# Synthesis and Cytotoxic Activity of a Series of Diacetylenic Compounds Related to Falcarindiol[1)]

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**The synthesis of a series of diacetylenic compounds related to the natural product falcarindiol has been carried out. Unsymmetrical diacetylenes were prepared by a modification of the Cadiot-Chodkiewicz coupling reaction, while a Glaser coupling was used to prepare symmetrical diacetylenes. These compounds have been tested for *in vitro* cytotoxic activity against Hep-G2, and H-4-II-E cell lines. Diacetylenes with additional unsaturation at C-1, 2, appended with hydroxyl groups at C-3 and C-8, or with long hydrophobic chains, exhibited $IC_{50}$ values in the micromolar range.**

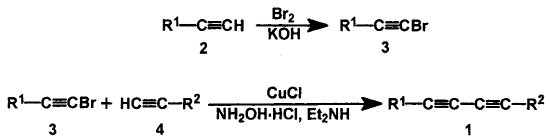
**Key words** antineoplastic; cytotoxin; diyne; natural product

Diacetylenic phytochemicals, most notably from members of the Araliaceae,[2)] have been shown to exhibit *in vitro* cytotoxic activity against various tumor cell lines.[3—6)] Even though these materials show cytotoxic activity against malignant cells, they tend to be less toxic to normal cells.[3,5)] While this class of compounds represents potential new antineoplastic agents, studies on them have been hampered by the difficulty in isolating them, their inherent instability, and low yields in their syntheses. In this work, we report the preparation of a series of diacetylenic compounds related to the above natural products and an examination of their *in vitro* cytotoxic activities against Hep-G2 (human hepatocellular carcinoma) and H-4-II-E (rat hepatoma) cell lines.

Unsymmetrical diacetylenes **1a**—**f** were prepared by a modification of the Cadiot-Chodkiewicz coupling reaction of an alkynyl bromide with a terminal alkyne (Chart 1).[7—9)] A competing side reaction in this coupling is the formation of the symmetrical diacetylene. In order to minimize this competing reaction, both the free cuprous ion concentration and the alkynyl bromide concentration were kept low. The alkynyl bromides, **3**, were prepared from the corresponding acetylene by reaction with hypobromite (95—100% yield). Propargyl alcohols were best prepared by reaction of lithium acetylide ethylenediamine complex with the appropriate aldehyde in a THF/DMSO solvent mixture.

A number of symmetrical diacetylenes were prepared by coupling of the corresponding terminal acetylenes using the Zal'kind modification of the Glaser coupling (Chart 3).[10)] Thus, 2,7-dimethyl-3,5-octanediyn-2,7-diol (**5a**) and 2,4-hexanediyn-1,6-diol (**5b**) were synthesized by this technique. Other symmetrical couplings using this method failed, apparently due to air sensitivity of the final products. Alternatively, symmetrical coupling has been achieved using the corresponding alkynyl bromides (Chart 3). Experimental details for these syntheses are available from the author of correspondence.

*In vitro* cytotoxic activities have been carried out on the diacetylenes using Hep-G2,[11)] and H-4-II-E[12)] cell lines. The MTS spectrophotometric technique[13)] was utilized to determine cell viability. $IC_{50}$ values were determined using the Reed-Muench method.[14)] The results are summarized in Table 1. It has already been reported that the diyne moiety is primarily responsible for the biological activity of these materials.[15,16)] The results of this present study suggest that di-




| | $R^1$ | $R^2$ | % yield |
|---|---|---|---|
| a: | $CH_2$=CH-CH(OH)- | -$(CH_2)_9$-$CH_3$ | 24 |
| b: | $CH_2$=CH-CH(OH)- | -CH(OH)-$(CH_2)_8$-$CH_3$ | 77 |
| c: | $CH_2$=CH-CH(OH)- | -CH(OH)-CH=C($CH_3$)-$(CH_2)_2$-CH=C($CH_3$)$_2$ | 18 |
| d: | $CH_3CH_2$-CH(OH)- | -$(CH_2)_9$-$CH_3$ | 25 |
| e: | $HOCH_2$- | -$(CH_2)_9$-$CH_3$ | 25 |
| f: | $HOCH_2CH_2$- | -$(CH_2)_9$-$CH_3$ | 8 |

