METABOLIC STUDIES OF TETRABENAZINE, A PSYCHOTROPIC DRUG IN ANIMALS AND MAN

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Abstract—Following administration of tetrabenazine (2-oxo-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo[a]quinolizine) to animals or man, 9 metabolites of the drug were detected in the urine by thin layer chromatography. Five of these compounds were found unconjugated and 4 conjugated with glucuronic acid.

The structures of all 5 unconjugated metabolites and of 2 aglucones were established either by comparison in thin layer chromatography with synthetic compounds or by NMR mass spectroscopy and elemental analysis of metabolites isolated by column chromatography on silicic acid.

The main steps of biological degradation of the drug are:

reduction of the keto group at C_2

oxidation at position 2′ of the isobutyl side chain

selective ether cleavage at C_9, followed by conjugation of the phenols to glucuronic acid.

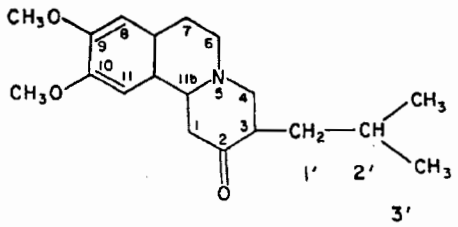
The same pattern of metabolite formation was observed in the rabbit, in the dog and in man. In all three species the glucuronides represent the prevailing form of excretion of the drug in the urine.

A general scheme for the biological degradation of tetrabenazine is suggested.

A description of the synthesis of several metabolites is given.

1. INTRODUCTION

THE PHARMACOLOGY of tetrabenazine, 2-oxo-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo [a] quinolizine, has been extensively investigated.[1] Like



reserpine, tetrabenazine depletes monoamine stores in tissues; unlike this drug, however, it acts more specifically on brain stores and does not affect gastric secretion, intestinal motility or blood pressure.

The distribution of the drug in animal tissues has been studied in the rabbit[2] and, using a highly sensitive and specific spectrofluorimetric method[3], in the guinea pig[4]. The present paper deals with the identification and characterization of a number of tetrabenazine metabolites; an attempt is made to specify the many biological pathways along which these substances are formed.

2. METHODS

1. *Species investigated and mode of administration*

The drug was administered intraperitoneally to male rabbits (30 mg/kg) and to female dogs surgically prepared for bladder catheterization (18 mg/kg) or subcutaneously to man (2 mg/kg) in the form of its methanesulfonate salt. In both animal species the urine was collected by catheterization. After urine collection specimens were immediately frozen.

2. *Extraction of tetrabenazine and its unconjugated metabolites I, II, III, IV and V from urine*

The first 24-hr urine specimen was concentrated to about $\frac{1}{5}$ its volume under reduced pressure at +40° and extracted twice with 6 volumes of benzene, an adequate amount of sodium sulfate and magnesium oxide mixture (5:1 w/w) being added to saturate the aqueous phase and to adjust its pH to 10. The benzene extracts were separated, filtered and combined.

3. *Isolation of conjugated metabolites of tetrabenazine*

A. *Separation of the glucuronides.* The aqueous phase left behind by the benzene extraction of the free metabolites was stored at +4°. The major portion of sodium sulfate which had crystallised was filtered off together with magnesium oxide. The pH of the filtrate was adjusted to 4 with glacial acetic acid and the glucuronides were isolated according to Kamil, Smith and Williams.[5]

B. *Enzymatic cleavage of the glucuronides.* The solution containing the glucuronides in their acid form was ice-cooled and brought to pH 4·5 with conc. ammonia, and buffered with $\frac{1}{10}$ volume of 0·1 molar sodium acetate. For each 10 ml solution 20,000 units β-glucuronidase in the form of Glusulase* were added in 3 portions over a 24-hr period of incubation at 37°.

C. *Extraction of the aglucones VI, VII, VIII and IX.* The incubated solution of the glucuronides was saturated under agitation with sodium sulfate, magnesium oxide was added to raise the pH to ~ 10 and the aglucones extracted with 6 volumes of benzene.

