INHIBITION OF ALDEHYDE REDUCTASE I BY SOME ISOQUINOLINE ALKALOIDS

Hana PAULOVÁ[a], Jan KOVÁŘ[b], Jiří PLOCEK[b] and Jiří SLAVÍK[a]

[a] *Department of Medical Chemistry and Biochemistry, Faculty of Medicine, University of J. E. Purkyně, 662 43 Brno and*
[b] *Department of Biochemistry, Faculty of Science, University of J. E. Purkyně, 611 37 Brno*

Received June 30th, 1986

Aldehyde reductase I has been found to be inhibited by certain isoquinoline alkaloids (protoberberines, protopines, benzylisoquinolines, benzyltetrahydroisoquinolines, phthalideisoquinolines, pavinanes) and narceine imide. The sensitivity of this enzyme to the compounds tested was compared with that of alcohol dehydrogenase and/or aldehyde reductase II to them; alcohol dehydrogenase proved more selective in binding the alkaloids. The kinetics of the inhibitory action of berberine and other results suggest that the binding site of aldehyde reductase I for alkaloids is relatively large, has a hydrophobic nature, and probably contains a group with a positive charge. This binding site is probably not identical with the active centre of the enzyme.

Isoquinoline alkaloids inhibit to some extent certain enzymes, such as ATPase[1], adenylate cyclase[1], monoaminooxidase[2], and choline esterases[3,4]. They also have an effect on NAD-dependent dehydrogenases, but these rather differ in their capacity to interact with isoquinoline alkaloids of different classes. Alcohol dehydrogenases from liver (LADH) and from yeast (YADH), kindred in both structure and function, behave similarly, but differences were observed[5-7]. Protoberberines form a rather firm linkage to both LADH and YADH (refs[5-7]), whereas aporphines bind to the former only[7-8]. Selected representatives of benzylisoquinolines, phthalideisoquinolines, protopines, and pavinanes had no inhibitory effects on YADH (refs[9]); their interaction with LADH has not been investigated yet. Protoberberines and aporphines were found[6,8] to bind to the active centre of LADH, which is located in a deep pocket, extending to the core of the enzyme molecule[10]. The presence of coenzymes tended to disrupt this bond[11]. With YADH, whose active centre is more shielded and thus less accessible[10], there was observed rather a surface interaction with alkaloids in proximity to the active centre[7]. Glutamate dehydrogenase, though an enzyme different in both structure and function, also proved capable of binding some isoquinoline alkaloids (aporphines, benzylisoquinolines, phthalideisoquinolines)[12]. The binding site of the enzyme for these compounds does not seem to coincide with the region of the active centre. However, other typical NAD-dependent

dehydrogenases (lactate dehydrogenase, malate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, hydroxybutyrate dehydrogenase) failed to interact with isoquinoline alkaloids[9].

The present paper deals with the inhibitory action of various isoquinoline alkaloids (different structural types) on aldehyde reductase I (ALR I, EC 1.1.1.2) and compares it with the effect on liver alcohol dehydrogenase (LADH, EC 1.1.1.1), which is functionally a kindred enzyme. Some basic data on the action of protoberberines, aporphines, and benzylisoquinolines on a similar enzyme, *viz.* aldehyde reductase II (ALR II, aldose reductase, EC 1.1.1.21) were published previously[13]. The main difference between ALR's I and II on the one hand, and LADH on the other consists in aldehyde reductases being monomeric enzyme specific to NADPH (ref.[14]), whereas LADH is a dimer utilizing NADH. Aldehyde reductases catalyse reduction of a number of aldehydic and other reducible substances; oxidation of the corresponding alcohols in the presence of $NADP^+$ takes a sluggish course[14].

