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Application of Electron Spin Resonance Spectroscopy to Problems of Structure and Conformation. VIII. Semidiones Derived from 9-Methyldecalones and 10-Methyl Steroidal Ketones. Assignment of Structure to A-Ring Steroidal Ketones[1]

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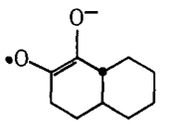
Radical anions (semidiones) are generated by exposure to air of basic solutions of 9-methyldecalones in dimethyl sulfoxide. This method readily produces semidiones from a variety of 10β- and 10α-methyl steroidal ketones having the carbonyl group in ring A, but fails when the carbonyl group is located in ring B or ring C. However, the α-bromo, and preferably the α-hydroxy, derivatives of B- and C-ring steroidal ketones give rise to semidiones in basic dimethyl sulfoxide solution, often in the absence of air. An analysis of the semidiones produced by electron spin resonance spectroscopy provides a convenient and rapid method for distinguishing between position and stereoisomers of steroidal ketones, and for determining the preferred conformations of 9-methyldecalones. Evidence is presented that published preparations of "lanosterone" yield mixtures of lanosterone and dihydroandrosterone.

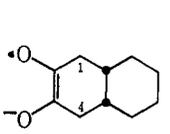
Treatment of a variety of aliphatic ketones (both open chain and cyclic) having α-methylene groups with potassium *t*-butoxide and oxygen in dimethyl sulfoxide (DMSO) has been shown to produce radical anions (semidiones).[2-9]

$$-COCH_2- + KOC(CH_3)_3 \xrightarrow[\text{trace of } O_2]{\text{DMSO}} .O>C=C<^{O^-}$$

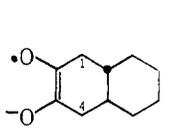
The electron spin resonance (esr) and hyperfine-splitting constants (hfsc) of the semidiones derived from monocyclic ketones can be used for conformational analyses of these systems.[2,9] When a ketone has two chemically different α-methylene groups, its oxidation in basic solution will generally produce two different semidiones. The proportions of the two semidiones as well as their hfsc will depend on the system being examined and can serve as a proof of structure for the ketone. For example, *cis*-2-decalone (1) can be easily differentiated from *trans*-2-decalone (2) from the fact that the former ketone yields a mixture of **3** and **4** in the ratio of 3:2, respectively, whereas the latter ketone yields a mixture of **3** and **5** in the ratio of 1:3, respectively.[3,9] In **5**, the α-carbon atoms



3
a^H = 13.17, 12.66, 7.09 gauss



4
a^H = 13.83, 12.72, 7.37, 6.55 gauss



5
a^H = 13.18, 13.18, 6.59, 6.59 gauss

(C-1 and C-4) are equivalent leading to hyperfine splitting by two pairs of magnetically equivalent α-hydrogen atoms (quasi-axial and quasi-equatorial hydrogens at C-1 and C-4). In **4**, C-1 and C-4 are conformationally nonequivalent and hyperfine splitting by four magnetically nonequivalent hydrogen atoms occurs.[9] Thus, the spectra of **4** and **5** immediately characterize the ring junction as being *cis* or *trans* without reference to model compounds. Moreover, 1-decalone can be differentiated unambiguously from either of the 2-decalones since oxidation of this ketone produces only a single semidione (**3**). This technique has been extended to steroids which contain the decalone system, that is, 19-nor- and 18-nor-D-homo steroids having the carbonyl function in the A and D rings, respectively.[9] Not only position isomers and *cis–trans* ring junctions but also the two possible *cis* forms (α,α and β,β) of the A/B or C/D ring junction can be distinguished. Moreover, changes in configuration at more distant centers, such as at C-9 in 19-nor-3-keto steroids, can also be detected. Thus, a combination of oxidation in basic solution and esr spectroscopy appears to be a promising tool for de-

(1) Reactions of Resonance Stabilized Anions. XXII. This work was supported by grants from the National Science Foundation and the National Institute of General Medical Sciences.

(2) G. A. Russell and E. T. Strom, *J. Am. Chem. Soc.*, **86**, 744 (1964).

(3) G. A. Russell and E. R. Talaty, *ibid.*, **86**, 5345 (1964).

(4) E. T. Strom, G. A. Russell, and R. D. Stephens, *J. Phys. Chem.*, **69**, 2131 (1965).

(5) G. A. Russell, R. D. Stephens, and E. R. Talaty, *Tetrahedron Letters*, 1139 (1965).

(6) G. A. Russell and E. R. Talaty, *Science*, **148**, 1217 (1965).

(7) G. A. Russell and K. Y. Chang, *J. Am. Chem. Soc.*, **87**, 4381 (1965).

(8) G. A. Russell, K. Y. Chang, and C. W. Jefford, *ibid.*, **87**, 4383 (1965).

(9) E. R. Talaty and G. A. Russell, *ibid.*, **87**, 4867 (1965).

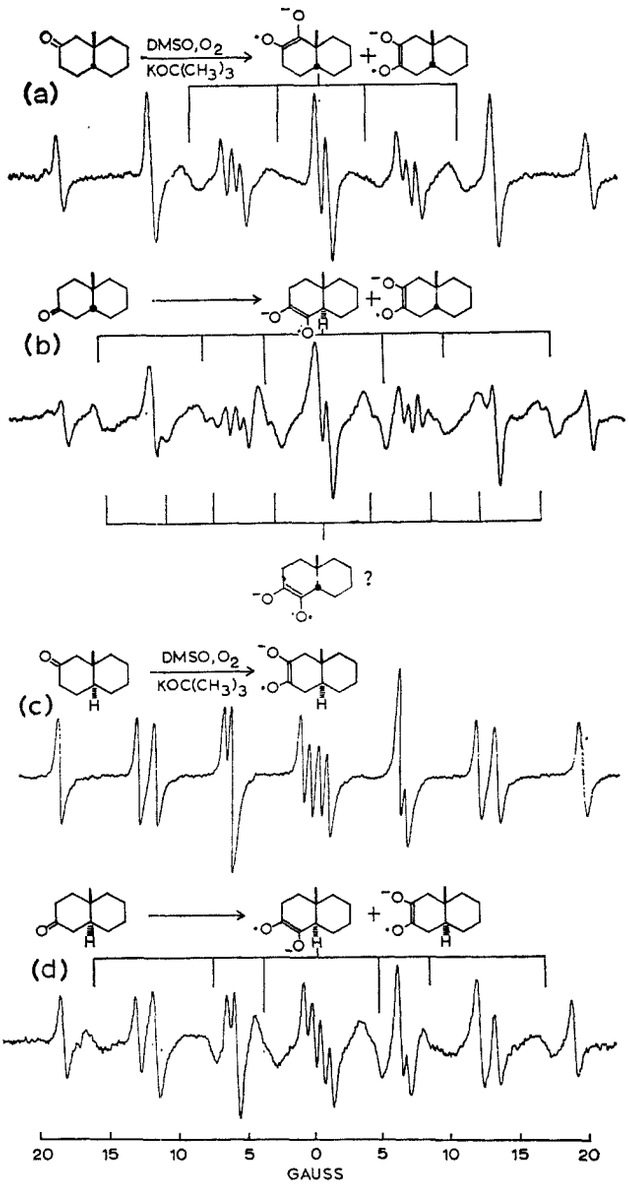


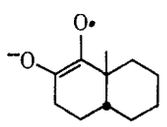
Figure 1.—First-derivative esr spectra of oxidation products of 9-methyldecalones in dimethyl sulfoxide at 25°: (a) *cis*-9-methyl-2-decalone; (b) *cis*-9-methyl-3-decalone; (c) *trans*-9-methyl-2-decalone; (d) *trans*-9-methyl-3-decalone.

termination of structure and conformation. We have now examined the applicability of this technique to a variety of trisubstituted cyclohexanones in the form of decalones and steroidal ketones having angular methyl groups.[10]

Results

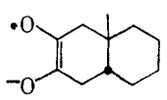
9-Methyldecalones.[11]—Oxidation of *cis*- or *trans*-1-keto- and 4-keto-9-methyldecalins in basic solution is expected to yield only a single semidione with hyperfine splitting by two and three α-hydrogen atoms, respectively. On the other hand, *cis*- or *trans*-2-keto- and 3-keto-9-methyldecalins are expected to give rise to two semidiones each, one of which will be identical with that obtained from the corresponding 1- and 4-keto derivatives, respectively. However, in contrast with the unsubstituted decalones, where *cis* and *trans* isomers of 2-decalone can be differentiated without reference to model compounds,[9] the presence of the 9-methyl substituent renders the α carbons (C-1 and C-4) nonequivalent in both the *cis*- and *trans*-2,3-semidiones. Hence, hyperfine splitting by four magnetically nonequivalent hydrogen atoms should occur in both cases, and a mere comparison of the hyperfine splitting patterns of the 2,3-semidiones will not allow an assignment of configuration to the ring juncture. Nevertheless, if there is a preferred position of enolization and oxidation for 2-keto- or 3-keto-9-methyldecalins, an empirical distinction between the *cis* and *trans* ketones can be made.

Treatment of *cis*-9-methyl-2-decalone (**7**) with potassium *t*-butoxide and oxygen in DMSO affords two semidiones, **8** and **9**, in an initial ratio of about 1:4, respectively (Figure 1a). The large and small hfsc are assigned to the quasi-axial and quasi-equatorial α-hydrogens, respectively.[2,9] The observation of a large difference between "axial" and "equatorial" hydrogen hfsc indicates a single populated conformation for **8** or **9**. Both of these semidiones could theoretically assume any one of two all-chair conformations: a "steroid" conformation (methyl group axial to the saturated ring) and a "nonsteroid" conformation (methyl group equatorial to the saturated ring).



8

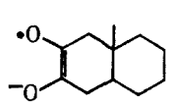
a^H = 12.95, 7.11 gauss



9

a^H = 13.11, 12.37, 6.83, 6.83 gauss

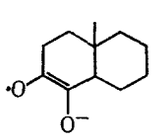
Oxidation of *trans*-9-methyl-2-decalone (**10**) yields only a single semidione (Figure 1c). The hyperfine-splitting pattern of 14 lines indicates that the radical anion is **11** and not the isomeric *trans*-1,2-semidione.



11

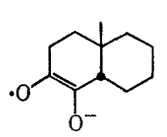
a^H = 13.05, 12.40, 7.11, 5.77 gauss

cis-9-Methyl-3-decalone (**12**) gives rise to a complex mixture of radical anions (Figure 1b). Along with the 12 lines expected for **9**, 14 more lines can be discerned. Six of these lines with relative intensities in the approximate ratio 1:1:2:2:1:1 are assigned to the 3,4-semidione (presumably *trans*, **13**), since the same semidione



13

a^H = 13.32, 12.40, 8.14 gauss

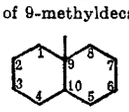


15

a^H = 19.75, 8.21, 4.33 gauss (?)

