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NEW METABOLITES OF ISOPROPYLANTIPYRINE IN THE RAT

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Isopropylantipyrine is an analgesic antipyrine analogue with the structure of 2,3-dimethyl-1-phenyl-4-isopropyl-pyrazolin-5-one (IPA). In a previous investigation on the metabolism of IPA (1), we identified one major and eight minor metabolites in rat urine. The enol glucuronide of N-desmethylisopropyl-antipyrine (3-methyl-1-phenyl-4-isopropylpyrazolin-5-one, desmethyl-IPA) was the major metabolite. All other identified metabolites were oxidation products of the phenyl or isopropyl group or the C-4 position, without the N-methyl group of the parent drug. During the course of a study on quantitative analysis of these metabolites, we have isolated two additional metabolites, which have been identified as the enol sulfate of 1-phenyl-3-methyl-pyrazolin-5-one and 1-phenyl-3-methyl-4-(1-hydroxyethyl)-pyrazolin-5-one. The present paper describes the isolation and identification of these two metabolites.

[H^3]-Isopropylantipyrine ([H^3]-IPA) was synthesized by tritiation of IPA by the method of Goromaru and Noda (2). [H^3]-IPA (s.a. 1.25 μCi/mg) was administered to three male Sprague Dawley rats (7-week old, 210 ± 3 g) at a single oral dose of 20 mg/kg as a suspension in 0.5% carboxymethyl cellulose. About 60-ml portion of the first 24-hr urine was applied on an Amberlite XAD-2 resin column (2 x 45 cm). The column was washed with 900 ml of distilled water and the metabolites of IPA were eluted with methanol (600 ml). The organic layer was evaporated under reduced pressure to give a brownish residue, which was dissolved in a small quantity of methanol. The solution was applied on t.l.c. plates (silica gel $60F_{254}$, 0.25 mm thick, Merck) and developed with a solvent system (A), ethylacetate-ethanol-acetic acid (6:3:1, v/v). A radioactive band on silica gel at Rf 0.88 was scraped from the plate under u.v. light and eluted with methanol. The eluted compounds were further separated on t.l.c. with a solvent system (B), chloroform-acetone-acetic acid (5:4:1, v/v). Three radioactive bands were detected at Rf values 0.42, 0.66 and 0.29, respectively and were extracted with methanol, separately.

The extract from the band at Rf 0.42 was subjected to high pressure liquid chromatography (Waters Model 6000A) on a μBondapack C_{18} column (3.9 mm ID x 3 cm). After elution with methanol-H_2O (1:4, v/v) at a flow rate of 1.8 ml/min, a radioactive peak appeared at 11.7 min and was collected (IM1). When IM1 was subjected to mass spectrometry (Hitachi 6MG Mass spectrometer), its spectrum showed intense peaks at m/e 174 (molecular peak), m/e 105 ($C_6H_5N_2$), m/e 91 (C_6H_5N) and m/e 77 (C_6H_5, base peak). These peaks suggested the presence of an intact phenyl group on pyrazolone ring (1) and the losses of both N-methyl and isopropyl groups from the parent drug. The fragmentation pattern was identical with that of authentic 1-phenyl-3-methyl-pyrazolin-5-one (desmethyl-desisopropyl-IPA, Tokyo Kasei, Tokyo). However, IM1 and desmethyl-desisopropyl-IPA exhibited different chemical characteristics in the following points. Firstly, IM1 was quite soluble in water whereas desmethyl-desisopropyl-IPA was soluble in chloroform and ethylacetate, with little solubility in water. Secondly, the migration on t.l.c. was different between IM1 and desmethyl-desisopropyl-IPA; Rf values with the solvent (B) were 0.42 and 0.78, respectively. Thirdly, unlike desmethyl-desisopropyl-IPA which can be eluted with water from an anion exchange column (Dowex 1 x 8, $HCOO^-$ form), IM1 was retained on the column during the washing and was eluted with 0.01N HCl. These findings suggested that IM1 was a conjugate of desmethyl-desisopropyl-IPA. In the mass spectrometer the conjugated moiety is likely to be split by heating. Upon hydrolysis with 6N HCl at 90°C for 1 hr, the hydrolysate was mixed with ethylacetate. The organic and aqueous layers were found to contain desmethyl-desisopropyl-IPA and sulfuric acid, respectively. The latter substance was confirmed by the formation of a white precipitate by an addition of $BaCl_2$ solution to the aqueous solution. In vivo incorporation of ^{35}S into the metabolite IM1 was confirmed by the methods of Dring et al (3) following an i.p. administration of $Na_2{}^{35}SO_4$ (The Radiochemical Center, Amersham) to rats prior to the administration of non-radioactive IPA. These results together would suggest that IM1 is sulfuric acid conjugate of desmethyl-desisopropyl IPA.

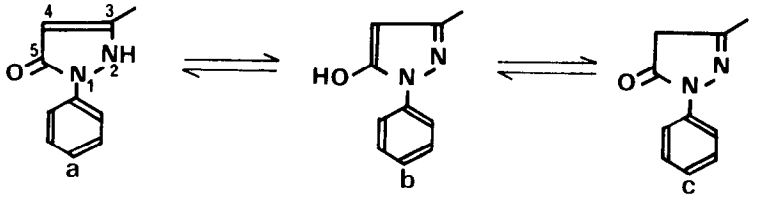

Fig. 1.

