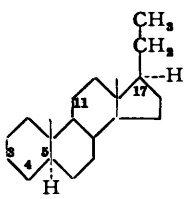
Recent Advances in the Chemistry of the Steroids.

TILDEN LECTURE, DELIVERED BEFORE THE CHEMICAL SOCIETY AT THE ROYAL INSTITUTION, LONDON, ON MAY 4TH, 1950.

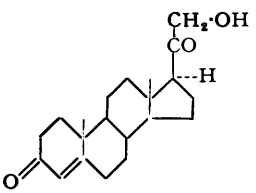
By F. S. SPRING.

THE considerable quickening in interest shown during the past year in the chemistry of the various steroid sub-groups makes a general review a matter of some difficulty and calls for a degree of selection from the mass of data available. For this reason, I intend to limit myself to a discussion of the chemistry of some of the adrenal cortical steroids, and specially to a discussion of some of the approaches being made to the partial synthesis of adrenal steroids bearing an oxygen function at positions 11 and 17. After the announcement by Hench and Kendall in 1949 of the effect of Kendall's adrenal compound E (cortisone) in the treatment of rheumatoid arthritis, such attempts at partial synthesis of 11 : 17-oxygenated adrenal steroids have been the main focus of interest in steroid chemistry.

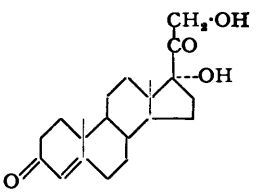
That extracts of adrenal cortical tissue will prolong the life of an adrenalectomised animal was established in 1930. Such extracts can be made in a variety of ways. For example, extraction of minced adrenal glands with cold acetone, followed by partitioning of the extracted material between a number of immiscible solvent pairs, gives a concentrate. This is extracted with ethyl acetate, and the solution washed with weak acid and alkali to yield a neutral concentrate. In a typical concentration process, 1000 lb. of beef adrenal yield approximately 10 g. of neutral concentrate. The isolation of individual compounds from the concentrate was undertaken about 1934 by Reichstein, Kendall, Pfiffner, and others using a variety of analytical procedures; among these may be mentioned the use of the Girard reagent to effect separation into carbonyl and non-carbonyl fractions, extensive use of partition techniques, and chromatography of acetylated extracts followed by hydrolysis of the separated components.



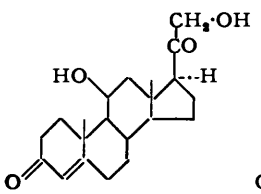
*allo*Pregnane.
(I.)



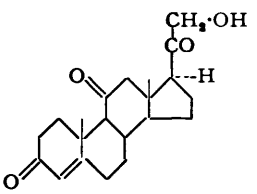
Deoxycorticosterone.
(II.)



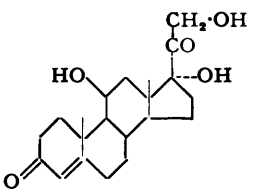
17-Hydroxydeoxycorticosterone (Substance S).
(III.)



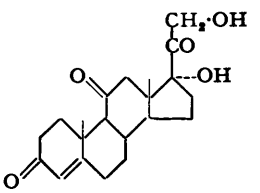
Corticosterone.
(IV.)



Dehydro-corticosterone.
(V.)



17-Hydroxy-corticosterone.
(VI.)



17-Hydroxydehydro-corticosterone (Cortisone).
(VII.)

As a result of these investigations just under thirty homogeneous compounds were isolated from adrenal extracts and, with one exception, their structures were determined by degradation and interconversion and in some cases by methods of partial synthesis from compounds of established structure by unambiguous methods. In addition to the isolated crystalline components, there remains an amorphous fraction which possesses to a markedly high degree the capacity to prolong the life of an adrenalectomised animal. Considerable variation in hormone content is observed in extracts from different animal species.

