Anal. Calcd. for $C_{11}H_{18}D_2$: C, 85.63; H + D as H, 13.21; R_D, 48.60. Found: C, 85.48, 85.63; H + D as H, 13.30, 13.12; R_D, 48.49 (see infrared spectrum 1).

Infrared Spectra.—The infrared spectra were measured on a Perkin-Elmer model 21 recording spectrophotometer. All optics were of rock salt, including the sample windows. The liquid intermediates were spread between two rock salt plates without spacers. The spectra so obtained are qualitative only because the thickness is unknown. Solids were measured as mulls in Nujol, again giving qualitative spectra. The angular acid VIII and the alcohols—IX, XX and XXIV—were dissolved in carbon tetrachloride for the region 2.0–7.5 m. and in carbon bisulfide for the region 7.0–15.5 m. Because of the volatility of these solvents, the concentrations probably are not accurate to more than about 5%. All of the hydrocarbons were observed in a cell 0.0565-mm. thick.

The principal purpose of the work was to ascertain what changes are produced in the spectrum of a hydrocarbon by the deuteration of a methyl group. Certain predictions could be made; the well-known band at 1380 cm.$^{-1}$ should disappear and new C–D stretching bands should appear. The fate of the fingerprint region was not certain, but some changes were to be expected.

An isolated methyl group has three stretching modes, a symmetric frequency and two doubly degenerate asymmetric frequencies.[25] On deuteration these bands should shift inversely as the square roots of the reduced masses, but because they occur in the same region as ordinary methylene stretching frequencies, the precise locations of the new C–D bands could not be predicted. Hence, the location of the deuterium bands could not be checked. The steric environment of the methyl group might influence these frequencies, perhaps causing splitting of the degenerate bands, but the maximum number of deuterium bands to be expected should be three. Experimentally, the hexahydroindans showed four and the decalins at least five bands. By the method of synthesis and the deuterium content of the hydrocarbons it is improbable that deuterium is located anywhere else in the molecules. No explanation for the extra bands is offered.

A methyl group also has three bending modes[25]—a doubly degenerate asymmetric frequency occurring in the same region as the methylene bending absorption near 1450 cm.$^{-1}$ and a symmetric bending frequency at 1380 cm.$^{-1}$. The deuteromethyl groups should exhibit the symmetric mode at about 1055 cm.$^{-1}$. All of the methyl-d_3 compounds show this band very near the correct frequency.

The infrared curves of the six decalins and of three hexahydroindans are shown in infrared spectra-1,2 and 4–10 (see footnote 5 for the spectra of 3a-methyl-*trans*- and -*cis*-hexahydroindans). Some similarities can be observed. For example, a few bands in the *trans* isomers are absent in the *cis* and *vice versa*. Some bands are common to all of the compounds, and others are present only in the deuterated molecules. From the curves only the following generalizations can be made: The symmetric bending frequency of the methyl group occurs near 1380 cm.$^{-1}$ in CH_3 and near 1055 cm.$^{-1}$ in CD_3. Introduction of even a single deuterium atom causes profound changes in the fingerprint region and disappearance of the CH_3 band at 1380 cm.$^{-1}$.

The decahydro-4a-naphthalenemethanols (infrared spectra 6, 12) illustrate an interesting feature in the methylene bending region. These compounds were studied in carbon tetrachloride solution in this region so that extinction coefficients could be compared. It is seen that the steric environment has a pronounced influence on the position of the absorption of the methylene group. The ordinary *trans* isomer has a peak at 1490 cm.$^{-1}$, absent in the deuterated analog. The ordinary *cis* isomer has no such peak, but the intensity of the band at 1470 cm.$^{-1}$ is markedly reduced in its deuterated analog. Therefore, in the *cis* isomer, the corresponding methylene group absorbs at 1470 cm.$^{-1}$. That the effect is real is further borne out by the very close similarity of the absorption at 1470 and 1450 cm.$^{-1}$ in the deuterated alcohols; this absorption is likely due to the ring methylenes, which would be virtually unaffected by going from two *trans*-fused chair forms of cyclohexane to two *cis*-fused forms. This observation lends support to the suggestion made earlier that the steric environment of the methyl group affects the frequency of some of its vibrational modes. The symmetric bending mode, however, seems entirely independent of steric factors, for it occurs at 1380 cm.$^{-1}$ in both isomers as well as in the hexahydroindans.

The extension of the above results to the sterols themselves did not prove fruitful. After studying a number of infrared spectra of sterols related to androstane and etiocholane,[26] no peaks were found that were common to either series. Thus, the only structural feature which can be deduced as a result of the present infrared study relates to the angular methyl group. It absorbs at 1380 cm.$^{-1}$ as an isolated methyl group does. Jones' assumption[27] has been confirmed within the limitations of the model approach.

Acknowledgment.—We hereby express our appreciation to Dr. R. N. Jones for discussions initiating this study and many additional conversations, and to the National Institutes of Health for their generous support.

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(25) N. Sheppard and D. M. Simpson, *Quart. Revs.* (*London*), **7**, 19 (1953).

(26) K. Dobriner, E. R. Katzenellenbogen and R. N. Jones, "Infrared Absorption Spectra of Steroids," Interscience Publishers, Inc., New York, N. Y., 1953.

(27) R. N. Jones and A. R. H. Cole, THIS JOURNAL, **74**, 5648 (1952).

BROOKLYN 1, NEW YORK

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN CO.]

Ozonolysis. II.[1] The Effect of Pyridine on the Ozonolysis of 4,22-Stigmastadien-3-one[2]

BY GEORGE SLOMP, JR., AND JAMES L. JOHNSON

RECEIVED SEPTEMBER 3, 1957

A quantitative infrared method was developed for determining the side-chain and nuclear double bonds of stigmastadienone. The rate of reaction of these double bonds with ozone was accordingly determined under various conditions. Presence of a small amount of pyridine had a striking effect on the selectivity of the reaction. A yield of 3-ketobisnor-4-cholen-22-al, the selective ozonolysis product, of over 90% is reported. Pyridine also caused the reaction to follow a different course. The formation of two aldehydes instead of the expected ozonolysis intermediates is attributed to a reaction of the zwitterion intermediate with the pyridine. Reductive "decomposition" with formaldehyde and a reaction of pyridine oxide and 3-ketobisnor-4-cholen-22-al are reported.

