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Reaction of (20*S*)-3β-Acetoxybisnorchol-5-enic Acid with Lead Tetraacetate; Structure of Pregnane Derivatives and a Dimer[1]

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Reaction of 3β-acetoxybisnorchol-5-enic acid (**1**) with lead tetraacetate in benzene gave Δ^{20} and $\Delta^{17(20)}$ compounds (**2a** and **2b**) and 20α- and 20β-acetoxy compounds (**3a** and **3b**), with a trace of 20-keto compound (**4**) and a dimer (**5**). The structure of **5** was determined as 3'β-acetoxypregn-5'-en-20'α-yl 3β-acetoxybisnorchol-5-enate (Chart 1). The use of toluene or xylene in place of benzene resulted in decreased formation of **2b** with the formation of the 17-ethyl compound (**2c**). The reactions were examined under various conditions (Table I) and the reaction mechanisms are discussed.

Keywords——3β-acetoxybisnorchol-5-enic acid; lead tetraacetate; Δ^{20}-pregnene derivative; $\Delta^{17(20)}$-pregnene derivative; 20α- and 20β-acetoxypregnene derivatives; 20-ketopregnene derivative; dimer (3'β-acetoxypregn-5'-en-20'α-yl 3β-acetoxybisnorchol-5-enate); isotope effect; solvent effects

The reaction of lead tetraacetate with carboxylic acids is known to effect oxidative decarboxylation.[2] As a part of our studies on steroid chemistry, we carried out the reaction of 3β-acetoxybisnorchol-5-enic acid (**1**) with lead tetraacetate, and the results are reported herein.

1 was treated with lead tetraacetate and the products were examined by thin-layer chromatography (TLC). Four spots were obtained, and the corresponding substances, **2a**, **b**, **c**, **3a**, **b**, **4**, and **5**, were separated by silica gel column chromatography. Their structures were determined to be as shown in Chart 1.

When **1** was refluxed with lead tetraacetate in benzene for 5 h, the separated products were a 3:1 mixture of **2a** and **2b**, a 3:1 mixture of **3a** and **3b**, **4**, and **5**.

Structures of 2a and 2b

Compounds **2a** and **2b** were separated by column chromatography over 10% silver nitrate-silica gel. **2a** was identified as 3β-acetoxypregna-5,20-diene[3] by analysis of the mass (MS) spectrum and proton nuclear magnetic resonance (PMR) spectrum, which indicated the appearance of new vinyl protons.

The structure of **2b** was identified as [17(20)*E*]-3β-acetoxypregna-5,17(20)-diene by comparison of physical and spectral data with the data of Tanabe *et al.*[4]

Conformation of the Side Chain of 2a

The conformation of the Δ^{20}-side chain of **2a** was examined by means of PMR spectroscopy. The multiplet of 20-H is probably due to long-range coupling with 16-H. In confirmation of this, irradiation of a multiplet signal at 2.2 ppm produced 20-H as an octet. Further, the Δ^{20}-compound (**6**) which is saturated at the 5-6 positions of **2a** showed a similar PMR pattern for the Δ^{20}-side chain and also on decoupling. The coupling constant of 17α-H and 20-H was 5 Hz.

Wellman *et al.*[5] and Nes *et al.*[6] reported that rotation about the 17—20 bond of 20-oxopregnenes is restricted. In the case of Δ^{20}-compounds (**2a** and **6**), the angle between 20-H and 17α-H would preferentially be either *ca.* 38° or 122° as judged from the coupling constant.[7] The four possible conformations, **a**, **b**, **c**, and **d** (Fig. 1) were examined with Büchi models. In **d**, the non-bonded interaction of 21-H_2 and 18-CH_3 is excessively large, while there is a non-bonded interaction between 21-H_2 and 16-H in **c**. Therefore, **a** and **b** were considered