Most of the thirty compounds are known to be pregnane derivatives. It is of interest to note that these either contain a 3-keto-Δ^4-unsaturated system or they are saturated, in which case they have the *allo*-configuration at $C_{(5)}$ (I). Furthermore, adrenal steroids hydroxylated at $C_{(8)}$

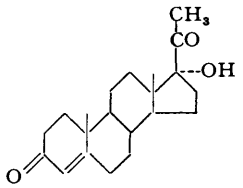
have, with one exception, the 3β-configuration. Although a number of the adrenal cortical steroids have not been available in sufficient quantity for physiological test, it is known that six of them (II—VII) are capable of prolonging the life of an adrenalectomised animal.

Physiological Effects of the Cortical Steroids.—An adrenalectomised animal can be maintained by the administration of a fresh cortical extract or by one of the six compounds (II—VII). In the dog life-maintenance test considerable differences in activity are observed. The amorphous fraction is the most active, being more active than deoxycorticosterone (II), which is the most potent among the crystalline compounds. Incidentally, deoxycorticosterone acetate is considerably more active than deoxycorticosterone itself. The four 11-oxygenated hormones (IV—VII) are less active than either deoxycorticosterone or the amorphous fraction. One important function of the adrenal gland is to exert a control of electrolyte balance, since adrenalectomy is followed by diminution in serum sodium involving excretion of sodium and chloride, and retention of potassium ions; a similar disturbed electrolyte balance is observed in Addison's disease (atrophy of adrenals). A comparison of the effect of adrenal steroids on sodium retention shows that this roughly parallels the life-maintenance test, deoxycorticosterone (II) and the amorphous fraction being highly active and the 11-oxygenated compounds (IV—VII) markedly less active.

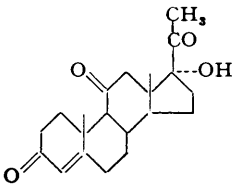
Another apparent function of the adrenal gland is an effect upon carbohydrate metabolism, since adrenalectomy is followed by a fall in liver glycogen which can be corrected by administration of adrenal extracts. The capacity of an adrenal hormone to promote glycogen formation in the liver of a starved adrenalectomised animal has been used as an assay method; by using this yardstick, a further interesting difference in effect between different adrenal hormones is observed. Of the six hormones the two bearing oxygen functions at positions 11 and 17, cortisone (VII) and 17-hydroxycorticosterone (VI), are the most active. Corticosterone (IV) and dehydrocorticosterone (V) are less active, whilst deoxycorticosterone (II) and substance S (III) are inactive. Thus, broadly speaking, deoxycorticosterone is highly active in sodium retention and inactive in glycogenesis, whereas the 11-oxygenated hormones are highly active in glycogenesis and inactive in sodium retention.

Finally, the profound effect of cortisone (VII) in rheumatoid arthritis, and to a lesser extent in rheumatic fever, appears to be highly specific. The adrenocorticotropic hormone of the pituitary (ACTH) is equally as effective as cortisone, and although sufficient 17-hydroxycorticosterone has not been available for reasonable assay, it is probable that it will prove to be as potent as cortisone in the treatment of arthritis. Although other compounds have been claimed to show similar effects or to lead to improvement in established cases of rheumatoid arthritis, these improvements are, according to Kendall, psychological; only cortisone has the effect of reducing the high erythrocyte sedimentation rate, a critical characteristic of arthritis.

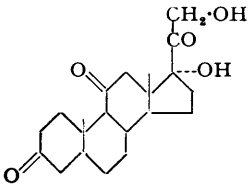
It has been reported that, in so far as rheumatoid arthritis is concerned, deoxycorticosterone (II) (cortisone with hydrogen in place of the 11- and 17-oxygen functions), 17-hydroxyprogesterone (VIII) (cortisone with hydrogen in place of the 21- and 11-oxygen functions), 17-hydroxy-11-ketoprogesterone (IX) (cortisone with hydrogen in place of the 21-hydroxyl group), 11-dehydrocorticosterone (V) (cortisone with hydrogen in place of the 17-hydroxyl



17-Hydroxyprogesterone.
(VIII.)



