J. Pharm. Pharmacol. 1982, 34: 771–776
Received February 25, 1982

0022–3573/82/120771–06 $02.50/0

Gas chromatographic determination of pargyline and pargyline amine metabolites after derivatization with isobutyl chloroformate

AFAF M. WELI*, NILS-OTTO AHNFELT** AND BJÖRN LINDEKE*†

Departments of Organic and Analytical** Pharmaceutical Chemistry, Biomedical Center, University of Uppsala, Box 574, S-751 23 Uppsala, Sweden*

Pargyline (I) and four of its major amine metabolites, *N*-benzylpropargylamine (II), *N*-methylpropargylamine (IV), *N*-benzylmethylamine (VI) and pargyline *N*-oxide (VIII), were determined by g.l.c. after reaction with isobutyl chloroformate in a two-phase system. Pargyline and metabolite IV which form the same derivative, were separated quantitatively by liquid–liquid distribution before derivatization with isobutyl chloroformate. Also, metabolites VI and VIII form identical derivatives, and as they could not be easily separated, VIII was reduced to I before derivatization. The analytical method was applied in studies of the metabolism of pargyline in microsomes from phenobarbitone pretreated rats. The metabolism was rapid, leading mainly to the formation of II, IV, VI and VIII. 92% of the substrate was consumed during the first min of incubation at an initial substrate concentration of 100 μM (32 nmol mg^{-1} protein), and II, IV, VI and VIII accounted for 83% of formed metabolites. The metabolite II was further metabolized while the levels of IV, VI and VIII remained constant after 5 min. It was possible to determine 0·5 μM of pargyline, II and VI in microsomal incubations, with an r.s.d. of <5% (n = 6). *N*-Methylpropargylamine (IV) was positively identified and quantitated as a pargyline metabolite for the first time.

Pargyline is an irreversible inhibitor of type B monoamine oxidase (Parkinson & Callingham 1980) but, as with many other MAO-inhibitors, it is no longer used clinically due to undesirable side effects—'the cheese effect'—(Fowler et al 1981). The drug, however, is a useful tool in experimental pharmacology, e.g. to determine MAO active centres (Parkinson & Callingham 1980). Also its possible conversion to reactive metabolites (De Masters & Nagasawa 1978; Lebsack & Anderson 1979; Hallström et al 1981a) makes it an interesting substrate for mechanistic investigations of drug metabolizing enzymes. Nevertheless, until recently few studies have been devoted to the metabolism of pargyline (Diehl et al 1976; De Masters & Nagasawa 1978; Pirisino et al 1979; Lebsack & Anderson 1979; Shirota et al 1979) and little is known about the enzyme mechanisms involved (Lebsack & Anderson 1979; Shirota et al 1979; Hallström et al 1981a; Coutts et al 1981). One reason for this has been the lack of suitable analytical assays for monitoring pargyline and its metabolites (Fig. 1). Apart from formaldehyde (III) and benzaldehyde (V) hitherto only the more lipophilic amines (pargyline (I), *N*-benzylpropargylamine (II benzyl PA) and *N*-benzylmethylamine (VI benzyl MA) have been quantitated, which renders for example material balance studies impossible.

As a continuation of our studies on the metabolism of α-acetylenic amines (Hallström et al 1981a,b; Lindeke et al 1981) we found it necessary to develop an analytical assay for pargyline and its major metabolites. Two-phase derivatization of the substrate and its amine metabolites with chloroformate esters and subsequent gas chromatographic (g.l.c.) analysis (Karlsson & Hartvig 1980) was then considered as a possible approach. The present paper describes an application of this technique in which pargyline together with four of its metabolites (benzyl PA (II), *N*-methylpropargylamine(methyl PA (IV), benzyl MA (VI) and pargyline *N*-oxide (VIII) in Fig. 1) were analysed after derivatization with isobutyl chloroformate (IBC).

MATERIALS AND METHODS

Instrumentation

Infrared and ^1H n.m.r. spectra of synthesized compounds were run for identification purposes on a Perkin-Elmer 157G spectrophotometer and a Perkin-Elmer R 12 B spectrometer, respectively.

