4. RECENT APPLICATIONS OF NMR TO THE INVESTIGATION OF STEROID STRUCTURES

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A detailed and clear understanding of the solution state structure and conformation of steroids is now possible.
This is achieved using high field Nuclear Magnetic Resonance (nmr). The commercial availability of super conducting magnets (9.4 Tesla - ^{1}H resonant frequency 400 MHz) and sophisticated computer control systems has led to the application of various two-dimensional (2D) nmr experiments and increased the utility of traditional assignment techniques such as nuclear Overhauser enhancement (nOe) and double resonance measurements. The combined application of these techniques and greater signal dispersion obtained at high magnetic field has resulted in complete unambiguous analysis of the proton spectra of several steroids[1,2,3]. The power of the various methods will be illustrated by their application to the solution conformation of various steroids, including 17α-acetoxy-6α-methylpregn-4-en-3,20-dione (Medroxyprogesterone acetate; MPA).

[1] Hall, L.D., Sanders, J.K.M., J.Amer.Chem.Soc., 1980, 102, 5703.
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5. A SIMPLE AND EFFICIENT METHOD FOR EXTRACTION AND FRACTIONATION OF STEROID HORMONES FROM A SMALL QUANTITY OF SERUM - Van den Berg, H., Floor, B. and Wilbrink, B.

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Extraction and further clean-up of serum or plasma samples are still essential prerequisites for reliable steroid hormone analysis. Most of the methods being applied up to now are rather tedious and time-consuming, involving liquid-liquid extraction as well as a column-chromatographic step. We developed a simple one-step extraction and purification procedure for the simultaneous assay of estrogens and progesterone from a small quantity of serum (0.5 ml) based upon the method originally described by Manlimos and Abraham (Anal. Letters 8 (1975), 403). Glass pipettes (5 ml) are packed with 3 ml of dry stationary phase (20% ethyleneglycol on Chromosorb-W.N.A.W., 60-80 mesh). 500 µl of undiluted serum is directly applied onto the column, which is then eluted with: a) 5 ml Isooctane (Isoc) → Progesterone (P); b) 6 ml 4% Ethylacetate (EA) in Isoc → Estrone (E_1); c) 5 ml 30% EA in Isoc → Estradiol (E_2); and d) 5 ml 75% EA in Isoc → Estriol (E_3) respectively.
Recoveries (n = 20): P > 95% (98 ± 2%); E_1 > 95% (100 ± 3%); E_2 > 95% (99 ± 0.5%); E_3 > 90% (92 ± 2%).

6. NYLON-TUBE IMMOBILIZED HYDROXYSTEROID DEHYDROGENASES FOR STEROID DETERMINATION

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Following previous work on cellulose-immobilized enzymes (Bovara, R., Carrea, G., Cremonesi, P. and Mazzola, G., 1981, Anal. Biochem. 112,239), some hydroxysteroid dehydrogenases (3α; 7α; 3β, 17β) were separately immobilized on nylon tubes for the continuous-flow determination of hydroxysteroids in biological fluids. Spectrophotometric monitoring in the visible region was obtained using Meldola Blue (8-dimethylamino-2,3-benzophenoxazine) that transfers the hydrogen of reduced coenzyme to tetrazolium salts. Immobilized enzymes were stable to intermittent use for at least two months. The operational range, using the "end point" approach, was 3-20 nmoles of steroid and the assay speed 10-15 samples per hour. With urinary steroids no purification was needed while serum bile acids had to be treated with Amberlite XAD-2 before analysis.

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