The fork of two large blood vessels on the C.A. membrane is located, and a circular area in the crotch is marked on the shell with a lead pencil. This area is disinfected with 95% alcohol, and a small circle ground through the shell with a dental engine equipped with a fine corborundum disk. Care must be taken not to damage the shell membrane. The circle of shell is removed with fine-pointed forceps, leaving the shell membrane intact.

The eggs are then inoculated by injecting 0.05 ml of the washed bacterial suspension just under the shell membrane and onto the C.A. membrane by means of a tuberculin syringe equipped with a ½-in., 27-gauge needle. The exposed shell membrane is then covered with sterile, melted paraffin-vaseline mixture, and the eggs are returned to the 37° C incubator.

The inoculated eggs are examined daily by transillumination, and embryos which appear to be dead are removed from the shell and examined grossly. The typical appearance is marked engorgement of blood vessels, and hemorrhage in the embryo, C.A. membrane, and sometimes in the yolk membrane. The fluids are usually clear, but the amniotic fluid may be tinged with hemolyzed blood. Cloudy fluids or embryos showing evidence of decomposition indicate contamination.

Toxigenic strains of *Coryn. diphtheriae* kill the embryos with remarkable uniformity. Ninety-nine per cent of 424 embryos were dead in 96 hr under the conditions described here. On the other hand, the majority of the embryos inoculated with nontoxigenic strains survived; i.e., 84% of 84 embryos were alive at 96 hr. Survival to the date of hatching gave essentially the same results (0.24% survival for embryos inoculated with toxigenic strains, and 81% survival for those inoculated with nontoxigenic strains).

A total of 32 strains was tested by this method. Of these, 9 were known *Coryn. diphtheriae,* and 23 had been isolated from cases of clinical or suspected diphtheria. Toxigenicity, as determined in guinea pigs, corresponded exactly with the results in the embryos. From these results, it appears that death of 80% or more of the embryos in test lots of 10 or more for each culture may be regarded as a specific indication of toxigenicity.

The response of the 9–10 day chick embryos is sufficiently uniform that it could be used as a preliminary method of differentiating between toxigenic and nontoxigenic cultures suspected of being *Coryn. diphtheriae.* The potential usefulness of this method lies in the fact that it may be used in small laboratories or in those not having provisions for the care and maintenance of animals.

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A Reevaluation of Steroid Nomenclature

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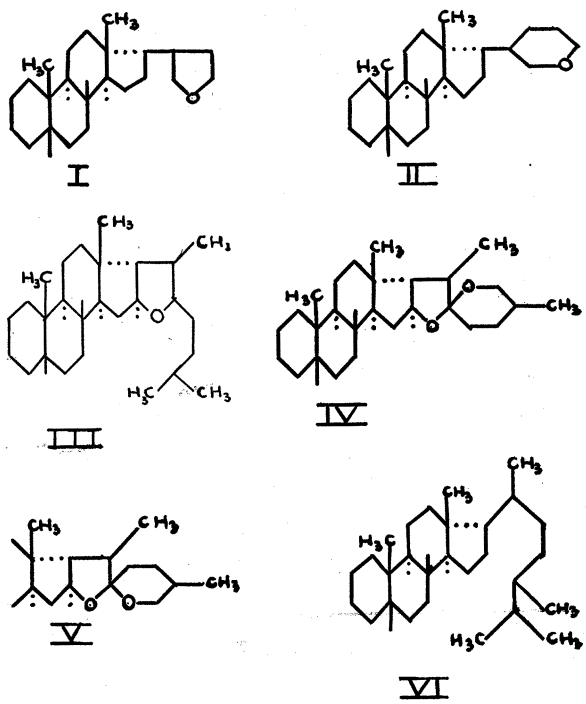
It must be obvious to those actively engaged in steroid studies that the various nomenclatures currently in use are by no means consistent and are not particularly facile in operation. The difficulties are noticeable when unnatural or unusual compounds are encountered, especially those differing in bridgehead configuration, those that have larger or smaller rings than usual, those that have one or more opened rings, and the genins, sapogenins, and their transformation products.

To attempt to bring some sort of order to this rather bewildering picture, a synthesis of the naming systems commonly used by authoritative workers has been made, which, with certain modifications, seems to lend itself to an adequate delineation of most compounds encountered in steroid chemistry.