(10) Preliminary results of this work have been reported.[5,6]

(11) The numbering system of 9-methyldecalin is given in structure **6**.



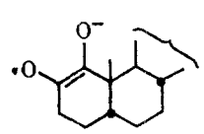
6

is also formed in the oxidation of *trans*-9-methyl-3-decalone (**14**). Hence, epimerization at C-10 can occur for the 3,4-semidiones, a phenomenon that was noted in the oxidation of **1** and **2**.[9] The origin of the remaining eight lines of approximately equal intensity in Figure 1b is not immediately obvious. One very likely semidione with three α-hydrogen atoms that can give an esr pattern of eight lines is **15**, the C-10 epimer of **13**. If the methyl group is axial to the unsaturated ring, one would expect hyperfine splitting by two "equatorial" hydrogens (at C-2 and C-10) and one "axial" hydrogen (at C-2) in **15**. The experimental hfsc for a pattern of eight lines are 4.33, 8.21, and 19.75 gauss. The absolute values of a^H, however, throw some doubt on this interpretation, particularly the abnormally large "axial" hfsc.[12] Moreover, **15** should also be present in the oxidation products of **14**. Although the same eight lines cannot be detected in the spectrum obtained from **14**, it is possible that the small percentage of attack at C-4 coupled with the large number of lines from the 2,3-semidione prevents the detection of **15**. The relative proportions of **9** and **13** formed initially in the oxidation of **12** are 3:1, respectively. On the assumption that the third semidione is **15**, the relative amounts of attack at C-2 and C-4 in **12** are 55 and 45 %, respectively.

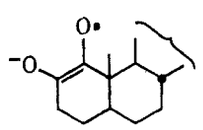
Oxidation of *trans*-9-methyl-3-decalone (**14**) gives a mixture of semidiones (Figure 1d) in which **11** predominates (93 % initially) over **13**. Semidione **13** decays much faster than **11** so that, upon standing for a few hours, only **11** can be detected in the oxidate from **14**.

From the hfsc of **13** it is obvious that two quasi-axial hydrogen atoms are present. Thus the conformation of **13** involves a quasi-axial hydrogen atom (relative to the unsaturated ring) at C-10 with the methyl group at C-9 occupying an axial position.

10β-Methyl Steroidal Ketones. A. 1-Ketones.—Oxidation of a typical *cis*-1-keto steroid, methyl 1-oxo-5β-etianate (**16**), yields immediately semidione **17**. Exposure of a basic solution of a *trans*-1-keto steroid, 5α-androstan-1-one (**18**), to air does not immediately yield an esr signal. However, after about an hour, a four-line spectrum consistent with **19** develops. The hfsc of **17** and **19** are not sufficiently



17
a^H = 13.18, 7.54 gauss
17a, 11-oxo



19
a^H = 12.29, 7.19 gauss

different to allow a distinction to be made between a *cis*- and a *trans*-1-ketone. However, the rates of oxidation are quite distinctive. 5α-Cholestan-1-one (**18a**) behaved similarly to **18**.

B. 2-Ketones.—2-Oxo-5β-spirostan (**20a**) gives rise to a mixture of two semidiones having partial structures **17** and **21**, of which the former semidione decays quite rapidly. An initial ratio of **17**:**21** of about

(12) The hfsc are more consistent with a distorted boat structure for **15** with the values of 4.33 and 19.75 gauss for the hydrogens at C-2 and 8.21 gauss for the distorted quasi-equatorial hydrogen at C-10.

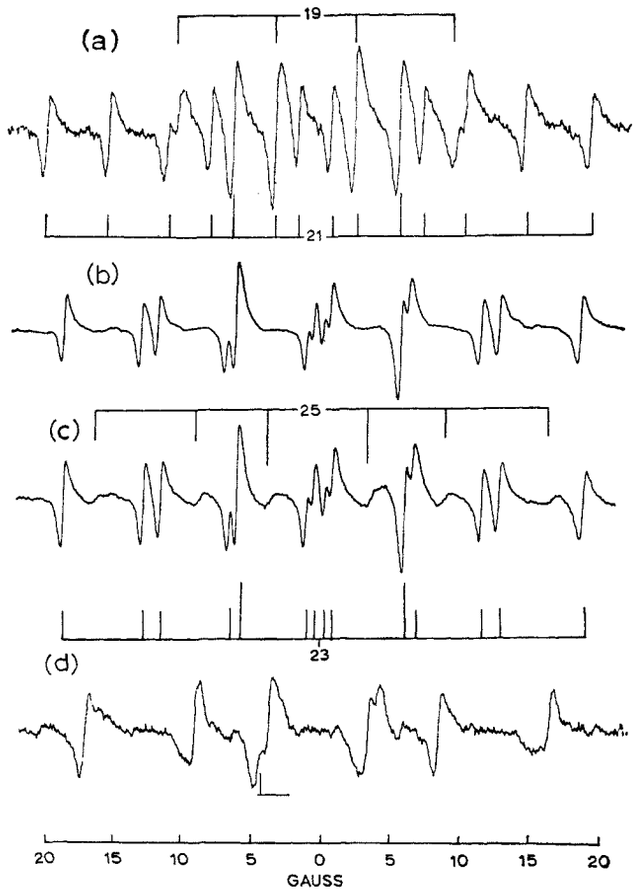
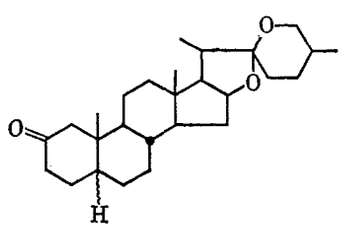
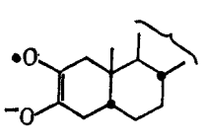


Figure 2.—First-derivative esr spectra of oxidation products of 10β-methyl 2- and 3-keto steroids in dimethyl sulfoxide at 25°: (a) 5β-spirostan-2-one; (b) 5α-androstan-17β-ol-2-one; (c) 5α-androstan-17β-ol-3-one; (d) 5β-androstan-17β-ol-3-one.

60:40 is found. The spectrum of **21** consists of 14 lines, two of which overlap two of the four lines of **17** (Figure 2a).

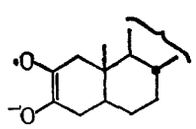


20a, 5β
b, 5β, 11-oxo
c, 5α, 12-oxo



21
a^H = 14.00, 12.19, 9.08, 4.79 gauss
21a, 5β-methyl
b, 4,4-dimethyl
c, 11-oxo

5α-Androstan-17β-ol-2-one (**22a**) and 17α-methyl-5α-androstan-17β-ol-2-one (**22b**) yield only a single semidione (partial structure **23**) exhibiting hfs by



23
a^H = 13.00, 12.37, 7.16, 5.81 gauss
23a, 12-oxo
b, 11-oxo
c, 9α-fluoro-11β-hydroxy
d, 7-oxo
e, 4α-ethyl
f, 4,4-dimethyl

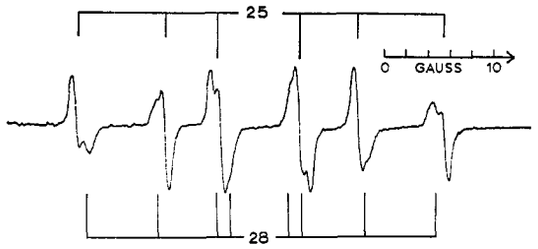
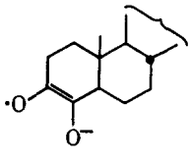


Figure 3.—First-derivative esr spectrum of mixture of semidiones formed by disproportionation of the 3,4-ketol of 17β-acetoxyandrostane in dimethyl sulfoxide in presence of potassium *t*-butoxide at 25°.

four nonequivalent hydrogen atoms (Figure 2b). The similarity of *trans*-2-keto steroids and *trans*-9-methyl-2-decalone, both in regard to the point of oxygenation and the esr spectrum of the 2,3-semidiones, is in agreement with the accepted conclusion that *trans*-9-methyl-2-decalone has a steroid-like conformation.

C. 3-Ketones.—The oxidation of 5α-androstan-3-one (**24a**), 5α-androstan-17β-ol-3-one (**24b**), 5α-cholestan-3-one (**24c**), and 5α-pregnane-3,20-dione (**24d**) gives an identical mixture of two radical anions (Figure 2c). The major radical anion is present to the extent of 92% of the total and is identical with the semidione **23** obtained by oxidation of a *trans*-2-keto steroid. Under low resolution the spectrum of the minor radical is an approximately 1:1:2:2:1:1 sextet. Under higher resolution eight lines of unit intensity are found. The minor radical is assigned partial structure **25** since the same radical anion is the major oxidation product of 5α-4-keto steroids. The similarity of the oxidation



25

a^H = 13.23, 12.43, 8.07 gauss
25a, 2,2-dimethyl
b, 11-oxo
c, 9-fluoro-11β-hydroxy
d, 7-oxo

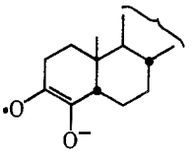
products from *trans*-3-keto steroids and *trans*-9-methyl-3-decalone supports the supposition that the preferred conformation of the decalone is steroid-like.

In the oxidation of 5α-spirostan-3-one (**24e**) the relative proportions of the 2,3- and 3,4-semidiones are initially the same as for **24a–d**, but the 2,3-semidione decays more rapidly than the 3,4-semidione. For the other 5α-3-keto steroids examined the 3,4-semidione decays more rapidly than the 2,3-semidione.

Oxidation of 5β-androstan-17β-ol-3-one (**26a**), 5β-pregnane-3,20-dione (**26b**), 5β-cholestan-3-one (**26c**), 5β-pregan-3-one (**26d**), and 5β-spirostan-3-one (**26e**) gives a mixture of radical anions having the spectrum shown in Figure 2d. The major radical anion is formed by attack at C-4 and is identical with **25**. A careful analysis of the spectrum shows the presence of approximately 20% of a minor radical anion corresponding to attack at C-2, *i.e.*, partial structure **21**. The presence of **21** makes it difficult to cleanly resolve the eight lines of **25** and the spectrum of **25** is usually found to be a 1:1:2:2:1:1 sextet (see Figure 2, ref 3). However, in the presence of a large excess of base, **21** is apparently preferentially destroyed to give a well-resolved eight-line spectrum. Thus, *cis*-3-keto steroids give quite a different oxidate than *cis*-9-methyl-3-decalone (slight preference for attack at C-2).

