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The absorption, metabolism and elimination of (±)-*N*-(2-benzoyloxyethyl)norfenfluramine (JP992) in man

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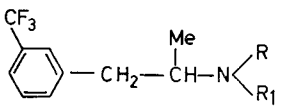
The urinary excretion of *N*-(2-benzoyloxyethyl)norfenfluramine (JP 992) was examined in man with normal and acidic urine after oral administration of the drug; 86–100% of the dose was excreted in 48 h as metabolites of the drug having *m*-trifluoromethylbenzyl and benzoyl moieties, but no unchanged drug was detected. Norfenfluramine, *N*-2-hydroxyethylnorfenfluramine, and a metabolite, which on treatment with zinc and hydrochloric acid gave norfenfluramine, were excreted in urine. *N*-2-Hydroxyethylnorfenfluramine and a second unidentified metabolite were also excreted as both sulphate and glucuronide conjugates.

N-(2-Benzoyloxyethyl)norfenfluramine (Id, JP 992) is as potent an anorectic but ten times less toxic in animals as fenfluramine (compound Ib) (Beregi, Hugon & others, 1970). In man with acid urine only 7–10% of the dose of Id was excreted in urine as *N*-2-hydroxyethylnorfenfluramine (Ic) and norfenfluramine (Ia), but no unchanged drug was detected (Brookes, 1968). Pronounced renal tubular reabsorption of Id is probable because it was almost completely absorbed between pH 4 and 9 in the buccal absorption test; its extensive metabolism is also likely.

We have determined by the oxidation method of Beckett, Shenoy & Brookes (1971) the metabolites of Id containing *m*-trifluoromethylbenzyl and -benzoyl moieties excreted in the urine in order to investigate the absorption, distribution and elimination of Id in man, and to determine quantitatively the excretion of various metabolites of the drug.



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Ia	$R=R_1=H$	Norfenfluramine
Ib	$R=H$; $R_1=Et$	Fenfluramine
Ic	$R=H$; $R_1=C_2H_4OH$	*N*-2-Hydroxyethylnorfenfluramine
Id	$R=H$; $R_1=C_2H_4OCOC_6H_5$;	*N*-(2-Benzoyloxyethyl)norfenfluramine (JP 992)
Ie	$R=COC_6H_5$; $R_1=C_2H_4OH$	*N*-(2-Hydroxyethyl)-*N*-benzoylnorfenfluramine (Rearranged form of JP 992)

MATERIALS AND METHODS

Urinary excretion trials

Three normal healthy males took 60 or 100 mg doses of compound Id hydrochloride (equivalent to 54·31 or 90·52 mg of the base respectively) by mouth in water (the drug was dissolved in 1 ml absolute ethanol and then diluted to 50 or 100 ml

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with water). The urinary pH was kept acid by ingestion of ammonium chloride (Beckett & Brookes, 1967). One subject was also given 2 × 150 mg tablets of compound Id (equivalent to 271·56 mg of the base) and the pH of the urine was not controlled.

In another experiment, two subjects with induced acid urine were given 180 mg base equivalent of Id in solution orally, or on other occasions 8 mg base of Id equivalent (as methane sulphonate) in 2 ml aqueous solution intravenously or 20 mg base equivalent of Ic orally in aqueous solution.

Urine samples were collected at every ½ h or 1 h for the first 4 h, hourly for the next 10 to 12 h and then at longer intervals up to 48 h. In all cases the exact time of urine collection was noted, the pH of the urine and volume was determined shortly after collection, and the urine was stored at 4° until analysis. A "blank" urine sample was collected at the time of drug administration.

Materials

Compounds Ia, b, c, d and e hydrochlorides were obtained from Servier Laboratories Ltd., U.K., as the racemic forms.

Analysis

Total *m*-trifluoromethylbenzyl and -benzoyl metabolites and compounds Ia and Ic were measured (Beckett & others, 1971). After large doses, aliquots of urine high in drug concentration were diluted to contain >50 μg/ml of drug with the subjects "blank" urine before analysis.

Investigations for other metabolites

The urine samples (5 ml) were made alkaline (pH 12) for the extraction of Ia and Ic. They were then made acidic with 6N HCl and zinc powder (about 0·05 g) added. After ½ h, the solution was made alkaline with 20% NaOH, the internal standard (1 ml, 10 μg/ml aletamine hydrochloride in water) added and the solution extracted with freshly distilled ether (3 × 2·5 ml) and analysed for compound Ia by gas liquid chromatography (g.l.c.).