In the preparation of aldehydes by the ozonolysis of olefins it is of utmost importance to add exactly the correct amount of ozone to the solution because excess ozone causes undesirable side reactions which decrease the yield. Aldehydes are oxidized by ozone to acids and peracids,[3] and alcohols,

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(1) Preceding paper, G. Slomp, Jr., *J. Org. Chem.*, **22**, 1277 (1957).

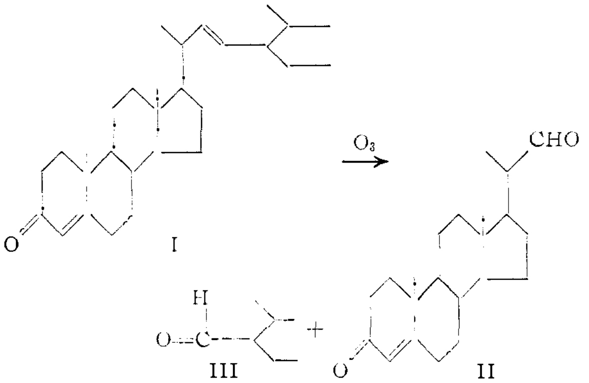
(2) Presented in part before the Division of Organic Chemistry at the 129th Meeting of the American Chemical Society, Dallas, Texas, April 8–13, 1956, Abstracts p. 30-N.

(3) (a) J. E. Leffler, *Chem. Revs.*, **45**, 400 (1949); (b) L. Long, Jr., *ibid.*, **27**, 437 (1940), and references cited therein.

ethers, and other functional groups which may be present in the molecule may also be attacked by ozone or by ozonized oxygen under certain conditions.[3b] Moreover, in some situations it may be desirable to cleave one double bond selectively over another less reactive double bond present in the same molecule.

An infrared absorption method which makes ozone control easy[4] has now been developed for following the course of certain ozonolysis reactions. Although this method has been applied to only three unsaturated steroids, it should be more widely applicable. This method, applied to ozonolysis reactions in the liquid phase, was an outgrowth of work reported in an earlier paper from these laboratories[5] in which an infrared method of analysis was used in a study of the selective and stereospecific hydrogenation of the nuclear double bond of 4,22-stigmastadien-3-one. The new method offers a convenient means for determining exactly when to stop the ozonolysis. It is especially useful for studying selective ozonolyses of compounds which possess two different double bonds since the extent of reaction of each one can be determined separately.

This method was applied to the ozonolysis of stigmastadienone and striking results were obtained. The nuclear and side-chain double bonds of stigmastadienone absorb at different wave lengths and are very different chemically. It appeared that the electrophilic ozone should attack the side-chain double bond much more rapidly than the nuclear one since the former possessed the greater electron density. Heyl and Herr[6] had already reported the above ozonolysis and the isolation of 3-ketobisnor-4-cholen-22-al in 60% yield and it seemed possible that by application of the infrared analysis method optimum conditions could be found so that better selectivity and higher yields could be attained.



The ozonolyses were performed in dry methylene chloride[7] with varying amounts of pyridine present and the rate of reaction of each double bond with ozone was determined by removing small samples from the reactor at timed intervals and analyzing them by the infrared method for the amount of each double bond remaining and the total amount of aldehyde present.

The Infrared Method.—Portions of the spectra obtained on the samples removed during an ozonolysis without any pyridine present are shown in Fig. 1. The absorption of the side-chain double bond at 10.26 μ decreased as shown until 1.52 molar equivalents of ozone had been added whereupon it remained nearly constant, the whole curve being displaced somewhat downward as additional ozone was added. This constant absorption was assumed to represent zero side-chain double bond remaining and with this as a base line, the amount of side-chain double bond present in each of the earlier samples easily was calculated.

The rate of reaction of the nuclear double bond accordingly was shown by the change in absorption of the conjugated ketone system at 6.0 μ (see Fig. 1). By the time 0.75 molar equivalent of ozone had been added this absorption band had diminished only slightly. A new band had appeared, however, at 5.82 μ and it was attributed to the formation of aldehydic carbonyls by the reaction of the side-chain double bond. It has recently been shown[8] that pure crystalline ozonides do not absorb in the carbonyl region. As the ozonolysis was continued, the conjugated-carbonyl absorption band diminished more rapidly and concurrent with the increasing attack on this system there appeared a new band at 5.67 μ and shoulders at 5.58 and 5.85. The identity of these new bands will be the subject of a later paper. The amount of nuclear double bond remaining in each sample was accordingly calculated from the 6.0 μ absorption band and the total amount of aldehydes (3-ketobisnor-4-cholen-22-al and 2-ethyl-3-methylbutanal) present in the reaction mixture was calculated from the 5.82 μ absorption band. All the results are compiled in Fig. 2.

When the reaction was repeated with a small amount of pyridine present, a new set of curves was obtained. Under these conditions the aldehyde band at 5.82 μ was much stronger and there was much less attack on the 3-keto-Δ^4-system.[9] These data also are included in Fig. 2.

Discussion.—The competitive rate curves derived from the infrared assays indicate where to stop the ozonolysis in order to achieve maximum selectivity and also give some indication of the order of selectivity obtained. In order to have the maximum yield of the desired unsaturated aldehyde the ozonolysis should be stopped at the point where the two double-bond-disappearance curves are the farthest apart. Without pyridine present this is at 1.1 ± 0.5 molar equivalents of ozone. The yield should be 70 to 85% (the dis-

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[4] R. D. Cadle and C. Schadt [*J. Chem. Phys.*, **21**, 163 (1953); THIS JOURNAL, **74**, 6002 (1952)] have determined the kinetics of the ozonolysis of ethylene in the gas phase by a similar method.

