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CYTOTOXICITY OF *ENT*-UDOTEATRIAL DIACETATE AND ITS ANALOGUES

Yuting Ge, Kazuhiko Nakatani, and Sachihiko Isoe*

Institute of Organic Chemistry, Faculty of Science, Osaka City University, Sugimoto 3-3-138, Sumiyoshi, Osaka 558, Japan

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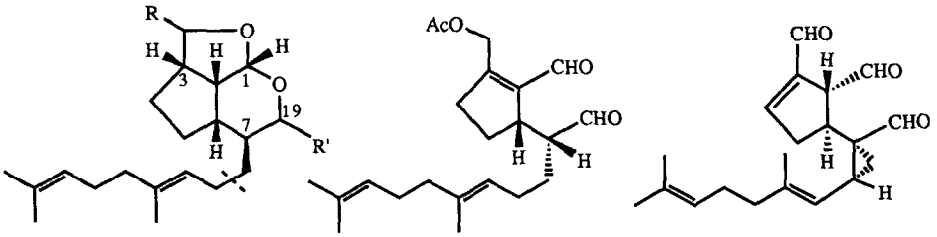
Abstract: *The analogues of ent-udoteatrial hydrate involving homogeranyl side chain synthesized from the key intermediate, the exo-methylene lactone, were found to be cytotoxic against human carcinoma KB and A-549 cells.*

In 1981, Faulkner *et al.* have reported the isolation of udoteatrial (**1**)[1] from the green algae *Udotea flabellum* as the compound that was responsible for the antimicrobial activity of the crude extract against *Staphylococcus aureus* and *Candida albicans*. Since **1** was a complex mixture of its monohydrate form, they could elucidate its structure to have the novel diterpene udoteane skeleton using the diacetates of **1** (**2** and **3**) as shown below. Although the stereochemistry at C_7 was erroneously assigned, Whitesell *et al.* have determined the stereostructure of **1** as (*2S*, 3R*, 6R*, 7R**).[2] This novel carbon framework assumed to be consisted of tricyclic monoterpene portion and geranyl group was later found in the related marine diterpene (-)-petiodial (**4**)[3] and (-)-halimedatrial (**5**)[4] isolated from *Udotea petiolata* and *Halimeda* species, respectively. Although these two compounds showed significant activities against several marine bacteria, inhibition of cell division in fertilized sea urchin eggs, and cytotoxicity to herbivorous damselfish causing death within one hour, the cytotoxicity as well as other biological activities of **1** were not reported. Because of the structural similarity of **1** to **4** and **5** it was considered that **1** might have some biological activities.



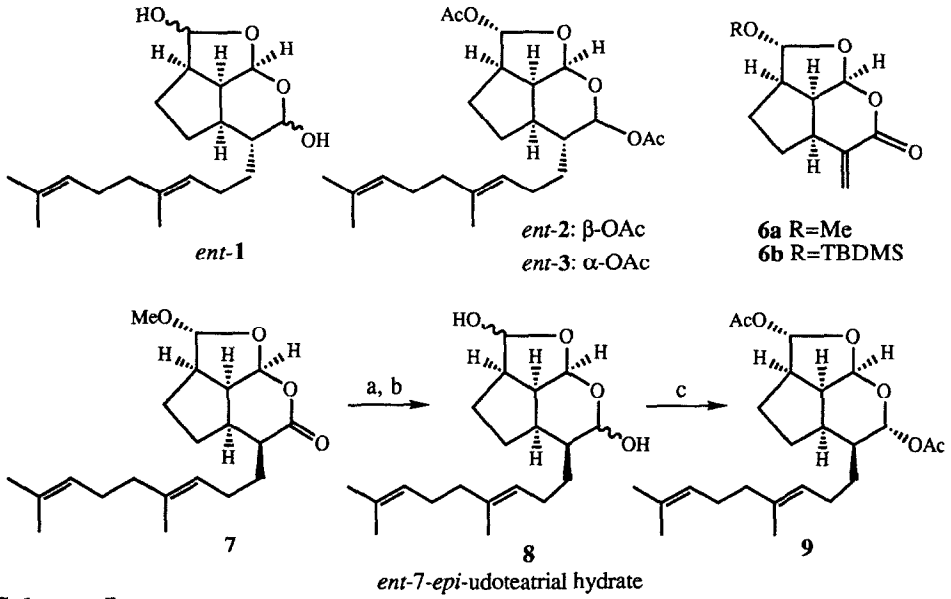
R=R'=OH **1**
udoteatrial hydrate
R=α-OAc, R'=β-OAc **2**
R=α-OAc, R'=α-OAc **3**

4
(-)-petiodial

5
(-)-halimedatrial

We have recently succeeded in the determination of the absolute structure of **1** as (*2R, 3S, 6S, 7S*) by the synthesis of *ent*-**1** followed by its conversion into its diacetates *ent*-**2** and *ent*-**3**.[5] Our synthesis employed the tricyclic exo-methylene lactone (**6**) as a key intermediate was designed to be applicable to obtain analogues involving a variety of side chains instead of geranyl group. We report, herein, the preliminary investigations of the structure and activity relationships of the synthetic analogues of *ent*-udoteatrial hydrate. In these studies, it was found that the diacetates of *ent*-**1** (*ent*-**2**) showed significant *in vitro* cytotoxicity against human carcinoma KB and A-549 cells. Furthermore, the activity was found to be dependent on the structure of side chain as well as the stereochemistry at the carbon bearing the acetoxy group at C_{19}.