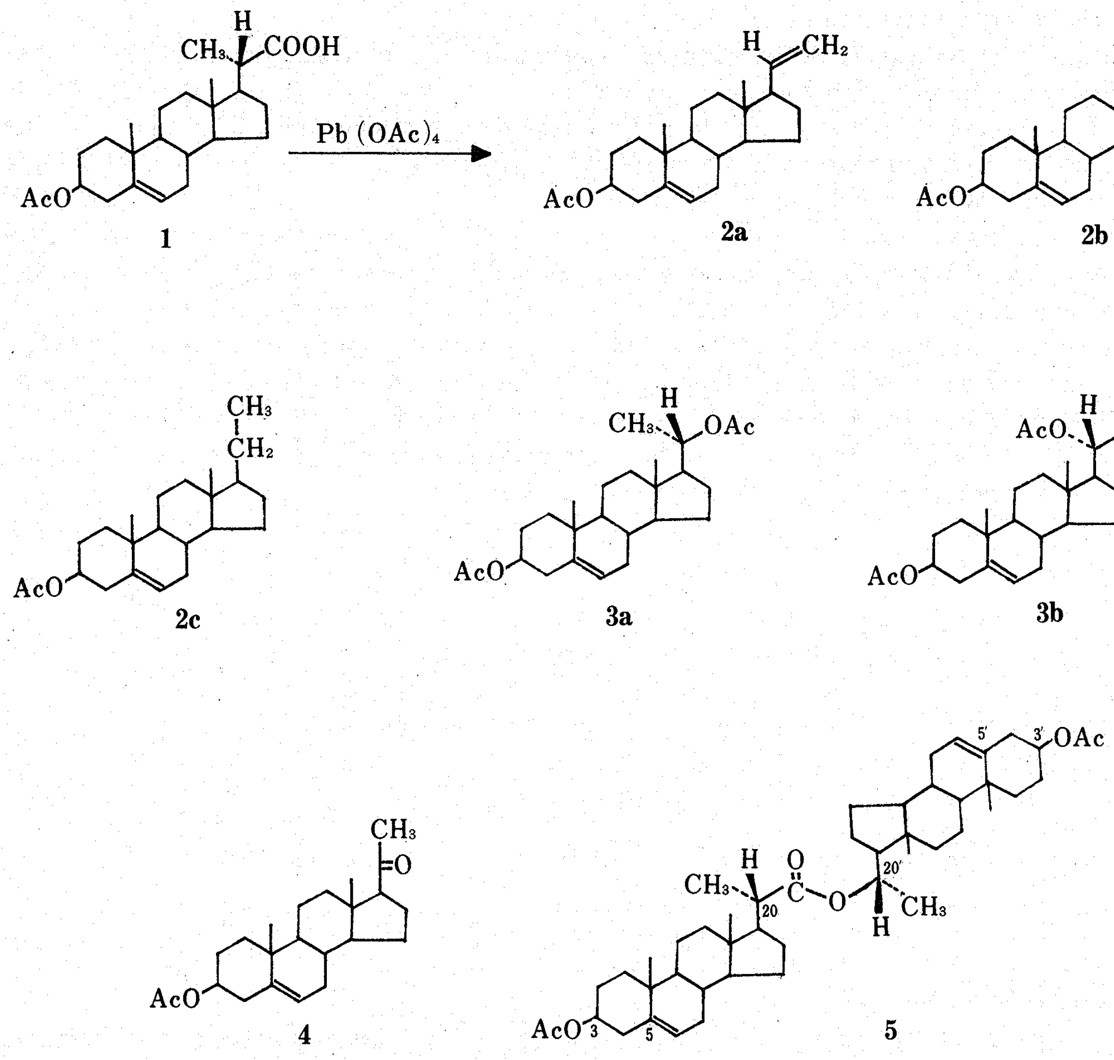


Chart 1. Reaction Products of 1 with Lead Tetraacetate

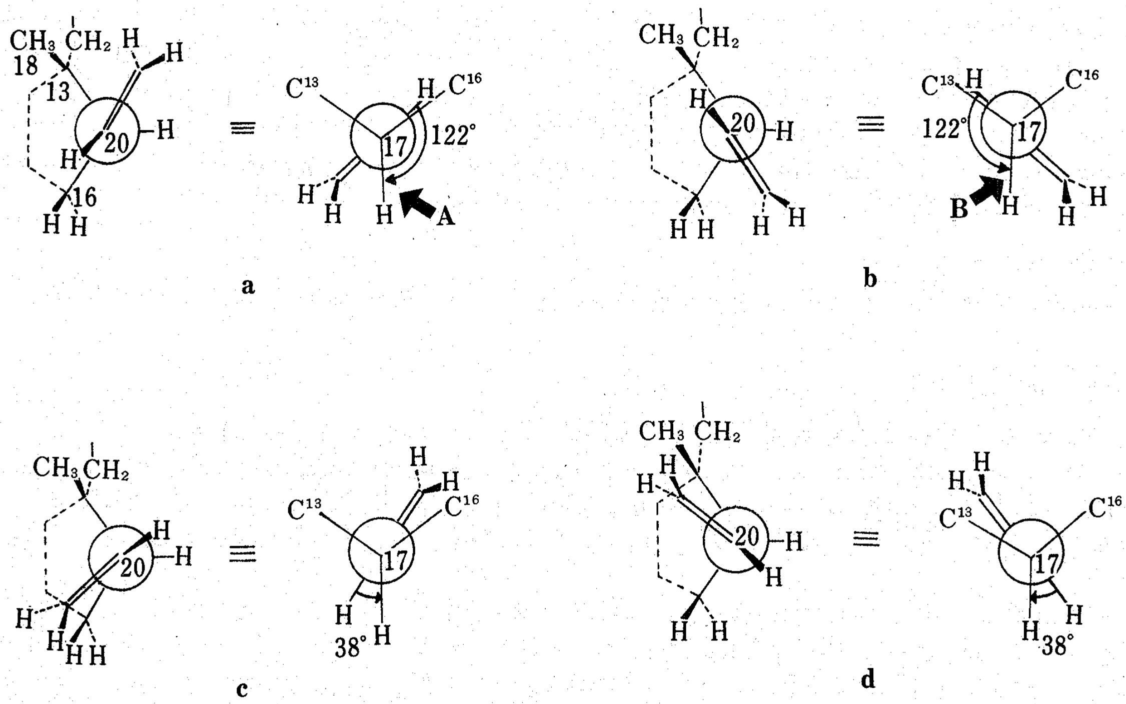


Fig. 1. Probable Conformations of Δ^{20}-Compounds

to be the preferred conformations.

Epoxidation of **6** was carried out in order to obtain further informations (Chart 2). Treatment of **6** with *m*-chloroperbenzoic acid gave colorless needles of the 20ξ,21-epoxides (**7a, b**). In the PMR spectrum of **7**, the signal of 18-CH_3 appeared as two peaks in a 2: 1 ratio. Further, **7** was reduced with lithium aluminum hydride ($LiAlH_4$) in ether to give the 3,20-diols (**8a, b**), whose structures were determined as 20α-OH and 20β-OH (2: 1 ratio) by comparison with authentic specimens.[8)] These results can be explained as follows. In conformation **a**, epoxidation of the double bond from the side of arrow A would occur preferentially, forming a larger amount of 20α-OH compound than 20β-OH compound in the reduction of **7** with $LiAlH_4$. In the case of conformation **b**, the reaction would proceed by epoxidation from the side of arrow B. The formation of both 20α-OH and 20β-OH compounds (2: 1) by epoxidation of the Δ^{20}-sterol followed by $LiAlH_4$ reduction would suggest the presence of the two rotational isomers (**a** and **b**) (Fig. 1), with conformer **a** being present in the larger amount. A similar phenomenon was also observed in the study of Nes *et al.*[9)]

