

T A D E U S R E I C H S T E I N

Chemistry of the adrenal cortex hormones*

*Nobel Lecture, December 11, 1950***

You have done me the great honour of awarding me a share of the Nobel Prize for Medicine, together with Dr. Hench and Dr. Kendall, although I possess no more than a layman's knowledge of either medicine or physiology. I hope, therefore, that you will not take it amiss if, in the following remarks, I have to make use of a number of chemical formulae that possibly not all of you understand. It is in this case the only adequate language.

If a chemist wishes to do research on the isolation of physiologically active materials, he is dependent upon the collaboration of physiologists who assist him by controlling the grading through experimentation on animals. When I began my researches on adrenal extracts with my colleague Herr J. von Euw, in the year 1934, we were not in a favourable position. The most important deficiency symptoms of adrenalectomy were certainly known, but there were only relatively few quantitative methods to determine the activity of the extracts. There were really only three:

- (1) The survival test (carried out especially on rats)⁷.
- (2) The dog test, after Swingle and Pfiffner (Increase of the non-protein nitrogen level in the blood^{8,9}).
- (3) The Everse-de Fremery test (adynamia after brief muscular stimulation)^{10,11}.

Of these, the dog test was the most sensitive and should also have given relatively accurate results, but it was not at our disposal. On the other hand, Tests 1 and 3 were available in laboratories with which we were on friendly terms. However, both methods require relatively large amounts of material. Hence the grading of extracts could only be controlled biologically in the

* Owing to lack of time, only a brief survey of the chemistry of these substances can be given here, for which reason I shall report almost exclusively on our own work. There are reviews in existence which discuss this field more exactly and give the contributions of various research groups, moreover in correct chronological order¹⁻⁶.

** I wish to thank Dr. H. Dahn for his help in the preparation of this manuscript.

first stage of purification. The following figures give an orientation for the process:

1,000 kg of adrenals from cattle gave in the case of industrial extraction* an extract with about 1 kg of dry residue. The activity of this extract could be concentrated to about 25 g by careful distributive operations, without noticeable loss. Further separating experiments by physical methods (available at this time) always gave a distribution of activity among several fractions. On the other hand, chemical methods were more successful. With Girard's and Sandulesco's¹³ ketone reagent T**, the same 25 g could be divided into the following two groups: about 7-8 g of a ketone group which contained practically the whole activity, and about 15-16 g of ketone-free material that was biologically inactive. From this point further biological control was no more possible for the reasons given above. The whole of the material available (ketone-free as well as ketone-group⁷ was therefore separated into single components as carefully as possible.***

For this the following were used:

Distributive operations (water, benzene, ether, chloroform).

Fractional crystallization.

Chromatography of the acetates on Al_2O_3 .

Only when pure, crystalline, homogeneous substances were produced were they tested, as far as possible, biologically. However, on account of the scarcity of material, the accurate determination of chemical structure was given priority. In nearly all cases this could be elucidated in all its details.

When I began my researches on adrenal extracts I believed that I was dealing with a hormone (or hormone mixture) that definitely did *not* represent a steroid. The solubilities were very different from those of other steroids. The assumption proved false. As soon as it was possible to perform the first degradation experiments it became apparent that we were dealing with

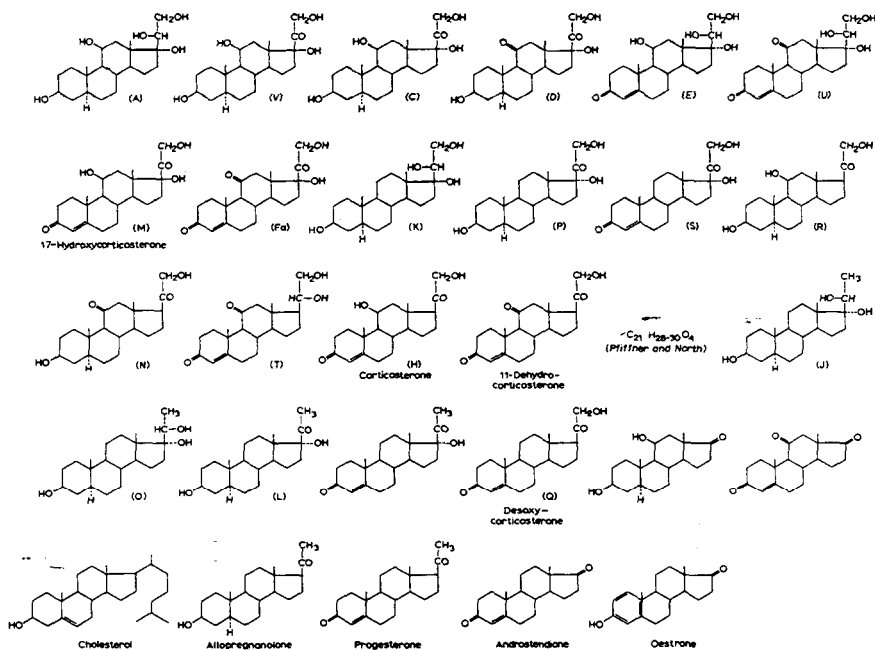
* Carried out more or less according to the method of Swingle and Pfiffner¹², but without the final wasteful purification through water.

