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IDENTIFICATION AND STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF OSTHOL, A CYTOTOXIC PRINCIPLE FROM *CNIDIUM MONNIERI*

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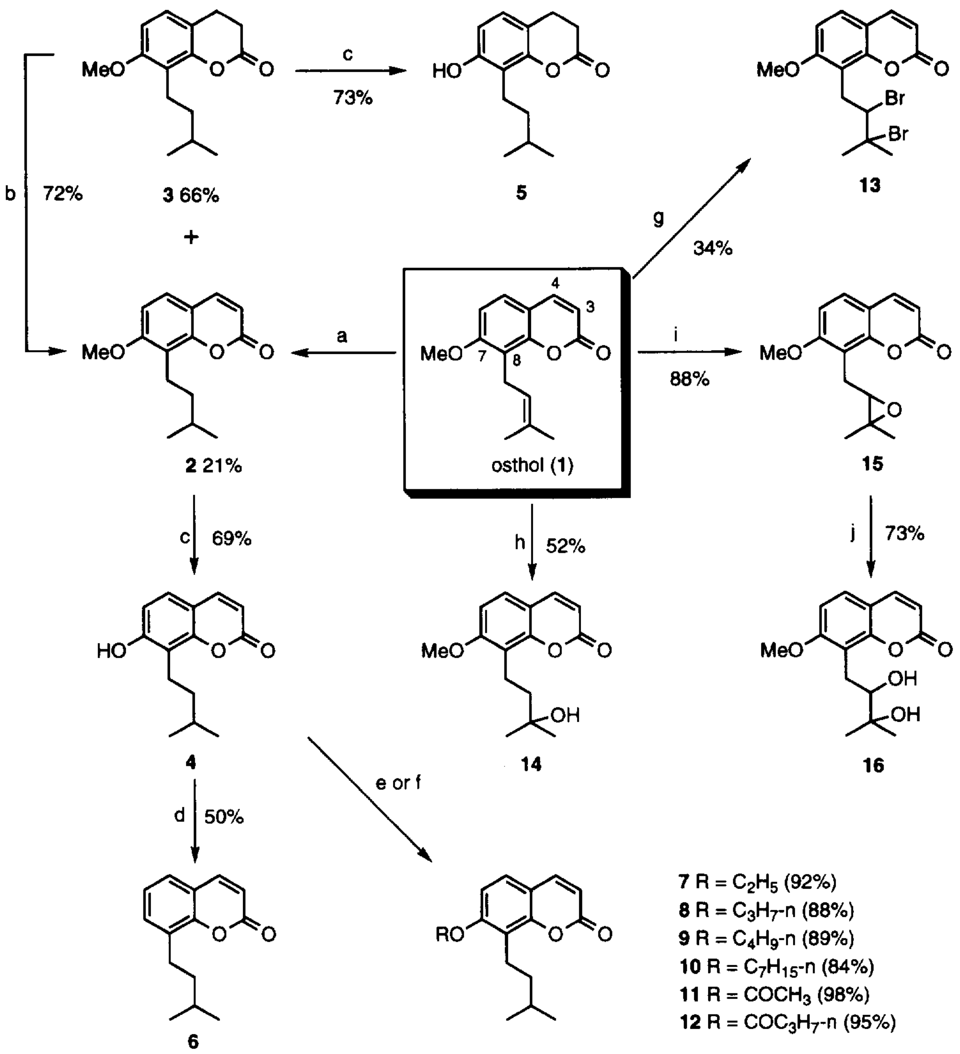
Abstract: Osthol (**1**) was isolated from the fruit of *Cnidium monnieri* as a cytotoxic principle. Its structure–activity relationship study reveals that the 3,4-olefinic bond is essential for its cytotoxic activity, and the prenyl (C_5) unit attached at the 8 position enhances the cytotoxicity. Analogues **7** and **8** that have a longer alkoxy unit at the 7 position showed ten times higher cytotoxicity than **1**. Copyright © 1996 Elsevier Science Ltd

During the course of identifying the cytotoxic substances from higher plants,[1] we found that the methanol extract of the fruit of *Cnidium monnieri* (Umberiferae) showed weak cytotoxicity (IC_{50} = 24 μg/mL) against V-79 Chinese hamster lung cells, and bioassay-guided fractionation/isolation yielded osthol (**1**)[2] as the active substance.[3] The IC_{50} values of **1** against V-79, KB, P-388 and MM46 cells[4] are 14, 12, 3.1 and 8.6 μg/mL, respectively. Although coumarins are a rather popular class of compounds from higher plants, and some of them have been reported to possess cytotoxic/antitumor activity,[2,5] their structure–activity relationship (SAR) is still unclear. Since osthol (**1**) is available from this plant in quantity, chemical modification of this compound was made in order to obtain information about the SAR of **1** and to obtain more potent analogue which may qualify the potential of this lead as an antineoplastic agent.