G.l.c. analysis was performed on a Hewlett-Packard 5730 A gas chromatograph with thermionic detector and equipped with a 1·2 mm × 2 mm i.d. glass column with a packing of 10% SP-1000 on

† Correspondence.

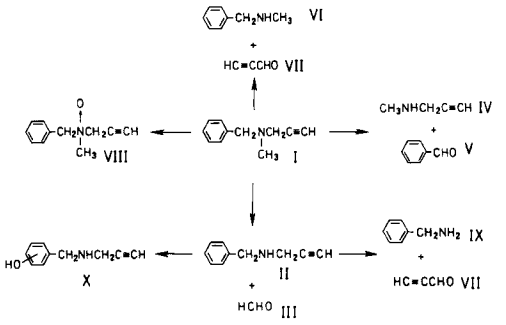

FIG. 1. Metabolic pathways of pargyline (I) in mammals. II, *N*-benzylpropargylamine: III, formaldehyde; IV, *N*-methylpropargylamine; V, benzaldehyde; VI, *N*-benzylmethylamine; VII, propiolaldehyde; VIII, pargyline *N*-oxide; IX, benzylamine and X, *N*(hydroxybenzyl)propargylamine.

Supelcoport 80/100 mesh. The column was operated at 130 and 190 °C with a carrier gas flow of 30 ml min^{-1} (8% H_2 in He).

Identification of the carbamates was done on an LKB 9000 g.l.c.–m.s. system with He as the carrier gas. The glass column—a 1·2 mm × 2 mm i.d.—had a packing of 3% OV-17 on Gas-Chrom Q. Mass spectra were recorded under electron impact conditions at an ionizing voltage of 70 eV. The trap current was 60 μA, the accelerating voltage 3·5 kV and the temperature of the ion source 270 °C.

A Bergman and Beving pHM 62 standard pH meter was used for pH measurements and a Beckman model 25 spectrophotometer was used in the protein and cytochrome P-450 assays.

Chemicals and reagents

Pargyline (I) and *N*-benzylpropargylamine (II) (Brit. pat. 1962), *N*-methylpropargylamine (IV) (Bogentoft et al 1973) and pargyline *N*-oxide (VIII) (Hallström et al 1981a) were synthesized according to the cited procedures.

Isobutyl carbamates prepared from *N*-methylbutylamine, *N*-methylamphetamine and benzylamine were used as internal standards. They were obtained by reacting the corresponding amine with IBC according to standard procedures (Ahnfelt & Hartvig 1980).

N-Benzylmethylamine (VI), *N*-methylbutylamine, benzylamine (IX) and isobutyl chloroformate (IBC) were purchased from Fluka AG, Buchs, Switzerland, *N*-methylamphetamine was supplied by ACO läkemedel, Solna, Sweden. NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and serum albumin were obtained from Sigma Chemical Co, St Louis, MO, U.S.A.

Determination of distribution constants

The extractions were made in centrifuge tubes at 25 °C using equal phase volumes. Aqueous solutions of different pH containing pargyline (I), benzyl PA (II) methyl PA (IV) or benzyl MA (VI) (5×10^{-5} M) were shaken with CH_2Cl_2 for at least 30 min.

Aliquots, 10 ml of each phase were pipetted into separate tubes and the amount of amine in both phases were determined by g.l.c. after reaction in a two-phase system with IBC according to the g.l.c.-method outlined below.

The distribution ratio, D_A, was calculated from the peak height ratio to internal standard obtained from the two phases, where

$$D_A = \frac{\text{amount in organic phase}}{\text{amount in aqueous phase}}$$

The distribution constants, $K_{D(A)}$, were calculated from the relation

$$\frac{1}{D_A} = \frac{1}{K_{D(A)}} + \frac{{}^{a}H^{+}}{K'_{HA} \times K_{D(A)}}$$

where

$$K'_{HA} = \frac{{}^{a}H^{+} \times [A]}{[HA^{+}]}$$

cf. (Schill 1978).

