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FULLY UNSATURATED DECAPRENOL FROM BOVINE PITUITARY GLANDS

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SUMMARY: Two types of bovine pituitary gland polyprenols were resolved by silica gel chromatography; i. e., the high molecular weight dolichols of 17 to 23 isoprene units with the OH-terminal isoprene residue saturated, and a fully unsaturated decaprenol. The latter compound was found to be a mixture of molecules differing in the proportion of cis- and trans- isoprene units.

INTRODUCTION: The dolichols of eukaryotes are all α-dihydropolyprenols (cf. Ref. 1, and references cited therein). They differ in the chain length and the degree of saturation; a family of $(\text{prenol})_{13\text{-}18}$ is characteristic for yeast (2,3) and a group of $(\text{prenol})_{17\text{-}22}$ for several mammalian tissues (3,4,5,6). A shorter chain $(\text{prenol})_{11}$ found in pig liver was also α-saturated (7). Even more saturated polyprenols occur in molds (8,9). On the other hand, fully unsaturated, shorter chain length polyprenols are characteristic for bacteria (2,5,10) and plants (3,8,11,12,13,14). In the present paper, the occurence in the pituitary gland of single, fully unsaturated $(\text{prenol})_{10}$ apart from the typical α-saturated dolichols, is described. This is the first demonstration of a fully unsaturated polyprenol in an animal tissue.

MATERIALS AND METHODS: Silica gel (70-200 mesh) was from Serva, Heidelberg. Hydroxy-(C_{15})alkoxypropyl Sephadex (trade name: Lipidex 5000) was from Packard-Becker, B.V.Groningen, The Netherlands. Pre-coated TLC plates of silica gel 60 F-254 (250 µm and

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500 µm) were from Merck, Darmstadt. Thin-layer chromatography was carried out in Solvent A (benzene:ethyl acetate, 95:5, v/v). Thin-layer reversed phase chromatography was performed on cellulose plates (Cellulose-TLC, Serva, Heidelberg) impregnated with paraffine (11) in Solvent B (acetone:water 98:2, v/v) and Solvent C (acetone:water, 88:12, v/v) saturated with paraffin.Spots of polyprenols were detected with iodine vapor.

For proton NMR, samples were dissolved in CCl_4 or C_6D_6 and spectra were recorded with a Jeol JNM-MH 100 instrument. Mass spectra were determined on a LKB 9000 mass spectrometer.

Bovine pituitary glands (5 kg) from adult animals were collected in the slaughterhouse and kept in acetone at room temperature for up to several months. The acetone extraction of whole glands was repeated three times. The partially delipidated glands were cut into pieces and air-dried at room temperature for two days. They were hydrolyzed together with the evaporated acetone extract according to Wellburn et al. (12). The saponification mixture was diluted with water, extracted three times with light petroleum ether, and evaporated to dryness. The yield of unsaponifiable lipid was 22.5 g. The lipid was dissolved in hexane and cholesterol was removed by crystallization at 0°C. The remaining lipid material (13.2 g) was applied on a 1 kg silica gel column and chromatographed under N_2 in benzene; 300 ml fractions were collected. Fractions containing polyprenols as revaled by TLC on silica gel in solvent A, were combined and chromatography repeated on a 80 g silica gel column eluted with 1% ethyl acetate in benzene; 15 ml fractions were collected.
Two peaks were obtained. The first, consisting of dolichols (about 20 isoprene units), had a yield of ca. 850 mg and was completely separated from the second. The material of this second, more polar peak was rechromatographed on a 80 g silica gel column as described above; the yield was 130 mg. It was further fractionated on Lipidex-5000 as described previously (15). 130 mg of the polyprenol fraction were applied to a column (1 x 100 cm) of Lipidex-5000, equilibrated with 75% aqueous acetone, and the column was eluted with a 2 x 1800 ml linear gradient of 75% to 95% acetone in water; 8 ml fractions were collected. The presence of polyprenol in the eluate was detected by reversed phase thin layer chromatography in solvent C.

