cleophile in the subsequent reactions of the 5-formamido intermediate.

Experimental Section

Materials. Adenosin, 2'-deoxyadenosine, 2',3'-*O*-isopropylideneadenosine, 9-β-D-arabinofuranosyladenine, adenine, inosine, and 4,6-diamino-5-formamidopyrimidine were commercial products of Sigma Chemical Co. They were used as received, after checking their purity by LC. [8-^{14}C]Adenosine was a product of NEN, which was prior to use mixed with unlabeled adenosine and crystallized from water. [1'-^{14}C]Adenosine was prepared from adenine and tetra-*O*-acetyl[1-^{14}C]ribofuranose by the method of Vorbrüggen et al.[18] The labeled ribofuranose tetraacetate was synthesized from [1-^{14}C]D-ribose (NEN) according to Guthrie and Smith.[19] The anomeric mixture of N^6-ribosyladenine was obtained by fusing adenine and D-ribose.[14] 5'-*O*-Methyladenosine and 9-(5'-*O*-methyl-β-D-arabinofuranosyl)adenine were gifts from the group of Prof. D. Shugar (University of Warsaw). Their preparation has been described elsewhere.[20]

Preparative Separation of the Intermediates. Intermediates 1βp, 2αp, 2βp, 3αp, and 3βp were separated preparatively on a Spherisorb RP-18 column (250 mm × 8 mm, particle size 5 μm) by using an acetic acid buffer (0.02 mol dm^{-3}, pH 4.3) containing 6% (v/v) of acetonitrile as eluant. The fractions obtained were lyophilized, and the buffer constituents were removed by passing the compounds through the column mentioned above with a mixture of water and acetonitrile as eluant. Finally the separated products were crystallized from a minimum volume of water.

Spectroscopic Measurements. The UV spectra were recorded on a Unicam SP 8100 spectrophotometer and the NMR spectra on a Jeol GX-400 spectrometer.

Isotopic Labeling Studies. The release of the ^{14}C8 atom from [8-^{14}C]adenosine and the [1-^{14}C]ribosyl group from [1'-^{14}C]-adenosine was followed as described earlier.[8]

Kinetic Studies by LC. The progress of the reactions of adenine nucleosides and compounds 1βp, 2αp, 2βp, 3αp, and 3βp with aqueous alkalies was followed by the LC technique described earlier.[8] The peak heights were transformed to concentrations with the aid of calibration solutions of known concentrations. The rate constants were calculated via the integrated first-order rate equation by using the data obtained during two half-lives. During this period the proportions of the possible reverse reactions were negligible and the first-order kinetics were strictly obeyed.

Acknowledgment. We thank Prof. D. Shugar and Dr. E. Darzynkiewicz for the generous gift of 5'-*O*-methyladenosine and 9-(5'-*O*-methyl-β-D-arabinofuranosyl)-adenine. Financial aid from the Academy of Finland, Research Council for the Natural Sciences, is gratefully acknowledged.

Registry No. 1βp, 10563-76-5; 2αp, 103960-07-2; 2βp, 103960-08-3; 3αp, 103960-09-4; 3βp, 103960-10-7; 4, 73-24-5; [8-^{14}C]adenosine, 3257-92-9; [1'-^{14}C]adenosine, 103960-06-1; adenosine, 58-61-7; 2'-deoxyadenosine, 958-09-8; 2',3'-*O*-isopropylideneadenosine, 362-75-4; 5'-*O*-methyladenosine, 20649-45-0; 9-(β-D-arabinofuranosyl)adenine, 5536-17-4; 9-(5'-*O*-methyl-β-D-arabinofuranosyl)adenine, 60738-17-2; D-ribose, 50-69-1.

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Direct Observation of the Reverse 1,5-Hydride Shift: The Mechanism of Acid-Catalyzed Isomerization at C-25 of Spirostanols

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Received April 5, 1986

(25*R*)-3β-Acetoxy-5α-[5,6,22-$^2H_3$]furostan-26-al (**9**) has been prepared by hydrogenation of diosgenin acetate under hydrogen gas in deuterated ethanol–ethyl acetate, containing a trace amount of deuterated perchloric acid, followed by oxidation. The deuterium atom at C-22 of the aldehyde **9** shifted to C-26 upon refluxing with hydrochloric acid in methanol giving 26α- and 26β-deuterated tigogenin in the ratio of 1:2 and 26α- and 26β-deuterated neotigogenin in the ratio of 1:4. The deuterium atom at C-26 of tigogenin acetate (**10b** and **10c**) shifted back to C-22 upon treatment with boron trifluoride in ethandithiol, giving (25*R*)-3β-acetoxy-5α-[5,6,22 or -26-$^2H_3$]furostan 26-dithioacetal (**13** and **14**). Evidence for the 1,5-reverse hydride shift in the overall acid isomerization reaction of a spirostanol came from the observation that the ratio of the deuterium atom at 26α to that at 26β changes from 1:4 for neotigogenin to 1:1 for the acid isomerization products tigogenin and neotigogenin.