In vitro metabolism of pargyline

To obtain high metabolic activity, microsomes from induced Sprague-Dawley rats (200–250 g)—pretreated with sodium phenobarbitone for 4 days (3×80 mg $kg^{-1} + 1 \times 40$ mg kg^{-1} i.p.) before being killed—were used. The microsomes were prepared as previously described (Cho et al 1974). Microsomal protein (Geiger & Bessman 1972) and cytochrome P-450 content (Omura & Sato 1964) were determined by the standard procedures as cited.

Incubations were conducted at 37 °C in 25 ml Erlenmeyer flasks. Microsomal suspension equivalent to 0·4 g of wet liver (~13 mg protein) was incubated in a total volume of 5 ml of 0·15 M potassium phosphate buffer pH = 7·5, containing 1·5 μmol $NADP^+$, 17·5 μmol of glucose-6-phosphate, 20 μmol of $MgCl_2$, 5 i.u. of glucose-6-phosphate dehydrogenase, 500 nmol of pargyline and 300 μmol KCl. The incubation was started by addition of the microsomes and terminated after 1, 2, 5, 10 and 15 min by transferring the incubation mixture to centrifuge tubes containing 0·5 ml of a 20% zinc sulphate solution. After centrifugation at 3000 rev min^{-1} for 4 min, the supernatant was transferred to another centrifuge tube. The precipitated

protein was washed with 2 ml 1·15% KCl and the pH of the combined supernatants and washing solution was adjusted to 6.

Analytical procedure

An equal volume of CH_2Cl_2 was added to the aqueous solution from above and the tube was shaken for at least 30 min. The organic phase which now contained pargyline and the metabolite II (benzyl PA) was pipetted into another tube and treated with IBC in the presence of 6 ml of an aqueous solution containing 0·1 M of NaCl in phosphate buffer pH = 6 (Karlsson & Hartvig 1980). Isobutyl *N*-butyl-*N*-methylcarbamate (12 nmol) and isobutyl *N*-methyl-*N*-(1-phenyl-2-propyl)carbamate (80 nmol) were included as internal standards. 5 ml of the aqueous phase remaining after extraction and containing the metabolites IV (methyl PA), VI (benzyl MA) and VIII (pargyline *N*-oxide), were treated with 0·5 ml of 5 M HCl and 0·5 ml of 15% $TiCl_3$ solution overnight, to reduce *N*-oxidized pargyline metabolites (Hallström et al 1981a). The pH was adjusted to 6 and extraction with an equal volume of CH_2Cl_2 was repeated. This CH_2Cl_2 phase containing pargyline was treated as above with IBC in the presence of isobutyl *N*-butyl-*N*-methylcarbamate (12 nmol) as internal standard.

5 ml of the remaining aqueous phase—containing the metabolites IV (methyl PA) and VI (benzyl MA) was then made alkaline (1 ml 0·5 M NaOH, pH above 10) and treated with IBC in the presence of an equal volume of CH_2Cl_2, containing isobutyl *N*-butyl-*N*-methylcarbamate (12 nmol) and isobutyl *N*-methyl-*N*-(1-phenyl-2-propyl)carbamate (80 nmol) as internal standards.

The IBC concentration was 1% and a reaction time of 10 min was used in all cases. The work-up procedure is summarized in Fig. 2. Four to eight μl of the CH_2Cl_2 solutions were injected on the gas chromatograph.

Standard curves were prepared by adding 20-500 nmol of each pargyline, pargyline *N*-oxide (VIII) and the amines II (benzyl PA), IV (methyl PA) VI (benzyl MA) to incubation mixtures without co-factors. These standard samples were quenched immediately and processed through the assay.

