

15. Steroid saponins from *Balanitis roxburghii* Planch

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The fruit pulp of *Balanitis roxburghii* Planch yielded five new steroid saponins of diosgenin named as Balanitisin A, B, C, D and E. The seed kernels, the roots and the stemwood contain respectively Balanitisin F and G.

Balanitisin H, and Balanitisin I. Balanitisin A contains 2 moles of glucose and 1 mole of rhamnose and has the structure, diosgenin-3-O- α -D-glucopyranosyl(1 \rightarrow 3)-O- α -D-glucopyranosyl(1 \rightarrow 4)-O- ϵ -L-rhamnopyranoside. Balanitisin C contains glucose and rhamnose (1:3) while Balanitisin E contains glucose, arabinose, xylose and rhamnose (4:1:2:2), the carbohydrate moiety being attached to the steroid sapogenin diosgenin, a starting material for the synthesis of sex hormones and oral contraceptives. Balanitisin D, F and G contain glucose and rhamnose in molar ratios of 2:1, 4:1 respectively.

2. ADVANCES IN ANALYTICAL METHODS

16. Application of antibodies against 12 α -derivative of progesterone to a direct radioimmunoassay of progesterone in plasma

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Antibodies against the succinyl-BSA derivative of 12 α -hydroxyprogesterone were obtained from 2 out of 3 immunized rabbits. One of those antisera was used to develop a sensitive and reproducible direct assay (without extraction), the practical range being 0.5–20 ng/ml plasma, when taking 20 μ l of plasma per assay tube. Transcortin present in human plasma had to be saturated with cortisol. The antiserum tested under assay conditions was homogenous and had a $K_A = 3 \times 10^9$ l/mol. With the exception of 5 α -dihydroprogesterone there was no significant interference from other progesterone metabolites or steroid hormones. Calibration and plasma dilution curves were parallel to each other. The results obtained for human menstrual cycle and pregnancy were fully compatible with those of other authors.

17. Universal reagent immunoassay. Labelled second antibody assay for norethisterone

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The theoretical advantages of using radioiodine labels for steroids in radioimmunoassay have not been fully realized. This preliminary communication describes a novel solid-phase system which employs radioiodine-labelled second antibodies. The method consists of two steps. (i) the competitive interaction between added norethisterone, specific antibody and norethisterone-bovine albumin conjugate linked to polystyrene-tube surfaces and (ii) the assessment of bound antibody by its interaction with radioiodine-labelled anti-rabbit-immunoglobulin antibodies (universal reagent). Standard curves could be generated over the range 7.8–1000 pg per tube. The coefficient of variation at each point ranged from 50–1.6%, respectively. The least dose detectable was 2 pg and a 50% reduction in binding was elicited by 60–100 pg of norethisterone. The recovery of norethisterone added to pooled human plasma was quantitative. Cross-reaction studies revealed non-parallelism between standards and related steroids. At low concentrations of metabolites the discrimination was less than observed in conventional assay systems.

18. Methods for GC/MS analysis of steroids based on exchange of oxime functions

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Simple and O-substituted oxime derivatives of steroids may be interconverted by an acid-catalyzed reaction of the

oxime with the appropriate substituted hydroxylamine hydrochloride in pyridine. The reaction involves cleavage of the carbon-nitrogen bond and follows pseudo-first order kinetics in the presence of excess reagent. The rate of conversion depends on the position of the oxime function and decreases in the order: C-3 > C-20 > C-17 > C-11. The proximity and configuration of other functional groups also influence the rates. Thus, determination of reaction rates with the aid of gas chromatography-mass spectrometry may be used to determine positions of functional groups. The rapid exchange of oximes at C-3 permits selective "labeling" of saturated 3-ketosteroids in analyses of complex mixtures. The exchange reaction has been used to convert unsubstituted oximes of 3-ketosteroids, which can be isolated from biological materials by ion exchange, into methoximes which are more suitable for GC/MS analysis.

19. A sensitive enzyme immunoassay for testosterone

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We report here the first sensitive enzyme immunoassay of testosterone. A testosterone-penicillinase conjugate was prepared using the carbodiimide method. Antiserum to testosterone was raised using testosterone-3-carboxymethyl-BSA. The separation of bound from free fraction of the label was achieved by the double antibody technique. The enzyme activity in the bound fraction was measured by the method of Novick (1962). The sensitivity of the assay was 15 pg/tube. The within and between assay errors, measured as a coefficient of variation, were within acceptable limits (<12%). A high degree of correlation was obtained ($r = 0.97$) between the assay reported here and RIA. This substantiates the validity of the assay.

20. Computerised gas chromatography-mass spectrometry (GC-MS) applied to the investigation of steroids in human seminal fluid

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Gas chromatography-mass spectrometry (GC-MS) has not been previously employed to analyze steroids in seminal fluid. For this purpose, ethereal extracts and ethyl acetate extracts from the solvolyzed aqueous phase (sulphate fraction) of human seminal fluid were purified by column chromatography on Sephadex LH-20 and thin layer chromatography and subjected to analysis by computerised GC-MS using a 25 m capillary column (OV-101). The steroid fractions were derivatised as their methoxime-trimethylsilyl and *t*-butyldimethylsilyl (TBDMS) ethers. The most important steroid found in both free and sul-