AUTOXIDATION *vs* HYDROLYSIS IN 16α-ACYLOXY STEROIDS+

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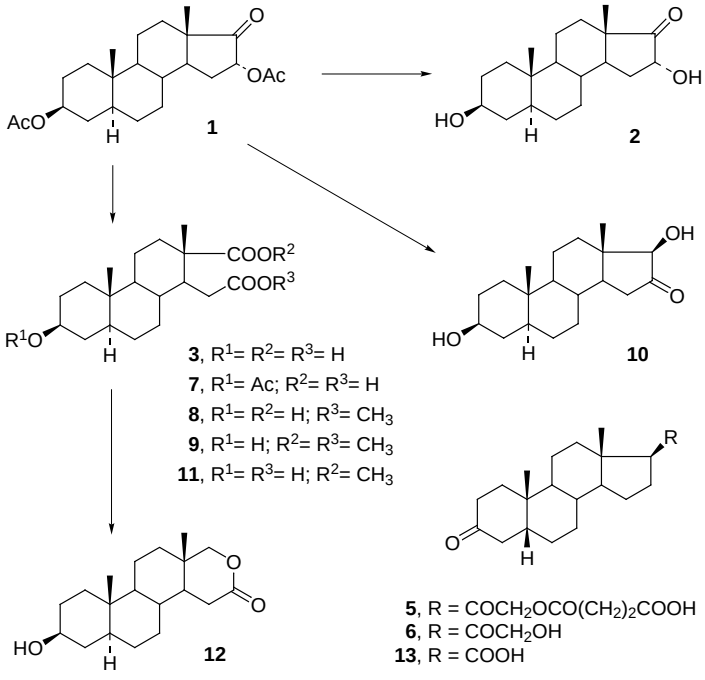
Some enolizable α-hydroxy ketones are extremely susceptible to oxidation with traces of air in a reaction vessel. Autoxidation can be used in synthesis of oxo acids or diacids and their derivatives. Yet alkaline hydrolysis of the substrate is possible though under strictly air-free conditions.

Key words: Steroids; Oxidation; Ketones; Mass spectrometry; ^{1}H NMR spectroscopy; Neighbouring group participation.

Some properties of neurosteroids have already been utilized. For example, derivatives of 3α-hydroxy-5α-pregnan-20-one were often more active than nonsteroidal tranquillizers in the treatment[2] of anxiety and pain. Neuroprotective properties of other neurosteroids, which have the potential of reducing the detrimental consequences of stroke, are not yet generally exploited in practice. A reason for this situation is their low solubility in water. Recently[3] we have prepared a few potential neurosteroids designed to act on $GABA_A$ receptors. For the synthesis of new water-soluble analogues, we needed first to hydrolyse diacetate **1** (Scheme 1) to diol **2**. Unexpected results obtained during this work are described here.

A methanolic solution of potassium hydroxide was used for a swift hydrolysis of simple esters. This cheap reagent was employed even with oxo esters[4] as long as the reaction was carried out under an inert atmosphere. However, when this reagent was used with compound **1**, diol **2** was isolated in a low yield only. The side product, which had first eluded detection, was identified as the product of autoxidation – acid **3**. A few additional experiments revealed that α-acetoxy ketone **1** was extremely susceptible to oxida-

+ Part CDI in the series On Steroids; Part CD see ref.[1]



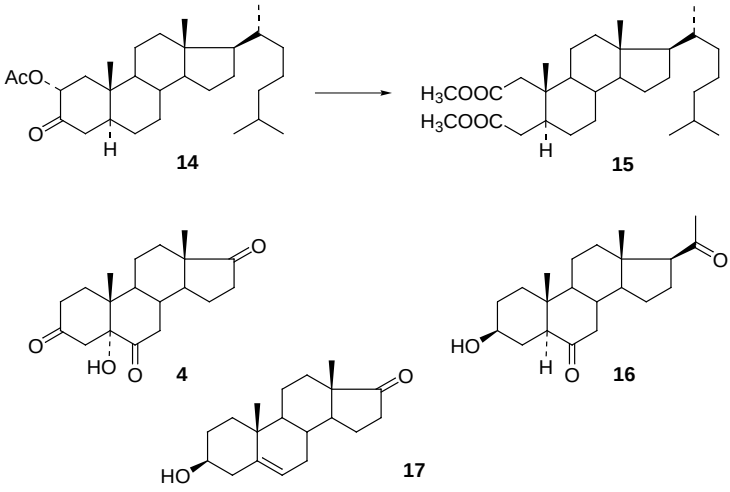
SCHEME 1

tion with traces of oxygen remaining in the vessel used: increased yield of acid **3** was obtained in the reaction carried out by standing in air, the stirring of the mixture in air accelerating the oxidation still further.