RESULTS AND DISCUSSION

Analytical method

Selectivity in the determination of secondary and tertiary amines together with amine *N*-oxides can in certain cases be accomplished by g.l.c. after suitable derivatization. In general, however, derivatives of

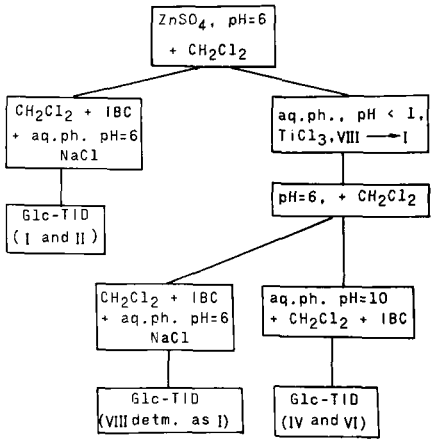

FIG. 2. Schematic representation of the batch separation of pargyline (I) and *N*-benzylpropargylamine (II) from *N*-methylpropargylamine (IV), *N*-benzylmethylamine (VI) and pargyline *N*-oxide (VIII). The second batch separation comprises the separation of VIII (as I) from IV and VI. IBC denotes isobutyl chloroformate, and g.l.c.–TID gas liquid chromatography with thermionic detection.

amine *N*-oxides are characterized by chemical instability and reproducible quantitation of decomposition products can be obtained in exceptional cases only (Berman & Spirtes 1971; Evans et al 1980; Lindberg et al 1980). Another approach comprises reduction of the *N*-oxide with Ti^{3+} after separation from the parent amine followed by analysis of the second batch of the amine formed in the reduction (Beckett et al 1971; Hallström et al 1981a).

The results from the present study substantiate previous findings (Ahnfelt & Hartvig 1980) showing that low concentration analysis of low molecular weight polar amines, like the pargyline metabolites, can be made after derivatization with chloroformate esters. Such derivatization minimizes adsorption and improves the chromatographic properties as noted especially for methyl PA (IV) which for the first time has been positively identified as a pargyline metabolite.

Isobutyl chloroformate (IBC) was chosen because of the good g.l.c. properties of the carbamate derivatives formed (Fig. 3). The use of this reagent was possible because of the inherent nitrogen of the compounds which allowed the use of the thermionic detector. Moreover, excess of reagent could be injected without complications.

As a benzylic substituent in tertiary amines is a good leaving group, pargyline is preferentially debenzylated to give isobutyl *N*-methyl-*N*-propargylcarbamate upon treatment with IBC. The formed quaternary ammonium intermediate is

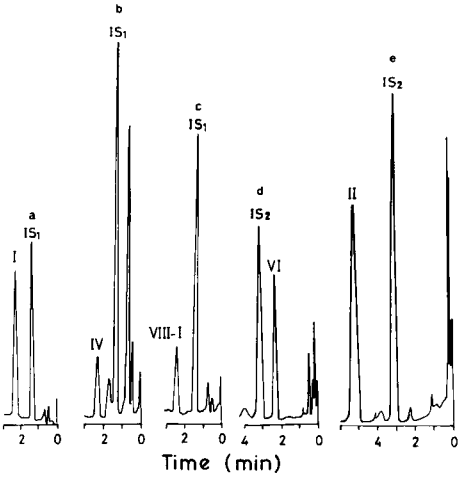

FIG. 3. G.l.c. of derivatized extracts of an incubation of pargyline with a rat liver microsomal preparation. Analysis (a) of pargyline (I) (2·8 nmol mg^{-1} protein) with isobutyl *N*-butyl-*N*-methylcarbamate (IS_1) as internal standard, (b) of *N*-methylpropargylamine (IV) (1·4 nmol mg^{-1} protein) with IS_1 as standard, (c) of pargyline *N*-oxide (VIII) (3·8 nmol mg^{-1} protein) after reduction to (I) with IS_1 as standard, (d) of *N*-benzylmethylamine (VI) (7·9 nmol mg^{-1} protein) with isobutyl *N*-methyl-*N*(1-phenyl-2-propyl)carbamate (IS_2) as internal standard and (e) of *N*-benzylpropargylamine (II) (9·1 nmol mg^{-1} protein) with IS_2 as standard. The column temperature was 130 °C in cases a through c and 190 °C in d and e. Further experimental details are given in Materials and Methods.

attacked by the chloride ion leading to the formation of benzyl chloride as a by-product (Karlsson & Hartvig 1980). The addition of an excess of chloride ions (addition of NaCl) will thereby direct the cleavage so that debenzylation is quantitative and the only reaction occurring. Also, pargyline *N*-oxide (VIII) is decomposed in the reaction with IBC but to isobutyl *N*-benzyl-*N*-methylcarbamate. The *O*-acylated *N*-oxide is the conceivable intermediate.

