A Direct, Low-Temperature ^1H, ^{13}C, and ^{19}F Nuclear Magnetic Resonance Study of Boron Trifluoride Complexes with Stigmasterol, Androstanolone, Androsterone, Testosterone, Nortestosterone, Androstenedione, and Progesterone[1,2]

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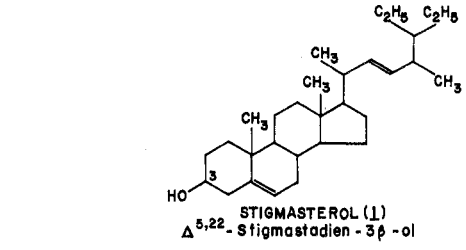
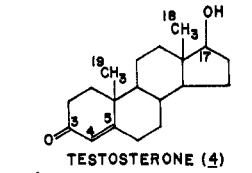
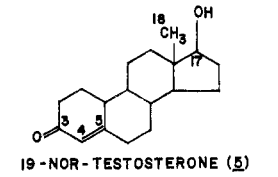
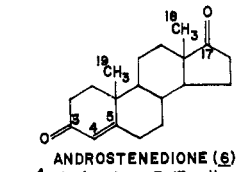
A study of boron trifluoride complexes with stigmasterol (1), androstanolone (2), androsterone (3), testosterone (4), nortestosterone (5), androstenedione (6), and progesterone (7) has been carried out by direct, low-temperature ^1H, ^{13}C, and ^{19}F nuclear magnetic resonance methods. A consideration of the ^1H and ^{19}F NMR spectra of the complexes led to an identification of the primary interaction site in each steroid. The steroid functional group basicities decrease in the order OH > C=O (α,β unsaturated) > C=O (saturated). In five of the systems, complex formation occurs at one site in the ligand, whereas competitive complexation was evident in solutions of 4 and 5. The ^{13}C NMR spectrum of BF_3–6 was interpreted in terms of possible electron density changes occurring in the molecule.

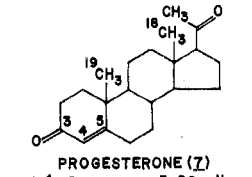
Solutions of a variety of organic bases with boron trihalides have been investigated by calorimetric[3-7] and spectroscopic[8-18] techniques to evaluate the heats of formation of the complexes and their structural features. The more recent measurements of these acid–base systems have utilized direct, low-temperature nuclear magnetic resonance (NMR) methods.[19-27] In the presence of excess base and at temperatures low enough to reduce the rate of exchange, it is possible to observe separate resonance signals for bulk and coordinated ligand. This observation leads to an accurate measure of the ^1H, ^{11}B, ^{13}C, and ^{19}F NMR chemical shift changes produced by complex formation and the stoichiometry of the complex, a qualitative estimate of steric factors, an evaluation of the ligand preference of a boron trihalide in a mixture of bases, and a determination of the most active site in complicated molecules. These features have been evaluated for amines and phosphines,[19] oxygen-containing bases,[20,21] pyridines[22,23] and other nitrogen heterocycles,[24] esters,[25] cyclic ketones,[26,28,29] several ethers,[27] and three steroids.[30] Steroids are of interest from the viewpoints of their physiological importance, and their structural features, particularly, the multiple potential sites for interaction with Lewis acids. Since it has been demonstrated that this low-temperature NMR technique is particularly well suited for identifying principal interaction sites in molecules,[25,30] this approach was used here. The steroids chosen were stigmasterol and several androgens, namely, androstanolone, androstenedione, androsterone, 19-nortestosterone, progesterone, and testosterone (see structures).