As an initiation into the system, it is suggested that the parent substances commonly met with be named as follows: estrane,[1] androstane, and etiocholane; pregnane and 5-allopregnane; genan (I) and the 5-allo derivative, cholane and 5-allocholane; *E*-homogenan (II) and the 5-allo derivative, cholestane and coprostane; pseudosapogenan (III) and the 5-allo derivative, sapogenan (IV); 22-isosapogenan (V) and the 5-allo derivatives, ergostane and copro-

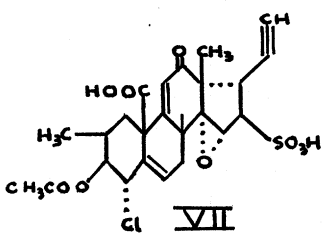


[1] Configuration undetermined at positions 5 and 10.

ergostane (VI); stigmastane; and coprostigmastane.

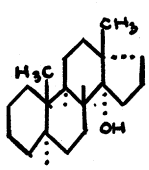
In naming substituted steroids, few modifications of current practice are suggested, but it is believed that the changes outlined will make for increased clarity. When double bonds are present, the symbol Δ should be used, followed by the usual cipher for locating the double bond. Only the lower figure necessary to fix the bond is employed, unless the bond culminates at a position that is not consecutively higher, in which case the two numbers necessary to fix the bond follow the Δ and are separated by a colon. In the event that a triple bond is part of the system, the symbol τ precedes the cipher. This new symbol should prevent confusion when double and triple bonds are present in the same compound. The usual "ene-yne" suffixes follow the name of the parent compound.

It is recommended[2] that the hydroxylic, epoxidic (ethereal, oxiranic), carbonylic, carboxylic, sulfonic, of other acidic functions shall follow, in that order, the name of the parent compound, each substituent being preceded by an appropriate number and the symbol α or β to indicate configuration in the conventional manner (*1*). Halogenic, aminoidal, alkyl, and aryl functions shall precede the name of the parent substance with appropriate number and symbol to indicate location and configuration, the substituent with the lowest number appearing first, followed by consecutively higher-numbered substituents. Thus, the systematic name for compound VII is 2β-methyl-4α-chloro-$\Delta^{5,9:11}$-τ^{20}-pregnadienyne-3β-ol-14:15-epoxide-12-one-19-oic-16β-sulfonic acid 3-acetate.[3]

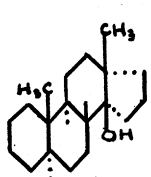


VII

If substituents are introduced at position 5, no further characterization other than the appropriate number is required, since the name of the parent compound is adequately descriptive. A substituent introduced at other bridgehead junctions requires no further characterization if the stereochemistry is normal. If, however, the substituent occupies a sterically "unnatural" position, the name of the parent compound is prefixed by a cipher locating the position, followed by the prefix "iso." Thus VIII is named androstane-14-ol, whereas IX is called 14-isoandrostane-14-ol (not androstane-14β-ol, or 14-alloandrostane-14β-ol).

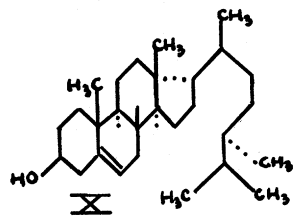


VIII



IX

Since it is known that stereoisomerism may occur in the side chain at positions 17, 20, and 24:28, the same rule may be applied as above. Campesterol (X) is named systematically as Δ^5-24-isoergostene-3β-ol (not Δ^5-24a-ergostene-3β-ol).

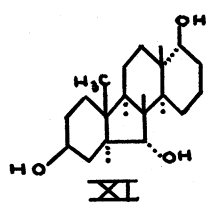


X

At nonbridgehead positions, or at positions adequately covered above, the usual α or β symbols may be used to denote configuration. The recommendation of Fieser (*1*) that hydroxylic substituents at position 20 be designated as "a" or "b" (rather than α or β) should be followed until spatial studies indicate actual configuration at that point. There seems to be little reason to deviate from the "iso" nomenclature in the case of carbon substituents in the side chain.

There is little justification for the use of "cis-trans" (referring to configuration at positions 3 and 17 with reference to positions 5 and 13) or "epi" designations. The former seems to be dropping from use, though the latter may have a certain degree of utility in the case of compounds of unknown or unproved structure, where "epi" may be used to indicate a configuration isomeric with that of the first-discovered material.

"Nor" and "homo" prefixes are used in the usual sense. If, as in XI, ring B has undergone contraction and ring D is expanded, the ring letter, followed by "nor" or "homo," is fully descriptive. In the event that a carbon atom in a side chain is missing, as, for example, in the 18-methyl group in XI, the numeral of the missing group, followed by "nor," is sufficient. Systematically, XI is named B,18-bisnor-D-homoandrostane-3β,6α,17aβ-triol[4] (not B,13-bisnor-D-homoandrostane-3β,6α,17aβ-triol).