We, at first, focused on the stereoisomer of *ent*-**2** and *ent*-**3** at the carbon bearing the homogeranyl side chain. The homogeranyl lactone (**7**)[5] was obtained by introduction of geranyl sulfone to **6a** followed by separation of its stereoisomer at C_7. After reduction of the lactone portion of **7**, acid hydrolysis of the resulting hemiacetal afforded the *ent*-7-*epi*-udoteatrial hydrate (**8**) (**Scheme I**). Acetylation of **8** was found to give the acetate (**9**) as a sole product.

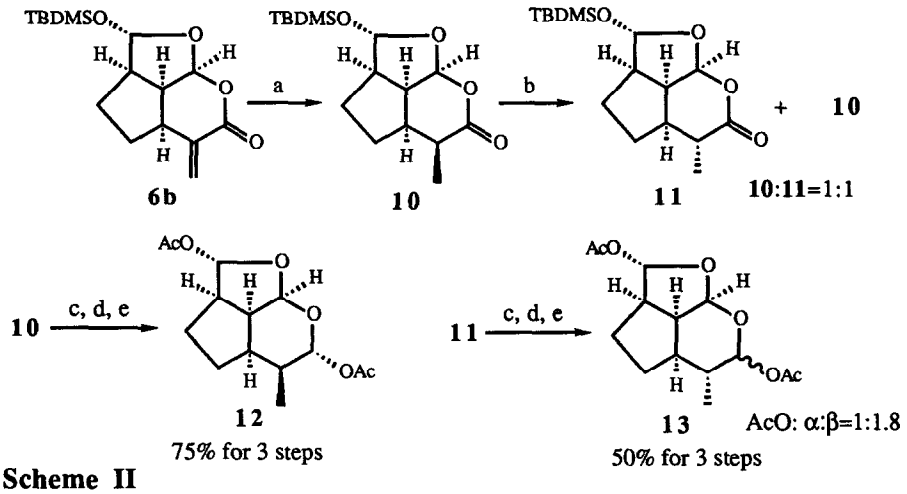


Scheme I

(a) DIBAL, CH_2Cl_2, -78°C, 99% (b) (0.1M) p-TsOH, THF:H_2O:acetone = 4:2:1, rt, 69%
(c) Ac_2O, Pyr, rt, 66%

To examine the effect of side chain on the biological activities, we chose the compound bearing the methyl group as a simple side chain to compare with those involving the homogeranyl group. Thus, hydrogenation of **6b** with Rh/Al_2O_3 stereoselectively afforded the β-methyl derivative (**10**) (**Scheme II**). The α-methyl isomer (**11**) could be obtained by base catalyzed isomerization of **10**. These **10** and **11** were converted into the diacetate (**12**) and (**13**), respectively, by the same reaction sequence mentioned for the preparation of **9** from **7**.

Since the monohydrate form of trialdehyde was not stable enough for biological tests, their diacetates were used instead. With analogues (*ent*-**2**, *ent*-**3**, **9**, **12** and **13**) in hand, we then examined their biological properties. Although the natural udoteatrial hydrate was reported to show antimicrobial activities against *Staphylococcus aureus* and *Candida albicans*, none of those analogues was active against various microogranisms. At this moment it was not clear whether protection of two hemiacetal portions of *ent*-**1** with acetate decrease the activities of natural **1**.



Scheme II

(a) cat. PtO_2, AcOEt, rt, 99% (b) DBU, benzene, reflux, 72 h, 70% for **10** and **11** (c) DIBAL, toluene, -78°C, 1h (d) (0.1M) p-TsOH, THF:H_2O:acetone = 4:2:1, rt (e) Ac_2O, Pyr, rt

Table 1: cytotoxicity of analogues of *ent*-udoteatrial hydrate against human oral epidermoid cacinoma KB and human lung carcinoma A-549

compound No.	IC_{50} (μg/ml)	
	human KB	human A-549
ent-**2**	0.4	0.5
ent-**3**	1.6	1.9
9	3.4	3.9
12	>25.0	>25.0
13	>25.0	>25.0