Experimental Section

Methods. The boron trifluoride was CP grade (J. T. Baker) and 99.5% pure. The dichloromethane solvent and 2-cyclohexeu-1-one were reagent grade and they were dried over $CaSO_4$ before use. The steroids were purchased from Steraloids, Inc., and they were used as received. The purity of the steroids was verified by the absence of extraneous carbon-13 (^{13}C NMR) signals and the dryness of each sample was checked by the absence of a ^{19}F NMR signal for the BF_3–H_2O adduct. The BF_3 was fractionated twice at −100° and condensed in vacuo in the NMR sample tube (Wilmad Glass Co., 504PP). The tube was sealed, warmed in an acetone–Dry Ice mixture to dissolve the components, and stored in liquid nitrogen until the spectrum could be recorded. With these precautions, sample decomposition was negligible as determined by ^1H, ^{13}C, and ^{19}F NMR spectra.

The ^1H and ^{19}F NMR chemical shift and area measurements were made on a Varian A-60 and a Varian HA-100 spectrometer, the latter operating at 94.1 MHz for the study of ^{19}F nuclei. The ^{13}C NMR spectra were recorded at 22.6 MHz with a Bruker HX-90-E instrument equipped with a Bruker-Nicolet Data System, Model B-NC-12. Pulse widths of about 3 μsec (7 μsec produces a 90° tip angle) were applied at 1-sec intervals. Hydrogen nuclei were noise decoupled at 90 MHz and 2000 Hz bandwidth. At the steroid concentration used, 5000 pulses were sufficient for a reasonable signal to noise ratio. Measurements over the temperature range of −150 to 200° are possible with the three instruments.

The procedure for carrying out the NMR measurement is described in more detail elsewhere[20-25] and it involves cooling the sample in the spectrometer probe to reduce the rate of ligand exchange. When separate ^1H or ^{13}C NMR signals are observed for bulk and coordinated ligand molecules, or multiple ^{19}F NMR signals for BF_3 when this molecule is bound at more than one site, the temperature is adjusted to maximize spectral resolution. The complete spectrum is recorded at this point for chemical shift data, and area integrations are measured electronically. Area data were obtained only from the ^{19}F NMR spectra, since spectral complexi-

Table I
^1H and ^{19}F Chemical Shift Data for BF_3–Steroid Complexes

Base	Mole ratios[a] BF_3:base:CH_2Cl_2	Temp, °C	δ, ppm[b,c] ^1H B[d,e]	^1H C	^{19}F
1	1.00:2.90:850	0			10.2
2	1.00:2.30:275	5	18 0.75	0.83	10.0
			19 1.25		
3	1.00:3.10:325	−5	18 0.88		10.8
			19 0.88		
4	1.00:2.70:250	5	18 0.80	0.88	10.1 (0.59)[f]
			19 1.22		13.4 (0.28)
					11.0 (0.13)
5	1.00:2.60:270	5	18 0.79	0.86	10.1 (0.59)[f]
					13.4 (0.31)
					10.9 (0.10)
6	1.00:3.10:325	−15	18 0.92		13.3
			19 1.20	1.36	
7	1.00:3.50:340	−5	18 0.66		13.3
			19 1.20	1.26	

[a] The accuracy of the mole ratios is 1–2%. [b] The ^1H chemical shifts (parts per million) are relative to internal tetramethylsilane (Me_4Si). [c] The ^{19}F chemical shifts are relative to internal hexafluorobenzene, which appeared at higher field in all cases. [d] The numbers 18 and 19 refer to the steroid methyl group carbon atoms. [e] The letters B and C refer to the signals of bulk and coordinated steroid molecules, respectively. [f] The relative signal areas are given in parentheses.

