Short Communication

Synthesis of Radioactive Aphidicolin,
a Potent Inhibitor of DNA Replication in Eukaryotic CellsSusumu IKEGAMI, Keiju OKANO and
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Much of our knowledge on cellular events governed by DNA replication has been obtained by experiments using inhibitors of cellular DNA synthesis. However, conclusions derived from these experiments are hardly considered as any more than suggestive or corroborative of data obtained by other means because the inhibitors so far used have undesirable effects on cellular functions aside from blocking DNA synthesis or inhibit DNA synthesis in indirect ways such as preventing deoxyribonucleoside triphosphate biosynthesis, thereby depriving DNA polymerases of necessary substrates.¹⁾

We have searched for chemicals directly and selectively blocking DNA synthesis in eukaryotic cells and found that aphidicolin, which is produced by several fungi,^{2~6)} is one such compound.^{7,8)} Aphidicolin prevents mitotic division but does not affect meiotic maturational divisions. Aphidicolin inhibits cellular DNA synthesis but does not inhibit protein and RNA synthesis. Aphidicolin inhibits the activity of DNA polymerase α without affecting the activity of DNA polymerases β and γ ,^{7,9)} suggesting that DNA polymerase α is the enzyme responsible for cellular DNA replication. The unusual and remarkable activity of aphidicolin on cellular and enzymatic functions as revealed by these experiments prompted us to report on the preparation of ¹⁴C- and ³H-labelled aphidicolin in order to study further the mode of inhibitory action of

the chemical on the complex process of DNA replication.

APHIDICOLIN

Aphidicolin was obtained from the culture filtrate of fungus *Harziella entomophila* by the procedure described elsewhere.^{6,8)} Aphidicolin was converted into 3 α ,18-isopropylidenedioxy-17-noraphidicol-16-one (**I**) in accordance with the procedure described by Dalziel *et al.*³⁾ and used as the starting material for the synthesis of ¹⁴C- and ³H-labelled aphidicolin.

A stirred suspension of [methyl-¹⁴C]methyltriphenylphosphonium iodide (197 mg, 1.7×10^6 cpm/mg) in tetrahydrofuran (3 ml) was treated with 15% *n*-butyllithium in *n*-hexane (0.3 ml) for 5 min at room temperature. **I** (20 mg) in tetrahydrofuran (4 ml) was added, and the stirred mixture was left for 20 min. The mixture was boiled under reflux for 3.5 hr, then cooled, diluted with water, and extracted with diethylether several times. The extracts were washed with water and dried over sodium sulfate. Evaporation of the solvent gave an oily solid. The product was applied to a Kieselgel HF₂₅₄ plate (20 by 20 cm), which was developed with ethyl acetate-carbon tetrachloride (15:85, v/v). The material of R_f 0.66 was recrystallized from methanol to give [17-¹⁴C]3 α , 18-isopropylidenedioxy-aphidicol-16-ene (**II**) (8.3 mg) as needles, mp 118~119°C, 2.0×10^6 cpm/mg.

Osmium oxide (15 mg) was added to a solution of [17-¹⁴C] **II** (7 mg) in pyridine (3 ml). After 3 days at room temperature, sodium hydrogen sulfite (0.36 g) in water (6 ml) and pyridine (4 ml) was added, and the mixture was stirred for 1 hr, and then extracted with chloroform. The combined extracts were

washed with water, dried and evaporated *in vacuo*. The product was applied to a Kieselgel HF₂₅₄ plate (10 by 20 cm) which was developed with ethyl acetate–benzene–methanol (50: 50: 1, v/v). The material (2.9 mg, 3.8×10^6 cpm/mg) of *R_f* 0.47 dissolved in methanol (2 ml) containing 2 N hydrochloric acid (0.1 ml) was left at room temperature for 2 days. Excess of solid sodium hydrogen carbonate was added, and the mixture was stirred for 1 hr, and then filtered. Removal of the solvent yielded a solid which was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate four times. The combined ethyl acetate extracts were dried and evaporated *in vacuo* to give crude [17-¹⁴C]-aphidicolin (2.9 mg) as an oily solid. The crude [17-¹⁴C]aphidicolin was treated with toluene-*p*-sulfonic acid and acetone in a conventional manner. The bisacetone formed was analyzed by gas liquid chromatography with a Hitachi K-53 instrument using a column (0.3 by 200 cm) of 2% Silicone OV-1 on Chromosorb W. The retention time of the major peak of the sample was 24.5 min (the column temperature, 210°C; nitrogen flow rate, 35 ml/min), which was identical to that of authentic 3 α ,18;16,17-bisopropylidenedioxy-aphidicolane.³⁾ The minor peak at 26.6 min is ascribable to 3 α ,18;16,17-bisopropylidenedioxy-16-epi-aphidicolane.³⁾

The crude [17-¹⁴C]aphidicolin (2.8 mg) was mixed with authentic aphidicolin (10 mg), and they were recrystallized four times from ethyl acetate–methanol to give needles (6.5 mg), mp 235~236°C (lit. *cit.*,³⁾ 227~233°C), 3×10^6 cpm/mg. The specific radioactivity of the sample was unchanged after eight cycles of recrystallization.

[17-³H]Aphidicolin was prepared in an analogous way. In brief, 3 α ,18-isopropylidenedioxy-17-methoxy-aphidicol-16-ene (III), which was prepared by Wittig reaction of triphenylphosphine-methoxymethylene with I, was treated with osmium oxide to give 3 α ,18-isopropylidenedioxy-aphidicol-17-al (IV) together with a C-16 epimer of IV. Silica gel column chromatography with benzene–ethyl

- (I) $R_1, R_2 = 0$
 (II) $R_1, R_2 = \text{CH}_2$
 (III) $R_1, R_2 = \text{CHOCH}_3$
 (IV) $R_1 = \text{OH}, R_2 = \text{CHO}$

acetate (100: 2, v/v) separated the fast-moving IV from the slow-moving epimer. Reduction of IV (2.9 mg) by [³H]lithium aluminum hydride (5 mCi, 6.6 mg), followed by 2 N hydrochloric acid treatment and recrystallization from ethyl acetate, afforded [³H]aphidicolin (1.8 mg), mp 235°C, 7×10^7 cpm/mg. The details of the experimental procedure will be described elsewhere.

The use of specifically labelled samples of aphidicolin as tracers provides a means for the investigation of many problems that have been difficult to explore. For instance, there is a possibility that aphidicolin is converted into a more active form in a target cell. An approach to the isolation and identification of such metabolite is possible by applying radio-labelled aphidicolin to the cell and determining the radioactivity and biological activity of each separated metabolite.

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