The choice of pH and other conditions for the derivatization were made according to the principles given by Karlsson & Hartvig (1980) and by Ahnfelt et al (1982). For derivatization of secondary and primary amines it is necessary to have such a pH that the amines are partly distributed to the organic solvent. Thus, the distribution ratio does not need to be high for quantitative derivatization, which will occur even at fairly low distribution ratios although at a slower rate (cf. Ahnfelt et al 1982). It can be shown theoretically that the reaction rate is highest at $pH \geq pK'_{HA} + 2$, i.e. when the distribution ratio for the amine is equal to the distribution constant, $K_{D(A)}$. For reactive compounds like secondary amines the distribution process will limit the rate of the reaction providing the chloroformate concentration is sufficient. A reaction time of 10 min (pH = 6 and 0·1 M NaCl) at ambient temperature was found to be sufficient for the derivatization of pargyline and benzyl PA (II) whereas a pH of at least 10 was found necessary for the derivatization of the more polar amine metabolites IV (methyl PA) and VI (benzyl MA).

The structures of the carbamates formed from pargyline and the various metabolites (II, IV, VI and VIII) were confirmed by their mass spectra (Table 1).

Table 1. Mass spectrometric characteristics of the isobutyl carbamates formed from pargyline (I), *N*-benzylpropargylamine (II), *N*-methylpropargylamine (IV), *N*-benzylmethylamine (VI) and pargyline *N*-oxide (VIII). The carbamates were identified on a LKB 9000 g.l.c.–m.s. system using a 3% OV 17 column. The gas chromatograph was temperature programmed from 80–150 °C. For further experimental particulars see Materials and Methods.

	Relative intensity (%) at 70 eV*				
m/z	I	II	IV	VI	VIII
57	100	30	100	24	22
68	40	3	37		
91	4	100	2	100	100
96	41		55		
106		21		2	2
113	45		68		
114	48		71		
120	0·3	3		44	49
150		58		2	2
164		0·6		37	44
165				35	43
169	4		10		
189		12			
221				20	28
245		14			

* Only peaks of diagnostic importance have been included.

Since pargyline is debenzylated to give the same derivative as IV (methyl PA), it was necessary to separate these amines from each other before derivatization, and for similar reasons, also separation of the *N*-oxide (VIII) from VI (benzyl MA) would be desirable. The distribution for all the amines and the *N*-oxide was therefore studied in order to evaluate possible conditions for batch separation. As shown in Table 2, the difference in log $K_{D(A)} \times K'_{HA}$ enabled quantitative separation of pargyline from IV (methyl PA) at pH 6. Pargyline is extracted to more than 99% to CH_2Cl_2 at this pH but IV to less than 1%. The *N*-oxide, (VIII), however, could not be separated from VI (benzyl MA), so reduction of VIII back to pargyline was the only option to distinguish between VIII and VI. The

Table 2. pK_a-values and distribution constants between CH_2Cl_2 and aqueous phase for pargyline (I), *N*-benzylpropargylamine (II), *N*-methylpropargylamine (IV) and *N*-benzylmethylamine (VI). For experimental details see Material and Methods.

Compound	pK_a	$-\log K_{D(A)} \times K'_{HA}$	$\log K_{D(A)}$
I	6·6*	3·4	3·2
II	7·3**	4·9	2·4
IV	8·4**	8·3	0·16
VI	9·5***	8·0	1·5

* (Hallström et al (1981a). ** (Martin et al 1975). *** (Perrin 1965).

reduction of VIII gives pargyline as the only amine-containing product (Hallström et al 1981a).

Although, the choice of stationary phase is not critical in the g.l.c. analysis of the IBC-derivatives of pargyline, benzyl PA (II) methyl PA (IV) and benzyl MA (VI), the use of less polar columns like OV-17 is not suitable for the separation of products of such polarity as the derivative of the secondary metabolite, benzylamine.