Table II
Fluorine-19 Chemical Shift and Area Data for BF_3–Steroid Mixtures

Base mixture (A:B)	Mole ratios[a] (BF_3:A:B:CH_2Cl_2)	Temp, °C	δ, ppm[b]	Assignment[c]
2:6	1.00:0.90:0.80:55	−10	13.4 (0.11)[d]	6
			11.0 (0.07)	2 or 6
			10.0 (0.82)	2
2:7	1.00:0.70:0.90:75	−10	10.0	2
3:6	1.00:0.60:0.60:120	−15	13.3 (0.32)	6
			10.7 (0.68)	3
3:7	1.00:1.90:2.00:130	0	10.9	3
4:6	1.00:1.30:1.30:85	−10	13.4 (0.19)	4 or 6
			10.6 (0.16)	4
			9.2 (0.65)	4
4:7	1.00:0.70:0.70:85	0	10.0	4
5:6	1.00:0.90:0.90:170	−10	13.4 (0.16)	5 or 6
			10.5 (0.09)	5
			10.0 (0.75)	5
5:7	1.00:0.80:0.80:50	0	13.4 (0.36)	5 or 7
			10.0 (0.64)	5

[a] The mole ratios are accurate to 1–2%. [b] The chemical shifts were measured with respect to internal C_6F_6, which appeared at higher field in all cases. [c] The steroid molecules giving rise to the signals in the preceding column are listed. [d] The relative signal areas are given in parentheses.

ty (^1H) and the nature of the Fourier transform pulse experiments prevented reliable intensity measurements with the other nuclei.

Results

The ^1H and ^{19}F NMR chemical shift and area results for the BF_3 complexes are given in Table I, and representative spectra for these nuclei are shown in Figures 1 and 2, respectively. The NMR data for each steroid were obtained

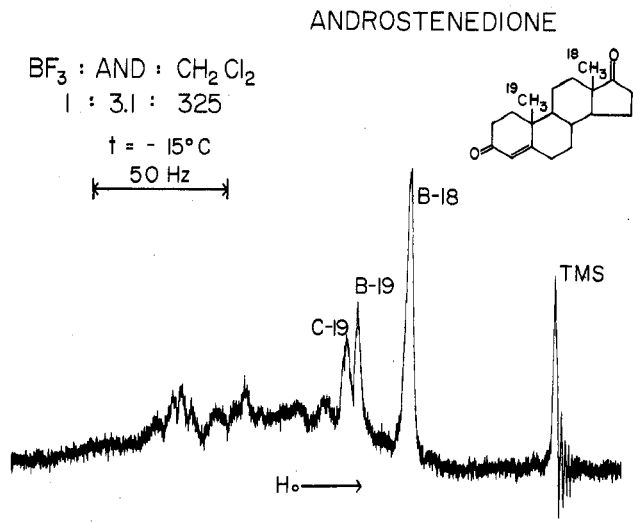


Figure 1. A portion of the ^1H NMR spectrum of a solution of BF_3 and androstenedione in methylene chloride, recorded on a Varian A-60 spectrometer, is shown. The signals arising from the 18- and 19-CH_3 groups of bulk (B) and coordinated (C) steroid are shown. Concentrations are in mole ratios.

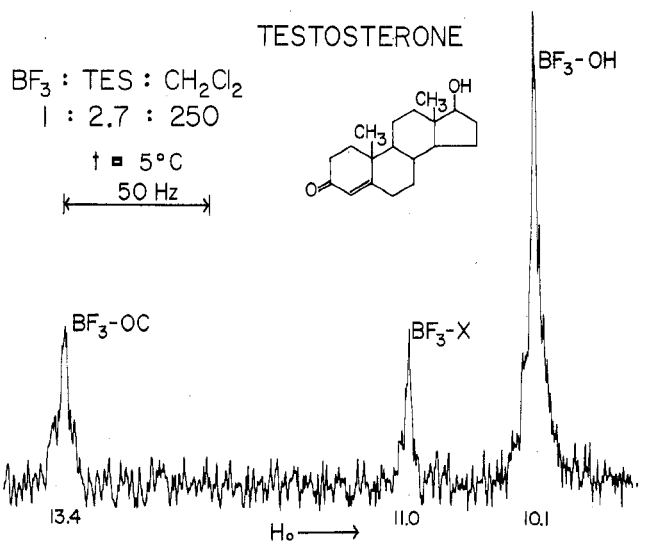


Figure 2. The ^{19}F NMR spectrum of a solution of BF_3 and testosterone in methylene chloride, recorded on a Varian HA-100 at 94.1 MHz, is shown. The linkage giving rise to each signal is identified (carbonyl and hydroxyl), although the origin of one peak (BF_3–X) is not clear (see text). Concentrations are in mole ratios.

