

- Acad. Sci. U.S.A.* 71, 1356-1360.
- Hartman, K. A., Lord, R. C., and Thomas, Jr., G. J. (1973), in *Physico-Chemical Properties of Nucleic Acids*, Vol. 2, Duchesne, J., Ed., New York, N.Y., Academic Press.
- Lafleur, L., Rice, J., and Thomas, Jr., G. J. (1972), *Biopolymers* 11, 2423-2437.
- Livramento, J., and Thomas, Jr., G. J. (1974), *J. Am. Chem. Soc.* 96, 6529-6531.
- Lord, R. C., and Thomas, Jr., G. J. (1967), *Spectrochim. Acta, Part A* 23, 2551-2591.
- Maslova, R. N., Lesnick, E. A., and Varshavsky, Ya. M. (1969a), *Biochim. Biophys. Res. Commun.* 34, 260-265.
- Maslova, R. N., Lesnick, E. A., and Varshavskii, Ya. M. (1969b), *Mol. Biol. (Engl. Transl.)* 3, 728-737.
- McConnell, B., and Seawell, P. C. (1972), *Biochemistry* 11, 4382-4392.
- McDonald, C. C., Phillips, W. D., and Penswick, J. (1965), *Biopolymers* 3, 609-616.
- McKay, H. A. C. (1943), *J. Am. Chem. Soc.* 65, 702-706.
- Medeiros, G. C., and Thomas, Jr., G. J. (1971), *Biochim. Biophys. Acta* 247, 449-462.
- Osterman, L. A., Adler, V. V., Biblaskhvil, R., Savochkina, L. P., and Varshavskii, Ya. M. (1966), *Biokhimiya* 31, 398-404.
- Pearson, R. G., and Dillon, R. L. (1953), *J. Am. Chem. Soc.* 75, 2439-2443.
- Prescott, B., Gamache, R., Livramento, J., and Thomas, Jr., G. J. (1974), *Biopolymers* 13, 1821-1845.
- Rimai, L., Cole, T., Parsons, J. L., Hickmott, Jr., J. T., and Carew, E. B. (1969), *Biophys. J.* 9, 320-329.
- Schweizer, M. P., Chan, S. I., Helmkamp, G. K., and Ts'o, Sundaralingam, M. (1969), *Biopolymers* 7, 821-860.
- Sundaralingam, M. (1973), *Proceedings of the Vth Jerusalem Symposium on Quantum Chemistry and Biochemistry*, pp 417-456.
- Sundaralingam, M., and Abola, J. (1972), *Nature (London)* 235, 244-245.
- Thomas, Jr., G. J. (1970), *Biochim. Biophys. Acta* 213, 417-423.
- Thomas, Jr., G. J., and Barylski, J. R. (1970), *Appl. Spectrosc.* 24, 463-464.
- Thomas, Jr., G. J., and Hartman, K. A. (1973), *Biochim. Biophys. Acta* 312, 311-322.
- Tomasz, M., Olson, J., and Mercado, C. M. (1972), *Biochemistry* 11, 1235-1241.
- Tsuboi, M., Takahashi, S., and Harada, I. (1973), in *Physico-Chemical Properties of Nucleic Acids*, Vol. 2, Duchesne, J., Ed., New York, N.Y., Academic Press.
- Yu, Nai-Teng (1969), Ph.D. Thesis, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Mass.
- Watenpugh, K., Dow, J., Jensen, L. H., and Furberg, S. (1968), *Science* 159, 206-207.
- P. O. P. (1964), *J. Am. Chem. Soc.* 86, 696-700.
- Searcy, D. G. (1968), *Biochim. Biophys. Acta* 166, 360-370.
- Shelton, K. R., and Clark, J. M., Jr. (1967), *Biochemistry* 6, 2735-2739.
- Shimanouchi, T., Tsuboi, M., and Kyogoku, Y. (1964), *Adv. Chem. Phys.* 6, 435-498.
- Shoemaker, D. P., Garland, C. W., and Steinfeld, J. I. (1974), *Experiments in Physical Chemistry*, 3rd ed, New York, N.Y., McGraw-Hill, p 47.