EXPERIMENTAL

13-Alkylberberines were obtained from berberine sulphate (E. Gurr, England), adhering to a described procedure[15]. The natural isoquinoline alkaloids used were isolated from species of the family *Papaveraceae* at the Department of Medical Chemistry and Biochemistry, Faculty of Medicine, University of J. E. Purkyně. Narceine imide was a gift from Dr J. Trojánek, Research Institute for Pharmacy and Biochemistry, Prague. *p*-Nitrobenzaldehyde was a product from the firm Fluka (Switzerland), NADH from Merck (F.R.G.), NADPH from Reanal (Hungary); purity of the two coenzymes was determined chromatographically[16] as c. 90%. Alcohol dehydrogenase (LADH) was isolated from equine liver by a reported procedure[17], aldehyde reductase I (ALR I) from porcine liver as described in ref.[18].

The initial rates of the reactions catalysed by the two enzymes were measured spectrophotometrically (Cary 118, Varian, U.S.A.) at 340 nm in cells with an optic path of 0·5 or 1 cm. The reactions ran in a Na-phosphate buffer pH 7, 0·1 mol l^{-1}, at 25°C, unless otherwise stated. The standard reaction mixture for LADH (1 ml) was 0·2 mmol l^{-1} NADH and 1·5 mmol l^{-1} acetaldehyde; the standard reaction mixture for ALR I (1 ml) was 0·2 mmol l^{-1} NADPH and 1 mmol l^{-1} *p*-nitrobenzaldehyde. The reactions were initiated by small volumes of the enzyme preparations, the final activity of the enzymes in the cell being 0·1 μkat. The inhibitory effects of the compounds tested are expressed in reaction rate decreases (%) due to given concentrations of these compounds; they are also expressed by values of $K_{0\cdot5}$ (inhibitor concentrations decreasing the reaction rate to a half under standard conditions), or by inhibition constants K_{is} (slope) and K_{ii} (intercept). The values of K_{is} and K_{ii} were determined for saturating concentration of one of the reactants (coenzyme or substrate) and variable concentration of the other (substrate or coenzyme), using a linear regression from the equation:

$$1/v_0 = 1/V_m(1 + [I]/K_{ii}) + K_m/V_m \cdot 1/[S](1 + [I]/K_{is}),$$

where v_0 designates the initial reaction rate, V_m the maximum reaction rate, K_m the Michaelis constant, [I] the inhibitor concentration, and [S] the variable concentration of either the coenzyme or the substrate.

RESULTS AND DISCUSSION

The inhibitory effects of selected isoquinoline alkaloids on ALR I and LADH are surveyed in Table I. It shows that ALR I can interact with every compound tested, with the exception of the representative of aporphines, *viz.* aporheine ((+)-roemerine); no inhibition was observed even with other aporphines, *viz.* bulbocapnine and glaucine. However, the degrees of inhibition at comparatively high concentrations of the alkaloids suggest that the interaction is rather weak. The only exceptions were berberine (a representative of protoberberines) and narceine imide (the latter case is probably an artifact, due to the isolation from *P. somniferum*[19]). These two compounds are relatively strong inhibitors of ALR I. The results in Table I also confirm the data obtained with ALR II (ref.[13]): Both protoberberines are stronger inhibitors than any of the benzylisoquinolines which, in turn, are more efficacious than aporphines. The inhibitory effects of these groups of alkaloids on ALR I and ALR II are comparable (*cf.* Table I and data in ref.[13]). These findings testify to a close relationship of ALR's I and II, even in their capability of interacting with isoquinoline alkaloids. There is some similarity, too, in the differences between the action of alkaloids on aldehyde reductases and on LADH. Even with LADH berberine has proved to be the strongest inhibitor of all the alkaloids tested, next in

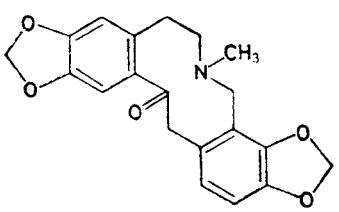
TABLE I

Inhibition of aldehyde reductase I (A) and alcohol dehydrogenase (B) by isoquinoline alkaloids. For conditions see Experimental, concentration of the alkaloids was 0·15 mmol l^{-1}; the results are averages from three measurements