* * Dr. A. Girard generously gave us the prescription for the preparation and use of this reagent before they were published. For this great kindness we wish particularly to express here also our thanks.

* * * Such a method for the isolation of biologically active material is not only unusual but extremely troublesome and unreliable. It was employed here only of necessity on account of the impossibility of a biological control. On the other hand, it was partly the cause why such a large number of different substances were isolated directly from the adrenals.

steroids and were able to prove this shortly afterwards. The relatively high solubility in water is due to the high oxygen content.

From the adrenal extracts the 29 steroids shown in Scheme 1 were isolated. *



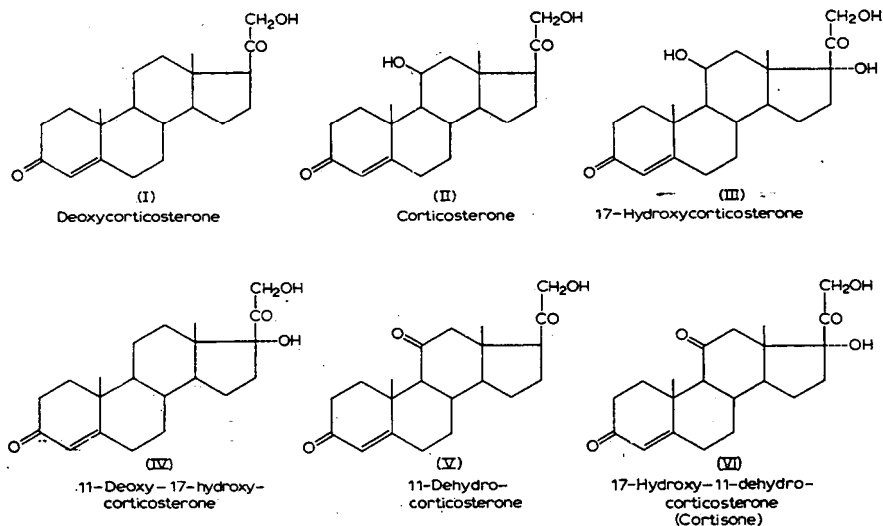
Scheme 1. Steroids isolated from adrenal extracts.

It is neither possible nor necessary to go into details of these formulae here. They should show in the main that all these substances are very closely related to each other. The similarity of the formulae diagrams is an expression of this. Of these 29 steroids, five (the bottom row) were already known;

* In this table, to save space, the alphabetical designations have been employed as we originally used them. A larger number of the same substances has been isolated from the adrenals by Wintersteiner and Pfiffner, as well as by Kendall and his colleagues, partly before and partly afterwards, who used different alphabetical designations for some of them. Such alphabetical designations are only of use to identify a substance when used in conjunction with the writer's name. Today it is better to use systematic names. It was possible to establish by direct comparison the identity of a number of substances isolated independently in the three laboratories mentioned. I would like to thank sincerely here Dr. Kendall and Dr. Wintersteiner for letting me have material for comparison.

they occur in other natural substances. The rest are characteristic of the adrenals. The difference between them is due mainly to the presence or absence of certain oxygen atoms and to the presence or absence of a double bond. Of these 29 steroids, 6 were biologically active in the sense that they prolong the life of adrenalectomized animals and are able to eliminate one or more of the deficiency symptoms*; these are shown in Scheme 2.

In the life maintenance test, and in the effectiveness on the electrolyte and



Scheme 2. The six active substances.

water metabolism, deoxycorticosterone (I: referred to here as DOCA) is by far the most effective, and III and VI the weakest; II, IV, and V are somewhere in between. In other tests, such as those connected with changes in carbohydrate metabolism**, the relationship is reversed; I and VI are by far the most effective and I the weakest.

