

Inhibition of Phenylalanine Ammonia-Lyase *in vitro* and *in vivo* by (1-Amino-2-phenylethyl)phosphonic Acid, the Phosphonic Analogue of Phenylalanine

Bernd Laber, Hans-Herrmann Kiltz*, and Nikolaus Amrhein

Lehrstuhl für Pflanzenphysiologie und *Lehrstuhl für Biochemie, Ruhr-Universität Bochum, D-4630 Bochum

Z. Naturforsch. **41c**, 49–55 (1986); received June 25, 1985

Dedicated to Professor Hans Grisebach on the occasion of his 60th birthday

Phenylalanine Ammonia-Lyase, (1-Amino-2-phenylethyl)phosphonic and -phosphonous Acids, Amino Acid Metabolism, *Fagopyrum esculentum*

The phosphonic analogue of L-phenylalanine, (R)-(1-amino-2-phenylethyl)phosphonic acid (APEP), inhibits buckwheat phenylalanine ammonia-lyase (PAL) competitively with a K_i value of 1.5 μM . The K_i value for the (S)-enantiomer is 11.6 μM . The corresponding values for the enantiomers of the phosphonous analogue are 35 and 205 μM , respectively. APEP inhibits the light-induced synthesis of anthocyanin in hypocotyls of etiolated buckwheat seedlings and causes a specific increase in the endogenous phenylalanine concentration in buckwheat hypocotyls as well as other plant tissues. Kohlrabi seedlings develop normally in the presence of APEP, while their anthocyanin content is greatly reduced. These results indicate that APEP inhibits PAL *in vivo*.

Introduction

Phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) catalyses the first committed step in the biosynthesis of phenylpropanoid compounds and their derivatives in higher plants. Continuing interest in this enzyme, which occupies a central position in plant secondary metabolism, is reflected by periodic reviews [1–5]. In 1977, we introduced the O-hydroxylamine analogue of L-phenylalanine, L- α -aminooxy- β -phenylpropionic acid (L-AOPP), as a potent competitive inhibitor of PAL [6]. AOPP inhibited PAL from buckwheat and *Rhodotorula glutinis* with K_i values which were lower than the respective K_m values for L-phenylalanine by more than four orders of magnitude [6]. Subsequently, it was proposed [7] that the enantiomers of AOPP pack into the active site of PAL in a mirror image relationship and act as transition state analogues in the elimination reaction. Soybean PAL was apparently irreversibly inhibited by L-AOPP [8], which can presumably be explained by the slow dissociation of the enzyme-ligand complex [9].

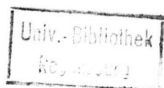
Inhibition by L-AOPP of PAL *in vivo* is evident

Abbreviations: AOPP, L- α -aminooxy- β -phenylpropionic acid; APEP, (1-amino-2-phenylethyl)phosphonic acid; PAL, phenylalanine ammonia-lyase (EC 4.3.1.5.).

Reprint requests to Prof. Dr. N. Amrhein.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/86/0100–0049 \$ 01.30/0

from the observation that L-AOPP blocks the formation of phenylpropanoid compounds in plant cells and tissues [6, 10–18] and causes the accumulation of the substrate of PAL, L-phenylalanine [12, 13, 15, 19]. L-AOPP has been employed with considerable success in studies on the regulation of the level of PAL in plant tissues [20–23], on the turnover of isoflavone derivatives [16, 24], on the function of lignin in xylem vessels [25, 26], and of products of PAL activity in the regulation of cell elongation [27] as well as of phytoalexin function in pathogen resistance in soybean [28]. Generally, for effective suppression of phenylpropanoid synthesis *in vivo*, relatively high concentrations of L-AOPP, *i.e.* ≥ 0.1 mM, have to be employed. Considering that the K_i values for the inhibition of PAL *in vitro* are in the nanomolar range [6, 7], L-AOPP appears a relatively inefficient inhibitor *in vivo*. Low rates of uptake and/or rapid inactivation of the inhibitor in plant tissues may explain this phenomenon. We have observed that the biological activity of L-AOPP, as well as of other aminooxy compounds, is completely abolished in the presence of carbonyl compounds, such as pyruvate or acetone (approx. 50% inactivation at equimolar concentrations in the incubation medium; Amrhein, unpublished results). Using [2- ^{14}C]acetone, L-AOPP can, in fact, conveniently be quantitated in the assay originally devised for the quantitation of a naturally occurring aminooxy compound, L-canaline(2-amino-4-(aminooxy)-butyric acid) [29]. Similar reactions with cellular metabolites may thus decrease the con-



