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METABOLISM OF DRUGS—LIX.
A NEW METABOLITE OF ANTIPYRINE

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Abstract—The metabolism of antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) has been investigated in various animal species (rats, mice, guinea pigs and rabbits) and in men. It was found that all of the species examined, including man, excreted a considerable amount of a new metabolite together with the known metabolite, 4-hydroxyantipyrine, and the unchanged drug. This new metabolite was isolated as crystals from the urine of medicated rats and was characterized as 3-hydroxymethyl-2-methyl-1-phenyl-3-pyrazolin-5-one on the basis of analytical and spectral data (ultraviolet, infrared and nuclear magnetic resonance). From the results of thin-layer chromatography (TLC), it was concluded that this metabolite is excreted into urine more in the free than in the conjugated form.

ANTIPYRINE (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) has been used for a long time as an antipyretic and analgesic, and its metabolic fate has been investigated by several workers. The early work of Lawrow showed that in dogs antipyrine was excreted as conjugates of hydroxyantipyrine.[1] More recently, Brodie and Axelrod reported that in man 30–40 per cent of this drug was oxidized to 4-hydroxyantipyrine, which was excreted almost entirely in a conjugated form, and about 5 per cent was eliminated unchanged.[2] Halberkann and Fretwurst obtained similar results.[3] Tanaka presented chromatographic evidence which supported the concept that a sulfate ester of 4-hydroxyantipyrine as well as a glucuronide is formed in rabbits.[4] However, no information has been available to date as to the fate of the remainder, which represents more than half of the drug ingested. The present investigation was initiated in order to determine whether antipyrine follows any other metabolic pathway.

It will be shown in this paper that humans as well as experimental animals (rats, mice, guinea pigs and rabbits) oxidize a considerable part of ingested antipyrine to a new metabolite, 3-hydroxymethyl-2-methyl-1-phenyl-3-pyrazolin-5-one, in addition to the well known 4-hydroxyantipyrine.

METHODS

Materials. Antipyrine (m.p. 111–113°) was obtained from a commercial source. 4-Hydroxyantipyrine (m.p. 182–183°) was synthesized according to the method described by Knorr and Pshorr.[5]

The experimental animals were rats (SD strain, body wt. about 210 g), mice (CF No. 1 strain, about 22 g), guinea pigs (about 950 g) and rabbits (about 3 kg); only male animals were used. Some experiments were also performed with human volunteers (male).

Administration of antipyrine. Antipyrine (300 mg/kg body wt.) was dissolved in water and administered to the laboratory animals s.c. It was administered orally to the human volunteers in a dose of 0·3 g. In the experiment designed for the isolation of metabolic products, 300 mg antipyrine per kg body wt. was injected daily for 7 days to 20 rats (a total of 11·06 g antipyrine).

Extraction of the metabolites. After the administration of antipyrine, 48-hr urine samples from each species (about an 8-hr urine from man) were extracted continuously for 9 hr with $CHCl_3$ at pH 6·6 (fraction A). To this urine was then added a 1/5 vol. of concentrated HCl, and the mixture was heated on a boiling water bath for 45 min to hydrolyze the conjugated metabolites. The hydrolyzed urine was then extracted again continuously with $CHCl_3$ for 9 hr at pH 6·6 (fraction B). From these two fractions, A and B, the solvent was evaporated to dryness under reduced pressure after drying over anhydrous Na_2SO_4. Urine collected from each species before medication was used as a negative control.

Thin-layer chromatography. (TLC) was carried out on silica gel plates, 0·25 mm thick (Silica gel G, Merck), which were activated at 110° for 30 min. The solvent system used was benzene–ethyl acetate–ethanol (4:5:1).

The resulting chromatograms were visualized by spraying with Dragendorff, $FeCl_3$ and Ehrlich reagents. Antipyrine and its metabolites give orange, brown and red colors, respectively, with the above three reagents. A little heating is necessary, however, to produce color with the Ehrlich reagent. 4-Hydroxyantipyrine does not give a color with either $FeCl_3$ or Ehrlich reagents, but it does produce a color with the Dragendorff reagent.

RESULTS

Detection of urinary metabolites. The metabolites in two fractions (A and B) of the urine extracts from various animals, including man, were studied by means of TLC as described above.

It appeared from the chromatograms that the mouse excreted 4-hydroxyantipyrine (R_f 0·45) only in the free form together with unchanged antipyrine (R_f 0·23), whereas the other three animal species excreted 4-hydroxyantipyrine almost equally in both free and conjugated forms.

Similarly, it was concluded that man, after a 300 mg dose of antipyrine, also excreted 4-hydroxyantipyrine. This was, however, mostly in a conjugated form in the 0–8 hr urine, since its presence could hardly be detected before hydrolysis (fraction A), but was readily detected after hydrolysis (fraction B). The excretion of unchanged antipyrine and of 4-hydroxyantipyrine was confirmed, not only by this type of thin-layer chromatography, but also by the isolation of crystalline compounds which will be described below.

