Biochemical Pharmacology, Vol. 16, pp. 2421–2429. Pergamon Press Ltd. 1967. Printed in Great Britain

BIOCHEMISTRY OF DRUGS—XI.*

THE METABOLIC FATE OF A NEW PSYCHOTROPIC DRUG 11-(3-DIMETHYLAMINOPROPYLIDENE)-6,11-DIHYDRODIBENZ(b,e)-THIEPINE (PROTHIADENE)

O. HOREŠOVSKÝ, Z. FRANC and P. KRAUS

Research Institute for Pharmacy and Biochemistry, Prague, Czechoslovakia

(*Received* 21 *March* 1967; *accepted* 17 *July* 1967)

Abstract—The absorption, distribution, excretion, and metabolism of 11-3-dimethylaminopropylidene)-6,11-dihydrodibenz(b,e)thiepine (prothiadene) has been studied in rats with use of ^{35}S-labeled drug. Immediately after the i.v. application of the labeled drug, high amounts of ^{35}S were found in the lungs and later in the large intestine, too. In addition, when prothiadene was given orally, high concentration of radioactive compounds were found in liver and kidneys as well. In any case, only small amounts of ^{35}S were found in the skin, muscle and blood. There are certain sex differences in the organ fixation of prothiadene. The drug and its metabolites are mainly excreted by the urine (about 2/3) and by faeces (about 1/3). Approximately one third of the dose is excreted during 24 hr, regardless to the way of application. Considerable amounts are eliminated through bile. The drug and its metabolites which are excreted into the intestine, are partly reabsorbed. There are at least seven metabolites of prothiadene in the chloroform extract of the alcalinated urine. They were identified as unchanged prothiadene, mono- and *bis*-demethylated product and sulphoxide. The composition of remaining three compounds is unknown. In addition, two glucuronides and free plus bound sulphates were found in the native urine.

RAJŠNER and Protiva[1] synthetized 11-(3-dimethylaminopropylidene)-6,11-dihydrodibenz(b,e)thiepine (prothiadene; see Fig. 1). With regard to the results of pharmacological and clinical studies it has been suggested to use this drug as an antidepressant.

FIG. 1. The structure of prothiadene.

Some observations indicate that prothiadene can substitute imipramine in cases of clinical resistance to this drug.[2]

* Preliminary articles: *Čs. Gastroenterologie* **13**, 572 (1959); *Československ. Farm.* **11**, 291 (1962), *ibid.* **12**, 234 (1963), *ibid.* **12**, 230 (1963), *ibid.* **13**, 55 (1964); *Physiol. Bohemoslov.* **10**, 390 (1961), *ibid.* **12**, 150 (1963); *Neoplasma* **10**, 193 (1963). *ibid.* **11**, 165 (1964); *Čs. Gynekologie* **28**, 301 (1963).

EXPERIMENTAL AND RESULTS

All attempts to work out a suitable chemical method for the determination of prothiadene were unsuccessful. For this reason, a ^{35}S-labeled compound was used in the present study. It has been prepared by a slight modification of the original method.[1]

The activity of ^{35}S in the tissues was determined after Horešovský and Franc.[3]

The results were statistically evaluated by means of the Student's *t*-test.

Animals

Unless otherwise stated, adult male Wistar rats were used. The animals were maintained on a Larsen diet with a free access to water up to the sacrifice.

The distribution of radioactivity in tissues after i.v. and oral administration of labeled prothiadene.

Prothiadene-^{35}S was applied either i.v. (15 mg/kg; 0·2 mCi/mg) or *per os* by a gastric tube (100 mg/kg; 0·22 mCi/mg). At indicated intervals, the animals were killed and the radioactivity in tissues was determined. The results are summarized in Figs. 2 and 3.

