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# METABOLIC STUDIES OF VERSIDYNE*, A NEW ANALGESIC, IN THE RABBIT AND IN MAN

D. E. SCHWARTZ, H. BRUDERER, J. RIEDER and A. BROSSI

Experimental Medicine and Chemical Research Departments,
F. Hoffman-La Roche & Co. Ltd., Basle

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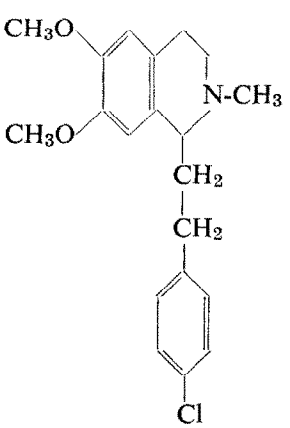
**Abstract**—In man and rabbit, four metabolites of Versidyne (racemic 1-*p*-chlorophenethyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline) were identified by comparison with synthetic substances. One of these metabolites, N-desmethyl Versidyne, was demonstrated to be present in small quantities in the free form in blood and urine. The other three are 6-O-desmethyl derivatives of Versidyne and of N-desmethyl Versidyne. Two of these are found in large quantities in urine, chiefly in the form of their glucuronides. In both species they account for the elimination of a major portion of the drug. The corresponding 7-O-desmethyl derivatives were not found. This suggests that Versidyne and at least one of its metabolites are selectively demethylated by the organism at the 6-position.

On the basis of the present findings, a pathway for the biological degradation of Versidyne in man and rabbit is suggested.

## INTRODUCTION

VERSIDYNE is racemic 1-*p*-chlorophenethyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (see formula below). It has been clinically investigated in the form of the free base (Ro 4-1778/1) and as its hydrochloride (Ro 4-1778).




Partially hydrogenated 1-aralkyl-2-methyl-isoquinolines closely related in chemical structure to the opium alkaloid laudanosine possess analgesic activity in animals.[1, 2] In the case of Versidyne the analgesic effect has been confirmed in man.[3, 12–16] These findings led us to investigate more closely the metabolism of the drug. It is known that

* Versidyne = registered trade mark for Ro 4-1778.

tetrahydro-isoquinolines are dehydrogenated when treated with mercuric acetate, giving rise to fluorescent compounds.[4] Whatever the products formed in this reaction may be, we found that, under appropriate conditions, a number of tetrahydro-isoquinolines can be converted into fluorophors of definite structure, many of which have a high molecular fluorescence intensity.[5]

In the studies here reported this highly sensitive and specific method has been applied to gain insight into the metabolism of Versidyne.

## I. SPECIES INVESTIGATED

1. *Rabbits*

Male rabbits weighing 2–3 kg received a single intraperitoneal injection of 20 mg Versidyne base per kg.

*Urine and feces.* The animals were maintained on water only in metabolic cages which permitted exact separation of urine and feces. They were catheterized twice daily, after which they were fed outside the metabolic cages for 1 hr. Each animal's 24-hr urine was pooled and immediately frozen. Subsequently, a 3-day pool was made of these 24-hr specimens, using an aliquot of each specimen.

2. *Humans*

Healthy male volunteers on normal diet were given a single oral dose of 120 mg Versidyne base.

*Urine.* Quantitative 24-hr specimens were collected for 3 days following administration of the drug and immediately frozen. Subsequently a 3-day urine pool was prepared using an aliquot of each 24-hr specimen.

*Blood.* Blood collected 30, 60, 120 and 240 min after administration was rendered incoagulable by addition of 1·5 ‰ potassium oxalate.

## II. QUALITATIVE EVIDENCE FOR THE PRESENCE OF THE DRUG AND SOME OF ITS METABOLITES IN BIOLOGICAL SAMPLES

1. *Extraction of Versidyne and its free metabolites from urine*

A portion of the 3-day urine pool (see I.1 and I.2) was concentrated to about 1/10 of its original volume under reduced pressure at 40°. The concentrated urine was extracted with 3 vol. of peroxide-free isopropylether*, an adequate amount of a sodium sulfate and magnesium oxide mixture (5 : 1 w/w) being added to saturate the aqueous phase and to set its pH at 10.

2. *Isolation of the tetrahydro-isoquinoline moieties from their conjugates in the urine*

These substances were extracted either after enzymatic cleavage or after acid hydrolysis of the conjugates. For detailed description of conditions see under III. (*c*). The glucuronides were also isolated according to the procedure of Kamil, Smith and Williams.[6]

3. *Identification of metabolites*

Identification of Versidyne metabolites was carried out by comparison with synthetic compounds (see Figs. 1–4) using thin-layer chromatography and electrophoresis.

* Isopropylether was used immediately after passage through an aluminium oxide column (activity I)

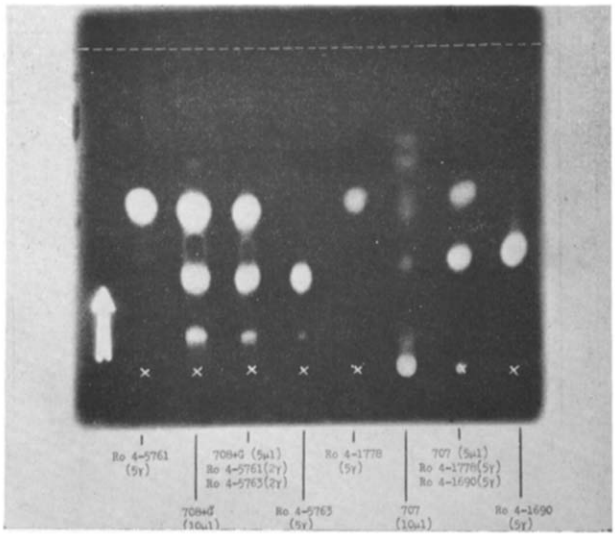



FIG. 1. Chromatoplate: silicic acid. System (a): *t*-butylalcohol–di-*n*-butylether–2% aq. $NH_4OH$ (70:20:7·5)

Urine extracts of Versidyne-treated rabbit:

707 = Free metabolites of 3-day pooled urine sample. Extraction with isopropylether at pH 10.