It was possible to determine 0·5 μM pargyline, II and VI with a relative standard deviation of <5% (Table 3). The metabolic studies performed so far have, however, comprised higher concentrations (Fig. 4). Investigation of the mass spectra of the carbamates (Table 1) suggests that the sensitivity of the assay, if needed, can be further improved by conversion to g.l.c.–m.s. analysis with selected ion monitoring.

Application of the assay in studies of the metabolism of pargyline

The metabolism of pargyline is rapid and essentially all the substrate is consumed within the first few minutes when the initial concentration is 100 μM (Fig. 4). The metabolic activity is associated with the microsomal fraction, requires oxygen and is NADPH-dependent. None of the metabolites could be identified in derivatized extracts of the soluble fraction or when co-factors were omitted.

Table 3. Precision in the determination of pargyline (I), *N*-benzylpropargylamine (II), *N*-methylpropargylamine (IV), *N*-benzylmethylamine (VI) and pargyline *N*-oxide (VIII), when taken through the analytical procedure. The relative standard deviation (r.s.d. %) was determined at 0·5 μM concentration (n = 6) except for pargyline *N*-oxide (VIII) (1 μM). For experimental details see Materials and Methods.

Compound	R.s.d. % (n = 6)
I	3·2
II	3·3
IV	15
VI	4·8
VIII	20

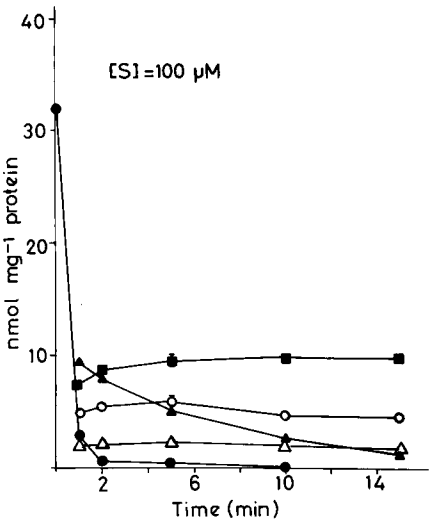

FIG. 4. Levels of pargyline (I, ●——●) and of *N*-benzylpropargylamine (II, ▲ - - - ▲), *N*-methylpropargylamine (IV, △——△) *N*-benzylmethylamine (VI, ■——■) and pargyline *N*-oxide (VIII, ○——○), as a function of time in liver microsomes from phenobarbitone pretreated rats. The initial substrate concentration was 100 μM (32 nmol mg^{-1} protein). The curves represent the mean of six experiments comprising three different microsomal preparations. The bars included at 5 min denotes s.e.m.-values.

N-Demethylation appears to be the most important metabolic route in liver microsomes from phenobarbitone pretreated rats, this is followed by *N*-depropargylation. *N*-Benzyl PA (II)—the metabolite that is most rapidly formed—is the most lipophilic of the metabolites (Table 2), and should be the best substrate for further metabolism (Lindeke & Cho 1982). That II undergoes further *N*-depropargylation (cf. Diehl et al 1976) is supported by the formation of benzylamine (IX) which, although not quantitated, was identified as an IBC-derivative. *N*-(Hydroxybenzyl)propargylamine (X) has been reported as another secondary metabolite of pargyline (Diehl et al 1976) but could not be positively identified in this study. Contrary to benzyl PA (II), none of the primary metabolites IV, VII and VIII appear to be further metabolized at an appreciable rate.

Of the metabolites that were identified, *N*-methyl PA (IV) has not been previously proven as a pargyline metabolite, although its formation has been inferred from the identification of benzaldehyde (V) (Hallström et al 1981a).

Material balance studies showed that the predominating part of the pargyline metabolites can be determined with this assay. Thus, in the example shown (Fig. 4) 27 out of 32 nmoles could be accounted for in the extracts from a 1 min incubation. Part of the 5 nmoles not accounted for will constitute secondary metabolites, formed in the further metabolism of benzyl PA (II). Moreover, non-enzymic decomposition of the *N*-oxide (VIII) (Hallström et al 1981a), within the microsomal membrane (Lindeke 1982), could contribute to a reduced recovery of this metabolite.