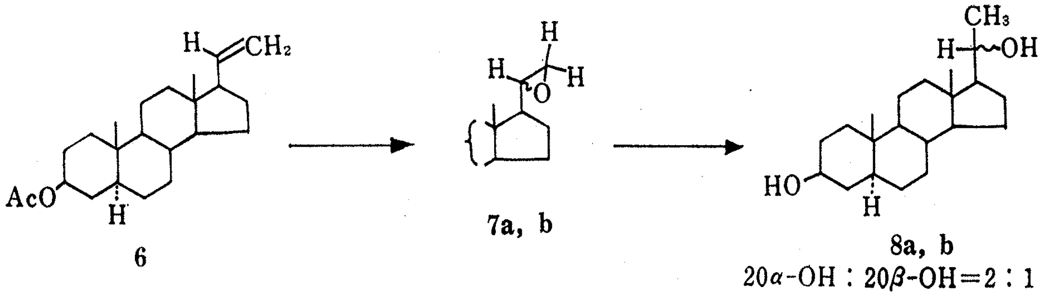


Chart 2

Structures of 3a and 3b

Comparison of the PMR signal intensities of 18-CH_3 and 21-CH_3 indicated that **3** contained diastereomers in a ratio of 3: 1. Recrystallization of **3** from MeOH provided **3a** as crystals, which were assumed to be 3β,20α-diacetoxypregn-5-ene. Alkaline hydrolysis of **3a** gave a product which was identical with authentic 3β,20α-dihydroxypregn-5-ene (**9a**).[10)] The mother liquor after recrystallization of **3** was hydrolyzed and the product was analyzed by gas chromatography–mass spectrometry (GC–MS). The spectrum showed a fragmentation pattern consistent with that of authentic 3p,20β-dihydroxypregn-5-ene (**9b**),[10)] besides that of **9a**. This result indicated that the ratio of α-configuration to β-configuration of the 20-acetoxy group introduced by the reaction of **1** with lead tetraacetate was 3: 1.

Structure of 4

4 was identified as 3β-acetoxypregn-5-en-20-one by comparison with an authentic specimen.

Structure of 5

Recrystallization of **5** from CH_2Cl_2–MeOH gave colorless granular crystals of 313—314°C. Elemental analytical values and the mode of fragmentation in the high-resolution mass spectrometry (Fig. 2) of **5** suggested that it is a steroid dimer with the composition $C_{47}H_{70}O_6$. The largest ion of its MS, *m/e* 610.4754 is consistent with $C_{43}H_{62}O_2$, which is considered to be $[M-2AcOH]^+$.

A signal ascribed to the proton on carbon substituted with oxygen appeared at 4.92 ppm in the PMR spectrum of **5**, and its intensity was one-half of that of the signal which would be expected for 6-H or 3α-H proton. Doublets at 1.15 and 1.19 ppm showed one-half of the intensity of the 18- or 19-methyl signal. From their chemical shifts, these signals were

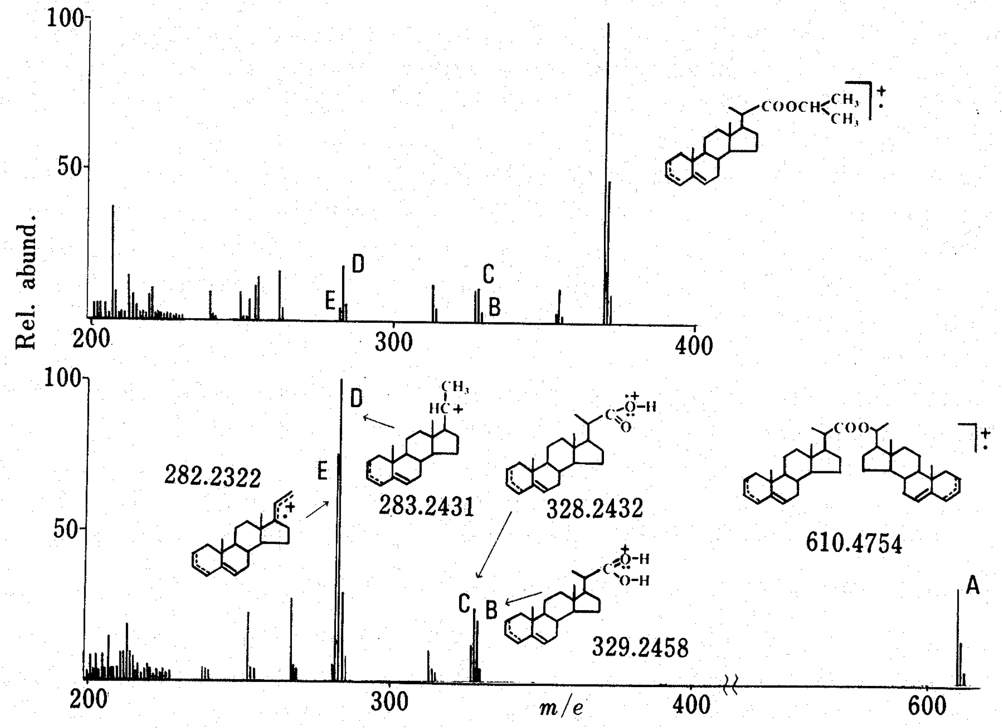
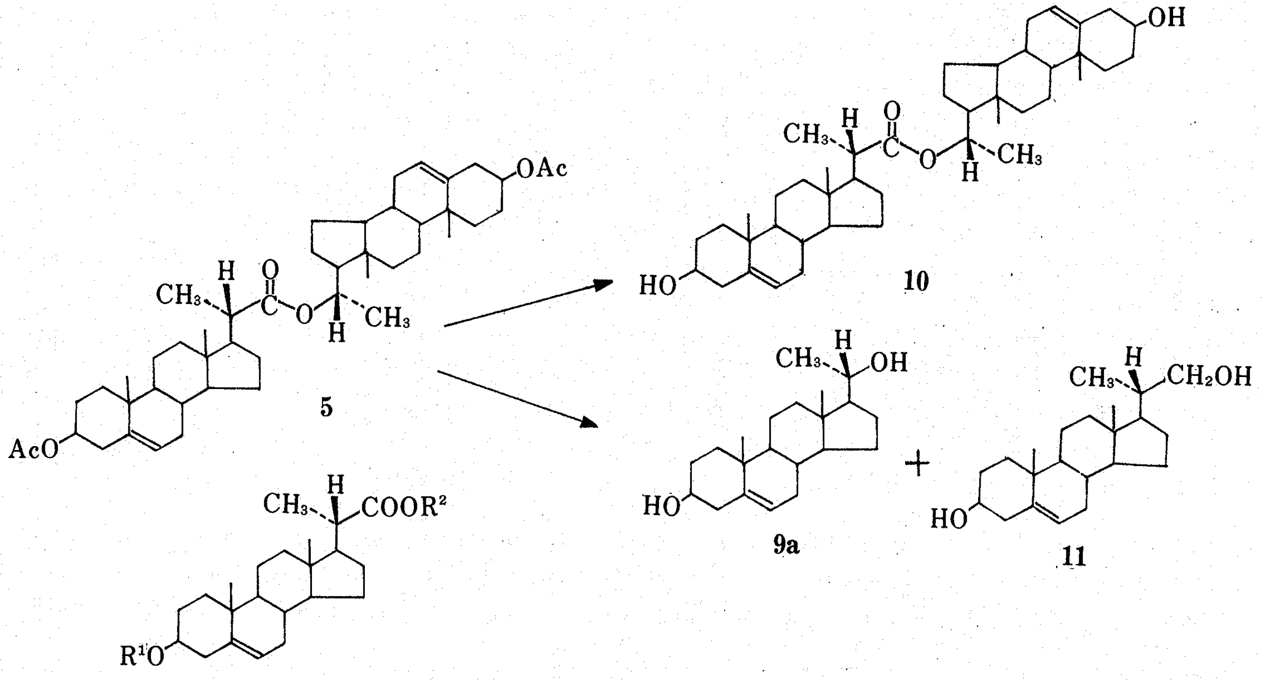


