Peptido-aminobenzophenones – novel latentiated benzo-1,4-diazepines

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Summary. It has been shown that cleavage of the N-terminal L-amino acids of a novel series of dipeptide derivatives of 2-aminobenzophenones occurs readily in vivo to give benzo-1,4-diazepines. Such compounds may serve as useful pro-drug forms of minor tranquillizers such as Valium®.

Among minor tranquillizers, the benzo-1,4-diazepines are, without doubt, the most widely used. The group includes such well-established medicinal products as Librium® (chlordiazepoxide, **I**) and Valium® (diazepam, **XIII**), the hypnotics Mogadon® (nitrazepam, **II**) and Rohypnol® (flunitrazepam, **III**), the anticonvulsant Rivotril® (clonazepam, **IV**) and others[1].

Early in the history of benzodiazepines, it was observed that strong acids opened the heterocyclic ring[2], but the scope of the reaction for different members of the series was not investigated in detail. We have studied this process for several series of benzodiazepines and have found a wide range of variation in the ease of opening of the ring. In the case of compounds with a second basic centre, e.g. bromazepam (**V**) and flurazepam (**VI**), spectroscopic evidence indicated that ring opening occurred under very mild acid conditions but the benzodiazepine reformed rapidly on neutralisation. Moreover, ring closure studies by UV. and ^{1}H-NMR. measurements established for a variety of ring-opened forms (**X**) that, depending on the nature of substituents (table 1, a) and solvents (table 1, b), they had half-lives ranging from 73 sec to 230 h. Electron-withdrawing groups at the 7-, and 2'-, positions inhibit ring closure, whereas N-1 alkylation and polar solvents promote this reaction.

Evidently, a compound (**VIII**) that released, in vivo, the precursor form (**X**) could serve as a pro-benzodiazepine. Such a pro-drug could have favourable characteristics, such as rate of metabolism or water solubility at physiological pH that could provide an improved presentation of the benzodiazepine, in vivo. We have prepared a series of peptides with the general formula **VIII** to serve in this

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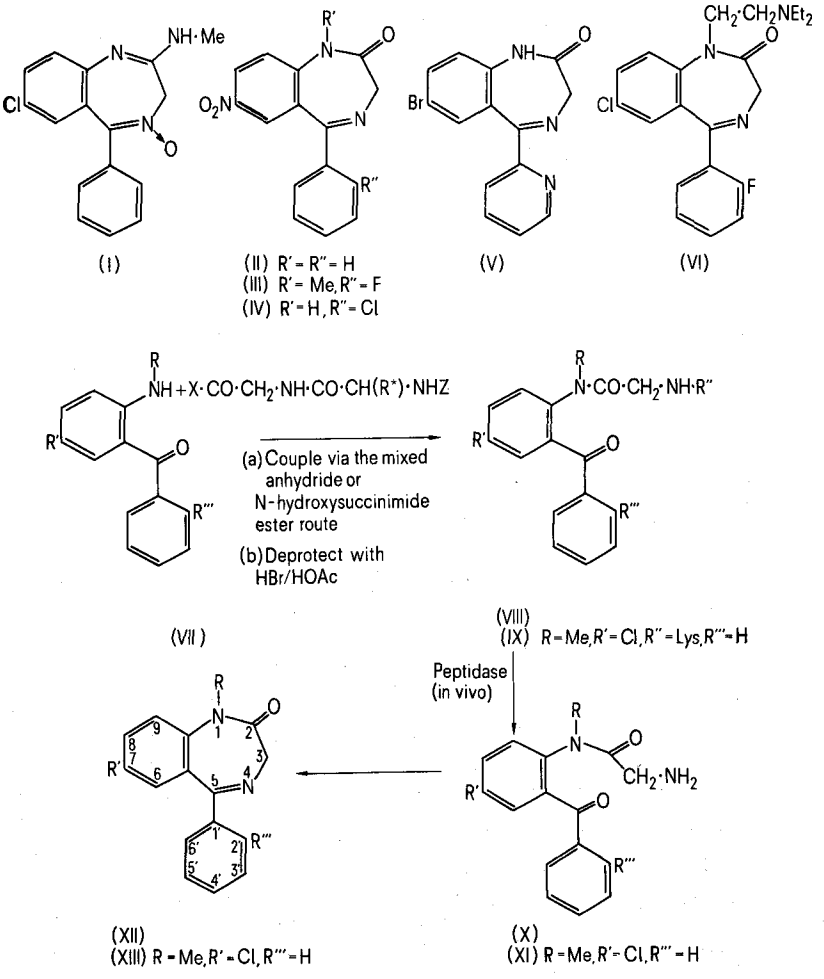

