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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.

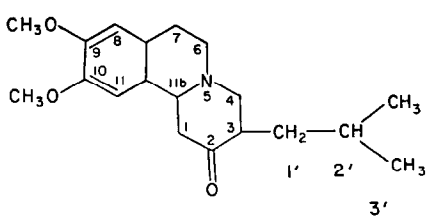
The same pattern of metabolite formation was observed in the rabbit, in the dog and in man. In all three species the glucuronides represent the prevailing form of excretion of the drug in the urine.

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 $\sim$ 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

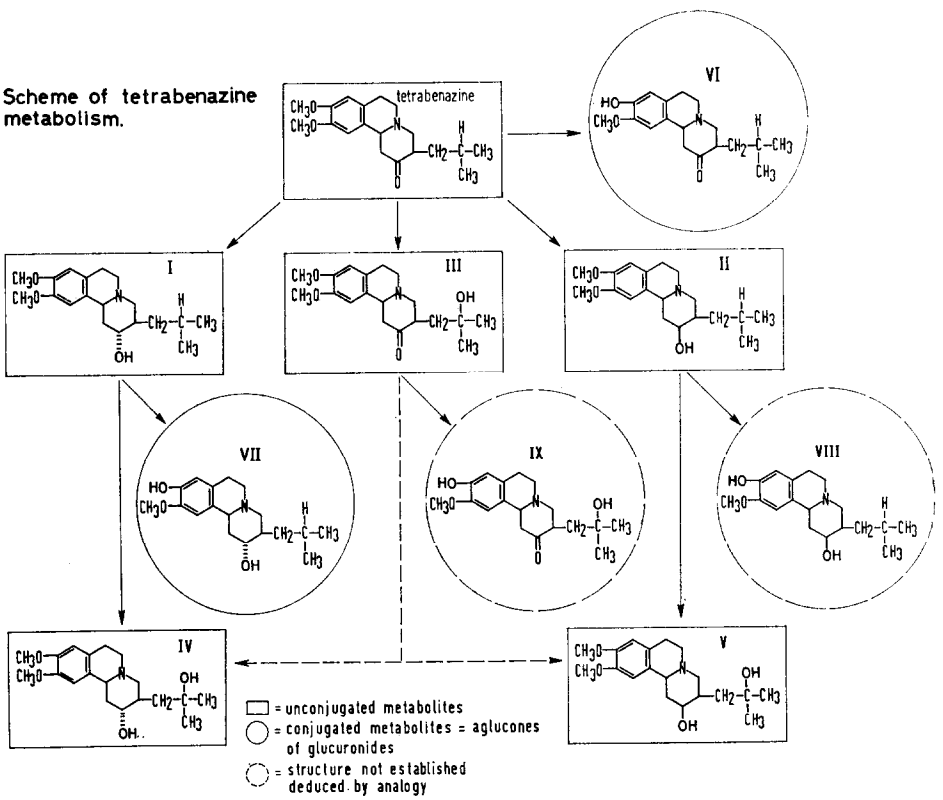
$C_{19}H_{29}NO_4$ + 0·35 mole H_2O (mol. wt. = 341·76)

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

The correct molecular weight of IV was determined by high resolution mass spectroscopy. Due to the high intensity of the peak at m/e 334 (M–H), the measurement was done on this peak:

found	334·2016	±0·0013
calc.	334·2018	for C_{19} H_{28} N O_4

The elemental composition of the molecule is therefore C_{19} H_{29} N O_4 and its molecular weight = 335·209



On the basis of these results the presence of two alcoholic groups in the molecule must be assumed. One alcoholic group is that already present in metabolite I, the metabolic precursor of IV, whereas the position of the second alcoholic group could be ascertained by nuclear magnetic resonance. The NMR spectra of metabolite I and metabolite II show doublets for the isopropyl groups at 0·85 and 0·92 ppm (J = 6·5 c/s), whereas in the case of metabolite IV the doublet is replaced by a single band at 1·20 ppm (J = 6·0 cps). One must therefore assume that in metabolite IV the second alcoholic group is located in the side chain, as indicated above.