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

centration of L-AOPP in a tissue and reduce its efficiency as an inhibitor. Furthermore, other biosynthetic pathways, such as ethylene synthesis [30], are affected by these high concentrations of L-AOPP. In addition, tyrosine decarboxylases from certain plants are strongly inhibited by L-AOPP (B. E. Ellis, personal communication). Thus, the specificity of L-AOPP for PAL must be interpreted with caution, and the search for new inhibitors of PAL is worthwhile. Inhibition of PAL by cinnamic acid derivatives and related compounds has been investigated more recently [31], but the K_i values even of the most active compounds were in the same range as the K_m values, and little information was given on the specificity of the compounds for PAL *in vivo*. In this communication, we wish to report that, of the many putative inhibitors which we screened over the years, the phosphonic analogue of L-phenylalanine, (R)-(1-amino-2-phenylethyl)phosphonic acid (APEP), is another promising inhibitor of PAL, both *in vitro* and *in vivo*.

Materials and Methods

The phosphonic and phosphonous analogues of phenylalanine were provided by Ciba-Geigy AG., Basel, Switzerland. Plant material was grown and processed for analysis of anthocyanin and amino acid content as described previously [11, 12], except that, rather than floating excised buckwheat hypocotyls in petri dishes, derooted seedlings were placed into scintillation vials (20 seedlings/vial) containing 5 ml 10 mM potassium phosphate buffer, pH 5.5, with the compound to be tested at the appropriate concentration. After a 24 h incubation in the light, the anthocyanin content of the hypocotyls only was determined. PAL was extracted from an acetone powder prepared from the hypocotyls of 6-day old etiolated buckwheat seedlings which had been illuminated for 10 to 12 h [32]. PAL activity was determined spectrophotometrically [32]. An aromatic amino acid transaminase was partially purified from mungbean shoots and assayed according to the procedure described in [33].

Results

Inhibition of PAL in vitro

The effect of the phosphonic and phosphonous analogues of phenylalanine (Fig. 1) on the reaction catalysed by buckwheat PAL was evaluated by

kinetic analysis. Double-reciprocal plots of initial velocities versus variable substrate concentration (30 – 1000 μM) clearly indicated that the inhibition was linear competitive in each case (data for (R)-APEP shown in Fig. 2; other data not shown). Re-

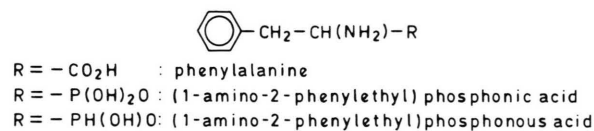


Fig. 1. Structures of phenylalanine and its phosphonic and phosphonous analogues.