Autoxidation of α-hydroxy ketones is known to proceed in the presence of peroxides[5] and under catalysis by light[6]. However, the above reaction was reported even for experiments run in the absence of peroxides: 2α-hydroxy-5α-cholestan-3-one was converted[7] to a mixture of oxidized products, mainly esters of 2,3-seco-5α-cholestane-2,3-dioic acid; derivatives of androstane-17β-carboxylic acid[8] were found among some hydrolytic products of 21-acetoxypregnan-20-one derivatives (the oxidation reportedly appeared only with 11β-acetoxy derivatives). Our reaction was first proposed as an explanation of the unexpected "hydrolysis" of 16β-amino-3β-hydroxy-5α-androstan-17-one[9] into methyl ester of 3β-hydroxy-16,17-seco-5α-androstane-16,17-dioic acid (**3**).

We did not observe the presence of peroxides in the mixture (the iodine-starch test) nor any dependance of the oxidation rate on light. Thus we believe that the mechanism of the above oxidation is similar to that of

an enol ether reaction with oxygen[10] rather than the "peroxide" mechanism[5]. Accordingly, no oxidation was observed in α-hydroxy ketones lacking hydrogen atoms geminal to the hydroxyl (*e.g.* **4**, Scheme 2).



SCHEME 2

Finally, we established experimental conditions for the preparation of either products of hydrolysis or autoxidation. Thus hydrolysis of esters **1** and **5** (Scheme 1) to yield corresponding alcohols **2** and **6** was achieved by the rigorous elimination of oxygen from the reaction mixture. Alternatively, the C–C bond in α-hydroxy or α-acyloxy ketones was cleaved when the potassium hydroxide treatment was carried out in air, preferably under stirring.

Though solvent only plays a minor role in the oxidation, its modification can be synthetically useful: since bulky alcohol (*e.g.* propan-1-ol, 2-methylpropan-2-ol) is less likely to interfere with the oxidation, free acids **3** and **7** are formed. On the other hand, the reaction in methanol, leading to the acid **3** and esters **8** to **9**, was slower (see Experimental) and allowed for the isolation of a product of hydrolysis and intramolecular hydride ion migration, leading to ketol **10**.

The structure of the products were deduced from analysis of their IR, MS and NMR spectra or by correlation with authentic samples. Mass spectrum of the monoester **8** contained a fragment (*m/z* 74) typical of esters of a 16-carboxylate. The structural assignment of monoesters **8** and **11** was confirmed by NMR spectroscopy as follows: very similar substitution effects of a COOH and COOMe group and their sterical proximity did not allow this

problem to be solved from routine ^{1}H NMR spectra. Therefore a complete analysis of high-field ^{1}H and ^{13}C spectra was performed. Structure assignment of protons was obtained from homonuclear 2D-COSY spectra (for data of selected protons, see Table I). The second-order effects observed in proton subspectra of critical structure fragment –C(14)H–C(15)H_2–COOR required simulation-iteration analysis for obtaining correct values of chemical shifts and coupling constants. Calculated subspectra are shown in Fig. 1. Very similar values of J(14,15α) and J(14,15β) indicate close conformational similarity of given fragment in compounds **3**, **8**, **9** and **11**. Chemical shifts of carbon atoms were assigned using the "attached proton test" spectra and the literature data on similar compounds (Table II). Among the spectra, two pairs should always reflect the effect of esterification of either C(13)–COOH or C(15)–COOH. Chemical shift differences for all pairs of compounds are in general rather small (for selected carbon atoms and protons they are

TABLE I
Selected proton NMR data of compounds **3**, **8**, **9**, **11** and **12** in $CDCl_3$–CD_3OD (9 : 1)