Similarly *metabolite V* was isolated by column chromatography from the urine of dogs which had received II. Nuclear magnetic resonance of this compound showed a single band at 1·30 ppm indicating that in this metabolite also the proton in the isobutyl side chain is replaced by a tertiary alcoholic group.

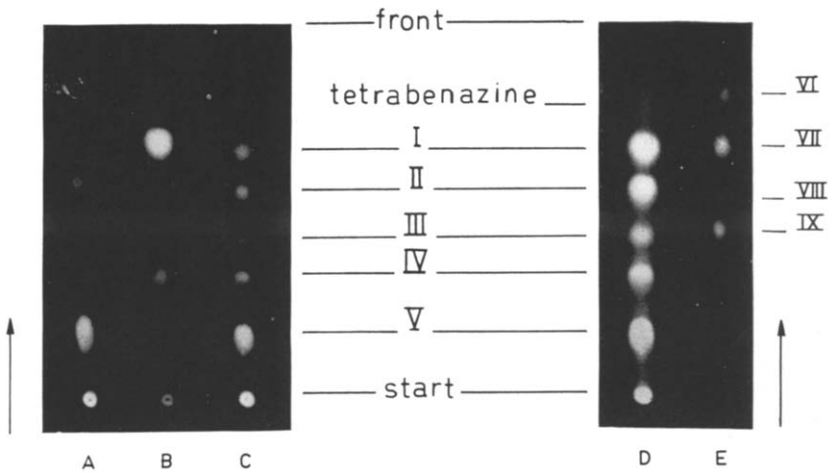


FIG. 1.

A. Rabbit urine: unconjugated metabolite following administration of II
B. Rabbit urine: unconjugated metabolite following administration of I
C. and D. Rabbit urine: unconjugated metabolites following administration of tetrabenazine.
E. Rabbit urine: aglucones of glucuronides following administration of tetrabenazine.

Spot at start was not always observed and may be an artifact.

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Metabolite III

Since upon administration of compound I or compound II no formation of metabolite III was observed one may conclude that III must be metabolically derived from tetrabenazine to which it is structurally related in a different way than are I and II. In fact when the bulk of the free metabolites was treated with Girard T reagent a ketonic fraction was isolated which contained III together with unchanged tetrabenazine, while all other free metabolites (I, II, IV and V) remained in the non-ketonic fraction. One may therefore conclude that the ketonic group of tetrabenazine remains unaltered in metabolite III. This is further substantiated by the fact that reduction of III with lithiumaluminiumhydride gives rise to the formation of IV and V. On the other hand when IV was oxidised with aluminium isopropylate under drastic conditions (see 2.7) the main reaction product could be separated from the unchanged material by thin layer chromatography. Chromatographic analysis in 3 systems showed it to be identical with metabolite III. Its molecular weight was determined by high resolution mass spectroscopy. Due to the high intensity of the peak at m/e 332 (M–H), the measurement was done on this peak:

found	332·1866 ± 0·0030
calc.	332·1861 for $C_{19}H_{26}NO_4$

and its molecular weight = 333·194.

The elemental composition of the molecule is therefore $C_{19}H_{27}NO_4$. The cracking pattern, similar to that of tetrabenazine, was found to be in agreement with the proposed structure (see scheme p. 650).

The same general pattern of metabolite formation was found when the urine of rabbits, dogs or humans was examined in thin layer chromatography.

2. *The conjugated metabolites (aglucones)*

The bulk of the aglucones obtained after enzymatic hydrolysis of the glucuronides isolated from the urine of animals which had received tetrabenazine (see under 2,3) showed the presence of at least 4 compounds which after treatment with mercuric acetate fluoresce intensely (see Fig. 1) These aglucones are not found in control urine of the same animals. We shall designate them as metabolites VI, VII, VIII and IX (formula: see scheme p. 650).

The bulk of the aglucones as well as the single aglucones, isolated by column chromatography, show in the u.v. spectrum a shift of the maximum from 285 mμ in acid or neutral ethanol to 300 mμ in alkaline ethanol, indicating the presence of a phenolic group.