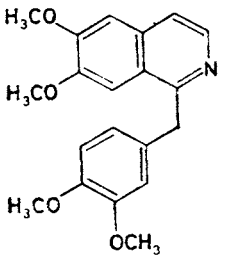
	Alkaloid	Class	Inhibition, %	
			A	B
I	protopine	protopines	24	<10
IIa	papaverine	benzylisoquinolines	26	10
IIb	escholamine	benzylisoquinolines	17	<10
IIIa	laudanosine	benzyltetrahydroisoquinolines	20	20
IIIb	pseudorine	benzyltetrahydroisoquinolines	18	—[a]
IVa	narcotine	phthalideisoquinolines	10	<10
IVb	hydrastine	phthalideisoquinolines	21	10
Va	eschscholtzine	pavinanes	22	<10
Vb	californidine	pavinanes	22	<10
VI	aporheine	aporphines	<10	60
VII	berberine	protoberberines	65	78[b]
VIII	narceine imide	seco-phthalideisoquinolines	30	35

[a] Not measured; [b] determined at alkaloid concentration 60 μmol l^{-1}.

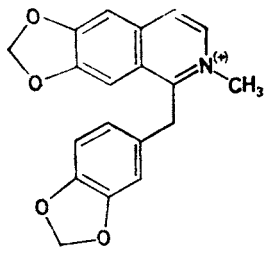
efficacy being narceine imide. LADH was also rather inhibited by aporheine, whereas the majority of the other alkaloids tested had practically no effect on this enzyme (Table I). Hence it seems that interaction of isoquinoline alkaloids with LADH is more selective than with aldehyde reductases.