In 1939, Marker et al.[1] reported the acid-catalyzed isomerization of spirostanols ("iso reaction"), when isomers were still considered to differ in the configuration at C-22. In 1953, Scheer et al.[2] showed that the "normal" and "iso" spirostanols differ in configuration at C-25 and this was confirmed by Callow et al.[3] using the deuterium–hydrogen exchange reaction at C-25 under the acid isomerization reaction condition. A mechanism via furostan-26-al (**3**) was proposed by Woodward et al.,[4] who synthesized 3β-hydroxy-5α-furostan-26-al (**3**) which was transformed to tigogenin (3β-hydroxy-5α-spirostan) by acid treatment, suggesting that the hydrogen at C-22 of the aldehyde **3** migrates to C-26 to form a spirostanol. Soon after that, Djerassi et al.[5] trapped the aldehyde **3** as a dithioacetal from a spirostanol and suggested that the hydrogen at C-26 of tigogenin migrates to C-22. Although the 1,6-hydrogen

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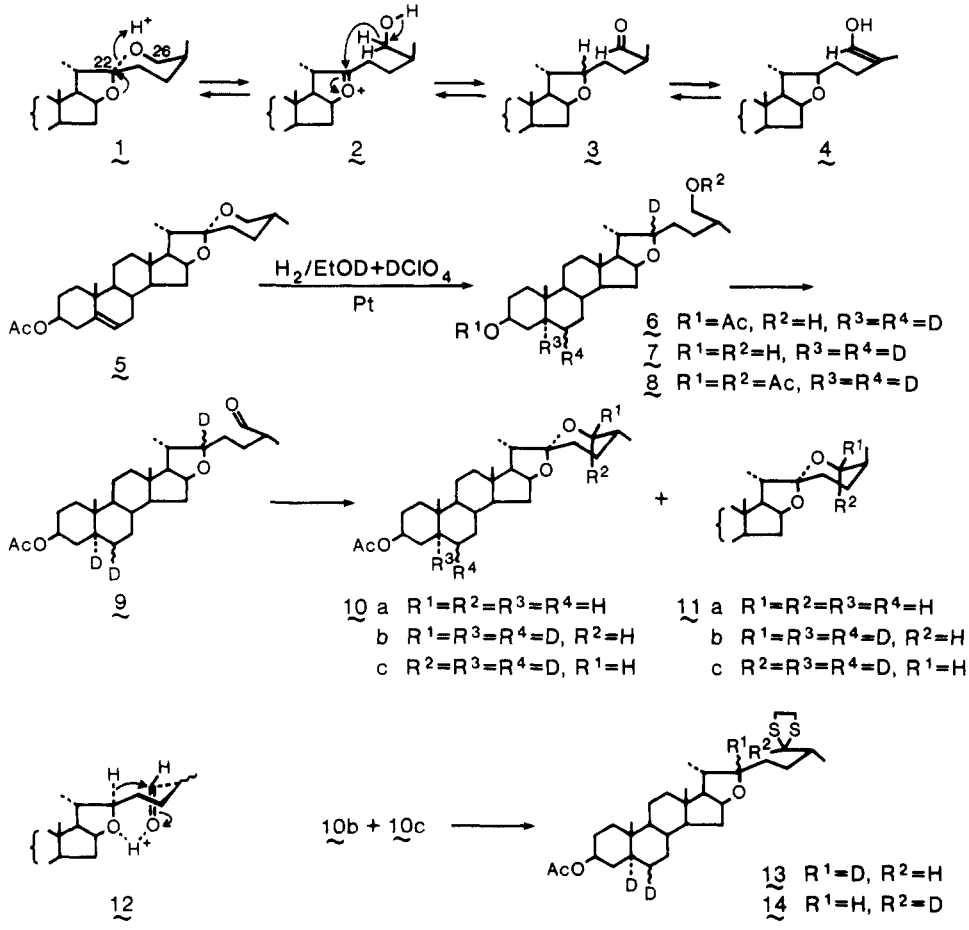
0022-3263/86/1951-3823$01.50/0

Chart I



abstraction reaction of furostan-26-al has been recently shown to give spirostan[6] and a base-catalyzed 1,5-hydride shift on 26-hydroxy-22-oxocholesterol was also reported,[7] there has been no direct evidence on the acid-catalyzed reverse 1,5-hydride shift on acid isomerization at C-25 of spirostanols.

In connection with our recent study on the biosynthesis of steroidal saponins, especially on the stereospecificity of the 24(25) double bond reduction followed by stereospecific oxidation of one of the methyl groups at C-25 of the cholesterol side chain,[8] we studied the "iso reaction" to avoid scrambling the configuration at C-25 when treating biosynthetically labeled compounds in acid solution such as acid hydrolysis of saponins. The methyl groups at C-25 of the 25*R* and 25*S* series of furostanol glycosides were found to originate from C-6 and C-2 of mevalonic acid, respectively.[8] Using deuterated solvent and deuteriochloride, we confirmed that the isomerization reaction in methanol is very slow and also found that the hydrogens at C-26 are not exchanged for a deuterium from the solvent. The deuterated positions were determined by ^{13}C NMR spectroscopy.[9]

Results and Discussion

We used a deuterium-labeled experiment to closely examine the reverse 1,5-hydride shift between C-22 and C-26

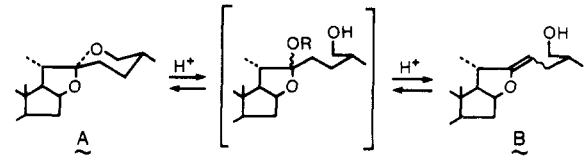


Figure 1.

