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Cytotoxicity of Cucurbitacins *in vitro*

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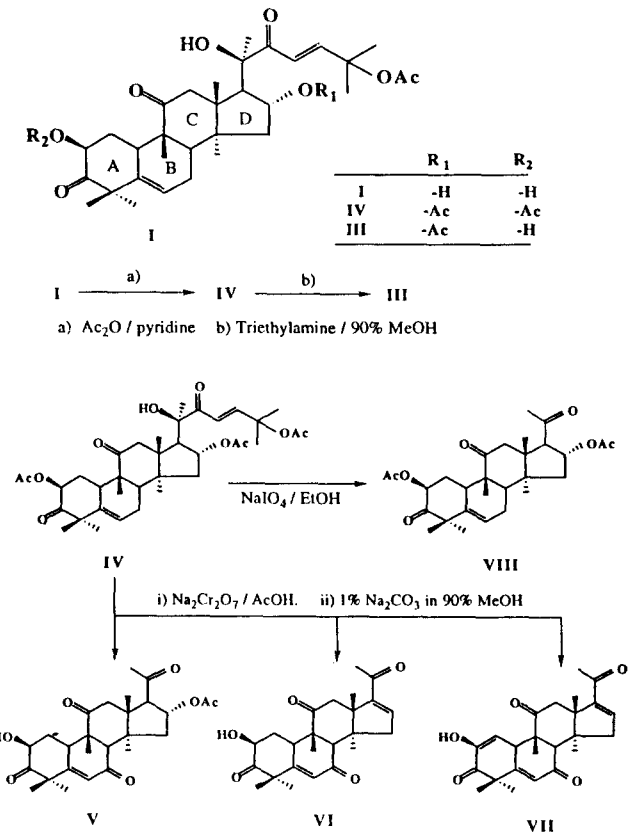
The cucurbitacins are a group of highly oxygenated tetracyclic triterpenes having an unique 19(10→9β) abeo-10α-lanostane skeleton, and distributed in several species of Cucurbitaceae, Cruciferae and Euphorbiaceae, etc. (Shrotria, 1976). The common characteristics of these compounds are an α-hydroxy ketone in ring A and two carbonyls at C-11 and C-22, in some case, the latter being conjugated to a $\Delta^{23,24}$ double bond as depicted in Scheme 1. Due to their cytotoxicity especially against various tumor cell lines with an unusually high potency, they have been investigated extensively during last two decades by many reseach groups (Ryu *et al.*, 1994a, *references are therein*). Recently, we have reported (Ryu *et al.*, 1994a) the isolation of eight kinds of cucurbitacins including cucurbitacin B and D from the root of *Trichosanthes kirilowii* (Cucurbitaceae). On the scrutiny of their cytotoxicity, we had found that the activity of cucurbitacin B **I**, the most potent one among isolates from *Trichosanthes kirilowii*, was remarkably reduced by the saturation of double bond (*i.e.*, 23,24-dihydrocucurbitacin B **II**), which strongly suggested that the C-23,24 olefinic feature of cucurbitacin presumably including the adjacent 22-carbonyl, so actually α,β-unsaturated ketone, might exert a cytotoxic activity. And it was supported by the postulation that the cytotoxicity of cucurcitacins against cultured tumor cells might be due to their alkylating activity upon the biological macromolecule which played an important physiological role on cell growth, e.g., enzymes or receptors usually bearing thionyl residues.(Lien *et al.*, 1984., Kupchan *et al.*, 1970). Therefore, we tried to investigate the mode of action of **I** *in vitro* on the aspect of the interaction between



Scheme 1. The preparation of some cucurbitacin analogues.

cucurbitacins and the cellular biomolecules which consequently caused an cytotoxic effect. Thus, the cytotoxicity of some derivatives of **I** was examined and their cytotoxic potencies were compared with that of **I**. All six kinds of derivatives were prepared for this purpose by the following Scheme 1, so as to be converted to compounds lacking in the side chain moiety of **I**(**V**~**VIII**) or were blocked hydroxy substitutions by the simple esterification (**III** and **IV**).

The peracetate (**IV**) and partial acetate (**III**, fabacein) of **I**, which were prepared respectively by the method of Kupchan *et al.* (1972), demonstrated relatively low cytotoxicity toward each tested tumor cells in constrast with the exceptionally high potency of **I** (Table I). According to just this result, it seemed that the α,β-unsaturated ketone (enone) moiety in the side chain of **I** might apparently not be requisite for the cytotoxicy because **III** and **IV** were still retained the enone structure. However, none of other derivatives (**V**~**VIII**), which were prepared by the oxidation with $NaIO_4$ or $Na_2Cr_2O_7$ (Kock *et al.*, 1963, Shlegel *et al.*, 1961), thus resulted in the elimination of the side chain (C-22~C-27), but in the retention of the tetracyclic backbone of **I**, were exhibited any significant activity toward all

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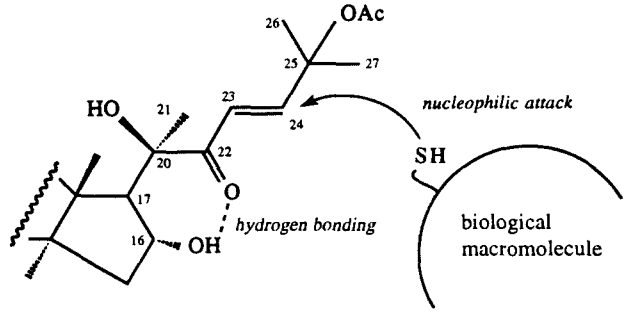
Table I. Anticancer activity of cucurbitane triterpenes from *Trichosanthes kirilowii in vitro*

Compound	ED_{50} (μg/ml)				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
I	0.04×10^{-3}	0.06×10^{-3}	0.01×10^{-3}	0.07×10^{-3}	0.08×10^{-3}
II	0.7	3.7	0.2	2.2	1.8
III	>100	4.4	>100	>100	>100
IV	3.1	0.2	0.5	5.3	4.6
V	>100	>100	>100	>100	>100
VI	>100	>100	>100	>100	>100
VII	>100	>100	>100	>100	>100
VIII	>100	>100	>100	>100	>100

ED_{50} value of compound against each cancer cell line, which was defined as a concentration (μg/ml) that caused 50% inhibition of cell growth *in vitro*.



Scheme 2. The postulated mode of action in the cytotoxicity of cucurbitacins

tested cell lines at the concentration below 100 μg/ml (Table I, Ryu *et al.*, 1994b). These results suggested more clearly that the cytotoxic activity of cucurbitacins were predominantly due to the partial structure of its side chain which comprised of the enone moiety rather than the tetracyclic skeleton itself. These also supported the postulation that the profound lessening of the cytotoxicity in **III** and **IV** could be accompanied by the reduction of elctrophilicity of enone moiety, which was resulted from the acetylation of C-16 hydroxyl group that was originally linked with C-22 ketone by a hydrogen bonding interaction in case of **I** (Kupchan *et al.*, 1972).

In conlusion, it seemed that the C-16 hydroxy group in ring D of cucurbitacins could activate the electophilicity of enone in side chain by way of a strong hydrogen bonding with C-22 ketone as depicted in Scheme 2, thus facilitate the nucleophilic attack by the cellular macromolecule, which consequently caused the cytotoxic activity of cucurbitacins against the tumor cells, at least in *in vitro* experiment.

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