[5] G. Slomp, Y. F. Shealy, J. L. Johnson, R. A. Donia, B. A. Johnson, R. P. Holysz, R. L. Pederson, A. O. Jensen and A. C. Ott, *ibid.*, **77**, 1216 (1955).

[6] F. W. Heyl and M. E. Herr, *ibid.*, **72**, 2617 (1950).

[7] Methylene chloride was used instead of chloroform, since the superiority of it over chloroform as an ozonolysis solvent had already been demonstrated.[1]

[8] (a) R. Criegee, A. Kerckow and H. Zinke, *Chem. Ber.*, **88**, 1878 (1955); (b) E. Briner and E. Dallwigk, *Helv. Chim. Acta*, **39**, 1446 (1956).

[9] The absorption actually increased slightly. This was partly because the stigmastadienone was not all in solution at the start of the reaction but dissolved as the reaction progressed. In addition, there developed a slight overlap of the 5.82 and 6.00 μ bands which introduced a small error in the determination of the latter. This error was neglected.

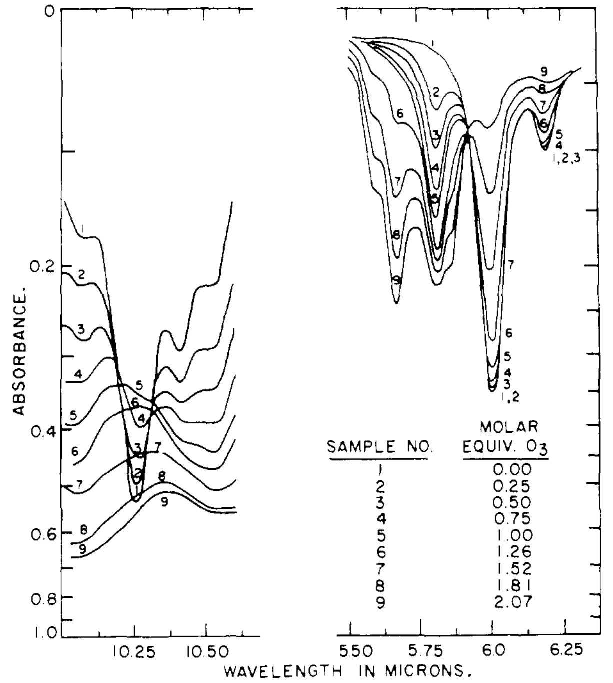


Fig. 1.—Portions of the infrared absorption spectra of samples from the ozonolysis of stigmastadienone in methylene chloride.

tance they are apart) depending on whether nuclear double bond was attacked in the product or in the unreacted starting material that remained. Similarly, with about one volume per cent. pyridine present (an optimum concentration chosen from many variable experiments) a maximum yield of about 95% should be obtained if 1.6 to 1.7 molar equivalents of ozone were used. These conclusions were confirmed when the reactions were repeated using the required amounts of ozone and the 3-ketobisnor-4-cholen-22-al was isolated by reductive decomposition and crystallization. The results agreed well with the predictions based on the rate curves. Thus, by the inclusion of pyridine it was possible to prepare the desired selective ozonolysis product in a high yield and purity.

The large difference observed in the amount of aldehyde detected with and without pyridine present prompted further investigation of this unusual result. In the absence of pyridine, the amount of aldehydes formed was about 50% or stoichiometrically about one aldehyde from one steroid molecule. The rate of their formation corroborated the assignment of the 5.82 μ band to the aldehyde formed from the cleavage of the side-chain double bond, since the rate of formation of aldehyde was diminishing just as the nuclear double bond was disappearing most rapidly. This was understood, according to the mechanism of Criegee,[10] to indicate that one end of the side chain double bond was converted to an aldehyde and that the zwitterion formed from the other end had dimerized or polymerized. Although this behavior was more common when the intermediate was a ketone, it might also be expected when the aldehyde was sterically hindered, as either of these would be.

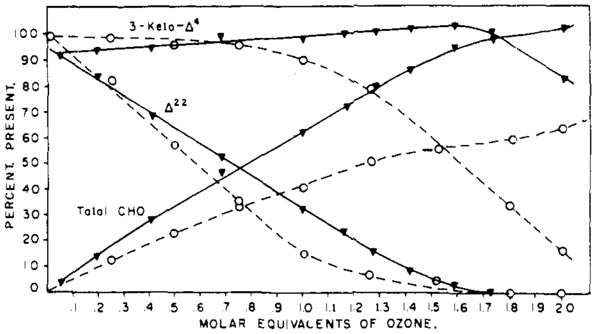
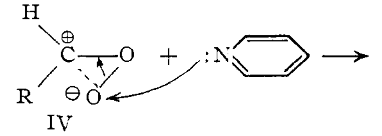


Fig. 2.—The ozonolysis of stigmastadienone in methylene chloride–pyridine mixtures. Competitive rate of reaction at Δ^{22} and Δ^4 and rate of appearance of aldehyde products: ○, 0.0 vol. %; ▽, 0.94 vol. % pyridine present.

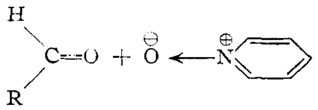
For the reaction with pyridine present the rate of appearance of aldehydes was approximately equal to that for disappearance of the side-chain double bond. The curve showed a plateau at about 95% present (or stoichiometrically about *two* aldehydes from one steroid molecule) when the 22,23-double bond was completely reacted. This indicated that *both* ends of the double bond were being converted to aldehydes during the ozonolysis, prior to zinc reduction steps.