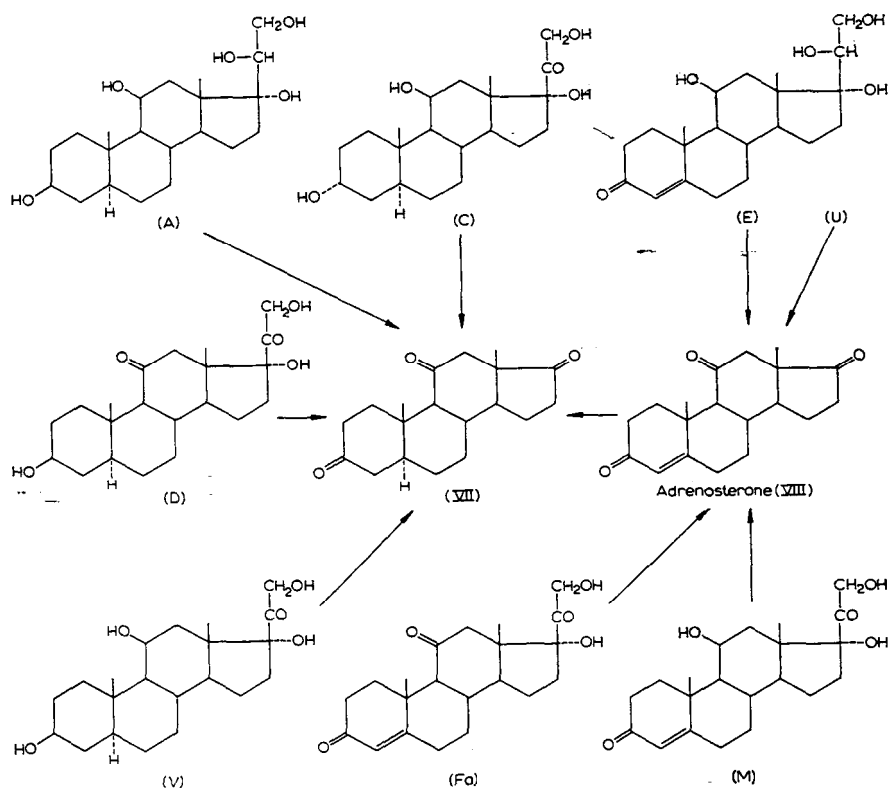
Reciprocal relationship and proof of the steroid nature

(a) $C_{21}O_5$ groups A whole series of substances from the adrenals contains 21 C-atoms and 5 O-atoms. Out of the eight substances of this group, four

* Subsequently referred to as "cortin-activity", for short.

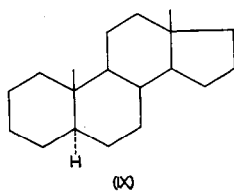
** Including Ingle's Test¹.

were saturated and four were unsaturated. The four saturated representatives (substances A, C, D, and V) all gave the same triketone VII on oxidation with CrO_3 , the four unsaturated members (E, Fa, M, and U) gave an analogous unsaturated triketone VIII, which was named adrenosterone and which can be converted to VII by catalytic hydrogenation. This is proof of

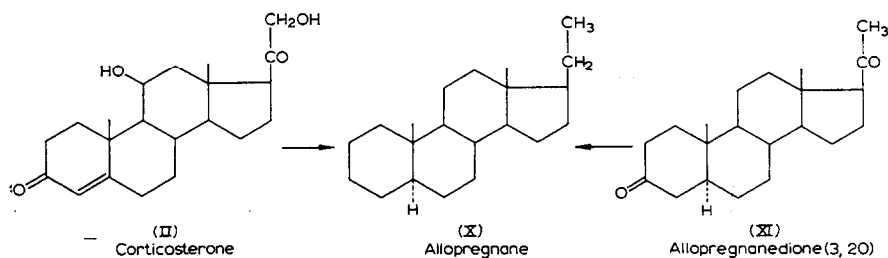


Scheme 3. Chemical relationship of C_{21}O_5 substances.

the close chemical relationship of all substances of the C_{21}O_5 group. The two triketones VII and VIII showed distinct androgenic activity with the cock's comb test, which gave rise to the suggestion that we were dealing with steroid derivatives. Shortly afterwards, the reduction of the triketone VII by Clemmensen's method was accomplished, whereby the crystalline hydrocarbon androstane IX was obtained, which was already known through the work of Butenandt¹⁴. In this way the steroid nature was unequivocally proved.



(b) $C_{21}O_4$ group - It was possible to proceed similarly with representatives of the $C_{21}O_4$ group, which carry oxygen in the Π position (see below)*. This will only be indicated for the most important representative of this group, corticosterone (II). It was possible to convert this substance in several stages to the hydrocarbon allopregnane (X), which was also ob-



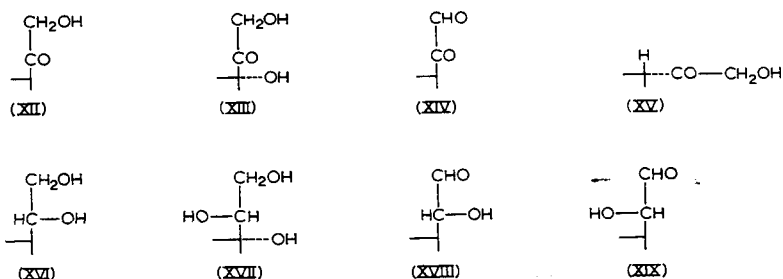
tainable from allopregnanedione-3,20 (XI), by which the steroid structure was proved unequivocally here.