Table I. Catalytic Reduction of Diosgenin Acetate (5) and Tigogenin Acetate (10a)

H_2 or D_2	solvent		yield (%)[a]	deuteration ratio[b] C-5	C-6	C-22
	Reduction of Diosgenin Acetate (5)					
a H_2	EtOD[c]					
	AcOEt	$DClO_4$[c]	98	100	100	75
b D_2	AcOH	$HClO_4$	90	~0	~0	~0
c H_2	AcOD[c]	$HClO_4$	94	50	50	60
	Reduction of Tigogenin Acetate (10a)					
d H_2	CD_3CO_2D[c]	$DClO_4$	98			80
e D_2	AcOD	$DClO_4$	90			90

[a] Total yield of furostanols. [b] The ratios were based on ^{13}C NMR analysis. [c] The deuterium atom % of ethan[^2H]ol, acetic [^2H]acid, [$^2H_4$]acetic acid, and perchloric [^2H]acid were 97% 98%, and 99%, respectively.

and made an interesting observation on catalytic hydrogenation over Adam's catalyst in preparing 22-deuterated dihydrotigogenin 3-*O*-acetate (**6**) (see Table I). Reduction of diosgenin acetate (**5**) in a solvent mixture of ethan[^2H]ol and ethyl acetate with a trace amount of perchloric [^2H]acid under nondeuterated hydrogen gas gave [5,6,22-$^2H_3$]dihydrotigogenin 3-*O*-acetate (**6**) together with deuterated dihydrotigogenin (**7**) and its 3,26-*O*-diacetate (**8**). The signal due to C-20 of **6** showed two peaks at δ_C 37.8 (75%) indicating a deuterium atom at the β position[10]

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Table II. Acid Treatment of 22-Deuterated 3β-Acetoxyfurostan-26-al (9)

reaction condition	yield (%)			
	10b	**10c**	**11b**	**11c**
a 5% HCl/MeOH, reflux	8.8	4.4	4.9	1.2
b 5% HCl/EtOH, reflux	15.0	15.0	3.7	3.7
c BF_3–Et_2O/C_6H_6, rt[a]	10.7	10.7	1.1	1.1

[a] rt = room temperature.

(C-22) and at δ_C 37.9 (25%) which is the nonlabeled signal in proton-decoupled ^{13}C NMR. The signals due to C-5, -6, and -22 (δ_C 44.7, 28.5, and 90.1, respectively) collapsed when a deuterium atom was attached to the C-5, -6, and -22, respectively. In contrast, hydrogenation carried out under deuterium gas in nondeuterated solvent afforded the same products but ^{13}C NMR and mass spectra showed that the introduction ratio of deuterium was very poor. Catalytic reduction of tigogenin acetate (**10a**) in acetic [^2H] acid with a trace amount of perchloric acid under nondeuterated hydrogen gas gave **6** ($R^3 = R^4 = H$) and **8** ($R^3 = R^4 = H$). These results seemed to suggest that the hydrogen from protic solvents plays an important role on catalytic reduction over Adam's catalyst in acidic solution and hydrogenates not only a ketal but also a double bond. The reaction mechanism remains to be deduced.

Collin's oxidation of the 26-alcohol (**6**) gave the aldehyde (**9**) with retention of the deuterium atoms. This compound is the expected intermediate **3** in the mechanism for the "iso reaction" proposed by Woodward.[4] As shown in Table II, the aldehyde (**9**) deuterated at C-22 was treated with hydrochloric acid in methanol or ethanol or with boron trifluoride etherate in benzene. Acetylation and chromatography of the products gave a mixture of 26β- and 26α-deuterated tigogenin acetate (**10b** and **10c**) and a mixture of 26β- and 26α-deuterated neotigogenin acetate (**11b** and **11c**). The deuterium-labeled positions were supported by ^1H NMR spectral analysis. The axial proton, 26α-H, of **10b** appeared at δ_H 3.36 as a doublet (J = 11 Hz) and the equatorial proton, 26β-H, of **10c** appeared at δ_H 3.45 as a doublet (J = 4 Hz), while nonlabeled tigogenin (**10a**) showed 26α-H and 26β-H at δ_H 3.37 (triplet, J = 11 Hz) and δ_H 3.47 (doublet of doublets, J = 11 and 4 Hz, respectively). In the case of neotigogenin, H-26α of **11b** appeared at δ_H 3.92 (doublet, J = 3 Hz) and 26β-H of **11c** at δ_H 3.27 (doublet, J = 3 Hz), while the nonlabeled specimen (**11a**) showed a doublet of doublets (δ_H 3.96, J = 9 and 3 Hz, 26α-H) and a broad doublet (δ_H 3.29, J = 9 Hz, 26β-H). The ratios of 26β-^2H to 26α-^2H were 2:1 in tigogenin and 4:1 in neotigogenin obtained from hydrochloric acid–methanol treatment. This indicates that the conformation of intermediate **12** may contribute significantly to the stereospecific hydride shift from C-22 to C-26. On treatment with boron trifluoride–benzene or with hydrochloric acid–ethanol, the ratios of 26α-^2H to 26β-^2H in the products were 1:1 because the "iso reaction" was fully equilibrated under those conditions. In hydrochloric acid–methanol, the equilibration is extremely slow. Thus we determined the 1,5-hydride shift from C-22 to C-26.