RESULTS AND DISCUSSION: A crude polyprenol fraction was isolated from total pituitary neutral lipids, obtained by acetone extraction, after saponification of triglycerides and removal of cholesterol and other substances by crystallization and silica gel chromatography. The NMR spectrum of this crude polyprenol showed, in addition to typical dolichol signals, a broad doublet (J = 5 Hz at δ = 3.95 (Fig. 1). This suggested that the dolichol might be accompanied by its unsaturated counterpart. Subsequently, the crude polyprenols were preparatively separated into two fractions. This sepa-

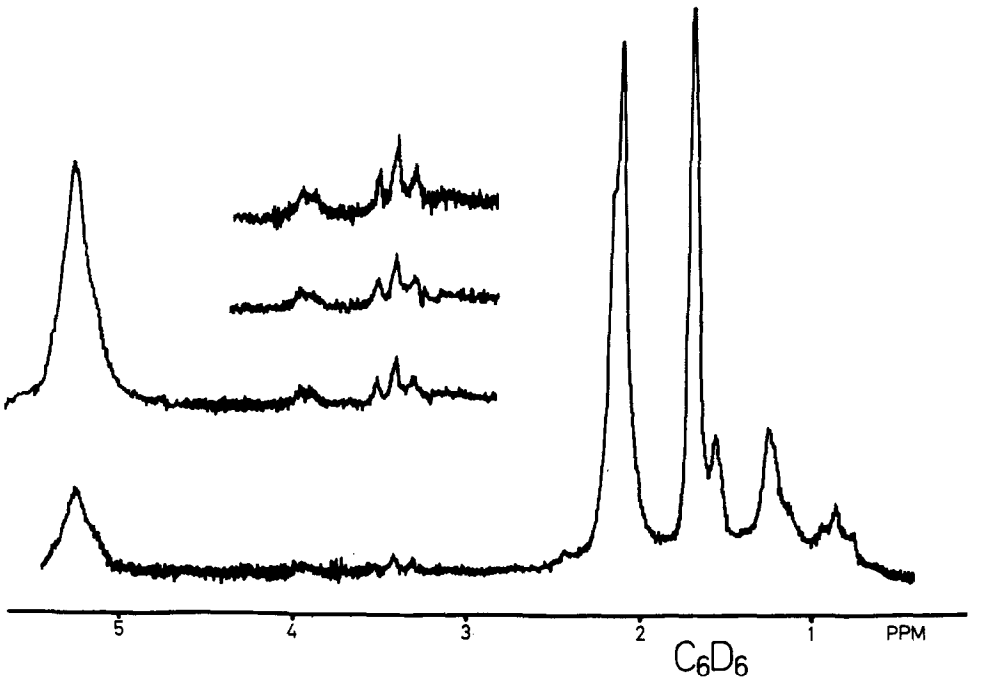

Fig. 1. NMR spectrum of the crude polyprenol fraction from bovine pituitary glands. The spectrum was recorded at 60 MHz; the sample was dissolved in deuterated benzene.

ration was possible because of the use of silica gel, rather than aluminum oxide, for column chromatography. The major (~ 85%) fraction contained dolichol-like, α-saturated polyprenols with chain lengths of 17 to 23 isoprene units, with $(prenol)_{20}$ dominating as shown by TLC in Solvent B. The second, more polar fraction (~ 15% of total) showed a chromatographic mobility in Solvent C similar to fully unsaturated $(prenol)_{10}$; proton NMR spectra supported this similarity.

The final resolution of the short-chain, unsaturated polyprenol fraction was achieved by reversed-phase chromatography on Lipidex 5000, as described in "Methods". Two partially overlapping peaks (A and B) were obtained. Their mobilities, although relatively close on the Lipidex 5000 column, were distinctly different on the

reversed-phase TLC plate. The NMR spectra of these peaks are shown in Fig. 2A and 2B. Both fractions have a fully unsaturated polyprenol structure, as documented by the already mentioned characteristic $-CH_2-OH$ doublet at δ = 3.95 and a low field triplet of the α-terminal olefinic proton at δ = 5.37. The spectra of both fractions are almost exactly superimposable with the most striking exception of the intensity ratio of cis to trans methyl group signals. From the integration (not shown) it was concluded that compound A (see Fig. 2A) contains two, and compound B three internal trans isoprene units. This is confirmed by the appearence of two signals of internam trans methyl groups (at δ = 1.63 and 1.64) for compound A and three such signals (δ = 1.62, 1.63, and 1.64) for compound B, apart from a broad signal of cis methyl groups (δ = 1.75) and signals of ω-terminal methyl groups (δ = 1.69 for cis, d = 1.57 for trans) for both compounds (16).