Acknowledgements

This work was supported by the Swedish Medical Research Council, grants No 03136-09A and 5645-03. We are indebted to Dr P. Hartvig, Prof. G. Schill and Prof. J. Vessman for valuable discussions.

REFERENCES

Ahnfelt, N.-O., Hartvig, P. (1980) Acta Pharm. Suec. 17: 307–318

Ahnfelt, N.-O., Hartvig, P., Karlsson, K.-E. (1982) Chromatographia in the press

Beckett, A. H., Gorrod, J. W., Jenner, P. (1971) J. Pharm. Pharmacol. 23: 555–615

Berman, H. B., Spirtes, M. A. (1971) Biochem. Pharmacol. 20: 2275–2286

Bogentoft, C., Svensson, U., Karlén, B. (1973) Acta Pharm. Suec. 10: 215–222

Brit. pat. (1962) 906.245 issued to Abbott lab. Sept. 19, 1962. CA 58: 5570h

Cho, A. K., Lindeke, B., Sum, C. Y. (1974) Drug. Met. Dispos. 2: 1–7

Coutts, R. T., Faster, B. C., Pasutto, M. (1981) Life Sci. 29: 1951–1958

De Masters, E., Nagasawa, T. H. (1978) Res. Commun. Chem. Pathol. Pharmacol. 21: 497–505

Diehl, E., Najm, S., Wolff, R.-E., Ehrhardt, J.-D. (1976) J. Pharmacol. (Paris) 7: 563–575

Evans, J. V., Daley, S. K., McClusky, G. A., Nielsen, C. J. (1980) Biomed. Mass Spectrom. 7: 65–73

Fowler, C. J., Norqvist, A., Oreland, L., Saramies, E., Wiberg, A. (1981) Comp. Biochem. Physiol. 68c: 145–149

Geiger, J., Bessman, S. P. (1972) Anal. Biochem. 49: 467–473

Hallström, G., Lindeke, B., Khuthier, A. H., Al-Iraqi, M. A. (1981a) Chem.-Biol. Interact. 34: 185–200

Hallström, G., Lindeke, B., Andersson, E. (1981b) Xenobiotica 11: 459–471

Karlsson, K.-E., Hartvig, P. (1980) Acta Pharm. Suec. 17: 249–261

Lebsack, M. E., Anderson, D. (1979) Res. Commun. Chem. Pathol. Pharmacol. 26: 263–275

Lindberg, C., Bondesson, U., Hartvig, P. (1980) Biomed. Mass Spectrom. 7: 88–92

Lindeke, B. (1982) Drug Metab. Rev. 13: 71–121

Lindeke, B., Cho, A. K. (1982) in: Jacoby, W. B., Bend, J. R., Caldwell, J. (eds) Metabolic Basis of Detoxication. Academic Press, New York p 105

Lindeke, B., Hallström, G., Johansson, C., Ericsson, Ö., Olsson, L.-I., Strömberg, S. (1981) Biomed. Mass Spectrom. 8: 506–513

Martin, Y. C., Martin, W. B., Taylor, J. D. (1975) J. Med. Chem. 18: 883–888

Omura, T., Sato, R. (1964) J. Biol. Chem. 239: 2370–2378

Parkinson, D., Callingham, B. A. (1980) J. Pharm. Pharmacol. 32: 49–54

Perrin, D. D. (1965) Dissociation Constants of Organic Bases in Aqueous Solution. Butterworths, London

Pirisino, R., Ciottoli, G. B., Buffoni, F., Anselmi, B., Curradi, G. (1979) Br. J. Clin. Pharmacol. 7: 595–598

Schill, G. (1978) Separation Methods for Drugs and Related Organic Compounds, Ch. 3. The Swedish Academy of Pharmaceutical Sciences, Stockholm, Sweden

Shirota, F. N., De Masters, E. G., Nagasawa, H. T. (1979) J. Med. Chem. 22: 463–464