In the next step, we investigated the 1,5-hydride shift from C-26 to C-22. The equal mixture of [26β-^2H]- and [26α-^2H]tigogenin acetate (**10b** and **10c**) obtained above was refluxed with ethanedithiol in the presence of boron trifluoride etherate according to Djerassi[5] and gave a mixture of [22-^2H]- and [26-^2H]-5α-furostan 26-dithioketals (**13** and **14**, respectively). No deuterium loss was shown by mass spectrometry during the hydride shift reaction. The distribution of deuterium atoms was determined by ^1H NMR. The signals due to H-22 (δ_H 3.28, multiplet) and H-26 (δ_H 4.54, doublet, J = 6 Hz) decreased in intensity. The ratio of ^2H-22 to ^2H-26 based on ^2H NMR was 1:2. This may be due to an isotope effect on the hydride shift.

As mentioned above, we observed 1,5-hydride shifts stepwise from C-22 to C-26 and then from C-26 to C-22. Finally, in order to obtain direct evidence for the 1,5-reverse hydride shift, we chose the mixture of 26-deuterated neotigogenin acetate (**11b** and **11c**) (the ratio of 26α-^2H to 26β-^2H being 1:4). This mixture, when refluxed in 5% hydrochloric acid in ethanol and then subjected to acetylation, gave isomeric products at C-25, namely, deuterated tigogenin acetate (**10b** and **10c**) and deuterated neotigogenin acetate (**11b** and **11c**). The deuterium atom at C-26 was distributed equally on the axial and the equatorial positions after full equilibration of the reverse 1,5-hydride shift. The mass spectra of the products, the **10b** and **10c** mixture, the **11b** and **11c** mixture, and that of the starting material indicate that the reverse hydride shift proceeds with complete retention of all deuterium atoms.

Experimental Section

Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on Varian XL-200 and XL-100-12A spectrometers operated at 200.057 MHz and 25.16 MHz in [^2H]chloroform, respectively. Chemical shifts are given in δ (ppm) downfield from internal tetramethylsilane. The accuracies of δ_H and δ_C are ±0.01 and ±0.02 ppm, respectively. Mass spectra were determined on a Hitachi RMV-8GN spectrometer.

Hydrogenation of Diosgenin Acetate (1) to 3β-Acetoxy-22ξ-(25*R*)-5α-[5,6,22-$^2H_3$]furostan-26-ol (6). (a) Under H_2 Gas in Ethan[^2H]ol. Diosgenin acetate (**1**) (2 g) was hydrogenated under H_2 gas at atmospheric pressure for 18 h over platinum dioxide (400 mg) in ethan[^2H]ol (20 mL) and ethyl acetate (30 mL) with three drops of perchloric [^2H]acid. After the inorganic material was filtered off, the solution was diluted with ethyl acetate (220 mL). The resulting ethyl acetate solution was washed successively with water (100 mL), dilute sodium hydrogen carbonate (150 mL), water (2 × 100 mL), and then evaporated. Chromatography of the resulting residue (1.7 g) on silica gel (Lobar C, hexane–chloroform–ethyl acetate, 1:1:1) gave 3β-acetoxy-22ξ-(25*R*)-5α-[5,6,22-$^2H_3$]furostan-26-ol (**6**)[8b] (1.01 g) (d_0 0%, d_1 5%, d_2 28%, d_3 49%, and d_4 18%), 22ξ-(25*R*)-5α-3,26-dihydroxy[5,6,22-$^2H_3$]furostan (**7**) (186.7 mg), and 3,26-diacetoxy-22ξ-(25*R*)-5α-[5,6,22-$^2H_3$]furostan (**8**)[8b] (446 mg), mp 116–117 °C from methanol. ^{13}C NMR of nonlabeled **6**: δ_C 12.28 (C-19), 16.64 (C-18 and C-27), 18.94 (C-21), 20.87 (C-11), 21.44 (3-*O*-CO*CH*$_3$), 27.51 (C-2), 28.53 (C-6), 30.15 (C-24), 30.46 (C-23), 32.15 (C-7 and C-15), 34.06 (C-4), 35.30 (C-8), 35.61 (C-10), 35.77 (C-25), 36.81 (C-1), 37.97 (C-20), 39.69 (C-12), 41.05 (C-13), 44.73 (C-5), 54.33 (C-9), 56.74 (C-14), 65.32 (C-17), 68.08 (C-26), 73.70 (C-3), 83.27 (C-16), 90.32 (C-22), 170.68 (3-*O*-*CO*CH$_3$). ^{13}C NMR of nonlabeled **7**: δ_C 12.37 (C-19), 16.63 (C-18 and C-27), 18.90 (C-21), 20.92 (C-11), 28.66 (C-6), 30.05 (C-24), 30.27 (C-23), 31.53 (C-2), 32.11 (C-15), 32.22 (C-7), 35.30 (C-8), 35.49 (C-25), 35.73 (C-10), 37.05 (C-1), 37.80 (C-20), 38.12 (C-4), 39.75 (C-12), 41.04 (C-13), 44.50 (C-5), 54.44 (C-9), 56.82 (C-14), 65.32 (C-17), 68.01 (C-26), 71.25 (C-3), 83.26 (C-16), 90.32 (C-22). ^{13}C NMR of nonlabeled **8**: δ_C 12.29 (C-19), 16.62 (C-18), 16.78 (C-27), 18.94 (C-21), 20.87 (C-11), 20.96 (27-*O*-CO*CH*$_3$), 21.44 (3-*O*-CO*CH*$_3$), 27.51 (C-2), 28.52 (C-6), 30.47 (C-24), 30.82 (C-23), 32.16 (C-7 and C-15), 32.81 (C-25), 34.04 (C-4), 35.31 (C-8), 35.61 (C-10), 36.81 (C-1), 37.95 (C-20), 39.69 (C-12), 41.02 (C-13), 44.71 (C-5), 54.33 (C-9), 56.74 (C-14), 65.33 (C-17), 69.40 (C-26), 73.68 (C-3), 83.26 (C-16), 90.19 (C-22), 170.66 (3-*O*-*CO*CH$_3$), 171.25 (26-*O*-*CO*CH$_3$).