The chain length of the unsaturated polyprenols was estimated by integration of the olefinic region of the NMR spectrum and its comparison with the integration of the $-CH_2-OH$ signal, and pointed to ten isoprene units for both compounds. This results was confirmed by mass spectra at an ionization energy of 70 and 15 eV. Both compounds gave clear $M-H_2O$ ions at m/e = 680, accompanied by successive cleavage fragments of the polyprenol chain at 68 mass unit intervals (16). It should be stressed that the shorter-chain, fully unsaturated polyprenol fraction isolated from the pituitary gland contained a single iso-prenologue of 10 isoprene units $(prenol)_{10}$ within the sensitivity limits of the methods used. The only heterogeneity of this fraction consisted in the different number of trans isoprene units in sub-fractions A and B, pointing to a different metabolic origin of these compounds (17). The significance of the fully unsaturated

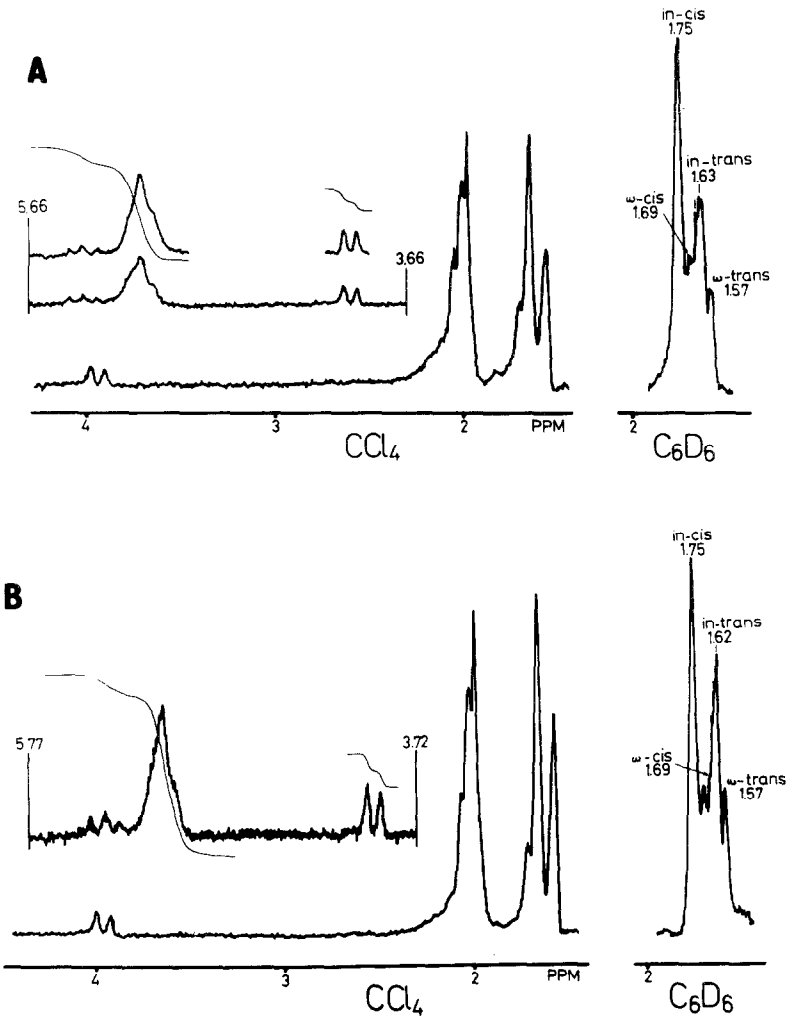

Fig. 2. NMR spectra of two polyprenol fractions (A and B) obtained by chromatography on Lipidex-5000. For details see text. The spectra were recorded at 100 MHz; the samples were dissolved in deuterated benzene or CCl_4, as indicated in the Figure. Internal double bonds are indicated by "in-cis" or "in-trans"; terminal double bonds by "ω-cis" or "ω-trans".

in the pituitary is not known. It seems unlikely that the unsaturation is simply due to an inefficiency of the system responsible for saturation of the α-isoprene unit, since polyprenols of only one chain length $(\text{prenol})_{10}$ are affected; moreover, compounds of this chain length do not occur in the α-saturated

form in this tissue. Therefore, some degree of specificity must be assumed, implying the existence of an - yet unknown - function. Fully unsaturated, shorter polyprenols are typical for bacteria. However, bacterial contamination of the starting material of the preparation can be clearly ruled out. Bactoprenols are heterogenous in chain length, whereas one-chain-length polyprenol is present in preparation obtained from bovine pituitary gland. Moreover, the amount of the $(prenol)_{10}$ found in the pituitary gland (> 25 mg kg tissue) would require a mass of bacteria as high as the mass of the glands used, given the low concentration of polyprenols in bacteria (10).

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