Investigations for conjugated metabolites

(a) With β-glucuronidase (Ketodase, Warner–Chilcott). The following samples were placed on a water bath at 37·5° with constant shaking for 24 h: 1. A "blank" urine (5 ml) adjusted to pH 4·5 with acetic acid, acetate buffer, B.P. 1963 (1 ml) and containing Ketodase (0·5 ml). 2. Aliquots of urine (5 ml) from subjects given oral doses of JP 992; these were adjusted to pH 4·5 with acetic acid and acetate buffer (1 ml) and Ketodase added to some.

(b) With sulphatase (Clarase 300; Takamine). The above experiments were repeated at pH 5·5 using sulphatase (20 mg) instead of β-glucuronidase. After incubation, the samples were analysed by g.l.c. for bases as described.

Thin-layer chromatography

Glass plates (20 × 20 cm) coated with a layer (0·5 mm) of silica gel G (Merck) and activated at 105° for 1 h were stored in a desiccator over silica gel. The reference samples of compounds Ia, Ic, Id and Ie and ethereal extracts of the bases from urine were spotted on plates and the chromatograms developed with the following solvent systems: (a) methanol–acetone (1:1), (b) methanol–chloroform (20:80), (c) methanol–chloroform–ammonia 25% (90:10, 5 drops), and (d) methanol–

chloroform (1 : 1). Dragendorff's reagent (Stahl, 1962) and a solution of bromothymol blue in ethanol were used to visualize the spots.

Buccal absorption test

The general procedure of Beckett & Triggs (1967) was adopted. Drug solution (0·5 ml) equivalent to 1 mg base of compounds Ia, Ib, Ic and Id was diluted with appropriate buffer solution to 25 ml (pH range 4–9·18) and examined by the test. The expelled solutions were combined, the volume adjusted to 250 ml with distilled water and an aliquot (5 ml) analysed by g.l.c. as for the bases described and the findings were related to calibration graphs prepared from diluted saliva solutions. All curves were plotted as a percentage of the base absorbed against the mean pH of the buffer solution before and after the test.

RESULTS AND DISCUSSION

Total m-*trifluoromethylbenzyl and -benzoyl-containing metabolites*

The drug Id was excreted almost completely after an oral dose under conditions of acidic and uncontrolled urinary pH as *m*-trifluoromethylbenzyl and -benzoyl metabolites (Table 1); unchanged drug was not detected in the urine. These meta-

Table 1. *Urinary excretion in* 48 *h of total* m-*trifluoromethylbenzyl and -benzoyl-containing metabolites and of norfenfluramine* (*Ia*), N-2-*hydroxyethylnorfenfluramine* (*Ic*) *and other metabolites after oral administration of* (±)-N-(2-*benzoyloxyethyl*)-*norfenfluramine* (*Id*) *hydrochloride to subjects under conditions of acidic urine.*

Subject	Dose (mg)	% dose excreted as†					Total drug moieties by oxidation to trifluoromethyl benzoic acid as % of dose
		1	2	3	4	5	
1	60	6·2	3·0	19·4	12·3	8·9	101
1	60	—	—	—	—	—	102
2	100	3·2	2·2	4·3	12·7	5·0	87
3	100	5·0	1·5	2·7	7·5	7·5	86
4	300	*4·7	0·3	3·9	5·7	—	101

1. Norfenfluramine (Ia).
2. *N*-2-Hydroxyethylnorfenfluramine (Ic).
3. Norfenfluramine on treatment with Zn/HCl.
4. *N*-2-Hydroxyethylnorfenfluramine as glucuronide.
5. *N*-2-Hydroxyethylnorfenfluramine as sulphate.

* 2 × 150 mg tablets but urine not kept acidic.