XI

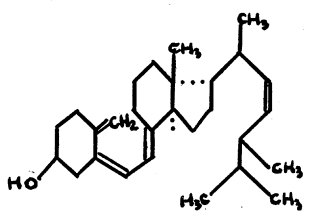
Nomenclature of steroids with open rings has long presented a problem, and most representatives of this class are saddled with trivial names.[4] It is recom-

[2] The suggestions in this paragraph violate certain of the Geneva rules, but there seems little reason to change the system used by many steroid workers.

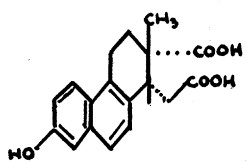
[3] May be named as 14:15-α-epoxide, but not required.

[4] It is realized that compounds of this class may be named according to the Geneva rules or those of the Ring Index (*2*). However, adherence to these rules may cause obscuration of the significant relationships to the parent steroids.

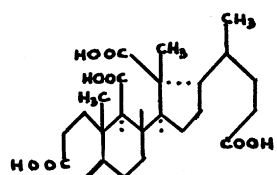
mended that Jaffe's (*3*) suggestion of using the prefix "seco" to indicate an open ring be adopted. "Seco" is to be preceded by the two numbers, separated by a colon, indicating the point of disjunction and is to antecede the name of the parent compound. Thus, calciferol (XII) is named 9 : 10-seco-$\Delta^{5,7,10:19,22}$-ergostatetraene-3β-ol; cis-bisdehydromarrianolic acid (XIII) is 16 : 17-seco-$\Delta^{1,3,5:10,6,8}$-14-isoestrapentaene-3-ol-16,17-dioic acid; and prosolannelic acid (XIV) is 3 : 5,11 : 12-diseco-4-norcholane-5-one-3,11,12,24-tetroic acid.



XII



XIII



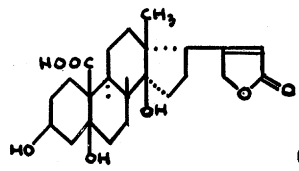
XIV

The current nomenclature in the realm of genins and sapogenins is most confusing. The suggestions outlined in the earlier part of this article should aid in bringing some sort of order to this area. Thus, strophanthidinic acid (XV) is to be called $\Delta^{20:22}$-14-isogenen-3β,5,14-triol-23-one-19-oic acid; anhydrodihydrostrophanthidin (XVI) is Δ^{14}-genen-3β,5-diol-19-al-23-one; isoperiplogenin (XVII) is 14-isogenan-3β,5-diol-14 : 21-epoxide-23-one;[5] episarsapogenin (XVIII) is sapogenan-3α-ol; and gitogenic acid (XIX) is 2 :3-seco-5-allo-22-isosapogenan-2,3-dioic acid.

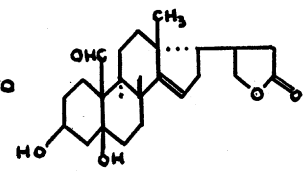
The pseudosapogenins, dihydrosapogenins and dihydrostrophanthidin (XVI) is Δ^{14}-genen-3β,5-diol-ilar ring structures, are named as pseudosapogenans.

The steroidal alkaloids represent an incompletely solved structural problem, with indications that a considerable number of these compounds are similar to solanidine. It is suggested (*4, 5*) that the parent compound be named solanidan (XX). For conformity with other parent compounds, it is indicated that

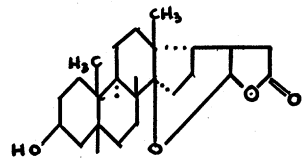
[5] May be named as 14 :21-β-epoxide, but not required.



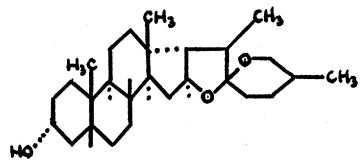
XV



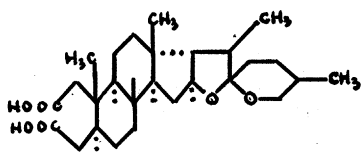
XVI



XVII

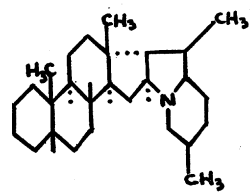


XVIII



XIX

the compound with a normal (coprostane) orientation at position 5 be called solanidan, whereas the compound similar to cholestane be named 5-allosolanidan.[6]



XX

It is obvious that the naming system proposed is cumbersome. It is one that is useful in most applications, however, and one that is reasonably consistent. It is believed that its utility outweighs its unwieldiness, and its routine application, therefore, is suggested.

[6] This contravenes Fieser (*5*), who has named XX as 5-iso-solanidan, and has reserved the name solanidan for the 5-allo compound.

References

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4. Prelog, V., and Szpilfogel, S. *Helv. Chim. Acta,* **27**, 390 (1944).
5. Fieser, L. F., and Fieser, M. *Op. cit.,* 646.