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The Conversion of 4-Hydroxypyrazolo[3,4-*d*]pyrimidine (Allopurinol) into 4,6-Dihydroxypyrazolo[3,4-*d*]pyrimidine (Oxipurinol) *in vivo* in the Absence of Xanthine–Oxygen Oxidoreductase

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1. A patient with congenital deficiency of xanthine oxidase (EC 1.2.3.2) (xanthinuria) excreted the xanthine isomer 4,6-dihydroxypyrazolo[3,4-*d*]pyrimidine (oxipurinol) in his urine when the hypoxanthine isomer 4-hydroxypyrazolo[3,4-*d*]-pyrimidine (allopurinol) was given by mouth. 2. The identity of the oxipurinol that the patient excreted was established by mass spectrometry. 3. The mass spectra and infrared spectra of allopurinol, oxipurinol, hypoxanthine and xanthine are compared. 4. A mechanism for the fragmentation of these compounds that occurs during their mass-spectrometric investigation is proposed. 5. A possible metabolic pathway for the oxidation of allopurinol to oxipurinol in the absence of xanthine oxidase is discussed.

4-Hydroxypyrazolo[3,4-*d*]pyrimidine (allopurinol) is a structural isomer of hypoxanthine (6-hydroxypurine) that is both an inhibitor and a substrate for xanthine oxidase (xanthine–oxygen oxidoreductase, EC 1.2.3.2). It is rapidly oxidized *in vivo* to 4,6-dihydroxypyrazolo[3,4-*d*]pyrimidine (oxipurinol) (Elion, Kovensky, Hitchings, Metz & Rundles, 1966), which is a structural isomer of xanthine (2,6-dihydroxypurine), and which is excreted in the urine. Oxipurinol also inhibits xanthine oxidase (Elion, Taylor & Hitchings, 1964).

Patients with xanthinuria (Dent & Philpot, 1954) are grossly deficient in xanthine oxidase (Watts, Engelman, Klinenberg, Sjoerdsma & Seegmiller, 1964), and therefore would not be expected to oxidize allopurinol to oxipurinol *in vivo*. The present paper reports the conversion of allopurinol into oxipurinol in a xanthinuric patient, and in particular the mass-spectrometric identification of the metabolite. A possible metabolic pathway for the reaction in the absence of xanthine oxidase is suggested.

METHODS

Patient. The patient was a 31-year-old male with xanthinuria. His serum uric acid was consistently less than 0·5mg./100ml., the serum concentration of hypoxanthine and xanthine, measured together, was about 0·5mg./100ml., and the urinary excretions of uric acid, hypoxanthine and xanthine were 13–32, 4–94 and 209–394mg./24hr. respectively when he was on a purine-free diet. (The values for hypoxanthine and xanthine are expressed as the equivalent amount of uric acid.) These results are characteristic of xanthinuria. The uric acid was determined by the method of Liddle, Seegmiller & Laster (1959), and the other purines were determined as described by Chalmers & Watts (1968, 1969). Lack of xanthine oxidase was demonstrated histochemically in a jejunal mucosa biopsy (Dr M. Johnson).

Isolation of the material for mass spectrometry and infrared spectrophotometry. The urine was part of a 24hr. collection that was made when the patient had been taking 200mg. of allopurinol every 8hr. for 10 days. It contained 33mg. of allopurinol and 101mg. of allopurinol riboside together with 420mg. and 45mg. respectively of u.v.-absorbing substances having electrophoretic and spectroscopic properties compatible with oxipurinol and oxipurinol riboside respectively (Simmonds, 1969*b*). A 50ml. sample of this specimen was chromatographed on a column of Dowex 1 (X8; acetate form; 100–200 mesh) as described by Simmonds & Wilson (1967). The purines, pyrimidines and pyrazolopyrimidines were eluted in three separate u.v.-absorbing peaks, *A*, *B* and *C*. Fractions from peak *C*, which was shown, on the basis of electrophoretic and u.v.-spectral data, to contain almost exclusively oxipurinol (Simmonds, 1969*a*), were pooled and evaporated to dryness under reduced pressure. The residue was then dissolved in 10ml. of approx. 0·1M-NH_3, the pH adjusted to 8·0, and the solution applied to a column of Dowex 1 (X8; acetate form; 100–200 mesh) resin. Gradient elution was carried out with 1–10mM-HCl, the effluent being monitored at 254nm. The u.v. spectra of the fractions contained in the u.v.-absorbing elution peak were checked in a Unicam SP.800