Fig. 2. Mass spectra of **12** (upper) and **5** (lower)



	R^1	R^2
12:	Ac	$CH\langle^{CH_3}_{CH_3}$
13:	H	$CH\langle^{CH_3}_{CH_3}$
14:	Ac	CH_3
15:	H	H

Chart 3

assumed to be those of methyl groups in the partial structures $CH_3\text{-}\underset{|}{C}H\text{-}CO\text{-}$ and $CH_3\text{-}\underset{|}{C}H\text{-}O\text{-}$, respectively. In view of these results together with the results of MS and the mode of reaction with lead tetraacetate, this dimer seemed to be a steroid with an ester linkage between positions 22 and 20′. This ester linkage was supported by the infrared (IR) spectrum of **5** which showed a new absorption at 1720 cm^{-1}.

Therefore, cleavage of this dimer (**5**) by hydrolysis was attempted by refluxing with 2% sodium hydroxide solution on a water bath. The product (**10**) thereby obtained was found to have resulted from hydrolysis of the acetyl groups at the 3- and 3′-positions in **5**. The cleavage into the component steroid units was not observed under other conditions either (see experimental section).

Reduction of **5** with $LiAlH_4$ in tetrahydrofuran was then attempted and its reaction products were identified as 3β,20α-dihydroxypregn-5-ene (**9a**)[10] and 3β,22-dihydroxybisnorchol-5-ene (**11**)[11] by GC–MS, mixed mp determination and PMR spectroscopy (Chart 3).

These results suggest that **5** is an ester compound linked at 22 and 20′. Therefore, isopropyl 3β-acetoxybisnorchol-5-enate (**12**) was synthesized as a model compound and its PMR spectrum and MS were compared with those of **5**. In the PMR spectrum of **12**, the methyls in the isopropyl ester $(\text{-COO-CH}\begin{smallmatrix}\diagup CH_3\\ \diagdown CH_3\end{smallmatrix})$ appeared as a doublet at 1.15 ppm and the methine proton as a septet at 4.95 ppm. These values agreed well with the chemical shifts of the corresponding signals in the dimer (**5**). The fragmentation pattern of **12** in its MS also agreed well with that of **5**, except for the M^+ ion (Fig. 2). Fragment ions B and C appeared in the spectra of both, while ions D and E appeared in the spectrum of **12**, though they were relatively less intense than those in **5**, in which the ion D is the base peak. These observation suggest that ions D and E originate from the pregnene skeleton forming the dimer.

As stated earlier, this dimer is resistant to hydrolysis in acid and alkali solution. Thus, hydrolysis of model compounds (Chart 3, **12** and **14**) in 2, 5, and 20% alkali solution was attempted. The methyl ester (**14**) was hydrolyzed in 2% alkali to give 3β-hydroxybisnorchol-5-enic acid (**15**), while the isopropyl ester (**12**) gave **13** on hydrolysis of the 3ρ-acetyl ester alone. In 5% alkali, on the other hand, **12** gave **13** and **15** in 40% and 60% yields, respectively. In 20% alkali, **12** gave only **15** formed by the hydrolysis of both esters.

In view of these results, the structure of **5** was considered to be 3′β-acetoxypregn-5′-en-20′α-yl 3β-acetoxybisnorchol-5-enate with the ester linkage at positions 22 and 20′.

Comparison of Product Yields under Other Conditions in the Reaction of 1 with Lead Tetraacetate

Change of the solvent from benzene to toluene, xylene, dimethylformamide (DMF), or tetrahydrofuran (THF) resulted in different yields of the products, as shown in Table I, with the formation of a 17-ethyl compound (**2c**), whose structure was confirmed by comparison with the authentic specimen[12] obtained by synthesis.[13] In addition, the yield of 20-keto

Table I. Yields (%) of Products according to Solvents Used

Solvent	Refluxing time (h)	**2a**	**2b**	**2c**	**3a**[a]	**3b**[a]	**4**	**5**	Recovered **1**
Benzene	5	17	5	0	43	14	Trace	13	0
Benzene/O_2	5	5	2	0	13	4	3	5	55
Toluene	5	14	Trace	17	32	10	13	7	0
Xylene	5	14	Trace	21	26	8	15	7	0
Xylene/He	5	16	Trace	22	32.5	11	0	8.5	0
DMF	15	11	6	22	11	3	7	10	25
THF	30	0.5	0	5.5	Trace	Trace	12	Trace	70
$CHCl_3$	30	38	Trace	0	10	3	Trace	Trace	33
Benzene–Cu–Py	1	48	16	0	16	5	Trace	Trace	0
Toluene–Cu–Py	1	51	17	Trace	7.5	2.5	9	Trace	0

[a] The yields of **3a** and **3b** were calculated from the PMR data of the mixture of **3a, b**.

compound (**4**) increased greatly, in contrast to the trace amount found when benzene was used. In the reaction in benzene under a stream of oxygen gas, 3% of **4** was obtained with 55% recovery of the starting material (**1**). Kochi[14] reported that treatment of pentanoic acid with lead tetraacetate resulted in the inhibition of decarboxylation by oxygen, and our result is in agreement. In contrast, the formation of **4** was not observed in the reaction in xylene under a stream of helium gas.

