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Metabolism of 9-(*S*)-(2,3-dihydroxypropyl)adenine, an antiviral agent, in mice

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9-(*S*)-(2,3-Dihydroxypropyl)adenine (DHPA, I) exhibits significant antiviral activity against both DNA and RNA viruses [1]. Within the series of acyclic nucleoside analogues, the drug is stucturally unique; both the adenine base and the 2,3-dihydroxypropyl chain form an absolute requirement for the antiviral effect [2]. DHPA displays a relatively low toxicity for cell cultures and little or no acute or subacute toxicity in mice [1]. It also acts as a powerful adjuvant of adenine arabinoside [1] and 6-azauridine [3], allowing the use of lower doses of these drugs. In chicken embryo fibroblasts DHPA inhibits cellular transformation induced by Rous sarcoma virus concomitantly with the inhibition of the activity of protein kinases [4]. The drug also specifically affects the development of chick embryos, and in these embryos it is metabolized to 9-(*S*)-(2,3-dihydroxypropyl)hypoxanthine [5]. Recently it has been established that (*S*)-DHPA strongly inhibits *S*-adenosyl-L-homocysteine hydrolase [6]; this activity could be one of the main reasons for the antiviral effect of the drug.

In mice, DHPA is rapidly excreted from the organism, mostly unchanged. However, the drug is accumulated in the liver [7]. In addition to DHPA, the hypoxanthine derivative (II) which results from the aminohydrolase action upon DHPA, has been detected in the liver extract of mice treated with DHPA. This compound was accompanied in the liver by yet another acidic compound of an unknown structure. This catabolite might be a phosphorylation product of DHPA, an oxidation product of the purine base or an oxidation product of the aliphatic chain of DHPA. Our attention was drawn to the 2,3-dihydroxypropyl derivatives of hypoxanthine, xanthine and uric acid, and DHPA 3′-phosphate and 3-(adenin-9-yl)lactic acid.

9-(*S*)-(2,3-Dihydroxypropyl)adenine (I), its 3′-phosphate and 9-(*S*)-(2,3,dihydroxypropyl)hypoxanthine (II) were synthesized according to [8], 3-(adenin-9-yl)lactic acid according to [9]. Adenine-U-^{14}C-DHPA (266 μCi/μmole) and thymidine-2-^{14}C (48 μCi/μmole) were purchased from the Institute for Research, Production and Uses of Radioisotopes in Prague. Cow milk xanthine oxidase was purchased from Boehringer (Mannheim, West Germany). Separation of compounds was performed on Whatman No. 3 MM paper and the compounds were detected in u.v.-light and by means of a Frieseke-Hoepfner scanner. Paper electrophoresis was carried out for 1 hr at 20 V/cm in 0.1 M triethylammonium hydrogen carbonate (pH 7.5) and paper chromatography in solvent systems shown in Table 1.

The mixture of 9-(*RS*)-(2,3-dihydroxypropyl)guanine [cf. 10] (0.225 g, 1 mmole), sodium nitrite (10 mmole), water (10 ml) and concentrated hydrochloric acid (10 mmole) was left overnight at 0°, evaporated *in vacuo* and the residue chromatographed on Dowex 1 × 2 column (100–200 mesh, 100 ml) by a gradient of 0–0.5 M acetic acid (0.5 l. each). The fractions containing xanthine derivative III were pooled, evaporated *in vacuo* and the residue crystallized from 50 per cent aqueous ethanol. Yield was 150 mg (66.5 per cent), m.p. higher than 260°. Elemental analysis corresponded to calculated data. u.v.-Spectra (pH 2): λ_{max} 234 nm (ε 9200), 246 nm (ε 11,000); λ_{min} 218 and 248 nm.

Xanthine oxidase activity was assayed in an incubation mixture (150 μl) containing 5.10^{-4} M or the compounds tested and 0.04 e.u. xanthine oxidase (from cow milk) in 0.05 M phosphate buffer pH 7.4 with 10^{-4} M EDTA or 10^{-3} M potassium ferricyanide. After incubation at 37° for 1 hr, the mixture was analyzed by HPLC on Separon SI C18 reversed phase column (7 μm, 25 × 0.4 cm) in 0.01 M

Table 1. Chromatographic and electrophoretic mobilities of synthesized purine 2,3-dihydroxypropyl derivatives and detected catabolites of DHPA*

Compound	R_F		E_{Up}
	S_1	S_2	
9-(*S*)-(2,3-Dihydroxypropyl)adenine	0.52	0.28	0
9-(*S*)-(2,3-Dihydroxypropyl)hypoxanthine	0.38	0.18	0.20
9-(*RS*)-(2,3-Dihydroxypropyl)xanthine	0.30	0.11	0.55
Adenine	0.44	0.40	0
9-Carboxymethyladenine	0.32	0.40	0.70
3-(Adenin-9-yl)lactic acid	0.40	0.20	0.68
DHPA 3′-phosphate	0.08	0.03	1.00
Uric acid	0.25	0.08	0.75
Compound 2 (Fig. 1)	0.38	0.18	0.20
Compound 3 (Fig. 1)	0.30	0.11	0.55 (+ 0.67)†
Compound X [see Ref. 7]	0.30	0.11	0.55

* Paper chromatography was performed in the solvent system S_1: 2-propanol-concentrated aqueous ammonia-water (7:1:2), and S_2: *n*-butanol-acetic acid-water (10:1:3). E_{Up}, electrophoretic mobility in 0.1 M triethylammonium hydrogen carbonate (pH 7.5) referred to uridine 3′-phosphate.