with at least two samples, and each spectrum was recorded in triplicate. Although the composition of the BF_3–steroid complexes was not determined from the ^1H NMR signal areas, a 1:1 mole ratio was assumed for the adducts. This assumption is reasonable in view of the numerous studies of BF_3 complexes,[20-26] including those with steroids,[30] which have invariably indicated 1:1 complex formation. Since the 18-C and 19-C methyl group ^1H NMR signals were readily observed and assigned by a comparison to published spectra,[31] they were used to identify the steroid interaction site. An assignment was precluded by signal overlap only in solutions of **3**. In all cases the formation of BF_3 complexes produced low-field displacements of the methyl group ^1H NMR signals. The ^{19}F NMR chemical shift data also were used to identify the steroid interaction sites, and in solutions of **4** and **5**, in which BF_3 was bound at more than one functional group, signal areas provided a quantitative measure of this competition.

In Table II, ^{19}F NMR chemical shift and area data for

solutions of BF_3 and pairs of steroids are listed. The only mixtures given are those in which at least one ^{19}F NMR signal could be identified unambiguously. By reference to the chemical shift data of Table I, ^{19}F NMR signal assignments were made readily in the first four base mixtures listed. Signal overlap prevented a completely quantitative consideration of the remaining four mixtures.

Discussion

The condition of slow intermolecular exchange on the NMR time scale, sufficient to produce sets of resonance signals for bulk and coordinated ligand molecules, generally requires lifetimes of the order of approximately 0.1 sec. With the bases previously studied, exchange rates were inversely proportional to the ligand basicity and, consequently, the strength of the complex.[20-27] Since these experiments with alcohols and ketones require temperatures in the range of −100°, it is somewhat surprising that the slow exchange condition can be achieved here in the 0° range. The slower exchange in these solutions can be attributed to the larger size of the steroid molecules.

The identification of the principal interaction site in steroids 2–7 was accomplished primarily by a correlation of the proton and ^{19}F NMR chemical shift data, and in one case the results were supplemented by ^{13}C NMR measurements. With the exception of the complex of **3**, the observation of a separate ^1H NMR signal for the 18- or 19-CH_3 group of the coordinated steroid was possible. Since a change in diamagnetic shielding is the most significant factor causing ^1H NMR signal displacements in these complexes, it is safe to assume that the effect would be attenuated with distance from the interaction site. Thus, the signal of the methyl group closer to the site of BF_3 complexation would undergo the greater shift change. For example, in base **2**, the A-ring carbonyl and the D-ring hydroxyl are the two possible sites for complex formation with BF_3. The observation of a separate resonance signal for only the 18-CH_3 group, displaced approximately 0.1 ppm downfield from the signal of unbound steroid, indicates that binding is occurring primarily at the hydroxyl group oxygen atom. The structures of **4** and **5** differ by only one methyl group, and they are similar in that each contains an α,β-unsaturated keto group in the A ring and a D-ring hydroxyl. Again, a separate ^1H NMR signal was observed for the 18-CH_3 group of complexed steroid in both cases, implying a dominant interaction at the D-ring hydroxyl. In bases **6** and **7**, which contain two carbonyl groups as possible interaction sites, the principal interaction site is the A-ring carbonyl, as reflected by the appearance of a signal for the 19-CH_3 of coordinated steroid.