Kochi[2b,14] has found that oxidative decarboxylation of carboxylic acids by lead tetraacetate is catalyzed by cupric acetate-pyridine. Addition of a catalytic amount of cupric acetate-pyridine in benzene and toluene resulted in a reverse yield ratio of the olefin compounds (**2a**, **b**) and the acetoxy compounds (**3a**, **b**), with increase of the former. This is in accord with the results of Kochi.

With chloroform as a solvent, the product was the Δ^{20} compound (**2a**), together with the acetoxy compounds (**3a**, **b**).

Discussion

It is considered that the olefin compounds (**2a**, **b**) and the acetoxy compounds (**3a**, **b**) are formed *via* the 20-carbonium cation (D).[2b] When benzene was used as a solvent, the formation ratio of **2a** and **2b** was 3:1 and that of **3a** and **3b** was 3:1. This must be due to the steric interaction between the side chain and the steroid skeleton.

The dimer was considered to have been formed by bonding of the acyloxy radical (B) and the radical (C) at the 20-position.

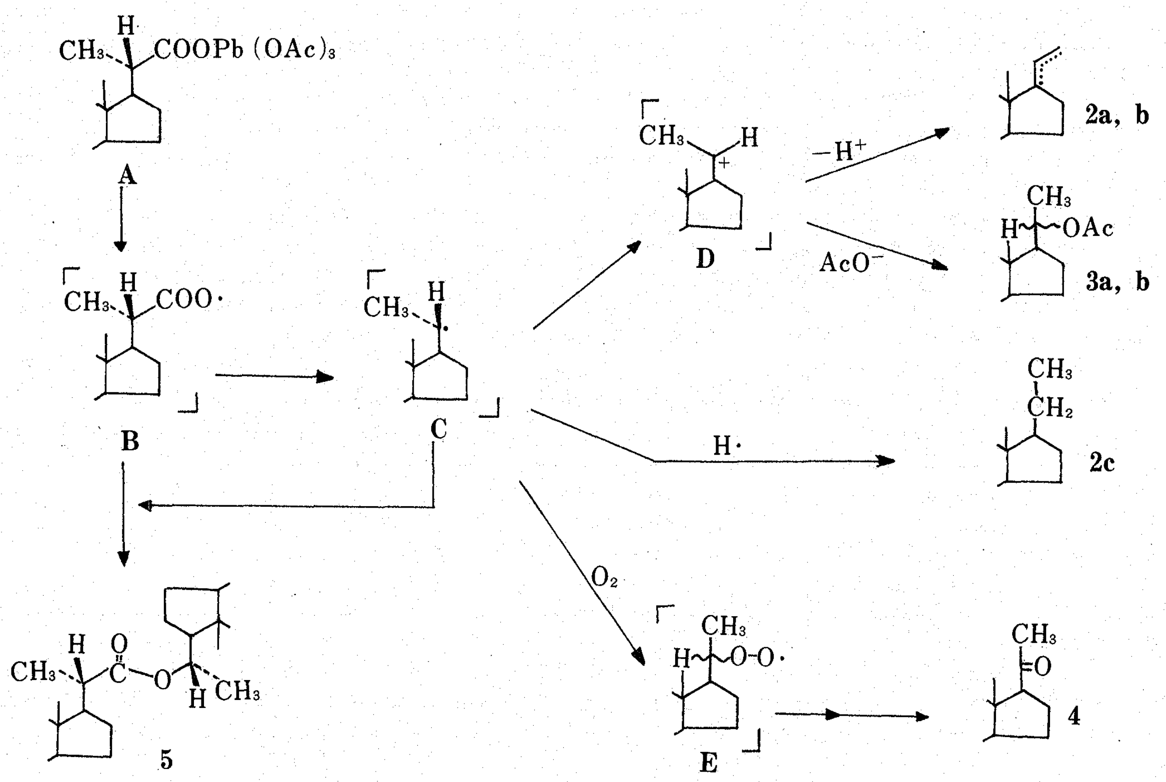


Chart 4. Proposed Reaction Mechanism

The 17-ethyl compound (**2c**) was not formed when benzene was used as a solvent, but was formed when toluene, xylene, DMF, or THF was used. Therefore, the hydrogen to be bonded to the radical (**C**) at the 20-position originates from the solvent. In order to confirm this, the reaction was carried out in [$^2H_8$]tolene. The less polar products obtained were found to be a 1:1 mixture of Δ^{20}-olefin compound (**2a**) and $\Delta^{17(20)}$-olefin compound (**2b**) and only a minute amount of the [$^2H_1$]ethyl compound by MS and PMR spectral analyses. The above results clearly prove that hydrogen of the solvent is utilized in the formtion of the ethyl compound (**2c**). The formation in low yield of the [$^2H_1$]ethyl compound in [$^2H_8$]toluene may be explained in terms of isotope effect.

The 20-keto compound (**4**) was considered to have been formed by the bonding of dissolved oxygen with the radical (C) at the 20-position, and the peroxy radical (E) thereby formed underwent decomposition to form **4**. This hypothesis is supported by the fact that **4** was not formed at all when the reaction was carried out in a helium atmosphere.

Change of the solvent from benzene to toluene, xylene, or THF resulted in the formation of **2c**, a decrease in the yield of **2b**, and an increase in the yield of **4**, but these results cannot be accounted for at present.

The results mentioned above indicated that the solvent used has a major effect on the reaction of 3β-acetoxybisnorchol-5-enic acid (**1**) and lead tetraacetate, altering the kind of products formed and their formation ratio.

Further, the formation of the dimer (**5**) is interesting in connection with the report[15] that steroid dimers showed a considerable prolongation of the duration of activity as compared to the corresponding monomers.

Experimental

All melting points were obtained on a micro-melting point determination apparatus (type MM2, Shimadzu Seisakusho Ltd.) and are uncorrected. PMR spectra were recorded on a JEOL JNM-MH-100 spectrometer at 100 MHz, with tetramethylsilane as an internal standard in deuteriochloroform. Abbreviations used: s=singlet, d=doublet, bd=broad doublet, t=triplet, q=quartet, m=multiplet. IR spectra were recorded on a Hitachi EPI-G3 machine as $CHCl_3$ solutions. Mass spectra (MS) were determined on a JEOL JMS D-100 spectrometer. Column chromatography was performed with Kanto Kagaku silica gel (100 mesh above), aluminum oxide 90 (E. Merck, Darmstadt), or Sephadex LH-20 (Pharmacia Fine Chemical). Thin layer chromatography (TLC) was done on Merck precoated Kieselgel 60 F_{254} plates (0.25 mm thick). Gas chromatography–mass spectrometry (GC–MS) was performed on a JEOL JMS D-100 spectrometer with a 1.5% OV-17 (2 m × 3 mm) column.