† The metabolites constituting the difference between the total of 1–5 and the total drug by oxidation are under investigation.

bolites were excreted rapidly during the first 12 h with a peak at about 2 h (Fig. 1), but small amounts continued to be excreted up to 48 h; smooth rate of excretion: time curves were obtained in subjects with acid urine (Fig. 1) but not when urine pH was uncontrolled. The semi log plots after the maxima showed an exponential decrease and were parallel (Fig. 2); the "apparent" half life of the total drug moieties was about 2 h. Thus, the drug is rapidly and completely absorbed from the gastro-

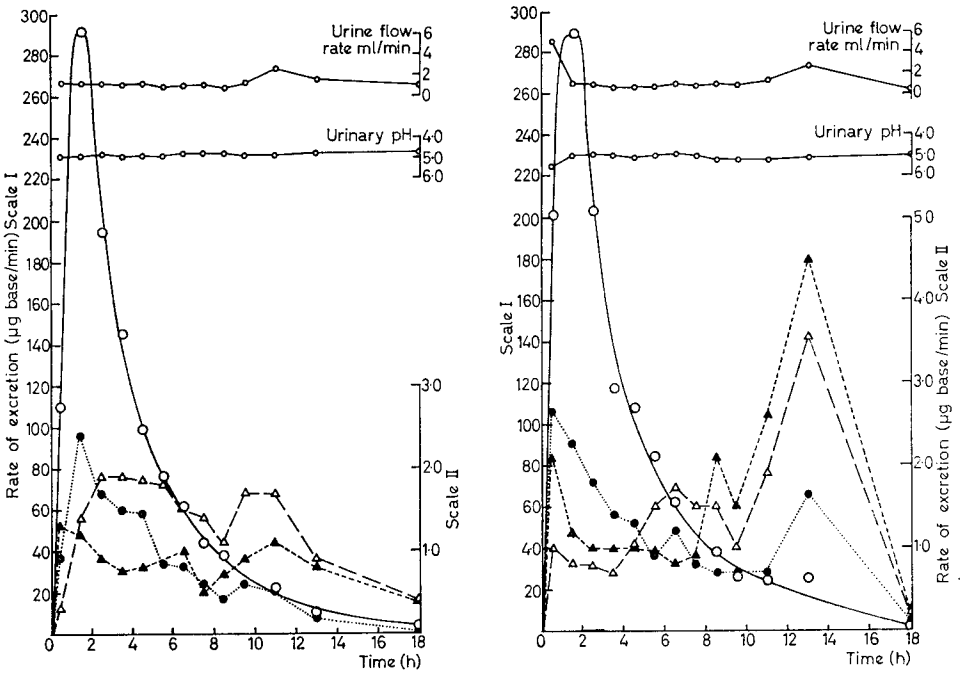


FIG. 1. Urinary excretion of *m*-trifluoromethyl-benzyl and -benzoyl metabolites ○—○ (scale I), and the metabolites: compound Ic ●---●, Ia ▲---▲ △---△ (all scale II) produced on treatment with Zn/HCl, after the oral administration of 100 mg of compound Id HCl under acidic urine condition to two subjects.

