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The discrimination between human, porcine and bovine insulin with ^1H NMR spectroscopy

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Abstract—The ^1H- and ^1H-^1H correlation (COSY) NMR spectra of human, porcine and bovine insulin have been recorded. The 1·1–1·5 ppm regions of these spectra show the methyl signals of the alanine and threonine residues. The number of alanines and threonines differs for the three insulins and thus allows an easy discrimination with NMR.

Insulin consists of two peptide chains, the A chain of 21 amino acids and the B chain of 30 amino acids, linked by two intermolecular disulphide bonds. Human and porcine insulin differ in only one amino acid, whereas human and bovine insulin differ in three amino acids (see Table 1). Although the biological activity and binding to cell membrane receptors is essentially the same for human and porcine insulin (Sonnenberg & Berger 1983), there are immunological (Falholt et al 1983; Wu et al 1983) and psychological reasons that favour human insulin over non-human insulin for the treatment of diabetes. Because of this, a method to discriminate between the various insulins is needed.

Table 1. Structural differences and position of the alanine and threonine residues in human, porcine and bovine insulin.

Insulin species	Amino acid residue at chain position				
	A-8	A-10	B-14	B-27	B-30
Human	Thr	Ile	Ala	Thr	Thr
Porcine	Thr	Ile	Ala	Thr	Ala
Bovine	Ala	Val	Ala	Thr	Ala

Since NMR spectroscopy allows the direct observation of the hydrogen atoms of organic molecules, the spectra of the various insulins should, in principle, be different. To find whether these differences would allow the discrimination between human, porcine and bovine insulin, we have recorded their ^1H and ^1H-^1H correlation (COSY) NMR spectra.

Materials and methods

Porcine and bovine insulin (Diosynth B.V., Oss) were extracted from animal pancreases. Semisynthetic human insulin (Diosynth B.V., Oss) was synthesized by coupling threonine to [des-30-alanine] porcine insulin. Insulins from both animal sources were subsequently purified by chromatography to EP quality. The human insulin, for which there is no EP monograph yet, was purified by chromatography to USP quality.

The insulins were added to 0·45 mL D_2O (99·75% deuterium, Merck) and the apparent pH was adjusted to 3·0–3·1 by addition of ca 0·06 mL CD_3COOD (99·5% deuterium, Aldrich). The solutions thus obtained were filtered through cotton-wool into a 5 mm o.d. NMR-tube.

360 MHz spectra were obtained at 310 K using a Bruker AM-360 spectrometer and standard spectral parameters. The ^1H-^1H correlation spectra (COSY-45, N-type selection) were obtained using a sweep width of 1400 Hz, a resolution of 2·7 Hz/point, a relaxation delay of 2 s, and 128 scans/FID. A

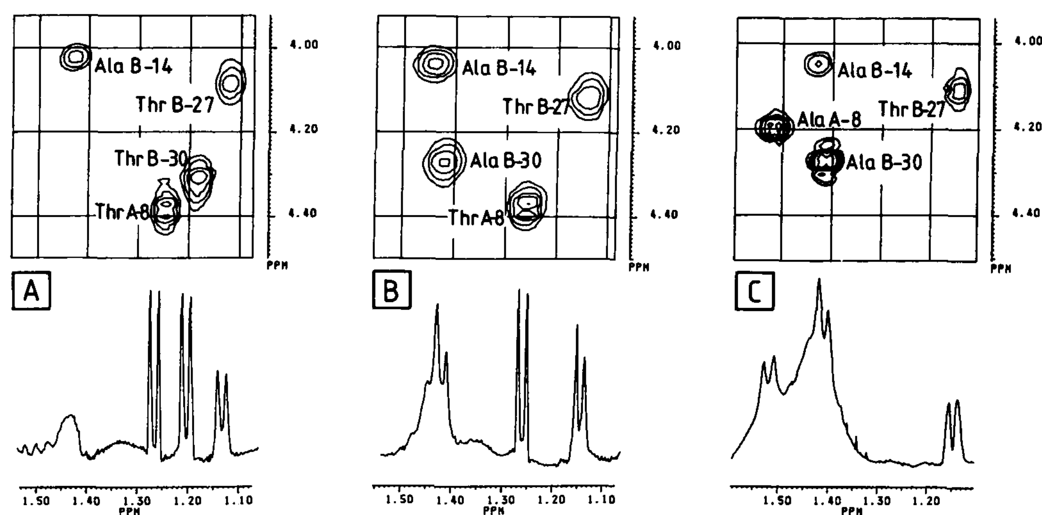

FIG. 1. Expanded regions of the contourplots of ^1H correlation spectra of (A) human-, (B) porcine- and (C) bovine insulin showing the correlations between the methyl and methine protons of the alanine and threonine residues. For reference purposes the respective one-dimensional spectra are plotted below the contourplots. The signal of ethanol originally present in these spectra has been eliminated by computer manipulation.

