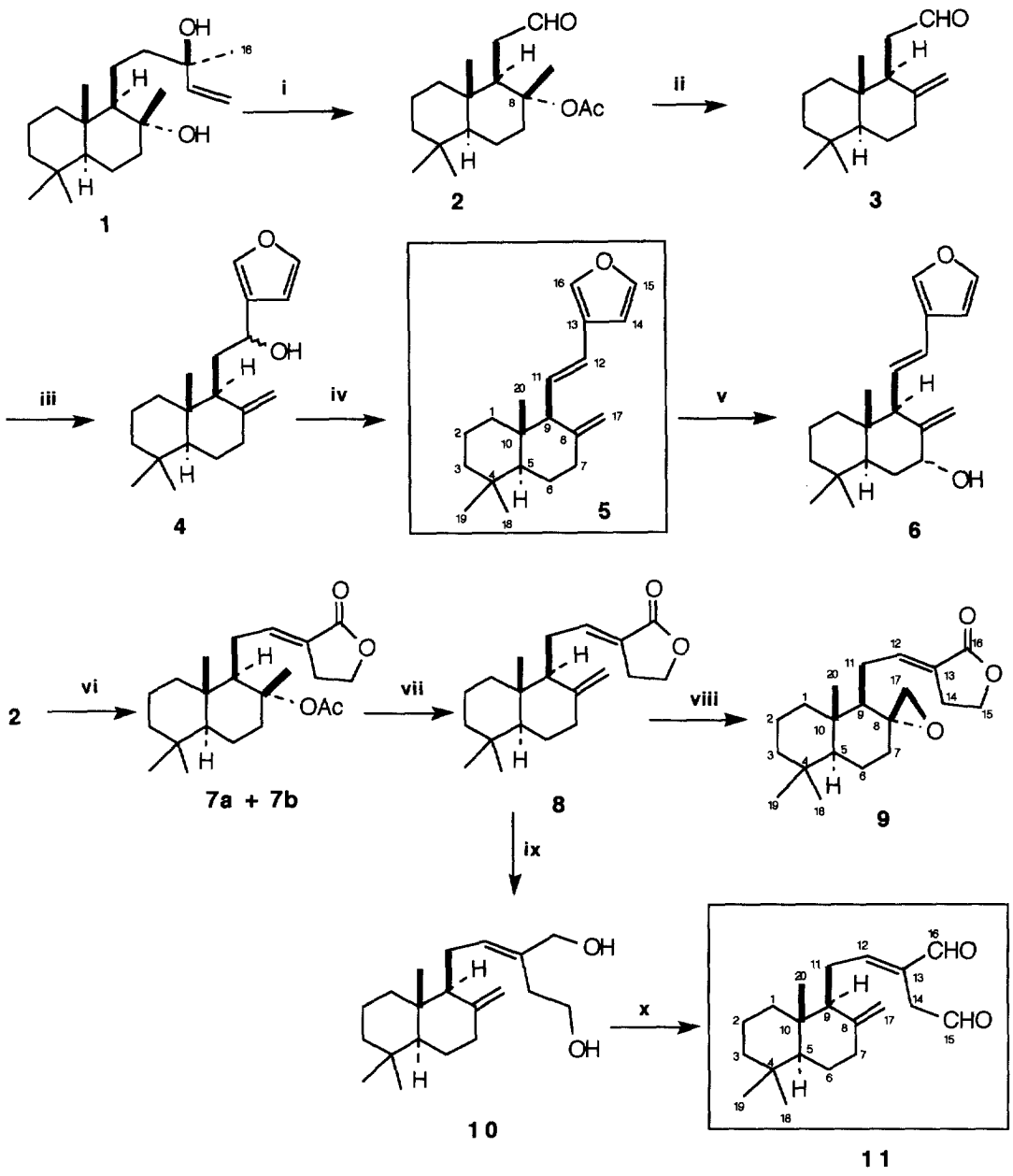
Abstract: A new series of labdane-related diterpenes have been synthesized from (-)-sclareol and assayed *in vitro* cytotoxicity against mouse and human cancer cells. A key intermediate, homodrimane and furanolabdane derivatives show good *in vitro* cytotoxicity comparable to those of mitomycin C and adriamycin.

