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Note

Identification of 1,3-diphenyl-2-aminopropane metabolites by gas chromatography, gas chromatography with Fourier transform infrared spectroscopy and gas chromatography—mass spectrometry

Z. JUVANCZ*, M. LEDNICZKY, L. IMRE, M. BIHARI* and M. VAJDA

Central Research Institute for Chemistry of the Hungarian Academy of Sciences, Pusztaszeri ut 57—69, H-1025 Budapest (Hungary)

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1,3-Diphenyl-2-aminopropane (DAP), which was synthesized for use as a central nervous stimulant [1], has been used as a model compound for the study of the liver monooxygenase system [2].

In the present study, the metabolites of DAP were isolated from bile and urine, and after enzymatic hydrolysis purified by column chromatography and thin-layer chromatography (TLC). The prepurified sample was analysed by gas chromatography (GC), gas chromatography with Fourier transform infrared spectroscopy (GC—FTIR) and gas chromatography—mass spectrometry (GC—MS). The following metabolites have been identified by comparison with authentic samples: 1-(4′-hydroxyphenyl)-3-phenyl-2-aminopropane, 1,3-diphenylpropane-2-on-oxime, 1,3-diphenylpropane-2-on, 1,3-diphenyl-2-hydroxypropane.

EXPERIMENTAL

Samples of bile (0—5 h) and urine (0—24 h) were hydrolysed enzymatically with β-glucuronidase—arylsulphatase (isolated from *Helix pomatia*, Calbiochem) [3] in an acetate buffer (0.1 *M*, pH 4.5). The metabolites were then prepurified on Amberlite XAD-2 resin, using methanol for the final elution [4]. The resin was purified before use. One volume of resin was stirred for 1 h with each portion of the following washing solutions: twice with 5 vols. of methanol—concentrated hydrochloric acid (100:1), twice with 5 vols. of

*Present address: Chemical Works of Gedeon Richter Ltd., Gyömröi ut 17, H-1475 Budapest, Hungary.

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methanol—water—acetone (45:45:10), and finally three times with 10 vols. of water (distilled twice from glass, or HPLC quality) [5]. The purified resin was stored under methanol.

The methanolic eluates of the samples were divided into two parts, and evaporated to dryness in a nitrogen stream. The first part of the sample was dissolved in 200 μl of ethyl acetate, and chromatographed on a short column. The column, constructed in the laboratory from PTFE, was 5 × 0.5 cm and packed with Merck LiChroprep Si 60 (25—40 μm). The column was first eluted with 10 ml of ethyl acetate to eliminate impurities, followed by 5 ml of ethanol which eluted the metabolites. The samples obtained were analysed by on-line GC—FTIR [6, 7] after derivatization. For this purpose flash methylation with Methelute (Pierce) was used [8].

The second part of the divided sample was chromatographed on home-made TLC plates (Kieselgel HF_{254}, Merck 20 × 20 cm, 0.25 mm thickness). The metabolites were separated from polar impurities using benzene—ethanol (5:1) as solvent. Separation from apolar compounds was achieved after elution of the spots and rechromatography in benzene—diethyl ether (1:1) on another plate. Spots were detected by ultraviolet light. The spots were eluted from the plates and the solutions analysed by capillary GC. The metabolites were identified by GC—MS.

Apparatus

For GC—FTIR a Varian 3700 GC coupled with a Nicolet 7199 FT-IR was used [9—11]. The column was of silanized glass, 2 m × 2 mm, packed with 3% SE-30 on Chromosorb W AW DMCS (100—120 mesh). The carrier gas was nitrogen at a flow-rate of 20 ml/min. Temperatures were: injector 300°C, transfer line 305°C, light pipe 305°C; temperature programme was 210—290°C (8°C/min).

A Perkin-Elmer F22 was used for capillary GC with flame-ionization or nitrogen—phosphorus detection. The column, 25 m × 0.25 mm, was of Pyrex glass coated with SE-30. Carrier gas was nitrogen at a flow-rate of 3 ml/min. Injections were made in the splitless mode (split closed 30 sec) [12]. Temperatures were: injector 240°C, detector 240°C; temperature programme was 1 min at 70°C, then 70—140°C (30°C/min), 140—205°C (4°C/min), 16 min at 205°C.