This unusual result can be attributed to a reaction of the zwitterion intermediate with the pyridine. Based on the ozonolysis mechanism formulated by Criegee and co-workers,[10] a new mode of stabilization of the zwitterion IV is now postulated to explain the action of pyridine. The pyridine which probably solvates the zwitterion, could react with the latter to form pyridine oxide plus a second molecule of aldehyde. This may happen by attack on the oxygen of the zwitterion



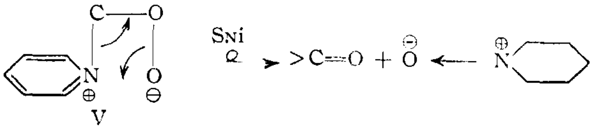
IV

R = $C_{21}H_{31}$ or C_6H_{13}



II or III

or, more likely, by addition to the zwitterion, followed by rearrangement of the resulting intermediate V.



V

That the pyridine was not simply reacting with the dimeric (or polymeric) aldehyde peroxide, converting it to additional aldehyde, was shown by adding pyridine to a solution of this material prepared by an ozonolysis with pyridine absent. The aldehyde

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(10) For leading references see footnote 2 of P. S. Bailey, THIS JOURNAL, **78**, 3811 (1956).

assay showed no change after addition of the pyridine.

Unfortunately, pyridine oxide could not be isolated from the reaction mixture because, as was shown in a separate experiment, the pyridine oxide reacted readily with the formed aldehyde if the temperature was raised to that of the room. Obviously, mechanical difficulties hamper the isolation of this reactive material at low temperatures, but there is further evidence that it is formed.

The observation was made that at the end of the ozone addition an oxidant could be iodimetrically titrated in the reaction mixture. When no pyridine was present, the amount of oxidant was equal to the amount of ozone added (see Table I) indicating that all of the active oxygen which was added as ozone was still present in a titratable form, presumably the dimeric (or polymeric) aldehyde peroxide of Criegee.

TABLE I

IODOMETRIC TITRATION OF OZONOLYSIS REACTION MIXTURES

Pyr. concn., vol. %	Mmoles double bond ozonized	Ozone added		Mmoles of active oxygen titrated
		Mmoles	Molar eq.	
0	24.06	25.18	1.07	25.8
0.94	24.35	41.15	1.69	12.37

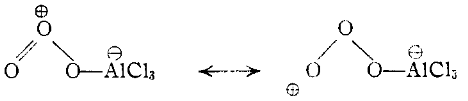
With pyridine present, on the other hand, more ozone was added but the titration showed much less oxidant or about the amount of the excess ozone added. Of the one molecule of ozone that was used to open the double bond, two oxygens were used to form aldehyde (this was evident from the infrared results already described). The third oxygen, therefore, must have been present in a form not titratable iodimetrically, which is true of pyridine oxide. When the linear portions of the curves representing the rate of disappearance of the side-chain double bond (Fig. 2) were extrapolated to complete reaction, it was apparent that about 0.3 molar equivalent more ozone was needed to cleave the side-chain double bond when pyridine was present than in its absence. Pyridine is not cleaved by ozone under these conditions[1]; neither is pyridine oxide. The titration shows that most of this excess ozone was still present in a form which was titratable as active oxygen. This oxidant was believed to be the pyridine oxide–ozone complex which was described earlier.[1] The rest of the missing ozone may have reacted with methylene chloride with pyridine catalysis. Further investigation showed that this oxidant was formed at a uniform rate, was stable on storing at −78°, but on warming was very rapidly consumed and large quantities of 3-ketobisnor-4-cholenic acid were formed. These observations are all in agreement with the properties of the pyridine oxide–ozone complex investigated earlier.[1]

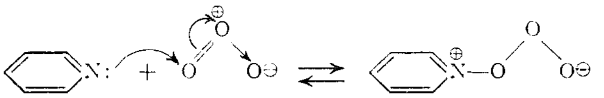
This proposed mechanism also allows a possible explanation for the increased selectivity of the ozonolysis reaction which was observed in the presence of pyridine. Pyridine has been used by others[6,11] in ozonolysis reactions, especially when chloroform was used as solvent, to combine with any acids which were formed by oxidation of the solvent. But, the present role of the pyridine appeared to be more complex than that of a simple acid-binding agent alone.

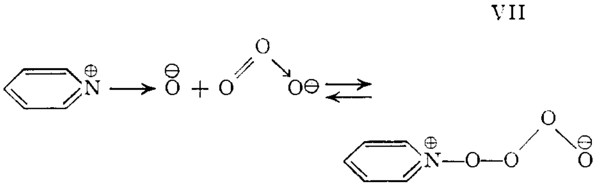
The possibility that the pyridine promotes selectivity of the ozonolysis of the side-chain double bond over that of the nuclear one by reacting with the conjugated carbonyl system, thus blocking it from further reaction with ozone, was considered. That this was not probable was shown from the examination of the infrared absorption spectra at low temperatures (*ca.* −50 to −60°) of a solution of stigmastadienone in methylene chloride with and without the pyridine present. The conjugated ketone absorption band was identical in both samples.

A possible explanation is that the pyridine slows the ozonolysis reactions enough so that differences in electronegativity of the two double bonds becomes important. A decrease in both the immediate ozone concentration and the electrophilic activity of the ozone could result from the formation of ozone complexes and the latter may have different selectivity characteristics than ozone itself.

The electrophilic nature of the ozone molecule has already been observed by Wibaut and coworkers.[12] They also showed[13] that the electrophilic activity of ozone can be catalyzed by acids of the Lewis type. This may happen in much the same way as in the more familiar Friedel–Crafts reaction, the acid combining with one of the terminal negatively charged oxygen atoms yielding a salt-like compound VI thereby enhancing the electrophilic nature of the ozone molecule. Nucleophilic agents such as pyridine and pyridine oxide, on the other hand, should accordingly react with ozone[14] to form compounds such as VII and VIII




VII


VIII

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[11] (a) W. Logemann and H. Dannenbaum, U. S. Patent 2,344,992; (b) W. L. Ruigh, U. S. Patent 2,413,000; (c) F. W. Heyl, A. P. Centolella and M. E. Herr, THIS JOURNAL, **69**, 1957 (1947); (d) F. W. Heyl and M. E. Herr, U. S. Patents 2,554,986 and 2,601,287; (e) F. W. Heyl and A. P. Centolella, U. S. Patent 2,623,052.