† Compound 4 in Fig. 1(B).

ammonium dihydrogen phosphate pH 5.1 with varying concentration of methanol. Under the assay conditions, hypoxanthine and xanthine afforded quantitatively uric acid, whereas inosine and xanthosine, as well as DHPHX and DHPX remained unchanged.

Sodium periodate degradation was performed with 1 per cent solution (0.1 ml) at 37° for 1 hr, followed by addition of 50 μl 2 M sodium hydroxide and incubation as above. The mixture was analyzed by paper chromatography and electrophoresis (cf. Table 1).

The animals were injected intraperitoneally with ^{14}C-DHPA, killed by cervical dislocation, bled, and the liver was homogenized in a cooled glass homogenizer with a tight fitting Teflon pestle in 3 vol. of cold 0.25 mM KCl. The extraction of radioactive compounds from the cellular pool with 0.4 M $HClO_4$ was carried out as described previously [7]. Urine was collected on Whatman No. 3 paper (inserted in the cages used to house the mice), eluted with water and analysed as indicated in Fig. 1.

The comparison of the labelled fractions isolated from the urine and the liver pool of DHPA-treated mice with authentic compounds revealed (Table 1) that two of the compounds can be identified as the hypoxanthine derivative II and the 9-(2,3-dihydroxypropyl)xanthine (III). The latter derivative was detected in both the liver and the urine. The third, still more acidic, component which was detected in urine, was supposed to be the corresponding 2,3-dihydroxypropyl derivative of uric acid.

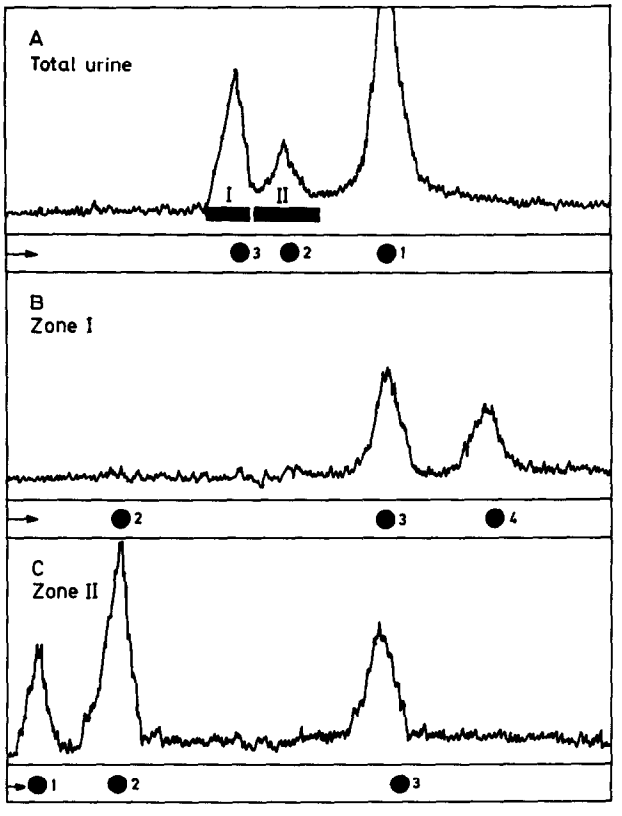
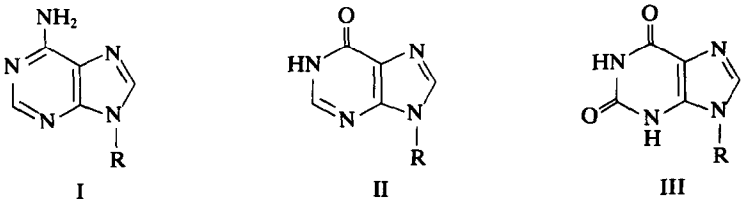


Fig. 1. Separation of metabolites of intraperitoneally injected ^{14}C-labelled DHPA excreted into urine of mice. Three female mice (24 g) placed in metabolic cages received adenine-U-^{14}C-DHPA (25 μCi/0.1 μmole/mouse) and their urine was collected for 8 hr and analyzed. (A) Paper chromatography of an aliquot sample in the solvent system composed of 2-propanol-concentrated aqueous ammonia-water (7:1:2); (B) and (C) paper electrophoresis of zones I and II in 0.1 M triethylammonium hydrogen carbonate (pH 7.5). 1, 9-(*S*)-(2,3-dihydroxypropyl)adenine; 2, 9-(*S*)-(2,3-dihydroxypropyl)hypoxanthine; 3, 9-(*S*)-(2,3-dihydroxypropyl)xanthine; and 4, 9-(*S*)-(2,3-dihydroxypropyl)uric acid.



