METABOLISM OF 8-METHOXYPSORALEN IN MAN:
IDENTIFICATION AND QUANTIFICATION OF 8-HYDROXypsoralen

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SUMMARY
8-Hydroxypsoralen (8-HOP) has been identified as a metabolite of 8-methoxypsoralen (8-MOP) in man. 8-HOP was present mainly as conjugates, the overall-urinary excretion being about 1% of given dose of 8-MOP. Less than 0.1% of 8-MOP was excreted unmetabolized. Enzymatic hydrolysis of urine liberated about 2% of 8-MOP.

INTRODUCTION
8-Methoxypsoralen (8-MOP, Figure 1) has during the last few years successfully been used in the therapy of psoriasis (1, 2, 3). Little information is available concerning the metabolism of 8-MOP in man. About 80% of given dose is reported to be excreted as hydroxyl or glucuronide derivatives (4) but the detailed structure of the metabolites has not been established. The present study is focused on the identification and quantification of 8-hydroxypsoralen (Figure 1) in man after oral administration of 8-MOP in therapeutic doses.

Fig. 1. – Structural formulae:
8-Methoxypsoralen (R = CH₃)
8-Hydroxypsoralen (R = H)

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MATERIALS AND METHODS
Apparatus
The gas chromatographic analysis was performed using a Varian 1400 instrument equipped with an electron capture detector (⁶³Ni). The chromatographic conditions are the same as used previously (5).

The mass spectrometric analysis was carried out with an LKB 2091 instrument (ionization energy 70eV). The gas chromatographic column was packed with 3% OV-17 on Gas Chrom Q 80/100 mesh.

Photometric measurements were performed with a Zeiss PMQ III Spectrophotometer.

Chemicals
8-Methoxypsoralen was supplied by AB Draco, Lund, Sweden.
8-Hydroxypsoralen was prepared by demethylation of 8-methoxypsoralen (6).
8-Ethoxypsoralen and 8-butoxypsoralen were prepared from 8-hydroxypsoralen and the corresponding alkyl iodide using the extractive alkylation technique (7).
8-Glucuronidase (Type L-11) and sulphatase (Type III) were obtained from Sigma Saint Louis, Missouri, U.S.A.

Tetabutylammonium hydrogen sulphate from Labkemi (Gothenburg, Sweden) was neutralized with...
sodium hydroxide prior to use. Phosphate and citrate buffers used had an ionic strength of 0.1 unless otherwise stated.

All solvents and reagents were of analytical grade.

**Determination of acid dissociation constant \( (k_{HA}) \) and partition coefficient \( (k_d) \)**

The acid dissociation constant of 8-hydroxypsoralen (8-HOP) was determined by photometric measurements (cf. 8) at 280 nm using phosphate buffers pH 6.3-7.0 (25°). The absorbance of the unionized and the ionized forms of 8-HOP were obtained in 0.1 M phosphoric acid and carbonate buffer pH 10.2 respectively.

The partition properties of 8-HOP were studied using methylene chloride as organic phase and phosphate buffers pH 7.7-8.3 as aqueous phases. The ratio \( k_{HA} \cdot k_d^{-1} \) was evaluated graphically from the slope of the linear plot \( D_{HA}^{-1} \) (partition ratio) versus \( a_{H+}^{-1} \). The partition coefficient was calculated from the ratio \( k_{HA} \cdot k_d^{-1} \) and the acid dissociation constant determined as described above.

**Hydrolysis of conjugates**

The hydrolysis of glucuronide conjugates was performed by mixing 1.00 ml of urine and 1.00 ml of 0.1 M citrate buffer pH 3.8 (containing 0.1 M phosphate) with 4 mg of \( \beta \)-glucuronidase. The mixture was incubated for 48 h at 37°.

Sulphate conjugate were determined after mixing 1.00 ml of urine with 1.00 ml of citrate buffer pH 4.95 and 1 mg of sulphatase. The mixture was incubated for 48 h at 37°.

**Identification of 8-hydroxypsoralen**

Urine (10.0 ml) was hydrolyzed as described above using 40 mg of \( \beta \)-glucuronidase and extracted with methylene chloride (20.0 ml). The organic phase was re-extracted with carbonate buffer pH 10.9 (1.5 ml) and the aqueous phase was mixed with 1 M tetrabutylammonium sulphate (0.10 ml), methylene chloride (0.20 ml) and ethyl iodide (0.050 ml). The mixture was shaken for 60 min at 25° and the organic phase was analyzed by gc-ms.

**Quantification of 8-hydroxypsoralen**

Urine (2.00 ml) was mixed with 1M phosphoric acid (0.100 ml) and extracted with methylene chloride (6.00 ml). The organic phase (5.00 ml) was separated and extracted with carbonate buffer pH 10.9 (1.50 ml). The aqueous phase (1.00 ml) was mixed with 1 M tetrabutylammonium sulphate in buffer pH 10.9 (0.100 ml), 1.00 ml of methylene chloride containing 8-butoxypsoralen as internal standard and 0.100 ml of methyl iodide. The mixture was shaken for 60 min and the organic phase was evaporated. Methanol (0.025 ml) and 0.200 ml of 0.1 M NaOH were added and the solution was left for 10 min at 25°. The solution was extracted once with 1 ml of methylene chloride (discarded) and twice with 1 ml of toluene (discarded). The aqueous phase was mixed with 0.050 ml of toluene and 0.025 ml of 12 M HCl and extracted for 3 min. Part of the organic phase (2 µl) was injected into the gas chromatograph equipped with an electron capture detector.

**Quantification of 8-methoxypsoralen**

Urine (2.00 ml) was mixed with 0.50 ml of phosphate buffer pH 7.0 and 5.00 ml of methylene chloride. The organic phase (3.00 ml) was mixed with 0.200 ml methanol containing 8-butoxypsoralen and evaporated to dryness. The analysis was then performed as above: methanol (0.025 ml) and...

**Administration of 8-methoxypsoralen**

8-Methoxypsoralen tablets (Neomeladinine 15 mg) were obtained from Memphis Chemical Co. (Cairo, Egypt). 45 mg were given orally to five healthy volunteers (weight 60-75 kg). Urine was collected during 24 h.

**RESULTS AND DISCUSSION**

**Identification of 8-hydroxypsoralen (8-HOP)**

A total ion current recording of a urine extract analyzed according to the experimental section is given in Figure 2. The mass spectra of peak 1 (Figure 3A) and the ethyl derivative of synthetically prepared 8-HOP (Figure 3B) are almost identical thus establishing 8-HOP as a metabolite of 8-MOP in man.

**Quantification of 8-hydroxypsoralen**

8-HOP was separated from 8-MOP by solvent extraction. The determinations were performed after methylation using gas chromatography with electron capture detection according to the method given for 8-MOP (5).

**Quantification of 8-methoxypsoralen**

8-HOP was extracted from urine at low pH with methylene chloride and separated from 8-MOP by re-extraction into buffer pH 10.9. A quantitative recovery was obtained in both steps as calculated from the partition coefficient \( (k_d=32) \) and the acid dissociation constant \( (pK_{HA}=6.72) \).

The handling of 8-HOP in alkaline solutions should be kept to a minimum. About 1% of 8-HOP is decomposed at pH 10.9 and 30% in 0.1 M NaOH in 5 min (25°) as determined by photometric measurements.