For GC—MS a Pye-104 gas chromatograph was coupled with an MM-12 F 1A (V.G. Micromass) type single-focusing mass spectrometer. The column, 10 m × 0.25 mm, was of Pyrex glass coated with OV-101. Carrier gas was helium at 2 ml/min. Injections were splitless. Temperatures were: injection 205°C, ionization chamber 200°C; temperature programme was 5 min at 95°C, then 95—145°C (25°C/min), 145—200°C (5°C/min), 20 min at 200°C. The mass spectra of the samples were recorded using electron-impact ionization of 70 eV and 3 kV accelerating voltage.

RESULTS

GC and TLC data suggested that the major metabolic pathway produced an aromatic hydroxylated metabolite. We could not determine the exact position

of the hydroxyl group in the aromatic ring by GC—MS. In order to determine the exact position of the hydroxyl group we used on-line GC—FTIR; however, the sensitivity of the GC—MS method was better by two orders of magnitude than GC—FTIR in our hands. The GC—FTIR results were obtained as Chemigrams™. The Chemigram is produced by the Nicolet instrument on-line, in real time, by monitoring five preselected wavelength regions (windows) and these are recorded both on the screen and by the plotter [9, 10]. Five windows were selected corresponding to the expected bands of functional groups (e.g. 850—750 ν window corresponds to *para*-substitution), and the infrared absorbances were monitored in these windows (Fig. 1). The complete infrared spectra of the main metabolite and 1-(4′-hydroxyphenyl)-3-phenyl-2-aminopropane standard were identical.

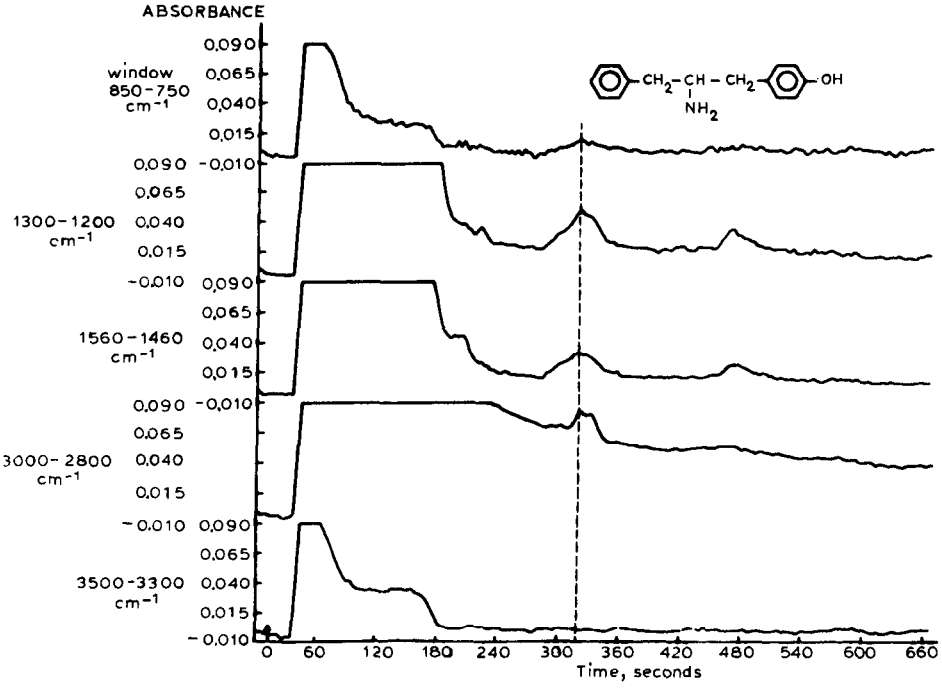

Fig. 1. Chemigram of DAP main metabolite (t_R = 310 sec) 1-(4′-hydroxyphenyl)-3-phenyl-2-aminopropane. Windows correspond to the expected functional groups. For parameters see text.