708 + G = Aglucones of glucoronides from 3-day pooled urine sample: isolation of glucuronides using technique of Kamil, Smith and Williams, incubation with β-Glucuronidase, extraction with benzene at pH 10.

Ro 4-5761, 4-5763, 4-1778 and Ro 4-1690 = synthetic reference compounds.

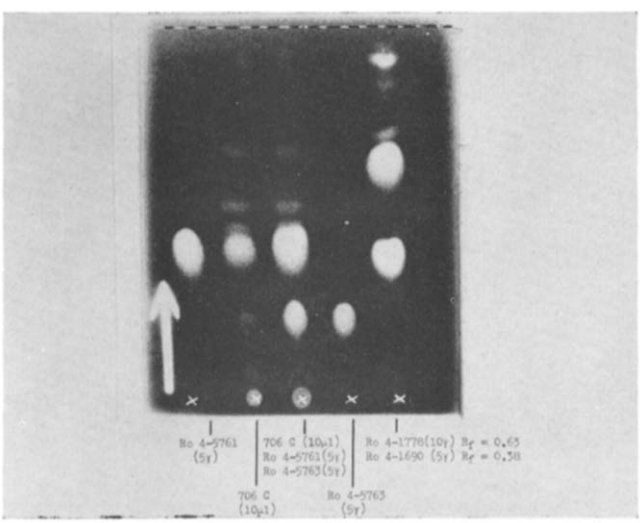



FIG. 2. Chromatoplate: silicic acid. System (b): chloroform–*n*-butanol–2% aq. $NH_4OH$ (70:30:0·6)

Urine extracts of Versidyne-treated rabbit:

706 C = Aglucones of glucoronides (3-day pooled urine sample); isolation of glucuronides using technique of Kamil, Smith and Williams, followed by hydrolysis with N hydrochloric acid and extraction at pH 10.

Ro 4-5761, 4-5763, 4-1778 and Ro 4-1690 = synthetic reference compounds.

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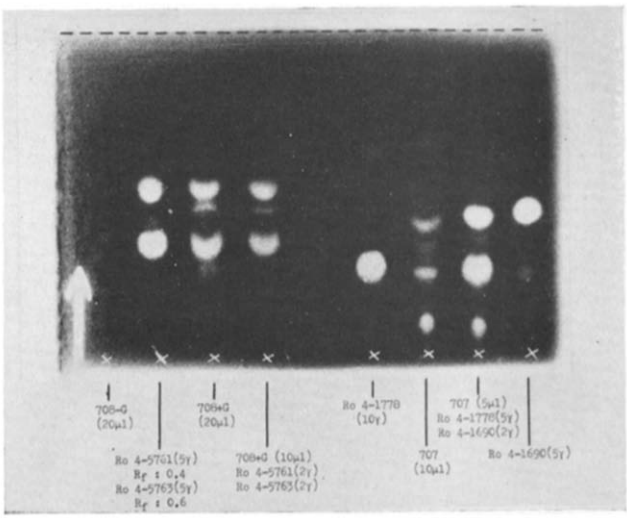



FIG. 3. Chromatoplate: silicic acid. System (d): *n*-butanol–formic acid–water (84:8:8).
Urine extracts of Versidyne-treated rabbit
707 – Free metabolites of 3-day pooled urine sample. Extraction with isopropylether at pH 10.
Glucuronides isolated from 3-day pooled urine sample:
708 – G – Extracted with benzene at pH 10 after incubation *with* β-Glucuronidase.
708 – G – Extracted with benzene at pH 10 after incubation *without* β-Glucuronidase.
Ro 4-5761, 4-5763, 4-1778 and Ro 4-1690 – synthetic reference compounds.

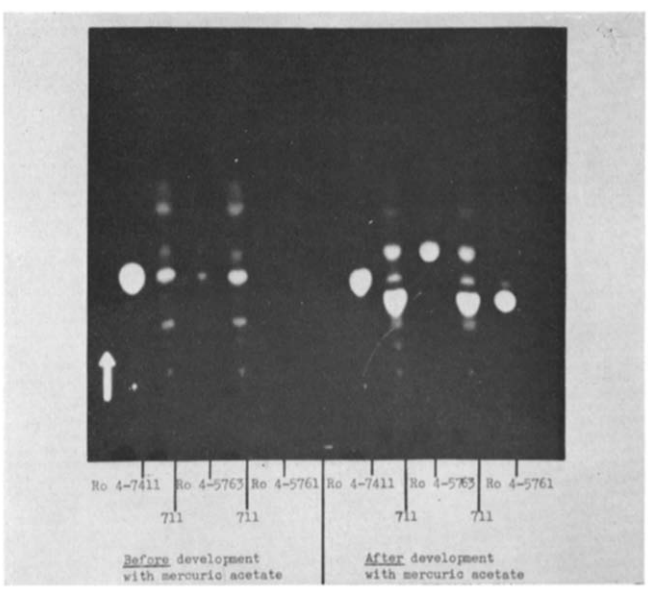



FIG. 4. Chromatoplate: silicic acid. System (d): *n*-butanol–formic acid–water (84:8:8).
711 – Urine extract of Versidyne-treated rabbit:
Extraction with benzene at pH 10 after incubation of urine with Glusulase.
Ro-5671, Ro 4-5763 and Ro 4-7411 – synthetic reference compounds.

*Preparation of samples*

Immediately after extraction, isopropylether or benzene extracts (see II. 1 and II.2) were evaporated to dryness at room temperature and under low pressure. Weighed residues or reference compounds were dissolved in methanol–benzene (1 : 1) to 0·2–1 % final concentration.