The strong dependence of the ^{19}F NMR signal position of the BF_3 complex on the nature of the ligand functional group[24-26] was used to interpret the spectral data for this nucleus in Table I. Base **1** was chosen to provide a reference ^{19}F NMR signal, in this case +10.2 ppm from C_6F_6, for the BF_3–OH linkage. Since **6** and **7** have only carbonyl groups as potential interaction sites, the ^{19}F NMR signal at +13.3 ppm is indicative of complexation at this functional group. Moreover, the similarity of the ^{19}F NMR chemical shifts for the solutions of **6** and **7** identifies the A-ring carbonyl as the principal site. This conclusion also is consistent with the ^1H NMR data of Table I for these molecules. From these reference ^{19}F NMR data, and again, taking into account the ^1H NMR results previously discussed, interaction at the D-ring hydroxyl group of **2** and the A-ring hydroxyl of **3** can be inferred from the ^{19}F NMR spectra of these complexes. Competitive complexing at more than one steroid site is indicated by the three ^{19}F NMR signals produced by complexes of **4** and **5**. The signals at +10.1 and +13.4 ppm, respectively, can be assigned to the D-ring hydroxyl group and A-ring carbonyl of these molecules. The signal at about +11 ppm in both cases may correspond to the BF_3 complex of the enol form (HO–C_3=C_4–C_5=C) of these bases, or perhaps the complex which remains after proton dissociation of the D-ring hydroxyl group (BF_3^-–OC). This latter process may account for the somewhat higher resonance observed for the BF_3–**3** complex, but, if so, it is not clear why it does not occur with the complexes of **1** and **2**. The relative areas of the ^{19}F NMR signals for the BF_3 complexes of **4** and **5** show that the D-ring hydroxyl group is the primary interaction site, with significant competition from the α,β-unsaturated keto group of the A ring. Low signal intensity is probably responsible for the lack of an observable 19-CH_3 ^1H peak for the BF_3–**4** adduct. In those cases where competitive complex formation occurs, it was not possible to determine whether sites in the same or different molecules were utilized.

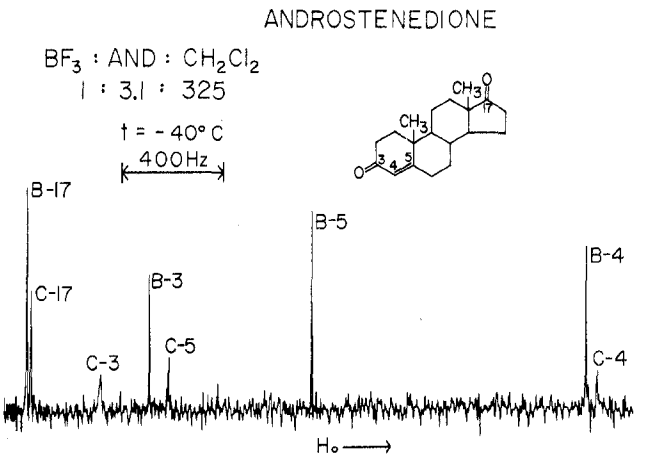


Figure 3. The low-field portion of the ^{13}C NMR spectrum of a solution of BF_3 and androstenedione in methylene chloride is shown. The spectrum is the accumulation of 5000 pulses, and it was recorded at 22.6 MHz on a Bruker HX-90-E Fourier transform spectrometer. The signals arising from carbon atoms of bulk (B) and coordinated (C) steroid are identified.