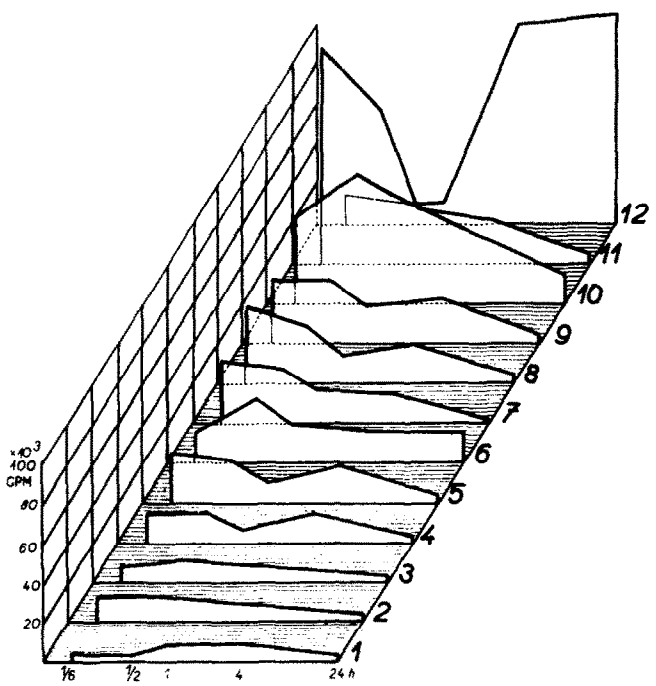

FIG. 2. The radioactivity in some organs following an i.v. application of ^{35}S-labeled prothiadene. The rats were injected with 15 mg/kg of ^{35}S-prothiadene (0·2 mc/mg) and killed at indicated intervals. The radioactivity is expressed as cpm/1 g wet wt. 1—blood, 2—muscle, 3—skin, 4—heart, 5—spleen, 6—stomach, 7—brain, 8—kidney, 9—liver, 10—small intestine, 11—lung, 12—large intestine.

Sex differences

Male and female rats were treated orally with 100 mg/kg of labeled prothiadene. They were killed 6 hr later and the specimens from the organs were treated as before. The differences in absorption and distribution are evident from Fig. 4.

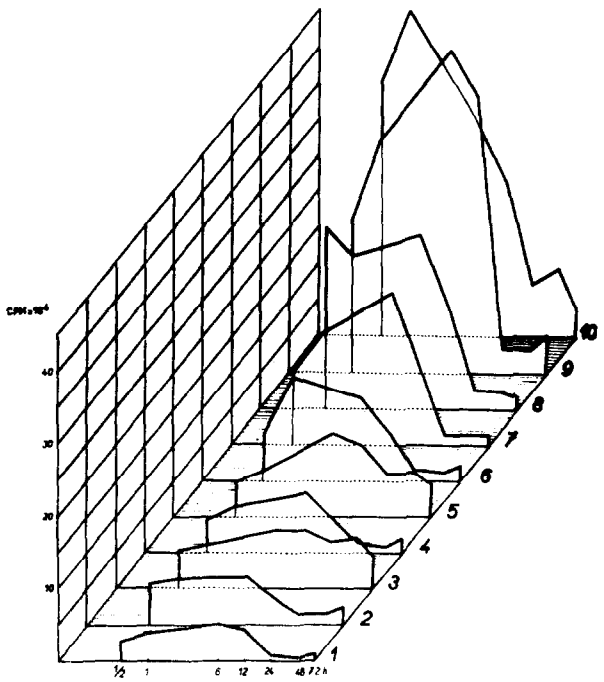

FIG. 3. The radioactivity in some organs following an oral application of ^{35}S-labeled prothiadene. The rats obtained 100 mg/kg of prothiadene *per os* (0·22 mc/mg) and were killed at indicated intervals. The radioactivity is expressed as cpm/1 g wet wt. 1—brain, 2—blood, 3—skin, 4—muscle, 5—residue, 6—heart, 7—spleen, 8—kidney, 9—lungs, 10—liver.