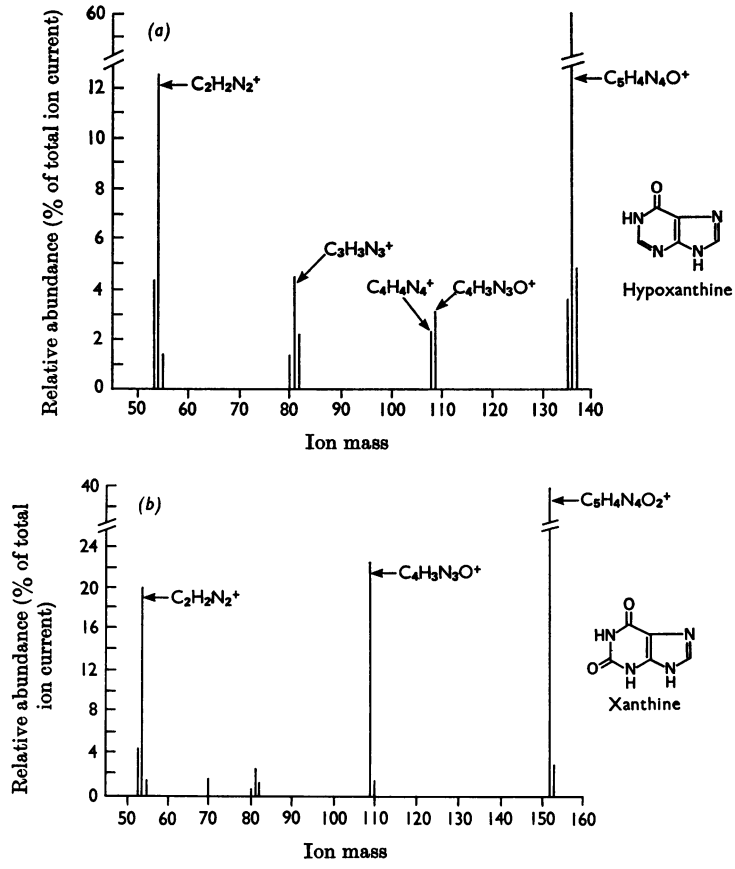


Fig. 1. Mass spectra of hypoxanthine (*a*) and xanthine (*b*). The ion masses of the main peaks, and those for the formulae allocated to them, were as follows. $C_2H_2N_2^+$: found, 54·021±0·001; theory, 54·0218. $C_3H_3N_3^+$: found, 81·033±0·001; theory, 81·0327. $C_4H_4N_4^+$: found, 108·043±0·001; theory, 108·044. $C_4H_3N_3O^+$: found, 109·026±0·002; theory, 109·0276. $C_5H_4N_4O^+$: found, 136·039±0·001; theory, 136·0386. $C_5H_4N_4O_2^+$: found, 152·034±0·001; theory, 152·0334.

spectrophotometer, and fractions giving the characteristic u.v. spectra for oxipurinol were pooled and evaporated to dryness under reduced pressure.

Mass spectrometry. Spectra were obtained on a Varian MAT model SM1 double-focussing mass spectrometer. All samples were admitted by using a direct insertion probe at 220°. Other operating conditions were: ionizing energy, 70 v, ionizing current, 300 μA; source temperature, 150°.

Infrared spectrophotometry. Spectra were obtained on a Perkin–Elmer model 237 grating spectrophotometer. All samples were examined as KBr disks.