For chromatography 10–20 $\mu$l, for electrophoresis 20–30 $\mu$l of solutions of the extract or the reference compounds were deposited on the chromatoplate.

*Thin-layer chromatography*

Silicic acid chromatoplates according to Stahl[7] were used. Development of the chromatograms was carried out in the following systems:

(a) *t*-butanol—di-*n*-butylether—2 % aq. $NH_4OH$ (70 : 20 : 7·5).

(b) chloroform—*n*-butanol—2 % aq. $NH_4OH$ (70 : 30 : 0·6).

(c) cyclohexanol, water saturated.

(d) *n*-butanol—formic acid—water (84 : 8 : 8).

*Electrophoresis*

Clean electrophoretic separation of Versidyne and its metabolites on paper is difficult because the capillarity of the paper causes diffuse spreading of spots during migration. This disadvantage is avoided when chromatoplates are used. Moreover, silicic acid chromatoplates in acid pH range exhibit a smaller electro-osmotic effect than paper, so that the distance for migration can be more fully utilized for separation. The following conditions were finally selected:

*Support:* Silicic acid chromatoplate.

*Electrolyte:* (Johnson and Lindsay's Universal buffer, modified):
2·003 g citric acid monohydrate, 0·589 g boric acid, 1·298 g $KH_2PO_4$ and 1·755 g diethylbarbituric acid were dissolved in 500 ml *n*-propanol + 400 ml distilled water. After adjusting the pH to 5·0 with 0·2 N decarbonated NaOH, the solution was brought to a volume of 1 l. with distilled water.

*Current:* 0·2–0·3 mA; potential: 450 V; running time: 6 hr.

*Paper electrophoresis apparatus:* Pfleuger Electrorheophor, Pfleuger Ltd., Brussels, Belgium.

Substances to be separated were applied on the chromatoplate. The chromatoplate was sprayed with the electrolyte and immediately placed horizontally in the electrophoresis apparatus. The plate was then connected to the electrode vessels by means of filter paper strips impregnated with the electrolyte.

*Visualisation of Versidyne and its metabolites*

Mercuric acetate spray reagent: 200 mg of mercuric acetate were dissolved in 90 ml methanol + 10 ml glacial acetic acid (the spray reagent can be stored at room temperature for months).

After complete removal of the solvent from the plate by a current of dry air, the plate was sprayed with the reagent and heated for 10 min at 100° in a ventilated drying oven. Verisdyne and the metabolites mentioned below are converted by this procedure to dehydro-compounds,* which fluoresce strongly in long-wave u.v. light

* Although some of the substances under consideration appear as slightly fluorescent spots after the plate has been dried, use of the mercuric acetate reagent mentioned is necessary. Metabolite I, for example, becomes visible only under its influence, while the fluorescence of the other spots is greatly intensified.

(350–360 mμ). The limit of sensitivity after chromatographic or electrophoretic separation for Versidyne lies between 0·5 and 1 μg, for its metabolites even lower. Phenols were made visible with a freshly prepared *Dibromoquinone chlorimide spray reagent:* 100 mg dibromoquinone chlorimide were dissolved in 100 ml methanol.

Phenolic substances of this group also appear as yellow spots when mercuric acetate spray reagent is used (see above). This is not the case for the corresponding non-phenolic compounds.

## III. QUANTITATIVE DETERMINATION OF VERSIDYNE AND SOME OF ITS METABOLITES*

The spectrofluorimetric method of determination used here is based on dehydrogenation with mercuric acetate. This enables all compounds bearing an intact tetrahydro- or dihydro-isoquinoline ring structure, including a number of Versidyne metabolites, to be identified.

Versidyne and its metabolites differ in their solubility in water (conjugated and unconjugated), in their $pK_a$ values (Versidyne = 6·8 ± 0·1 and metabolite I = 8·1 ± 0·1) as well as in the ease with which they undergo dehydrogenation with mercuric acetate. On the basis of all these differences it was possible (see methods below) to determine separately each of these compounds with adequate accuracy and specificity.

### (*a*) *Unchanged Versidyne*

Unchanged Versidyne was selectively extracted from blood plasma or urine with peroxide-free isopropyl ether after adjusting the pH accurately to 5·0 (phosphate buffer). The drug was re-extracted from isopropyl ether with 0·1 N $H_2SO_4$. An aliquot of this extract was heated with an equal volume of mercuric acetate reagent† for 20 min at 100°. Fluorescence was measured at activation wavelength 365 mμ and fluorescence wavelength 460 mμ. This procedure allowed 80–85% recovery with a sensitivity of 0·3 μg for 1-ml samples.

### (*b*) *Unchanged Versidyne + metabolite I*

Urine was extracted directly, blood after dilution with 3 vol. of 0·3 M *o*-phosphoric acid. Whole animals were homogenized after removal of fur and paws in a blendor-type homogenizer with 5 parts (w/v) chilled 0·3 M *o*-phosphoric acid. The homogenate was agitated for 20 min, heated for another 20 min in a boiling water bath, cooled and centrifuged. The supernatant alone was used in the subsequent extraction.

Samples (urine, blood or tissue) were extracted with 3 vol. of peroxide-free isopropyl-ether, an adequate amount of sodium sulfate and magnesium oxide (5 : 1 w/w) being added to saturate the aqueous phase and to adjust its pH to 10.

Both substances were re-extracted from isopropylether with 0·1 N $H_2SO_4$. An aliquot of this extract was then heated for 30 min at 130° in a sealed ampule with an equal volume of mercuric acetate reagent. In this way both substances developed their maximum fluorescence intensity. Samples were measured with simultaneously treated standard solutions of Versidyne and Ro 4-1690 (metabolite I) at activation wavelength 365 mμ and at fluorescence wavelength 460 mμ. The procedure gives for both substances

* For lack of space only the principle of these determinations will be given. A more detailed description of these methods can be supplied upon request.