Oxidation of 5α-androstane-3,17-dione (**24f**) and 5β-androstane-3,17-dione (**26f**) with limited amounts of oxygen forms almost exclusively the radical anions derived from the A-ring diones. Competitive experiments involving 5α- and 5β-3-keto steroids (**24a** and **26d**) indicate that the 5β-3-keto steroid is oxidized about six times as readily as the 5α isomer.

D. 4-Ketones.—The esr spectra of the oxidates of 5α-androstan-17β-ol-4-one (**27a**), 5α-cholestan-4-one (**27b**), and 5β-cholestan-4-one (**27c**) are very similar to the spectrum obtained by the oxidation of a 5β-3-ketone (Figure 2d). The major semidione is identified as **25**. However, a more detailed perusal of the spectra indicates that, although the major semidiones are indeed identical, some of the lines of the minor radical in Figure 2d (positions identified with reference to Figure 2a) are consistently missing in the spectra from the 4-ketones. In particular, the two wing and the two center peaks of semidione **21** are missing in the oxidate of a 4-keto steroid. However, there is definitely a minor radical anion present in the oxidates of the 4-keto steroids which may well be the C-5 epimer of **25**, *i.e.*, **28**.

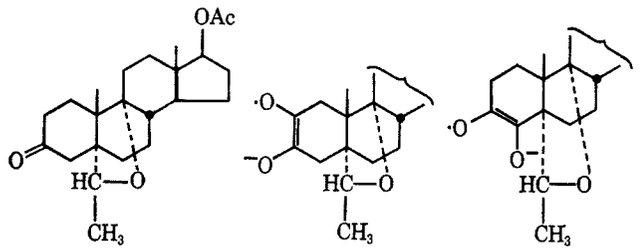


28

a^H = 13.2, 12.0, 6.6 gauss
28a, 5β-methyl

To settle the question of the existence of epimeric (5α and 5β) structures for the 3,4-semidione, we prepared the 3,4-diketone (which exists as the Δ^4-enol) in the 17β-hydroxyandrostane series. Surprisingly, this diketone does not spontaneously disproportionate in DMSO in the presence of potassium *t*-butoxide,[9] nor is it reduced to the semidione by propiophenone enolate anion. Treatment of 4-bromo 3-ketones in the 5β-pregnane or 17β-acetoxy-5β-androstane series with potassium *t*-butoxide in DMSO gives, at best, a very low concentration of the desired semidione. We prepared the α-ketol(s) from the 3,4-epoxide of 17β-acetoxy-5α-androstane by reaction with boron trifluoride in DMSO solution (see the Experimental Section). The α-ketol(s) gives a strong signal from disproportionation in DMSO in the presence of potassium *t*-butoxide. Two radical anions are present as shown in Figure 3. We assign the major 1:1:2:2:1:1 sextet to the 5α-3,4-semidione (**25**) and the minor octet of unit intensity to the 5β-3,4-semidione (**28**). With the spectrum of Figure 3 in hand, the lines of **28** can be identified in the spectra of the oxidation products of the 3- and 4-ketones (for example, in Figure 2d). The ratio of 5α- to 5β-3,4-semidiones is about 6:1. The steroid conformation requires a quasi-axial hydrogen atom at C-5 with respect to ring A in both of these semidiones, since the methyl group at C-10 will

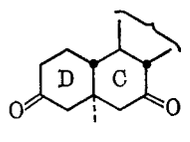
be quasi-axial and quasi-equatorial to ring A in **25** and **28**, respectively. Hence, the observation of two large hfsc and one small hfsc (assigned to quasi-axial hydrogens at C-2 and C-5, and quasi-equatorial hydrogen at C-2, respectively) in both **25** and **28** supports our contention that these two semidiones involve *cis–trans* isomerization about the A/B ring juncture. The above assignment of hfsc is in accord with the observation that oxidation of 5β-methylcholestan-3-one (**26g**) gives a mixture of two radical anions of which the major one (85%) is the 3,4-semidione (**28a**) having a^H = 12.40 and 7.22 gauss. Also, 2,2-dimethyl-5α-androstan-17β-ol-3-one (**24g**) yields a single semidione (**25a**), as far as can be ascertained, with a 12.96-gauss doublet splitting by the axial hydrogen at C-5. Further support for this assignment is provided by examination of the 5α-substituted ketone **29** which yields 10% of **31** in which a^H = 12.58 and 6.92 gauss.



29 **30** **31**

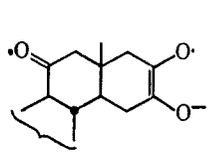
The great similarity in the hfsc of **25** and **28** suggests that differences in the hfsc of C-5 epimeric 3,4-semidiones may not always be resolved, and even under conditions of high resolution the presence of another radical anion, such as a 2,3-semidione, may very well prevent the detection of the minor C-5 epimeric semidione.

E. D-Homo Analogs.—A comparison of the behavior of D-homo steroidal ketones having the carbonyl group in ring D with that of the A-ring ketones is interesting since both classes of steroids are derived from 9-methyldecalones, although their partial structures are not enantiomeric. In fact, the D-homo ketones are enantiomeric in this sense with 5-methyl-19-nor ketones having the carbonyl group in ring A. 3α-Acetoxy-D-homo-5β-androstane-11,17-dione (**32**), an enantiomer of 5α-methyl-19-nor-3,7-dioxo steroids (**32E**) in its partial structure, yields initially a single

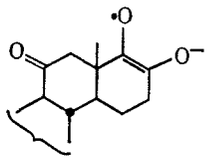


32E

semidione (**33**) with hfsc similar to **23**. A second semidione with hfs by two hydrogen atoms, presumably the 17,17a-semidione **34** (hfsc similar to **19**), is also



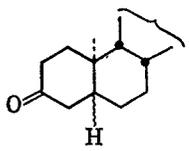
33
a^H = 12.94, 11.96
7.27, 5.51 gauss



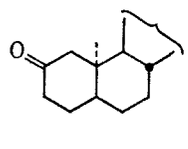
34
a^H = 12.80, 6.79
gauss

observed when the solution is allowed to stand for 1 hr after the admission of more oxygen. Hence, oxidative attack at C-17a must be considerably slower than at C-16, as was found to be the case for 5α-2-keto steroids.

10α-Methyl Steroidal Ketones.—We have examined the oxidation in basic solution of 5β-lumistanone (lumistanone A, **35**), 5α-lumistanone (lumistanone B, **36**), 17β-acetoxy-5α,10α-androstan-2-one (**37**), and 3α,17β-diacetoxy-5α,10α-androstan-2-one (**37a**).

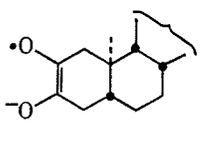


35, 5β
36, 5α

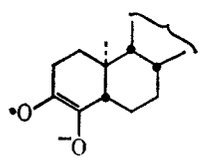


37
37a, 3α-acetoxy

Oxidation of **35** yields a mixture of **38** and **39** in an initial ratio of 88:12, respectively. There are small



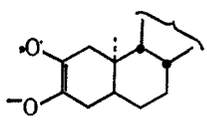
38
a^H = 12.47, 11.89
6.99, 5.58 gauss



39
a^H = 12.73, 12.73,
7.68 gauss

differences in the hfsc of **38** and the corresponding 2,3-semidione obtained in the oxidation of 5α-androstan-3-ones (**23**). Actually partial structure **35** can be considered to be a C-8 epimer of the enantiomer of the partial structure for a 5α-androstan-3-one. The results indicate that epimerization at C-8 does not significantly affect the point of oxidation or the hfsc (*i.e.*, the conformation) of the resulting 2,3-semidione for a 10β-methyl-5α-3-keto steroid.

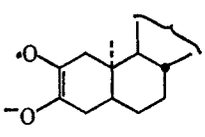
Oxidation of **36** produces about 80% of **39** and 20% of a minor radical anion presumed to be **40**. Partial



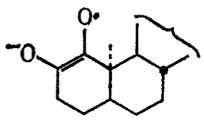
40

structure **36** is the C-8 epimer of the partial structure for the enantiomer of 5β-androstan-3-one. Again, epimerization at C-8 does not significantly affect the preferred point of oxidation.

Compound **37a** affords a single semidione upon treatment with potassium *t*-butoxide in DMSO. This semidione (**41**) can be considered to be the C-9 epimer of **40** and the C-8,9 epimer of the enantiomer



41
a^H = 12.90, 12.17,
6.86, 6.86 gauss



42
a^H = 12.80, 7.58
gauss

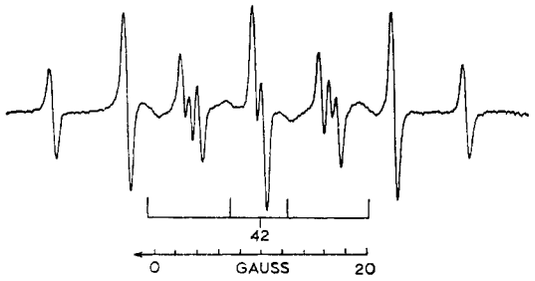
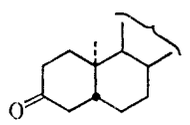


Figure 4.—First-derivative esr spectrum of mixture of semidiones formed by oxidation of 17β-acetoxy-10α,5α-androstan-2-one in dimethyl sulfoxide at 25°.

of **21**. The 12-line spectrum of **41** resembles very closely that of *cis*-9-methyldecalin-2,3-semidione (**9**). The spectrum of **41** is considerably different from the spectrum of **21** or the partially resolved spectrum of **40**. The similarity of **41** and **9** suggests that **9** exists in a "nonsteroid" conformation with the methyl group axial to the unsaturated ring.

Oxidation of **37** gives a mixture of **41** and **42** in which **41** is present to the extent of about 97.5 % (Figure 4). Hence, there is a sharp contrast between **37** and the corresponding 5β,10β-2-keto steroid which gives predominant oxidation at C-1. The preferred attack at C-3 in **37** and also in *cis*-9-methyl-2-decalone is consistent with the decalone possessing a predominant "nonsteroid" conformation (the methyl group axial to the cyclohexanone ring). These results also indicate that the position of oxygenation of A-ring ketones and the hfsc (conformations) of the resulting semidiones can vary considerably when epimerization at C-9 occurs.