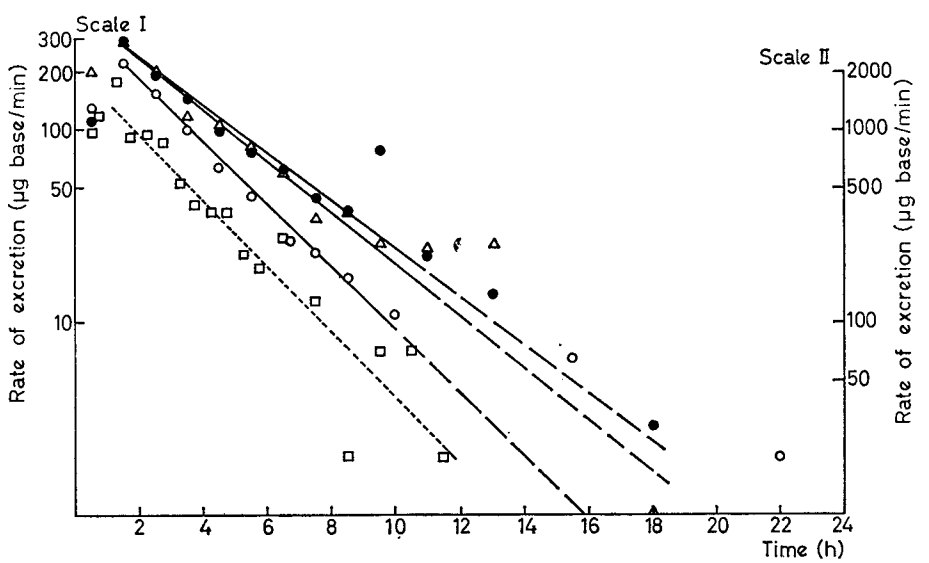


FIG. 2. Urinary excretion of *m*-trifluoromethyl-benzyl and -benzoyl metabolites after oral administration of compound Id HCl. Acidic urine control: Subject 1, 60 mg, ○—○; Subject 2, 100 mg, △—△; Subject 3, 100 mg, ●—●. Fluctuating urinary pH (all scale I): Subject 4, 300 mg (2 × 150 mg tablets) □---□ (scale II).

intestinal tract and is eliminated rapidly and almost completely via the urine; the predominant metabolites must therefore be highly water soluble compounds and their solubility must be independent of pH since they were recovered from acid and alkaline urine in similar amounts (Table 1). Because virtually quantitative amounts of *m*-trifluoromethylbenzoic acid equivalent to the drug given were obtained after oxidation of total urinary metabolite, a negligible amount of *p*-hydroxylation could have been involved since *p*-hydroxylated metabolites would not give the above acid on oxidation.

Basic metabolites

N-2-Hydroxyethylnorfenfluramine Ic and its dealkylated product norfenfluramine Ia were shown to be present in urine by g.l.c. (see Beckett & others, 1971) and t.l.c. (Table 2); less than 4% of Ic and only about 5% of Ia were recovered after an oral dose of Id under conditions of acid urine (Table 1). By comparison with the oral route, the amount recovered of Ic was doubled (6·7–8·7%) and Ia halved (1–1·7%) when

Table 2. R_F *values for compound Ia, Ic, Id and Ie using various solvent systems.*

Solvent system	R_F values			
	Ia	Ic	Id	Ie
a	0·55	0·45	0·69	0·69
b	0·32	0·29	0·70	0·71
c	0·35	0·46	0·67	0·70
d	0·28	0·51	—	—

Ia and Ic from urine gave similar R_F values to those of authentic samples of Ia and Ic.

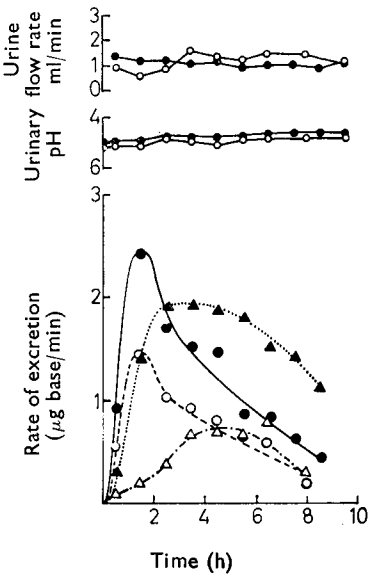


FIG. 3. Comparison of the urinary excretion of compounds Ic –●– and Ia ▲– – –▲ after the oral administration of 100 mg of compound Id HCl, and of compounds Ic ○– – –○ and Ia △–·–△ after the oral administration of 20 mg of compound Ic under acidic urine conditions to Subject 6. (Similar results were obtained with Subject 5.)

a small amount (8 mg base) of Id was administered intravenously to the same subject this was probably a result of a partial bypass of the liver.

The recovery of Ia and Ic in the urine after doses of 180 mg base of Id and 20 mg base of Ic orally were respectively 1·5–3·5 and 1·2–3·7% (5 subjects) and their profiles (Fig. 3) were similar, indicating rapid *in vivo* hydrolysis of Id to Ic; Fig. 4 shows that hydrolysis is virtually complete within 2 h since the semi log rate of excretion:

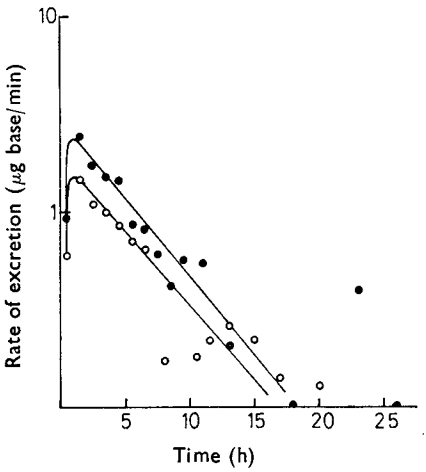


FIG. 4. Comparison of the urinary excretion of compound Ic after the oral administration of 100 mg of compound Id HCl ●—● and 20 mg base of compound Ic ○—○ under acidic urine conditions to Subject 6. (Similar results were obtained with Subject 5.)