Flash methylation was chosen as the derivatization method for several reasons. In our laboratory flash methylation is routinely used. Preliminary experiments were made with trifluoroacetyl derivatives, but experiments were not promising in case of the biological samples, of which we had only limited quantitaties. We favoured flash methylation against trifluoroacetylation to avoid evaporation of a further solvent, which further contaminated the samples. The samples were already contaminated with the residues of several solvents and chemicals used during preceding operations. Methylation was not complete under our experimental conditions. The amino group was only partially derivatized, therefore double peaks were found. This did not influence

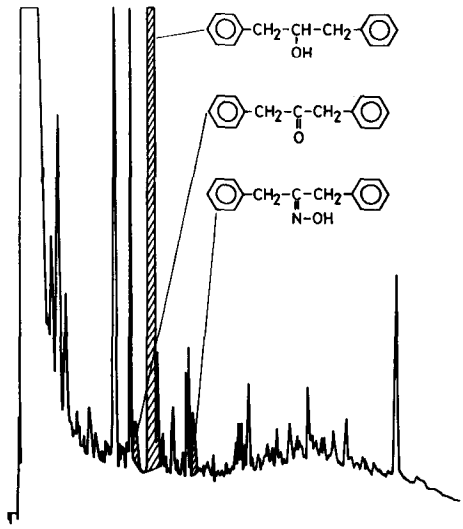

Fig. 2. One of the typical glass capillary GC analyses (flame-ionization detection) for minor metabolites in a urine extract. For parameters see text.

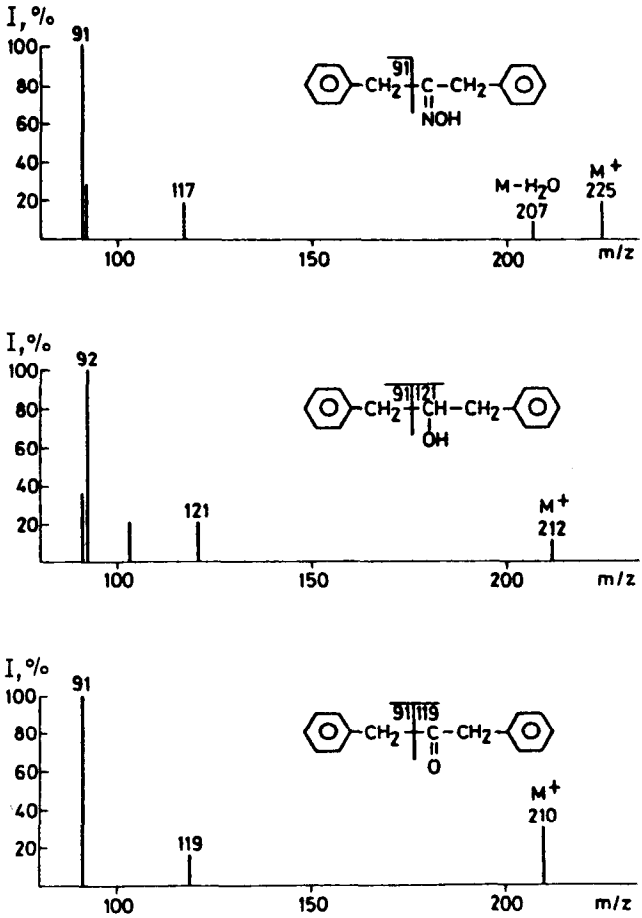

Fig. 3. Mass spectra of the minor metabolites. For parameters see text.

the identification of 1-(4′-hydroxyphenyl)-3-phenyl-2-aminopropane. The presence of another hydroxylated compound was shown by GC—MS, but its amount was insufficient for identification by GC—FTIR. According to TLC R_F values and GC retention times, one of the trace materials is probably 1-(2′-hydroxyphenyl)-3-phenyl-2-aminopropane by comparison with standard compound.

Some other minor metabolites eluted from TLC spots were analysed by glass capillary GC (Fig. 2) and GC—MS. The identification was based on comparison with synthetic standards. We found the following compounds: 1,3-diphenylpropane-2-on-oxime, 1,3-diphenylpropane-2-on, 1,3-diphenyl-2-hydroxypropane. The mass spectra of these compounds are shown in Fig. 3.

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