4. *Thin layer chromatographic analysis of tetrabenazine metabolites*

Benzene extracts of the free metabolites (see under 2.2.) or the aglucones (see under 2.3.C.) were evaporated to dryness under reduced pressure. For thin layer chromatography weighed residues or the reference compounds were dissolved in methanol:benzene (1:1) respectively to 0·5 or 0·1 per cent concentration, and 10–20 μl applied on the chromatogram.

Chromatography was done on silicic acid chromatoplates (silica gel G, Merck) prepared according to Stahl.[6] The following systems were used for development:

(a) t-amylalcohol† : di-*n*-butylether : 0·25 % aq. NH_4OH (80:7:13)
(b) chloroform : *n*-butanol : 2·5% aq. NH_4OH (80:20:0·6)
(c) toluene : acetone : 25% NH_4OH (50:50:1)
(d) cyclohexanol saturated with water.

* Glusulase (suc d'Helix pomatia): Industrie biologique française, Gennevilliers, Seine, France.
† t-Amylalcohol: "Chemische Fabrik Schweizerhalle".

Visualization and identification. Spots were made visible in u.v. light (350–360 mμ) by spraying the chromatogram with a mercuric acetate reagent (200 mg mercuric acetate in 90 ml methanol + 10 ml acetic acid). The chromatoplates were then heated at 110° for 10 min in a drying oven. Under these conditions tetrabenazine and all mentioned metabolites are converted to dehydro-compounds of high molecular fluorescence intensity, so that even amounts as small as 0·1 μg can be detected on the chromatogram. Furthermore, the difference in the colour of fluorescence desplayed by each compound provides further means of identification (see Table 1). While the ketonic compounds, tetrabenazine, metabolite III and their corresponding phenolic aglucones VI and IX show a clear blue fluorescence, the alcoholic compounds II and III and their corresponding phenolic aglucones VII and VIII appear as gray or yellow spots. Metabolites IV and V on the other hand are of a darker blue (see Table 1.)

The phenolic aglucones show in chromatographic system (a) very similar R_f values to those of their corresponding methyl ethers: in system (b) however they can easily be differentiated from one another. Dibromoquinone chlorimide reagent was also used to detect phenols.

Column chromatography. When larger amounts of the metabolites were needed for the purpose of identification, column chromatography on silicic acid was used. Columns were packed with silicic acid* according to Marvel and Rands,[7] except that instead of chloroform a mixture consisting of equal portions of peroxide free isopropylether† and chloroform saturated with water were used and the column washed with isopropylether saturated with water.

The benzene extracts from the urine containing the free metabolites the aglucones respectively (see under 2.2 and 2.3.) were evaporated to dryness. The residue left by the extract containing the free metabolites was taken up in the minimum quantity of isopropylether–chloroform (1:1)‡, and put on the top of the column. Development of the chromatogram was carried out with isopropylether, isopropylether–chloroform, chloroform and chloroform–*n*-butanol mixtures of increasing polarity. All phases were saturated with water. Single fractions were collected, evaporated to dryness and analysed separately by thin layer chromatography (see under 2.4.). In a typical chromatogram tetrabenazine was eluted with isopropylether–chloroform (2:1)

I with isopropylether-chloroform (1:2)
II with chloroform
III with chloroform + 2% *n*-butanol
IV with chloroform + 10% *n*-butanol
V with chloroform + *n*-butanol (1:1).

5. *Separation of ketonic and non-ketonic material*

The material present in the urine extracts (free metabolites or aglucones) can be separated into a ketonic and a non-ketonic fraction, according to Girard[8] using (Carbazoylmethyl) trimethylammonium chloride.

* Silicic acid, 100 mesh, Mallinckrodt, anal. reagent.

† Isopropylether Shell was passed through a column of neutral aluminium oxide activity I the day of use.

‡ In the case of the aglucones the residue was dissolved in chloroform to which was added a few drops of methanol.