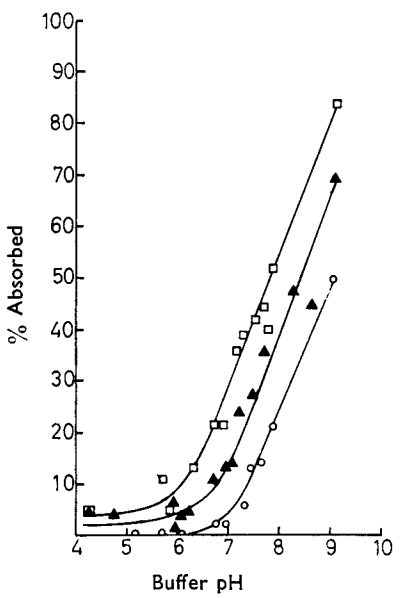


FIG. 5. Buccal absorption of norfenfluramine (Ia) ▲—▲, fenfluramine (1b) □—□ and *N*-2-hydroxyethylnorfenfluramine (Ic) ○—○.

time plots of Ic from Id and Ic after that time are parallel. The peak excretion rate of Ia occurs later than that of Ic and the urine concentrations remain high for longer (Fig. 3), although Ia is only slightly more lipophilic than Ic in the buccal absorption test (see Fig. 5) at pH 5 and only a slight difference in the kidney tubular reabsorption would then be expected at this urinary pH. The results in Fig. 3 indicate that Ia is produced by metabolism of Ic which itself is formed rapidly *in vivo* from Id.

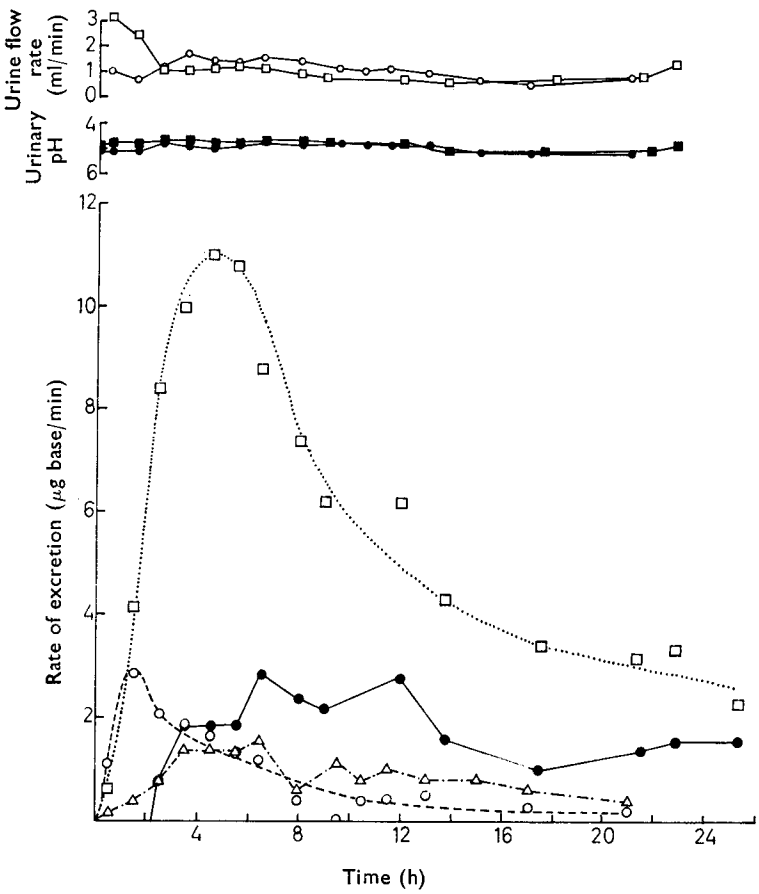
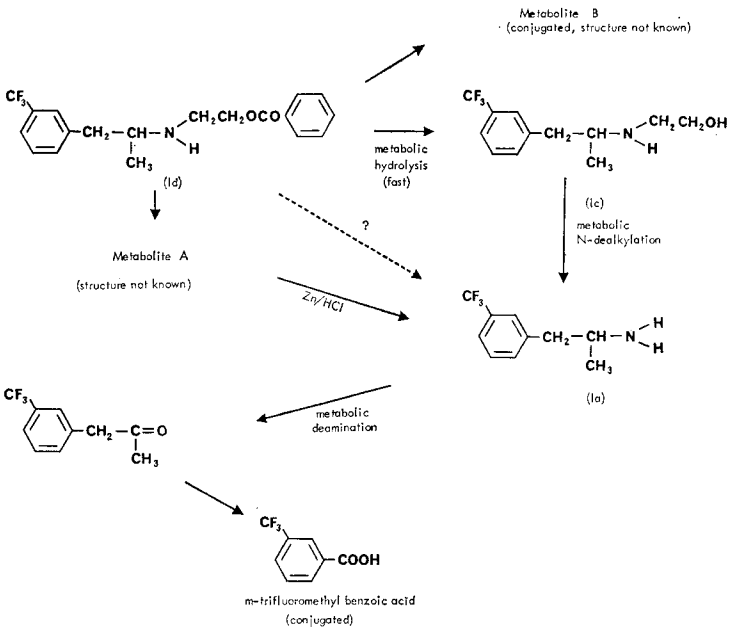


