peaks, one with a K_{av} of 2.67 (840–960 ml) and the other with a K_{av} of 3.12 (1000–1100 ml). The pooled fraction with the K_{av} of 2.67 or 3.12 was adjusted to pH 2.5 and stored in a dark bottle at 5°C. The gel filtration was repeated until all of the solution of the C18 fraction had been resolved.

For the recovery of yellow components, the fraction with a K_{av} of 2.67 or 3.12 was separately pumped through a column of C18 (2.5 × 22 cm). The column was washed with 400 ml deionized water, followed by elution with a 400 ml methanol gradient from 20% to 80% methanol, collecting 7 ml/tube/5 min. The absorption at 255 mμ was read manually. Both materials were eluted as a single peak between 32% and 42% methanol with the peak at 36% methanol. The solvent was removed by lyophilization to yield a yellow crystalline powder. The yields of the materials with the K_{av} of 2.67 and 3.12 were 16.8 mg and 12.2 mg, respectively, representing recoveries of 0.11% and 0.07%, respectively, of the C18 fraction. The yellow component with the K_{av} of 2.67 was readily soluble in water, whereas the yellow component with the K_{av} of 3.12 was only sparingly soluble in water. Alkali greatly increased the solubility of the latter component.

Identification of active yellow components. The component with the K_{av} value of 3.12 gave analytical results as follows: m.p., 275–281°C with decomposition; mass spectrum revealing m/e 376, 374, 360, 315, 285, 256, 242 (base), 213, 171, 152 and 43; absorption spectrum with characteristic maxima at 445, 371, 266 and 220 mμ; infrared spectrum with characteristic maxima at 3500, 3400, 3200, 2950, 1740 and 1650 cm^{-1}. These data matched identically those of B_2. Analytical data of authentic B_2 obtained from Sigma confirmed that the yellow component with the K_{av} of 3.12 was indeed B_2. The authentic B_2 also had a K_{av} value of 3.12 on Bio-Gel P-2.

The active yellow component with the K_{av} value of 2.67 had an identical absorption spectrum to that of B_2; it is very likely a B_2 derivative. FMN is only detectable in a much smaller quantity relative to B_2 in the urine, according to Gatautis and Naito (11). Therefore, it cannot be FMN. Besides, FMN had a totally different K_{av} value on Bio-Gel P-2, which was 0.77. A definitive identification of the yellow component with the K_{av} of 2.67, however, remains to be established.

Anticancer effect of B_2 and its derivative. Both B_2 and the yellow component (possible the derivative of B_2) with the K_{av} value of 2.67 were equally effective in the inhibition of colony formation of HBL-100 cells. At 0.11 mg/ml, both attained 100% inhibition. FMN was as active as B_2. The amounts of B_2 and its derivative recoverable from A2 and A5 were usually below 0.2%. Consequently, the contribution of anticancer activity by these materials was below 1.1%. Therefore, B_2 and its derivative are very minor active components of Antineoplaston formulations. B_2 was, however, the most inhibitory component among the active components of Antineoplaston formulations tested on human promyelocytic leukaemia HL-60 cells. The growth of these cells was significantly inhi-

Table I *Inhibitory effect of B_2 on the growth of HL-60 cells.*[a]

Treatment	Duration	Viable cells/ml	NBT positive cells/ml
None	48 h	6.3×10^5	0.25×10^5
B_2 (5 μg/ml)	48 h	3.9×10^5	0.47×10^5
B_2 (25 μg/ml)	48 h	0.19×10^5	–
None	72 h	10.0×10^5	0.46×10^5
B_2 (5 μg/ml)	72 h	6.1×10^5	0.57×10^5
B_2 (25 μg/ml)	72 h	0.09×10^5	–

[a] At time zero, a cell suspension of 3×10^5 HL-60 cells/ml was divided into six flasks, each with 10 ml suspension. B_2 was added to make 5 μg/ml to two flasks, and 25 μg/ml to the other two flasks. These were then divided into two groups; one was incubated for 48 h and the other was incubated for 72 h. At the end of each time period, viable cells were counted and NBT positive cells were assayed as previously described (10).