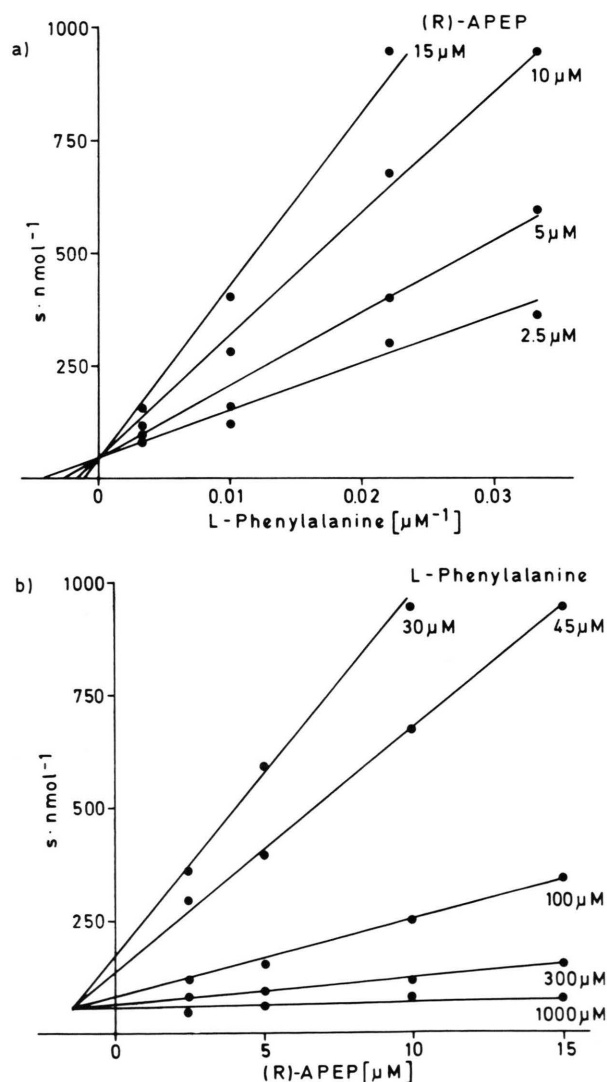


Fig. 2. Kinetic analysis of PAL inhibition by (R)-APEP. a) Lineweaver Burk plot; b) Dixon-Webb plot.

plots of the slopes versus analogue concentration were linear in each case, too, and confirmed the competitive inhibition. The apparent K_i values obtained from these secondary plots are summarized in Table I. As the apparent K_m value of buckwheat PAL for L-phenylalanine is 45 μM [6], it is obvious that the (*R*)-enantiomer of the phosphonic analogue, (*R*)-APEP, has a considerably higher affinity for PAL than the substrate, (*S*)-phenylalanine*, while the (*R*)-enantiomer of the phosphonous analogue has only a slightly increased affinity. In both cases, the (*S*)-enantiomers were less inhibitory than the (*R*)-enantiomers. When 2 μmol of any of the analogues were incubated with 45 pkat of PAL activity in a volume of 1 ml for 5 h under standard assay conditions, no significant changes in the ultraviolet absorption spectra (230–350 nm) were recorded. This result indicates that none of the analogues can serve as a substrate for buckwheat PAL.

The effects of the analogues on another enzymatic reaction involving phenylalanine as substrate, *i.e.* transamination, were analysed by using an aromatic amino acid transaminase from mungbean shoots [33]. (*R,S*)-APEP inhibited the transamination of phenylalanine competitively with an apparent K_i value of 3.2 mM (apparent K_m for phenylalanine ca. 1 mM), while the phosphonous analogue did not appear to be inhibitory at all.

Evidence for Inhibition of PAL *in vivo*

Specific interference of an inhibitor with PAL *in vivo* is expected to reduce the accumulation of phenylpropanoid compounds, such as anthocyanins

Table I. Inhibition of buckwheat PAL by the phosphonic and phosphonous analogues of phenylalanine. Apparent K_i values were obtained from replots of the slopes (see Fig. 2a) versus analogue concentration.

