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Semisynthesis of C-ring modified triptolide analogues and their cytotoxic activities

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Abstract—Several C-ring modified analogues of a potent antileukemic diterpene, triptolide (**1**), were synthesized and their structure–activity relationships were studied.

Triptolide (**1**) and the relating compounds (**2**–**4**), isolated from *Tripterygium wilfordii* (Celastraceae), are unusual diterpenes having two or three epoxides on the B/C ring system (see Fig. 1).[1–3] Although compounds **1**–**3** are known to have a significant antileukemic activity,[1,2] no systematic structure–cytotoxic activity relationship (SAR) studies have been reported.

In the present study, we synthesized two epoxide-transposition analogues of triptolide (**1**), that is, compounds **6** and **7** having 13,14-β-epoxide and 12-hydroxyl groups instead of the 12,13-α-epoxide and 14-hydroxyl groups in compound **1** and an epoxide-transposition analogue of triptonide **2**, that is, compound **5** (see Fig. 2). The SAR studies of these triptolide analogues were performed by using A549 human lung and HT29 human colon tumor cells.

Synthesis of compounds **6** and **7** was performed as described in Scheme 1. The reaction of compound **1** with thiocarbonyldiimidazole (TCDI) in 1,2-dichloroethane gave the corresponding thiocarbonylimidazole **8** in 94% yield. Subsequent reductive elimination of the imidazoyl-thiocarbonyloxy group in **8** with Bu_3SnH and AIBN caused simultaneous cleavage of 12,13-epoxide to give an allyl alcohol **9** in 91% yield. By the analogous

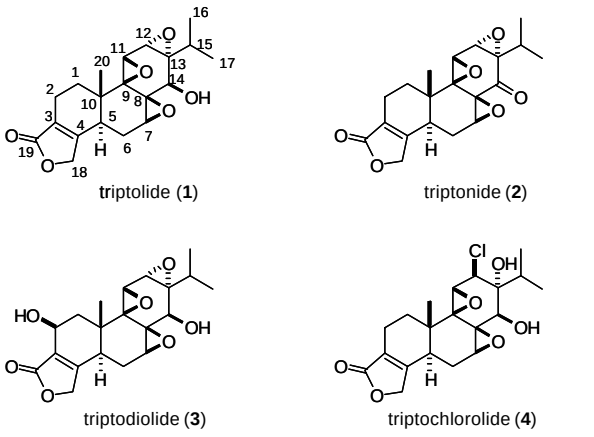

Figure 1. Triptolide families from *Tripterygium wilfordii*.

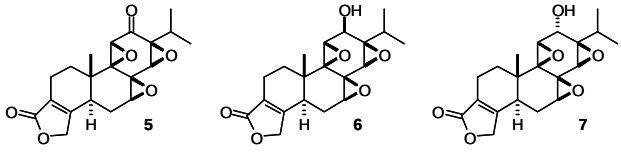

Figure 2. Triptolide epoxide-transposition analogues **5**–**7**.

procedure, 14-*epi*-triptolide (**11**),[4] which was easily prepared from compound **1** by Dess–Martin periodinane oxidation followed by reduction with $NaBH_4$, was converted to compound **9** in 59% overall yield. Direct epoxidation of compound **9** with some oxidizing agents, such as dimethyldioxorane, mCPBA, or hydrogen peroxide–