We have also examined 5β,8α,9α,10α-ergost-22-en-3-one (**43**), which is a C-9 epimer of the enantiomer of



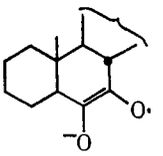
43

5α-androstan-3-one (**24a**) and a C-8,9 epimer of **35** as far as partial structures are concerned. Oxidation of **43** gives a mixture of the 2,3- and 3,4-semidiones in an initial ratio of about 60:40, respectively. The hfsc of the two semidiones (12.65, 12.65, 7.49, 5.04 and 12.67, 12.67, 7.55 gauss, respectively) are not very much different from those of the corresponding semidiones from **24a** or **35**. However, the large proportion of attack at C-4 in **43** is in marked contrast to the behavior of **24a** or **35** under similar conditions. The low solubility of **43** in DMSO prevents the resolution of any differences in the quasi-axial hfsc of the derived semidiones. In order to offset this difficulty, oxidation of **43** in the presence of a large excess of potassium *t*-butoxide (0.3 *M*) was attempted, but this time only the 3,4-semidione was observed initially (again as a 1:1:2:2:1:1 sextet), presumably by preferential destruction of the 2,3-semidione. Even at the usual base concentration of 0.1 *M*, the 2,3-semidione decays more rapidly than the 3,4-semidione. This preferential destruction can be detected during a 30-min period. This situation is exactly the reverse of that encountered with the C-9 epimer of **43**, *e.g.*, **24a** (when considered as its mirror image), wherein the 3,4-semidione preferentially decays as a function of time.

A-Ring Ketones with Substituents in Rings A–C.—From the foregoing results it is obvious that substituents located in ring D, or at more remote positions, have no effect on the behavior of A-ring ketones upon oxidation in basic solution. However, for substituents closer to the site of the reaction one may expect some changes in the point of oxygenation and the hfsc of the resulting semidiones. The results of examination of such substituted ketones are summarized in Table I.

The only appreciable effect of a substituent upon the point of oxidation occurs with the 11-oxo substituent which decreases the oxidation at C-1 for a 5β-2-keto steroid and increases the amount of oxygenation at C-4 for a 5α-3-keto steroid. The 11-oxo substituent has little effect upon the oxidation of 5β-3-keto steroids.

B-Ring Semidiones.—Treatment of 6- or 7-keto steroids with oxygen in basic DMSO solutions fails to produce paramagnetic products. 6α-Bromo-5α-cholestan-7-one (**46a**) furnishes an oxidate whose esr spectrum consists initially of four prominent lines of unit intensity (a^H = 13.59 and 11.08 gauss), superimposed on an eight-line spectrum consistent with a^H = 4.91, 8.71, and 11.08 gauss or alternately on a second four-line spectrum, a^H = 10.70 and 3.80 gauss. The initial four-line pattern decays rapidly to leave only eight lines of approximately equal intensity. The four-line spectrum is consistent with the 6,7-semidione (**47**) formed by oxidation of the 6-hydroxy 7-ketone.



47
a^H = 13.59, 11.08 gauss

The second four-line pattern could involve epimerization of **47** at C-8 or C-5 to give a new radical anion (**48**).

3β-Acetoxy-7α-bromo-5α-cholestan-6-one (**46b**) gives a complex esr spectrum composed of 14 lines and probably due to a mixture of radical anions. It appears that the eight-line spectrum tentatively assigned to a mixture of **47** and **48** is present in the 14-line multiplet. Further experiments with substituents at C-5 and C-8 are required to clarify the nature of the radicals.

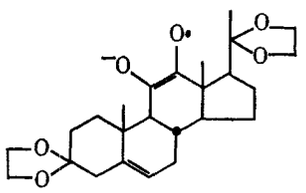
C-Ring Semidiones.—Oxidation of 11- or 12-keto steroids in basic DMSO solution does not yield a paramagnetic intermediate. Treatment of the 11-bromo-12-keto steroid, 11,23-dibromohecogenin acetate (**49**), with potassium *t*-butoxide in DMSO does not generate an appreciable quantity of a semidione within 2 hr. Exposure of the solution to oxygen yields a weak doublet absorption, a^H = 12.7 gauss. That the doublet is due to the 11,12-semidione is confirmed by the oxidation of 3,20-bisethylenedioxy-12β-hydroxypregn-5-en-11-one (**50a**) and 3,20-bisethylenedioxy-11α-hydroxypregn-5-en-12-one (**50b**) both of which furnish a high concentration of a semidione (**51**) with a major doublet splitting, a^H = 12.60 gauss, due to the quasi-

TABLE I

OXYGENATION PRODUCTS OF SUBSTITUTED STEROIDAL KETONES

Carbonyl position	Configuration at C-5	Compound	Semidiones (%); hfsc in gauss	Comments
C-2	β	2,11-Dioxo-5β-spirostan (**20b**)	**21c** (>95); 13.98, 12.70, 8.99, 5.31 **17a** (<5); 14.00, 6.34	**21c** decays rapidly
C-2	α	2,12-Dioxo-5α-spirostan (**22c**)	**23a** (100); 12.77, 12.39, 7.06, 5.76	10 resolved peaks with shoulders on 4th and 5th peaks from ends
C-3	α	3,11-Dioxo-5α-spirostan (**24h**)	**23b** (74); 12.48, 12.48, 7.19, 5.88 **25b** (26); 12.56, 12.56, 7.36	**23b** has 10 resolved peaks with shoulders on 4th peak from ends, **25b** decays first
C-3	α	Bismethylenedioxy derivative of 9α-fluoro-11β,17α,21-trihydroxy-5α-pregnane-3,20-dione (**24i**)	**23c** (91); 12.96, 12.96, 7.60, 1.76 **25c** (9)	**23c** intermediate in stability between **23** and **23b**
C-3	α	5α-Cholestane-3,7-dione (**24j**)	**23d** (89); 12.37, 11.89, 6.89, 5.64 **25d** (11); 12.82, 11.77, 7.94	
C-3	α	4α-Ethyl-5α-cholestan-3-one (**24k**)	**23e** (100); 12.97, 11.27, 5.17	Further oxidation yields a semidione with a^H = 11.58, 5.75 gauss
C-3	α	2,2-Dimethyl-5α-androstan-17β-ol-3-one (**24g**)	**25a** (100); 12.96	Oxygenation occurs slowly
C-3	α	4,4-Dimethyl-5α-cholestan-3-one (**24l**)	**23f** (100); 13.16, 5.10	
C-3	α	29	**30** (90); 12.56, 12.56, 7.93, 5.88 **31** (10); 12.58, 6.92	
C-3	α	Lanosterone	2,3-Semidione (100); 12.77, 4.80	
C-3	...	4,4-Dimethylcholest-5-en-3-one (**44**)	2,3-Semidione (100); 12.89, 3.71	
C-3	β	5β-Methylcholestan-3-one (**26g**)	**28a** (85); 12.40, 7.22 **21a** (15); 13.35, 12.32, 7.44, 5.56	**28a** more stable than **25**
C-3	β	4,4-Dimethyl-5β-cholestan-3-one (**26h**)	**21b** (100); 12.26, 5.31	Esr signal developed 2 hr after oxygenation
C-3	β	17β-Acetoxy-5β-androstane-3,11-dione (**26i**)	**25b** (80) **21c** (20)	
C-3	β	5β-Spirostan-3,11-dione (**26j**)	**25b** (80) **21c** (20)	
C-3	5β,10α	Stachen-3-one (**45**)[a]	2,3-Semidione (100); 12.75, 4.85	

[a] W. H. Baarschers, D. H. S. Horn, and L. F. Johnson, *J. Chem. Soc.*, 4046 (1962).



51

$a^{H\alpha}$ = 12.60 gauss

axial hydrogen atom at C-9. The spectrum is shown in Figure 5. Each line of the doublet is further split into quintets, average separation 0.89 gauss. The relative intensities of these five lines are 1:3.3:3.5:3.4:1 and may be the result of a doublet of 1:3:3:1 quartets. Probably, hfs by the methyl group attached to C-10 or C-13 is being observed. 3-Ethylenedioxy-12β-hydroxypregn-5-ene-11,20-dione (**50c**) yields a semidione with a 12.46-gauss doublet splitting, and a 1:3:3:1 quartet splitting, $a^H \cong$ 0.8–1.0 gauss. Possibly, for the 3,20-bisethylenedioxy compound hfs by the hydrogen at C-17 is observed, but in the 20-ketone this hydrogen atom is enolized or ionized.

A-Homo and B-Homo Steroidal Ketones.—A-Nor-B-homo-4α-cholestan-5-one (**52a**) and its 4β isomer (**52b**) readily yield semidione **53** upon oxidation in basic solution. The hfs by only two hydrogen atoms is consistent with a derivative of cycloheptane semidione for in the parent system hfs by "equatorial" hydrogen atoms is very small.[2,13] Thus the hfs in **53** is due to "axial" hydrogen atoms at C-4 and C-7. This analysis leads to a *trans*-A/B ring juncture for **53**.

(13) In cycloheptane-1,2-semidione hfs by "axial" hydrogen atoms, 6.70 gauss, and "equatorial" hydrogen atoms, 1.97 gauss, is observed.[2]

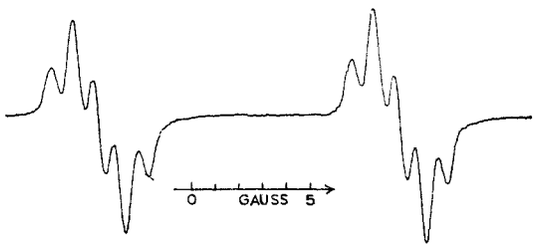
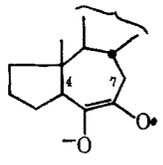


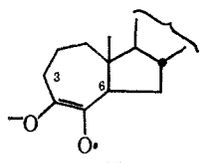
Figure 5.—First-derivative esr spectrum of semidione formed by oxidation of 3,20-bisethylenedioxy-11α-hydroxypregn-5-en-12-one in dimethyl sulfoxide at 25°.

Oxidation of A-homo-B-nor-6α-cholestan-5-one (**54a**) and its 6β isomer (**54b**) gives identical spectra. Initially a weak four-line pattern (a^H = 6.42 and 8.35 gauss) is observed which gradually gives rise to a doublet (a^H = 10.16 gauss) spectrum. Possibly the initial spectrum is due to **55** with hfs by the "axial" hydrogen atoms at C-3 and C-6. The doublet splitting may involve a further oxidized product, for example, the 6-hydroxy derivative of **55**.



53

a^H = 6.76, 6.76 gauss



55

a^H = 8.35, 6.42 gauss (?)

Overoxidation Products of Steroidal Ketones.—Throughout this work considerable attention has been paid to obtaining the esr spectrum of the first detect-

able paramagnetic oxidation product. We recognize that in many cases the first observable oxygenated product can be converted to other radical anions by exposure to additional oxygen. For example, upon further oxygenation of **36** the 1:1:2:2:1:1 sextet attributed to **39** is gradually replaced by a 1:1:1:1 quartet, a^H = 12.78 and 5.00 gauss, over a period of 3 hr. This new radical anion may well have an oxygen function at C-5, for example, a hydroxy or hydroperoxy group.[14] A similar change in spectrum is also observed in the oxidation of 5β-3-ketoandrostanes. This behavior is most pronounced when the initial major semidione has a methine hydrogen atom α to the dicarbonyl system. Thus, 2α-methyl-5α-androstan-17β-ol-3-one (**56**) is readily overoxidized to give a doublet (12.21 gauss) spectrum.

