ISOLATION AND INHIBITORY EFFECTS ON KB CELL CULTURES OF 3'-DEOXYADENOSINE FROM ASPERGILLUS NIDULANS (EIDAM) WINT.*

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The fermentation broth of Aspergillus nidulans was found to have an inhibitory effect on KB cell cultures. Using this system as a biological test, an active component of the broth was isolated as a crystalline compound having the composition $C_{10}H_{13}N_5O_3$.

The analytical composition and ultraviolet absorption spectrum of this metabolite was suggestive of a deoxyadenosine. A comparison of the IR and NMR spectra of the unknown and 2'-deoxyadenosine showed them to be different compounds. The NMR spectrum of the unknown compound showed a two proton multiplet with a center of gravity at 160 c.p.s. relative to tetramethylsilane as external reference (60 mc.) and precluded by its position, the unknown compound from having two protons on C.4[1] or adjacent (α) to the ring oxygen atom. Rather, it suggested that the two protons in question must be located on C.3[1] or β to the ring oxygen atom or that the unknown compound was 3'-deoxyadenosine.

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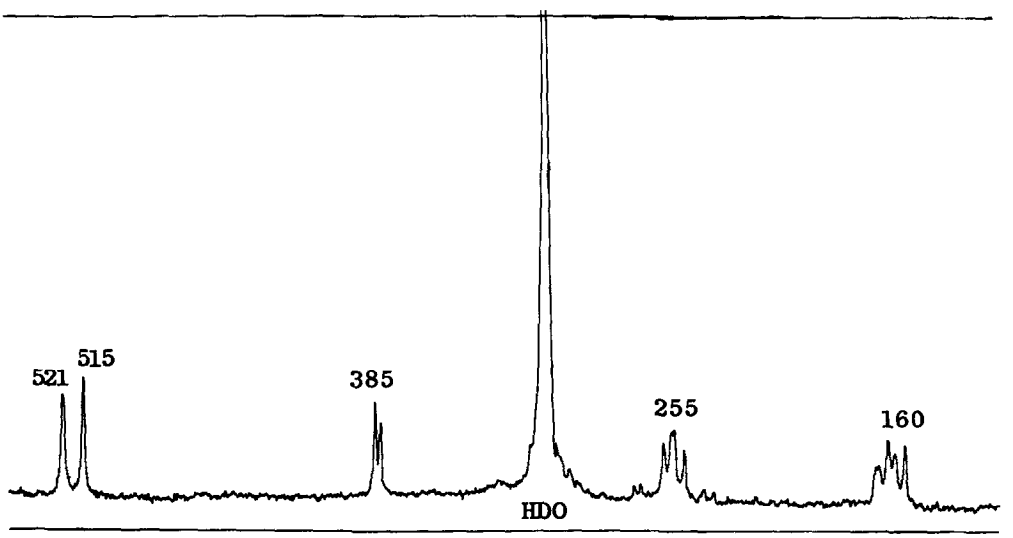
The above conclusion was shown to be correct when the complete IR and NMR spectra of synthetic 3'-deoxyadenosine (Lee, et al. 1961), kindly furnished by Dr. L. Goodman of the Stanford Research Institute, were compared with the spectra of the unknown compound. Both the IR and NMR spectra of the two compounds are identical.

3'-Deoxyadenosine was tested against KB cells as described previously (Gitterman, et al., 1963). The cytotoxic end-point was 10-25 µg/ml; the lethal end-point was 50-100 µg/ml.



Nuclear Magnetic Resonance of 3'-Deoxyadenosine (isolated from Aspergillus nidulans) in D_2O (60 mc.) using a Varian Associates Model 4300 B Spectrometer.

Isolation of 3'-Deoxyadenosine

Spores from a slant culture of Aspergillus nidulans were shaken and scraped free in 10 ml of salts solution. Three ml of this suspension were added to 50 ml of medium in 250 ml Erlenmeyer flasks which were incubated for 7 days at 28°C on a rotary shaker moving at 220 rpm. (Dulaney and Gray, 1962). The contents of each flask were poured into 400 ml of

medium in 2 liter baffled Erlenmeyer flasks which were incubated another 7 days at 28°C on a rotary shaker moving at 120 rpm. The medium used in both the inoculum and production stages contained 40 g dextrose, 10 g corn steep liquor, and 20 g hydrolyzed lactalbumin per liter of distilled water. The pH was adjusted to 6.9 with 6N NaOH prior to autoclaving.

Three liters of filtered fermentation broth was lyophilized to yield 34 grams of solids. These solids were triturated with two 250-ml portions of methanol and the combined methanol solution was evaporated to dryness in vacuo. The residue was tritrated with 40 ml of methanol and the methanol solution chromatographed on 120 g of acid-washed alumina. The elution of 3'-deoxyadenosine from the column with methanol was followed by measuring the intensity of the ultraviolet absorption at 260 mμ. The rich fraction was evaporated to dryness in vacuo to yield 327 mg of solids. Three recrystallizations from water gave 3'-deoxyadenosine; m.p. 230-231° (micro-block). Anal. calcd. for $C_{10}H_{13}N_5O_3$ (251.24): C, 47.8; H, 5.2; N, 27.9. Found: C, 47.2; H, 5.3; N, 27.9. $[\alpha]_D^{25}$ -35 (C, 9.425 in H_2O), (Carl Zeiss Photoelectric Precision Polarimeter 0.005°). The ultraviolet-absorption spectrum of a pH 4 solution showed a maximum at 259 mμ. (ϵ 13,100). The spectrum of a pH 11 solution showed a maximum at 260 mμ. (ϵ13,700).

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REFERENCES

Dulaney, E. L., and Gray, R. A., Mycologica 54, 476 (1962).

Gitterman, C. O., Dulaney, E. L., Kaczka, E. A., Campbell, G. E., Hendlin, D., and Woodruff, H. B., In Press (1963).

Lee, W. W., Benitz, A., Anderson, C. D., Goodman, L., and Baker, B. R., J. Am. Chem. Soc., 83, 1906 (1961).