Desmethyl-desisopropyl-IPA can assume, theoretically, one of three tauto-

meric forms (Fig. 1). In these tautomers either N-2 (Fig. 1, a) or C-hydroxy group (Fig. 1, b) is capable of accepting sulfuric acid. If the N-2 position is linked to sulfuric acid, its u.v. spectrum should be similar to that of 1-phenyl-3,4-dimethyl-pyrazolin-5-one because of the fixed 3,4-double bond in both structures. In our study, however, the u.v. spectrum of IM1 in methanol showed an absorption peak at 242 nm, being identical with that of 1-phenyl-3-methyl-5-ethoxy-pyrazoline in which one double bond is fixed between N-2 and C-3 and another double bond between C-4 and C-5 (4). Based on these results IM1 was assigned to the enol sulfate of 1-phenyl-3-methyl-pyrazolin-5-one (Fig. 2).

The band at Rf 0.66 was separated into two radioactive metabolites on a short column of Dowex 1 ($HCOO^-$ form): one metabolite (IM2) was eluted with water and another (IM3) with 0.1 M HCl solution. Extraction of the metabolites from the respective fractions with ethylacetate and evaporation of the solvent gave colourless solid substances.

The nmr spectrum (Japan Electron Optics FX-100 Spectrometer, 99.6 MHz, I.S.: tetramethylsilane) of IM2 in $CDCl_3$ showed the presence of an intact phenyl group (multiplet, 7.27 - 7.67 ppm, 5H) and C-3 methyl group (singlet, 2.25 ppm, 3H). This spectrum is different from the nmr spectrum of IPA in 1) that the singlet peak found for IPA at 3.07 ppm due to N-methyl group disappeared in the spectrum of the metabolite, 2) that six methyl protons on isopropyl group were found at 1.28 ppm (doublet, J = 7.0 Hz) for IPA, whereas in the metabolite the number of methyl protons, which appeared at 1.28 ppm as a doublet signal (J = 7.0 Hz), decreased to three and 3) that instead of the septet signal centered at 2.84 ppm found in IPA due to the methyne proton on the isopropyl group, a quartet signal (J = 7.0 Hz) centered at 3.66 ppm was observed for metabolite IM2. The mass spectrum of IM2 showed a molecular ion at m/e 218 which indicated an addition of one oxygen atom and elimination of two methyl groups from IPA, and an intense peak at m/e 174 ($M - CH_3CHOH + H$). The latter peak would be derived via a 'McLafferty type' rearrangement. These spectral data are indicative that the structure of IM2 is 1-phenyl-3-methyl-4-(1-hydroxyethyl)-pyrazolin-5-one (Fig. 2).

For identification of IM3, the isolated metabolite was methylated with diazomethane. The mass spectrum of the methylated metabolite was identical with that of 1-phenyl-3-methyl-4-methylisopropionate-pyrazolin-5-one which was identified in our previous study (1).

Formation of both IM1 and IM2 requires cleavage of a C-C bond. This type

of biotransformation is relatively rare for xenobiotics as compared with the removal of alkyl groups attached to hetero atoms.

At present we have no direct evidence for the mechanism of formation of these two metabolites. However, the metabolite IM1 is unlikely to be produced by an oxidative cleavage of the C-C bond as was observed for the metabolism of stilbene (5) or β-oxidation, since the C-4 position of IM1 does not have an oxygen atom attached. An alternative mechanism, therefore, is responsible for the removal of the isopropyl group. One possible mechanism is an oxidative deethylation of hydroxyethyl group from IM2 (Fig. 2, route a) and another is an oxidative elimination of isopropyl group from a metabolite containing t-isopropanol group (Fig. 2, route b) although such a metabolite has not been isolated in the present study. A similar type of reaction has been described for the metabolism of 17α-ethynyl-17β-hydroxyestr-4-en-3-one (6).

The metabolite IM2 seems to be derived from the carboxylic acid (metabolite IM3) via a two step reaction (Fig. 2) involving both decarboxylation and hydroxylation of ethyl group. It has been reported that a carboxylic acid is an intermediate in the demethylation of 4α-methyl-5α-cholest-7-en-3β-ol (7) and N-t-butyl-norchlorcyclizine (8).

Further investigation on the mechanism of the cleavage reaction and elucidation of the structure of metabolite having Rf 0.29 are in progress.

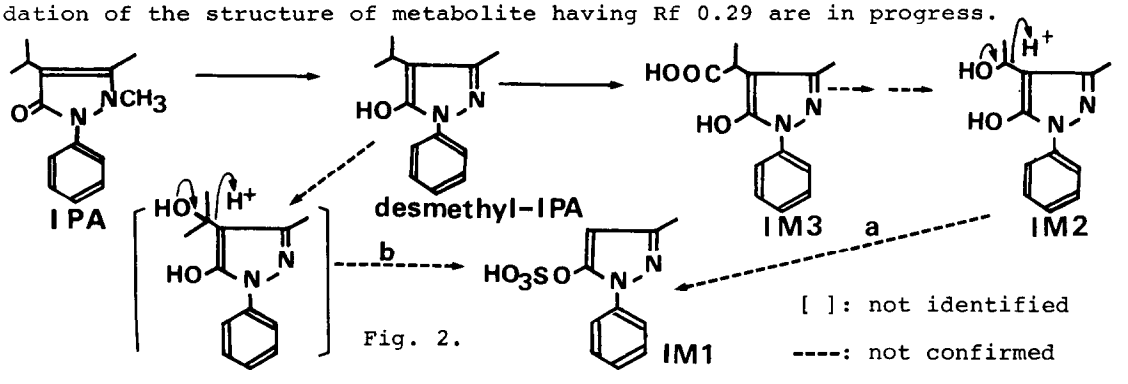

Fig. 2.

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