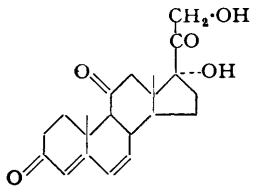
17-Hydroxy-11-ketoprogesterone.
(IX.)



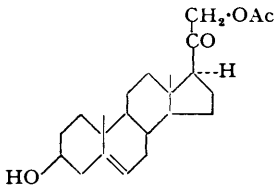
(X.)

group), substance S (III) (cortisone with hydrogens in place of the 11-oxygen atom), and 4 : 5-dihydrocortisone (X) (cortisone with hydrogen saturating the double bond) are all without effect in arthritis. Furthermore, none of them affects the erythrocyte sedimentation rate. As a further pointer to the remarkably high specificity of cortisone in this respect, Kendall has described experiences with the $\Delta^{4:6}$-diene (XI). This diene can be obtained from cortisone (as acetate) by bromination and dehydrobromination, and conversely it is readily reduced to cortisone (VII) by zinc-dust reduction. Although the diene has extremely high glycogenic

activity and again like cortisone causes atrophy of the adrenal gland, it has no effect on arthritis. It is interesting to compare this result with the pronounced progestational activity of the corresponding diene from progesterone.



(XI.)



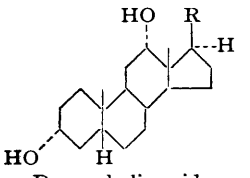
(XII.)

Among compounds related to cortisone which have been claimed to have a cortin-like effect is 21-acetoxypregnenolone (21-acetoxy-3β-hydroxy-20-keto-Δ^5-pregnene) (XII) which is highly active in the life-maintenance test with rats. It has also been claimed that 21-acetoxypregnenolone (artisone) has anti-arthritic properties although the clinical evidence has not been detailed. Again it has been claimed, and disclaimed, that deoxycorticosterone acetate together with a massive dose of ascorbic acid effects some relief of the symptoms of rheumatoid arthritis.

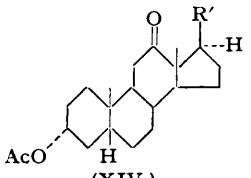
Partial Synthesis of Cortisone.

The isolation of cortisone from adrenal extracts for a clinical evaluation on a suitable scale is not feasible, the yield being of the order of a few hundred milligrams per 1000 lb. of adrenal gland after a most tedious isolation procedure. Mention may be made of the recent isolation by Schneider of cortisone from normal male urine in amounts of the order of 50 mg. per 1000 litres. In the same connection the observations of Pincus and his associates are of considerable interest; they find that when an isolated adrenal gland is perfused with a solution containing deoxycorticosterone (or its acetate) the perfusate is highly glycogenic and from it corticosterone was isolated, *i.e.*, the gland has hydroxylated deoxycorticosterone at the 11-position. The enzymic oxidation of steroids in the 11-position by means of an extract of minced adrenal gland has also been reported.

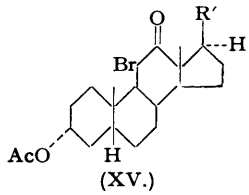
The need to provide a reasonably adequate source of cortisone is pressing. The prospect of preparing analogues, which will have a similar effect, whilst still present, is growing dimmer, and leaving aside the equally distant prospect of total synthesis as feasible approach to reasonable supplies of cortisone, the partial synthesis of cortisone from available steroids appears to be the most rational approach to clinical supplies. The demand for access to cortisone and related compounds is independent of any progress which may be made in the clinical treatment of rheumatoid arthritis with the adrenocorticotropic hormone of the pituitary (ACTH).



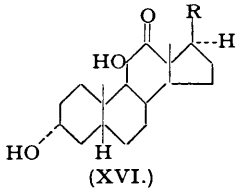
Deoxycholic acid.
(XIII.)