On the other hand, assay of *in vitro* cytotoxicity of these analogues indicated considerable results. Thus, the compounds involving homogeranyl side chain (*ent*-**2**, *ent*-**3** and **9**) were found to be cytotoxic against KB human oral epidermoid carcinoma and human lung carcinoma A-549 as summarized in the **Table**.[6] *Ent*-2 was most toxic among analogues we examined at the concentration of $4x10^{-1}$ μg/ml. The effect of side chain was apparent that the methyl derivatives **12** and **13** were much less toxic relative to *ent*-**2**, *ent*-**3** and **9**.[7]

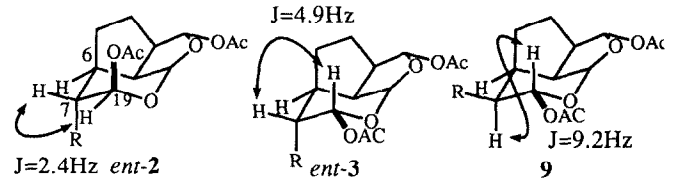
Furthermore, *ent*-**2** involving the acetate with axial orientation[8] at C_{19} exhibited at least 4 fold more enhanced cytotoxicity than those having the equatorial acetates. From stereoelectronic point of view, it was suggested that compound with the better leaving ability of acetoxy group showed stronger cytotoxicity, although the mechanism of the inhibition of cell growth with these compounds was not understand at all.[9]

In conclusion, we have found that the analogues of *ent*-udoteatrial hydrate were cytotoxic against human carcinoma *in vitro*. For the exhibition of cytotoxicity the presence of homogeranyl side chain as well as the stereochemistry of acetoxy group at C_{19} were seemed to be important factors. Our finding reported here may have values for the evaluation of new lead-compounds for the cancer chemotherapy. The question we are facing is that if the diacetates of natural udoteatrial hydrate could show comparable cytotoxicity. To answer this the synthesis of natural enantiomer of **1** is now in progress in our laboratory. These results as well as their biological properties will be reported in due course.

Acknowledgments: We gratefully acknowledge to the Takeda Chemical Industries, LTD. for carrying out the biological assay.

References and Notes

1. Nakatsu, T.; Ravi, B. N.; Faulkner, D. J. *J. Org. Chem.* **1981**, *46*, 2435.
2. Whitesell, J. K.; Fisher, M.; Jardine, P. D. S. *J. Org. Chem.* **1983**, *48*, 1557.
3. Isolation: Fattorousso, E.; Magno, S.; Novellino, E. *Experientia* **1983**, *39*, 1275; Paul, V. J.; Fenical, W. *Tetrahedron* **1984**, *39*, 2913; Synthesis: Isoe, S.; Ge, Y.; Yamamoto, K.; Katsumura, S. *Tetrahedron Lett.* **1988**, *29*, 4591.
4. Isolation: Paul, V. J.; and Fenical, W. *Tetrahedron* **1984**, *40*, 3053; Synthesis: Nagaoka, K.; Miyaoka, H.; Yamada, Y. *Tetrahedron Lett.* **1990**, *31*, 1573.
5. Ge, Y.; Kondo, S.; Odagaki, Y.; Katsumura, S.; Nakatani, K.; and Isoe, S. *Tetrahedron Lett. in press.*
6. Cytotoxicity assay was conducted by using suspensions of human lung carcinoma, A-549 (ATCC CCL-185) in Ham's F12K medium with 10% fetal bovine serum (FBS) and human oral epidermoid cacinoma, KB (ATCC CCL-17) in Eagle's MEM with no-essential amino acids and 10% FBS. These suspensions were distributed in a 96-well microtiter plate, which were cultivated at 37°C in an atmosphere of 5% carbon dioxide, 7% oxygen, and 88% nitrogen. After 24 hours, human recombinant basic FGF (endotherial cell growth factor) was added thereto in the final concentration of 2 ng/ml and DMF solution of a test compound was further added, followed by cultivation for 3 days. After cultivation, growth rate of these cells were measured by MTT method (Cancer Treatment Reports, Vol. 71, page 1141-1149, 1987). IC_{50} value of the test compound was determined from a graph of growth curve of these cells.
7. Increase of cytotoxicity by substitution with longer alkyl chain was sometimes observed. For a recent example, see Herscovici, J.; Bennani-Baiti, M. I.; Montserret, R.; Frayssinet, C.; and Antonakis, K. *BioMed. Chem. Lett.* **1991**, *1*, 721.
8. The coupling constants between C_7-H and C_{19}-H ($J_{H7\text{-}H19}$) observed in ^{1}H-NMR of *ent*-**2**, *ent*-**3** and **9** were 2.4, 4.9 and 9.2 Hz, respectively. Considering those values as well as their stereostructures it was considered that only the acetoxy group at C_{19} of *ent*-**2** occupied the axial position.



9. This observation suggested that the generation of oxonium species by elimination of acetoxy group might concern the exhibition of cytotoxicity of these compounds. Such oxonium species might be an alkylating agent as well known the case of iminium species generated from naphthyridinomycin/saframycin class of antitumor antibiotics.