Analogue	app. K_i [μM]
(<i>R,S</i>) (1-amino-2-phenylethyl)phosphonic acid	2.6
(<i>R</i>) (1-amino-2-phenylethyl)phosphonic acid	1.5
(<i>S</i>) (1-amino-2-phenylethyl)phosphonic acid	11.6
(<i>R,S</i>) (1-amino-2-phenylethyl)phosphonous acid	110
(<i>R</i>) (1-amino-2-phenylethyl)phosphonous acid	35
(<i>S</i>) (1-amino-2-phenylethyl)phosphonous acid	205

* Note that, due to the rules of the RS designation, the (*R*)-enantiomers of the phosphonic and phosphonous analogues have the same configuration as (*S*)-(1-amino-2-phenylethyl)carboxylic acid, *i.e.* L-phenylalanine.

and other flavonoids, in a tissue and to specifically increase the concentration of soluble phenylalanine. The following experiments were carried out, therefore, in analogy to those conducted previously with AOPP [6, 11, 12]. (*R*)-APEP was found to inhibit light-induced anthocyanin synthesis in buckwheat hypocotyls with a potency comparable to that of L-AOPP (Fig. 3). An inhibition of anthocyanin synthesis by 50% was obtained with a ca. 50 μM concentration of (*R*)-APEP (I_{50} value), while the I_{50} value was nearly 20-fold higher for the (*S*)-enantiomer. The phosphonous analogue (racemic mixture) produced little, if any inhibition at 1 mM concentration, but ca. 35% inhibition was obtained with a 3 mM solution.

When the effect of the phosphonic and phosphonous analogues (applied as their respective racemic mixtures) on the level of the soluble amino acids in buckwheat hypocotyls was assessed, it became clear that APEP caused a dramatic and specific increase in the level of phenylalanine, while the levels of the other amino acids were in the range of the control levels, with the possible exception of glutamate, the level of which decreased (Table II). In the (*R,S*)-APEP treated tissue, phenylalanine alone constituted 43% of the total soluble amino acids after a 24 h incubation as compared to less than 1% in the control. The phosphonous analogue also produced

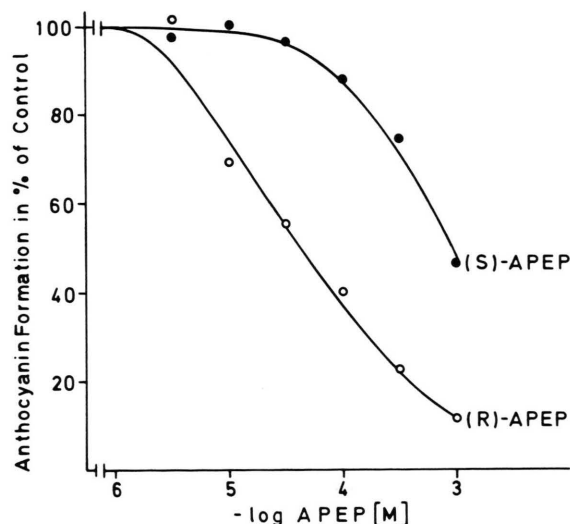


Fig. 3. Inhibition of light-induced anthocyanin accumulation in buckwheat hypocotyls as a function of the concentration of the APEP enantiomers.

Table II. 80% Ethanol soluble amino acids in buckwheat hypocotyls as affected by a 24 h incubation in the light \pm the phosphonic and phosphonous analogues of phenylalanine (racemic mixtures, 3 mM).*

Amino acid	Amino acid content (nmol \cdot g ⁻¹ fresh weight)			
	Initial	After 24 h incubation:		
		Control	Phosphonic analogue	Phosphonous analogue
asp	149	110	90	86
thr	82	122	98	110
ser	167	288	225	278
glu	298	287	197	211
asn	49	73	44	48
gly	135	151	141	162
ala	192	230	171	292
val	90	97	92	88
ile	99	109	117	116
leu	68	72	83	81
tyr	32	31	42	42
phe	48	27	1334	288
trp	9	18	26	18
lys	21	25	35	25
his	78	152	115	152
arg	76	128	103	128

* Values are the means of two replicates.