[12] J. P. Wibaut and H. Boer, *Rec. trav. chim.*, **74**, 241 (1955), and earlier papers, especially J. P. Wibaut, F. L. J. Sixma, L. W. F. Kampschmidt and H. Boer, *ibid.*, **69**, 1355 (1950).

[13] F. L. J. Sixma, H. Boer and J. P. Wibaut, *ibid.*, **70**, 1005 (1951).

[14] This attack is similar to that proposed by J. Meinwald [*Ber.*, **88**, 1888 (1955)] for nucleophilic attack on ozone by carbon.

which would be expected to show greatly reduced electrophilic activity and enhanced nucleophilic activity.[3a] It is noteworthy, in support of this theory, that the best selectivity was obtained when the amount of pyridine was about one mole per mole of stigmastadienone, and that at high pyridine levels the yield was actually observed to decrease. Here the nucleophilic activity of the terminal oxygen atom of the ozone complexes may have become of such importance that nucleophilic attack on aldehyde carbonyls may have occurred, yielding acid by hydride ion migration, thus lowering the yield of aldehyde.

This theory leads to the conclusion that the zinc reduction step is still necessary not to decompose ozonides as usual but to reduce the ozone complexes and the pyridine oxide. The zinc reduction also should be started at as low a temperature as possible, while the pyridine oxide–ozone complex is still stable, in order to obtain high yields of bisnoraldehyde. This was indeed found to be the case. If the above be true, then it should be possible also to decompose by adding a more reactive aldehyde to be attacked as the temperature of the mixture is raised. This prediction was tested by adding gaseous formaldehyde to an ozonolysis reaction mixture while allowing it to warm to room temperature. The yield and rotation are compared in Table II to that obtained by the usual zinc reduction, no reduction, trioxymethylene and dimethylsulfoxide treatment. Formaldehyde was distinctly better than the other methods of treatment and was nearly comparable to zinc reduction. The following reducing agents were also tried: aqueous sodium bisulfite, aqueous ferrous sulfate, sulfur dioxide,[15] aqueous sodium thiosulfate, catalytic hydrogenation (pyridine oxide is difficult to hydrogenate[16]), calcium hydride and acetic acid, and stannous chloride and acetic acid. Only the last one was effective. It gave very good quality material but the yield was slightly less (84–85%) than that obtained by the usual zinc decomposition method.

TABLE II

DECOMPOSITION METHODS AS APPLIED TO OZONOLYSIS REACTION MIXTURES

Treatment	Yield, %	$[\alpha]_D$ of product[a]
Formaldehyde	91.8	+81.4°
Zinc reduction	93.8	+83.5
No reduction	77.2	+80.1
Dimethylsulfoxide	82.2	+80.5
Trioxymethylene	75.9	+80.8

[a] $[\alpha]_D$ of pure 3-ketobisnor-4-cholen-22-al is +85° ($CHCl_3$).

Acknowledgment.—The authors are indebted to Mrs. G. S. Fonken, Mrs. E. Jensen and Mr. M. Grostic for the measurement and both qualitative and quantitative interpretation of infrared spectra; and to Mr. William A. Struck and his associates for analytical data.

Experimental[17]

Infrared Assays.—Measurements were made on a Perkin-Elmer model-21 spectrometer equipped with a sodium chloride prism. The following instrument settings were employed: resolution, 2; response, 1; gain, 5; speed, 2.5 μ per minute. Samples of about 1 to 2 ml. volume were taken from the reaction vessel and warmed quickly to room temperature. Determinations were also made by injecting the cold sample into the precooled cell (wrapped in Saran wrap, packed in Dry Ice). The cell was quickly unwrapped, placed in the instrument, and the absorption determined. The results were the same as those obtained at room temperature when corrected for methylene chloride shrinkage.

a. Side-chain Double Bond.—A portion of each sample was loaded into a 0.489-mm. cell and placed in the sample beam. A 0.475-mm. cell containing the methylene chloride solvent was placed in the reference beam. The spectrum was recorded twice over the range 9.75 to 10.50 μ. The amount of double bond present was determined using the base-line technique.[18] It was necessary to vary the slope of the base line to fit the particular solvent system used. The slope for a given system was determined by carrying an exploratory ozonolysis well past completion of the side-chain double bond reaction. The slope for the particular solvent system was fixed when the slope of a line drawn through points on the spectra at 10.15 and 10.26 μ become constant. The amount of double bond present in each sample was determined by drawing the base line with the required slope from the point on the spectrum at 10.15 μ. The percentage of unreacted double bond was then calculated according to the equation

$$\% \text{ C=C} = 100 \times \frac{A_B \text{ of sample at time } T}{A_B \text{ of sample at zero time}}$$

where A_B = base line absorbance at 10.26 μ. The accuracy was determined on synthetic mixtures and was ±2%.

b. Nuclear Double Bond and Total Aldehyde.—The samples were diluted with an equal volume of methylene chloride, loaded into a 0.125-mm. cell, and placed in the sample beam. A 0.112-mm. cell containing methylene chloride solvent was placed in the reference beam. The spectra were recorded twice over the range 5.5 to 6.5 μ. Standard samples of 3-ketobisnor-4-cholen-22-al and 2-ethyl-3-methylbutanal of equivalent molar concentrations in methylene chloride–pyridine solvent were run for each series.