(b) Under $^2H_2$ Gas in Nondeuterated Acetic Acid. Diosgenin acetate (245 mg) was hydrogenated for 2 h under $^2H_2$ gas in acetic acid (5 mL) and perchloric acid (1 drop) over platinum dioxide (100 mg). The inorganic material was filtered off, and

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the solution was diluted with benzene (70 mL), washed with water (3 × 50 mL), saturated sodium hydrogen carbonate (2 × 40 mL), and then water (2 × 40 mL). Chromatography of the residue gave compound **6** (95 mg) (d_0 84%, d_1 16%) and compound **8** (141 mg) (d_0 80%, d_1 20%).

(c) Under H_2 Gas in [$^2H_3$]Acetic [^2H]Acid. Diosgenin acetate (80 mg) was hydrogenated for 4 h under H_2 gas over platinum dioxide (40 mg) in [$^2H_3$] acetic [^2H]acid (3.5 mL) and perchloric acid (1 drop). Workup of the reaction described above gave 3-*O*-acetyl-26-*O*-[$^2H_3$]acetyl-5α-22ξ-[5,6,22,23-$^2H_4$]furostan[12] (55 mg) (d_0 0%, d_1 0%, d_2 0%, d_3 2%, d_4 9%, d_5 22%, d_6 30%, d_7 26%, d_8 8%).[13]

Hydrogenation of Tigogenin Acetate (10a). (d) Under H_2 Gas in Acetic [^2H]Acid. Tigogenin acetate (60 mg) was hydrogenated for 10 min over platinum dioxide (38.2 mg) under H_2 gas in acetic [^2H]acid (3.5 mL) and perchloric [^2H] acid (1 drop). Workup as described above gave compound **6** ($R^3 = R^4 = H$) (44.5 mg) (d_0 0%, d_1 16%, d_2 48%, d_3 36%)[13] and compound **8** ($R^3 = R^4 = H$) (12.4 mg) (d_0 0%, d_1 14%, d_2 44%, d_3 32%, d_4 10%).[13]

(e) Under $^2H_2$ Gas in Acetic [^2H]Acid. Tigogenin acetate (54 mg) was hydrogenated for 6 min over platinum dioxide (36 mg) under $^2H_2$ gas in acetic [^2H]acid (2.5 mL) and perchloric [^2H]acid (1 drop). Workup as described above gave **6** ($R^3 = R^4 = H$) (37 mg) (d_0 18%, d_1 0%, d_2 53%, d_3 25%)[13] and compound **8** ($R^3 = R^4 = H$) (11 mg) (d_0 9%, d_1 0%, d_2 46%, d_3 45%).[13]

Oxidation of 6 to 3β-Acetoxy-22ξ-(25*R*)-5α-[5,6,22-$^2H_3$]-furostan-26-al (9). The alcohol **6** (200 mg), obtained from experiment a, in dichloromethane (5 mL) was added to freshly prepared CrO_3–pyridine complex (600 mg of CrO_3 and 1 g of dry pyridine in 10 mL of dichloromethane were stirred for 0.5 h in an ice bath under nitrogen), and the mixture was stirred for 1.5 h at room temperature and then diluted with dichloromethane (10 mL). The solution was passed through silica gel (activity V) eluted with dichloromethane to remove the inorganic material. Concentration of the eluate at 30 °C gave a noncrystalline residue (200 mg). Chromatography of the residue on silica gel (Lobar A, hexane–chloroform–ethyl acetate, 15:1:1) gave 26-aldehyde **9** (150 mg) (d_0 0%, d_1 5%, d_2 23%, d_3 47%, d_4 25%). ^1H NMR of nonlabeled **9**: δ_H 0.77, (s, 18-H), 0.83 (s, 19-H), 0.98 (d, J = 6.5, 21-H), 1.10 (d, J = 7.0, 27-H), 2.02 (s, 3-*O*-Ac), 2.37 (m, 25-H), 3.25 (m, 22-H), 4.30 (dt, J = 8 and 5, 16-H), 4.68 (tt, J = 11 and 5, 3-H), 9.61 (d, J = 2, 26-H); ^{13}C NMR of nonlabeled **9**: δ_C 12.26 (C-19), 13.41 (C-27), 16.60 (C-18), 18.81 (C-21), 20.85 (C-11), 21.43 (3-*O*-CO*CH*$_3$), 27.49 (C-2), 27.77 (C-24), 28.24 (C-6), 30.74 (C-23), 32.17 (C-7 and C-15), 33.93 (C-4), 35.27 (C-8), 35.47 (C-10), 36.77 (C-1), 37.91 (C-20), 39.66 (C-12), 41.01 (C-13), 44.60 (C-5), 46.36 (C-25), 54.29 (C-9), 56.73 (C-14), 65.24 (C-17), 73.67 (C-3), 83.31 (C-16), 89.69 (C-22), 170.67 (3-*O*-*CO*CH$_3$), 205.13 (C-26).

