angegebenen Temperatur weiter, bis das Reaktionsgemisch zu einem Kristallbrei erstarrt ist, saugt ab und wäscht die Kristalle mit Aceton. Das Rohprodukt kann aus Acetonitril/Aceton, Äthylenchlorid oder Petroläther/Äthanol umkristallisiert werden. Farblose Nadeln. F. ca. 170–180°, Zers. (Kofler Heizbank). Rohausbeute: 120–145 g.

$C_4H_{12}ClNS_2$	Ber.	C 27,65	H 6,96	Cl 20,41	N 8,06	S 36,91%
	Gef.	„ 27,55	„ 7,08	„ 20,39	„ 8,09	„ 36,36%
MG	Ber.	173,73				
	Gef.	174				

Bromid des Tetramethyl-dischwefel (IV)-nitrids: 31,2 g Dimethylsulfoxid werden mit 15,9 g Bromcyan versetzt und mit einem Vibromischer während 120 h bei 26–30° intensiv durchmischt. Die entstandenen Kristalle werden abfiltriert und mit Aceton und Äther gewaschen. Umkristallisieren aus Acetonitril/Aceton. F. ca. 170–180°, Zers. (Kofler Heizbank). Rohausbeute: 11–13 g.

$C_4H_{12}BrNS_2$	Ber.	Br 36,63	N 6,42%
	Gef.	„ 36,57	„ 6,40%
MG	Ber.	218,18	
	Gef.	219	

Résumé. Lorsqu'on fait agir des halogénures de cyanogène sur le diméthylsulfoxide, on observe le dégagement d'acide carbonique et la formation de nitrures de soufre substitués.

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On the Biosynthesis of Testicular Steroids in vitro and its Inhibition

In the course of our studies on the selective inhibition of various steps in the adrenal biosynthesis of steroids by 3- and 4-substituted pyridine derivatives[1–3], further questions have arisen, including in particular the following: Is it possible to demonstrate similar examples of inhibition in other species and in other types of endocrine tissue, such as the gonads or the placenta? Are there any substances capable of blocking preferentially the biosynthesis of androgens and/or oestrogens without interfering with the adrenal 17α- and 11β-hydroxylases? In other words, are there any substances which directly inhibit either the side-chain splitting of 17α-hydroxylated C_{21} steroids into C_{19} steroids or the aromatisation of 19-hydroxylated C_{19} steroids into oestrogens, thereby exerting an influence on fertility?

Described below are the results of experiments in which an attempt was made to inhibit the side-chain splitting of appropriate radioactive C_{21} steroids in rat testicular tissue in vitro[4].

Methods. For a series of 12 incubations, 13 g of fresh testes taken from normal rats (aged 2 months) were homogenized for 4 min at 0°C in 220 ml buffer-saline solution[5]; for each incubation, 17 ml (corresponding to 1 g tissue) were then added in a special flask to 17 ml incubation solution[5] together with the radioactive precursor. After the test substances, dissolved in 0.25–0.5 ml water or ethanol, had been added, the incubate was gassed for 2 h at 35°C with O_2/CO_2 (95/5%; 0.5 l/min) at pH 7.1–7.25. The composition of each incubate was as follows: vol 34 ml, fresh tissue 1 g, NADP 14.5 μmoles, 49.3 m*M* NaCl, 14.7 m*M* KCl, 5.9 m*M* Na_2HCO_4, 10 m*M* $NaHCO_3$, 20 m*M* K-fumarate, 4 m*M* $MgSO_4$, 59 m*M* sucrose, 0.12–2.0 μC steroid precursor, i.e. for routine procedure 0.12 μC 4-^{14}C-17-hydroxyprogesterone, sp. act. 35.9 mC/mmole, weight ratio steroid/tissue $2.6 \cdot 10^{-6}$. After having been worked up as described in[5], the acetone-soluble portion of the $CHCl_3$ extract was paperchromatographed either free or following acetylation; for routine procedure 1/40 of the acetylated extract was run on strips in the 'Decanit' system[5]; the paper chromatograms were quantitated either in the Packard TRICARB liquid scint. counter Model 314 Ex or in the Packard Radiochromatogram scanner Model 7201. If the net radioactivity (NRA) of the peaks of androstenedione and testosterone in % of the NRA of the toal strip = U, and the remaining percentage of the NRA of the precursor = V, then the degree of the side-chain split D corresponds to $(U-V)/U \cdot 100$ (D = 100 in the control experiments without addition of blockers). A significant inhibition is thought to have a value of 25–50, and is expressed as the 'effective concentration' in μg substance/ml incubate: EC_{25-50}.