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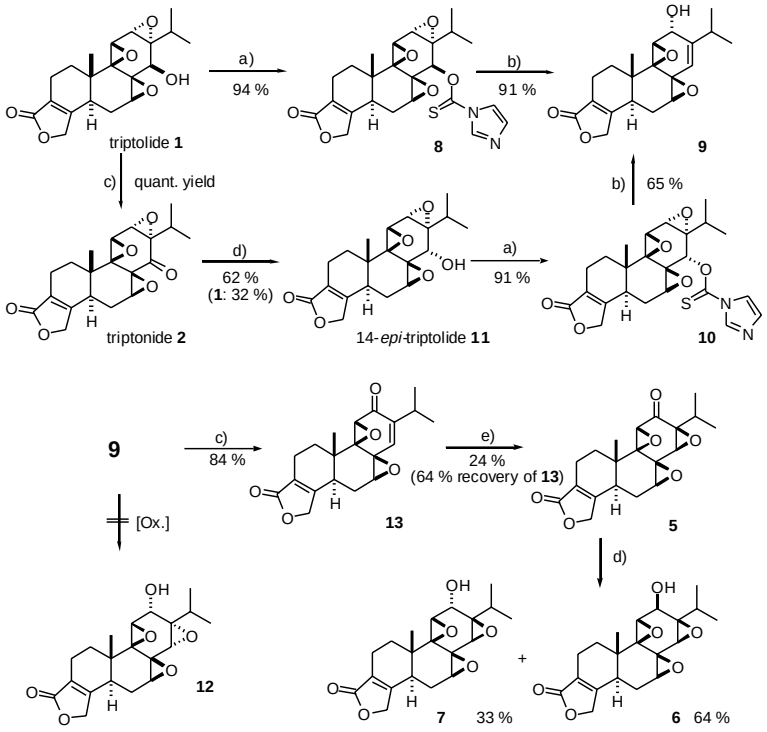


Scheme 1. Preparation of compounds **5**–**7**. Reagents and conditions: (a) TCDI/DMAP/$ClCH_2CH_2Cl$/50 °C; (b) Bu_3SnH/AIBN/CH_2Cl_2/50 °C; (c) Dess–Martin periodinane/CH_2Cl_2/rt; (d) $NaBH_4$/EtOH/rt; (e) 1 N NaOH/H_2O_2/MeOH/rt.

sodium hydroxide, failed to give a triepoxy compound **12**, probably due to the presence of the allylic 12-hydroxyl group. Then, the 12-hydroxyl group of compound **9** was converted to a carbonyl group with Dess–Martin periodinane to give an enone **13** in 84% yield. When the enone **13** was subjected to nucleophilic oxidation with hydrogen peroxide–sodium hydroxide,[5] it gave compound **5** having triepoxides, in 24% yield, as the sole epoxidation product. The starting material **13** was recovered in 64% yield. The X-ray crystallographic analysis of compound **5** confirmed its structure to be as shown in Figure 3.[6] The stereoselective epoxidation took place probably because the reaction proceeded via the β(convex)-facial nucleophilic attack by the hydrogen peroxide anion. Finally, compound **5** was reduced with $NaBH_4$ in EtOH to give a mixture of a pair

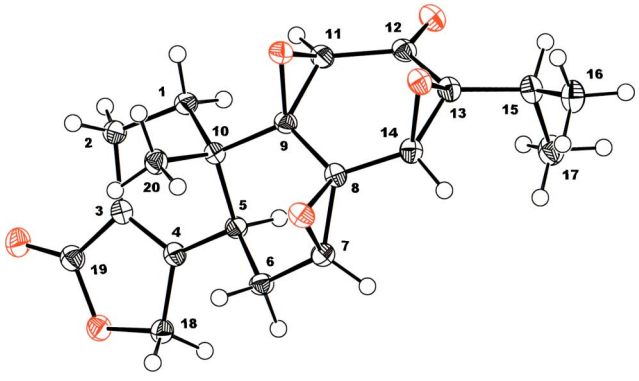


Figure 3. ORTEP representation of compound **5**.