In addition to the spots corresponding to antipyrine and 4-hydroxyantipyrine, the chromatograms clearly showed the presence of another compound which had an R_f value of 0·12 and gave the same color reactions as those of antipyrine with the Dragendorff, ferric chloride and Ehrlich reagents. This metabolite was isolated in a crystalline form from the urine of rats and its structure was later shown to be 3-hydroxymethyl-2-methyl-1-phenyl-3-pyrazolin-5-one. As judged from the chromatograms, this compound was undoubtedly one of the major metabolites in the urine of all of the species examined, and it seemd to be excreted more in the free than in the

conjugated form. This was especially true in man and in the mouse, although the result in man could not be compared strictly with those in the animal species because of the differences in size of dose and in time of sampling.

Isolation and characterization of the metabolites. After the administration of antipyrine to 20 male rats in a daily dose of 300 mg/kg for 7 days, the extraction of the combined urine (from the time of medication to 48 hr after the last dose) by the procedure described under Methods gave 7·52 g of brown gum for fraction A (free metabolites) and 5·36 g of a similar gum for fraction B (conjugated metabolites). Each fraction was purified by silica gel column chromatography as follows. Fraction A was dissolved in a small volume of benzene–chloroform (1:1) and the solution was poured onto the top of the column, which was packed with 150 g of silica gel (100–200 mesh, Kanto Chem. Co.). The column was eluted stepwise with benzene–chloroform, chloroform and chloroform–methanol as shown in Table 1.

TABLE 1. CHROMATOGRAPHY OF FRACTION A

Fraction No.*	Solvent	Nature of residue on evaporation
1– 11	Benzene–$CHCl_3$(1:1)	Oily substance
12– 20	Benzene–$CHCl_3$(1:2)	Oily substance
21– 22	Benzene–$CHCl_3$(1:3)	Oily substance
23– 29	Benzene–$CHCl_3$(1:3)	Crystals (M-1)
30– 41	Benzene–$CHCl_3$(1:3)	Crystals (M-1, M-2)
42– 45	$CHCl_3$	Crystals (M-2)
46– 49	$CHCl_3$–MeOH(99:1)	Crystals (M-2)
50– 71	$CHCl_3$–MeOH(99:1)	Oily substance
72– 79	$CHCl_3$–MeOH(97:3)	Oily substance
80– 95	$CHCl_3$–MeOH(97:3)	Crystals (M-3)
96–125	$CHCl_3$–MeOH(95:5)	Oily substance (M-3)
126–146	$CHCl_3$–MeOH(90:10)	Brown gum

* Each fraction = 100 ml.

From fractions 23–29 and fractions 42–49, metabolite 1 (M-1) and metabolite 2 (M-2) were obtained respectively as nearly pure compounds. M-1 was recrystallized from a mixture of chloroform–petroleum ether–ether to give colorless needles (m.p. 181–183°). The u.v. and i.r. absorption spectra and the R_f value (TLC) of this compound were identical with those of the authentic sample of 4-hydroxyantipyrine. Identity was also proved by taking a mixed m.p. M-2 was recrystallized from benzene–ether to give colorless plates (m.p. 111–113°). This compound was identified as unchanged antipyrine by use of the mixed m.p. test and by identity of spectral and chromatographic data.

Metabolite 3 (M-3), which was obtained from fractions 80–95 and which showed the same reaction as antipyrine to the three color reagents, was recrystallized from chloroform–ether to give colorless prisms (m.p. 143–144°). The u.v. absorption spectrum in 0·1 N NaOH showed absorption maxima at 243 and 260 mμ. This spectrum was similar to that of antipyrine (maxima at 242 and 256 mμ) and suggested that the pyrazolinone skeleton should be considered as remaining intact in the structure of this metabolite. The i.r. spectrum had a characteristic band at 3215 cm^{-1},

which strongly suggested the presence of a hydroxyl group. Elementary analysis indicated that M-3 had the empirical formula $C_{11}H_{12}O_2N_2$. It therefore possessed an additional oxygen as compared to antipyrine ($C_{11}H_{12}ON_2$).

Anal. Calcd. for $C_{11}H_{12}O_2N_2$: C, 64·69; H, 5·92; N, 13·72. Found: C, 64·67; H, 5·88; N, 13·59.

The location of the extra oxygen atom was finally decided on the basis of its n.m.r. spectrum in $CDCl_3$ (see Fig. 1). Note that the signal of the 3-methyl group of antipyrine