At a weight ratio to tissue of 10^{-6}, 17-hydroxyprogesterone was completely used up, yielding 50–80% testosterone and 5–25% androstenedione, as well as 11-deoxycortisol, its 20-dihydro derivative (20α) and pregn-4-ene-17,20α-diol-3-one in a yield of approx. 0.2–2%; no 11β-hydroxylated derivatives or testololactone were found. Qualitatively similar results were obtained from the transformation of 4-^{14}C-progesterone, which yielded 17-hydroxyprogesterone in addition. When tritiated pregnenolone, 17-hydroxypregnenolone, and androst-5-ene-3β,17β-diol were incubated, the main product obtained was again testosterone, with some androstenedione and dehydroepiandrosterone; by-products consisting partly of 20-dihydro derivatives (20α) and partly of Δ^4-3-keto analogues occurred in very much lower concentrations; for their identification see [5]. To this extent our results were in accordance with those reported in the literature[6–8] and indicated that in our set-up the testicular

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enzymes, including particularly androstenedione-synthetase, were functioning well. We also noted that degradation to testosterone and androstenedione could be obtained with 17-hydroxypregnenolone sulphate and 11-deoxycortisol (12.5%), but not with pregnenolone sulphate, pregn-5-ene-3β,17β,20α + β-triol, DOC, corticosterone, cortisol, or cortisone; the latter was merely reduced chiefly to cortisol.

In this connection, a possible substrate inhibition was to be considered. Actually the degradation of $3 \cdot 10^{-7} M$ 4-^{14}C-17-hydroxyprogesterone was already partially inhibited by inactive substrate or by progesterone[9] and

[9] This is in good agreement with the findings of D. K. MAHAJAN and L. T. SAMUELS, Fed. Proc. *21*, 209 (1962).

Table I. Weight ratio of steroid precursors to testicular tissue effecting inhibition of the transformation of exogenous C_{21}-steroids to testosterone

	Radioactive precursor		
	7α-^{3}H-17-hydroxypregnenolone $4.2 \cdot 10^{-9} M$ Substrate/tissue ratio $2.1 \cdot 10^{-7}$		4-^{14}C-17-hydroxyprogesterone $3 \cdot 10^{-7} M$ Substrate/tissue ratio $3 \cdot 10^{-5}$
Unlabelled precursor steroid added	Strong (80–100%) inhibition	Intermediate (25–80%) inhibition	Intermediate (25–80%) inhibition
Pregnenolone	$3 \cdot 10^{-4}$	$3 \cdot 10^{-5}$	
Progesterone	$3 \cdot 10^{-4}$	$3 \cdot 10^{-5}$	$1 \cdot 10^{-4}$
17-Hydroxypregnenolone		$3 \cdot 10^{-4}$	
17-Hydroxyprogesterone		$3 \cdot 10^{-4}$	$3 \cdot 10^{-4}$
Dehydroepiandrosterone		$3 \cdot 10^{-4}$	
Androstenedione		$3 \cdot 10^{-4}$	
Pregn-4-en-20α-ol-3-one			$3 \cdot 10^{-4}$
Pregn-4-en-20β-ol-3-one			$8 \cdot 10^{-5}$

Table II. Inhibition of various steps of the steroid biosynthesis in vitro. EC_{25-50} in μg/ml[a]

	Rat testicular tissue		Beef adrenal tissue	
	Side chain split[b]	17α-Hydroxylation[c]	17α-Hydroxylation[d]	11β-Hydroxylation[e]
Su-4885 (Metopirone®)	60	150	300	10
Su-9055 R = H	3	3	2.4	500[f]
Su-10,603 R = Cl	1.5	1	1.2	500[f]
Su-12,054	15	2	1.5	300
Su-13,572	3	10	150	150
Ba-36,581	3		60	150
Ba-16,848 R = H	1.5		3	300
Ba-21,773 R = $C_2H_5N(C_2H_5)_2 \cdot HCl$	6		60	300

[a] Effective concentration for testicular tissue as defined in this paper; for adrenal cortex tissue (150 mg/ml) = concentration reducing the biosynthesis from *endogenous* precursor(s) to 25–50% of the controls. [b] Of 4-^{14}C-17-hydroxyprogesterone. [c] Of 4-^{14}C-progesterone. [d] Measured by the formation of cortisol or 11β-deoxycortisol. [e] Measured by the formation of corticosterone. [f] Increase instead of inhibition between 30 and 150 μg/ml.

pregn-4-en-20β-ol-3-one (Table I) when the corresponding steroid/tissue ratio was raised to $3 \cdot 10^{-4}$, $1 \cdot 10^{-4}$ and $8 \cdot 10^{-5}$ respectively. It proved even easier to inhibit the degradation of $4.2 \cdot 10^{-9} M$ 7α-^{3}H-17-hydroxypregnenolone by employing pregnenolone or progesterone; with a steroid/tissue ratio of $3 \cdot 10^{-5}$ the inhibition was partial, but complete at a ratio of $3 \cdot 10^{-4}$. Testosterone had no effect at a ratio of up to $3 \cdot 10^{-4}$.