Differential absorbancies at 5.82 and 6.0 μ were tabulated. The amounts of nuclear double bond and of total aldehyde formed were calculated as

$$\% \text{ 3-keto-}\Delta^4 = 100 \times \frac{A_{S,\,6.0} - Y}{K_{a,\,6.0}}$$

$$\% \text{ total aldehyde} = 100 \times \frac{A_{S,\,5.82} - Y}{K_{b,\,5.83} + K_{c,\,5.82}}$$

where

S = sample
a = stigmastadienone
b = 3-ketobisnor-4-cholen-22-al
c = 2-ethyl-3-methylbutanal
A = absorbance
K = absorbance of the std. solns. (specific absorbance)

$$X = A_{S,\,6.0} \times \frac{A_{a,\,5.82}}{A_{a,\,6.0}}$$

$$Y = \frac{K_{b,\,6.0} \times \% \text{ total aldehyde}}{100}$$

The accuracy was determined on synthetic mixtures and was ±3%. Samples from the ozonolysis of blanks of methylene chloride containing pyridine and pyridine oxide showed a negligible infrared absorption at 5.82 μ.

Ozonolysis of Stigmastadienone.—For the rate studies a mixture of 10.00 g. (24.35 millimoles) of stigmastadienone (m.p. 128–129.5°, $[\alpha]_D$ +58°) in 250 ml. of dry methylene chloride and the indicated amount of dry pyridine in a 250-ml. tubular reactor equipped with a magnetic stirrer was ozonized at Dry Ice temperature. The ozonized oxygen was introduced at a constant ozone addition rate through a sparger having about fifteen tiny holes and reaching nearly to the bottom of the vessel. Samples, 2 ml. in volume, were withdrawn at about 15-minute intervals and assayed imme-

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[15] B. Witkop and S. Goodwin, THIS JOURNAL, **75**, 3374 (1953).

[16] C. C. J. Culvenor, *Rev. Pure and Appl. Chem.*, **3**, 83 (1953).

[17] Rotations, $[\alpha]_D$, were determined in chloroform at 23 to 25°.

[18] J. J. Heigl, M. R. Bell and J. U. White, *Anal. Chem.*, **19**, 293 (1947).

diately. The amount of ozone charged was calculated from the concentration meter[19] readings.

When the reaction was repeated using the optimum amount of ozone, the 3-ketobisnor-4-cholen-22-al was isolated as follows: The reaction mixture was removed and added without delay to 12.5 g. of zinc dust in a 500-ml., three-necked, round-bottomed flask. At the same time 25 ml. of glacial acetic acid was added. The temperature was raised quickly to 20° by means of a water-bath and the mixture was stirred for two hours. After filtration the solution was diluted with 250 ml. of Skellysolve B and washed with three 200-ml. portions of water. Crushed ice was added and washing was continued with three 50-ml. portions of 5% sodium hydroxide and then to neutrality with water, three 200-ml. washes being required. Each wash was backwashed with the same 250 ml. solution of 1:1 methylene chloride and Skellysolve B. The combined solution was dried over sodium sulfate and concentrated at reduced pressure, at a temperature below 60°, to about 50 ml. at which volume the product began to crystallize from the solution. The mixture was stored at $-10°$ overnight, filtered, washed with 5 ml. of cold Skellysolve B and dried in an oven at 40° and 20 mm. pressure. The yield when 2.37 ml. of pyridine and 40.42 millimoles of ozone were used was 7.50 g. (93.8%) of 3-ketobisnor-4-cholen-22-al, $[\alpha]_D$ +83.5°, 0.19% titratable acid present, 1 ± 2% dienone remaining and 100 ± 3% purity by infrared analysis.

When the ozonolysis was carried out at higher temperature, $-55°$, the yields were lower, the optimum amount of ozone was less, and larger quantities of 3-ketobisnor-4-cholenic acid were formed even when less than the optimal amount of ozone was used, *i.e.*, aldehyde was being oxidized to acid.

The ozonolysis was repeated as above but without pyridine present using 26.16 millimoles of ozone (1.07 molar equivalents). The product was isolated as in the previous example and yielded 7.14 g. (89.3% yield) of neutral fraction, $[\alpha]_D$ +81.2°, 5.4% titratable acid present, 2 ± 2% dienone remaining and 92 ± 3% bisnoraldehyde. The yield of bisnoraldehyde was calculated to be 82%.

2-Ethyl-3-methylbutanal (III).—4,22-Stigmastadien-3-one was ozonized and the 3-ketobisnor-4-cholen-22-al was isolated according to the procedure described above. The mother liquors from several runs were combined and steam distilled. The impure aldehyde (232 g.) was separated from the water, dried over anhydrous sodium sulfate, and fractionated through a packed column under reduced pressure. The 2-ethyl-3-methylbutanal was collected at 83–83.5° at 71 mm. (reported[20] b.p. 65–70° (754 mm.)). It had a mint-like odor and was optically active. It was observed to racemize and autoxidize on storing and the rate was accelerated by acid and slowed greatly by presence of a small amount of pyridine.[21] A center-cut fraction showed the following properties: $[\alpha]_D$ +37° (no solv.), d^{20}_4 0.83, n^{23}_D 1.4102, 2,4-dinitrophenylhydrazone m.p. 121.5–122° (reported[20] m.p. 115–117°).

Anal. Calcd. for $C_{13}H_{18}O_4N_4$: C, 53.05; H, 6.16; N, 19.04. Found: C, 53.61, 53.48; H, 5.79, 5.70; N, 18.92.

The semicarbazone melted at 132–133° (reported[22] m.p. 127–128°).

Anal. Calcd. for $C_8H_{17}ON_3$: C, 56.11; H, 10.01; N, 24.54. Found: C, 55.66; H, 9.64; N, 23.58.

The attempted preparation of the oxime yielded instead crystalline material, m.p. 128–132°, believed by infrared analysis to be the hydroxamic acid. Infrared analysis of the distillation residue showed the presence of 3-ketobisnor-4-cholenic acid, 3-keto-20α-bisnor-4-cholen-22-al,[23] 2-ethyl-3-methylbutanoic acid and steroids which were opened in the A-ring absorbing at 1805 cm.$^{-1}$.