I must content myself with these indications and can only add that not only could the fundamental skeleton be unequivocally proved, but also all the details of the further formulae given above. This relatively rapid elucidation of the chemical constitution and spatial configuration, with partly very limited quantities of the substance, was only possible because a few years ago the correct structure of steroids and bile acids was established through the work of Wieland, Windaus, Diels, and others, and that of the steroid sex hormones through the work of Butenandt, Laqueur, Ruzicka, and others, so that a link with substances of known configuration was possible.

* The establishment of the structure of representatives carrying no oxygen at position Π was easier because degradation as a rule leads to substances which are known or easily obtainable through partial synthesis.

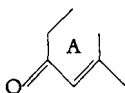
Relationship between chemical structure and biological activity

Desoxycorticosterone (I) is the simplest known compound with "cortin" activity (as defined above). If the double bond or the ketone group is hydrogenated, the activity is lost. Alteration of the lateral chain acts in a similar manner.



Two conditions must, then, be fulfilled for the activity:

- (1) a, b-Unsaturated ketone grouping in ring A in accordance with the partial formula

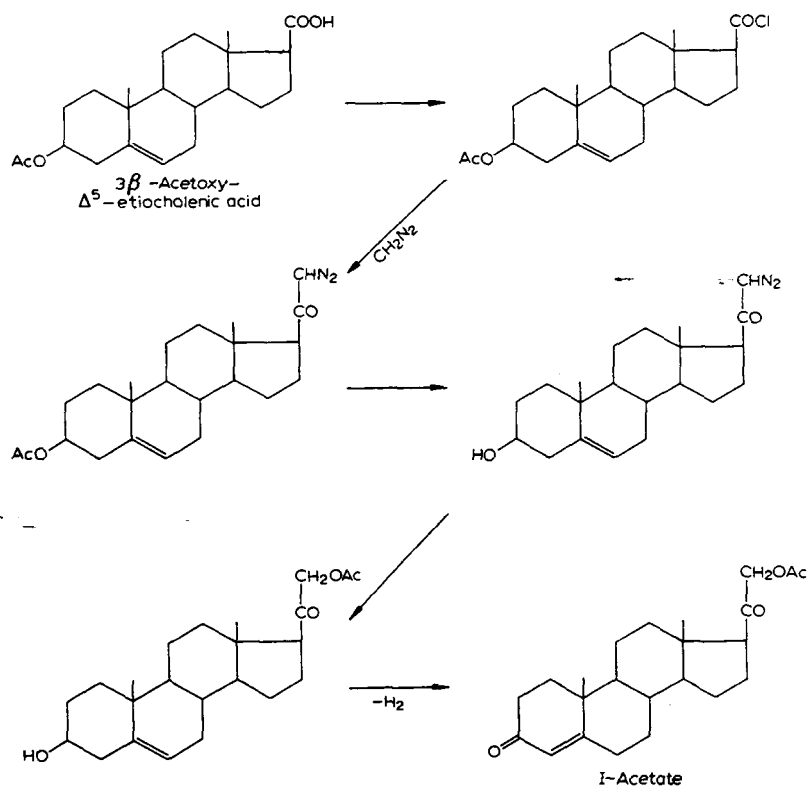


- (2) Correct lateral chain. Up to now only the two natural ketol groupings XII and XIII have shown themselves to be active.

We have tested with particular care whether other activesubstances with other lateral chains exist. This was done by means of partial synthesis. Analogues of I were prepared with various lateral chains. Of these only the ketoaldehyde XIV showed unequivocal activity, but its effect was weaker than that of I. It was indeed noteworthy that a reversion in the asymmetric centre at C(17) was sufficient to destroy the activity of I (XV was inactive). Also the different diols and triols XVI and XVII, of which more stereoisomers exist, and which occur to some extent in adrenal extracts, were inactive. The same holds for the hydroxyaldehydes XVIII and XIX, of which XIX at least is easily convertible to the active substance XIII with a dihydroxyacetone grouping.