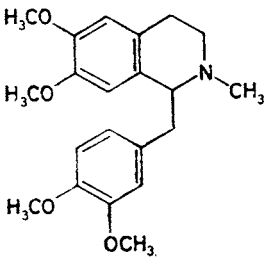
I



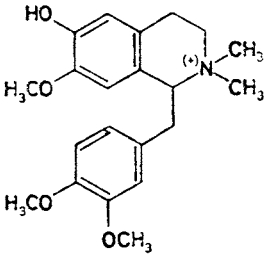
II a



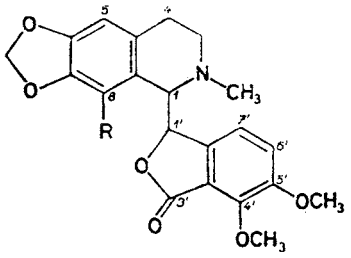
II b



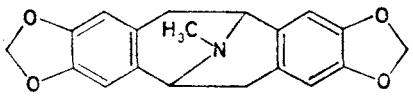
III a



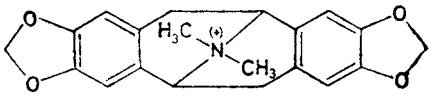
III b



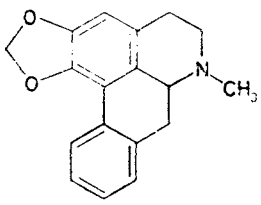
IV a, R = OCH_3
IV b, R = H



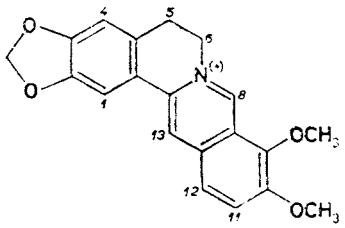
V a



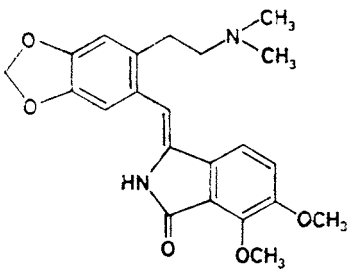
V b



VI



VII



VIII

The results in Table I further indicate that the positive charge of the alkaloid molecules somewhat suppresses their inhibitory effect on ALR I (*cf.* papaverine *vs* escholamine, and laudanosine *vs* pseudorine). The influence of charge was studied in greater detail on eschscholtzine (its pK is c. 6·3, ref.[20]). This alkaloid, in a concentration of 0·15 mmol l^{-1}, exhibited its strongest inhibitory effect in an alkaline medium (25% at pH 8), a weaker one in a neutral medium (22% at pH 7), and the lowest in an acid medium (15% at pH 6). In the case of berberine, the pK of which is in a strongly alkaline region[20], the inhibitory effect was practically independent of pH. These facts testify to a stronger bond to the chargeless form of the alkaloid, which may be due to the positive charge being present in the binding site of ALR I for these alkaloids. ALR I was found to have two positively charged groups important for binding certain ligands[21]. One of them, more remote from the active centre, constitutes a part of the binding site of the enzyme for some negatively charged inhibitors[21]. It can not be ruled out that the charge of this group will interfere with the binding of isoquinoline alkaloids. Since the compounds tested represent a rather small series, it is impossible to assess safety the effects of substituents attached to the molecular skeleton on the inhibitory efficacy of an alkaloid against ALR I. It is only the phthalideisoquinoline alkaloids that seem to allow of some enlightening comparisons (Table I). The inhibitory effect of hydrastine substantially exceeded that of narcotine. Either alkaloid (α-narcotine and β-hydrastine) has the *erythro* configuration[22], the only structural difference being the presence of a methoxyl group in the molecule of narcotine; steric interactions of this group hinder rotation of the isoquinoline and the phthalide rings. This suggests that in the binding site these rings of hydrastine can take a more convenient constellation than in narcotine. It seems noteworthy that similar phenomena were observed in the interaction of these two alkaloids with glutamate dehydrogenase[12].

TABLE II

Inhibition constants of intercept (K_{ii}, μmol l^{-1}) and slope (K_{is}, μmol l^{-1}), characterizing interactions of berberine with ALR I and LADH. The data were taken from Figs 2 and 3 as averages of two values (except K_{ii} and K_{is} for variable substrate concentration in the case of LADH, where the measurements refer to only one inhibitor concentration — *cf.* Fig. 3*a*)

Enzyme	Variable concentration of substrate		Variable concentration of coenzyme	
	K_{ii}	K_{is}	K_{ii}	K_{is}
ALR I	115	65	95	95
LADH	85	85	140	33

Since berberine has proved to be the strongest inhibitor of ALR I (Table I), we have investigated some semi-synthetical berberine derivatives with alkyl groups bound to C(13). Fig. 1 illustrates the inhibitory effects of these compounds on ALR I and LADH. The more hydrophobic derivatives (*i.e.* those with longer alkyl chains) appear to be capable of stronger interactions with both enzymes, the inhibitory efficacy for ALR I increasing in the substituent succession —H, —CH_3, —C_2H_5, the slope being approx. 0·5 (for the plot of $-\log K_{0.5}$ *vs* parameter of hydrophobicity, π, ref.[23]). With LADH the slope of an analogous plot is close to 1 (Fig. 1, ref.[6]). The derivatives with longer alkyl group had practically the same inhibitory efficacy on ALR I as the ethyl derivative, whereas with LADH the efficacy rapidly decresed with increasing length of the chain (Fig. 1). As has been shown before[6], the binding site of LADH for alkaloids is hydrophobic, deep and sterically confined, whereas this site in ALR I, though hydrophobic too, is much more extensive and rather superficial. These facts accord with the finding that aldehyde reductase bind a number of bulky (hetero)aromatic substances — the binding site is supposed to be rather broad and hydrophobic[24]. This site is likely to bind even the isoquinoline alkaloids.