Acid Treatment of [5,6,22-$^2H_3$]-26-Aldehyde 9 to [5,6,22-$^2H_3$]-Tigogenine Acetate (10b and 10c) and [5,6,26-$^2H_3$]Neotigogenine Acetate (11b and 11c). (a) With Hydrochloric Acid in Methanol. The 22-deuterated 26-aldehyde **9** (69 mg) in methanol (8 mL) with concentrated hydrochloric acid (1 mL) was refluxed under nitrogen. After 20 h, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (2 × 70 mL). This extract was washed with 1% sodium hydroxide (30 mL) and water (3 × 30 mL), and then the solvent was evaporated. Chromatography of the residue on silica gel (Lobar B, hexane–chloroform–ethyl acetate, 1:1:1) gave a mixture of tigogenin and neotigogenin, which was acetylated with acetic anhydride and pyridine and purified by chromatography (Lobar B, hexane–chloroform–ethyl acetate, 10:1:1), giving 26-deuterated tigogenin acetate (a 2:1 mixture of **10b** and **10c**, 17.5 mg) (d_0 0%, d_1 6%, d_2 26%, d_3 53%, d_4 15%) and 26-deuterated neotigogenin acetate (a 4:1 mixture of **11b** and **11c**, 8.4 mg) (d_0 0%, d_1 5%, d_2 24%, d_3 52%, d_4 19%).

(b) With Hydrochloric Acid in Ethanol. The 22-deuterated 26-aldehyde **9** (150 mg) in ethanol (50 mL) with concentrated hydrochloric acid (20 mL) was refluxed for 17 h under nitrogen. Workup as described above gave a 1:1 mixture of **10b** and **10c** (80.2 mg) (d_0 0%, d_1 4%, d_2 27%, d_3 49%, d_4 20%) and a 1:1 mixture of **11b** and **11c** (22.3 mg) (d_0 0%, d_1 2%, d_2 24%, d_3 52%, d_4 22%).

(c) With Boron Trifluoride Etherate in Benzene. Boron trifluoride etherate (0.75 mL) was added to 22-deuteriated 26-aldehyde **9** (150 mg) in dry benzene (15 mL) and the mixture was left at room temperature for 40 h under nitrogen. Workup as described above gave a 1:1 mixture of **10b** and **10c** (57.2 mg) (d_0 0%, d_1 3%, d_2 26%, d_3 50%, d_4 21%) and a 1:1 mixture of **11b** and **11c** (6.8 mg) (d_0 1%, d_1 5%, d_2 26%, d_3 49%, d_4 19%). ^1H NMR of **10b** and **10c**: δ_H 0.76 (s, 18-H), 0.79 (d, J = 6.2, 27-H), 0.83 (s, 19-H), 0.96 (d, J = 6.7, 21-H), 2.02 (s, 3-*O*-Ac), 3.37 (t, J = 11, 26α-H of the nonlabeled specimen), 3.36 (d, J = 11, 26α-H of **10b**), 3.47 (dd, J = 11 and 4, 26β-H of the nonlabeled specimen), 3.45 (d, J = 4, 26β-H of **10c**), 4.38 (dt, J = 8 and 6, 16-H), 4.66 (tt, J = 16.5 and 5, 3-H). ^{13}C NMR of **10b** and **10c**: δ_C 12.25 (C-19), 14.50 (C-21), 16.48 (C-18), 17.13 (C-27), 21.03 (C-11), 21.43 (CO*CH*$_3$), 27.49 (C-2), 28.84 (C-24), 30.24 (C-25, $\Delta\delta_{C(\beta\text{-}^2H)}$ −0.09), 31.43 (C-23), 31.80 (C-15), 32.05 (C-7, $\Delta\delta_{C(\beta\text{-}^2H)}$ −0.12), 33.94 (C-4, $\Delta\delta_{C(\beta\text{-}^2H)}$ −0.12), 35.03 (C-8 $\Delta\delta_{C(\beta\text{-}^2H)}$ −0.08), 35.11 (C-8), 35.49 (C-10, $\Delta\delta_{C(\beta\text{-}^2H)}$ −0.11), 36.74 (C-1), 40.04 (C-12), 40.59 (C-13), 41.66 (C-20), (44.68 C-5 was collapsed), 54.26 (C-9), 56.27 (C-14), 62.28 (C-17), (66.85, C-26, was collapsed), 73.67 (C-3), 80.85 (C-16)109.21 (C-22), 170.63 (*CO*CH$_3$). ^1H NMR of **11b** and **11c**: δ_H 0.76 (s, 18-H), 0.83 (s, 19-H), 1.07 (d, J = 6.6, 21-H), 0.98 (d, J = 7.1, 27-H), 2.02 (s, 3-*O*-Ac), 3.29 (dd, J = 9 and 2, 26β-H of the nonlabeled specimen), 3.27 (d, J = 2, 26β-H of **11c**), 3.96 (dd, J = 9 and 3, 26α-H of the nonlabeled specimen), 3.92 (d, J = 3, 26α-H of **11b**), 4.39 (dt, J = 8 and 6.5, 16-H), 4.66 (tt, J = 11 and 5, 3-H). ^{13}C NMR of **11b** and **11c**: δ_C 12.26 (C-19), 14.33 (C-21), 16.05 (C-27), 16.50 (C-18), 21.05 (C-11), 21.45 (CO*CH*$_3$), 25.83 (C-24), 26.01 (C-23), 27.02 (C-25, $\Delta\delta_{C(\beta\text{-}^2H)}$ −0.11), 27.50 (C-2), (28.53, C-6 was collapsed), 31.76 (C-15), 32.08 (C-7, $\Delta\delta_{C(\beta\text{-}^2H)}$ −0.12), 33.95 (C-4, $\Delta\delta_{C(\beta\text{-}^2H)}$ −0.12), 35.11 (C-8, br), 35.50 (C-10, $\Delta\delta_{C(\beta\text{-}^2H)}$ −0.13), 36.75 (C-1), 40.06 (C-12), 40.58 (C-13), 42.18 (C-20), (44.69, C-5 was collapsed), 54.27 (C-9), 56.27 (C-14), 62.11 (C-17), (65.16, C-26 was collapsed), 73.69 (C-3), 80.95 (C-16), 109.72 (C-22), 170.67 (*CO*CH$_3$).