Deviation from the mean did not exceed 20%.

an, albeit much lower, increase in the level of phenylalanine (Table II). It was demonstrated that phenylalanine which accumulated in tissue treated with (*R,S*)-APEP originated from *de novo* synthesis, because the accumulation of phenylalanine was suppressed by glyphosate, an inhibitor of the shikimate pathway enzyme 5-enolpyruvylshikimate 3-phosphate synthase [34] (Table III). As is evident from Table IV, phenylalanine was found to accumulate in the leaves or shoots, respectively, of a number of other plant species which were exposed to (*R,S*)-APEP, and the generality of the response is therefore obvious. When seeds of Kohlrabi (*Brassica oleracea* var. caulo-rapa DC, "Delikateß blauer") were allowed to germinate and seedlings allowed to grow on filter paper soaked with solutions containing increasing concentrations of the phosphonic and phosphonous analogues (Fig. 4), their anthocyanin content was reduced in a degree which was correlated with the potency of the analogues to inhibit buckwheat PAL *in vitro* (Fig. 5): 50% inhibition of anthocyanin synthesis was produced by 5 μ M (*R*)-APEP, 30 μ M (*S*)-APEP, 100 μ M and 1000 μ M of the (*R*)- and (*S*)-enantiomers, respectively, of the phosphonous analogue. Growth, as indicated by the fresh weight of the seedlings (Fig. 4), was affected only

Table III. Phenylalanine content of buckwheat hypocotyls as affected by (*R,S*)-APEP and glyphosate.

	Phenylalanine (nmol \cdot g ⁻¹ fresh weight)
Initial	59
24 h, control (buffer only)	31
24 h, 1 mM (<i>R,S</i>)-APEP	1321
24 h, 1 mM glyphosate	19
24 h, 1 mM (<i>R,S</i>)-APEP + 1 mM glyphosate	105

Table IV. Levels of phenylalanine in seedlings and leaves after a 24 h incubation \pm 1 mM (*R,S*)-APEP. The inhibitor solution was supplied through the cut end of the hypocotyl (seedlings) or through the petiole (leaves).

	Phenylalanine (μ mol \cdot g ⁻¹ fresh weight)	
	- APEP	+ APEP
<i>Leaves</i>		
Spinach	0.26	4.01
Wheat	0.21	1.35
Maize	0.32	1.55
Tomato	0.15	2.80
Henbane	0.85	1.73
<i>Seedlings</i>		
Buckwheat	0.03	1.33
Mungbean	1.11	2.92

little. However, longitudinal root growth was reduced, and the roots tended to thicken. This effect was not investigated in further detail.

Discussion

The phosphonic analogue of phenylalanine (APEP) has been shown here to be a competitive inhibitor of buckwheat PAL. Both enantiomers are inhibitory but the (*R*)-enantiomer, which has the same configuration as the PAL substrate, L-phenylalanine, has a higher affinity ($K_i = 1.5 \mu\text{M}$) for PAL than the (*S*)-enantiomer ($K_i = 11.6 \mu\text{M}$; Table I). While our work on PAL inhibition by APEP was in progress, we were informed of the results of similar experiments conducted in the laboratory of Dr. J. S. Knypl, University of Lodz, Poland [35]. It was reported that the levorotatory enantiomer had a stronger inhibitory activity than the dextrorotatory enantiomer. As the dextrorotatory APEP was found to have the *S* configuration [36], our results are in qualitative agreement with the results of the Polish group. Of the phosphonous analogues of phenylalanine, the (*R*)-enantiomer was also more inhibitory ($K_i = 35 \mu\text{M}$) than the (*S*)-enantiomer ($K_i = 205 \mu\text{M}$). Among the phosphorus analogues of amino acids the monobasic α -amino phosphonous acids are considered the closest analogues (isosteres) of the α -amino carboxylic acids [37, 38]. The similarity of the K_m of buckwheat PAL for L-phenylalanine, and of the K_i of (*R*)-(1-amino-2-phenylethyl)phosphonous acid, *i.e.* $45 \mu\text{M}$ and $35 \mu\text{M}$, respectively, is in agreement with this conception. From the p*K* values given in [38] for valine and its phosphonic and phosphonous analogues, it can be concluded that at pH 8.8 (pH used in assay of PAL) the phosphonic analogue of phenylalanine is doubly ionized. It is possible, therefore, that the additional charge carried by the phosphonic analogue is responsible for this analogue's higher affinity for PAL in comparison with the phosphonous and carboxylic acids, because the active site of PAL must contain a counter ion, possibly a protonated amino group [39], to the carboxyl group of phenylalanine. Non-acidic, or weakly acidic analogues of phenylalanine, such as its amide or methyl ester, the tetrazole or methylphosphinic analogues, or phenylethylamine, are not, or only slightly inhibitory (Amrhein and Laber, unpublished results) which clearly shows that the carboxyl group, or an equivalent, is required for binding of the ligand by PAL.