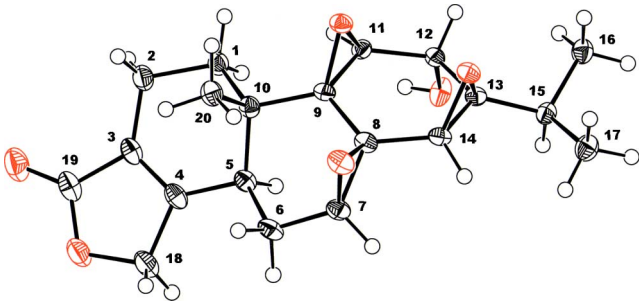
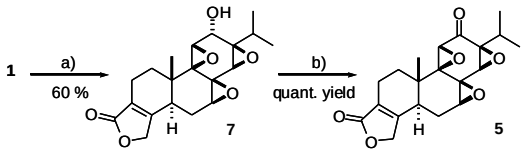


Figure 4. ORTEP representation of compound **7**.

of epimers, **6** and **7**, in a ratio of 66:34, whose separation was made by ODS-HPLC. The X-ray crystallographic analysis of compound **7** confirmed its structure to be as shown in Figure 4.[6]

Although Yu et al. reported an alternative conversion procedure of **1** to **7** by prolonged treatment of **1** with diethylamine in phosphate buffer (pH 7.4),[7] the reported spectral data including the ^1H and ^{13}C NMR spectra of compound **7** were not identified with those of our present product **7**. Therefore, we decided to examine the method described by Yu et al.[7] As a result, the thus obtained product was shown to be the same as our present product **7** by the spectral data including the ^1H and ^{13}C NMR spectra. The identity was confirmed also by obtaining its keto derivative by the oxidation of their conversion product with Dess–Martin periodinane, which was identical to our present compound **5** (Scheme 2).



Scheme 2. Alternative synthesis of **5**. Reagents and conditions: (a) Et_2NH/MeOH/phosphate buffer (pH 7.4); (b) Dess–Martin periodinane/CH_2Cl_2/rt.

Table 1. Cytotoxicity of triptolide analogues **1**, **2**, **5–11**, and **13** against A549 human lung and HT29 human colon tumor cells

Compound	IC_{50} (μg/mL)	
	A549	HT29
1	0.0013	0.00010
2	0.0035	<0.00046
5	0.85	0.70
6	>10	>10
7	0.27	0.15
8	0.024	0.004
9	0.081	0.017
10	5.0	0.88
11	0.059	0.0091
13	1.2	0.67

The cytotoxic activities of triptolide **1** and its analogues **2**, **5–11**, and **13** on A549 human lung and HT29 human colon tumor cells are shown in Table 1. Comparison of the activities of compounds **1** and **8** with those of compounds **11** and **10**, respectively, may imply the importance of the stereochemistry of the 14-oxygen functional group: those compounds with 14β-oriented substituents are more cytotoxic than those with 14α-oriented ones. Kupchan et al.[8] suggested that the cytotoxic activity of compound **1** is due to the activation of the 9,11-epoxide group by the hydrogen bonding between the 14β-hydroxyl group hydrogen atom and the 9,11-β-epoxide oxygen atom. However, compounds **8** and **11**, which cannot form the corresponding hydrogen bonding, are still cytotoxic, and compound **9**, with a diepoxide system on the B/C-ring and no 14-oxygen functional group, has almost the same cytotoxic activity as 14-*epi*-triptolide **11**. The epoxide-transposition analogues **6** and **7**, with 7,8-, 9,11-, and 13,14-β-epoxides, and 12-hydroxy group, showed only very low to marginal activity. The present result suggested that the cytotoxic activity of triptolide (**1**) series may not be explained so simply as done by Kupchan et al. The activity seems to owe more to the 3D-alignment of the C-ring substituents, which may easily and inevitably be affected by the introduction of new groups or by changes of the groups attached to ring C or by the changes of C-ring structure itself, which may then affect the cytotoxic activity of the compounds.

In summary, in the present study, epoxide-transposition analogues (**6** and **7**) of triptolide (**1**) and some semisynthetic analogues of triptolide (**1**) were prepared. The results suggested the possible effect of the 3D-alignment of the C-ring substituents on the cytotoxic activity of the compounds of this series.

Acknowledgment

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