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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the water-soluble substances extracted by grinding with sand and water. The extract was centrifuged and the purple supernatant fluid removed and shaken vigorously with an equal volume of chloroform for 5 min.

Centrifugation of the mixture produced three layers, of which the clear purple upper one was retained. This was reduced in volume to about 10 ml and was placed on a 15 cm × 1.75 cm column of Sephadex 50, previously equilibrated with distilled water. Elution with water produced three fractions, in order of appearance: a cloudy one containing carbohydrate material (1), a slightly yellow fraction (2), and a third fraction (3) containing the purple pigment. Fractions 1 and 3 were discarded.

Fraction 2 gave an intense colour with the Elson and Morgan reaction. However, about 90% of this colour was produced without incubation with alkaline acetyl-acetone and had absorption spectrum peaks at 525 and 567. Under the same conditions, pure glucosamine gave a single peak at 528. The intensity of this interfering colour markedly increased on standing; that of glucosamine did not. Later work showed that the interfering colour resulted from the presence of two substances, substance A which was retained by the cation exchange resin Zeo-Karb 225 and substance B which was retained by De-Acidite FF (an anion-exchange resin). Substance A could be eluted from the Zeo-Karb 225 with 2*N* NaOH and recombined with substance B to give the interfering colour with the Elson and Morgan reagent. (It is, of course, well known that a mixture of certain amino acids with a neutral sugar produces a colour in the Elson and Morgan reaction[5]. However no neutral sugar appears to be present in either substance A or substance B.)

Fraction 2 was therefore always treated with Zeo-Karb 225 to remove this source of interference. The clear effluent was treated with De-Acidite FF, which retained the amino sugar compound. It was released from the De-acidite FF with 2*N* HCl. The HCl was removed under vacuum over phosphorus pentoxide and solid NaOH and the residue dissolved in water.

Hydrolysis of this final solution with *N* HCl released amino sugar, maximum release being obtained after about 4–5 h at 98°C. No free acetyl-glucosamine was present and none could be liberated by mild conditions such as would release it from a nucleotide. The absorption spectrum of the colour produced with the Elson and Morgan reagent was identical with that given by pure glucosamine. Paper chromatography gave a single spot corresponding to glucosamine[6].

The properties of the compound do not suggest its identity with any of the main forms in which amino sugars have hitherto been reported to occur in plants. Its behaviour on ion-exchange columns suggests an acidic substance; its behaviour with Sephadex 50 suggests a compound of intermediate molecular weight[7].

Résumé. On a découvert que certaines plantes contiennent de petites quantités de sucres aminés, les feuilles épigées paraissant en contenir les plus grandes concentrations. Dans les feuilles épigées de la *Sinapis alba*, ce sucre se trouve sous la forme combinée d'une substance acide de poids moléculaire intermédiaire. On mentionne ici également la présence de substances interférentes qui sont aussi colorées par la réaction Elson et Morgan.

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Présence d'une α_2-globuline lente au cours de la polyarthrite immunologique déterminée par l'adjuvant de Freund

L'injection d'adjuvant de Freund – qu'il s'agisse d'une émulsion de mycobactéries tuées, ou mieux de certaines fractions extraites des cires D – détermine chez le rat, une polyarthrite immunologique[1] qui s'accompagne de modifications quantitatives de l'albumine et des α_1-, α_2-, β-globulines sériques[2].

On sait d'autre part que l'électrophorèse en gel d'amidon peut révéler, dans le sérum de cet animal, la présence d'une α_2-globuline *lente* située immédiatement après l'α_1-globuline *lente*. Mais cette α_2 est très particulière puisqu'elle n'apparaît qu'au cours de certains états physiologiques: période néonatale, gestation[3,4], ou dans diverses conditions expérimentales telles que: processus tumoraux[5], exérèse chirurgicale[6], injection d'endotoxine ou d'adjuvant de Freund[7].

Ces données nous ont incité à préciser par électrophorèse – sur papier, en gel de gélose et en gel d'amidon – l'α_2-dysprotéinémie de la polyarthrite immunologique.

Matériel. Nos recherches ont porté principalement sur deux séries de rats Long Evans de même portée, âgés de 9 semaines, ayant reçu dans le coussinet adipeux plantaire d'une patte postérieure, 0,5 ml d'une émulsion contenant 1 mg/ml de fraction extraite de cire D obligeamment fournie par E. Lederer et P. Jolles. Première série: 6 animaux injectés avec la fraction Canetti Dp 35[8] et 4 animaux témoins. Deuxième série: 6 animaux injectés avec la fraction Atypique Dp 35[9] et 3 animaux témoins. Tous les animaux injectés ont présenté des polyarthrites: celles-ci sont apparues pour la première série entre le 12e et le 20e jour et pour la seconde entre le 8e et le 20e jour. Les prises de sang ont été effectuées avant l'injection

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