While APEP was only a weak inhibitor of the mungbean transaminase, it has previously been reported [40] that racemic APEP is a competitive in-

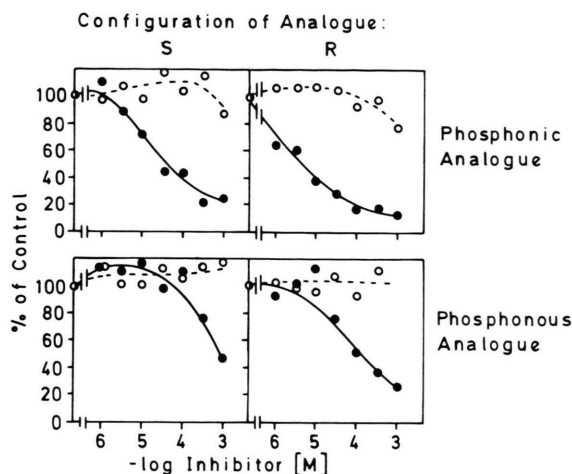


Fig. 4. Effect of phosphonic and phosphonous analogues of phenylalanine on fresh weight (---○---○---) and anthocyanin content (---●---●---) of Kohlrabi seedlings grown for 8 days in the presence of various concentrations of the inhibitors.

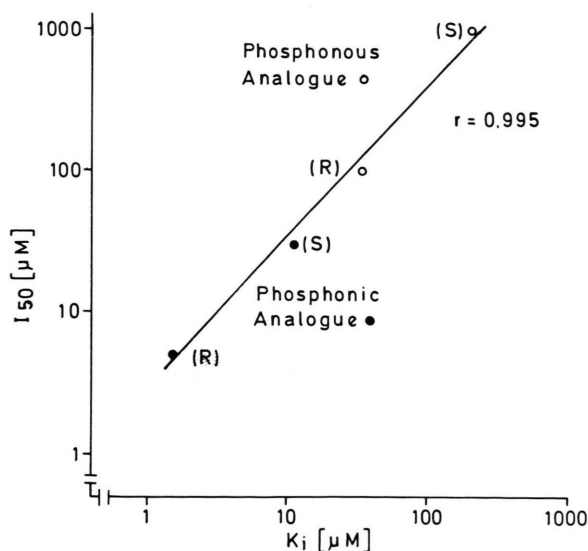


Fig. 5. Relationship between inhibition of buckwheat PAL (K_i) and of anthocyanin synthesis (I_{50}) by phosphonic and phosphonous analogues of phenylalanine. Data are presented in a double-logarithmic plot.