2-Ethyl-3-methylbutanoic Acid.[20,24]—The acid was prepared by oxidation of the aldehyde with potassium permanganate. A center-cut fraction showed b.p. 105° (17 mm.), n^{25}_D 1.4180. The acid chloride was prepared from racemic acid with thionyl chloride, b.p. 80–81.5° (75 mm.), n^{25}_D 1.4276, d^{25}_4 0.957.

The anilide, prepared from the acid chloride, was obtained as white needles, m.p. 125–126°.

Anal. Calcd. for $C_{13}H_{19}ON$: C, 76.05; H, 9.24; N, 6.82. Found: C, 75.93; H, 8.97; N, 6.87.

The amide also was prepared from the acid chloride, m.p. 133–134°.

Anal. Calcd. for $C_7H_{15}NO$: C, 65.06; H, 10.70; N, 10.84. Found: C, 66.10; H, 10.62; N, 10.63.

The acid was prepared also by blowing air through acidified (*p*-toluenesulfonic acid) mother liquors. The acid was isolated by distillation. It was preceded by an impure fraction of 2-ethyl-3-methyl-1-butanol[20] which may have been formed during the zinc reduction of the ozonolysis reaction mixtures.

Dimeric (or Polymeric) Aldehyde Peroxide plus Pyridine.—To the cold reaction mixture after completion of the ozonolysis of stigmastadienone without pyridine present was added 2.37 ml. of pyridine, and the solution was stored three hours at $-78°$. Infrared analysis showed no increase in aldehyde content and hence no reaction of pyridine with the aldehyde peroxide was apparent.

Pyridine Oxide Plus Bisnoraldehyde.—A 6.66-g. (20.28 mmoles) sample of 3-ketobisnor-4-cholen-22-al, $[\alpha]_D$ +84°, 0.12% titratable acid, 0.5 ± 2% dienone, 103 ± 3% purity (by infrared assay), was dissolved in 250 ml. of methylene chloride. To this solution was added 0.96 g. (12.18 mmoles) of pyridine oxide (Reilly Tar) containing 0.39 mmole of hydrogen peroxide as impurity (by iodometric titration) and the solution was allowed to stand at room temperature for three days. The solution was subjected to the usual zinc decomposition and washing procedure (described for ozonolysis with pyridine, above), four alkali washes being necessary to remove all the acid present. The neutral fraction crystallized from 30 ml. of solution and was collected by filtration. The yield after drying was 1.56 g. (23.4% by weight from aldehyde). The product contained no bisnoraldehyde by infrared assay, but showed new absorption bands at 1710 and 1194 cm.$^{-1}$ which were attributed to the 20-formate. There was some loss of conjugated ketone in the A-ring and a new band at 1754 cm.$^{-1}$ was believed to be due to opening of the latter to a six-membered enol-lactone. Recrystallization of 0.31 g. from acetone yielded 0.08 g., m.p. 206–210°. This material was saponified by sodium hydroxide in methanol to yield a steroidal alcohol with intact 3-keto-Δ^4-system (by infrared analysis), m.p. 190–200°.

The alkali washes were acidified with concentrated hydrochloric acid and extracted with three 250-ml. portions of chloroform. The organic phase was dried and evaporated to yield 3.8 g. of acidic fraction which was largely bisnoracid by infrared analysis but also contained a small amount of impurity believed to be the 3,5-seco-4-nor-3-oic acid.

A sample of material having the same infrared spectrum as the above formate was isolated[25] from the chromatography, over 100 g. of Florisil, of 35 g. of bisnoraldehyde produced by ozonolysis in the presence of pyridine. There was obtained 0.1 g., $[\alpha]_D$ +73° (chf.), m.p. 189–205°.

Titration for Oxidant.—An erlenmeyer flask was prepared with 25 ml. of glacial acetic acid, 2.5 g. of potassium iodide and 1.5 g. of sodium bicarbonate. A pipet was precooled in Dry Ice and a 10-ml. sample of the reaction mixture (from the ozonolysis of 10 g. of stigmastadienone and 2.37 ml. of pyridine in methylene chloride) was removed and titrated in the above solvent mixture with 0.1 *N* sodium thiosulfate. A volume correction of 1.128 was applied for the expansion of methylene chloride when the temperature was raised from $-78°$ to room temperature. The oxidant was calculated as peroxide oxygen. The titers increased at a uniform rate as the ozonolysis progressed. The final value is recorded in Table I. On storing the reaction mixture at $-78°$ no change in titer was observed. A sample was allowed to warm. When it reached room temperature (about one hour later) the titer was reduced to about one-half. It became zero in a few days.

Formaldehyde Decomposition of Ozonolysis Mixture.—Upon completion of a standard ozonolysis reaction the ozone-rich oxygen stream was replaced with a stream of gaseous formaldehyde (generated by heating trioxymethylene poly-

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(19) C. Alway and G. Slomp, *Advances in Chem. Series*, **21**, in press.

(20) W. Dirscherl and H. Nahm, *Ber.*, **76B**, 635 (1943).

(21) Base-catalyzed enolization of this aldehyde should be slow for both steric and electronic reasons.

(22) A. Guiteras, *Z. physiol. Chem.*, **214**, 89 (1933).

(23) M. E. Herr and F. W. Heyl, THIS JOURNAL, **74**, 3627 (1952).

(24) (a) A. W. Crossley and H. R. Lesueur, *J. Chem. Soc.*, **77**, 83 (1900); (b) C. D. Nenitzescu and I. Chicos, *Ber.*, **68B**, 1584 (1935); and (c) J. C. Shivers, B. E. Hudson and C. R. Hauser, THIS JOURNAL, **66**, 309 (1941).

(25) Unpublished work by B. A. Johnson of these laboratories.

mer)[26] for one hour. The solution was allowed to warm to room temperature. The next day a small sample was negative to potassium iodide–starch solution, hence the reaction product was diluted with 300 ml. of Skellysolve B and worked up by the usual washing and crystallization procedure. There was obtained 7.35 g. (91.8% yield) of bisnoraldehyde, $[\alpha]_D$ +81.4° (chf.), 8.37% titratable acid, 4 ± 2% dienone, and 85 ± 3% purity by infrared analysis.