Chemical Synthesis of N^{β} -Oxalyl-L- α,β -diaminopropionic Acid and Optical Specificity in Its Neurotoxic Action[†]

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ABSTRACT: A practical procedure is described for the bulk synthesis of the neurotoxin N^{β} -oxalyl-L- α,β -diaminopropionic acid (OA_2pr^3), a potential dicarboxylic amino acid antagonist of *Lathyrus sativus* seeds. L-Aspartic acid was reacted with sodium azide in 30% fuming sulfuric acid and L- α,β -diaminopropionic acid hydrochloride ($A_2pr^3 \cdot HCl$) was isolated in yields greater than 75%. Potassium methyl oxalate was found to react selectively with the β -amino group of A_2pr^3 resulting in near quantitative yields of

OA_2pr^3 . D- OA_2pr^3 has been made for the first time by this procedure. Unlike L- OA_2pr^3 the naturally occurring neurotoxin, D- OA_2pr^3 , is not neuroactive even in high doses. The microsynthesis of L-[2,3- 3H] A_2pr^3 from L-[2,3- 3H]aspartic acid is also described, and the same procedure could also be used to prepare the neurotoxin with other labels. The availability of the neurotoxin in bulk and in labeled form should further experimental approaches to the understanding of its mechanism of action.

N^{β} -Oxalyl-L- α,β -diaminopropionic acid (OA_2pr^3)¹ is the major neurotoxic amino acid isolated from the seeds of *Lathyrus sativus* (Rao et al., 1964; Murti et al., 1964) and de-

tected in several other species of *Lathyrus* (Bell and Donovan, 1966). Recent studies indicate that OA_2pr^3 is a potential antagonist of the dicarboxylic amino acids (Mehta et al., 1972; Magalhaes and Packer, 1972; Laxmanan and Padmanaban, 1974). Despite the well documented neurotoxic property of OA_2pr^3 (Nagarajan et al., 1965; Rao et al., 1967; Rao and Sarma, 1967) and its effects on certain biological systems (Cheema et al., 1970, 1971; Mehta et al.,

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¹ Abbreviations used are: A_2pr^3 , α,β -diaminopropionic acid; OA_2pr^3 , N^{β} -oxalyl-L- α,β -diaminopropionic acid.

1972; Magalhaes and Packer, 1972; Balcar and Johnston, 1972; Laxmanan and Padmanaban, 1974), the mechanism of its action in human neurolathyrism is still not clear. The neurotoxin used in all the studies so far has only been isolated in small amounts from the seeds. The nonavailability of the neurotoxin in large quantities has been a serious limitation in undertaking systematic studies with it. It has also not been possible to test several of the hypotheses that have been advanced to explain the mechanism of toxicity of OA_2pr^3 , since the labeled neurotoxin has not been made available.

The present paper describes for the first time a simple and practical procedure for the large-scale synthesis of the neurotoxin and also a microsynthesis for the labeled neurotoxin. The synthesis and some properties of the D isomer of OA_2pr^3 , the optical antipode of the naturally occurring neurotoxin, are also reported.

Materials and Methods

L- and D-Aspartic acid were obtained from Sigma Chemical Co. L-[2,3- 3H]Aspartic acid was obtained from New England Nuclear. Potassium methyl oxalate (mp 194–196°) was prepared from dimethyl oxalate and potassium acetate according to the procedure of Efimovsky (1959).

One-dimensional ascending paper chromatography on Whatman No. 1 paper was carried out in the following solvent systems (v/v): (a) 1-butanol-acetic acid-water, 4:1:1; (b) 1-butanol-1-propanol-0.1 N HCl, 1:1:1; (c) 2-propanol-water-ammonia (0.88 specific gravity), 80:20:2. Paper electrophoresis on Whatman No. 1 paper strips was carried out with a horizontal open strip paper electrophoretic apparatus at 400 V for 2 hr at room temperature. The following buffer systems were employed (v/v): (1) pH 3.6, pyridine-acetic acid-water, 10:100:1900; (2) pH 4.5, pyridine-acetic acid-water, 100:10:1900; (3) pH 2.2, 10% acetic acid. Amino acids were revealed by spraying ninhydrin in acetone.

Radioactivity on paper was determined by counting the strips in a toluene medium consisting of 0.5% 2,5-diphenyloxazole and 0.03% 1,4-bis[2-(5-phenyloxazolyl)]benzene in a Nuclear Chicago (Model 2211) scintillation counter. Optical rotations were determined in Schmidt Haensch (Model 13427) polarimeter using a 2-dm tube. Solid state (KBr disc) infrared spectra were recorded in a Perkin-Elmer (Model 221) infrared spectrophotometer.