The modification was made on the 3,4-olefinic bond, the substituent at position 7 and the prenyl group at position 8, which yielded analogues **2**—**16** as shown in Scheme 1.[6] Catalytic hydrogenation of **1** over palladium on carbon gave the dihydro-(**2**) and tetrahydro-(**3**) derivatives in a ratio of 1:3, and the latter was re-dehydrogenated to **2** in 72% yield. The des-*O*-methylation of **2** and **3** using boron tribromide gave phenols **4** and **5**, respectively. The dehydroxylation of **4** occurred through the reduction of the intermediary triflate using 1,1-bis(diphenylphosphino)ferrocene, $Pd(OAc)_2$ and formic acid[7] to afford compound **6**. The *O*-alkylation of **4**

Scheme 1



Reagents (a) H_2, Pd/C, CH_2Cl_2–EtOH, (b) Pd/C, mesitylene, reflux, (c) BBr_3, CH_2Cl_2, –78°C, (d) $(CF_3SO_2)_2O$, Et_3N, CH_2Cl_2, 0°C; 1,1-bis(diphenylphosphino)ferrocene, $Pd(OAc)_2$, HCO_2H, DMF, 60°C, (e) ROH, Ph_3P, diethyl azodicarboxylate, PhMe, room temp., (f) RCOCl, Et_3N, CH_2Cl_2, room temp., (g) Br_2, CH_2Cl_2, 0°C, (h) BF_3•OEt_2, H_2O–CH_2Cl_2, room temp., (i) *m*-CPBA, CH_2Cl_2, 0°C, (j) H_2SO_4, THF–H_2O, room temp.

Table 1. Cytotoxicity of coumarins against V-79 cells

compound	IC_{50} (μM)	compound	IC_{50} (μM)
osthol (**1**)	57	**11**	60
2	58	**12**	58
3	>100	**13**	60
4	40	**14**	>100
5	>100	**15**	>100
6	60	**16**	>100
7	5.8	coumarin	>100
8	5.6	7-hydroxycoumarin	58
9	16	7-methoxycoumarin	>100
10	17		

under Mitsunobu conditions[8] with the corresponding alcohols produced ethers **7**—**10**, and the *O*-acylation with the corresponding acyl chlorides gave esters **11** and **12**. The bromination of **1** yielded dibromide **13**, and the acid catalyzed hydration of **1** gave tertiary alcohol **14**. The epoxidation of **1** using *m*-chloroperbenzoic acid (*m*-CPBA) yielded epoxide **15**, which was subjected to acid hydrolysis affording diol **16**.

The prepared compounds were evaluated using V-79 cells, and the results are summarized in Table 1. The values are given in molar concentration (μM) for comparison. Coumarin, 7-hydroxycoumarin and 7-methoxycoumarin were also evaluated for comparison. The unsaturation of the prenyl group is not essential to the activity (**1** *vs.* **2**). Since compounds **2**, **4** and **6** showed more potent activity than 7-methoxycoumarin, 7-hydroxycoumarin and coumarin, respectively, the C_5 unit attached at the position 8 proved to enhance the activity. Contrary to dibromide **13** retaining activity, the introduction of a hydrophilic moiety (**14**, **15** and **16**) into the prenyl group resulted in a loss in activity. With regard to the substituent at position 7, although the substitution of the methoxy group of **2** by a hydrogen (**6**) did not alter the activity, a hydroxy group (**4**) slightly enhanced the activity. The substitution of the methyl group of the 7-methoxy group by a longer alkyl chain (C_2—C_7) resulted in the enhancement of the activity (**7**—**10**), and the ethoxy (**7**) and the *n*-propoxy (**8**) analogues showed the most potent activity. The substitution by an acyloxy group did not alter the activity (**11** and **12**) in spite of the close similarity in length (**7** *vs.* **11**). Since the dihydrocoumarins **3** and **5** showed no activity, the 3,4-unsaturated bond is essential for such activity.

Since analogues **7** and **8** showed ten times more potent cytotoxicity than **1**, we currently intend to evaluate these analogues using other cell lines and prepare more potent analogues through these lines of modification.

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