† *Mercuric acetate reagent of pH* 6·0: 53·6 g $CH_3COO/Na.3H_2O$ + 3 ml glacial acetic acid, make up to 100 ml with dist. water, then add 250 mg $Hg(CH_3COO)_2$.

an extraction yield between 90 and 95% with a sensitivity better than 0·3 mμ for 1-ml samples.

The concentration of metabolite I in the sample can be calculated by substraction of the fluorescence value obtained following procedure III (*a*) from that obtained following procedure III (*b*).

(*c*) *Conjugated metabolites II, III and IV* (*structure see p.* 785)

In order to free the tetrahydro-isoquinoline moiety of these conjugated metabolites, the urine was first treated with glusulase or acid.

For *enzymatic cleavage* of the conjugates, 10-ml urine samples were mixed with 5 ml 0·2 M sodium acetate buffer solution (pH 4·5). Glucuronides or glucuronides + sulfates were quantitatively hydrolysed by the action of 20,000 units of β-glucuronidase* or glusulase† (containing 20,000 units β-glucuronidase and 10,000 units sulfatase) respectively. These amounts of enzymes were added in 3 equal portions during an incubation period of 24 hr. Samples were incubated in a Dubnoff bath at 37°.

For *acid hydrolysis* of the conjugates (glucuronides and sulfates), 10-ml samples of urine were mixed with 2·5 ml of 5N $H_2SO_4$ solution and heated for 4 hr at 100° in sealed, nitrogen-filled ampules.

*Extraction of Versidyne metabolites after hydrolysis of the conjugates.* Acid hydrolysates were ice-cooled and neutralized with concentrated ammonia with continuous stirring. Extraction of the neutralized acid hydrolysates or of samples incubated with enzyme was performed with 3 vol. of benzene after adjusting the pH to 10 with MgO and saturating the aqueous phase with sodium sulfate.

Benzene extracts were used for both qualitative (see II.2) and quantitative assays of the metabolites. For quantitative determination benzene extracts were re-extracted with 0·1 N $H_2SO_4$. An aliquot of the sulfuric acid extract was mixed with an equal volume of mercuric acetate and the fluorescence measured ($F_a$). This value allowed estimation of the amount of metabolite IV present in the sample. The same solution was then transferred to an ampule which was sealed and heated for 30 min at 130°. After cooling, the fluorescence developed in the sample was compared with those of simultaneously developed standard solutions of Ro 4-5761 (metabolite II) and of Ro 4-5763 (metabolite III) at activation wavelength 365 mμ and fluorescence wavelength 460 mμ ($F_b$). The difference between the two fluorescence values ($F_b - F_a$) can be ascribed to the metabolites II and III. The contribution of each of these two metabolites to this fluorescence difference was further determined spectrofluorimetrically following their separation by thin layer chromatography.‡

## IV. RESULTS

(*a*) *Qualitative results*

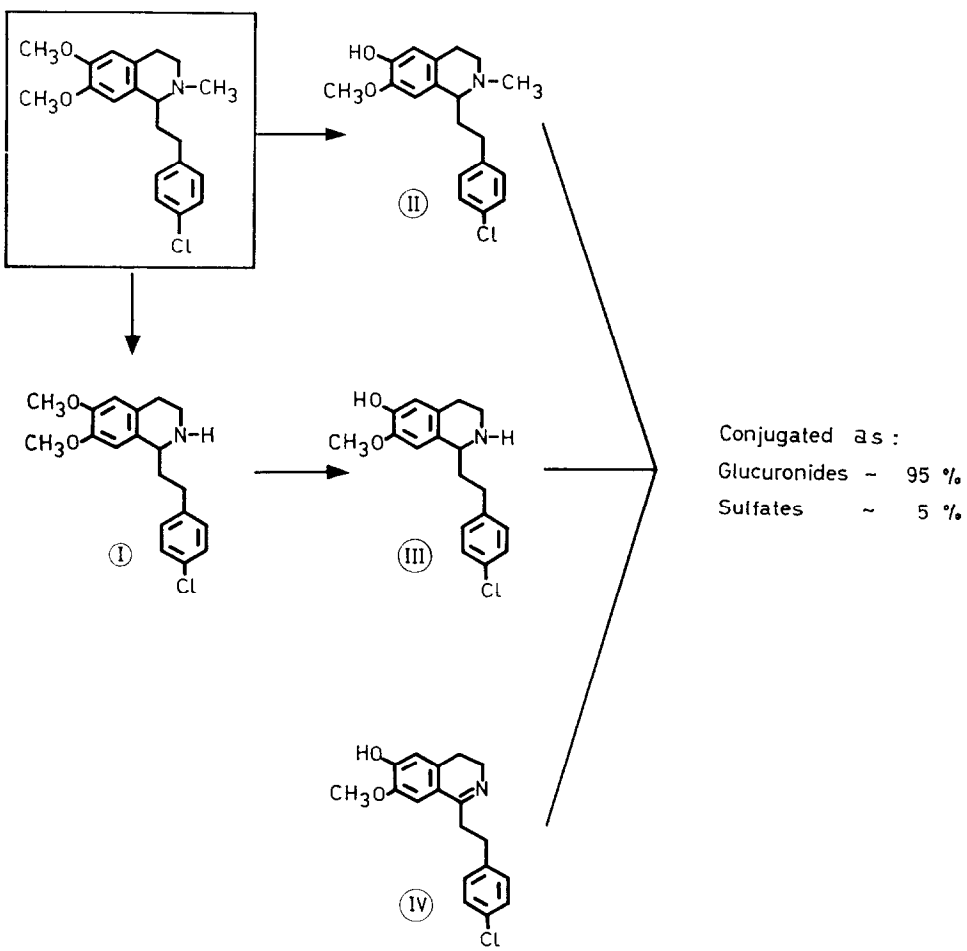
In addition to Versidyne, two free metabolites were found in blood and in urine. One of these, metabolite I, has been identified by chromatography as N-desmethyl Versidyne (see Figs. 1 and 3). The second of these free metabolites represents a much more polar compound, of still unknown structure.