TABLE 1

Compound		Tetra-benazine	Unconjugated metabolites I	II	III	IV	V	Conjugate metabolites Aglucones VI	VII	VIII	IX
Separation with Girard T reagent		ketonic			ketonic			ketonic			ketonic
Fluorescence after treatment with mercuric acetate (see under 2.4.)	In solution: Activation maxima (mμ):	330	385	385	360	380	380				
	Fluorescence maxima (mμ):	450	500	500	500	500	500				
	in TLC, system (a): colour	blue	yellow	white gray	blue	dark blue	dark blue	blue	ye'low	yellow	blue
R_x values in TLC, system (a)*		1	0·83	0·69	0·53	0·41	0·2	1	0·83	0·65	0·55
R_x values in TLC, system (b)		1	0·69	0·33	0·44	0·20	0·10	0·97	0·34	0·2	0·2
R_x values in TLC, system (c)		1	0·77	0·85	0·57	0·38	0·32	0·85	0·55	0·64	0·36
R_x values in TLC, system (d)		1	0·38	0·30	0·23	0·12	0·06	1·2	0·54	0·51	0·33

* R_x values can vary to a large extent in this system according to the source of *t*-amylalcohol used. *t*-amylalcohol from 'Chemische Fabrik Schweizerhalle', Switzerland, was used in these experiments.

6. *Spectra*

(a) Ultra-violet spectra were taken in analytical grade ethanol or ethanol made up to contain 0·01 N HCl or NaOH resp.

(b) NMR spectra were taken in $CDCl_3$ using a Varian A-60 NMR-spectrometer.

(c) Mass-spectra were taken with an MS 9 from AEI, Manchester, England.

7. *Oxidation of metabolite IV with aluminium isopropylate*

Eight mg of metabolite IV obtained by column chromatography (see under 2.4.) and 400 mg aluminium isopropylate were dissolved in 2 ml toluene and 2 ml cyclohexanone and heated in a sealed tube for 36 hr at 140°C. After cooling the reaction mixture was extracted with N H_2SO_4. The sulfuric acid extract was then re-extracted with benzene after adjusting the pH to 10 with magnesium oxide. The reaction product was separated from unchanged material by thin layer chromatography. The proper band was eluted by agitation with 2 ml dist. water, 0·5 g of MgO + Na_2SO_4 (1:5) and 20 ml of benzene. The benzene extraction was repeated and the combined benzene extracts concentrated under vacuum. The residue (5 mg), a colourless film, was found pure when analysed by thin layer chromatography.

3. RESULTS AND DISCUSSION

1. The direct benzene extract from the urine of tetrabenazine treated animals (rabbit, dog or man), when analysed by thin layer chromatography, showed 5 distinct fluorescent spots which will be designated here as metabolites I, II, III, IV and V.

Metabolites I and II

When this extract was chromatographically analysed, metabolites I and II were found to be identical in 4 chromatographic systems with the two synthetic isomers of 2-hydroxy-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo [a] quinolizine.

When I was administered to rabbits or to dogs instead of tetrabenazine, only one unconjugated metabolite was formed which corresponded chromatographically to metabolite IV of tetrabenazine; similarly following administration of II only one unconjugated metabolite was formed which corresponded to metabolite V of tetrabenazine (see Fig. 1 and scheme of tetrabenazine metabolism, on p. 650).

Metabolite IV

This was isolated, from the urine of dogs which had received I, by column chromatography on silicic acid (see under 2.5.). It was found pure when analysed by thin layer chromatography. It formed a colourless oil and when dried under high vacuum solidified to a slightly coloured film. The substance showed a strong affinity for water. Analysis was therefore performed after 48 hr drying at 50° in high vacuum over phosphorus pentoxide

$C_{19}H_{29}NO_4 + 0{\cdot}35$ mole H_2O (mol. wt. = 341·76)

	calc.	found
C	66·77	67·04
H	8·76	8·90
O	20·36	20·07
H_2O	1·84	2·14

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