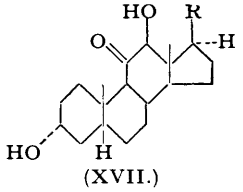
(XIV.)



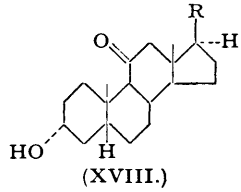
(XV.)



(XVI.)



(XVII.)



(XVIII.)

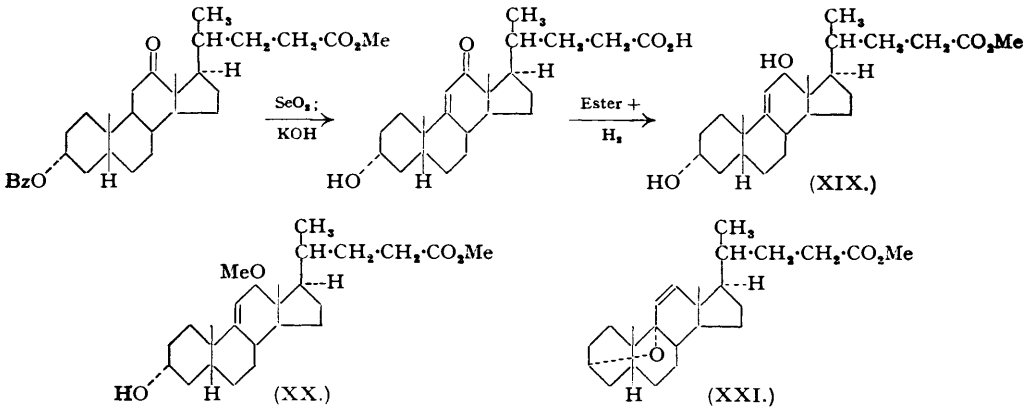
(R = CHMe·CH_2·CH_2·CO_2H; R′ = CHMe·CH_2·CH_2·CO_2Me.)

Cortisone from Deoxycholic Acid.—The compound which has been the centre of attraction as starting material, and in fact, bears the honour of being the starting material for the only existing method of manufacture, is deoxycholic acid (XIII), obtained from ox-bile or from the

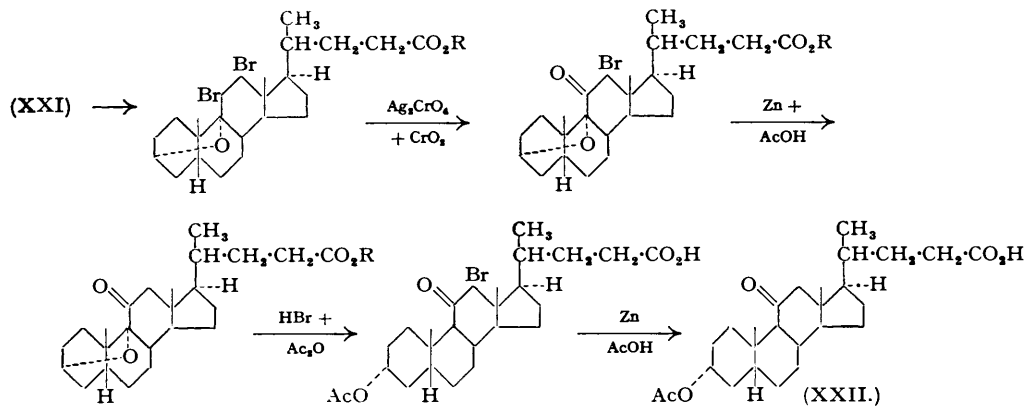
more abundant bile acid, cholic acid. The conversion of deoxycholic acid into cortisone involves the following four groups of reactions : (i) replacement of oxygen at $C_{(12)}$ by oxygen at $C_{(11)}$, (ii) degradation of the side chain to the pregnane type, (iii) development of the dihydroxyacetone grouping, and (iv) development of the αβ-unsaturated 3-ketone group.