Discussion

The positional isomers of an A-ring steroidal ketone with a 10β-methyl group can be readily distinguished by observation of the semidione or mixture of semidiones formed by oxygenation in basic DMSO solution. Moreover, for the 2- and 3-ketones the *cis* or *trans* nature of the A/B ring juncture can be specified by a comparison with the oxygenation products of reference compounds.

The semidiones observed in the oxidation of a ketone having different α-methylene groups, or a diketone such as androstane-3,17-dione, presumably reflect the relative rates and degrees of ionization of the α-hydrogen atoms. Oxygenation of the respective enolate anions will give rise to α-diketones,[14b,15-17] which can be reduced by enolate anions to yield the semidiones.

$$RC(O^-)=CHR' \longrightarrow RCOCHR'OO^- \rightleftharpoons RCOCHR'OOH$$

$$RCOCHR'OOH + OH^- \longrightarrow RCOCOR' + H_2O + OH^-$$

$$RCOCOR' + RC(O^-)=CHR' \longrightarrow RC(O\cdot)=C(O^-)\,R' + RCOCHR'\cdot$$

Radicals such as RCOCHR'$\cdot$ are too reactive to be detected by ordinary esr techniques. In view of this analysis of the oxidation mechanism it is apparent that the observed semidiones can also reflect the ease of reduction of the intermediate α-diketones involved. This discrimination may well be involved in the observation that 5α- or 5β-androstane-3,17-diones yield mainly A-ring semidiones. This conclusion is supported by the finding that the addition of cyclohexane-1,2-dione to a solution of cyclopentane-1,2-semidione in DMSO (generated spontaneously from 2-acetoxycyclopentanone) leads to the preferential formation of cyclohexane-1,2-semidione. The ratio of cyclohexane:cyclopentane semidiones is 84:16 2 min after the addition of the cyclohexane-1,2-dione, while after 20 min the cyclohexane semidione is almost the exclusive radical anion present (>95%) (initial concentrations of cyclohexanedione and α-acetoxycyclopentanone, 0.025 *M*).

Although the observed esr spectra of the steroidal semidiones do not show excessive line broadening from electron transfer, it is likely that the semidiones are in equilibrium with α-diketones. If one of the diketones

$$RCOCOR + R'C(O\cdot)=C(O^-)\,R' \rightleftharpoons RC(O\cdot)=C(O^-)\,R + R'COCOR'$$

is more susceptible to destruction by base (by cleavage or benzilic acid rearrangement) then one of the semidiones will be found to decay more rapidly than the other. Thus, for a mixture of 5α-2,3- and 5α-3,4-semidiones it is observed that the 3,4-semidione decays more rapidly than its 2,3 isomer. Results of this type may reflect the greater susceptibility to destruction by base of the 3,4-dione than the 2,3-dione. It is thus apparent that the change with time of the esr spectrum of the oxidate of a steroidal ketone should be a function of base concentration. Moreover, the possibility exists that in certain cases (*e.g.*, 5β-2-ketones) the concentration of base may actually affect the initial ratio of semidiones. The results reported herein were determined at a relatively low base concentration (0.1 *M*) chosen to provide reasonably optimum conditions for the formation of semidiones.

Regardless of whether the ratio of semidiones formed from a 2- or a 3-keto steroid is determined by the stability of the intermediate enolate anions, or by the stability of the semidiones themselves, it is obvious that the ratios of semidiones observed reflect the relative stabilities of a double bond introduced into the A ring of the substituted perhydrophenanthrene nucleus.[18] In fact, the ratio of semidiones observed for 2- and 3-ketones is in very good agreement with a variety of synthetic reactions whose courses are determined by similar thermodynamic considerations. Table II summarizes pertinent results.

It is obvious from Table II that observation of the semidiones formed by oxidation of a steroidal ketone in basic solution can be used to predict the preferred point of attack on the ketone in reactions such as methylation, bromination, or enol acetylation.

In the present and preceding papers we have examined the oxygenation of a number of β-decalones and derivatives. It is of interest to examine the factors that control the position of oxygenation. The pertinent data are summarized in Figure 6. The following generalizations can be made in regard to the data summarized in Figure 6.

A. *trans*-**2-Ketones.**—The greater selectivity in the reactions of the 5α-2-keto steroid relative to *trans*-β-decalone is due entirely to the angular methyl group. *trans*-9-Methyl-2-decalone has a steroid-like conformation.

B. *trans*-**3-Ketones.**—The greater selectivity in the reactions of the 5α-3-keto steroid relative to *trans*-β-decalone is due mainly to the angular methyl group although the rigidity of the steroid ring system

(14) For example, see (a) E. J. Bailey, J. Elks, and D. H. R. Barton, *Proc. Chem. Soc.* (London), 214 (1960); (b) E. J. Bailey, D. H. R. Barton, J. Elks, and J. F. Templeton, *J. Chem. Soc.*, 1578 (1962); (c) L. Crombie and P. J. Godin, *ibid.*, 2861 (1961).

(15) R. Hanna and G. Ourisson, *Bull. Soc. Chim. France*, 1945 (1961).

(16) (a) B. Camerino, B. Patelli, and R. Sciaky, *Tetrahedron Letters*, 554 (1961); (b) *Gazz. Chim. Ital.*, **92**, 676 (1962).

(17) S. Nakajima and K. Takeda, *Chem. Pharm. Bull.* (Tokyo), **12**, 1530 (1964).

(18) For a discussion of the factors that determine these relative stabilities, see A. S. Dreiding, *Chem. Ind.* (London), 1419 (1954); E. J. Corey and R. A. Sneen, *J. Am. Chem. Soc.*, **77**, 2505 (1955); T. G. Halsall and D. B. Thomas, *J. Chem. Soc.*, 2431 (1956); R. B. Turner, W. R. Meador, and R. E. Winkler, *J. Am. Chem. Soc.*, **79**, 4122 (1957); B. Berkoz, E. P. Chavez, and C. Djerassi, *J. Chem. Soc.*, 1323 (1962); R. Bucourt, *Bull. Soc. Chim. France*, 1983 (1962), 1262 (1963), 2080 (1964); R. Bucourt and D. Hainaut, *ibid.*, 1366 (1965), and the references cited therein.

TABLE II

COMPARISON OF OXIDATION AND SUBSTITUTION REACTIONS OF 2- AND 3-KETO-9-METHYLDECALINS AND STEROIDS

Compound	Percentage of reaction—Away from ring junction	Percentage of reaction—Toward ring junction	Type of reaction[a]	Conditions	Ref
cis-9-Methyl-2-decalone	100	0	OA	Cleavage by HNO_3	*b*
	80	20	Esr		
cis-9-Methyl-3-decalone	100	0	B	Br_2 in $CHCl_3$	*c*
	75	25	Esr		
trans-9-Methyl-3-decalone	100	0	B	Br_2 in HOAc	*d, e*
	93	7	Esr		
5β-Androstan-2-one	33	67	OB		*f*
	60	40	Esr		
5α-Androstan-2-one	100	0	B	Br_2 in HOAc[g]	*h, i*
	100	0	EA	Ac_2O, $HClO_4$	*b*
	100	0	OB		*j*
	100	0	Esr		
5β-Androstan-3-one	0	100	OB		*f, k*
	0	100	M		*l*
	0	100	EA	Isopropenyl acetate, H_2SO_4	*m*
	Minor	Major	B	Br_2 in $CHCl_3$–HOAc	*n*
	100	0[o]	C	$NaOCH_3$, $HCO_2C_2H_5$, C_6H_6	*p*
	20	80	Esr		
5β-Androstane-3,11-dione	0	100	OB		*f*
	20	80	Esr		
5α-Androstan-3-one	100	0	OB		*q, f, j*
	100	0	M		*l*
	100	0	B	Br_2 in HOAc	*r*
	100	0	EA	Isopropenyl acetate, H_2SO_4	*m*
	92	8	Esr		
5α-Androstane-3,11-dione	90	10	OB		*j*
	74	26	Esr		
9α-Fluoro-11β-hydroxy-5α-pregnan-3-one	Major	Minor	OB		*s*
	91	9	Esr		
5α,8β,9α,10α-Androstan-2-one	100	0	EA	Ac_2O, $HClO_4$	*t*
	85	15	EA	Isopropenyl acetate, p-$CH_3C_6H_4SO_3H$	*t*
	98	2	Esr		
5β-Lumistanone	100	0	B	Br_2 in HOAc	*u*
	88	12	Esr		
5α-Lumistanone	Minor	Major	B	Br_2 in HOAc	*u*
	20	80	Esr		

[a] OA, oxidation in acidic solution; esr, present study; B, bromination; OB, oxidation to α-diketone with oxygen in a *t*-butyl alcohol, potassium *t*-butoxide solution with or without pyridine; EA, enol acetylation; M, methylation with methyl iodide in a benzene, *t*-butyl alcohol, potassium *t*-butoxide solution; C, condensation. [b] A. J. Birch and R. Robinson, *J. Chem. Soc.*, 501 (1943). [c] M. Yanagita and K. Yamakawa, *J. Org. Chem.*, **22**, 291 (1957). [d] B. Riniker, J. Kalvoda, D. Arigoni, A. Fürst, O. Jeger, A. M. Gold, and R. B. Woodward, *J. Am. Chem. Soc.*, **76**, 313 (1954). [e] M. Yanagita and K. Yamakawa, *J. Org. Chem.*, **21**, 501 (1956). [f] S. Nakajima and K. Takeda, private communication. [g] Reaction reported to proceed rapidly in contrast to exceedingly slow bromination of 5α-1-keto steroid: H. P. Sigg and C. Tamm, *Helv. Chim. Acta*, **43**, 1402 (1960). Note similarity of rates of bromination and formation of semidiones. [h] C. Djerassi and T. Nakano, *Chem. Ind.* (London), 1385 (1960); T. Nakano, M. Hasegawa, and C. Djerassi, *Chem. Pharm. Bull.* (Tokyo), **11**, 465 (1963). [i] C. W. Shoppee and T. E. Bellas, *J. Chem. Soc.*, 3366 (1963). [j] Reference 17. [k] Reference 16. [l] Y. Mazur and F. Sondheimer, *J. Am. Chem. Soc.*, **80**, 5220 (1958). [m] W. G. Dauben, R. A. Micheli, and J. F. Eastham, *ibid.*, **74**, 3852 (1952). [n] H. H. Inhoffen, G. Kölling, G. Koch, and I. Nebel, *Ber.*, **84**, 361 (1951). [o] About 40% reaction at C-4 observed for a stigmastenone. [p] R. O. Clinton, R. L. Clarke, F. W. Stonner, D. K. Phillips, K. F. Jennings, and A. J. Manson, *Chem. Ind.* (London), 2099 (1961); R. O. Clinton, R. L. Clarke, F. W. Stonner, A. J. Munson, K. F. Jennings, and D. K. Phillips, *J. Org. Chem.*, **27**, 2800 (1962). [q] Reference 14b. [r] A. Butenandt and A. Wolff, *Ber.*, **68**, 2091 (1935). [s] Reference 16b. [t] J. A. Settepani, M. Torigoe, and J. Fishman, *Tetrahedron*, **21**, 3661 (1965). [u] J. Castells, G. A. Fletcher, E. R. H. Jones, G. D. Meakins, and R. Swindells, *J. Chem. Soc.*, 2627 (1960).

also contributes (*cf.* **2** and **57**). The angular methyl group has the same effect when introduced at C-10 or C-5 (*cf.* **24** and **32E**). *trans*-9-Methyl-3-decalone has a steroid-like conformation. Epimerization at C-8 does not significantly affect the preferred point of reaction of a 5α-3-keto steroid (*cf.* **24** and **35E**), whereas epimerization at C-9 has a profound effect (*cf.* **24** and **43E**).