Synthesis of L- α,β -Diaminopropionic Acid. The reaction conditions were adopted from the procedure of Kitagawa et al. (1969) by using smaller amounts of fuming sulfuric acid and sodium azide to suit a nearly 20-fold scale up of A_2pr^3 synthesis. The isolation procedure for A_2pr^3 from the reaction mixture has been simplified considerably.

L-Aspartic acid (100 g, 0.75 mol) was dissolved in 350 ml of 30% fuming sulfuric acid with cooling followed by the addition of 400 ml of dry chloroform. The mixture (50–55°) was treated with powdered sodium azide (100 g, 1.53 mol) in small amounts under reflux with efficient stirring, during 5 hr. The entire operation was carried out under anhydrous conditions. The mixture was stirred for a further 2 hr and after cooling in a ice bath the chloroform layer was poured off. The thick paste like residue was poured onto 3–4 kg of crushed ice and made up to about 10 l. The solution was passed through a Dowex 50-X8 column (H^+ form, 100–200 mesh, 7.5 \times 26 cm) and washed with 1 l. of 0.5 N HCl followed by water to neutrality. The absorbed A_2pr^3 was eluted with 10% ethanolic ammonia (4 N). The pooled

ninhydrin positive fractions (800 ml) was decolorized with carbon and concentrated to about 300 ml. The fraction was treated with 6 N HCl to pH 2.2–2.4 when most of the A_2pr^3 ·HCl crystallized out. After the addition of methanol (500 ml), A_2pr^3 ·HCl was filtered and washed successively with 80% methanol, methanol, and acetone and dried, yield, 85–92 g. The compound recrystallized from methanol-water had a mp 236–237° and $\alpha^{27D} +25.2^\circ$ (c 2, 0.5 N HCl) and was chromatographically and electrophoretically homogeneous.

The same reaction was carried out on D-aspartic acid when D- A_2pr^3 ·HCl was obtained. $\alpha^{27D} -25^\circ$ (c 2, 0.5 N HCl).

Preparation of L- OA_2pr^3 . A solution of L- A_2pr^3 ·HCl (60 g, 0.42 mol) and lithium hydroxide (24 g, 0.6 mol) in 300 ml of 50% methanol was added slowly with stirring to 108 g of potassium methyl oxalate (0.76 mol) in 350 ml of 50% methanol. The solution was filtered and after 18 hr the crystalline mass that had separated was filtered and washed successively with 50% methanol (200 ml), methanol, and acetone; yield, 80 g of an undefined salt of OA_2pr^3 . The crystals were dissolved in 250 ml of water and the pH was adjusted to 2.1–2.2 with ice-cold 2 N HCl when OA_2pr^3 separated out as an amorphous white powder. After cooling for a few hours OA_2pr^3 was filtered, washed by suspending in ice-cold water and then with methanol and acetone, and dried; yield, 50–60 g of chromatographically and electrophoretically pure OA_2pr^3 ; mp 206°; $\alpha^{27D} -28.7^\circ$ (c 2, 0.5 N HCl). Anal. Calcd for $C_5H_8O_5N_2$: C, 34.09; H, 4.57; N, 15.9. Found: C, 34.22; H, 4.46; N, 15.68.

Preparation of D- OA_2pr^3 . The above procedure was repeated with D- A_2pr^3 ·HCl when D- OA_2pr^3 was obtained. D- OA_2pr^3 was indistinguishable from L- OA_2pr^3 by paper chromatography and electrophoresis: mp 206°; $\alpha^{27D} +28^\circ$ (c 2, 0.5 N HCl). The solid state infrared spectra of D- and L- OA_2pr^3 were indistinguishable.

Synthesis of L-[2,3- 3H] OA_2pr^3 . With slight modifications the procedure described above for the bulk synthesis of A_2pr^3 and OA_2pr^3 was also used to synthesize millimolar quantities and less of labeled OA_2pr^3 .