(i) A method for the replacement of the 12-oxygen function of deoxycholic acid by an 11-oxygen function was first described by Reichstein and his collaborators. More recently alternative methods have been developed by Gallagher and by Kendall. Gallagher's method consists in the bromination of methyl 3-acetoxy-12-ketocholanate (XIV), which gives a mixture of epimeric 11-bromo-12-keto-esters (XV). Hydrolysis of the latter with alkali at room temperature gives a mixture of epimeric 3 : 11-dihydroxy-12-ketocholanic acids (XVI), whereas when alkaline hydrolysis is effected under more drastic conditions the Marker–Lawson acid (XVII) (3 : 12-dihydroxy-11-ketocholanic acid) is obtained. The reaction sequence is completed by protection of the 3-hydroxyl group by succinoylation followed successively by esterification, replacement of the 12-hydroxyl group with bromine, and reduction to give 3-hydroxy-11-ketocholanic acid (XVIII).

Kendall's method is dependent upon the high degree of reactivity of the allylic system in methyl 3 : 12-dihydroxy-$\Delta^{9(11)}$-cholenate (XIX), which is obtained from methyl 3-benzoyloxy-12-ketocholanate by the steps shown below :

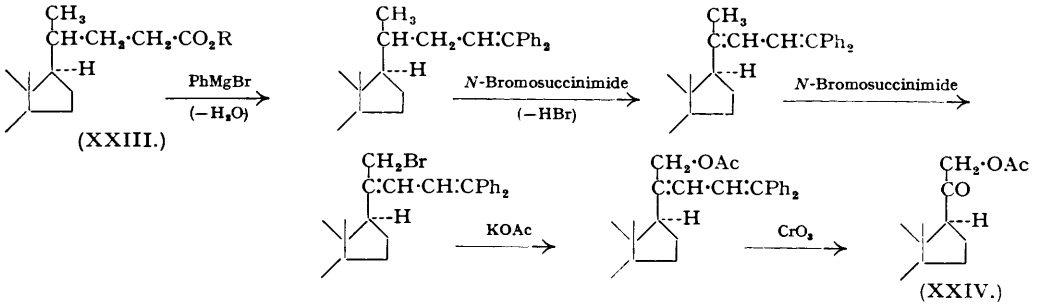


Treatment of the unsaturated diol (XIX) with methanol in the presence of acid gives the 12-methoxy-derivative (XX) which when treated successively with hydrochloric acid and sodium hydrogen carbonate yields the 3 : 9-epoxide (XXI) ; this series of reactions proceeds in uniformly high yield. The conversion of the 3 : 9-epoxy-ester into 3-acetoxy-11-ketocholanic acid (XXII) was effected as shown below :



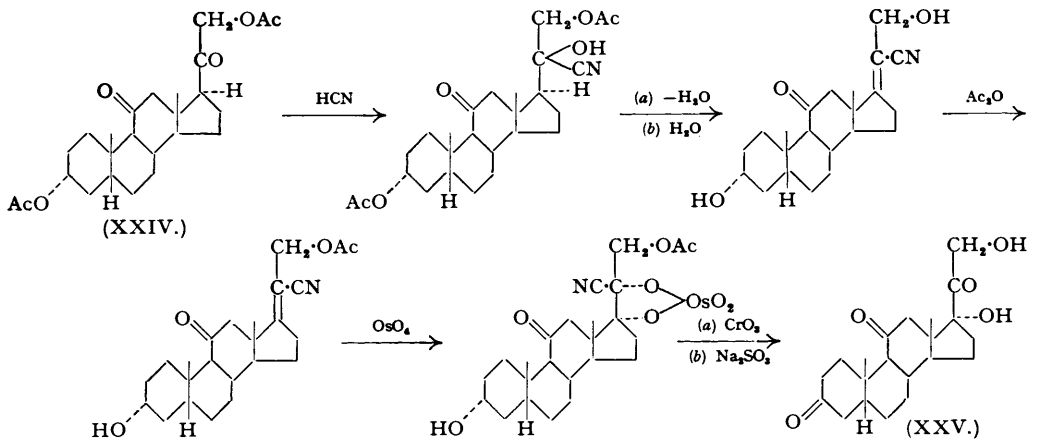
(ii) The degradation of the bile-acid side chain can be efficiently effected by the method of Miescher, Meystre, and Wettstein, the illustration of which (shown below) depicts the