In present work we compared the kinetics of inhibition of ALR I and LADH by berberine (Figs 2 and 3). The effect of this inhibitor was investigated at different concentrations of the coenzyme and the substrate (and saturating concentrations of the other reaction component). The very fundamental data have revelaed that different inhibition mechanisms operate with the two enzymes. Berberine was non-competitive toward NADPH and a mixed competitive behaviour of berberine toward *p*-nitrobenzaldehyde was observed with ALR I (Fig. 2), whereas with LADH it was nearly competitive toward NADH and nearly non-competitive toward acetaldehyde. The (hetero)aromatic inhibitors of aldehyde reductase so far known are usually non-competitive toward substrates[21], but berberine and other isoquinoline alkaloids are virtually competitive toward the substrate (glyceraldehyde) with ALR II

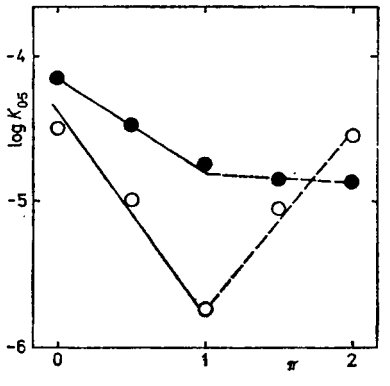


FIG. 1

Comparison of inhibitory efficacy of 13-alkyl derivatives of berberine on ALR I (●) and LADH (○). The efficacy is expressed by log $K_{0.5}$, the lengths of the alkyl substituents are expressed by the parameter of relative hydrophobicity[23], π. The data for LADH are taken from ref.[6]

(ref.[13]). The inhibitory action of berberine on ALR I was found to be between these two principal types of inhibitory behaviour.

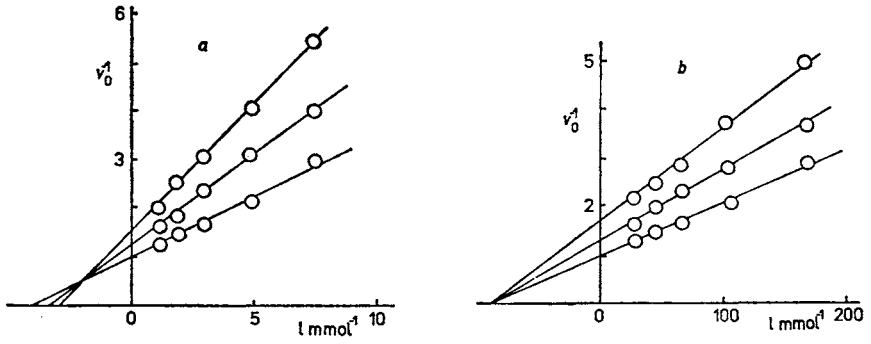


FIG. 2

The kinetics of inhibition of ALR I by berberine. v_0^{-1} denotes the reciprocal relative initial rate of the enzyme-catalysed reaction (at saturating concentrations of the coenzyme and substrate the rate is taken as 1); *a* reciprocal variable concentration of *p*-nitrobenzaldehyde (1 mmol^{-1}) at a saturating concentration of NADPH (0·15 mmol l^{-1}), the berberine concentrations corresponding to the respective lines from below were 0, 30, and 60 μmol l^{-1}); *b* reciprocal variable concentration of NADPH (1 mmol^{-1}) at a saturating concentration of *p*-nitrobenzaldehyde (2 mmol l^{-1}), the inhibitor concentrations being the same as in *a*