Partial syntheses

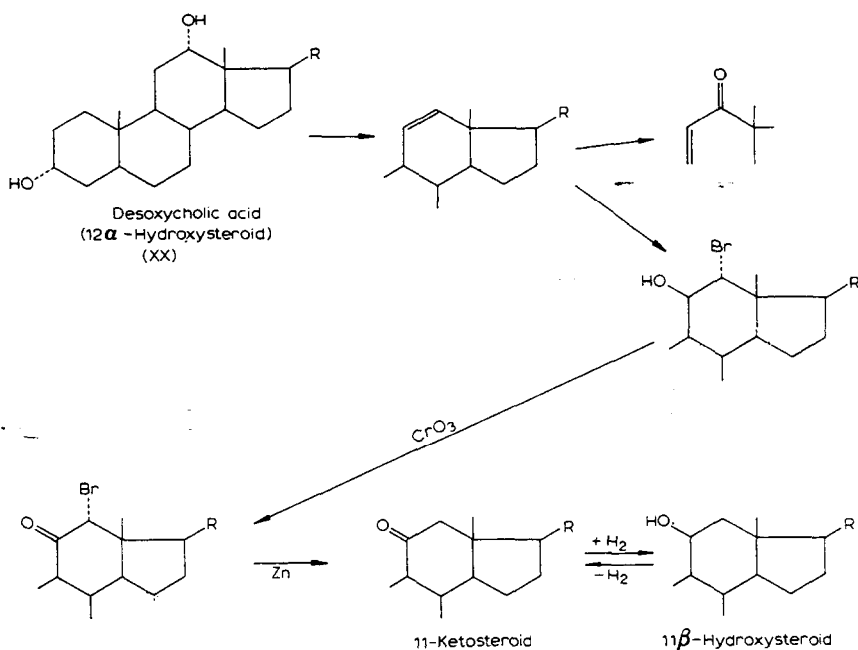
Naturally we have endeavoured to make the active corticosteroids more easily accessible by means of partial synthesis. This was achieved first with desoxycorticosterone.¹⁵



Scheme 4. Synthesis of desoxycorticosterone.

This partial synthesis is relatively simple and has been carried out on industrial scale for a considerable time. DOCA was used successfully in some cases of adrenal insufficiency. Many patients with Addison's disease could be maintained in relatively good condition even if they cannot be considered as completely adjusted. I was interested to hear briefly from Dr. G. W. Thorn of Boston that some patients with Addison's disease, who have recently been experimentally maintained on cortisone alone with no DOCA, showed fairly serious deficiency symptoms. He has been able to obtain the best substitute for the organ with DOCA plus cortisone.

Further partial syntheses were concerned above all with the introduction of an HO or ketone group in the 11 position of the steroid configuration. Before the discovery of the corticosteroids no natural steroid derivatives certainly carrying oxygen in the 11 position were known. For evidence of the constitution and for the synthesis such substances had first to be artificially prepared. This was first achieved as shown in Scheme 5, starting from desoxycholic acid (XX)¹⁶.

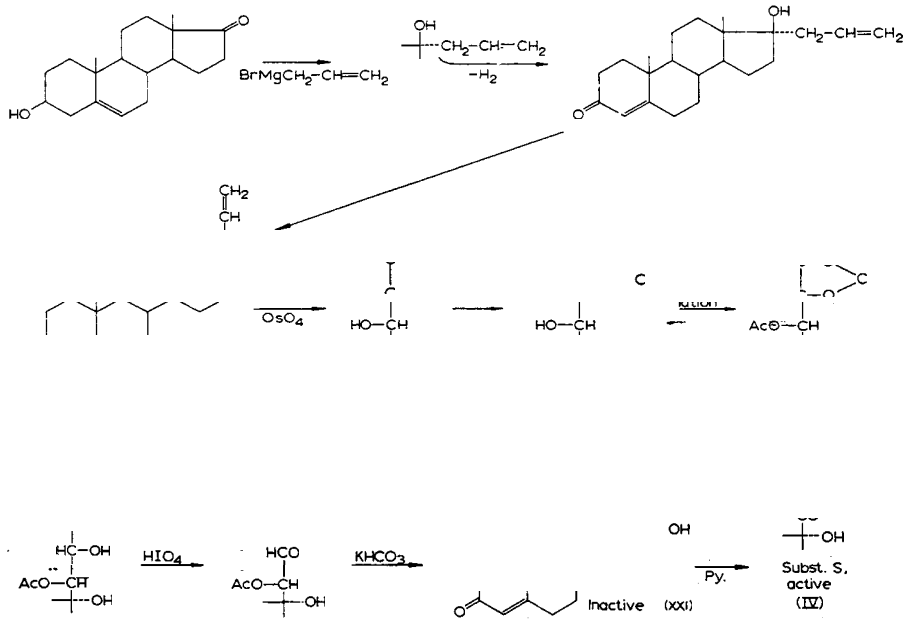


Scheme 5. Introduction of hydroxyl or ketone group in 11 position.