* β-Glucuronidase: Schering A.G., Berlin.

† Glusulase (suc d'Helix pomatia): Industrie biologique française, Gennevilliers, Seine, France.

‡ Silicic acid chromatoplate, solvent system b (see 11.3), elution with chloroform and re-extraction with 0·1 N $H_2SO_4$.

Three further metabolites of Versidyne, largely conjugated with glucuronic acid, were found in rabbit and human urine; their aglucones were identified chromatographically and electrophoretically with the synthetic compounds Ro 4-5761, Ro 4-5763 and Ro 4-7411 (see Figs. 1–4). Metabolite IV has a *dihydro*-isoquinoline structure, which explains its native fluorescence, whereas metabolites II and III are, like Versidyne, *tetrahydro*-isoquinolines and therefore fluoresce only after dehydrogenation with mercuric acetate (see Fig. 4). In this connection, it should be particularly emphasized that the three last-mentioned metabolites (II, III and IV) have a 6-hydroxy-7-methoxy structure in common. The corresponding compounds with methoxy in 6 and hydroxy in 7 position (Ro 4-5595 and Ro 4-5762 see formulae, p. 785) were not found either in free or conjugated form in our extracts. The present results suggest the following scheme for the biological degradation of Versidyne in man and rabbit:




*(b) Quantitative results*

*Blood.* After oral administration of 120 mg Versidyne base to healthy male volunteers, mean concentrations (4 individuals) of unchanged Versidyne ranged between 0·02 and 0·1 μg/ml whole blood when determined on large volume samples in the

time interval between 30 and 240 min after treatment. The half-life in whole blood in these subjects was about 2 hr (procedure: see III. (*a*).*

The amount of metabolite I appearing in the blood was of the same order as that of Versidyne (procedures: see III (*a*) and (*b*)).

*Urine.* In rabbit and human urine* the unchanged drug and its free metabolite I appeared only in minute quantities. On the other hand a large proportion of the drug was found to be eliminated in the form of glucuronides of metabolites II, III and IV (see Table 1). The amount of these metabolites excreted in the urine fell rapidly, as seen from Table 2. Metabolite II formed the major fraction of the known Versidyne metabolites (33–55% of Versidyne administered), while III, which is formed via I, occurred in somewhat smaller quantities, and IV only to about 2–5%. The ratio of metabolite II: metabolite III was larger in man than in the rabbit (see ratio Table 1). (Procedure: see III (*c*)).

In both species about 95% of the metabolites II, III and IV occurred in the form of the glucuronides, the remaining 5% being probably bound to sulfuric acid. Formation of 6-0-desmethyl derivatives of Versidyne and their subsequent glucuronidation therefore represents a major pathway of biological degradation of the drug in both species.

*Discussion of results*

Versidyne is subject to N- and O-desmethylation in the human and rabbit organism. These two reactions occur under the influence of enzymes probably mainly in the liver.

It has been shown by Axelrod[10] that enzymes which degrade various aromatic ethers are present in the microsome fraction of the liver of a number of vertebrates. It is of interest in this respect to note that in the case of Versidyne and some of its metabolites, the methyl ether at $C_6$ is selectively demethylated, while that at $C_7$ is not.

From Table 1 it can be seen that in man and in the rabbit more than half of the administered drug is eliminated in the urine within 3 days. The question arises, however, as to the fate of the remainder. It may be that the tetrahydro-isoquinoline ring is opened. Metabolites of this type, if formed, would not be detected by our spectrofluorimetric method.

Little is yet known as to the significance of the occurrence of metabolite IV (Ro 4-7411). From the chemist's point of view it seems reasonable to assume that dehydrogenation of the isoquinoline ring in position 1–2 would favor demethylation of the 6-methoxy group and that therefore dehydrogenation in position 1–2 might precede O-demethylation. Should this represent the true sequence of reactions which occurs in the organism it would then be necessary to assume that O-demethylation is followed by reduction of the double bond of the isoquinoline ring, since the amount of tetrahydro-compound (metabolite III) formed is much larger than that of the corresponding dihydro-compound (metabolite IV) (see IV (*b*)). However, the following observation militates against such a chemical assumption: if, instead of Versidyne, compound Ro 4-4461 (see formula p. 785) is administered intraperitoneally to rabbits, considerable amounts of metabolite IV, but no metabolite III are found among the glucuronides present in the urine. This result seems to indicate that reduction of the

* Further determinations of Versidyne in human blood and its metabolites in urine have been carried out by B. Koechlin *et al.* as well as by H. Wehinger and R. Clotten. They will be reported separately elsewhere.

TABLE 1. AMOUNTS OF VERSIDYNE AND CERTAIN OF ITS METABOLITES EXCRETED IN THE URINE OVER A 3-DAY PERIOD FOLLOWING ADMINISTRATION OF A SINGLE DOSE OF THE DRUG (expressed in % of dose).

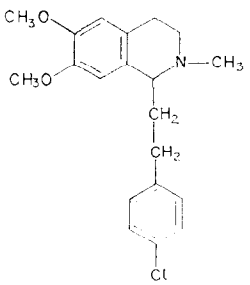
| | | Route of administration | Dose (Versidyne base) | Free: Versidyne + metabolite I | Conjugated*: metabolite II | Conjugated*: metabolites III + IV | Total: free + conjugated: Versidyne + known Versidyne metabolites | Ratio: metabolite II / metab. III + IV |
|---|---|---|---|---|---|---|---|---|
| Humans | A.B. (♂) | oral | 120 mg | 0·2 | 42·0 | 13·3 | 55·5 | 3·15 |
| | P.S. (♂) | oral | 120 mg | 0·2 | 50·6 | 15·2 | 66·0 | 3·33 |
| | A.R. (♂) | oral | 120 mg | † | 33·5 | 9·4 | 42·9‡ | 3·55 |
| Rabbit | T 687 (♂) | i.p. | 20 mg/kg | 0·5 | 35·0 | 19·0 | 54·4 | 1·84 |

* Values were obtained after enzymatic hydrolysis with glusulase of urine samples.
† Not determined.
‡ Urine samples of subject A.R. were analyzed on the first 2 days after administration only.