hibitor ($K_i = 17 \mu\text{M}$) of a phenylalanyl-tRNA synthetase from *Aesculus hippocastanum* ($K_m \text{ phe} = 31 \mu\text{M}$). Surprisingly, other aminoacyl-tRNA synthetases of *A. hippocastanum* were totally insensitive to phosphonate analogues of their natural amino acid substrates [40]. We found that yeast phenylalanyl-tRNA synthetase is not subject to strong inhibition by APEP (Leubner and Amrhein, unpublished result), and a systematic investigation of the inhibition of phenylalanyl-tRNA synthetases from a larger number of species is therefore indicated. The massive and selective accumulation of phenylalanine in APEP-treated buckwheat hypocotyls (Table II) is unlikely to be the result of the inhibition of protein synthesis, and, likewise, germination of seeds and growth of Kohlrabi seedlings in the presence of APEP concentrations which greatly reduce their production of anthocyanin (Fig. 4) is hardly consistent with the notion that phenylalanyl-tRNA synthetase might be the primary target of APEP action *in vivo*. It is clearly necessary, however, to investigate the specificity of APEP action in more detail.

The K_i values of L-AOPP (1.4 nM) and of (R)-APEP (1.5 μM) for buckwheat PAL differ by a factor of 10^3 . Nevertheless, under *in vivo* conditions, the potency of the two compounds is quite comparable, presumably due to the inactivation of AOPP in the

plant cell (see Introduction). In future attempts to selectively block PAL activity *in vivo* it may prove useful to use either of the two inhibitors, AOPP and APEP, to double-check the specificity of the response, since it seems unlikely that their affinities to targets other than PAL are identical.

Acknowledgements

Support of our investigations by the Deutsche Forschungsgemeinschaft is gratefully acknowledged. Mrs. S. Hochstein, Mr. L. Andert, and Mr. J. Schab provided excellent technical assistance with some of the experiments. We are indebted to Ciba-Geigy, Agricultural Division in Basel, Switzerland, and Central Research Laboratories in Manchester, U.K., for providing us with phosphorus analogues of phenylalanine used in this study. We thank Prof. O. Pongs for providing the facilities to carry out the amino acid analyses. Finally, we wish to thank Dr. J. S. Knypl, University of Lodz, Poland, for sending us the abstracts of his reports on the biological activity of APEP presented at meetings in Poland and Czechoslovakia.

Note added in proof: The detailed report of the Polish group on the inhibition of PAL by APEP *in vitro* appeared while this work was in print: K. M. Janas, A. Filipiak, J. Kowalik, P. Mastalerz, and J. S. Knypl, *Acta Biochim. Polon.* **32**, 131–143 (1985).