A mixture of L-[2,3- 3H]aspartic acid (2 mCi/250 mg), 1 ml of 30% fuming sulfuric acid, and 2 ml of dry chloroform was treated during 90 min with 300 mg of sodium azide with stirring at 50–55°. After stirring for 2 hr the mixture was diluted to 60 ml and the aqueous phase was passed through a Dowex 50-X8 column (H^+ form, 11 \times 1.5 cm). Traces of unreacted aspartic acid was eluted with 100 ml of 0.5 N HCl and the absorbed A_2pr^3 was eluted with aqueous ammonia. The A_2pr^3 fraction was taken to dryness and dissolved in 2.5 ml of 50% methanol containing 30 mg of lithium hydroxide. This solution was added, while stirring, to 600 mg of potassium methyl oxalate in 2 ml of 50% methanol. After 18 hr the solution was applied on to a Dowex 50-X8 column (H^+ form, 2.2 \times 0.8 cm) and elution was carried out with water. The first 100 ml of the eluate was rejected and the next 240 ml of the eluate having most of the radioactivity was lyophilized. The residue was collected in ethanol and recovered by centrifugation, yield 198 mg (72% radioactive recovery). The compound had a specific activity of 1283 $\mu Ci/mmol$ and was homogeneous by paper chromatography and paper electrophoresis and all radioactivity was associated with OA_2pr^3 .

Results

Synthetic L- OA_2pr^3 was identical with the neurotoxin

isolated from the seeds in all respects studied. When administered intraperitoneally to day-old chicks, the synthetic compound produced typical neurological symptoms at a dosage of 0.22 mg/g which is similar to that reported for the natural toxin (Rao et al., 1964; Roy and Narasinga Rao, 1968).

Unlike OA_2pr^3 isolated from the seeds, synthetic OA_2pr^3 was free from traces of N^α -oxalyl- α,β -diaminopropionic acid when examined by paper electrophoresis at pH 2.2 (Malathi et al., 1967). Yields of OA_2pr^3 were similar when the synthesis was carried out on much smaller amounts of A_2pr^3 (5–10 g). However, in this case OA_2pr^3 was isolated by chromatography on Dowex 50 and precipitating it with acetone from the lyophilized water eluate.

D- OA_2pr^3 was tested for toxicity in day-old chicks as they have been found susceptible to L- OA_2pr^3 . D- OA_2pr^3 failed to produce any neurological or visible toxic symptoms, when administered intraperitoneally even at a high dose of 11 mg/g.

Discussion

A preparative procedure for OA_2pr^3 had not been attempted earlier due to lack of a simple procedure for the synthesis of optically pure A_2pr^3 , although, enzymatic procedures for resolution of racemic A_2pr^3 are known (Greenstein and Winitz, 1961). Adamson's method (1939) of synthesis of diaminomonocarboxylic acids from monoaminodicarboxylic acids has been carried out only with glutamic acid and its higher homologs. However, Kitagawa et al. (1969) later achieved a novel synthesis of A_2pr^3 by carrying out the reaction on small amounts of aspartic acid in 30% fuming sulfuric acid instead of the conventional concentrated sulfuric acid. The method described now is adopted from this procedure, enabling a 20-fold scale up synthesis of optically pure A_2pr^3 , with considerable simplification of its isolation from the reaction mixture. Other procedures for synthesis of optically pure A_2pr^3 have employed Hoffmann's degradation of either carbobenzoxy- (Benoiton, 1968) or acetyl- (Karrer and Schlosser, 1923; Rajagopal Rao et al., 1974) asparagine. However, these latter procedures are tedious and chromatographic fractionation procedures have to be employed to isolate A_2pr^3 as other side products are encountered. In the present procedure, since A_2pr^3 was the only basic amino acid product it could be isolated by a simple desalting step on a Dowex 50 column.

The only procedure described in the literature for the synthesis of L- OA_2pr^3 (Rao et al., 1964; Mehta et al., 1972) is of theoretical interest and it involved treatment of the copper complex of A_2pr^3 with ethyl oxalyl chloride under controlled pH conditions. The yields by this procedure were extremely low (10%) and had no preparative value. In the present method, potassium methyl oxalate was found to react selectively with the β -amino group of A_2pr^3 under slightly alkaline conditions to yield OA_2pr^3 , almost quantitatively. The di- N -oxalyl derivative of OA_2pr^3 was not formed in detectable amounts even with a large excess of the ester. In other studies this reagent was also found to give good yields of N^δ -oxalyl-L-ornithine, N^γ -oxalyl-L- α,γ -diaminobutyric acid (*L. latifolius* neurotoxin), and N^ϵ -oxalyl-L-lysine (S. L. N. Rao, unpublished results).