C. *cis*-2-Ketones.—The rigid 5β-2-keto steroid and the flexible *cis*-β-decalone are amazingly similar in reactivity. Possibly the rigidity of the steroid nucleus and the angular methyl at C-10 have opposite effects which cancel. Evidence has already been presented that *cis*-9-methyl-2-decalone may have a nonsteroid conformation (*cf.* **7** and **37E**), at least in the transition state for oxygenation or ionization. Epimerization at C-9 greatly affects the preferred reaction center (*cf.* **20** and **37E**).

D. *cis*-3-Ketones.—The rigidity of the steroid nucleus has little effect on the reaction site (*cf.* **1** and **58**). The angular methyl group at C-10 in a 5β-3-keto steroid has but a small effect on the position of preferred reaction (*cf.* **26** and **58**). Apparently a β-methyl group at C-5 also has little effect (*cf.* **26** and **26g**). The data are consistent with the recent conclusions that *cis*-9-methyl-3-decalone (*cis*-10-methyl-2-

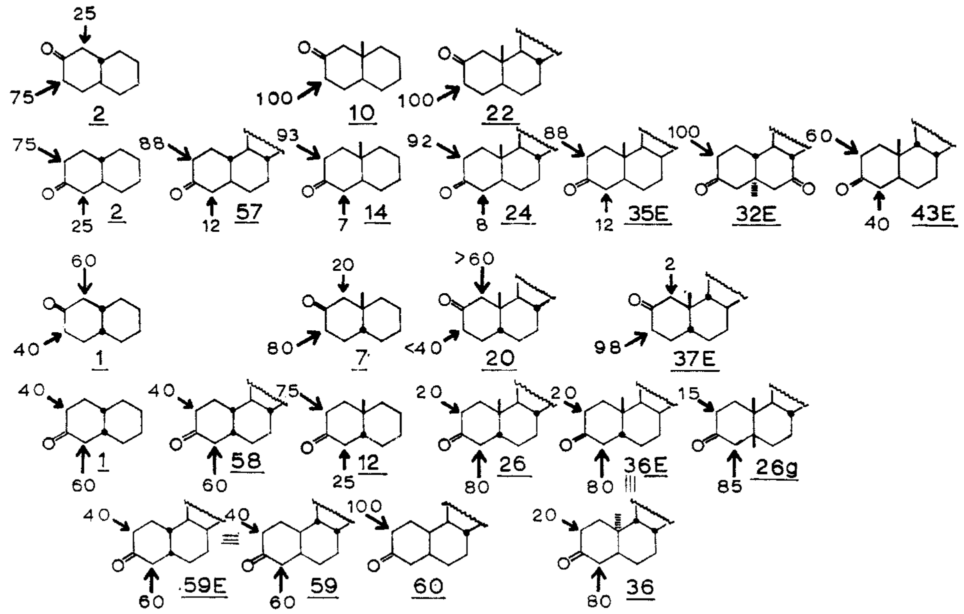


Figure 6.—Initial proportions of semidiones formed in the oxidation of 2-decalones and steroidal ketones in dimethyl sulfoxide solution in the presence of potassium *t*-butoxide. Partial structures with the identification E were examined in the enantiomeric form.

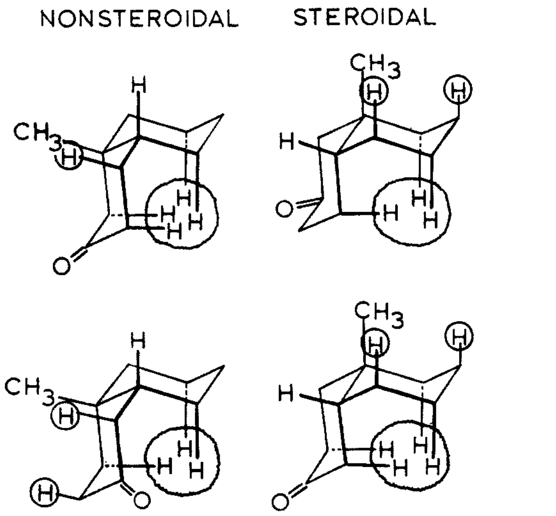


Figure 7.—Conformations of 2- and 3-keto-*cis*-9-methyldecalins.

decalone) has a preference for the nonsteroid conformation[19] (*cf.* **12** and **26**); *i.e.*, the methyl group is axial to the cyclohexanone ring. Epimerization at C-8 has no effect on the preferred reaction site (*cf.* **26** and **36E**, **58** and **59E**), while epimerization at C-9 has a profound effect (*cf.* **59** and **60**).

An elementary consideration of nonbonded interactions suggests the reason for the preference of nonsteroidal conformations for the 2- and 3-keto-*cis*-9-methyldecalins. In Figure 7 the relevant conformations are shown. Considering first the 2-ketones we find two methyl–hydrogen interactions in the steroidal conformation and only one such interaction in the nonsteroidal conformation. We believe that these nonbonded interactions are more important than *axial* hydrogen–hydrogen interactions at the back of the molecules. For the steroidal conformation of the 2-ketone there are three hydrogens at the back of the molecule, while for the nonsteroidal conformation there are four hydrogens in close proximity.

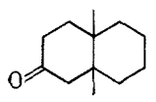
When the 3-ketones are considered we find equivalent methyl–hydrogen interactions in the steroidal and nonsteroidal conformations. Now, the preferred conformation is determined by interaction of *axial* hydrogen atoms at the back of the molecule. In the steroidal conformation there are four hydrogens in close proximity while for the nonsteroidal conformation there are only three hydrogen atoms.

The oxidation products can be rationalized by a consideration of relief of steric strain upon introduction of two adjacent trigonal atoms into the A ring. For the steroidal 3-ketone three hydrogen–hydrogen interactions are relieved by making C-4 trigonal, whereas only two such interactions are relieved when C-2 is made trigonal. Predominant oxidation occurs at C-4. For the nonsteroidal ketone a 1,3-diaxial methyl–hydrogen interaction is directly relieved by attack at C-2 or C-4. Attack at C-2 indirectly (by general flattening of the A ring) relieves not only the second methyl–hydrogen interactions but also two of the hydrogen–hydrogen interactions at the back of the molecule. For the 3-ketone in the nonsteroidal conformation we observe predominant attack at C-2.

For the 2-ketone in a steroidal conformation, no interaction is directly relieved by attack at either C-1 or C-3 and we find approximately equal attack at these centers. For the nonsteroidal 2-ketone, attack at C-3 directly relieves two hydrogen–hydrogen interactions at the back of the molecule and also indirectly relieves an important methyl–hydrogen diaxial interaction. We believe the latter relief in strain is very important and leads to preferred attack at C-3. It should be noted that attack at C-1 actually relieves more strain at the back of the molecule since it removes three *axial* hydrogen–hydrogen interactions.

We have applied this technique to some substituted *cis*-9-methyl-3-decalones. Compounds **61–64** have been

(19) D. R. Elliott, M. J. T. Robinson, and F. G. Riddell, *Tetrahedron Letters*, 1693 (1965); R. L. Williamson and T. A. Spencer, *ibid.*, 3267 (1965); W. G. Dauben, R. M. Coates, N. D. Vietmeyer, L. J. Durham, and C. Djerassi, *Experientia*, **21**, 565 (1965); K. L. Williamson, L. R. Sloan, T. Howell, and T. A. Spencer, *J. Org. Chem.*, **31**, 436 (1966).



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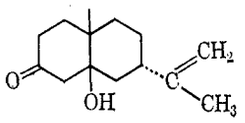

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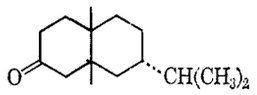
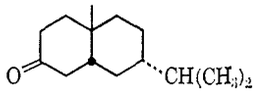
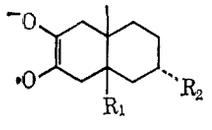


63



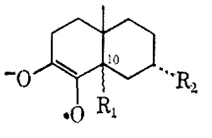
64

examined. Compounds **61–63** gave highly preferred oxygenation at C-2 (96, 98, and 92%, respectively), and from the arguments given above we conclude that these ketones, when considered as 3-ketodecalins, exist predominantly in a nonsteroidal conformation. This is particularly apparent when 5β,10β-dimethylcholestan-3-one (**26g**), which oxidizes preferentially at C-4, is compared with **61**. The hfsc for the major and minor semidiones are listed in structures **65** and **66**. The



65

$R_1 = CH_3$, $R_2 = H$; $a^H = 13.22, 12.85, 6.88, 6.88$ gauss
$R_1 = OH$; $R_2 = C(=CH_2)CH_3$; $a^H = 13.65, 12.47, 7.13, 6.53$ gauss
$R_1 = CH_3$; $R_2 = CH(CH_3)_2$; $a^H = 13.31, 13.04, 6.82, 6.82$ gauss
$R_1 = H$; $R_2 = CH(CH_3)_2$; $a^H = 12.93, 12.23, 6.75, 6.75$ gauss



66

$R_1 = \beta\text{-}CH_3$; $R_2 = H$; $a^H = 12.74, 7.22$ gauss
$R_1 = \beta\text{-}OH$; $R_2 = C(=CH_2)CH_3$; $a^H = 13.6, 6.0$ gauss
$R_1 = \beta\text{-}CH_3$; $R_2 = CH(CH_3)_2$; $a^H = 12.94, 7.31$ gauss
$R_1 = \alpha\text{-}H$; $R_2 = CH(CH_3)_2$; $a^H = 12.6, 12.6, 8.6$ gauss (?)

spectrum of **65** ($R_1 = CH_3$; $R_2 = H$) is very nearly a triplet of triplets on account of hfs by the α quasi-axial and quasi-equatorial hydrogen atoms (Figure 8). However, the second-derivative spectrum clearly indicates a slight difference in the hfsc of the two types of quasi-axial hydrogen atoms ($a^H = 13.22$ and 12.85 gauss). Since **61** exists in a nonsteroidal conformation when considered as a 3-ketone, it follows that when considered as a 2-ketone **61** exists in a steroidal 2-ketone conformation. The same analysis can be applied to *cis*-2-decalone (Figure 6). Since **1** and **58** give identical oxidative selectivity, it is concluded that the preferred conformation of **1** is similar to **58**; *i.e.*, **1** possesses a steroidal conformation when considered as a 3-ketone and a nonsteroidal conformation when considered as a 2-ketone.