R= (S)—CH_2—CH—CH_2OH
OH

The attempts to synthesize an authentic specimen of this material either by chemical synthesis [10] or by enzyme oxidation of compound III by xanthine oxidase were unsuccessful. Therefore, the freeze-dried eluate of the radioactive spot was treated with sodium periodate followed by alkaline hydrolysis. The product of this degradation cochromatographed with uric acid in both solvents, confirming thus the suggested structure.

Though both the hypoxanthine (II) and xanthine (III) derivatives are excreted by urine, the xanthine analogue III is retained to some extent in the liver of mice [5]. Twenty four hours after the intraperitoneal injection of ^{14}C-labelled DHPA (0.1 μmole) the residual radioactivity present in the liver, consisted from about 75 per cent of unchanged DHPA, 25 per cent of the xanthine analogue III, and only trace amounts of the hypoxanthine derivative II. In the urine collected during the period of 4 hr following the administration of the drug (50 per cent excretion of the total radioactivity) the level of unreacted DHPA amounted to more than 90 per cent, whereas only 3–4 per cent of the administered radioactivity appeared as hypoxanthine and xanthine derivatives. Neither radioactive adenine, nor hypoxanthine, nor xanthine, nor uric acid have been detected in the liver or urine, which points to the stability of the C_1—N_9 bond under *in vivo* conditions. Neither DHPA 3′-phosphate nor the lactic acid derivative of DPHA were detected.

The only catabolites of DPHA so far identified are products of enzyme degradation of the purine moiety of DHPA. The 2′,3′-dihydroxypropyl residue remains intact. 9-(2,3-Dihydroxypropyl)hypoxanthine (II) results from the aminohydrolase action upon DHPA. It is interesting that the liver enzyme behaves quite differently from its counterpart in chicken intestinal mucosa [1] and bacteria, where DHPA not only does not serve as a substrate but even acts as an inhibitor [11].

9-(*S*)-(2,3-dihydroxypropyl)xanthine (III) is evidently formed by the action of xanthine oxidase. It is assumed that the formation of this compound is preceded by the deamination of DHPA, since hypoxanthine is a much better substrate of the enzyme than adenine [12]. In an *in vitro* assay performed with milk xanthine oxidase, neither DHPA nor DHPHX were transformed to DHPX or uric acid derivative, though hypoxanthine and xanthine afforded uric acid quantitatively. Thus, the xanthine oxidase in liver *in vivo* differs from the purified enzyme. Xanthine oxidase recognises as a substrate hypoxanthine or xanthine but not their nucleosides or nucleotides. However, under *in vivo* conditions, this enzyme also seems to accept as a substrate 9-(2,3-dihydroxypropyl)hypoxanthine (II).

9-(*S*)-(2,3-Dihydroxypropyl)adenine injected intraperitoneally to mice, affords 9-(*S*)-2,3-dihydroxypropyl derivatives of hypoxanthine, xanthine and uric acid as the only catabolites. The linkage of the aliphatic substituent to the base moicty of the drug and the substituent itself were not affected.

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REFERENCES

1. E. De Clercq, J. Descamps, P. De Somer and A. Holý, *Science, New York* **200**, 563 (1978).
2. E. De Clercq and A. Holý, *J. Med. Chem.* **22**, 510 (1979).
3. B. Rada and A. Holý, *Chemotherapy* **26**, 184 (1980).
4. J. Kára, P. Vácha and A. Holý, *FEBS Lett.* **107**, 187 (1980).
5. R. Jelínek, A. Holý and I. Votruba, *Theratology*, in press.
6. I. Votruba and A. Holý, *Collect. Czech. Chem. Commun.* **45**, 3039 (1980).
7. A. Čihák and A. Holý, *Collect. Czech. Chem. Commun.* **43**, 2082 (1978).
8. A. Holý, *Collect. Czech Chem. Commun.* **40**, 187 (1975).
9. A. Holý, *Collect. Czech. Chem. Commun.* **43**, 3444 (1978).
10. A. Holý, *Collect. Czech. Chem. Commun.* **43**, 3103 (1978).
11. H. J. Schaeffer, D. Vogel and R. Vince, *J. Med. Chem,* **8**, 502 (1965).
12. T. A. Krenitsky, S. M. Niel, G. B. Elion and G. H. Hitchings, *Arch. Biochem. Biophys.* **150**, 585 (1972).