Administration of I or II to rabbits or dogs showed that VII is issued from I, VIII from II.

When the aglucones are treated with Girard T reagent compounds VI and IX are found predominantly in the ketonic, VII and VIII in the non-ketonic fraction.

The presence of a ketonic function in VI and IX indicates that they originate from tetrabenazine and metabolite III.

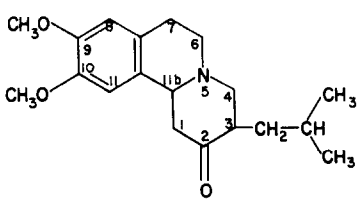
Phenols corresponding to the metabolites IV and V were usually not seen (Fig. 1). One can assume that these metabolites because of their more polar character are excreted as such, and thus escape further metabolic transformation.

Structure. The aglucones isolated from the urine of tetrabenazine-treated animals were partially separated by column chromatography on silicic acid. One of the main fractions obtained deposited crystals upon standing for several weeks in the refrigerator. The mother liquor was decanted and the crystals washed several times with ice-cold petroleum ether and benzene. Thin layer chromatography of this material showed a single component corresponding to the aglucone VII. After reaction with diazomethane in ether the alkaline-acid shift was no longer observed and the product of the reaction was found to be identical in thin layer chromatography to metabolite I.

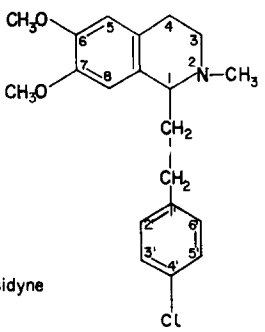
The definitive structure of these crystals (=VII) was established by comparison in thin layer chromatography: it was found identical in all systems to the synthetic compound 2,9-dihydroxy-3-isobutyl-10-methoxy-1,2,3,4,6,7-hexahydro-11bH-benzo-[a]quinolizine with which it melted without depression.

On the other hand VI was found identical in thin layer chromatography with the synthetic compound 2-oxo-3-isobutyl-9-hydroxy-10-methoxy-1,2,3,4,6,7-hexahydro-11bH-benzo[a]quinolizine hydrochloride (see scheme p. 650). One may assume that it is metabolically formed directly from tetrabenazine.

On the basis of the established structure for VI and VII the same phenolic structure may be postulated for VIII and IX, aglucones which would derive from II and III respectively. We have shown previously[9] that Versidyne® (=racemic 1-*p*-chlorophenethyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline) and one of its metabolites (N-desmethyl-Versidyne) undergo in the organism a selective ether cleavage in position 6 of the isoquinoline ring structure.



Tetrabenazine



Versidyne

More recently Koe and Pinson[10] studied the metabolism of Quantril (=2-acetoxy-3-diethylcarbamoyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo[a]quinolizine) and, on the basis of theoretical considerations, tentatively placed the phenolic OH of their aglucones at C-9. In view of our own findings with Versidyne and tetrabenazine we feel confident that the same selective ether cleavage may occur *in vivo* in the case of a larger number of isoquinoline compounds.

According to Axelrod[11] enzymes which degrade various aromatic ethers are present in the microsome fraction of the liver of a number of vertebrates. Such enzymes may well be responsible for the selective cleavage of ether linkage in this series of compounds, as was observed for Versidyne[9] and tetrabenazine. These aglucones could not be detected free in the urine, one may therefore conclude that they are immediately