+*Dedicated to Professor Sir Jack E. Baldwin on the occasion of his 60th birthday.*

Labdane diterpenes and drimanes are of special biological interest due to their significant antifeedant, antitumor, and antifungal activities shown, for example, in polygodial,[1] warburganal,[2] coronarin A,[3] and galanolactone.[4] Furanolabdane coronarin E **5** isolated from the aerial parts of *Alpinia chinensis* in 0.002 % yield[5] are biogenetically related to cytotoxic labdienedial **11**[3] and coronarin A. **5** could be derived by dehydration of **11** and the allylic oxidation of **5** could provide coronarin A. No information is available on the synthesis and the structure-cytotoxicity relationship of coronarin E **5**, its related compounds and labdane terpenes. Coronarin A showed significant cytotoxic activity (IC_{50} = 1.65 μg/mL) against Chinese hamster V-79 cells.[3] Interesting biological activities, natural scarcity, novel heterolabdane-type structure of **5**, and nontoxicity[6] of sclareol in humans as a lead compound prompted us to synthesize it in concise steps and screen coronarin E and its related labdane compounds in search of a new antitumor agent with a unique mode of action. In this letter, we would like to report the first synthesis and structure-activity relationship of optically active coronarin E, its novel labdane analogs and homodrimanes.

(-)-Sclareol **1**[7,8] is a readily available nontoxic natural product with a structure and established stereochemistry which is suitable as a chiral synthon in the semi-synthesis of new labdane terpenes, including coronarin E **5**, 7-epi-coronarin A **6**[8] and galanolactone derivatives. Labdane terpenes, **4**-**11**[9] were prepared by an efficient and regiospecific synthesis as outlined in Scheme 1. Thus, the Corey oxidative degradation[10] of (-)-sclareol **1**, with osmium tetroxide and sodium periodate in aqueous *t*-butanol, cleanly afforded the acetoxyaldehyde **2**[7-10] in one step (65 %) (scheme 1). The homodrimane **2**[8] could serve as a versatile intermediate for the synthesis of a variety of biologically active natural products including galanal A,[4] warburganal,[2] and galanolactone.[4] Regiospecific deacetylation at position 8 of **2** with collidine gave **3** (68 % yield) by known procedure.[8,9] Coupling of **3** with the 3-bromofuran in the presence of *n*-butyllithium in anhydrous ether at -78 °C for 2 hours provided the two diastereoisomeric furanolabdane hydroxides **4**[9] with a ratio of 3 to 1 respectively (yield 71 %). Without separation of the isomers of **4**, dehydration of **4** with 2, 6 - lutidine in the presence of methanesulfonyl chloride afforded exclusively coronarin E, **5**[9] (70

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00603-9



Scheme 1 *Reagents and conditions:* (i) OsO_4(cat.)/$NaIO_4$(1.8 eq.), *t*-butanol, THF, 25 °C, 5.5 h (65 %); (ii) Collidine, reflux, 8 h, 68 %; (iii) 3-Bromofuran (1.2 eq.), *n*-BuLi (1.5 eq.), ether, -78 °C, 2 h., (71 %); (iv) MsCl (5 eq.), 2, 6-lutidine (10 eq.), CH_2Cl_2, rt, 30 h. (70 %); (v) SeO_2 (4eq.), *tert*-BuOOH (8 eq.), CH_2Cl_2, rt, 6 h., (73 %); (vi) diethylphosphono-2-butyrolactone (1.0 eq.) , NaH(1.2 eq.), dry toluene, 0 °C, 4 h. then 80 °C, 1 h. (67 %, E/Z=3/1); (vii) quinoline, reflux, 2 h (71 %); (viii) *m*CPBA (1.0 eq.), $CHCl_3$, 0 °C, 2 h. (45 %); (ix) LAH (5 eq.), ether, r. t., 2 h, [85 %] ; (x) PCC (2.5 eq.), CH_2Cl_2, r. t., 50 min., [81 %].