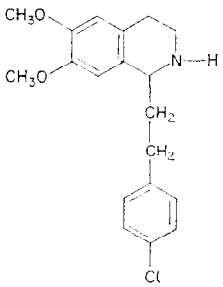
TABLE 2. RATE OF EXCRETION OF VERSIDYNE + METABOLITES I, II, III AND IV IN HUMAN URINE* FOLLOWING A SINGLE ORAL DOSE OF 120 mg VERSIDYNE BASE. (expressed as % of administered dose)

| | 1st day | 2nd day | 3rd day |
|---|---|---|---|
| A.B. (♂) | 42·5 | 10·2 | 2·8 |
| P.S. (♂) | 46·3 | 16·1 | 3·6 |
| A.R. (♂) | 36·6 | 6·3 | † |

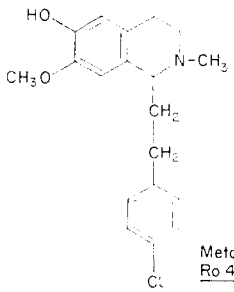
* Determined from urine samples of same subjects as in Table 1.
† Not determined.



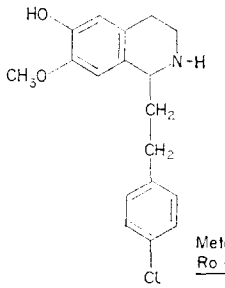

Versidyne
Ro 4-1778



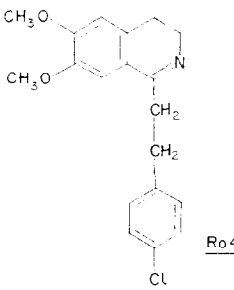

Metabolite I
Ro 4-1690



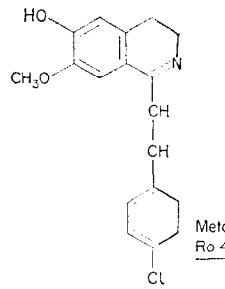

Metabolite II
Ro 4-5761



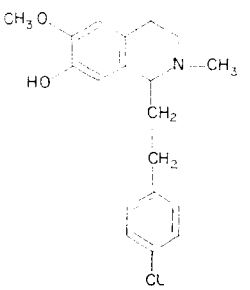

Metabolite III
Ro 4-5763



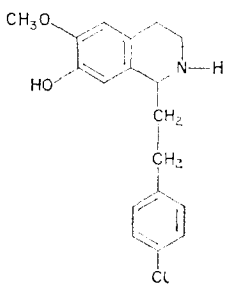

Ro 4-4461




Metabolite IV
Ro 4-7411




Ro 4-5595

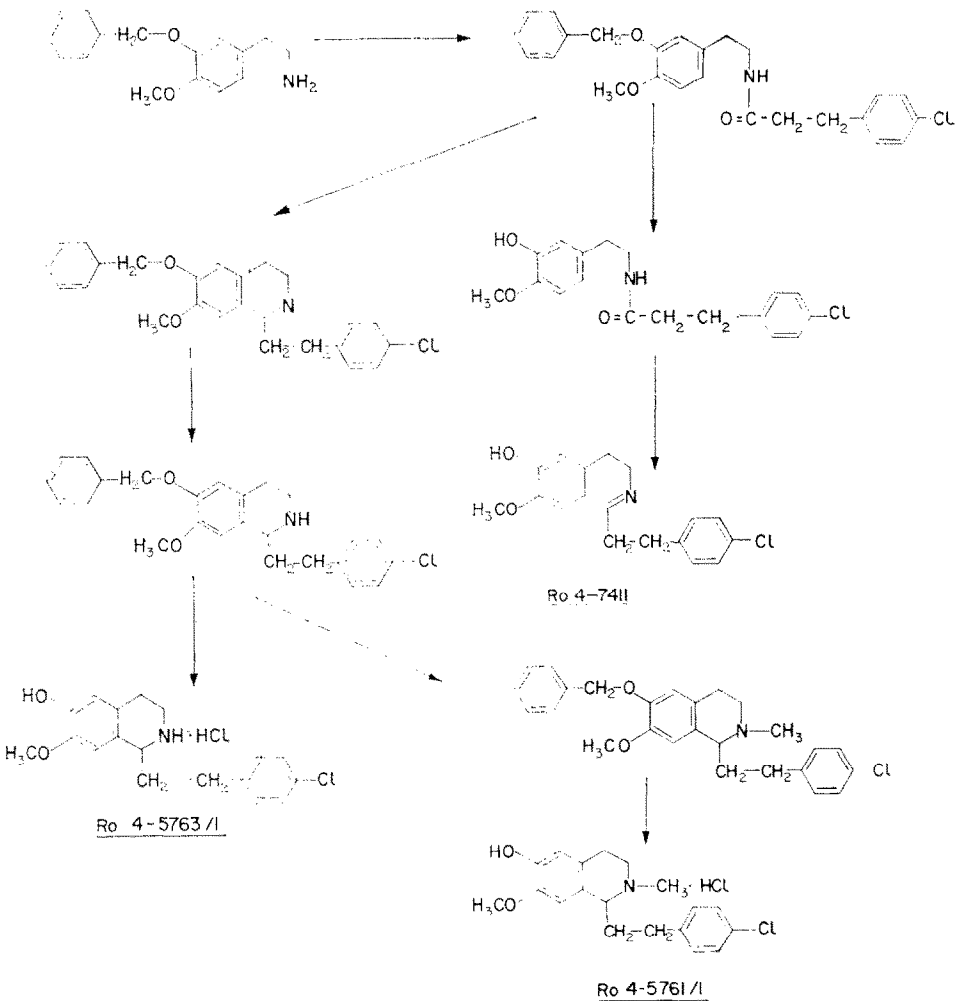



Ro 4-5762

double bond in this type of compound does not occur to any appreciable extent in the organism. However, it still does not exclude the possibility that the small amount of metabolite IV present might play a significant part in the biological degradation of Versidyne, e.g. as intermediate in the formation of still unknown metabolites of the drug. Investigations with labelled compounds are planned in an effort to elucidate this question.