Stannous Chloride Decomposition of Ozonolysis Mixture.—The cold ozonolysis mixture was poured onto 20 g. of anhydrous stannous chloride, 8 ml. of concentrated hydrochloric acid was added with stirring, and the mixture was quickly warmed to 25°, stirred for 4.5 hours and allowed to stand overnight. To the orange-brown mixture was then added 300 ml. of Skellysolve C and 250 ml. of water. It was stirred for 5 minutes and then transferred to a separatory funnel. The organic phase was separated and washed again with 150 ml. of water, three times with cold 5% sodium hydroxide at 5°, and then with water to neutrality, each wash being backwashed with a single solution of 125 ml. of methylene chloride and 250 ml. of Skellysolve C. The combined organic phase was dried over anhydrous sodium sulfate and concentrated at reduced pressure (temperature between 40–50°) to 50 ml. volume. The product crystallized from the mixture overnight. The product was collected on a filter and dried at room temperature at 5–10 cm. pressure over concentrated sulfuric acid. There was obtained 6.65 g. (85.2% of theory), $[\alpha]_D$ +87.1° (chf.), 98.1% pure by infrared assay, 0.51% acid by titration and 2% dienone by infrared assay.

KALAMAZOO, MICHIGAN

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(26) J. F. Walker, "Formaldehyde," Reinhold Publ. Corp., New York, N. Y., 1953, p. 36.

[CONTRIBUTION FROM THE INSTITUTE OF APPLIED MICROBIOLOGY, UNIVERSITY OF TOKYO]

Steroid Studies. VI.[1] Studies on the Constitution of Sargasterol

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Fucosterol was isolated from brown algae with the exception of *Sargassum ringgoldianum* Harvey which yielded sargasterol (I), which was shown to have two double bonds in the 5,6- and 24,28-positions. Upon Oppenauer oxidation compound I yielded sargastadienone (II) and upon ozonolysis the acetate Ib gave 20-iso-24-ketocholesteryl acetate (VIII). Our results indicate that I is C_{20}-isofucosterol: Wolff–Kishner reduction of VIII gave 20-isocholesterol (IX). The treatment of II with N-bromosuccinimide and subsequent dehydrobromination gave the trienone III from which 3-keto-Δ^4-20-iso-bisnorcholen-22-al (IV) was obtained by ozonolysis. The dienones of sargasterol and fucosterol (XI and XVIII, respectively) yielded the same trienone XIII which upon ozonolysis gave progesterone.

Fucosterol which was isolated from *Fucus vesiculosus* by Heilbron, *et al.*[3], is the only well-characterized sterol obtained from brown algae (*Phaeophyceae*). We have extracted sterols from *Phaeophyceae* growing in the seas off Japan; the yields of crude sterols are given in Table I.

TABLE I

Algae	Crude oil from dry algae, %	Unsaponifiable matter from crude oil, %	Crude sterol from unsap. matter, %
A. *Sargassum ringgoldianum* Harvey[a]	1.86–2.95[e]	22.8–30.0[e]	32.2–63.0[e]
B. *Eisenta bicyclis* (Kjellman) Setchell[a]	0.65	20.4	56.6
C. *Cystophyllum hakodatenese* Yendo[b]	0.50	24.8	40.6
D. *Fucus evanescens* Agardh[c]	3.6	14.0	40.8
E. *Pelvetia wrightii* (Harvey) Yendo[c,d]	3.0	12.5	54.3
F. *Costaria costata* (Turner) Sanders[b]	2.3	7.7	12.7

[a] From Aburatsubo Bay (May). [b] From Atsukeshi in Hokkaido (June). [c] From Fuyushima in Hokkaido (June). [d] K. Shirahama, *J. Agricul. Chem. Soc. Jap.*, **11**, 980 (1936); **12**, 521 (1937). [e] T. Kaneda and S. Ishii, *Bull. Jap. Soc. Scient. Fisheries*, **15**, 608 (1950); T. Kaneda, *ibid.*, 17, 20 (1952).

The sterols obtained from B–F were identified as fucosterol, since upon Oppenauer oxidation they yielded fucostadienone[4] and upon ozonolysis they gave 24-ketocholesterol[5-7] and acetaldehyde.

Since the sterol from A yielded a stadienone (II) and a 24-keto-steryl acetate (VIII) which were not identical with the corresponding fucosterol derivatives (m.p. and α_D), it was named sargasterol (I) and its structure investigated.

Perbenzoic acid oxidation and bromination of sargasterol showed the presence of two double bonds; the differences in rotation between the sterol and its derivatives, and its oxidation to a conjugated ketone showed the presence of a 5,6-double bond. That the second double bond in sargasterol is located in the side chain between carbon atoms 24 and 28 was shown by the ozonolysis fragments. Sargasteryl acetate (Ib) was readily reduced with platinum oxide in ethyl acetate to 5,6-dihydrosteryl acetate (V)[8] and with palladium black in ethyl acetate to 24,28-dihydrosteryl acetate (VI). Both dihydrosteryl acetates (V and VI) were reduced to the same stanyl acetate (VII) in an acid medium with platinum oxide. Although this stanyl acetate (VII) seemed to be pure reduction gives rise to a new asymmetric center.

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(8) The referee suggested that the fact that the reduction of Ib with platinum attacks selectively the 5,6-double bond rather than the more reactive double bond of the side chain is an abnormal phenomenon. The observed Δ-value in going from Ib to the V is +154. This is in disagreement with the accepted value of about +300. However, the infrared spectrum of V has maxima (823 and 820 cm.$^{-1}$) corresponding to the $\Delta^{24(28)}$ (all the compounds of $\Delta^{24(28)}$-fuco and sarga series have one or two maxima in the region 824–819 cm.$^{-1}$) and has no absorption bands of Δ^5 (840 and 800 cm.$^{-1}$). The above problem is under study.