Reaction of 3β-Acetoxybisnorchol-5-enic Acid (1) with Lead Tetraacetate in Benzene——A suspension of **1** (0.5 g) and lead tetraacetate (1.5 g) in benzene (200 ml) was refluxed for 5 h. Insoluble lead salts were filtered off at room temperature and the filtrate was washed with saturated $NaHCO_3$ solution and water, and dried over anhydrous Na_2SO_4. The benzene was evaporated off under reduced pressure. The residue was chromatographed over silica gel (50 g). The results are shown in the table below.

Eluting solvent	Yield (mg)	Product	*Rf* (developed with CH_2Cl_2)
hexane–benzene (1: 1)	96	**2a, b**	0.61
benzene	295	**3a, b**	0.48
benzene–CH_2Cl_2 (5: 1)	Trace	**4**	0.40
benzene–CH_2Cl_2 (4: 1)	61	**5**	0.39

The hexane–benzene (1: 1) fraction, a 3: 1 mixture of Δ^{20}- and $\Delta^{17(20)}$-olefins was chromatographed over 10% $AgNO_3$-impregnated silica gel (30 g). Elution with hexane–benzene (1: 1) and recrystallization from MeOH afforded [17(20)*E*]-3β-acetoxypregna-5,17(20)-diene (**2b**), colorless needles, mp 143—144°C (lit.[4] 143—143.5°C). MS *m/e*: 282 (M^+−AcOH). PMR δ (ppm): 0.74 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.55 (3H, d, J=7 Hz, 21-CH_3), 2.00 (3H, s, 3-$OCOCH_3$), 4.59 (1H, m, 3-H), 5.02 (1H, m, 20-H), 5.35 (1H, bd, 6-H).

Elution with benzene and recrystallization from MeOH afforded 3β-acetoxypregna-5,20-diene (**2a**), colorless needles, mp 131—133°C (lit.[3] 132.5—135°C). MS *m/e*: 282 (M^+−AcOH). PMR δ (ppm): 0.60 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 2.00 (3H, s, 3-$OCOCH_3$), 4.59 (1H, m, 3-H), 4.95 (1H, q, J=2.5 and 18 Hz, 21-H), 4.96 (1H, q, J=2.5 and 9 Hz, 21-H), 5.35 (1H, bd, 6-H), 5.60—5.94 (1H, m, 20-H).

The benzene fraction, a 3: 1 epimeric mixture of 20α- and 20β-acetoxy compounds was recrystallized from MeOH to afford pure 3β,20α-diacetoxypregn-5-ene (**3a**), colorless needles, mp 140—141°C (lit.[10] 142°C). MS *m/e*: 342 (M^+−AcOH). PMR δ (ppm): 0.68 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.22 (3H, d, J=6.5 Hz, 21-CH_3), 1.98 (3H, s, 3-$OCOCH_3$), 2.02 (3H, s, 20-$OCOCH_3$), 5.00 (1H, m, 20-H).

3a was hydrolyzed with 2% KOH in 10% aq. MeOH, and the hydrolysis product was identified as 3β,20α-dihydroxypregn-5-ene (**9a**) by comparison with an authentic sample.

The mother liquor was hydrolyzed with 2% KOH in 10% aq. MeOH, and the neutral product was proved to contain **9a** and 3β,20β-dihydroxypregn-5-ene (**9b**) by GC–MS. The authentic sample of **9a** was obtained by reduction of pregnenolone with sodium and ethanol.[16] **9b** was obtained by reduction of pregnenolone with $LiAlH_4$.[17]

The residue of the benzene–CH_2Cl_2 (5: 1) fraction was identified as 3β-acetoxypregn-5-en-20-one (**4**) by GC–MS comparison with an authentic sample.

The benzene–CH_2Cl_2 (4: 1) fraction was recrystallized from CH_2Cl_2–MeOH to give 3′β-acetoxypregn-5′-en-20′α-yl 3β-acetoxybisnorchol-5-enate (**5**), colorless granular material, mp 313—314°C. *Anal.* Calcd for $C_{47}H_{70}O_6$: C, 77.22; H, 9.65. Found: C, 77.04; H, 9.53. MS *m/e*: Calcd for $C_{43}H_{62}O_2$ ($M^+-2AcOH$): 610.4749. Found: 610.4754. PMR δ (ppm): 0.67 (6H, s, 18- and 18′-CH_3), 1.00 (6H, s, 19- and 19′-CH_3), 1.15 (3H, d, $J=6.5$ Hz, 21-CH_3), 1.19 (3H, d, $J=6.5$ Hz, 21′-CH_3), 2.00 (6H, s, 3- and 3′-$OCOCH_3$), 4.60 (2H, m, 3- and 3′-H), 4.92 (1H, m, 20′-H), 5.40 (2H, bd, 6- and 6′-H). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (22—20′-ester), 1730—1732 (3- and 3′-ester).

Preparation of 3β-Acetoxy-5α-pregn-20-ene (6) from 3β-Acetoxybisnor-5α-cholanic Acid——A suspension of 3β-acetoxybisnor-5α-cholanic acid (0.4 g), cupric acetate (0.06 g), pyridine (0.05 g) and lead tetraacetate (1.2 g) in benzene (160 ml) was refluxed for 1 h. The reaction mixture was treated in the same way as in the case of the reaction of **1** with lead tetraacetate to give 0.16 g of **6**. Recrystallization from MeOH gave colorless needles of mp 78—80°C. *Anal.* Calcd for $C_{23}H_{36}O_2$: C, 80.18; H, 10.53. Found: C, 79.86; H, 10.39. MS *m/e*: 344 (M^+), 284. PMR δ (ppm): 0.58 (3H, s, 18-CH_3), 0.82 (3H, s, 19-CH_3), 1.99 (3H, s, 3-$OCOCH_3$), 4.70 (1H, m, 3-H), 4.90—5.93 (3H, m, 20-H and 21-H_2).

