

## Materials and methods

**Materials.** Antineoplaston A2, A3 and A5 were prepared in the Biochemistry Pilot Plant of Burzynski Research Institute, Inc., as previously described (7). L-[Methyl-<sup>3</sup>H]methionine was obtained from Amersham/Searle Corporation. Octadecasilysilica (Porasil C18) (50–80 μ) and Prep PAK-500/C18 cartridge were obtained from Waters Associates. Bio-Gel (polyacrylamide) P-2, 200–400 mesh, was obtained from Bio-Rad Laboratories. B<sub>2</sub> and FMN were obtained from Sigma.

**Preparation of active yellow components from urine by C18 fractionation.** Urine was freshly collected, immediately adjusted to pH 2.5, and stored in a dark bottle at 5°C at least overnight. Precipitates were removed by filtration. The clear supernatant was then processed through a Prep PAK-500/C18 cartridge by means of a Waters Prep LC/System 500A for the fractionation of C18 retentive materials. One litre of urine, which was equivalent to two volumes of C18, was pumped through the cartridge, and the C18 cartridge was washed with 1 l 0.1% trifluoroacetic acid and 1 l deionized water. C18 retained materials were successively eluted with 300 ml each of 15%, 40% and 80% methanol. The C18 cartridge was then washed with 2 l deionized water to be ready for the next batch of urine. Ten litres of urine were processed as described above. Active yellow components were found predominantly in the fraction eluted with 40% methanol. The pooled 40% methanol fraction was adjusted to pH 7, and the solvent was removed by lyophilization.

**Purification of active yellow components by gel filtration on Bio-Gel P-2.** The dried materials obtained as described above were dissolved in water to make a solution of approximately

250 mg/ml. Insoluble materials were removed by centrifugation. The supernatant (5 ml) was applied to a column of Bio-Gel P-2 (2.5 × 96 cm) and the elution was carried out with deionized water, collecting 15 ml/tube/20 min. A 50 μl aliquot from each tube was diluted with water to 1 ml for the manual determination of absorption at 255 mμ. Active yellow components were eluted between 840 and 1100 ml as two absorption peaks with  $K_{av}$  values of 2.67 and 3.12, respectively.

**Determination of melting points.** Melting points were determined by a Fischer-John apparatus.

**Determination of absorption spectra.** The sample was dissolved in water to make 12.5 μg/ml for the determination of absorption spectra by a Perkin-Elmer Lambda-3 UV/VIS spectrophotometer equipped with an automatic recorder.

**Determination of infrared absorption spectra.** A small amount of sample, approximately 1 mg, was mixed with a drop of Nujol mineral oil for the determination of infrared absorption spectra by a Perkin-Elmer infrared spectrophotometer.

**Determination of mass spectra.** Mass spectra were obtained on a Finnigan 4000 gas chromatograph-mass spectrometer with a Finnigan INCOS computer data system. Spectra were matched by computer with those in the National Bureau of Standards spectral library.

**Assessment of anticancer activity.** Anticancer activity was assessed by the inhibition of colony formation of cultured human breast carcinoma cell line HBL-100, as previously described (8), or by the inhibition of MAT<sup>LT</sup> purified from human promyelocytic leukaemia HL-60 cells, as described in a separate paper (9). The inhibitory effect of the active yellow components on HL-60 cells and the assay of NBT positive cells were conducted as described in a separate paper (10).