The distribution of radioactivity in the brain

In this experiment, ^{35}S-labeled prothiadene was given orally. The animals were killed 8 hr after the intake of 100 mg/kg of the drug. The brains were dissected into the main parts. The radioactivity was also measured in some other organs, as enumerated in Table 1. It is evident that there were no significant differences in the activity of ^{35}S in different parts of brain.

Biliary excretion

The rats were anesthetized by 1 g/kg body wt. of urethane i.p. Fifty mg/kg of prothiadene have been given 2 hr later by a stomach tube. The abdomens of experimental animals were then opened and plastic canulles were inserted into their biliary ducts. At indicated intervals, specimens were taken and their radioactivity was determined. The results are presented in Fig. 5.

The elimination of radioactivity after oral and i.v. application of labeled prothiadene

In this experiment, similar doses were used as in the distribution studies, i.e. 15 mg/kg i.v. or 100 mg/kg orally. The animals were placed in metabolic cages and the excrements were collected separately. The amounts of radioactivity eliminated by urine and faeces are given in Fig. 6.

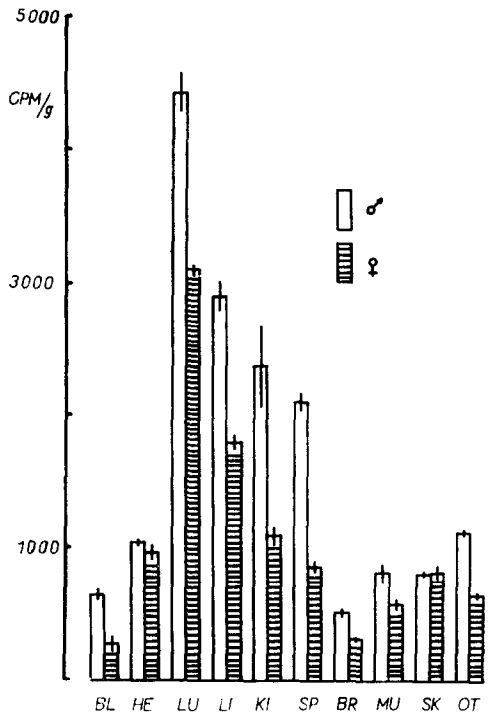

FIG. 4. Sex differences.

The animals obtained 100mg/kg of labeled prothiadene (0·22 mc/mg). They were killed 6 hr after the application of the drug. The results are presented as cpm/1 g wet wt. HE—heart, LU—lung, LI—liver, KI—kidney, SP—spleen, BR—brain, MU—muscle, SK—skin, OT—other tissues (residue, incl. the contents of intestines).

TABLE 1. THE DISTRIBUTION OF RADIOACTIVITY IN DIFFERENT PARTS OF BRAIN, COMPARED WITH THAT OF SOME OTHER ORGANS

Sample	CPM per 1 g of wet wt. of the sample	CPM in the whole organ	% of the dose
Telencephalon	1040	483	0·01
Diencephalon	1125	834	0·02
Mesencephalon	1080	453	0·01
Liver	13,664	89,922	1·92
Gastrointestinal tract	232,000	3 421,600	72·96
Residue	4556	806,600	17·20
Total unexcreted radioactivity			92·12
Urine*	76,150	105,200	2·24
Stool*	22,575	1360	0·03
Bile*†	124,508	262,994	5·58
Total excreted radioactivity			7·85
Recovery			99·97

* Cumulative excretion 0–8 hr.

† The bile was taken by a canulle inserted in the biliary duct.

The animals were treated with 100 mg/kg of ^{35}S-labeled protiaden (0·22 mCi/mg) *per os* and killed 8 hr later. The radioactivity in organs and excrements was determined after ref. 3. The data are averages from 6 values.