FIG. 6. Comparison of the urinary excretion of (a) unchanged drug □—□ and metabolite compound Ia ●—● after the oral administration of 20 mg of compound Ib HCl and (b) unchanged drug ○– – –○ and compound Ia △–·–△ after the oral administration of 20 mg of compound Ic under acidic urine conditions to Subject 6.

Under acidic urine conditions, about 25% (19–32·5%, 5 subjects) of an oral dose of compound Ia (20 mg base) was recovered from urine unchanged and about 13% (9·7–19·1%, 5 subjects) of Ia was recovered after an oral dose of Ib (20 mg base), thus indicating about 50% *in vivo* conversion of Ib to Ia. However, only about 3% (1·2–3%, 2 subjects) of Ia was recovered in urine after an oral dose of Ic (20 mg base), indicating about 10% metabolism of Ic by this route; the introduction of the hydrophilic OH group into fenfluramine (Ib) to give *N*-2-hydroxyethylnorfenfluramine (Ic) has thus led to a significant reduction in *N*-dealkylation. Elimination of Ic as

conjugates cannot account for the difference since only about 15–20% of Ic is excreted as combined glucuronide and sulphate conjugates (Table 1). The introduction of the hydroxyl group into the *N*-ethyl group facilitates metabolism of *N*-2-hydroxyethylnorfenfluramine other than by dealkylation since less of this compound as well as of norfenfluramine is excreted when this change is made (see Fig. 6).



Neither compound Id nor its rearranged form Ie (see Beckett & others, 1971) were detected in urine. Two unknown metabolites were excreted. Metabolite A in urine on treatment with zinc and hydrochloric acid give Ia and had an excretion profile similar to that of Ia excreted as free base (Fig. 1). Metabolite B with a g.l.c. retention time of 23 min on Column A (see Beckett & others, 1971) was excreted as glucuronide and sulphate conjugates. Neither the ketone nor the oxime, free or conjugated were excreted in the urine, but conjugated *m*-trifluoromethylbenzoic acid was excreted.

Acknowledgement

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REFERENCES

BECKETT, A. H. & BROOKES, L. G. (1967). *J. Pharm. Pharmac.*, **19**, 425–495.

BECKETT, A. H., SHENOY, E. V. B. & BROOKES, L. G. (1971). In the press.

BECKETT, A. H. & TRIGGS, E. J. (1967). *Nature, Lond.*, **19**, 31S–41S.

BEREGI, L. G., HUGON, P., LE DOUAREC, J. C., LAUBIE, M. & DUHAULT, J. (1970). *International Symposium on Amphetamines and Related Compounds*, pp. 21–61. Editors: Costa, E. and Garattini, S. Milan.

BROOKES, L. G. (1968). Ph.D. Thesis, University of London.

STAHL, E. (1962). *Thin-Layer Chromatography*. Berlin: Verlag-Chemi.