3β-Acetoxy-22ξ-(25*R*)-5α-[5,6,22- or -26-$^2H_3$]furostan 26-Dithioacetal (13 and 14). A 1:1 mixture of [5,6,26α-$^2H_3$]- and [5,6,26β-$^2H_3$]tigogenin acetate (**10b** and **10c**) (21 mg) was suspended in ethanedithiol (0.4 mL) and boron trifluoride etherate (0.1 mL). The mixture was stirred for 1 h at room temperature, then another portion of ethanedithiol (0.5 mL) was added, and stirring was continued for another hour. Aqueous sodium hydrogen carbonate (5%, 10 mL) was added and the solution was extracted with benzene (3 × 20 mL) and then washed with water. Evaporation of the solvent gave a residue, which when chromatographed on silica gel (hexane–chloroform–ethyl acetate, 10:1:1) gave a 1:2 mixture of 26-thioacetal **13** and **14** (10.9 mg) (d_0 0%, d_1 5%, d_2 28%, d_3 50%, d_4 17%), mp 126–127 °C, from methanol. ^1H NMR: δ_H 0.78 (s, 18-H), 0.83 (s, 19-H), 1.00 (d, J = 6.5, 21-H), 1.05 (d, J = 6.5, 27-H), 2.02 (s, 3-*O*-Ac), 3.17 (m, SCH_2CH_2S), 3.28 (m, 22-H with reduced intensity), 4.29 (dt, J = 7.5 and 5, 16-H), 4.54 (d, J = 6, 26-H with reduced intensity), 4.69 (tt, J = 11 and 5.5, 3-H).

Acid Treatment of [5,6,26-$^2H_3$]Neotigogenin (11b and 11c) to [5,6,26β-$^2H_3$]- and [5,6,26α-$^2H_3$]Tigogenin Acetate (10b and 10c) and [5,6,26β-$^2H_3$]- and [5,6,26α-$^2H_3$]Neotigogenin Acetate (11b and 11c). A 1:4 mixture of [5,6,26α-$^2H_3$]- and [5,6,26β-$^2H_3$]neotigogenin acetate (5.8 mg) in ethanol (10 mL) and concentrated hydrochloric acid (2.0 mL) was refluxed under nitrogen. After 67 h the reaction mixture was worked up and acetylated, giving [5,6,26β-$^2H_3$]- and [5,6,26α-$^2H_3$]tigogenin acetate (3.24 mg) (**10b** and **10c**) (d_0 0%, d_1 6%, d_2 27%, d_3 51%, d_4 16%) and [5,6,26β-$^2H_3$]- and [5,6,26α-$^2H_3$]neotigogenin (0.44 mg) (**11b** and **11c**) (d_0 0%, d_1 2%, d_2 26%, d_3 55%, d_4 17%). The ratio of **10b** to **10c** and the ratio **11b** to **11c** was 1:1 based on the basis ^1H NMR spectral analysis.

Acknowledgment. We thank Mrs. Y. Yoshimura of these laboratories for her helpful discussions and NMR measurements and also thank Drs. H. Ishii and H. Tada of these laboratories for their encouragement.

(12) Prolonging the reaction time increased the yield of this compound and the 26-*O*-acetyl group arose from the solvent ([$^2H_4$]acetic acid). The ^{13}C NMR signal assignments for the acetyl groups of **8** were based on this compound.

(13) The second and the third deuterium atoms were probably introduced at C-23 in acetic acid by equilibration between A and B as shown in Figure 1.[9,11] Deuteration at C-23 was avoided in (a) when ethanol–ethyl acetate was employed as a solvent.

Registry No. 1, 16653-41-1; 6, 104034-85-7; 6 (R^3 = R^4 = H), 104034-89-1; 7, 104034-86-8; 8, 104034-87-9; 8 (R^3 = R^4 = H), 104034-90-4; 9, 104034-91-5; 10a, 2530-07-6; 10b, 104034-96-0; 10c, 104034-97-1; 11b, 104034-98-2; 11c, 104034-99-3; 13, 104035-00-9; 14, 104051-49-2; 3-*O*-acetyl-26-*O*-[$^2H_3$]acetyl-5α-22ξ-[5,6,22,23-$^2H_4$]furostan, 104034-88-0; tigogenin-d_3 (isomer 1), 104034-92-6; tigogenin-d_3 (isomer 2), 104034-93-7; neotigogenin-d_3 (isomer 1), 104034-94-8; neotigogenin-d_3 (isomer 2), 104034-95-9.