These results show that an unambiguous assignment of the principal steroid sites for interaction with Lewis acids can be deduced by a consideration of the ^1H and ^{19}F NMR spectra of their BF_3 complexes. In some cases, the availability of ^{13}C data can be a valuable supplement. For example, although the choice of the A-ring carbonyl as the interaction site in base **6** is reasonable based on the ^1H and ^{19}F NMR results, this was verified conclusively by the ^{13}C NMR spectrum of its BF_3 complex, the pertinent portion of which is shown in Figure 3. The steroid ^{13}C chemical shift assignments were made by a comparison to published spectra.[32] This spectrum clearly demonstrates a distinct advantage of ^{13}C NMR spectroscopy; that is, even nonprotonated functional groups, in this case the two carbonyl groups, can be studied. As seen in Figure 3, two sets of steroid ^{13}C NMR signals, arising from bulk and coordinated molecules, are evident. Two sets of high-field signals also were observed, but the complexity of the spectrum prevented an unambiguous assignment. From spectra such as that in Figure 3, chemical shift differences, $\delta_{complex} - \delta_{bulk}$, of +8.4, −2.2, +24.9, and −0.6 ppm were measured for the 3-, 4-, 5-, and 17-C signals, respectively, of the coordinated and bulk steroid molecules. The larger chemical shift displacement of the 3-C signal (+8.4 ppm), and the deshielding which the positive sign represents, confirm the A-ring carbonyl as the interaction site. It is surprising that com-

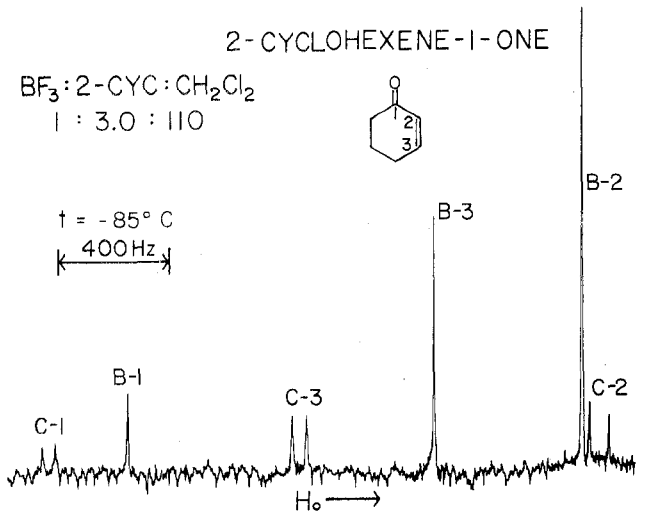


Figure 4. The low-field portion of the ^{13}C NMR spectrum of a solution of BF_3 and 2-cyclohexen-1-one in methylene chloride is shown. The spectrum is the accumulation of 5000 pulses, and it was recorded at 22.6 MHz on a Bruker HX-90-E Fourier transform spectrometer. The signals arising from carbon atoms of bulk (B) and coordinated (C) ligand are identified.

plexation at this site causes an observable shift at the 17-C position, ten bonds removed. A through-space interaction mechanism can be ruled out by the inflexibility of this steroid structure, leaving the transmission of the electronic changes through the molecular framework as the likely cause of this small shift. Also, the extremely large chemical shift displacement, +24.9 ppm, of the 5-C signal upon complex formation must reflect extensive charge withdrawal at this site. Interaction of the carbonyl oxygen and BF_3 would increase the contribution of the resonance form, $^{-}O-C_3{=}C_4-C_5^{+}$, and thus deshield the 5-C atom. This could account for the low-field shift. These features also were evident in the ^{13}C NMR spectrum of a mixture of BF_3 and 2-cyclohexene-1-one, which comprises the A ring of **6**. Only the low-field portion of the spectrum is shown in Figure 4, although bulk and complexed base signals were also evident in the high-field region. From such spectra, chemical shift differences of +12.5, −2.7, and +21.2 ppm were measured for the 1-, 2-, and 3-C signals, respectively, between the BF_3 complex and the free base. The magnitudes and signs of these shift differences compare well with those of Figure 3, and they again confirm complex formation at the A ring of steroid **6**. The appearance of doublets for all the ^{13}C NMR signals of complexed base is probably the result of slow cis–trans isomerization of BF_3 at the oxygen atom. This feature will be discussed in more detail in a subsequent paper dealing with a series of cyclic ketones.[33]