Table 1. Effect of a) substitution and b) solvent on ring closure of open forms of benzo-1,4-diazepines

a) Substitution (structure **X** → **XII**, in pyridine)

R	R′	R‴	$T^1/_2$*
H	NO_2	H	33 h
H	NO_2	Cl	230 h
H	Cl	H	7 h
Me	Cl	H	4.5 min

b) Solvent effect (structure **XI** → **XIII**)

Solvent:	Water (pH 7)	Pyridine	Dimethoxyethane
$T^1/_2$**:	73 sec	5.5 min	160 min
Chloroform	Dioxan		
280 min	307 min		

*Based on ^{1}H-NMR-measurements. **Based on UV-measurements.

Table 2. Relative rates of formation of diazepam, in vivo*, from latentiated forms

Compound	Molar activity at peak, relative to diazepam	Time of peak activity (min)
Diazepam (**VIII**), R′=Cl, R=Me, R″=H, R‴:	1	immediate
Gly	0.4	15
L-Phe	0.8	2–3
D-Phe	< 0.1	–
L-Leu	0.6	2–3
L-Ala	0.3	2–3
D-Ala	< 0.1	–
L-Lys	1.0	2–3
L-Arg	1.0	2–5
L-Glu	0.5	5–15

*Measured by anti-pentylenetetrazole test for anti-convulsant activity in mice, i.v. administration.

way as latentiated benzodiazepines. The synthesis was achieved by coupling the appropriate 2-aminobenzophenone derivative (**VII**) with a suitably protected and activated dipeptide derivative, followed by deprotection, or by similar attachment of a single amino acid residue to the appropriate glycylaminobenzophenone, as illustrated in the figure below.

The compounds **VIII** with the terminal L-amino acid residues were cleaved by peptidases, in vivo, to release the precursor (**X**) which cyclised at physiological pH to the benzodiazepine; they were also cleaved at a comparable rate in whole blood of various species: rodents, cat, dog, primates and man. The sequence of reactions is illustrated by the diazepam series: pro-drug (**IX**) → open form (**XI**) → diazepam (**XIII**). As expected, compounds with D-terminal amino acids were not cleaved and had no benzodiazepine-like pharmacological activity. The results of the anti-pentylenetetrazole test for anti-convulsant activity in mice, following i.v. administration, were compared with those of parent benzodiazepines for potency (ED_{50}) and time of onset for peak activity.

Measurements using various terminal amino acids combined with the same nucleus established that there were substantial differences in the rate of peptidase cleavage, in vivo. This is illustrated for the diazepam series in which the order for L-amino acids is Phe,Lys,Arg > Leu > Ala > Glu > Gly (table 2). Similar variations were observed for members of other series of latentiated benzodiazepines. These investigations have established that appropriate peptidoaminobenzophenones are novel minor tranquillizers that depend for their pharmacological activity on rapid release of a ring-open precursor of benzodiazepines. Physical properties, such as enhanced solubility in aqueous media compared with the corresponding benzodiazepines and different half-lives, in vivo, provide for novel applications of these compounds.

Ultrastructural changes in dog thyroid follicular cells elicit by concanavalin A in vitro

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Summary. Dog thyroid follicular cells exposed to concanavalin A (Con A) in vitro showed changes in cell shape, induction of colloid droplets and alterations in the distribution of microvilli. Cells exposed to Con A plus suboptimal concentrations of TSH (thyroid stimulating hormone) showed pseudopods and their cytoplasm was virtually occupied with colloid droplets. This findings suggest that Con A potentiated pseudopod and colloid droplet formation induced by TSH.

Recently, Concanavalin A (Con A) has been found to mimic insulin effects on isolated adipocytes[2,3]. It also mimicked the action of a gonad-stimulating peptide in inducing the production of 1-methyladenine in isolated starfish ovary follicle cells[4]. These findings suggest that Con A has the same capacity as a peptide hormone to stimulate certain cell functions. Therefore, one might expect Con A to exert certain TSH-like effects on thyroid follicular cells. Here we report on the effects of Con A on the ultrastructure of dog thyroid follicular cells and on the ultrastructural response to TSH.

Materials and methods. 1. Materials. The compounds used in this study and their sources are listed elsewhere[5]. Con A was purchased from Sigma Chemical Co. (St. Louis, Missouri). 2. Treatment of animals. 12 mongrel dogs, weighing between 16 and 20 kg and previously maintained on a normal iodine, balaced diet and in a controlled environment, were fasted overnight and anesthetized with sodium pentobarbital 50 mg/kg b.wt i.v. These dogs were divided into the following groups: Group A, non-

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