- [1] E. L. Camm and G. H. N. Towers, *Phytochemistry* **12**, 961–973 (1973).
- [2] E. L. Camm and G. H. N. Towers, *Progr. Phytochem.* **4**, 169–188 (1977).
- [3] K. R. Hanson and E. A. Havir, *Rec. Adv. Phytochem.* **12**, 91–137 (1979).
- [4] K. R. Hanson and E. A. Havir, in: *Secondary Plant Products* (E. E. Conn, ed.) **Vol. 7** of *The Biochemistry of Plants: A Comprehensive Treatise* (P. K. Stumpf and E. E. Conn, eds.), pp. 577–625, Academic Press, New York 1981.
- [5] D. H. Jones, *Phytochemistry* **23**, 1349–1359 (1984).
- [6] N. Amrhein and K. H. Gödeke, *Plant Sci. Lett.* **8**, 313–317 (1977).
- [7] K. R. Hanson, *Arch. Biochem. Biophys.* **211**, 575–588 (1981).
- [8] E. A. Havir, *Planta* **152**, 124–130 (1981).
- [9] D. H. Jones and D. H. Northcote, *Arch. Biochem. Biophys.* **235**, 167–177 (1984).
- [10] D. Strack, N. Tkotz, and M. Klug, *Z. Pflanzenphysiol.* **89**, 343–353 (1978).
- [11] N. Amrhein and H. Holländer, *Planta* **144**, 385–389 (1979).
- [12] H. Holländer, H. H. Kiltz, and N. Amrhein, *Z. Naturforsch.* **34c**, 1162–1173 (1979).
- [13] J. Berlin and B. Vollmer, *Z. Naturforsch.* **34c**, 770–775 (1979).
- [14] B. E. Ellis, S. Remmen, and G. Goeree, *Planta* **147**, 163–167 (1979).
- [15] W. Noé, C. Langebartels, and H. U. Seitz, *Planta* **149**, 283–287 (1980).
- [16] E. Diederich and N. Amrhein, *Naturwissenschaften* **67**, 40 (1980).
- [17] R. Tutschek, *Planta* **155**, 307–309 (1982).
- [18] N. Ishikura, M. Iwata, and S. Mitsui, *Bot. Mag. Tokyo* **96**, 111–120 (1983).
- [19] S. O. Duke, R. E. Hoagland, and C. D. Elmore, *Plant Physiol.* **65**, 17–21 (1980).
- [20] N. Amrhein and J. Gerhardt, *Biochim. Biophys. Acta* **583**, 434–442 (1979).
- [21] W. Noé and H. U. Seitz, *Planta* **154**, 454–458 (1982).
- [22] W. Noé and H. U. Seitz, *FEBS-Letters* **146**, 52–54 (1982).
- [23] S. E. Shields, V. P. Wingate, and C. J. Lamb, *Eur. J. Biochem.* **123**, 389–395 (1982).
- [24] U. Jacques, J. Köster, and W. Barz, *Phytochemistry* **24**, 949–951 (1985).
- [25] N. Amrhein, G. Frank, G. Lemm, and H.-B. Luhmann, *Eur. J. Cell Biol.* **29**, 139–144 (1983).
- [26] C. C. Smart and N. Amrhein, *Protoplasma* **124**, 87–95 (1985).

- [27] L. Barnes and R. L. Jones, *Plant Cell Environ.* **7**, 89–95 (1984).
- [28] P. Moesta and H. Grisebach, *Physiol. Plant Pathol.* **21**, 65–70 (1982).
- [29] M. Sugii, H. Miura, and K. Nagata, *Anal. Biochem.* **92**, 265–269 (1979).
- [30] N. Amrhein and D. Wenker, *Plant Cell Physiol.* **20**, 1635–1642 (1979).
- [31] T. Sato, F. Kiuchi, and U. Sankawa, *Phytochemistry* **21**, 845–850 (1982).
- [32] H. Scherf and M. H. Zenk, *Z. Pflanzenphysiol.* **56**, 203–206 (1967).
- [33] O. L. Gamborg and L. R. Wetter, *Canad. J. Biochem. Physiol.* **41**, 1733–1740 (1963).
- [34] N. Amrhein, J. Schab, and H. C. Steinrücken, *Naturwissenschaften* **67**, 356–357 (1980).
- [35] K. M. Janas, A. Filipiak, J. Kowalik, P. Mastalerz, and J. S. Knypl, 20th Meeting of the Polish Biochemical Society, Sept. 18–20, 1984, Olsztyn. Abstracts, ART, Olsztyn 1984, p. 105.
- [36] J. Kowalik, W. Sawka-Dobrowolska, and T. Glowiak, *J. Chem. Soc. Chem. Commun.* **1984**, 446–447.
- [37] E. K. Baylis, C. D. Campbell, J. G. Dingwall, and W. Pickles, *ACS Symposium Series* No. 171, L. D. Quin and J. Verkade eds., pp. 183–186 (1981).
- [38] E. K. Baylis, C. D. Campbell, and J. G. Dingwall, *J. Chem. Soc. Perkin Trans. I* **1984**, 2845–2853.
- [39] D. S. Hodgins, *Arch. Biochem. Biophys.* **149**, 91–96 (1972).
- [40] J. W. Anderson and L. Fowden, *Chem. Biol. Interact.* **2**, 53–55 (1970).