From other studies, Djerassi, Dauben, and their coworkers have concluded[19] that **64** exists predominantly in a nonsteroidal conformation. Our results with **64** strongly support this conclusion since a highly preferred ionization and oxygenation occurs at C-2 to yield **65**, which constitutes more than 92% of the total paramagnetic oxidation products. The presence of more then six lines in the spectrum of the minor oxidation products is reminiscent of the behavior of **12** and

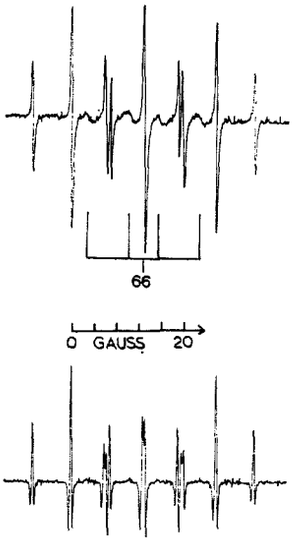


Figure 8.—First-derivative (top) and second-derivative (bottom) esr spectra (~9.5 gigacycles/sec) of oxidation product of *cis*-9,10-dimethyl-2-decalone. The second-derivative spectrum was obtained by the use of a Japan Electron Optics Laboratory Co., Ltd., spectrum accumulator, Model JNM-RA-1. Note that the minor semidione (**66**) can be detected in the first-derivative spectrum, but hardly at all in the second-derivative spectrum.

indicates the possible formation of both 10α- and 10β-3,4-semidiones. This complication coupled with the small amount of attack at C-4 renders the hfsc given for **66** tentative at best.

Experimental Section[20]

The details given in a previous paper[9] under the headings of "Materials" and "Formation and Detection of Radical Anions" pertain also to the present paper. The hfsc reported here are considered to be more accurate than those obtained previously[3,6] with a spectrometer having a 6-in. magnet. When secondary alcohols were available instead of the desired ketones, the latter compounds were obtained by oxidation with chromic acid as described below under "Lanosterone." Some liquid 9-methyldecalones, available as their semicarbazones, were obtained in the free state by refluxing the semicarbazones (100 mg) with 1 *N* hydrochloric acid (4 ml) in benzene (1.5 ml) for 90 min under nitrogen.

***cis*-9-Methyl-3-decalone (12).**[21]—To 31.6 g (0.28 mole) of 2-methylcyclohexanone, 14.9 g (0.10 mole) of freshly prepared 1-diethylamino-3-butanone,[22] and 40–50 mg of hydroquinone was added gradually 0.6 g of sodium metal at room temperature. After the sodium dissolved, the mixture was refluxed (a liquid starts to reflux at about 100°) under a blanket of nitrogen for 3 hr (pot temperature, 135°). The brown solution was then cooled by ice, carefully acidified with 10 % hydrochloric acid, and thoroughly extracted with ether. The ether layers were combined, washed with water, and dried (anhydrous sodium sulfate). Evaporation of the ether furnished an oil which was vacuum distilled through a short Vigreux column to yield 4.5 g (27 %) of 3-keto-9-methyl-Δ⁴-octahydronaphthalene, bp 155–164° (19 mm). By redistillation of this material, a fraction of bp 155–160° (19 mm), lit.[21] bp 140–150° (16 mm), was obtained.

A solution of 2.5 g of this octalone in 15 ml of absolute ethanol containing 2 drops of glacial acetic acid was shaken in a Parr hydrogenation apparatus with 0.25 g of 10 % palladium on carbon and hydrogen under an initial pressure of 13 psi. After 1 equiv of hydrogen was absorbed (7 min), the reaction mixture was filtered, and evaporated to dryness at room temperature under reduced pressure; the residue was steam distilled. The first distillate (75 ml) gave a colorless oil which solidified in a

(20) All melting points (capillary) and boiling points are uncorrected.
(21) See Table II, footnote *c*.
(22) A. L. Wilds and C. H. Shunk, *J. Am. Chem. Soc.*, **65**, 469 (1943).

refrigerator. The solid was filtered, washed with water, dried, and recrystallized three times from hexane–chloroform to afford 260 mg of *cis*-9-methyl-3-decalone, mp 48.0–49.2° (lit. mp 47°,[21,23] 46–48°,[24] 43.4–47.2° [25]). Gas–liquid partition chromatography (glpc) showed the presence of only one compound.

Lanosterone. A.[26]—A mixture of 2.1 g (4.9 mmoles) of commercial "lanosterol" (Aldrich Chemical Co. and Steraloids, Inc.) and 41 ml of reagent grade acetone was stirred and treated dropwise with Jones reagent[27] at room temperature until a distinct orange color persisted in the solution for at least 5 min. Dilution of the reaction mixture with 400 ml of water caused precipitation of the organic material, which was filtered, washed with water, and dried. The crude product, weighing 1.95 g (93%), was recrystallized once from ether–methanol (mp 87.5–93.0°) and then chromatographed on 60 g of alumina (Woelm neutral, activity I). Elution with 1200 ml of benzene–pentane (1:4, respectively, by volume) yielded 180 mg of product, mp 93.0–98.5°, which after four crystallizations from methanol–ether furnished 60 mg of white crystals, mp 108.5–111.0° (lit. mp 107–109°,[28] 111–114°,[26] 81–82°,[28] 79–80° [29]).

Anal. Calcd for $C_{30}H_{48}O$: C, 84.84; H, 11.39. Calcd for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.40; H, 11.81.

B.[28]—Commercial "lanosterol" (5 g) was oxidized with Jones reagent as described above, except that the crude product (4.65 g) was first subjected to extensive fractional crystallization from methanol–acetone. After seven stages of crystallization, two fractions consisting of well-defined crystals were collected: (1) 1.06 g, mp 109.5–112.0°; and (2) 0.66 g, mp 78–81°. Fraction 1 had a lower solubility than fraction 2 in methanol–acetone. Other fractions with greater solubility than these consisted of gummy materials. Fraction 2 was chromatographed on 20 g of alumina (same material as above), and the solid eluted with benzene–pentane (800 ml of 20:80, and 600 ml of 30:70, respectively, by volume) was recrystallized three times from methanol–acetone to afford 57 mg of white crystals, mp 85.5–88.5°.

The difference in melting point between the products obtained by the two methods warrants some comments. The first method is essentially that of Bancroft and his co-workers[30] and the second one is similar to that followed by Ruzicka and his co-workers. Despite the great pains taken by the Swiss workers to purify their product, Bancroft, *et al.*, consider their own product to be pure lanosterone while commenting that the same compound had been obtained previously "in an impure form" by Ruzicka, *et al.* However, Ruzicka, *et al.*, have shown that commercial "lanosterol" is a mixture of lanosta-8(9),24-dien-3-ol (lanosterol) and lanost-8(9)-en-3-ol, and have, in fact, separated two ketones from the oxidation product of such a sample of "lanosterol." They consider the two ketones, mp 81–82 and 119.5–120.5°, to be lanosterone and the 24,25-dihydrolanosterone, respectively. Hence, it would appear that the ketone of Bancroft, *et al.* (mp 107–109°), is actually a mixture of lanosterone and the dihydrolanosterone in which the latter compound predominates. In support of this argument, we have found by glpc that our product of mp 108.5–111.0 or 109.5–112.0° is really a mixture of two compounds with slightly differing retention times, the one having the shorter retention time being the major component. The mass spectrum of this sample exhibited a parent peak at *m/e* 426 and a much weaker peak at *m/e* 424 (relative intensities 2.3:1.0). Furthermore, we could not detect any vinyl proton by nuclear magnetic resonance (nmr) spectroscopy. On the other hand, the 60-Mc/sec nmr spectrum ($CDCl_3$) of the ketone having a mp of 85.5–88.5° had a triplet signal (coupling with methylene protons at C-23, $J \cong 6.7$ cps) at 5.12 ppm (vinyl proton) downfield from tetramethylsilane used as an internal standard. However, the ratio of the vinyl proton to the remaining protons was somewhat less than that to be expected from lanosterone, an observation which suggests some admixture with dihydrolanosterone; and, indeed, the presence of two components identical in retention times with those from the higher melting sample was detected by glpc, the major one this time having the longer retention time. As expected, the oxidates from both samples had identical esr spectra, and both materials exhibited strong infrared absorption (KBr) at 5.86–5.87 μ. Hence, it appears that neither Bancroft, *et al.*, nor Ruzicka, *et al.*, prepared pure lanosterone, although the product of the latter group of workers more closely approaches lanosterone in its properties.

Lumistanone A (35) and **lumistanone B (36)** were prepared from lumisterol essentially as described by Jones and his co-workers.[31,32] The alumina used during the preparation of the former ketone was Woelm neutral, activity II, and required a 1:1 (v/v) mixture of *n*-pentane and benzene to elute the dihydrolumisterol obtained by reduction of lumisterol with sodium in liquid ammonia.

6α-Bromo-5α-cholestan-7-one (46a),[33] **3β-acetoxy-7α-bromo-5α-cholestan-6-one (46b),**[34] **11,23-dibromohecogenin acetate (49),**[35,36] **2-acetoxycyclopentanone,**[37] **4,17β-dihydroxyandrost-4-en-3-one,**[16] **4-bromo-5β-pregnan-3-one,**[39] and **17β-acetoxy-4β-bromo-5β-androstan-3-one**[40] were prepared according to known procedures.