Synthesis of metabolites

Metabolites II (Ro 4-5761/1), III (Ro 4-5763/1) and IV (Ro 4-7411)* were prepared according to the method of Brossi *et al.*[1, 8] by the following route:




*N-(3-Benzyloxy-4-methoxyphenethyl)-β-(4-chlorophenyl)-propionamide*

72 ml β-(4-Chlorophenyl)-propionamide and 100 g 3-benzyloxy-4-methoxyphenethylamine[9] were dissolved in 750 ml xylene in a flask attached to a Dean-Stark

* For the synthesis of metabolite I (Ro 4-1690) see Brossi *et al.*[1]

apparatus for water removal and refluxed for 15 hr. After cooling the precipitate was filtered and crystallized from ethyl acetate. The amide was obtained as colorless needles, m.p. 153°, in a yield of 145 g.

$C_{25}H_{25}O_3NCl$ Required C 71·0 H 6·0%
(mol. wt. 422·9) Found C 70·9 H 6·1%
u.v. spectrum (ethanol) : $\lambda_{max}$ = 278 mμ, log = ε 3·55
i.r. spectrum (KBr pellet) : 2·98 mμ (NH)
6·11 mμ (C = O amide)
6·50 mμ (amide-2)

1-(4-*Chlorophenethyl*)-6-*benzyloxy*-7-*methoxy*-3,4-*dihydroisoquinoline*

97 g N-(3-Benzyloxy-4-methoxyphenethyl)-β-(4-chlorophenyl)-propionamide was dissolved in 500 ml benzene (absolute) and treated with 42·1 ml freshly distilled phosphorus oxychloride and the mixture was heated to 100° for 2 hr. After distillation of the solvent under water-pump vacuum the residue was treated with 500 ml water and was boiled for 15 min. The acid solution was filtered and made alkaline to phenolphthalein with 3N sodium hydroxide. The base was taken into ether, washed with water and dried ($Na_2SO_4$) and distilled. The residue crystallized on the addition of isopropyl ether. The isoquinoline base was purified by crystallization from ether–petrol ether and the pale yellow crystals (70 g) had m.p. 108°.

$C_{25}H_{24}NO_2Cl$ Required C 74·0 H 5·9 Cl 8·7%
(mol.wt. 405·9) Found C 74·2 H 6·0 Cl 8·7%
u.v. spectrum (ethanol) : $\lambda_{max}$ = 272 mμ, log ε = 3·95
$\lambda_{max}$ = 309 mμ, log ε = 3·88
(n/100 HCl) : $\lambda_{max}$ = 247 mμ, log ε = 4·20
$\lambda_{max}$ = 307 mμ, log ε = 4·00
$\lambda_{max}$ = 358 mμ, log ε = 4·00
(n/100 NaOH) : $\lambda_{max}$ = 272 mμ, log ε = 3·95
$\lambda_{max}$ = 309 mμ, log ε = 3·88

1-(4-*Chlorophenethyl*)-6-*benzyloxy*-7-*methoxyl*-1,2,3,4-*tetrahydroisoquinoline*

50 g 1-(4-Chlorophenethyl)-6-benzyloxy-7-methoxy-3,4-dihydroisoquinoline was dissolved in 1 l. of ethanol and treated with 10 g sodium borohydride and left at 20° overnight. The solution was distilled, the residue dissolved in ether, washed neutral with water, dried ($Na_2SO_4$) and distilled. The crystalline residue, after two crystallizations from acetone–petrol ether, had m.p. 89–89·5°. Yield: 42·5 g.

$C_{25}H_{26}NO_2Cl$ Required C 73·6 H 6·4 Cl 8·7%
(mol.wt. 408) Found C 73·8 H 6·4 Cl 8·6%
u.v. spectrum (ethanol) : $\lambda_{max}$ = 284 mμ, log ε = 3·61

1-(4-*Chlorophenethyl*)-2-*methyl*-6-*benzyloxy*-7-*methoxy*-1,2,3,4-*tetrahydroisoquinoline*

36 g 1-(4-Chlorophenethyl)-6-benzyloxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline was dissolved in 1350 ml methanol and treated with 9 ml of a 40% aqueous formaldehyde solution and left at room temperature for 2 hr. This was followed by hydrogenation with 10 g of Raney nickel. After hydrogenation the catalyst was filtered and the solvent distilled. The residue, a pale yellow oil was purified by chromatography in

benzene on an alumina column (activity II). The tetrahydroisoquinoline derivative was obtained as a colorless oil which crystallized with isopropyl ether, m.p. 76·5–77°.