conversion of a cholanic ester derivative (XXIII) into a 21-acetoxy-20-ketopregnane derivative (XXIV):



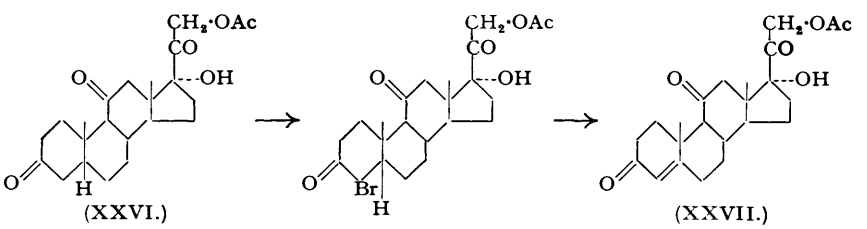
(XXIII.) (XXIV.)

(iii) The third series of changes involves the introduction of 17α-hydroxyl group for which Sarett's method was developed. In the illustration shown below, 3α : 21-diacetoxy-11 : 20-diketopregnane is converted into 17α : 21-dihydroxy-3 : 11 : 20-triketopregnane (XXV).



(XXIV.) (XXV.)

(iv) The final stages are concerned with the introduction of the αβ-unsaturated ketone grouping. Here Kendall's efficient method is employed; in this a 4-bromo-3-keto-steroid is treated with 2 : 4-dinitrophenylhydrazine, and the precipitated (labile) hydrazone is treated with excess of pyruvic acid. Starting from the acetate of 17α : 21-dihydroxy-3 : 11 : 20-triketopregnane (XXVI), the reaction sequence gives the acetate of cortisone (XXVII).

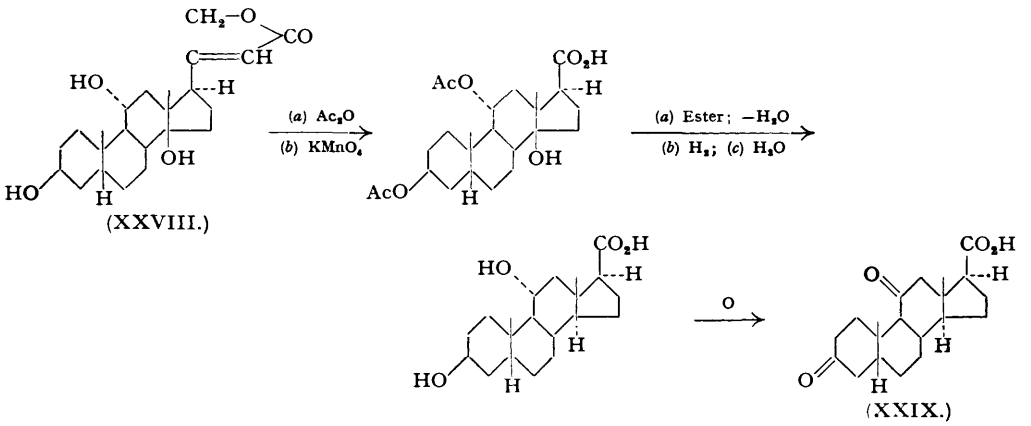


(XXVI.) (XXVII.)