Although a good estimate of the relative base strengths of the steroids toward BF_3 can be made from the single base data for Table I, direct competition of ^{19}F NMR experiments with pairs of bases was carried out to establish this trend conclusively. Only those combinations for which at least one unambiguous signal assignment could be made are included in Table II. As mentioned previously, the signals at +10 and +13 ppm are attributed to the hydroxyl and carbonyl group adducts, respectively, and, with the possible exception of the entries for **3**, the peaks at +10.5 to +11 ppm in Table II are assigned to an enol or CO^{-} linkage with BF_3. The spectra for the first four combinations of Table II were interpreted readily. For example, **2** and **3** clearly dominate the BF_3 interactions in mixtures with **7**, since the one peak observed in both cases corresponds closely to that listed in Table I for these ligands. In the **2–6** and **3–6** mixtures, some competition is noted but **2** and **3** complex the much larger fraction of BF_3. Thus, from the first four entries, the steroid complexing abilities decrease in the order **2, 3 > 6 > 7.** In the spectra of the remaining mixtures, because of some ambiguity in signal assignment, one can only state that the binding abilities of **4** and **5** with BF_3 also are greater than those of **6** and **7.** Although no explanation can be offered, it should be noted that in the **4–7** combination, BF_3 binding occurs exclusively at the D-ring hydroxyl of **4,** in contrast to the single base study of this molecule, wherein a competition from the A-ring carbonyl was observed.

The assignment of binding abilities to the specific steroid functional groups can proceed directly from a consideration of the data of Tables I and II. The functional group basicities toward BF_3 decrease in the order OH (six-membered ring), OH (five-membered ring) > C=O (α,β-unsaturated keto) > C=O (saturated keto). Where a comparison can be made, this trend agrees with the order of proton basicities for the steroid components containing these functional groups.[34] For example, cyclohexanol is more basic than 2-cyclohexen-1-one, which in turn is more basic than cyclopentanone and cyclohexanone. These results also show that the steric hindrance introduced by the proximity of a methyl group (18-CH_3) is not sufficient to overcome the intrinsic basic strength of a nearby hydroxyl group. These results also may have some bearing on the physiological behavior of the steroids. For example, the requirement of a 17-β hydroxyl group for binding to human serum globulins has been demonstrated for several steroids, including **2, 4,** and **5,** and the biological activity of **7** is attributed to the presence of the A-ring carbonyl of this base.[35] The results presented here also show the strong binding abilities of these functional groups.

This study has demonstrated the utility of the direct, low-temperature NMR method for identifying the primary interaction sites in complicated ligands such as steroids. The availability of ^{13}C spectral data for acid–base complexes provides an insight into the electron density changes which occur upon complexation. Although solubility problems restricted the experiments to the steroids described here, the use of ^{19}F Fourier transform NMR techniques in future work should expand the scope of this area of study.

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Registry No.—**1**–BF_3, 54293-13-9; **2**–BF_3, 54293-14-0; **3**–BF_3, 54293-15-1; **4**–BF_3, 54293-16-2; **5**–BF_3, 54293-17-3; **6**–BF_3, 54293-18-4; **7**–BF_3, 54293-19-5; 2-cyclohexen-1-one–BF_3, 50781-10-7.

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Kinetics of Oxidation of Aldo Sugars by Quinquevalent Vanadium Ion in Acid Medium

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The oxidation kinetics of glucose, considered as a model compound for reducing sugars, with vanadium(V) in sulfuric and perchloric acid solutions has indicated a rate-limiting bimolecular reaction between vanadium(V) and the reducing sugars leading to the formation of a free radical. There is a linear correlation between the observed rate constant k_1 and [sugar], $[H^+]^2$, and $[HSO_4^-]$. The oxidation is faster in sulfuric acid, and various correlations between the rate and acidity have been tested. The mutarotation equilibrium is immediately attained and therefore the rate of oxidation is independent of the rate of mutarotation. The linear correlation between the rates of oxidation of sugars and their concentrations present in the solution as free aldehyde helps to explain the observed reactivity of different sugars, which is in the order xylose > arabinose > galactose > mannose > glucose.