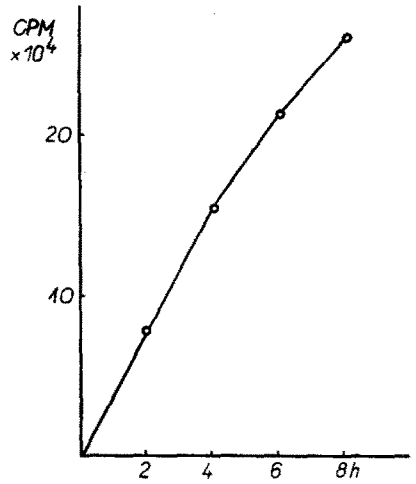

FIG. 5. Cumulative biliary excretion of radioativity after peroral dose of labeled prothiadene. For experimental details, see text.

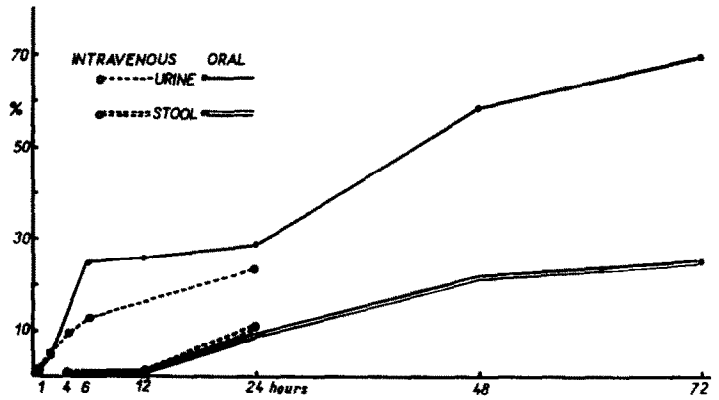

FIG. 6. The excretion of radioactivity after a single dose of ^{35}S-labeled prothiadene. The animals were treated with 15 mg/kg i.v. or with 100 mg/kg p.o. of the drug. The results are presented as cumulative values (in per cent of the applied dose).

The separation and identification of some urinary metabolites of prothiadene

The urine of prothiadene-treated rats (100 mg/kg p.o.) has been collected for 72 hr after the intake of the drug. Ten ml of filtered urine was acidified with acetic acid to pH 3 and extracted three times with 10 ml of ether. The organic phase was collected, the solvent evaporated and the dry residue was redissolved in 0·2 ml of ethanol. The aqueous phase was alcalinated with 1 ml of concentrated ammonium hydroxide and extracted repeatedly into chloroform. The organic phase was then treated as described before.

The aqueous phase was neutralized, mixed with an excess of benzidine reagent* and allowed to stand 2 hr at 4°. The precipitate (i.e. benzidine sulphate) was collected

* Five g of benzidine hydrochloride were dissolved in 40 ml of N HCl and introduced into 200 ml of 50% ethanol.

on a Büchner funnel, washed three times with ethanol and dried. Hydrochloric acid was added to the filtrate, in a quantity sufficient to obtain the final concentration 6 N. The mixture was hydrolyzed 2 hr under reflux and then treated with benzidine reagent again. Another portion of precipitate was obtained. The radioactivity of extracts, precipitates and of the residue has been determined. Relative amounts of ^{35}S in the fractions are evident from Table 2.

TABLE 2. QUANTITATIVE DISTRIBUTION OF RADIOACTIVITY OF SOME URINARY FRACTIONS AFTER ORAL APPLICATION OF PROTHIADENE-^{35}S

Fraction	%of total urinary ^{35}S-activity
Ether extract	13
Chloroform extract	19
Free sulphate	18
Bound sulphate	8
Residual activity	42

The metabolites of prothiadene contained in the chloroform extract were separated with use of two-dimensional paper chromatography. The composition of solvents and other experimental details are given in the legend to Fig. 7. It may be seen from this figure that there are at least seven urinary metabolites of prothiadene.

