SC 7058

Isolation and identification of 3'-amino-3'-deoxyadenosine from *Cordyceps militaris*

Homocitrullylaminoadenosine was recently isolated in this laboratory from the mold *Cordyceps militaris*[1]. We now have isolated the nucleoside component of homocitrullylaminoadenosine (3'-amino-3'-deoxyadenosine, Fig. 1) from the same organism. The nucleoside was extracted from mycelia grown 30 days in static culture in media composed of 0.5 % enzymically hydrolyzed casein and 1 % glucose. The mycelia were blotted dry and homogenized in a Waring blendor with 70 % ethanol (1 ml 70 % ethanol per g wet wt. of mycelia). The homogenate was centrifuged at 23 500 × *g* for 15 min and the resulting precipitate re-extracted in the same manner with a volume of

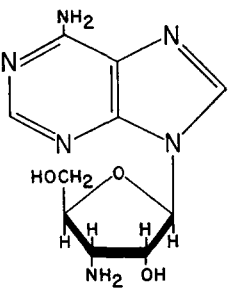

Fig. 1. 3'-Amino-3'-deoxyadenosine.

70 % ethanol equal to one-half that taken for the first extraction. The supernatant fluids from both extractions were combined and taken to dryness on a rotary evaporator under vacuum at a bath temperature not exceeding 45°. The tar-like residue was taken up in water and insoluble material remaining was removed by centrifugation. The turbid supernatant (representing the extract from 3.5 kg of mycelia) was adjusted to pH 3.5 by the addition of HCl and applied to a Dowex-50 (NH_4^+) column 9.5 cm in diameter and 35 cm high. The material was washed through the column with HCl (pH 3.5) until the eluate had an absorbancy at 260 mμ of less than 0.1. The eluting fluid was then changed to 0.1 N aq. ammonia resulting in the elution of 3 major peaks with considerable overlapping between them. The first of these contained cordycepin, the second 3'-amino-3'-deoxyadenosine, and the third homocitrullylaminoadenosine. Fractions containing the bulk of the second peak were pooled and concentrated on a rotary evaporator. In one of our preparations this procedure resulted directly in the crystallization of the nucleoside from solution. Our experience in other preparations has shown that as long as slight contaminants are present in the solution, the nucleoside tends to remain supersaturated, and crystallization is obtained only with difficulty. The crude material was recrystallized several times from water. Our recrystallized product melted at 275–278° while an authentic sample of 3'-amino-3'-deoxyadenosine[2] (kindly supplied to us by Dr. B. R. Baker of the Stanford Research Institute) melted at 274–277°.

The absorption spectra of the parent compound both at pH 2 and 7.5 were indistinguishable from those shown by adenosine. When the compound was heated in 1 N HCl at 100° for 1 h, the resulting ultraviolet-absorbing material had absorption spectra characteristic of free adenine both in acid and alkali. In addition the ultra-

TABLE I

R_F VALUES OF SYNTHETIC AND ISOLATED 3'-AMINO-3'-DEOXYADENOSINE.

Descending chromatography was used in each case. Solvents: (1) 86 % butanol–water (86 : 14, v/v); (2) isobutyric acid–conc. ammonia–water (66 : 1 : 33, v/v); (3) aq. ammonia (pH 10). The spots on the chromatogram were visualized with an ultraviolet lamp.

Solvent	*Authentic compound*	*Isolated compound*
1	0.07	0.07
2	0.90	0.88
3	0.50	0.51