The oxidation kinetics of reducing sugars with quadrivalent cerium in the presence of 0.5 *M* sulfuric acid has been discussed in a previous communication.[1] It is, therefore, of interest to us to investigate how the oxidation mechanism of reducing sugars is affected by a change in the one equivalent oxidant. The choice of quinquevalent vanadium ion is motivated by our additional interest in studying the correlation between the acid-catalyzed oxidation rate and various acidity scales.

The mechanism of the oxidation of various organic compounds by quinquevalent vanadium has been reviewed.[2] The kinetics of vanadium(V) oxidation of glucose and xylose in sulfuric, perchloric, and hydrochloric acid solutions was reported[3] after the review[2] was published. However, this study is not conclusive and needs a reinvestigation, as no attempt was made by the authors[3] to measure the rates of oxidation of other hexoses as well as to consider the effect of mutarotation equilibrium on the rate of oxidation of reducing sugars.

Experimental Section

Ammonium metavanadate was dissolved in sulfuric or perchloric acid solutions as required. The acid concentration of the stock vanadium(V) solution was taken as the difference between the amount initially added and the amount consumed by reaction 1.

$$NH_4VO_3 + 2H^+ \longrightarrow NH_4^+ + VO_2^+ + H_2O \quad (1)$$

The vanadium(V) solutions are quite stable. The sugar solutions were freshly prepared by direct weighing of the samples. The vanadium(V) solution was standardized against a freshly prepared standard solution of ferrous ammonium sulfate to a barium diphenylamine sulfonate end point in the presence of phosphoric acid.

The reaction has been studied in the presence of an excess of sugars and at 50° unless stated otherwise. The other experimental details for following the progress of the reaction from time to time and calculation of the observed rate constant k_1 with respect to vanadium(V) are similar to one described elsewhere.[4]

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Stoichiometry and Product Analysis. The reaction mixtures had an excess of glucose or mannose to ensure that there was no appreciable reduction of vanadium(V) by the more reactive oxidation products. Arabinose and formic acid were confirmed as the oxidation products in the glucose oxidation by paper chromatography using pure samples as reference.

In another set of experiments, the completely oxidized reaction mixtures were treated with barium carbonate to remove most of the sulfuric acid. The absence of formaldehyde, gluconic acid, and glucuronic acid in the reaction mixtures was established with color reactions of chromotropic acid[5a] and β,β'-dinaphthol,[5b] respectively. The filtrate and washings were subjected to fractional distillation in an all-glass apparatus fitted with standard joints. The distillate collected at 101–102° was made to a known volume and titrated against a standard alkali to a phenolphthalein end point. The distillate was confirmed to be formic acid by using chromotropic acid[5c] and paper chromatography.

The results of few quantitative estimations for the formic acid indicated that 2 equiv of vanadium(V) are used per mole of formic acid produced. The reaction is therefore expressed as in eq 2.

$$C_6H_{12}O_6 + 2V(V) + H_2O \longrightarrow C_5H_{10}O_5 + HCOOH + 2V(IV) + 2H^+ \quad (2)$$

Results and Discussion

The first-order dependence both in vanadium(V) and reducing sugars at any given acid concentration was established by effecting a tenfold variation in the respective concentrations at the constant concentration of the other. The rate increased with the increase in the ionic strength; lithium perchlorate was used for the purpose. (These data are available as supplementary material; see paragraph at end of paper.) There is no deviation from the first-order dependence in glucose even at 4 *M* sulfuric or perchloric acid as had been noted in the oxidation of butane-1,3-diol,[6] quinol,[7] and glycerol.[8] The linear plot (Figure 1) between the observed rate constant k_1 and [sugar] passes through the origin, thus confirming a first-order dependence in the reducing sugars.

The values of the second-order rate constant k_2 (Table I)