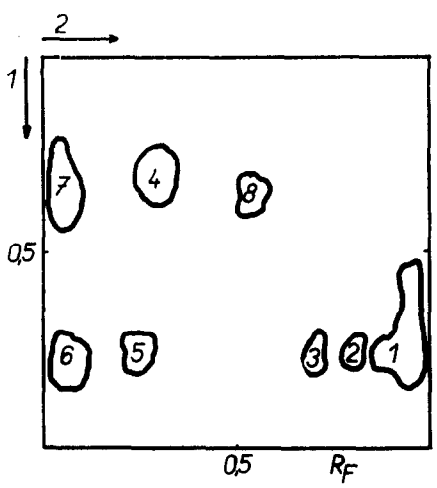

FIG. 7. Two-dimensional paper chromatography of prothiadene metabolites contained in the chloroform extract of urine.

Solvents: I: benzene—chloroform (2:3), II: Isoamylalcohol—ethanol—formic acid—water (100:15:1:100)

The Whatman No. 1. filter paper was impregnated with formamide. The spots were located by autoradiography. (In certain instances, u.v. light and Dragendoff reagent were used for the detection, too). 1—unchanged prothiadene, 2—demethylated prothiadene/11-(3-methylaminopropylidene)-6,11-dihydrodibenz/b,e/thiepine/,3-*bis*-demethylated prothiadene/11-(3-aminopropylidene)-6,11-dihydrodibenz/b,e/thiepine, 4-sulphoxide, 5-8 unknown.

The spots located by autoradiography were cut out and eluted into chloroform. U.V.-spectra of the metabolites were determined and compared with the spectra of prothiadene and some related compounds. This analysis revealed that compound I is unchanged prothiadene, compound II is 11-(3-methylaminopropylidene)6,11-dihydrodibenz(b,e)thiepine, compound III is 11-(3-aminopropylidene)-6,11-dihydrodibenz(b,e)thiepine and compound IV is prothiadene sulphoxide. The structure of compounds No. V, VI and VII was not identified. The results of quantitative determinations of the chloroform–extractable metabolites are summarized in Table 3.

TABLE 3. QUANTITATIVE DISTRIBUTION OF RADIOACTIVITY IN THE CHLOROFORM EXTRACT OF URINE AFTER THE ORAL APPLICATION OF PROTHIADENE-^{35}S

Compound	% of the activity of the chloroform extract
Prothiadene (I)	33
Desmethylprothiadene (II)	1
Desdimethylprothiadene (III)	12
Suphoxide (IV)	9
Unknown substances (V–VIII)	44

The formulae of these compounds see in Fig. 9. The metabolites in the chloroform extract of rat urine were separated by two dimensional paper chromatography. The spots were eluted and some of them were identified by u.v. spectra. (For the composition of solvent systems, seen the legend to Fig. 7).