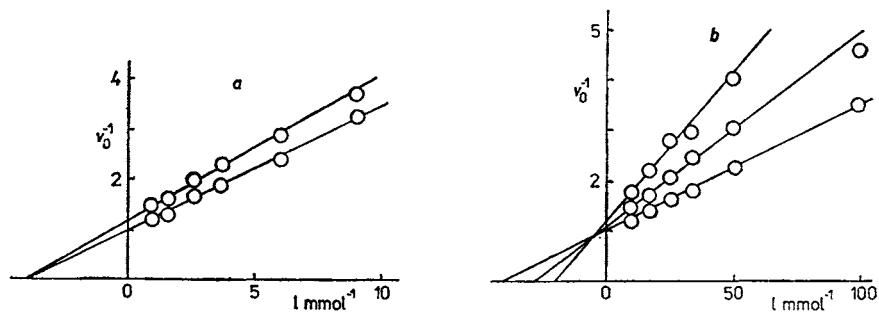


FIG. 3

Kinetics of inhibition of LADH by berberine; for the meaning of symbols see the legend for Fig. 2. *a* reciprocal variable concentration of acetaldehyde (1 mmol^{-1}) at a saturating concentration of NADH (0·2 mmol l^{-1}); concentrations of berberine: 0 μmol l^{-1} (lower line) and 16 μmol l^{-1} (upper line); *b* reciprocal variable concentration of NADH (1 mmol^{-1}) at a saturating concentration of acetaldehyde (2 mmol l^{-1}), the concentrations of berberine for the lines from below were 0, 16, and 40 μmol l^{-1}, respectively

A refined interpretation of the inhibition data (Figs 2 and 3) corroborates the stated differences in behaviour between ALR I and LADH toward berberine. The interpretation is based on the assumptions that *a*) with either enzyme the kinetics is essentially controlled by a mechanism, in which the binding of the coenzyme precedes the binding of the substrate[25,26], *b*) with either enzyme the transfer of hydride between the nicotinamide ring of the coenzyme and the substrate is rapid, and so is dissociation of the arising alcohol[25,27]. Consequently, with either enzyme the kinetics is close to Theorell-Chance's approximation[25]. Under these assumptions the inhibitory constants of the intercept and the slope (K_{ii} and K_{is}), determined from the data in Figs 2 and 3, can be interpreted as follows[7]: K_{is} for variable concentration of the substrate corresponds to the dissociation constant of the ternary complex enzyme–reduced coenzyme–inhibitor for dissociation of the inhibitor ($K_{E\text{-}NAD(P)H,I}$); K_{is} for variable concentration of the reduced coenzyme corresponds to the dissociation constant of the binary complex enzyme–inhibitor ($K_{E,I}$). The two constants K_{ii} correspond, assuming validity of Theorell - Chance's approximation, to the dissociation constant of the ternary complex, enzyme–oxidized coenzyme–inhibitor ($K_{E\text{-}NAD(P),I}$), or, assuming the ordered mechanism, the constants are of a complex nature (their values being most markedly dependent on $K_{E\text{-}NAD(P),I}$). The data on the inhibition of LADH by berberine (Fig. 3), interpreted as outlined above, essentially confirm the previous results, obtained by analysis of the inhibitory action of berberine on the inverse reaction, and by studying the reaction equilibria[5,6]. The linkage of berberine to the free enzyme (LADH) is firmer than those to the complexes LADH–NADH and LADH–NAD, because K_{is} for variable concentration of the coenzyme takes much lower values than for variable concentration of acetaldehyde, and is also lower than either K_{ii} (Table II). The value of K_{is} for variable concentration NADH, controlling the dissociation constant of the complex enzyme–berberine, equalled in this case 33 μmol l^{-1} (Table II), which very well accords with the value 36 μmol l^{-1}, determined for similar conditions by measurements of equilibria[5]. By contrast, in the action of berberine on ALR I the differences between the individual inhibition constants (slope and intercept) were not so great (Fig. 2 and Table II). The determined values of the inhibition constants ranged about 100 μmol l^{-1}; only K_{is} for variable concentration of *p*-nitrobenzaldehyde had lower values. Consequently, according to the above-described interpretation of the inhibition constants, the linkage of the alkaloid to ALR I seems somewhat stabilized by the reduced form of the coenzyme. This, rather inconspicuous coenzyme effect on the affinity of berberine to ALR I is in agreement with the idea that the binding site of ALR I for berberine (and other isoquinoline alaloids) is apart from the active centre of the enzyme.