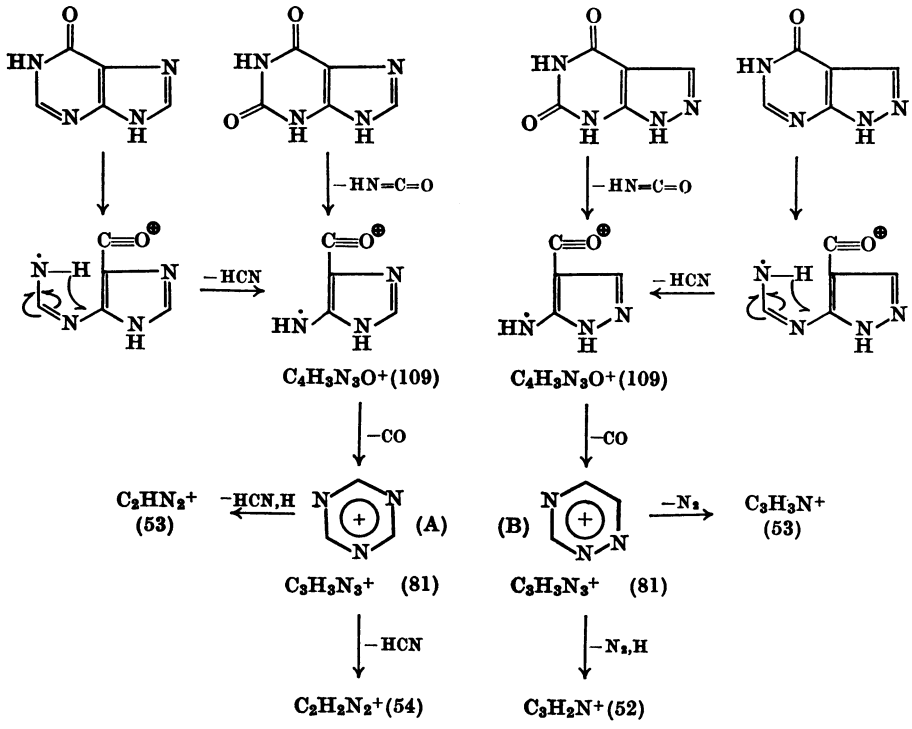
RESULTS

The mass spectra of the purines (hypoxanthine and xanthine), the pyrazolo[3,4-*d*]pyrimidines (allopurinol and oxipurinol) and of the unknown material isolated from the xanthinuric patient's urine when he was taking allopurinol are shown in Figs. 1, 2 and 3 respectively. The accurate masses and atomic compositions of certain major ions are given.

The i.r.-absorption spectra in the wave-number range 625–2000 $cm.^{-1}$ of the five substances are compared in Figs. 4, 5 and 6.

The mass spectra of the purines and their isomeric hydroxypyrazolo[3,4-*d*]pyrimidine derivatives are very similar. That of hypoxanthine has been examined previously (Rice & Dudek, 1967), but in the absence of further labelling studies the proposed fragmentation mechanisms for the other three compounds must be regarded as speculative.

The primary fragment obtained from each molecule ion is $C_4H_3N_3O^+$ of mass 109. In the process hypoxanthine and allopurinol lose HCN, and xanthine and oxipurinol undergo a retro-Diels–Alder reaction with elimination of



Scheme 1. Proposed fragmentation mechanisms for hypoxanthine, xanthine, allopurinol and oxipurinol.

HN=C=O. The $C_4H_3N_3O^+$ ions then lose CO to give $C_3H_3N_3^+$ of mass 81, probably with rearrangement to the fully aromatic structures (A) and (B). With the purines further fragmentation of the symmetrical triazine ion (A) yields the ions $C_2H_2N_2^+$ and $C_2HN_2^+$ of masses 54 and 53 with loss of a further molecule of HCN or HCN + H. The corresponding ion (B) from the hydroxypyrazolo[3,4-*d*]-pyrimidines has two nitrogen atoms adjacent, thus possibly facilitating the elimination of N_2 or N_2 + H rather than HCN, yielding the ions $C_3H_3N^+$ and $C_3H_2N^+$ of masses 53 and 52. The proposed fragmentation scheme is summarized in Scheme 1.

Although the ions of mass 53 have about the same relative intensity in both sets of compounds, there is a significant difference in the abundance of the ions $C_3H_2N^+$ and $C_2H_2N_2^+$ of masses 52 and 54. The former is more predominant in the hydroxypyrazolo[3,4-*d*]pyrimidine spectra and the latter in the purine spectra. Comparison of the reference spectra with that of the unknown indicates that the unknown material consists mainly of oxipurinol. However, the ion of mass 54 is more intense than is expected for pure oxipurinol, and hence a small amount of isomeric xanthine also appears to be present. There is no evidence of hypoxanthine, allopurinol or methylated purine bases in the unknown material.

These conclusions are confirmed by the i.r. data. All four reference compounds have characteristic spectra, particularly in the range 700–1400 cm.$^{-1}$ (Figs. 4 and 5). Only that of oxipurinol is a reasonable match for the spectrum of the unknown substance (Fig. 6).

