2. Analytical methods

2A. Gas liquid chromatography and g.l.c. mass spectrometry

27. Gas–liquid chromatographic studies of cholecalciferol and related compounds

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The preparation of derivatives for g.l.c. analysis of cholecalciferol and related compounds was investigated for reproducible conversion of the hydroxyl group(s) to the less polar heptafluorobutyryl ester function(s) by use of heptafluorobutyric anhydride and heptafluorobutyryl imidazole ester as derivatizing agents. Separation efficiency was checked on 15 different analytical columns of which 1% OV-25, DEGA, STAP and FFAP as liquid phases showed better performances. Detector systems used were FID and ECD (^{63}Ni) giving detection limits of 0·1 μg and 1 ng—absolute amounts injected on top of columns—respectively. Qualitive and quantitative analysis of ergocalciferol, cholecalciferol and its 25-hydroxylated metabolite was carried out in the presence of cholestanol and dihydrotachysterol as internal standards which were derivatized identically and simultaneously with compounds to be determined. Mass fragmentography by monitoring specific ions at m/e 378 for ergocalciferol and 366 for cholecalciferol heptafluorobutyryl ester is undoubtedly most specific. Such a system furthermore permits quantitative analysis at the subnanogram level which looks most promising for determination in human blood samples of the compounds mentioned.

28. Detection and isolation of unknown steroids in the urine of patients with hypertension and adrenal disorders

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The γ-lactone of aldosterone and 18-OH-DOC were estimated by gas chromatography in our laboratory in the urine of 168 patients, most of them with various forms of hypertension (labile hypertension, hypertension with vascular and adrenal disorders, malignant hypertension, renal hypertension) and adrenal disorders (cyclic oedema with hypokalemia and without hypertension). In the urine of 48 patients elevated amounts of aldosterone and/or variable amounts of 18-OH-DOC and/or of two unknown compounds called x and y (both more polar than aldosterone) were found. The latter compounds were detected in 22 patients. Generally, compounds x and y were not found at the onset but after some progression of the disease. The plasma potassium levels and the plasma renin activity are not necessary disturbed. Compounds x and y were extracted from the urine and the extract chromatographed on celite and Sephadex columns and on paper in the system benzene–acetone–water. The specificity of the binding of the isolated products to the soluble nuclear proteins of the rat kidney was estimated.

29. Application of high-resolution capillary gas chromatography to the evaluation of urinary steroid-spectra in different endocrine disorders of childhood

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The pathologic disturbances in secretion and metabolism of steroid hormones were studied in different endocrine disorders of childhood by fractionated determination of 29 urinary C_{19}- and C_{21}-steroids (adrenal carcinoma (n = 1), congenital adrenal hyperplasia of undetermined type (n = 1), AG-syndrome (n = 5), precocious puberty (n = 3), Addison's Disease (n = 1). The steroid patterns of 34 normal children of various ages served as controls. Trimethylsilylether derivates of 9 C_{19}- and 20 C_{21}-steroids were separated on a glass capillary column coated with methylsilicone and detected by FID. In adrenal carcinoma an excessive amount of pregnanetriol and pregnanetriolone was excreted together with a slightly increased excretion of C_{21}-steroids. After exstirpation, the metastases again produced high excretion of DHA and etiocholanolone. In a case of congenital adrenal hyperplasia one month after birth an excessive amount of pregnanetriol and pregnanetriolone was excreted together with a slightly increased excretion of corticosteroid metabolites (TH-DOC, THA, THS, THE, several cortols). A pathognomonically high excretion of pregnanetriol, pregnanetriolone, allo-pregnanediol, androsterone, and etiocholanolone and decreased amounts of tetra- and hexahydrocorticosteroid metabolites were observed in congenital 21-hydroxylase deficiency. In precocious puberty a consistently elevated excretion of C_{19}- and C_{21}-steroid fractions was found. The results showed that the fractionation of 29 different urinary C_{19}- and C_{21}-steroids by a method like capillary gas-chromatography with high sensitivity, specificity, precision, and practicability is a valuable means for rapid identification of various adrenal and/or gonadal disorders in childhood. (Supported by DFG, SFB 87, Project L/M/P).

30. Steroid hormone assays of human tumour tissue by high resolution mass fragmentography

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The technique of high resolution molecular ion detection during combined gas chromatography-mass spectrometry has been employed to measure steroid concentration in human diseased tissue. A simple solvent extraction procedure was used to minimise losses and the crude extracts containing the steroids were treated with bis (trimethylsilyl) acetamide prior to analysis. Standard solutions of the steroids of particular interest (as trimethylsilyl ethers) were prepared in the concentration range of 0·1–10 ng/μl and their mass fragmentograms used to calibrate the system. Similar fragmentograms obtained from the tissue extracts allowed determination of oestradiol-17β, oestriol and oestrone at levels greater than 1 ng/g wet weight tissue, while dehydroepiandrosterone, testosterone, androsterone, epiandrosterone and 5α-dihydrotestosterone were assayed when their levels exceeded 5 ng/g. Analysis of primary breast tumours from postmenopausal women has revealed oestradiol levels between 10 ng/g and 15 μg/g with oestrone