The position of the "inactive" oxygen in the corticosteroids was first definitely demonstrated with the help of this method. It also served for the partial synthesis of some corticosteroids with oxygen in the II position, also among others of corticosterone itself¹⁷. However, for practical purposes it is too laborious. As physiologists and physicians later demanded ever greater quantities of such corticosteroids for research and clinical use, better methods were developed especially by American research workers, and particularly Kendall, which today are already technically carried out in part (Merck) but which are still very laborious. They likewise start from desoxycholic acid (XX).

A further problem in the partial synthesis of stable corticosteroids was the

formation of the dioxyacetone lateral chain (XIII) which occurs, for example, in 17 hydroxycorticosterone (III) and cortisone (VI). We accomplished this first as shown in Scheme 6 in the partial synthesis of our substance S (IV)¹⁸.



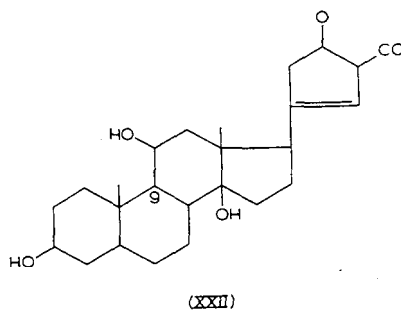
Scheme 6. Partial synthesis of substance S.

Originally it was not the partial synthesis of (IV) that was attempted in this way but that of the dihydroxyaldehyde (XXI). It became apparent that this substance was biologically ineffective. However, it could be rearranged to the active IV, which was thus obtained artificially for the first time. For practical purposes this method is much too laborious. In the last two years, again particularly in the U.S.A., at the cost of a considerable amount of time, much better methods have been discovered, of which I only mention here those of Sarett¹⁹, Gallagher and his collaborators²⁰, Julian and his collaborators²¹, Miescher and his collaborators²², and Wagner and Moore²³. From what I have heard, Dr. Kendall has also made important contributions, which are not yet published. It is unfortunately completely impossible for me to name the many other important studies whose aim is to simplify and cheapen the production of cortisone. For after the clinical results of Hench, Kendall and their colleagues^{24, 25} it can hardly be doubted that the future demand for these substances will be very great.

I will therefore indicate in just a few words in which direction we have sought to bring this wish nearer fulfilment. As stated, the introduction of an oxygen atom in position II of the steroid skeleton is one of the major difficulties in the synthetic production of cortisone. The preparation of this substance from desoxycholic acid still remains a long and laborious way, even when so many improvements to it have been discovered. If it is wished to obtain cortisone more simply, there remain two ways:

1. Total synthesis.
2. The discovery of a better qualified raw material.

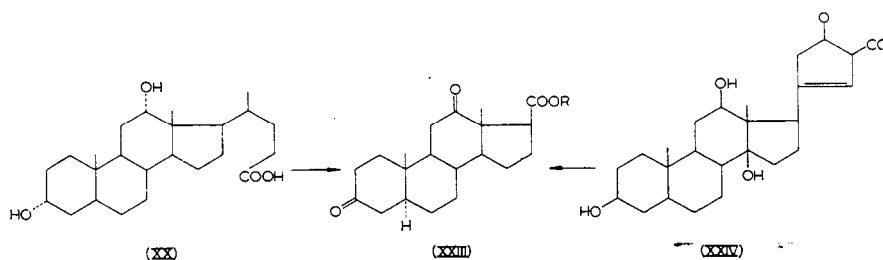
Both will be tried. The prospects of a total synthesis are difficult to assess*, possibly it will be achieved this year, perhaps it will take many years to make them practicable. I have, with some of my colleagues, attempted to make a contribution to the second possibility. As stated above there were no natural substances known before the discovery of the corticosteroids which were with certainty substituted with oxygen in the II position. However, two were known of which this was *suspected*. These were the aglycones of two cardiac glycosides, namely digoxigenin and sarmentogenin. Tschesche and Bohle^{26,27} have investigated both, and on the basis of their results have expressed the opinion that they are stereoisomeric at C(9) and that they both have the Formula XXII.



We were able to check digoxigenin first. This is the aglycone of a glycoside from *Digitalis lanata* which will be known to you as digilanid C and is manufactured in Basle. Professor Stoll as early as 1937 had the kindness to place 5 g of digoxigenin at our disposal. We degraded it and established

* R.B. Woodward referred to the partial synthesis of an acid which is already very closely related to cortisone, on the 9th April, 1951, in Boston (Mass.). Cf. R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W.M. McLamore, *J. Am. Chem. Soc.*, 73 (1951) 2403.

that the oxygen atom was not attached at C₍₁₁₎. Shortly afterwards, Hoehn and Mason²⁸ in the U.S.A. degraded desoxycholic acid (XX) to 3,12 diketo-etiocholic acid (XXIII). This proved to be identical with the acid obtained from the degraded of digoxigenin. The latter has thus the Formula XXIV and is little more suitable for the manufacture of cortisone than the much cheaper desoxycholic acid (XX).