Chart 1. Preparation of Unsymmetrical Diacetylenes

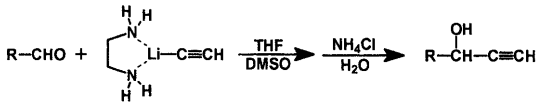
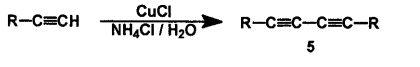
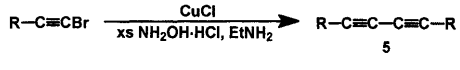



Chart 2. Preparation of Propargylic Alcohols




a: R = $(CH_3)_2$C(OH)- 78% yield

b: R = $HOCH_2$- 23% yield




c: R = $CH_2$=CH-CH(OH)- 65% yield

d: R = $CH_3CH_2$CH(OH)- 84% yield

e: R = $CH_3(CH_2)_8$CH(OH)- 5% yield

Chart 3. Preparation of Symmetrical Diacetylenes

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Table 1. Cytotoxic Activities of Diacetylenes

| Diacetylene | Cytotoxicity ($IC_{50}$, $\mu$M) | |
|---|---|---|
| | Hep-G2 | H-4-II-E |
| **1a** | 32.5±1.4 | 4.15±0.01 |
| **1b** | 0.95±0.07 | 1.96±0.06 |
| **1c** | 4.34±0.15 | 11.6±0.2 |
| **1d** | 309±35 | — |
| **1e** | 59.3±3.9 | 73.1±3.1 |
| **1f** | 42.8±3.6 | 27.6±1.0 |
| **1g**[a)] | 35.8±0.3 | 149.0±0.4 |
| **5a** | >750 | — |
| **5b** | 806±26 | 181±13 |
| **5c** | 10.3±1.0 | 41.5±2.0 |
| **5d** | 247±23 | — |
| **5e** | 12.8±1.2 | 40.2±1.4 |
| **5f** | 956±146 | — |
| Tingenone[b)] | 1.91±0.09 | 2.74±0.40 |

*a*) Ref. 5. *b*) Tingenone was used as a positive control (ref. 24).

acetylenes in which $R^1$ is an allyllic alcohol group and $R^2$ is a long hydrophobic chain are essential for high cytotoxicity. Thus, **1a**, **1b**, and **1c** are the most active in this study. The importance of the unsaturation at C-1, 2 is reflected in the relative cytotoxicities of **1a** and **1d**. Bernart *et al.*[6)] have reported a decrease in cytotoxicity with saturation at C-1, 2. Symmetrical diacetylenes with two allylic alcohol moieties (**5c**) or two long hydrophobic chains (**5e**) show intermediate cytotoxicities. Fujimoto and co-workers[17)] had observed that shorter chain lengths decrease the cytotoxic activity in diacetylenic compounds, and our results confirm this observation. Thus, cytotoxicities for symmetrical diacetylenes drops off dramatically as chain length shortens; **5b**<**5d**<**5e**. Unsaturation in the hydrophobic ($R^2$) chain seems to attenuate cytotoxic activity; dehydrofalcarindiol (the natural product, **1g**) is less toxic than **1b**. Park *et al.*[18)] and Bernart *et al.*[6)] have reported that unsaturation at C-16, 17 decreases cytotoxicity.

To our knowledge, the mechanism of cytotoxic activity of this class of compounds is not well understood. Because the compounds are lipophilic, they would be expected to associate strongly with membrane lipids,[19)] potentially leading to disorganization of membrane systems.[2)] Phototoxicity studies of polyacetylenes suggest that these materials may act as photosensitizers in lipid peroxidation, and this may be responsible for the biological activity.[20,21)] Antioxidant activity of polyacetylenes[22,23)] indicate free radical intermediates of these compounds may also be important. Further investigations into the mechanism of activity of these materials are currently underway in our laboratories.

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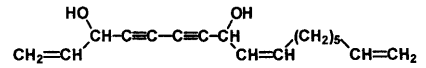


**1g**

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