Possible Routes to Cortisone.—The laborious nature of the route to cortisone from deoxycholic acid has naturally led to considerable effort being expended in the examination of other natural products as potential starting materials for a partial synthesis of the adrenal steroid; a suitable steroid derived from vegetable sources would for obvious reasons be a more attractive starting material than an animal steroid.

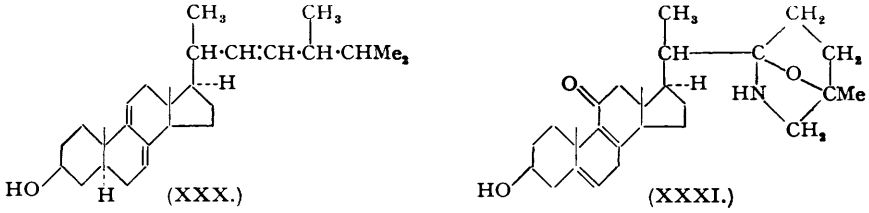
Among the cardiac-active glycosides sarmentocymarin is obtained in small yield from the seed of a *Strophanthus* species; the exact botanical identification of the species from which the glycoside was obtained by different workers is unfortunately obscure. Hydrolysis of the glycoside yields a sugar and an aglycone sarmentogenin (XXVIII), the structure of which was

established by Katz by degradation to the known 3 : 11-diketoætiocholanic acid (XXIX) by the steps shown below. Should sarmentocymarin become reasonably available, a relatively simple route to cortisone becomes a possibility.



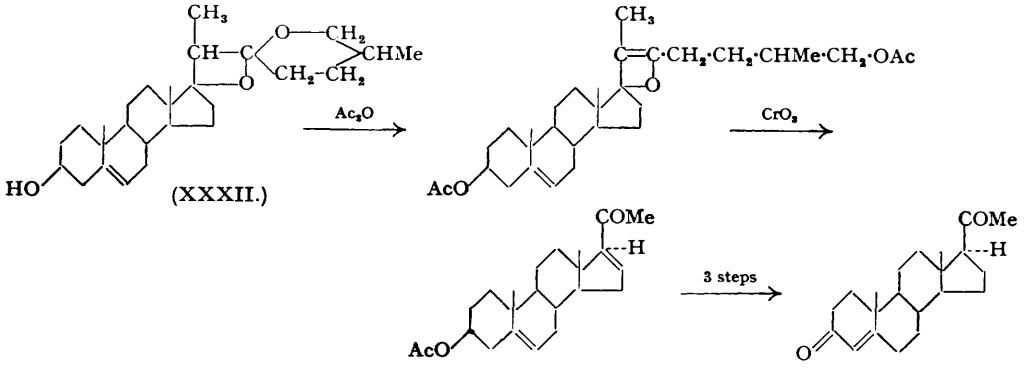
(XXVIII.) (XXIX.)

Among the sterols there are few which appear reasonable starting materials for the partial synthesis of cortisone. Ergosterol-D (XXX) appears to be worthy of investigation since it is reported to contain a $\Delta^{9(11)}$-ethylenic linkage which offers a means for the introduction of oxygen at $C_{(11)}$. Among the steroid alkaloids, jervine from *Veratrum viride* (green hellebore) is of considerable interest since a proposed structure (XXXI) for this alkaloid represents it as carrying an oxygen atom at $C_{(11)}$.



(XXX.) (XXXI.)

Probably the most attractive natural source for the part-synthesis of pregnane derivatives is the steroid sapogenins. The ease with which a sapogenin can be converted into a 20-keto-pregnane derivative (Marker) is illustrated by the conversion of diosgenin (XXXII), obtained from the roots of Mexican *dioscorea*, into progesterone :



(XXXII.)

A sapogenin of immediate interest for a possible partial synthesis of cortisone is botogenin, which, according to Marker, is a 12-ketodiosgenin, and the related hecogenin, which is probably a dihydro-12-ketodiosgenin.