There still remained sarmentogenin. Here the supply of material was the major difficulty. This aglycone was isolated by Jacobs and Heidelberger²⁹ from a sample of *Strophanthus* seeds which were falsely declared to be *S. hispidus*. The writers knew that it was definitely *not S. hispidus* which was before them and were able to bring forward very good evidence that it was probably *S. sarmentosus*. Later, Tschesche and Bohlea²⁷ also isolated sarmentogenin from some analogous seed samples, likewise falsely declared to be *S. hispidus*. The seeds of *Strophanthus sarmentosus* were never a commercial commodity, and we endeavoured first of all for a long time in vain to obtain samples of it. We then attempted to obtain *S. hispidus*, in the hope that we would also come across the falsely declared strain, but these seeds were also no longer commercially available. Meanwhile the war had broken out and a special expedition to Africa could no longer be considered. By chance in January, 1940, we obtained from the firm of A.G., formerly B. Siegfried, of Zofingen, their last reserves of 100 g of seeds, which they had received a long time before from London, under the designation "semen *Strophanthi hispidi*". Dr. Katz investigated them in my laboratory³⁰. They proved straightaway to be the desired strain. He was able to obtain 0.3 g of sarmentogenin. The breakdown ran relatively smoothly, and showed that sarmentogenin actually has Formula XXII (p. 305) - that is, carries an HO group in the 11 position and therefore would be very suitable as a raw material for the artificial production of cortisone if it could be had in greater quantities. Recognition of the parent plant was a necessary condition of procuring it.



Fig. 1. *Strophanthus sarmentosus* P.DC.: view of a liana in full flower, which simultaneously bears ripe fruit. The leaves have mostly fallen during the time of ripening. Transition forest near Abuentim, north of Mampong (Ashanti), Gold Coast, February, 1948. (Photo Dr. J. Schmutz.)

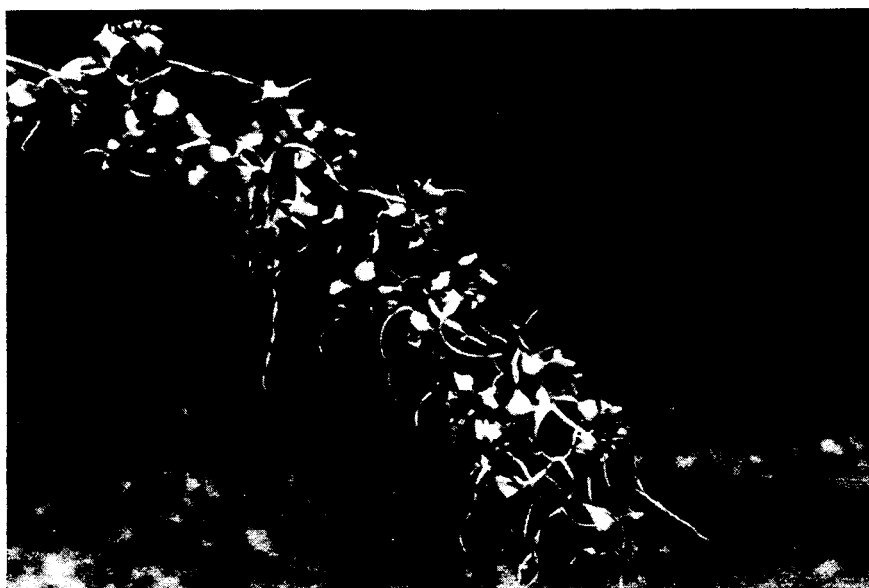


Fig. 2. *Strophanthus sarmentosus* P.DC.: flowering branch. Place and time as in Fig. 1. (Photo Dr. J. Schmutz.)



Fig. 3. *Strophanthus sarmentosus* P.DC.: view of a liana with fruits shortly before ripening. The leaves have for the most part fallen. The plant is flowering simultaneously. Place and time as in Fig. 1. (Photo Dr. J. Schmutz.)