DISCUSSION

The present observations establish that the material isolated from the patient's urine is oxipurinol. Elion *et al.* (1966) reported that only about 2% of the dose of allopurinol was excreted as oxipurinol by a xanthinuric patient, whereas the oxipurinol content of the urine in the present study was equivalent to about 63% of the allopurinol dose. We suggest that this discrepancy is due to the facts that Elion *et al.* (1966) studied a patient who had taken the drug for only 1 day whereas the present patient had taken it for 10 days, and that the conversion of allopurinol into oxipurinol, in the absence of xanthine oxidase, involves the metabolism of the compound by processes that include

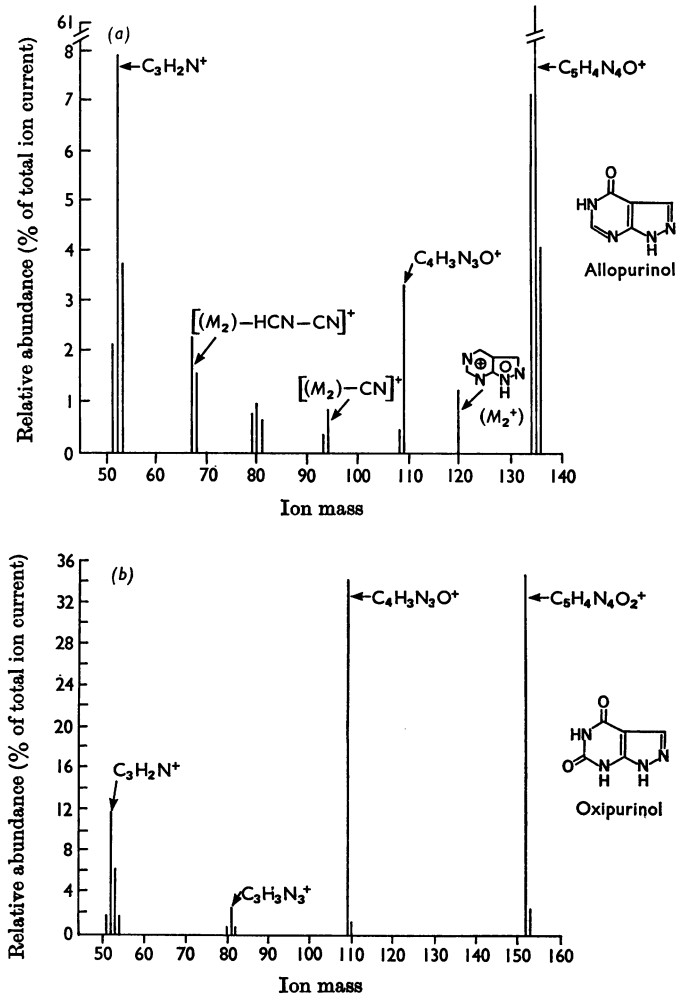


Fig. 2. Mass spectra of 4-hydroxypyrazolo[3,4-*d*]pyrimidine (allopurinol) (*a*) and 4,6-dihydroxypyrazolo[3,4-*d*]-pyrimidine (oxipurinol) (*b*). The ion masses of the main peaks, and those for the formulae allocated to them, were as follows. $C_3H_2N^+$: found, 52·017±0·002; theory, 52·0187. $C_3H_3N_3^+$: found, 81·031±0·002; theory, 81·0327. $C_4H_3N_3O^+$: found, (*a*) 109·029±0·002; (*b*) 109·026±0·002; theory, 109·0276. $C_5H_4N_4O^+$: found, 136·037±0·002; theory, 136·0386. $C_5H_4N_4O_2^+$: found, 152·034±0·001; theory, 152·0334.

one or more metabolic pools that are turning over relatively slowly.

The conversion of allopurinol into oxipurinol *in vivo* in the absence of xanthine oxidase suggests that allopurinol may follow a metabolic pathway analogous with the one that has been invoked to explain the excretion of both xanthine and hypoxanthine, rather than hypoxanthine alone, in xanthinuria (Engelman, Watts, Klinenberg, Sjoerdsma & Seegmiller, 1964). This scheme would involve the conversion of allopurinol into its ribonucleotide by IMP–pyrophosphate phosphoribosyltransferase (EC 2.4.2.8), oxidation into oxipurinol ribonucleotide by IMP–NAD oxidoreductase (EC 1.2.1.14) and the formation of oxipurinol via oxipurinol ribonucleoside. Kelley, Rosenbloom, Miller & Seegmiller (1968) suggested, on the basis of indirect evidence, that IMP–pyrophosphate phosphoribosyltransferase converts allopurinol into allopurinol ribonucleotide in man, and the enzymic synthesis of the compound was reported by McCollister, Gilbert, Ashton & Wyngaarden (1964). However, the activity of the enzymes of the pathway with the allopurinol and oxipurinol derivatives has not yet been tested directly, and Elion *et al.* (1966) did not mention the

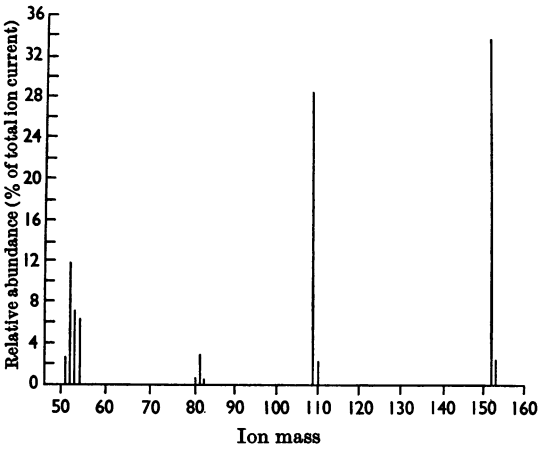


Fig. 3. Mass spectrum of the unknown substance isolated from the xanthinuric patient's urine when he was taking allopurinol.