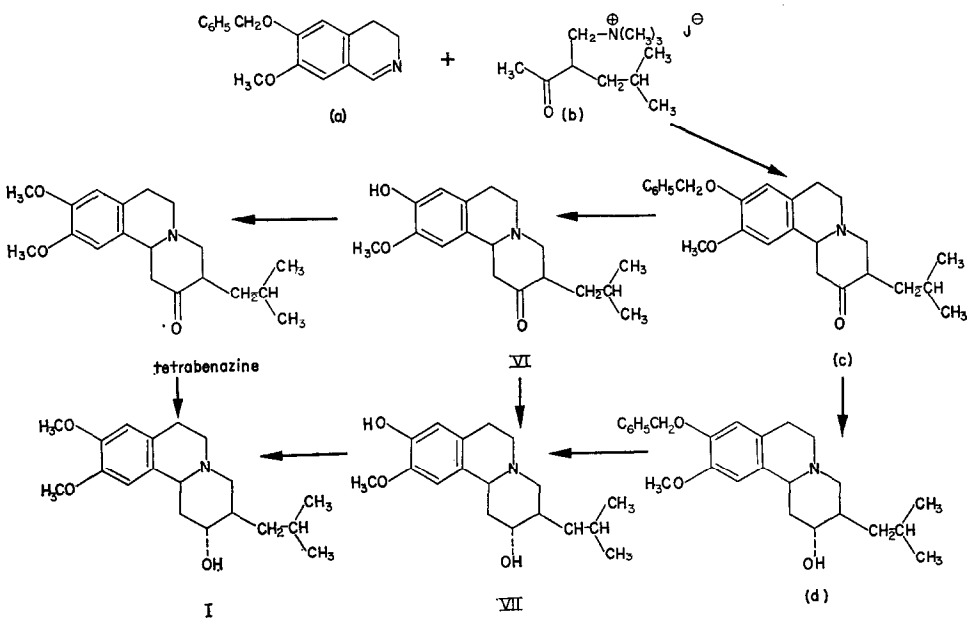
conjugated in the organism. A quantitative analysis of each metabolite was not attempted in the case of tetrabenazine since labelled material was not available for this study. We may say however that while tetrabenazine can be detected only in trace amount, its unconjugated metabolites in somewhat larger amounts the glucuronides represent the prevailing form of excretion of the drug in the urine.

Metabolites I, II and V, pharmacologically tested for ethanol potentiation, were found to be less active than tetrabenazine.[15]

SYNTHESIS OF METABOLITES

1. *Introduction*

The synthesis of both isomeric alcohols I and II has already been described.[12] Both the benzoquinolizines VII and VI were synthetized according to the following scheme.[13]



The condensation product of 6-benzyloxy-7-methoxy-3,4-dihydroisoquinoline (a)[14] with the quaternary salt (b) gives the benzoquinolizine-derivative (c), which after debenzylation gives the phenol VI. Etherification of this compound with diazomethane leads to tetrabenazine. The reduction of the ketone (c) with sodium borohydride yields only the trans-alcohol (d) (OH *trans* to the substituent at C-3), which after debenzylation leads to the phenolic compound VII. Alternatively one can obtain the phenolic compound VII by reduction of the ketone VI with sodium borohydride. Etherification of VII gives the *trans*-alcohol I.

2. *Experimental*

2-Oxo-3-isobutyl-9-benzyloxy-10-methoxy-1,2,3,4,6,7-hexahydro-11bH-benzo[a]quinolizine (*c*): 20 g 6-Benzyloxy-7-methoxy-3,4-dihydroisoquinoline (a) (m.p. 101°), 25·8 g 3-Dimethylaminomethyl-5-methyl-hexan-2-one methiodide (b) (m.p. 179–180°) were heated in 500 ml ethanol for 1 hr at 100° (oilbath).

After addition of potassium hydroxide (20 g) and water (200 ml) the solution was evaporated under reduced pressure. The residue was extracted twice with 500 ml of chloroform and the resulting extract was washed with saturated sodium chloride solution (2 × 50 ml), dried over sodium sulfate, filtered and distilled. The red-brown residual oil (26 g) was purified by column chromatography using 10 times the quantity of aluminium oxide (Act. II). 8·5 g of a lightbrown oil was eluted with benzene, it crystallized upon addition of a small amount of isopropyl ether. Two further crystallizations from ethyl acetate gave colourless crystals, m.p. 132·5–133° (5·8 g).