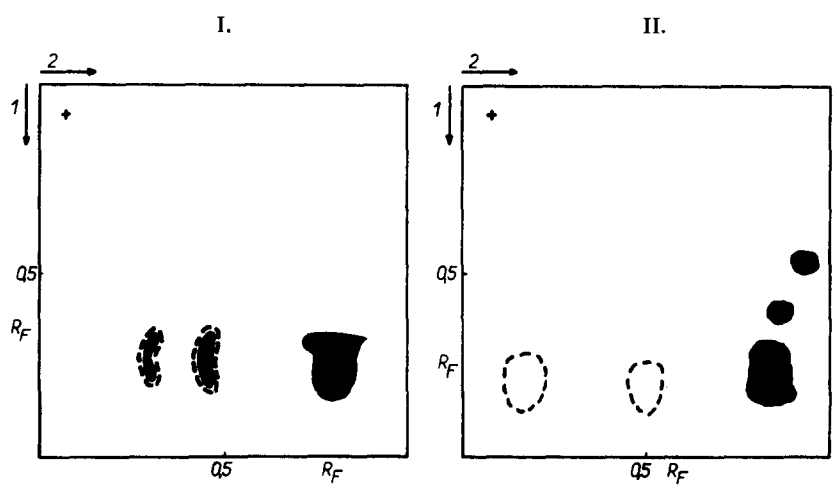

FIG. 8. The chromatography of urinary metabolites of prothiadene.
Native urine (Chromatogram No. I) and urine incubated with β-glucoronidase (chromatogram No. II), both were analyzed by two-dimensional paper chromatography. Solvents: I: *n*-butanol-acetic acid-water (12:3:5), II: 0,1 M veronal buffer (pH 8·7) saturated with ethylacetate. Black spots denote compounds detectable by autoradiography, the spots circumscripted by a dotted line are the compounds giving a positive reaction with naphtoresorcine.

In another set of experiments, 1 ml of the urine was enzymatically hydrolyzed with 15,000 units of β-glucuronidase in acetate buffer (pH 4·5), 36 hr at 37°. Native and hydrolyzed samples of urine were analyzed by paper chromatography. (Details are given in description to Fig. 8.) It is evident that two spots changed essentially their position after the incubation with β-glucuronidase. In addition, no radioactivity was associated with the naphtoresorcine coloured spots on the chromatogram of the enzymatically hydrolyzed urine. The result indicates that in the urine of rats treated with prothiadene, there are at least two glucuronides originating from this drug.

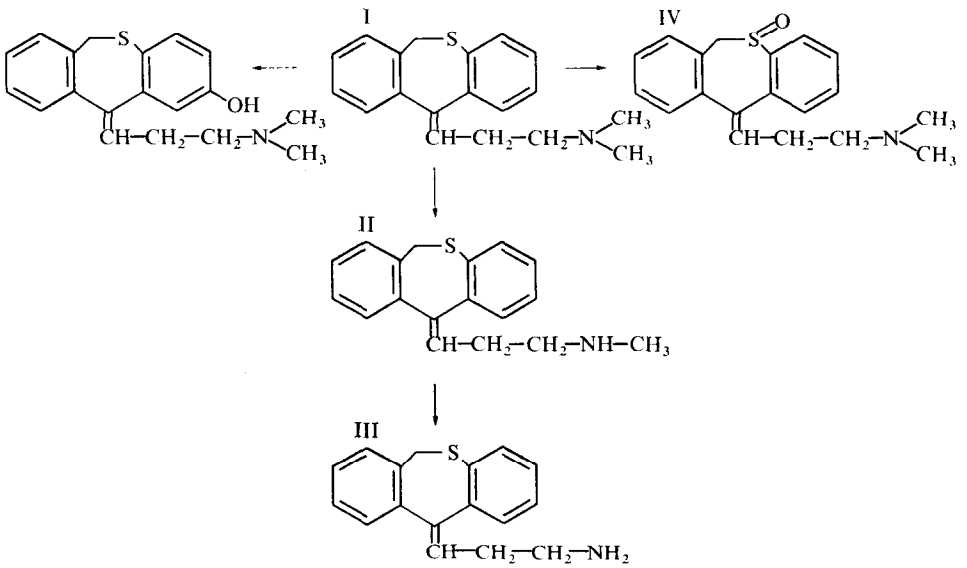

FIG. 9. Scheme of the biotransformation of prothiadene.
I—Prothiadene, II—demethylated prothiadene, III—*bis*-demethylated prothiadene, IV—sulphoxide. The compound without a number is presumable metabolite which may conjugate with glucuronic acid.

DISCUSSION

The distribution studies show marked differences in the organ fixation of prothiadene, particularly after the intravenous application. Initially, an extremely high level of radioactivity has been found in the lungs but already in the fourth hour, the radioactivity in all parenchymatous organs is of a similar magnitude. On the other hand, between the first and fourth hour after the application, a 9-fold increase of radioactivity has been found in the large intestine. At the same time, a lower but also statistically significant rise was noted in the heart, spleen and kidneys, whereas the increase in liver was insignificant. In our opinion, the observations presented here allow a hypothesis that prothiadene (free and/or metabolized) accumulated in the large intenstine is partly reabsorbed into the blood.