The isolation of the neurotoxin in large amounts from seeds of *L. sativus* poses problems, since, unless precautions are taken, under the usual handling procedures as much as 50% of the isolated toxin could be present as the α isomer (Bell and Donovan, 1966). Generally, the samples isolated

in small amounts from the seeds contain 7–10% of the α isomer (Roy and Narasinga Rao, 1968). The migration of the oxalyl group from the β -amino group to the α -amino group appears to be favored by heat (Bell and Donovan, 1966) and, once formed, there is as yet no method of separating the two isomers of OA_2pr^3 . Also, the results of studies using such a sample will be questionable as nothing is known of the toxicity of the α isomer. The present procedure of direct precipitation of OA_2pr^3 from a solution of its isolated salt in the pH range 2.1–2.2 resulted in a sample free from the α isomer. Pure OA_2pr^3 has a mp 206° dec and traces of α isomer if present depress the melting point to 196–197° and a sample with a melting point of 165–175° contains appreciable amounts of the α isomer. Although the optical purity of L- and D- OA_2pr^3 was established on the basis of their almost equal but opposite signs of rotation, attempts were also made to determine it enzymatically. However, it was seen that L- OA_2pr^3 is not appreciably oxidized at pH 7.2 by snake venom (*Crotalus atrox* and *Vipera russeli*) L-amino acid oxidase.

The present study also shows that the toxicity of synthetic L- OA_2pr^3 is similar to that of the natural neurotoxin. Since D- OA_2pr^3 is not neurotoxic, it implies that whatever the mechanism of toxicity of the L isomer is, it is the result of a stereospecific interaction. In this context it is interesting to note that OA_2pr^3 appears to be a structural antagonist of aspartic acid and/or glutamic acid in several systems (Mehta et al., 1972; Magalhaes and Packer, 1972; Laxmanan and Padmanaban, 1974). Synthetic L- and D- OA_2pr^3 should thus prove useful as potential antagonists of the dicarboxylic amino acids.

Since OA_2pr^3 can be made in large quantities it should be possible to undertake feeding studies in animals to establish its role in neurolathyrism. Several studies which were hitherto not possible can now be carried out with labeled OA_2pr^3 having high specific activity. [^{14}C] OA_2pr^3 with low specific activity has earlier been made by enzymatically oxalylating A_2pr^3 with labeled oxalic acid (Cheema et al., 1971). It should also be possible to label the oxalyl moiety of OA_2pr^3 using potassium [^{14}C]methyl oxalate. It is hoped that the synthetic procedures described will hasten toxicological and metabolic studies with this neurotoxin and help to establish its role in the crippling disease.

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References

- Adamson, D. W. (1939), *J. Chem. Soc.*, 1564.
- Balcar, V. J., and Johnston, G. A. R. (1972), *J. Neurobiol.* 3, 295.
- Bell, E. A., and Donovan, J. P. (1966), *Phytochemistry* 5, 1211.
- Benoiton, L. (1968), *Can. J. Chem.* 46, 1549.
- Cheema, P. S., Malathi, K., Padmanaban, G., and Sarma, P. S. (1969), *Biochem. J.* 112, 29.
- Cheema, P. S., Padmanaban, G., and Sarma, P. S. (1970), *J. Neurochem.* 17, 1295.
- Cheema, P. S., Padmanaban, G., and Sarma, P. S. (1971),