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total of 256 experiments in the t_1-domain were collected. In all spectra the HDO-peak was suppressed by presaturation.

Results and discussion

The ^1H spectra of the insulins show complex patterns of many overlapping broad lines and do not allow a detailed interpretation. The methyl protons of alanine and threonine, which are crucial for the discrimination between the insulins (see Table 1), are expected at ca 1·4 and 1·6 ppm, respectively (Bundi & Wüthrich 1979). Fig. 1 shows resolution-enhanced expansions of this region for the three insulins. The methyl signals can be easily assigned on the basis of the primary structural differences: 1·14 ppm Thr B-27, 1·20 ppm Thr B-30, 1·26 ppm Thr A-8, 1·42 ppm Ala B-30, 1·44 ppm Ala B-14 and 1·52 ppm Ala A-8.

A more profound characterization of the insulins can be obtained by two-dimensional spectroscopy (COSY). Fig. 1 shows the 1·1–1·6/3·9–4·5 ppm areas of these spectra. All correlations between the chemical shifts of the methyl protons, and the vicinal methine protons of the alanine and threonine residues are easily observed.

The chemical shifts of the methyl and methine protons of the alanine and threonine residues are virtually identical for all three insulins. Since in general proton chemical shifts are extremely sensitive to the protein conformation (Wüthrich 1976; Dwek 1973), this suggests similar tertiary structures for human, porcine and bovine insulin.

The methyl signal of Thr B-27 is broader than the other threonines; this suggests less mobility i.e. more molecular interaction for Thr B-27. At first sight this is unexpected for a residue so close to the end of the B-chain. However a closer look at the tertiary structure, as revealed by X-ray analysis (Blundell et al 1971), shows that the contact between the monomers in the insulin dimer involves Thr B-27 but not Thr A-8 and B-30. Since the dimeric form is the predominant species present at pH $\simeq$ 3 (Cheshnovsky et al 1983), the larger line-width for Thr B-27 can be understood.

Conclusion. Both the one- and two-dimensional ^1H spectra allow the discrimination between human, porcine and bovine insulin. The tertiary structures of the three insulins in D_2O-solutions are essentially the same.

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Antidiarrhoeal activity of bisnordihydrotoxiferine isolated from the root bark of *Strychnos trinervis* (Vell.) Mart. (Loganiaceae)

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Abstract—Bisnordihydrotoxiferine, a dimeric tertiary indole alkaloid obtained from the root bark of *Strychnos trinervis* (Vell.) Mart. (Loganiaceae), inhibited normal defaecation and castor oil, arachidonic acid, and magnesium sulphate-induced diarrhoea on intraperitoneal administration in mice. The effect may be related to the ability of the compound to decrease normal and castor oil-stimulated gastric emptying, small intestinal transit and water and electrolyte accumulation, and inhibition of normal colonic transit. As prostaglandins are involved in gastrointestinal functions, inhibition of their synthesis is likely to contribute to the anticathartic activity, which has never been reported before for an indole alkaloid.

The dimeric tertiary indole alkaloid bisnordihydrotoxiferine (bisnor) was isolated from the ethanolic extract of the root bark of a Brazilian plant, *Strychnos trinervis* (Vell.) Mart. Bisnor produced no obvious actions in the central nervous system or at the neuromuscular junction when tested in frog isolated rectus abdominis muscle and rat phrenic nerve-diaphragm preparation in concentrations up to 200 μg mL^{-1}. The antidiarrhoeal activity of bisnor and its effect on some of the pathological processes involved in the production of diarrhoea are presented in this communication. Bisnor had been isolated earlier by others (Verpoorte et al 1978; Massiot et al 1983). Some of the results were presented to the 4th Annual Congress of Brazilian Society of Pharmacology and Experimental Therapeutics (Melo et al 1986).

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Materials and methods

Male Wistar rats (140–180 g) and male albino mice (21–25 g) starved for 18–24 h were used. The animals had free access to water. Drugs and bisnor were prepared in 0·1% Tween 80 and administered i.p. in doses below the acute LD50 value of 237·0 mg kg^{-1} (95% confidence limits: 200·9–279·5). Bisnor was inactive by the oral route in doses up to 100·0 mg kg^{-1}.