%). Allyic oxidation of coronarin E **5** with selenium oxide and *t*-butylperoxide in methylene chloride cleanly provided (-)-7-*epi*-coronarin A **6**[9] (73 % yield). No coronarin A was generated in this oxidation. Coupling of the aldehyde **2** with the anion of diethylphosphono-2-butyrolactone provided the isomeric lactones **7a**[9] (*E*-form) and **7b**[9] (*Z*-form), respectively, with a ratio of 3:1(yield 67 %). Separation of the isomers by column chromatography gave **7a**.[9] In our synthesis, the acetoxy group at C-8 of **2** served as a protecting group for the later-generating exomethylene group at C-8 of **8**. Subsequent elimination of acetic acid at C-8 position of compound **7a** with quinoline (reflux, 2 h) provided the exomethylene compound **8**[9](71 %). The other regio-isomers ($\Delta^{8,9}$ and $\Delta^{7,8}$) were not detected. **8** is a versatile intermediate for useful synthesis of galanolactone-related compounds. Epoxidation of the exomethylene at C-8 of **8** with *m*CPBA (1 eq.) at 0 °C exclusively afforded (-)-8-*epi*-galanolactone **9**[9] (45 %). Steric hindrance by the butanolide ring in the β-face may force the mCPBA to approach in the direction of the less-hindered α-face, resulting in the epimer **9**. Reductive ring cleavage of **8** with excess LAH (5 eq.) in anhydrous diethylether (rt., 2 h.) afforded dienediol **10**[9] (yield 85 %). Subsequent oxidation of the diols of **10** with PCC (2.5 eq.) in methylene chloride cleanly provided the target compound, (+)-labdienedial **11**[1,9] (81 % yield) (Scheme 1). During this reduction-oxidation procedure, two olefin groups of **8** and **10** were left intact.

The *in vitro* cytotoxicity of labdane-related compounds on the murine and human cancer cells was defined by the microculture tetrazolium assay as described previously.[11] IC_{50} values are presented in Table 1. All labdane analogs **1** - **11** show a modest *in vitro* cytotoxicity (IC_{50} = 0.16 - 12.3 μg/mL) while homodrimane **3**[8], a key intermediate, is most active and comparable to adriamycin against P388 cell line. **3** is 240 times more active than galanolactone (IC_{50} = 38.5 μg/mL), the parent compound against P388 cell line. Most analogs

Table 1: *In vitro* cytotoxicities of labdane-related compounds.

	IC_{50} (μg/mL)		
	P388[a]	B16[b]	SNU-1[c]
1	10.1	28.0	11.9
2	6.9	31.9	4.8
3	0.16	30.8	3.9
4	3.6	47.2	3.9
5	10.0	18.8	8.0
6	10.3	27.3	4.8
7a	7.8	11.3	12.3
8	6.7	7.5	19.2
9	12.3	84.5	15.7
10	6.3	28.9	8.6
11	4.1	41.2	12.0
Mitomycin C	0.06	(nt)	2.32
Adriamycin	0.09	(nt)	2.15

[a]P-388: murine lymphocytic leukemia. [b]B16: murine melanoma.
[c](SNU-1): human gastric adenocarcinoma. (nt): not tested.

exhibit a moderate cytotoxicity (IC_{50} = 7.5 - 47.2 μg/mL) against B16 cell line. **8** is most active (IC_{50} = 7.5 μg/mL) and **7a** shows a modest cytotoxicity at the value of IC_{50} = 11.3 μg/mL. **9** shows a weakest *in vitro* cytotoxicity (IC_{50} = 84.5 μg/mL) against B16 cell line. Conversion of the olefin at C-8 and C-17 of **8** into the epoxide of **9** decreases the cytotoxicity by a factor of twelve. It is interesting to note that the electrophilic heterocycle at C-12 of labdane diterpenes generally increases cytotoxicity. Thus, **8** increases activity to four times that of the simple aldehyde of drimame, **3**. Electrophilic heterocycles of **7a** and **8** have greater cytotoxicity against B16 cells than non-electrophilic ones of **4**, **5**, and **6**. Most compounds, **1-11** show good activity against SNU-1 cells (IC_{50} = 3.9-19.2 μg/mL). **2**, **3**, **4** and **6** show a comparable *in vitro* cytotoxicity to those of clinically useful mitomycin C and adriamycin. Analogs **1-11** are generally active against P388 and SNU-1 cancer cell lines, while they are weakly active against B16 cell line. The unique structures of furanolabdane and homodrimane framework, with the established stereochemistry, are key backbones responsible for significant cytotoxicity.

In conclusion, these efficient and regiospecific syntheses make it possible to resolve the natural scarcity of coronarin E and labdadienedial and to further test the biological activities of coronarin E, (-)-7-*epi*-coronarin A, labdanedial and their related compounds. This structure-activity relationship of labdane derivatives and homodrimanes may be used as lead information for the possible development of the new antitumor agents related to virtually non-toxic sclareol[6] (LD_{50} > 5 g/Kg orally administered[12] to rats). **2**, **3**, **4**, and **6** deserve further evaluation as antitumor agents because of their low toxicity and the *in vitro* cytotoxicity comparable to those of mitomycin C and adriamycin.

Acknowledgment: This study was supported by Yonsei University Research Fund.

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