- J. Neurochem.* 18, 2137.
- Efimovsky, O. (1959), *J. Rech. C. N. R. S.* 47, 147.
- Greenstein, J. P., and Winitz, M. (1961), in *Chemistry of the Amino Acids*, Vol. 3, New York, N.Y., Wiley, p 2463.
- Karrer, P., and Schlosser, A. (1923), *Helv. Chim. Acta* 6, 411.
- Kitagawa, T., Ozasa, T., and Taniyama, H. (1969), *Yakugaku Zasshi* 89, 285.
- Laxmanan, J., and Padmanaban, G. (1974), *Nature (London)* 249, 469.
- Magalhaes, M. C., and Packer, L. (1972), *FEBS. Lett.* 23, 188.
- Malathi, K., Padmanaban, G., Rao, S. L. N., and Sarma, P. S. (1967), *Biochim. Biophys. Acta* 141, 71.
- Mehta, T., Hsu, A. F., and Haskell, B. E. (1972), *Biochemistry* 11, 4035.
- Murti, V. V. S., Sheshadri, T. R., and Venkatasubramanian, T. A. (1964), *Phytochemistry* 3, 73.
- Nagarajan, V., Mohan, V. S., and Gopalan, C. (1965), *Indian J. Med. Res.* 53, 269.
- Rajagopal Rao, D., Hariharan, K., and Vijayalakshmi, K. R. (1974), *Indian J. Biochem. Biophys.* 11, 265.
- Rao, S. L. N., Adiga, P. R., and Sarma, P. S. (1964), *Biochemistry* 3, 432.
- Rao, S. L. N., and Sarma, P. S. (1967), *Biochem. Pharmacol.* 16, 218.
- Rao, S. L. N., Sarma, P. S., Mani, K. S., Raghunatha Rao, T. R., and Sriramachari, S. (1967), *Nature (London)* 214, 610.
- Roy, D. N., and Narasinga Rao, B. S. (1968), *Curr. Sci.* 37, 395.

Structural and Thermodynamic Basis of Affinity in Anti-Dinitrophenyl Antibody[†]

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ABSTRACT: The thermodynamic quantities of the anti-dinitrophenyl antibody-hapten interaction are reported for rabbit, goat, and guinea pig antibodies. Rabbit and goat antibodies had similar exothermic enthalpy changes for their reaction with 2,4-dinitrophenyl-L-lysine (−13.9 and −14.8 kcal/mol, respectively). The enthalpy change with guinea pig antibody was much less exothermic (−8.7 kcal/mol), and this value was the same for two guinea pig antibody preparations that differed in affinity by almost two orders of magnitude. A heterogeneous goat anti-dinitrophenyl antibody preparation was fractionated on a molecular sieve column in the presence of a bivalent ligand, a procedure

that has been reported to separate antibodies according to differences in the depth of interaction with the ligand. The relationship of these differences in apparent site *depth* to changes in interactions with the hapten tail was examined by comparing the affinities of various fractions for two haptens. The results show that the presumed *deeper* sites have stronger interactions with the hapten tail. These studies suggest that the heterogeneity of anti-dinitrophenyl antibodies with respect to affinity results from differences in entropy driven lysyl side-chain interactions which arise from a heterogeneity in antigen binding site *depth*.

The physical-chemical principles governing antibody-antigen interaction will be revealed only when the continually emerging antibody structure information can be quantitatively correlated with sound thermodynamic data for interaction with various ligands. Such correlation will provide a "thermodynamic mapping" of the antigen binding site in terms of the energetics of specific ligand-antigen binding site interactions. In addition such studies will lead to an understanding of the thermodynamic basis of variation in affinity within a heterogeneous antibody population. Essential to any such efforts, however, is the acquisition of reliable and meaningful thermodynamic quantities.

Since most antibody preparations constitute a heteroge-

neous population of molecules, derived thermodynamic quantities generally represent a complex average value and their relationship to a true thermodynamic value is ambiguous. This is particularly true for enthalpy changes estimated by a van't Hoff analysis based on average association constants. Barisas et al. (1972) have shown, for example, that the van't Hoff ΔH for the binding of Dnp¹- and Tnp-lysine to their respective antibodies underestimated the true enthalpy change, determined calorimetrically, by as much as 10 kcal/mol. It is therefore apparent that meaningful enthalpy changes for hapten-antibody reactions can best be obtained by direct calorimetric measurements.

In this communication the results of thermodynamic studies on the binding of Dnp-lysine and dinitroaniline to anti-Dnp antibodies from rabbit, goat, and guinea pig are reported. These results support previous conclusions that

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¹ Abbreviations used are: Dnp, dinitrophenyl; Dnp-lysine, N-2,4-dinitrophenyl-L-lysine; Tnp, trinitrophenyl; Dnp-D-GL, copolymer of D-glutamic acid and D-lysine with dinitrophenyl groups on ϵ -amino; bis-Dnp-lysine, N,N'-didinitrophenyl-L-lysine.