17β-Acetoxy-3α,4α-epoxy-5α-androstane (mp 189.0–191.5°) was prepared from 17β-acetoxy-5α-androst-3-ene[41] in 80% yield as described in the literature.[42]

Reaction of 17β-Acetoxy-3α,4α-epoxy-5α-androstane with DMSO in Presence of Boron Trifluoride.[43]—A solution of 54 mg of the epoxide in 0.06 ml of dry DMSO and 2.0 ml of dry dioxane containing a trace of boron trifluoride–etherate was heated under a blanket of nitrogen for 9 hr on the steam bath, and let stand at room temperature for an equal length of time. A minute drop of boron trifluoride–etherate was added again and the solution heated under a blanket of nitrogen for a further period of 2.5 hr. After being allowed to stand at room temperature for 2.5 hr, the light yellow solution was poured into 18 ml of ice–water and extracted repeatedly with chloroform. The combined chloroform extracts were dried (anhydrous sodium sulfate) and evaporated to dryness under reduced pressure. Crystallization of the oily residue from ether–pentane at Dry Ice temperatures furnished 10 mg of white crystals, mp 158–159°, infrared absorption ($CHCl_3$) at 2.90 (br), 5.80, 5.83, and 7.98 μ. The parent peak in the mass spectrum occurred at *m/e* 348. Other principal peaks occurred at 288 (CH_3CO_2H) and 270 μ (CH_3CO_2H, H_2O). Weak absorption at 6.01 and 6.11 μ indicated that the product is probably contaminated with a small amount of the 3,4-diketone (in the form of the Δ^4-enol). The mass spectrum showed small peaks at *m/e* 346 and 286 ($-CH_3CO_2H$) but not at 268. Since the diketone would not interfere with our esr experiments, the product was used without further purification.[44] Without more experimental work, we cannot determine whether our product is a single α-ketol or a mixture of α-ketols. However, this uncertainty does not matter for our purposes since all the isomeric 3,4-ketols would yield the same radical anion(s). In fact, the crude, oily product gave the same esr spectrum as that obtained from the crystals.

(23) E. C. du Feu, F. J. McQuillin, and R. Robinson, *J. Chem. Soc.*, 53 (1937).

(24) R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler, and W. M. McLamore, *J. Am. Chem. Soc.*, **74**, 4223 (1952).

(25) W. G. Dauben, J. B. Rogan, and E. J. Blanz, Jr., *ibid.*, **76**, 6384 (1954).

(26) G. Bancroft, Y. M. Y. Haddad, and G. H. R. Summers, *J. Chem. Soc.*, 3295 (1961).

(27) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *ibid.*, 39 (1946); see also C. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

(28) L. Ruzicka, R. Denss, and O. Jeger, *Helv. Chim. Acta*, **28**, 759 (1945).

(29) M. Lindberg, F. Gautschi, and K. Bloch, *J. Biol. Chem.*, **238**, 1661 (1963).

(30) Bancroft, *et al.*, may have used Jones reagent, but they do not specify the concentrations of chromic acid and sulfuric acid.

(31) J. Castells, G. A. Fletcher, E. R. H. Jones, G. D. Meakins, and R. Swindells, *J. Chem. Soc.*, 2785 (1960).

(32) See Table II, footnote *u*.

(33) A. Nickon and J. F. Bagli, *J. Am. Chem. Soc.*, **83**, 1498 (1961).

(34) E. J. Corey and R. A. Sneen, *ibid.*, **78**, 6269 (1956); E. J. Corey and G. A. Gregoriou, *ibid.*, **81**, 3127 (1959).

(35) G. P. Meuller, R. E. Stobaugh, and R. S. Winniford, *ibid.*, **73**, 2400 (1951).

(36) See also G. P. Mueller, L. L. Norton, R. E. Stobaugh, L. Tsai, and R. S. Winniford, *ibid.*, **75**, 4892 (1953); R. Hirschmann, C. S. Snoddy, Jr., and N. L. Wendler, *ibid.*, **75**, 3252 (1953); J. Elks, G. H. Phillips, T. Walker, and L. J. Wyman, *J. Chem. Soc.*, 4330 (1956).

(37) From 2-bromocyclopentanone[38] by the method of I. V. Machinskaya and A. S. Podberezina, *Zh. Obshch. Khim.*, **28**, 1501 (1958) [*Chem. Abstr.*, **53**, 1184 (1959)].

(38) R. Mayer, *Ber.*, **89**, 1443 (1956).

(39) R. E. Marker and E. J. Lawson, *J. Am. Chem. Soc.*, **61**, 586 (1939).

(40) L. F. Fieser and W.-Y. Huang, *ibid.*, **75**, 4837 (1953).

(41) J. McKenna, J. K. Norymberski, and R. D. Stubbs, *J. Chem. Soc.*, 2502 (1959).

(42) M. E. Wolff, W. Ho, and R. Kwok, *J. Med. Chem.*, **7**, 577 (1964).

(43) T. Cohen and T. Tsuji, *J. Org. Chem.*, **26**, 1681 (1961).

(44) The 2α,3α- and 2β,3β-epoxy derivatives of 5α-cholestane have been reported[43] to yield a common ketol (3β-hydroxy 2-ketone), along with minor amounts of the 2,3-dione and the 2β,3α-diol.

cis-6α-**Isopropyl-9β-methyl-3-decalone.**—A solution of 2.347 g (0.0115 mole) of 10β-methyl-7α-isopropenyl-$\Delta^{1(9)}$-octal-2-one[45] in 40 ml of ethyl acetate was shaken with 230 mg of 10% palladium on charcoal and hydrogen under an initial pressure of 21.5 psi. After 2 equiv of hydrogen were absorbed (40 min), the reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure to afford 2.35 g of a viscous oil, which was chromatographed on 60 g of alumina (Woelm neutral, activity II). The product eluted with 230 ml of *n*-pentane was collected after 80 ml of the first pentane eluate was rejected on the basis of thin layer chromatography. The resulting product could not be sublimed at 42° (0.1 mm) as described in the literature.[46] However, by repeated low-temperature crystallization (acetone–Dry Ice) from *n*-pentane, a small amount (12 mg) of pure *cis*-6α-isopropyl-9β-methyl-3-decalone (*cis*-7α-isopropyl-10β-methyl-2-decalone) was obtained, mp 55.5–57.0° (lit.[46] 55–57°), single peak in glpc.[46]

(45) J. A. Marshall, W. I. Fanta, and H. Roebke, *J. Org. Chem.*, **31**, 1016 (1966).

(46) C. Djerassi, J. Burakevich, J. W. Chamberlin, D. Elad, T. Toda, and G. Stork, *J. Am. Chem. Soc.*, **86**, 465 (1964). The yield of the pure compound is not mentioned in this paper.

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The Synthesis of Steroidal Cyclopropano Ketones

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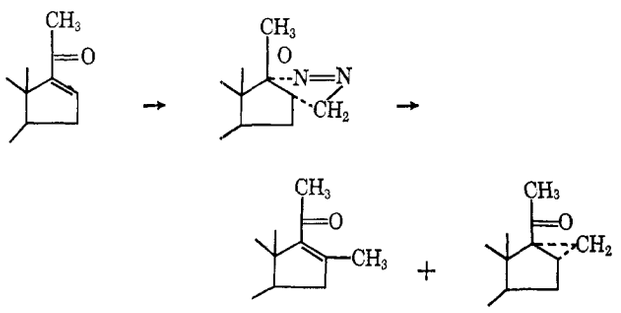
The ylide, dimethylsulfoxonium methylide, has been shown to react with steroidal $\Delta^{1,4,6}$-, $\Delta^{4,6}$-, and 19-nor-Δ^{4}-3-ones to give the corresponding 1α,2α-, 6α,7α-, and 4β,5β-methylene derivatives, respectively. The preparation of cyclopropano steroids by this method is compared with the sequence unsaturated ketone → pyrazoline → cyclopropano steroid. The ORD, nmr, and other physical properties of these compounds are described.

The formation of a steroidal pyrazoline by the addition of diazomethane to a Δ^{16}-20-one was first reported by Wettstein[1] in 1944. The thermal decomposition of this system gave as the major product the 16-methyl-Δ^{16}-20-one and a minor product which was subsequently shown[2] to be the 16,17-methylene-20-one (see Scheme I).

SCHEME I



In 1960, Wiechert and Kaspar[3] extended this reaction to the $\Delta^{1,4,6}$-3-one system. They found that the pyrazoline formed in this case could be converted in good yield to the 1,2-methylene-$\Delta^{4,6}$-3-one. The addition of diazomethane was assumed to take place from the less hindered α side of the molecule and therefore the placement of the pyrazoline and methylene rings was considered to be 1α,2α.

Our interest in modifying the structure of physiologically active steroids led us also to utilize the addition of diazomethane to the $\Delta^{1,4,6}$-3-one moiety as a means of preparing 1,2-methylene compounds. Thus, as was reported,[3] we found that the 1α,2α-pyrazoline derivative (**2**) formed from 16α,17α-(dimethylmethylenedioxy)-1,4,6-pregnatriene-3,20-dione[4] (**1**) could be converted thermally or by acid-catalyzed decomposition to 1α,2α-methylene-16α,17α-(dimethylmethylenedioxy)-4,6-pregnadiene-3,20-dione (**4**). The over-all yield of **4** prepared by the perchloric acid–acetone decomposition of the pyrazoline was 23%. The possible utility of this sequence for the preparation of substituted 1α,2α-methylene derivatives was investigated by treating diazoethane[5] with **1**. The resulting 5-methylpyrazoline derivative **3**, was obtained in 25% yield and, in analogy with the previous assignments, given the 1α,2α configuration. While the ultraviolet absorption spectrum of **2** had the expected[3] peaks at 235 mμ (ε 4000) and 293 mμ (ε 21,800), the more highly substituted pyrazoline **3** had only the higher wavelength peak at 289 mμ (ε 21,000). The infrared spectra of the pyrazolines showed the N=N stretching peak at 6.48 μ (1543 cm^{-1}) in **2** and 6.50 μ (1538 cm^{-1}) for **3**, in addition to the peaks expected for a $\Delta^{4,6}$-3-one system at 6.06, 6.19, and 6.33 μ.[6] The nmr spectrum of **3** exhibited a doublet at τ 8.71 (J = 7.7 cps) resulting from the methyl attached to the pyrazoline ring. This doublet collapsed to a singlet when a spin-decoupling experiment[7] was carried out and the sample irradiated 235 cps downfield. Spin decoupling also

(1) A. Wettstein, *Helv. Chim. Acta*, **27**, 1803 (1944).

(2) A. Sandoval, G. Rosenkranz, and C. Djerassi, *J. Am. Chem. Soc.*, **73**, 2383 (1951).

(3) R. Wiechert and E. Kaspar, *Chem. Ber.*, **93**, 1710 (1960).

(4) U. S. Patent 3,174,971 (March 23, 1965).

(5) A. L. Wilds and A. L. Meader, Jr., *J. Org. Chem.*, **13**, 763 (1948).

(6) G. R. Allen, Jr., and M. J. Weiss, *J. Am. Chem. Soc.*, **82**, 2840 (1960).

(7) We wish to thank Mr. G. D. Vickers, Olin Mathieson Chemical Corp., New Haven, Conn., for his assistance in performing this experiment on a Varian Associates HR-60 instrument.