Having determined the requisite condition for effective biosynthesis in our system, we were looking for inhibitors of a non-steroid type. We found that among compounds from the 3- and 4-pyridine series, in which adrenocortical inhibitors had earlier been discovered[1-3,10], there were indeed some which exerted a strong inhibitory effect on the testicular side-chain split, i.e. in concentrations of 1–3 μg/ml; for purposes of comparison, we also determined their inhibitory effect on 17α- and 11β-hydroxylation in adrenal tissue and, in some instances, on 17α-hydroxylation in testicular tissue as well. A selection of various types of inhibitor is given in Table II. Among these inhibitors, those most frequently encountered were compounds, such as Su-9055, Su-10,603 and Ba-16,848, which strongly inhibited both androstenedione-synthetase as well as 17α-hydroxylase while exerting little if any influence on 11β-hydroxylation. Metopirone, on the other hand, which is a potent 11β-hydroxylase inhibitor, had very little effect on the splitting of the side-chain[11]; the same applied to Su-12,054, which is a potent 17α-hydroxylase blocker. Of greater interest were compounds producing preferential inhibition of androstenedione-synthetase, such as Ba-21,773, Ba-36,581 and especially Su-13,572. These examples provided the answer which we were seeking, since they demonstrate that relatively small structural alterations are capable of producing marked shifts in the pattern of inhibition.

From among various categories of compound, we also investigated such substances as were already known to exert some sort of influence on male or female fertility. But neither ORF 1616, ICI 33,828, Clomiphene (MRL 41), U-11,555 A, Su-13,320, Wi-13,099, nor Largactil or Ergocornine, exerted any influence on androstenedione-synthetase in concentrations of 30 μg/ml.

As regards the question of specificity of effect in various species and various types of endocrine tissue in vitro, it has already been demonstrated qualitatively that 17α-hydroxylation can be inhibited by Su-8000 in adrenal tissue[1,10], canine testicular tissue[12], and human placental tissue[13]. With four other substances we have now been able to show that the 17α-hydroxylation appears to be inhibited at about the same concentration in bovine adrenal tissue as in rat testicular tissue, whereas with Su-13,572 there is a marked difference (Table II)[14].

Zusammenfassung. Der Seitenkettenabbau von C_{21}-Steroiden zu Androgenen in Rattentesteshomogenat erwies sich abhängig von der Konzentration der Vorstufen und konnte durch gewisse Pyridinderivate präferentiell blockiert werden.

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The Amino Sugar Content of Plant Tissues

Amino sugars are of widespread occurrence in both bacterial and animal tissues. Recent work has indicated that they probably occur in plant tissues too. UDP-acetylglucosamine has been identified in mung seedlings[1], polysaccharides and mucoproteins containing amino sugar have been extracted from higher plants[2], and an ascorbic acid oxidase preparation has been shown to contain glucosamine residues[3].

This note describes the occurrence of a glucosamine-containing acidic substance in higher plants. One of the species examined (*Sinapis alba*) contained, in addition to this, two substances, which, when present together, gave a marked Elson and Morgan reaction, although neither of them contained amino sugar.

For routine examination the tissues were extracted by grinding with acid-washed sand and four times their weight of distilled water. The extract was centrifuged and the supernatant retained. 5 ml samples were hydrolysed for 10 h with $2N$ HCl. The hydrochloric acid was removed from the hydrolysate under vacuum in the presence of phosphorus pentoxide and solid sodium hydroxide. The residue was dissolved in 3 ml distilled water, centrifuged, and the clear supernatant allowed to percolate through a 5 × 1 cm column of Zeo-Karb 225 (52–100 mesh size). The column was washed with 3 Vol of water and the amino sugar eluted with 5 ml $2N$ HCl. Amino sugar was determined in the eluate by the method of Elson and Morgan as described by BOAS[4].

The levels of amino sugar found in a variety of plant leaves by this method fell within the range 1–3 mg/100 g. Epigeal leaves of the sycamore (*Acer pseudo-platanus*) and the mustard (*Sinapis alba*) contained up to 8 mg/100 g.

A more detailed examination was made of the amino sugar substance of mustard. Mustard seed was germinated on moist filter paper at 70°C and the epigeal leaves harvested after five days' growth. They were weighed, and

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