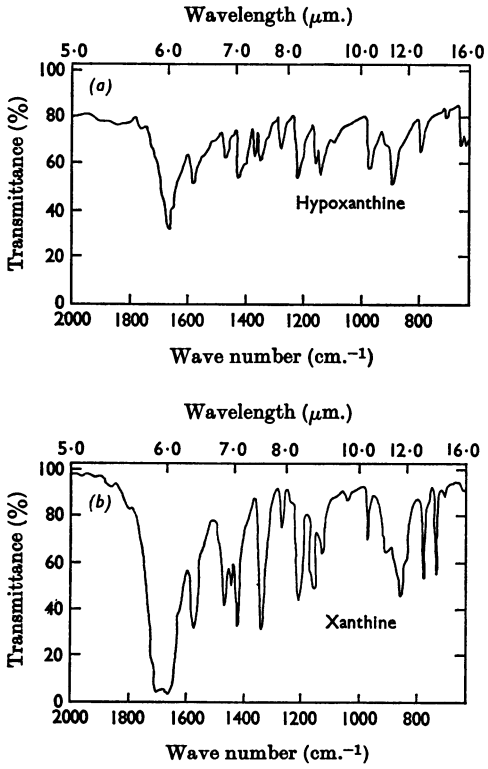


Fig. 4. Infrared spectra of hypoxanthine (*a*) and of xanthine (*b*).

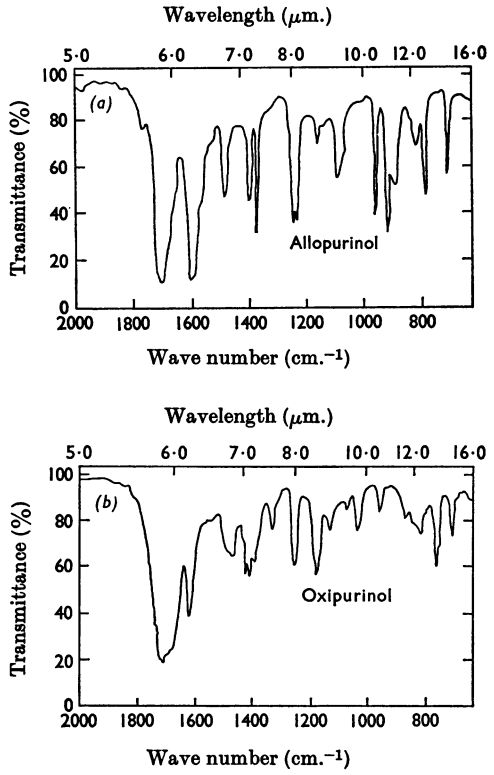


Fig. 5. Infrared spectra of 4-hydroxypyrazolo[3,4-*d*]-pyrimidine (allopurinol) (*a*) and of 4,6-dihydroxypyrazolo-[3,4-*d*]pyrimidine (oxipurinol) (*b*).

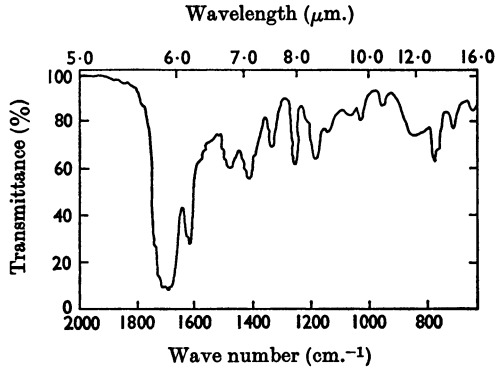


Fig. 6. Infrared spectrum of the unknown substance isolated from the xanthinuric patient's urine when he was taking allopurinol.

excretion of allopurinol ribonucleotide or oxipurinol ribonucleotide after allopurinol administration to non-xanthinuric human subjects. They also obtained no evidence for the incorporation of [6-^{14}C]allopurinol into liver nucleic acids in mice.

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