Epoxidation of 6 with *m*-Chloroperbenzoic Acid——A solution of *m*-chloroperbenzoic acid (40 mg) in $CHCl_3$ (50 ml) was added over 45 min to a solution of **6** (60 mg) in $CHCl_3$ (50 ml) at 5°C. After being stirred at room temperature for 48 h, the reaction mixture was washed with 10% Na_2SO_3 solution, saturated $NaHCO_3$ solution and water. The $CHCl_3$ solution was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed on aluminum oxide (10 g). Elution with benzene gave 30 mg of 3β-acetoxy-20,21-epoxy-5α-pregnane (**7a**, **b**). Recrystallization from MeOH gave colorless needles, mp127—128°C. *Anal.* Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07. Found: C, 77.00; H, 0.99. MS *m/e*: 360 (M^+), 300 (M^+-AcOH), 43 (, base peak). PMR δ (ppm): 0.75 and 0.80 (3H, s and s in a ratio of 2: 1, 18-CH_3 of **7a**, **b** isomers), 0.82 (3H, s, 19-CH_3), 1.99 (3H, s, 3-$OCOCH_3$), 2.40 (1H, q, 21-H), 2.65 (1H, t, 21-H), 2.84 (1H, m, 20-H), 4.70 (1H, m, 3-H).

Reaction of 7a, b with Lithium Aluminum Hydride——A suspension of **7a**, **b** (30 mg) and $LiAlH_4$ (30 mg) in abs. ether (50 ml) was refluxed for 1 h. The excess $LiAlH_4$ was destroyed by adding dil. H_2SO_4, then the organic layer was separated, washed with saturated $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. GC–MS analysis of the residue indicated that 3β,20α-dihydroxy-5α-pregnane (**8a**) and 3β,20β-dihydroxy-5α-pregnane (**8b**)[8] were present in the ratio of 2: 1.

Hydrolysis of the Dimer (5)——1) **5** (20 mg) was hydrolyzed with 2% NaOH in 20% aq. MeOH (50 ml) under reflux for 5 h and the hydrolyzate was extracted with CH_2Cl_2. The extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was recrystallized from CH_2Cl_2–MeOH to give colorless granular 3′β-hydroxypregn-5′-en-20′α-yl 3β-hydroxybisnorchol-5-enate (**10**) (15 mg) of mp 289—290°C. *Anal.* Calcd for $C_{43}H_{66}O_4$: C, 79.82; H, 10.28. Found: C, 79.77; H, 10.42. MS *m/e*: 646 (M^+), 628, 610. PMR δ (ppm): 0.67 (6H, s, 18- and 18′-CH_3), 1.00 (6H, s, 19- and 19′-CH_3), 1.15 (3H, d, $J=6.5$ Hz, 21-CH_3), 1.19 (3H, d, $J=6.5$ Hz, 21′-CH_3), 3.50 (2H, m, 3- and 3′-H), 4.92 (1H, m, 20′-H), 5.36 (2H, bd, 6- and 6′-H). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (22,20′-ester). The aqueous layer after extraction with CH_2Cl_2 was acidified with dil. HCl and again extracted with CH_2Cl_2. The extract was washed with water, and dried over anhydrous Na_2SO_4. Removal of the solvent by evaporation left no steroid product.

2) **5** (20 mg) was hydrolyzed with 5%, 20% NaOH or 7% HCl in 20% aq. MeOH (50 ml) by refluxing for 5 h and processed in the same way as in 1) above to give **10**.

Reduction of the Dimer (5) with $LiAlH_4$——A suspension of **5** (60 mg) and $LiAlH_4$ (120 mg) in abs. THF (50 ml) was refluxed for 5 h, then the excess $LiAlH_4$ was destroyed by adding dil. H_2SO_4. The reaction product was extracted with CH_2Cl_2, and the extract was washed with saturated $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. A part of the residue was analyzed by GC–MS, and exhibited two peaks corresponding to **9a** and **11**. The residue (50 mg) was chromatographed on Sephadex LH-20 (20 g). Elution with $CHCl_3$ gave 20 mg of 3β,20α-dihydroxypregn-5-ene (**9a**), which was identical with a standard sample.[10] Elution with $CHCl_3$–MeOH (9: 1) gave 20 mg of 3β,22-dihydroxybisnorchol-5-ene (**11**) which was identified by comparison with a standard sample.[11]

Preparation of Isopropyl 3β-Acetoxybisnorchol-5-enate (12) from 3β-Acetoxybisnorchol-5-enic Acid (1)——Oxalyl chloride (5 ml) was added to a solution of **1** (100 mg) in $CHCl_3$ (20 ml). The whole was stirred at room temperature for 24 h, then excess oxalyl chloride was removed by evaporation under reduced pressure. To the pyridine solution (30 ml) of the residue, 10 ml of isopropanol was added. The mixture was refluxed for 24 h. After the removal of the pyridine and isopropanol by evaporation under reduced pressure, the residue was extracted with CH_2Cl_2. The extract was washed with dil. HCl, saturated $NaHCO_3$ solution, water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (50 g). Elution with benzene gave 80 mg of white oil. Crystallization from MeOH gave **12** as colorless needles, mp 105—106°C. *Anal.* Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.77; H, 9.76. MS *m/e*: 370 (M^+-AcOH). PMR δ (ppm): 0.66 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.15 (3H, d, $J=6.5$ Hz, 21-CH_3), 1.19 (6H, d, $J=6.5$ Hz, CH_3 of isopropyl), 1.98 (3H, s, 3-$OCOCH_3$), 4.56 (1H, m, 3-H), 4.95 (1H, septet, methine of isopropyl), 5.36 (1H, bd, 6-H). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1724—1725 (22-ester).

Hydrolysis of Isopropyl 3β-Acetoxybisnorchol-5-enate (12)——1) **12** (20 mg) was hydrolyzed with 2% NaOH in 20% aq. MeOH (50 ml) under reflux for 5 h and the hydrolyzate was extracted with CH_2Cl_2. The extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was recrystallized from MeOH to give colorless needles of isopropyl 3β-hydroxybisnorchol-5-enate (**13**) of mp 126—127°C. *Anal.* Calcd for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 76.82; H, 10.33. MS *m/e*: 388 (M^+), 370. PMR δ (ppm): 0.66 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 1.15 (3H, d, $J=6.5$ Hz, 21-CH_3), 1.19 (6H, d, $J=6.5$ Hz, CH_3 of isopropyl), 3.45 (1H, m, 3-H), 4.95 (1H, septet, methine of isopropyl), 5.28 (1H, bd, 6-H). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1724 (22-ester).