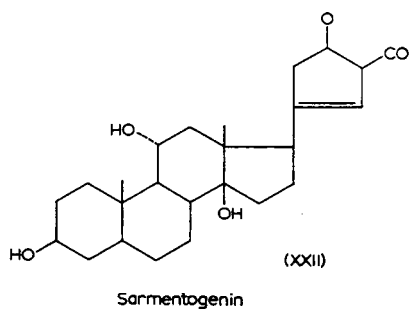
As soon after the war as it became possible, we began to search for it. It was soon clear that it was not possible to obtain suitable samples of plants from existing organizations, or state forest, or agricultural stations. The only possible way was to collect them ourselves. Through the co-operation of the Basle authorities and the liberal financial help of Basle industry, two assistants (Dr. A. Katz and Dr. J. Schmutz) of the Pharmaceutical Institute were first able, in 1947, to travel to Africa for nearly nine months, and examine many medicinal plants, including some seeds of species of *Strophanthus*. Naturally, of greatest interest was *S. sarmentosus*. Over twelve unquestionable samples of this species, some of them relatively large, were col-



Fig. 4. *Strophanthus sarmentosus* P.D.C.: ripe fruit. During the ripening, the fruit turns largely to wood. The follicles are only a little tapered towards the end; length of the single follicle is about 25 cm. Place and time as in Fig. 1. (Photo Dr.J. Schmutz.)

lected from various parts of the Gold Coast, Togo, the eastern Ivory Coast and Southern Nigeria. In Figs. 1-5 are shown some high lianas with fruits, further individual fruits, flowers and seeds of these plants. The major difficulty of harvesting the seeds is that the fruits only ripen once a year; the harvest must be gathered in a relatively short time. Unripe fruits are of little value; and after the fruits ripen they burst open and the seeds fly out.

The outcome of the 1947 expedition was negative from the point of view of cortisone. *Strophanthus sarmentosus* from the regions named, produced no,



or only traces of, sarmentocymarine, but instead a new glycoside, which we named sarverosid³¹ and which is not suitable for the manufacture of cortisone.

After this unexpected result we set ourselves the task of investigating as far as possible all *Strophanthus* species, as far as they were at all obtainable. This work goes far beyond what I can describe here. In botanical literature, over 45 different species of *Strophanthus* are described, some 35 of them in Africa alone. Up till now we have investigated twenty-one of them, some it is true insufficiently, on account of shortage of material. This work would be carried out without regard to practical ends; but we believe that it will also be of practical use. Some of the species examined contain sarmentogenin. We

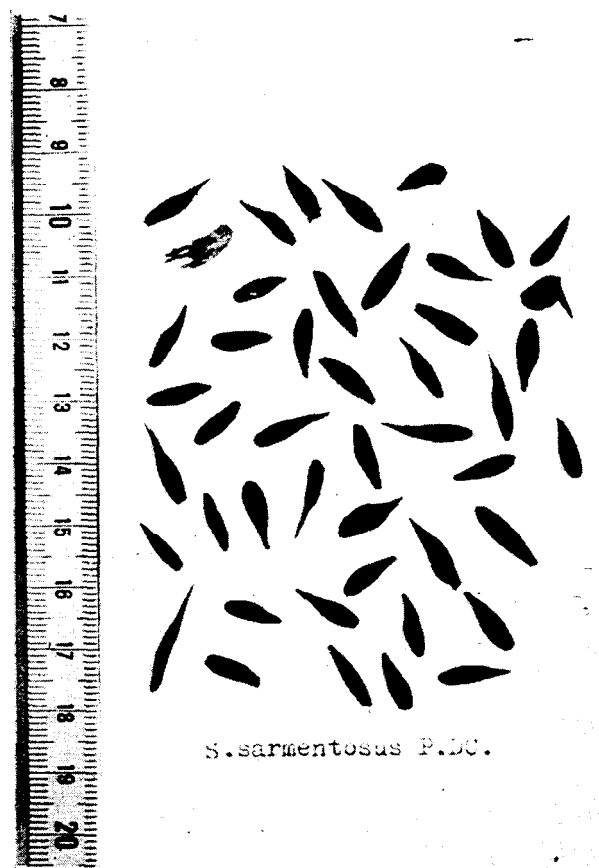


Fig. 5. *Strophanthus sarmentosus* P.DC.: ripe single seeds from the high plateau of Togo (sample Walkowiak³¹). (Photo Dr. L. Jenny.)

also hope very soon to be able to determine the best variant. The seeds of this plant will only be available in sufficient quantity for the production of cortisone if we succeed in cultivating them on a large scale. Whether that is possible only experimentation can show. At best, practical results will not be obtained for four or five years as the plants require at least three years before they bear their first fruits, and four to five years before the first useful harvest. Perhaps total synthesis will make these experiments unnecessary from a practical point of view. Nevertheless, we hope to be able to push forward undisturbed with the investigation of the various *Strophanthus* species for as long as possible. As with all research into the products of Nature, unexpected results are possible here also.

Allow me in closing to thank you sincerely for your friendly attention.

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