$C_{25}H_{31}NO_3$ (mol. wt. 393·5) calc. C 76·3 H 7·9%
found C 76·2 H 7·9%
u.v. spectrum (ethanol) $\lambda_{max} = 282$ mμ, log $\epsilon = 3{\cdot}62$
$\lambda_{max} = 286$ mμ, log $\epsilon = 3{\cdot}62$
i.r. spectrum (KBr pellet): 5·91 μ (C = O)

2-Oxo-3-isobutyl-9-hydroxy-10-methoxy-1,2,3,4,6,7-hexahydro-11bH-benzo[a]quinolizine, Hydrochloride (*VI*). 1 g (c) was dissolved in ethanol (100 ml) and hydrogenated in presence of 5% palladium on charcoal (200 mg) at room temperature. After the theoretical amount of hydrogen was absorbed (1½ hour) the catalyst was filtered and the filtrate evaporated. The hydrochloride was crystallized from ethanol-ether, white crystals, m.p. 217–219° (dec.) separated (940 mg).

$C_{18}H_{25}NO_3$.HCl calc. C 63·6 H 7·7 Cl 10·4%
(mol. wt. 339·9) found C 63·6 H 7·6 Cl 10·3%
u.v. spectrum (ethanol): $\lambda_{max.} = 286$ mμ, log $\epsilon = 3{\cdot}60$

The above phenolic base (20 mg) was dissolved in ether (20 ml) and treated with an excess of diazomethane overnight. After evaporation of the solvent the residue was crystallized from a small quantity of isopropyl ether to obtain colourless crystals, m.p. 126° which gave no depression with an authentic sample of tetrabenazine.

100 mg VI was dissolved in ethanol (5 ml) and reduced with sodium borohydride (100 mg) for 4 hr at room temperature. After evaporation of the solvent the oily residue was taken up in methylenechloride. The methylenechloride extract was dried over magnesium sulfate, filtered and evaporated. The crystalline residue, after two crystallizations from isopropyl ether, had m.p. 168° (VII).

2-Hydroxy-3-isobutyl-9-benzyloxy-10-methoxy-1,2,3,4,6,7-hexahydro-11bH-benzo[a]-quinolizine (*d*). 1·5 g of the benzyloxyketone (c) was dissolved in 100 ml ethanol and reduced with 500 mg sodium borohydride for 4 hr at room temperature. After evaporation of the solvent, the residue was treated with 50 ml water and extracted with chloroform (3 × 50 ml). The chloroform extract was washed with saturated sodium chloride solution (2 × 10 ml), dried over sodium sulfate and evaporated. The residue crystallized on the addition of isopropyl ether. The base was recrystalized twice

from a mixture of ethyl acetate and petroleum ether (40–60°) to give 1·25 g of light yellow crystals, m.p. 175–177°.

$C_{25}H_{33}NO_3$	calc.	C 75·9	H 8·4%
(mol. wt. 395·5)	found	C 75·6	H 8·3%

u.v. spectrum (ethanol): $\lambda_{max.} = 281$ mμ, log $\epsilon = 3{\cdot}61$
$\lambda_{max.} = 285$ mμ, log $\epsilon = 3{\cdot}61$
i.r. spectrum (KBr pellet): 3·22 μ (OH)

2,9-*Dihydroxy*-3-*isobutyl*-10-*methoxy*-1,2,3,4,6,7-*hexahydro*-11*bH*-*benzo*[*a*]*quinolizine* (*VII*). 1 g (d) was dissolved in ethanol (100 ml) and hydrogenated in presence of 5% palladium on charcoal (200 mg) at room temperature. After the theoretical amount of hydrogen was absorbed (1½ hr) the catalyst was filtered off and the filtrate evaporated. The crystalline residue was crystallized from methanol–ethyl acetate to give 745 mg whitish crystals, m.p. 192·5–193°.

$C_{18}H_{27}NO_3$. 0·5 CH_3OH	calc.	C 69·1	H 9·1	O 17·4	OCH_3 14·6%
	found	C 68·6	H 9·1	O 17·5	OCH_3 14·6%.

The above phenolic base (20 mg) was dissolved in ether (20 ml) and treated with an excess of diazomethane overnight. After evaporation of the solvent the residue crystallized upon addition of ethyl acetate to give pale yellow crystals, m.p. 168° which showed no depression with a sample of authentic I, prepared from tetrabenazine.

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