Comparable results were obtained when the drug was given orally, except of the levels in the kidneys and livers: they are nearly as high as the levels in the lungs. In any case, blood concentrations were extremely low even in the first minutes after the i.v. application of the drug. In addition, no correlations were found between the blood and organ levels of prothiadene.

It has been possible to demonstrate sex differences in the fixation of prothiadene in certain organs. These findings could not be explained as yet. When the drug was given i.v., the excretion rate remains constant for the first 6 hr and then it sinks, but on the other hand, the excretion of ^{35}S by the faeces starts at this time, so that about 32 per cent of the applied dose are excrete within the first 24 hr, i.e. 22 per cent by the urine and 10 per cent by the stool. It seems noteworthy that only about 8 per cent of the dose are excreted in the first 4 hr after the i.v. application and therefore that all changes in the organ levels of prothiadene which were noted during this period should be, before all, attributed to the redistribution of the drug in the body.

Similar elimination patterns were obtained after the oral application. At the 24–72nd hr after the drug intake, the ratio of amounts excreted by urine and by faeces is approximately 2·5:1 and it does not differ much from the corresponding ratio at the 24th hr after the i.v. application. Although there is no direct evidence, a hypothesis seems to be justified that high amounts of radioactive substances in the intestines after the oral application are to a considerable extent due to the excretion of prothiadene and/or its metabolites into the intestine. The fact that 24 hr after the i.v. application, about 35 per cent of the drug remaining in the organism is accumulated in the gastrointestinal tract, strongly supports this opinion.

The biotransformation of psychotropic drugs, imipramine, chlorpromazine and prothiadene shows certain similar features, i.e. demethylation, oxidation of the heteroatom in the ring and the formation of glucuronides.[4–12] The deep degradation of prothiadene to inorganic sulphur probably comprises following steps: The oxidation of prothiadene to sulphoxide, the oxidation of sulphoxide to sulphone (hypothetical) and the splitting of the heteroring, with the formation of inorganic sulphate. Although demethylated prothiadene has similar pharmacological properties as the original compound, there is actually no reason to suppose that this metabolite is the proper active substance.

REFERENCES

1. M. Protiva, M. Rašjner, V. Seidlovà, E. Adlerová and Z. J. Vejdělek, *Experientia* **18**, 326 (1962).
2. K. Náhůnek, D. Bártová and L. Ingerová, *Activitas nerv. sup.* **6**, 178 (1964).
3. O. Horešovský and Z. Franc, *Coll. Czechoslov. chem. Commun. Engl. Edn.* **30**, 3218 (1965).
4. T. Berti and L. Cima, *Farmaco* **12**, 159 (1957).
5. V. Fishman and H. Goldenberg, *Proc. Soc. exp. Biol. Med.* **104**, 99 (1960).
6. J. L. Emerson, and T. S. Miya, *J. pharm. Sci.* **52**, 411 (1963).
7. C. L. Huang and B. H. Ruskin, *J. nerv. ment. Dis.* **139**, 381 (1964).
8. A. H. Beckett, M. A. Beaven and A. E. Robinson, *Biochem. Pharmac.* **12**, 779 (1963).
9. B. Herrmann and R. Pulver, *Archs int. Pharmacodyn. Thér.* **126**, 454 (1960).
10. W. Schindler, *Helv. chim. Acta* **43**, 35 (1960).
11. J. A. Gillette, J. V. Dingell, F. Sulser, F. Kuntzman and B. B. Brodie, *Experientia* **17**, 417 (1961).
12. J. V. Dingell, F. Sulser and J. A. Gillette, *J. Pharmac. exp. Ther.* **143**, 14 (1964).