A Study of the Hydrolysis of Methoxysilanes in a Two-Phase System

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Received January 31, 1986

The hydrolysis rates of $(CH_3)_{4-x}Si(OCH_3)_x$, x = 1–3, in a two-phase system composed of a hydrocarbon layer and an aqueous buffer phase were found to be first order in substrate and specific acid or base catalyst for pH 4–11. In addition, evidence for catalysis by bicarbonate was noted. In contrast to what one would predict on the basis of literature precedence, under acidic conditions, the following reactivity was found: $(CH_3)_3SiOCH_3$ > $(CH_3)_2Si(OCH_3)_2$ > $CH_3Si(OCH_3)_3$, whereas the order was reversed with basic catalysis. Activation parameters are consistent with an associative mechanism with either acid or base catalysts. This study, coupled with results in other solvents, suggests the intermediacy of a five-coordinate silicon structure which in acidic media has both entering and leaving groups on the same face of the alkoxysilane. The transition state or intermediate in the base-catalyzed process is proposed to be analogous to that of the S_N2 reaction of carbon systems. Qualitatively, aqueous solutions of hydrolyzed trifunctional silicon species were found to be the most stable, as characterized by the absence of gel formation, at pH values between 3 and 8. Outside of this range condensation and/or phase separation was noted.

Solvolysis studies of the Si–O bond are amply represented in the literature.[1,2] The compounds investigated have primarily either been aryl/(aryloxy)silanes that have chromophores which facilitate the monitoring of the reaction by UV–vis spectroscopy or carboxysilanes which when hydrolyzed yield an acid that can be measured by simple titration. A systematic study of the hydrolysis of a series of alkylalkoxysilanes has not been published, nor is it easy to compare the available examples as different solvents and various catalysts have been used. The following report is a description of the specific acid/base-catalyzed hydrolysis chemistry of the series $R_{4-x}Si(OR')_x$ where x = 1–3 and R = R' = CH_3. These compounds, particularly dimethyldimethoxysilane (DMDM) and methyltrimethoxysilane (MTMS), are important to the silicone industry, and their hydrolysis is often viewed as the initial reaction in the so-called "cure"—the process that produces polysiloxane elastomers and resins.[3]

Results and Discussion

Initial studies of proton-catalyzed hydrolysis of MTMS were carried out in an acetone/water solvent mixture. From these experiments, in which water was in relatively large excess with respect to MTMS, were determined first-order dependence for substrate[4] and catalyst, *p*-toluenesulfonic acid, over a range of acid concentrations—10^{-3} to 10^{-5} M. In the study the disappearance of MTMS was monitored by either ^1H NMR spectroscopy (determination of MTMS order) or by GC analysis (determination of catalyst order) in which the method of initial rates was used—the reaction being monitored to only 20–30% completion. Although estimates for activation parameters ($\Delta H^\ddagger$ = 7.1 kcal mol^{-1}, $\Delta S^\ddagger$ = −30 eu) were obtained over a 35° range and were consistent with the report of an analogous compound,[2a] several limitations—such as the sampling rate being determined by GC cycle times,[5] the acidity of catalysts in these unbuffered, mixed solvent systems being difficult to measure,[6] and the possibility of competitive processes involving hydrolysis products—led the investigation down a different avenue as described below.

The MTMS–water system exists as two phases if agitation is minimized, and the location of the interface changes with time as the MTMS diffuses into the aqueous layer. A plot of the volume change vs. time is linear (r = 0.996 for the following expression: ΔV(mL) = (2.62×10^{-4} mL s^{-1}) t(s) + 7.9×10^{-2} mL, at pH 5.0 and where μ = 0.01 M), suggesting hydrolysis and/or diffusion is occuring at some constant rate in accord with the concentration of MTMS being constant at the interface (see supplementary material). By using aqueous buffer solutions it was also determined that there existed a pH dependence for the process—qualitatively, the rate was slowest at a pH close to 7.

In light of these observations, a series of reactions were examined in which methylalkoxysilanes dissolved in pentane were mixed with aqueous buffer solutions, and the disappearance of starting material in the pentane phase was monitored. A sequence (Scheme I) in which the reaction is presumed to take place in the aqueous layer is supported by the absence of significant hydrolysis when

(1) For a review, see: Prince, R. H. *M. T. P. International Reviews of Science Inorganic Series One*, Tobe, M. L., Ed.; Butterworths: London, 1972; Vol. 9.

(2) (a) Pratt, R. F.; McNeil, K. J.; DiCaprio, J. A.; Walsh, D. A. *J. Am. Chem. Soc.* **1980**, *102*, 1859 and references cited therein. (b) Boe, B. *J. Organomet. Chem.* **1972**, *43*, 275 and references cited therein.

(3) Noll, W. *Chemistry and Technology of Silicones*; Academic Press: New York, 1968.

(4) White, M. A.; Smith, K. A., unpublished results.

(5) Attempts to quench the reaction by the addition of base or by cooling the samples were unsuccessful.

(6) Paul, M. A.; Long, F. A. *Chem. Rev.* **1957**, *57*, 1–45. Though not a recent review of acidity functions, this paper gives an excellent treatment of the problem of determining acidity in mixed solvents and when weak acids are employed.

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