Acknowledgement is due to Dr J. Trojánek, Research Institute for Pharmacy and Biochemistry, Prague, for supplying a sample of narceine imide.

REFERENCES

1. Meyerson L. R., McMurtney K. D., Davis V. E.: Neurochem. Res. *3*, 239 (1978).
2. Meyerson L. R., McMurtney K. D., Davis V. E.: Biochem. Pharmacol. *25*, 1013 (1976).
3. Ulrichová J., Walterová D., Preininger V., Slavík J., Lenfeld J., Cushman M., ŠimánekV.: Planta Med. *48*, 111 (1983).
4. Ulrichová J., Walterová D., Preininger V., Šimánek V.: Planta Med. *48*, 174 (1983).
5. Pavelka S., Kovář J.: Collect. Czech. Chem. Commun. *40*, 753 (1975).
6. Kovář J., Dürrová E., Skurský L.: Eur. J. Biochem. *101*, 507 (1979).
7. Kovář J., Stejskal J., Matyska L.: J. Enzyme Inhibition *1*, 35 (1985).
8. Walterová D., Kovář J., Preininger V., Šimánek V.: Collect. Czech. Chem. Commun. *47*, 296 (1982).
9. Stejskal J.: *Thesis*. Purkyně University, Brno 1984.
10. Brändén C. I., Jörnavall H., Eklund H., Furungren B. in the book: *The Enzymes* (P. D. Boyer, Ed.), 3rd ed., Vol. 11, p. 103. Academic Press, New York 1975.
11. Kovář J., Skurský L.: Eur. J. Biochem. *40*, 233 (1973).
12. Kovář J.: Arch. Biochem. Biophys. *221*, 271 (1983).
13. Nakai N., Fujii Y., Kobashi K., Nomura K.: Arch. Biochem. Biophys. *239*, 491 (1985).
14. Branlant G., Biellmann J. F.: Eur. J. Biochem. *105*, 611 (1980).
15. Pavelka S., Kovář J.: Collect. Czech. Chem. Commun. *41*, 3654 (1976).
16. Orr G. A., Blanchard J. S.: Anal. Biochem. *142*, 232 (1984).
17. Skurský L., Kovář J., Čermák A.: *12th FEBS Meeting 1978*; Abstr. No. 2953.
18. Kovář J., Plocek J.: J. Chromatogr. *351*, 371 (1986).
19. Hodková J., Veselý Z., Koblicová Z., Holubek J., Trojánek J.: Lloydia *35*, 61 (1972).
20. Holubek J., Štrouf O.: *Spectral Data and Physical Constants of Alkaloids*. Czech. Acad. Sci., Prague 1965.
21. Branlant G., Tritsch D., Biellmann J. F.: Eur. J. Biochem. *116*, 505 (1981).
22. MacLean D. B. in the book: *The Alkaloids* (A. Brossi, Ed.), Vol 24, p. 260. Academic Press, New York 1985.
23. Leo A., Hansch C., Elkins D.: Chem. Rev. *71*, 525 (1971).
24. Kador P. F., Kinoshita J. H., Sharpless N. E.: J. Med. Chem. *28*, 841 (1985).
25. Dalziel K. in the book: *The Enzymes* (P. D. Boyer, Ed.), 3rd. ed., Vol. 11, p. 1. Academic Press, New York 1975.
26. Morpeth F. F., Dickinson F. M.: Biochem. J. *193*, 485 (1981).
27. Kovář J., Plocek J.: Biochem. J., in press.

Translated by J. Salák.