2) **12** (20 mg) was hydrolyzed with 5% NaOH in 20% aq. MeOH (50 ml) under reflux for 5 h and processed in the same way as above to give 8 mg of **13** and 10 mg of 3β-hydroxybisnorchol-5-enic acid (**15**). The acidic material (**15**) after acetylation with Ac_2O-pyridine was identified as **1** by mixed mp determination and comparison of PMR and MS spectra with those of a standard sample.

3) **12** (20 mg) was hydrolyzed with 20% NaOH in 20% aq. MeOH (50 ml) under reflux for 5 h and processed in the same way as above to give 18 mg of **15**.

Hydrolysis of Methyl 3β-Acetoxybisnorchol-5-enate (14)——**14** (20 mg) was hydrolyzed with 2% NaOH in aq. MeOH (50 ml) under reflux for 5 h and processed in the same way as above to give **15**.

Reaction of 3β-Acetoxybisnorchol-5-enic Acid (1) with Lead Tetraacetate in Various Solvents——A suspension of **1** (0.5 g) and lead tetraacetate (1.5 g) in toluene, xylene, DMF, THF or $CHCl_3$ (200 ml) was refluxed for 5 h in the cases of toluene and xylene, for 15 h in the case of DMF or for 20 h in the cases of of THF and $CHCl_3$. Insoluble lead salts were filtered off at room temperature and the filtrate was evaporated to dryness under reduced pressure. Benzene (200 ml) was added to the residue and the resulting benzene solution was washed with saturated $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4, and worked up as in the case of the reaction in benzene. These results are shown in Table I. In the cases of toluene and xylene, **2** was a mixture of **2a**, **2c** and trace amounts of **2b**. In the case of DMF, **2** was a mixture of **2a**, **2b** and **2c**. This mixture was chromatographed over 10% $AgNO_3$-impregnated silica gel (40 g). Elution with hexane and recrystallization of the product from MeOH afforded 3β-acetoxypregn-5-ene (**2c**), colorless needles, mp 150—151°C, which was identical with an authentic sample.[12] MS *m/e*: 284 (M^+-AcOH). PMR δ (ppm): 0.58 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 2.00 (3H, s, 3-$OCOCH_3$), 4.60 (1H, m, 3-H), 5.36 (1H, bd, 6-H). **4** was identified as 3β-acetoxypregn-5-en-20-one by mixed mp determination with an authentic sample.

Preparation of 2c from 3β-Acetoxybisnorchol-5-enic Acid (1)——Oxalyl chloride (5 ml)[13] was added to a solution of **1** (100 mg) in $CHCl_3$ (10 ml). The whole was stirred at room temperature for 12 h, then excess oxalyl chloride was evaporated off under reduced pressure. The residue was dissolved in pyridine (10 ml) and the solution was refluxed for 2 h. After removal of the pyridine by evapotation, the residue was dissolved in abs. dioxane (10 ml). $LiAlH_4$ (100 mg) was added to the resulting solution and the whole was refluxed for 24 h. The excess $LiAlH_4$ was destroyed by adding dil. H_2SO_4, and the product was extracted with CH_2Cl_2. The extract was washed with saturated $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on 10% $AgNO_3$-impregnated silica gel (50 g) after acetylation with Ac_2O-pyridine. Elution with hexane and recrystallization of the product (50 mg) from MeOH afforded **2c**, colorless needles, mp 150—151°C (lit.[12] 146.8—148.8°C).

Reaction of 1 with Lead Tetraacetate under Oxygen Gas——A suspension of **1** (0.1 g) and lead tetraacetate (0.3 g) in benzene (40 ml) was refluxed for 5 h under a stream of O_2 gas. The reaction mixture was treated in the same way as in the case of the reaction in benzene to give 6 mg of **2a**, **b**, 18 mg of **3a**, **b**, 3 mg of **4**, 5 mg of **5**, and 55 mg of the starting material. The ratios of **2a** and **2b** and of **3a** and **3b** were the same as in the reaction in benzene.

Reaction of 1 with Lead Tetraacetate under Helium Gas——A suspension of **1** (0.1 g) and lead tetraacetate (0.3 g) in xylene (40 ml) was refluxed for 5 h under a stream of helium gas. The reaction mixture was treated in the same way as in xylene to give 34 mg of **2a**, **c**, 45 mg of **3a**, **b**, 8 mg of **5** and a trace of **2b**. The ratios of **2a** and **2c** and of **3a** and **3b** were the same as in the reaction in xylene.

Reaction of 1 with Lead Tetraacetate in the Presence of Cupric Acetate and Pyridine in Benzene——A suspension of **1** (0.5 g), cupric acetate (0.075 g), pyridine (0.06 g) and lead tetraacetate (1.5 g) in benzene (200 ml) was refluxed for 1 h. The reaction mixture was worked up as usual to give 283 mg of **2a**, **b** and 107 mg of **3a**, **b**. Further, traces of **4** and **5** were detected on TLC. The ratio of **2a** and **2b** was 3:1, as observed in the reaction in benzene and other solvents. The ratio of **3a** and **3b** was also 3:1.

Reaction of 1 with Lead Tetraacetate in the Presence of Cupric Acetate and Pyridine in Toluene——The reaction in toluene was performed under almost the same conditions as in benzene to give 68% of **2a**, **b**, 10% of **3a**, **b**, 9% of **4** and a trace of **2c**.

Reaction of 1 with Lead Tetraacetate in [$^2H_8$]Toluene——A suspension of **1** (25 mg) and lead tetraacetate (75 mg) in [$^2H_8$]toluene (10 ml) was refluxed for 5 h. The reaction mixture was processed in the same way as in the case of the reaction in undeuterated toluene to give 4 mg of **2** (which was found to be composed of an equal mixture of **2a** and **2b**), a trace of [$^2H_1$]ethyl compound [MS *m/e*: 285 (M^+-AcOH)], 3 mg of **3a**, **b** (3: 1), 5 mg of **4**, and 5 mg of the starting material.

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