$C_{26}H_{28}NO_2Cl$ Required C 74·0 H 6·7 Cl 8·4%
(mol.wt. 422) Found C 74·3 H 6·7 Cl 8·3%
u.v. spectrum (ethanol) : $\lambda_{max}$ = 282 mμ, log ε = 3·61
i.r. spectrum (KBr pellet) : 3·49 mμ, 3·58 mμ (N-alkyl)

1-(4-*Chlorophenethyl*)-2-*methyl*-6-*hyroxy*-7-*methoxy*-1,2,3,4-*tetrahydroisoquinoline, hydrochloride*(*Ro* 4-5761/1)

10 g 1-(4-Chlorophenethyl)-2-methyl-6-benzyloxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline was dissolved in 100 ml acetic acid and hydrogenated with 1·5 g palladium charcoal (5%) at 50°. After the uptake of the calculated quantity of hydrogen the catalyst was filtered, the solution was removed, the residue was saturated with potassium carbonate and extracted three times with 500 ml ethyl acetate. The solution was dried over sodium sulfate and the solvent removed. The residue oil was treated with an excess of ethanolic hydrogen chloride thus precipitating the hydrochloride. After one crystallization from alcohol—ether pale yellow crystals, m.p. 234–235°, were obtained.

$C_{19}H_{23}NO_2Cl_2$ Required C 62·0 H 6·3%
(mol.wt. 368·3) Found C 62·0 H 6·1%
u.v. spectrum (ethanol) : $\lambda_{max}$ = 284 mμ, log ε = 3·55
(n/100 NaOH) : $\lambda_{max}$ = 245 mμ, log ε = 3·98
$\lambda_{max}$ = 300 mμ, log ε = 3·69

1-(4-*Chlorophenethyl*)-6-*hydroxy*-7-*methoxy*-1,2,3,4-*tetrahydroisoquinoline, hydrochloride* (*Ro* 4-5763/1)

1-(4-Chlorophenethyl)-6-benzyloxy-7-methoxy-1, 2, 3, 4-tetrahydroisoquinoline was debenzylated as before. The oil crystallized after the addition of ethanolic hydrogen chloride as the hydrochloride. After three crystallizations from ethanol–ether pale yellow crystals, m.p. 226–228°, were obtained.

$C_{18}H_{21}NO_2Cl_2$ Required C 61·0 H 6·0%
(mol.wt. 354·3) Found C 61·5 H 6·2%
u.v. spectrum (ethanol) : $\lambda_{max}$ = 285 mμ, log ε = 3·59
(n/100 NaOH) : $\lambda_{max}$ = 248 mμ, log ε = 3·99
$\lambda_{max}$ = 300 mμ, log ε = 3·82

3-*Hydroxy*-4-*methoxyphenethylamine*

50 g 3-Benzyloxy-4-methoxyphenethylamine[9] was dissolved in 500 ml ethanol and hydrogenated after the addition of 2·5 g palladium charcoal (5%). After 1½ hr the calculated amount of hydrogen was taken up. The catylyst was filtered and the solution distilled. The residue formed crystals, m.p. 152°.

*N*-(3-*Hydroxy*-4-*methoxyphenethyl*)-β-(4-*chlorophenyl*)-*propionamide*

33·4 g 3-Hydroxy-4-methoxyphenethylamine was dissolved in 250 ml dimethylformamide (absolute) and treated dropwise by stirring with a solution of 20·1 g β-(4-chlorophenyl)-propionylchloride (1 mole of acid was treated with 2 moles of thionyl chloride, kept for 30 min in an oil-bath at 80° and then the excess of thionyl

chloride removed at water-pump pressure) in 100 ml dimethylformamide whereby the temperature rose to 40°. After stirring for a further 30 min at room temperature the solvent was removed on the water-pump. The residue was made alkaline with potassium carbonate solution and extracted three times with 250 ml chloroform, washed once with 50 ml water and once with 50 ml 1N hydrochloride acid and then washed neutral with salt solution, dried with sodium sulfate and evaporated. The residue crystallized from isopropyl ether. The amide (11·6 g) m.p. 141°, was obtained as red shining crystals.

$C_{18}H_{20}NO_3Cl$ Required Cl 10·6%
(mol.wt. 333·83) Found Cl 10·8%
u.v. spectrum (ethanol) : $\lambda_{max}$ = 277 mμ, log ε = 3·51
$\lambda_{max}$ = 245 mμ, log ε = 3·93
(n/100 NaOH) : $\lambda_{max}$ = 294 mμ, log ε = 3·70
(n/100 HCl) : $\lambda_{max}$ = 277 mμ, log ε = 3·50)
i.r. spectrum (KBr pellet) : 2·87 mμ (OH); 3·04 (NH)
6·12 mμ (C = O amide)
6·48 mμ (amide 2 bond)
12·38 mμ (*p*-disubst. benzene)

1-(4-*Chlorophenethyl*)-6-*hydroxy*-7-*methoxy*-3,4-*dihydroisoquinoline* (Ro 4-7411)

10 g N-(3-Hydroxy-4-methoxyphenethyl)-β-(4-chlorophenyl)-propionamide was dissolved in a mixture of 250 ml benzene (absolute) and 25 ml chloroform and treated with 6·9 ml phosphorous oxychloride and kept for 1 hr at 80°. The solution was distilled, the residue was decomposed with 100 ml water and the resulting acid solution was filtered. After distillation of the solvent whitish crystals of the hydrochloride, m.p. 240° (under decomposition), separated.

$C_{18}H_{19}NO_2Cl_2$ Required C 61·3 H 5·4 Cl 10·1%
(mol.wt. 352·3) Found C 61·4 H 5·6 Cl 10·3%
u.v. spectrum (ethanol) : $\lambda_{max}$ = 248 mμ, log ε = 4·13
$\lambda_{max}$ = 308 mμ, log ε = 3·89
$\lambda_{max}$ = 364 mμ, log ε = 3·99
n/100 $N_aOH$ : $\lambda_{max}$ = 247 mμ, log ε = 4·00
$\lambda_{max}$ = 335 mμ, log ε = 4·22

*Methiodide*

By boiling the liberated base (obtained from the hydrochloride with potassium carbonate solution) in acetone solution with an excess of methyliodide yellow crystals, m.p. 230·5–231°, were obtained. The reduction of the methiodide with sodium borohydride in ethanolic solution gives Ro 4-5761.

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