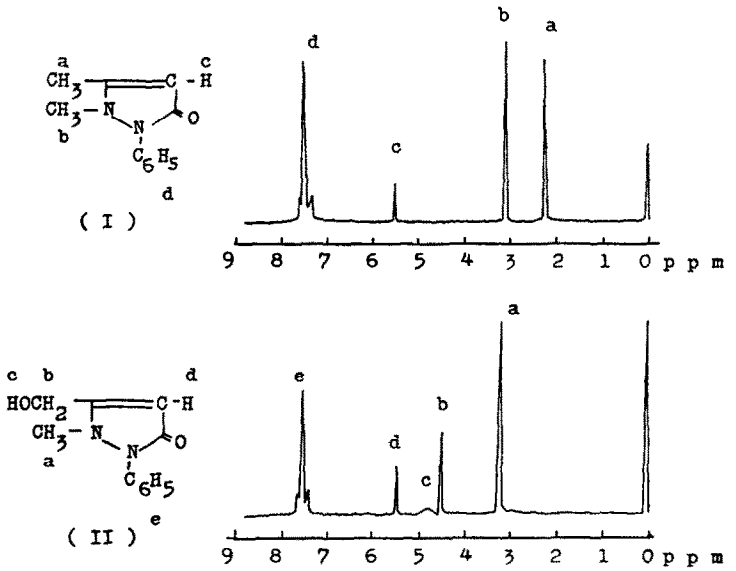

FIG. 1. Nuclear magnetic resonance spectra of antipyrine (I) and of a new metabolite, 3-hydroxymethyl-2-methyl-1-phenyl-3-pyrazolin-5-one (II) (in$CDCl_3$, 60 mc, JNM-C-60 H, int. ref. TMS).

at 2·26 ppm disappeared in the spectrum of M-3 and, in its stead, two new signals corresponding to two protons of a methylene (—$C\underline{H}_2OH$) and one proton of an alcoholic group (—$CH_2O\underline{H}$) appeared at 4·48 and 4·75 ppm respectively. All of the other signals of M-3, corresponding to five aromatic protons of 1-phenyl, three protons of 2-methyl and one proton of the 4-methine group remained at the same ppm as those of antipyrine. Considering these facts, the structure of M-3 must be 3-hydroxymethyl-2-methyl-1-phenyl-3-pyrazolin-5-one.

The crystalline material from fractions 30–41 was found to be a mixture of M-1 (4-hydroxyantipyrine) and M-2 (antipyrine) by thin-layer chromatography and it was rechromatographed. Fractions 96–125, which were indicated by their R_f values to contain M-3, were also rechromatographed. These procedures gave crystalline material of M-1, M-2 and M-3 in yields of 1·262, 0·346 and 2·049 g respectively.

Fraction B (the extract from hydrolyzed urine) was also purified by column chromatography as described for fraction A above, and 1·185 g of M-1 and 0·076 g of M-3 were isolated therefrom as pure crystals. Unchanged antipyrine was not obtained from this fraction.

DISCUSSION

It has been believed to date that antipyrine is mainly metabolized to 4-hydroxyantipyrine and its conjugates in animals and in man and that 4-hydroxyantipyrine is the only oxidized metabolite detectable in the urine.

From the present study it became evident that antipyrine is oxidized not only to 4-hydroxyantipyrine but also to 3-hydroxymethyl-2-methyl-1-phenyl-3-pyrazolin-5-one. We have not confirmed the structure of this new metabolite by comparison with a chemically synthesized authentic sample, but spectral measurements and elemental analysis have presented good evidence to support the assignment of this structure.

The amount of this hydroxymethyl metabolite in the 48-hr urine, as judged from the thin-layer chromatogram, seemed rather different among the species. It was, for example, excreted in relatively smaller amounts in the rabbit and guinea pig than in the rat and mouse. It can be said also that in the latter two animal species, the amount of this metabolite excreted is comparable with that of 4-hydroxyantipyrine.

This conculsion was also supported by direct isolation of the metabolites. The amounts of 4-hydroxyantipyrine and 3-hydroxymethyl metabolite obtained as crystals from the urine of rats accounted for about 20 and 18 per cent respectively of

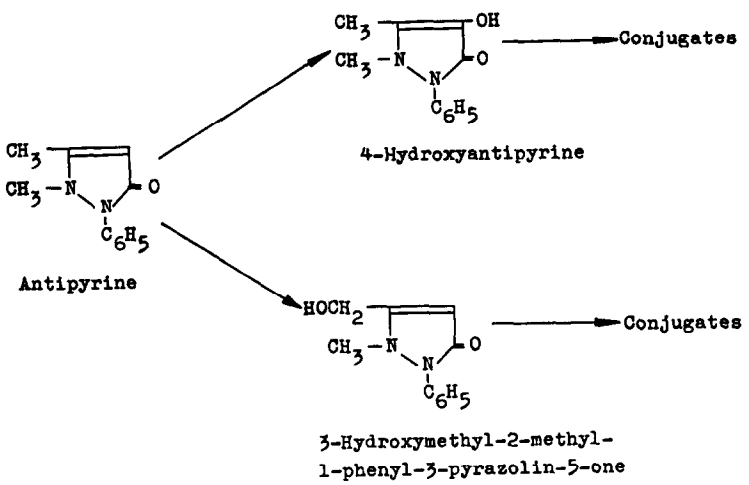

FIG. 2. The metabolic pathways of antipyrine.

the total dose administered. These values may not be the same as those obtained by quantitative estimation, but they do seem to reflect essentially the excretion pattern.

The presently demonstrated metabolic pathways of antipyrine are illustrated in Fig. 2.

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