violet-absorbing component had the same R_F value as adenine in paper chromatography. Comparison of the parent compound with authentic 3'-amino-3'-deoxyadenosine by paper chromatography in 3 solvent systems (Table I) indicated the two compounds were identical. When 4.6 μmoles of the nucleoside, as estimated utilizing a molar extinction coefficient of $1.5 \cdot 10^4$, was reacted with excess periodate at pH 5.0, 3.8 μmoles of periodate was consumed within the first 30 sec as determined in the Beckman spectrophotometer at 310 mμ according to the method of MARINETTI AND ROUSER[3]. As a result of the oxidation 4.4 μmoles of ammonia were found to be liberated as determined by the method of CONWAY[4] utilizing the modified diffusion chamber of ÖBRINK[5]. Hydrolysis of the compound by heating in 1 N HCl for 1 h at 100° liberated in addition to adenine, a compound which on paper was reactive both with the aniline phthalate spray reagent of PARTRIDGE[6] and with ninhydrin to produce a brown color. Authentic 3-aminoribose was obtained by acid hydrolysis of a commercial sample of 6-dimethylamino-9-(3'-amino-3'-deoxy-β-D-ribofuranosyl)-purine. When allowed to react with ninhydrin on paper, the authentic amino sugar also produced a brown pigment rather than the purple color customarily seen with 2-amino sugars. In addition it exhibited the same R_F value on paper (butanol–acetic acid–water; 3 : 1 : 1, v/v) as the sugar derivative obtained from hydrolysis of 3'-amino-3'-deoxyadeosine.

The absorption spectra in acid and alkali implicates Position 9 of the purine as the point of attachment of the sugar. The relative ease of hydrolysis and the concomitant liberation of adenine and a sugar reactive to aniline phthalate indicates that the linkage of the purine to the sugar is glycosidic. Whether this linkage is in the α or β configuration has not been independently determined, but the melting point and paper chromatographic behavior with authentic 3'-amino-3'-deoxyadenosine, which has the β configuration, suggests that the isolated nucleoside has the β configuration also.

The consumption of 1 mole of periodate with the production of 1 mole of ammonia implicates either the 2' or 3' position of the nucleoside as the point of attachment for the amino group. The periodate oxidation does not distinguish between these positions, but the behavior with ninhydrin corresponds to that of the authentic 3-amino sugar.

The amount of 3'-amino-3'-deoxyadenosine extractable from this organism appears to be one-twentieth that observed for homocitrullylaminoadenosine. Two possibilities come to mind for explaining the presence of 3'-amino-3'-deoxyadenosine in the mycelia of this organism: It is involved either in the synthesis or degradation of homocitrullylaminoadenosine. The preparative procedure was not drastic enough to cleave homocitrulline non-enzymically from the amino nucleoside. The bond between

these two fragments has the properties of an amide and required long hydrolysis in either strong acid or alkali to effect a significant cleavage. Enzymic hydrolysis during the isolation is rendered unlikely because the initial homogenization is carried out in 70 % ethanol. Therefore, we take the view that this material does not arise as a result of hydrolysis of homocitrullylaminoadenosine, but rather is probably involved in its biosynthesis. However, at the present time we have no experimental evidence to support this view.

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The metabolism of 6-methylaminopurine in cell-free systems

Recent reports from this laboratory have described an efficient utilization of 6-methylaminopurine for polynucleotide purine synthesis in normal tissues and tumors of rodents[1] and in bacteria[2]. The present report describes these conversions of 6-methylaminopurine and its metabolites by cell-free bacterial extracts and by purified enzymes which together presumably constitute the major salvage pathway for this compound.

When preliminary experiments with whole sonicates of *Escherichia coli* and *Salmonella typhimurium* had indicated the presence of a pyrophosphorylase activity toward 6-methylaminopurine, the following isolation and assay procedures were devised. Cultures of *S. typhimurium* LT_2 were grown on salts–glucose medium[3] to a density of $2 \cdot 10^9$ cells/ml, washed twice with cold medium, and disintegrated by sonic oscillation of 9 kcycles for 10 min. After centrifugation at $100\,000 \times g$ for 120 min, the supernatant was dialyzed stepwise against increasing concentrations of ammonium sulfate and each protein fraction thus obtained was dialyzed overnight against 0.01 N Tris buffer (pH 7.4). Protein was determined by the method of SUTHERLAND[4], and each preparation diluted to stock solutions of 2.0 mg/ml which were frozen until used. Following incubation of the purine-free base and 5-phosphorylribose-1-pyrophosphate (see legend of Table I), protein was precipitated by boiling, the supernatant evaporated to dryness, then redissolved in approx. 100 μl of solution which was 5 mM with respect to the purine and the corresponding 5'-nucleotide. After paper chromatography in