Parameter	**3**	**8**	**11**	**9**	**12**[a]
δ(H-3)	3.57 tt	3.57 tt	3.57 tt	3.57 tt	3.59 tt
δ(Me-18)	1.10 s	1.08 s	1.10 s	1.10 s	0.99 s
δ(Me-19)	0.78 s	0.78 s	0.78 s	0.78 s	0.81 s
δ(13-COOMe)	–	–	3.66 s	3.66 s	–
δ(15-COOMe)	–	3.64 s	–	3.65 s	–
δ(H-12α)	1.78	1.81	1.78	1.80	1.08
δ(H-12β)	1.72	1.69	1.66	1.56	1.49
δ(H-14)	2.21 ddd	2.28 ddd	2.23 ddd	2.28 ddd	1.37 ddd
J(14,8)	11.1	11.0	11.0	11.0	10.5
J(14,15A)	2.1	2.2	2.4	2.5	5.9
J14,15B)	7.8	8.1	7.7	7.8	12.7
δ(H-15A)	2.18 dd	2.18 dd	2.07 dd	2.09 dd	2.72 ddd
δ(H-15B)	2.13 dd	2.15 dd	2.12 dd	2.15 dd	2.09 dd
J(15A,15B)	–16.7	–16.7	–16.7	–16.7	–18.6

[a] δ 3.95 d, 1 H, J(17β,17α) = –10.7 (H-17β); δ 3.87 dq, 1 H, J(17α,17β) = –10.7, J(17α,18) = 0.8.

given in Table III). The only significant differences – *ca* 0.35 ppm in chemical shifts of the carbon C(13) in pairs **11**–**3** and **9**–**8** and *ca* –0.40 ppm in chemical shifts of the carbon C(15) in pairs **8**–**3** and **9**–**11** can be inter-

TABLE II
Carbon-13 NMR data of compounds **3**, **8**, **9**, **11** and **12** in $CDCl_3$–CD_3OD (9 : 1)

Carbon	**3**	**8**	**11**	**9**	**12**[a]
1	36.66	36.67	36.62	36.63	36.66
2	30.88	30.93	30.80	30.85	31.17
3	70.74	70.75	70.68	70.66	70.95
4	37.50	37.53	37.42	37.46	37.78
5	44.13	44.06	44.07	44.03	44.26
6	28.42	28.46	28.38	28.40	28.13
7	30.83	30.73	30.72	30.70	29.99
8	37.81	37.67	37.67	37.54	36.03
9	52.93	52.85	52.83	52.80	53.13
10	35.45	35.45	35.41	35.42	35.54
11	19.86	19.93	19.81	19.84	19.47
12	36.45	36.54	36.26	36.33	31.85
13	46.86	46.86	47.22	47.19	32.32
14	43.63	43.52	43.56	43.59	44.51
15	36.94	36.48	36.62	36.27	34.49
18	15.15	15.14	15.12	15.14	15.07
19	12.08	12.10	12.03	12.05	12.22
13-COOR:					
C=O	[b]	[b]	175.95	174.04	–
CH_3	–	–	51.80	51.80	–
15-COOR:					
C=O	[b]	174.37	178.90	178.52	171.23
CH_3	–	51.56	–	51.58	–

[a] C-17: 81.22; [b] not detected.

preted as the effect of the esterification of C(13)–COOH and C(15)–COOH, respectively. We would expect the strongest effect at the carbon atom in the α-position to a COOR group.

The above proof was further supported by chemical evidence: the major carboxy ester **8** was reduced by a carboxyl-reducing agent (borane); the primary reduction product underwent a spontaneous transesterification with the formation of a δ-lactone **12** (see IR spectrum in Experimental). Its ^{1}H NMR spectrum shows CH_2–O protons as doublets at δ 3.95 and 3.87 with a geminal coupling of 10.7 Hz. The absence of vicinal coupling and fine additional splitting of the second doublet at δ 3.87 by long-range coupling (0.8 Hz) to 18-methyl protons is in agreement with structure **12**.

In summary, the extreme sensitivity of some steroidal α-hydroxy and α-acyloxy ketones to air in the presence of alkali offers a warning to experimenters: all traces of oxygen have to be removed from the vessel. However, it also affords a mild method of choice for oxidation of the substrates to oxo acids or diacids (*e.g.*, in 2-methylpropan-2-ol) or their derivatives (*e.g.*, in methanol) without need to use more powerful oxidizing agents[11]: the isolation of compound **7** shows that the oxidation is faster than hydrolysis of acetates.

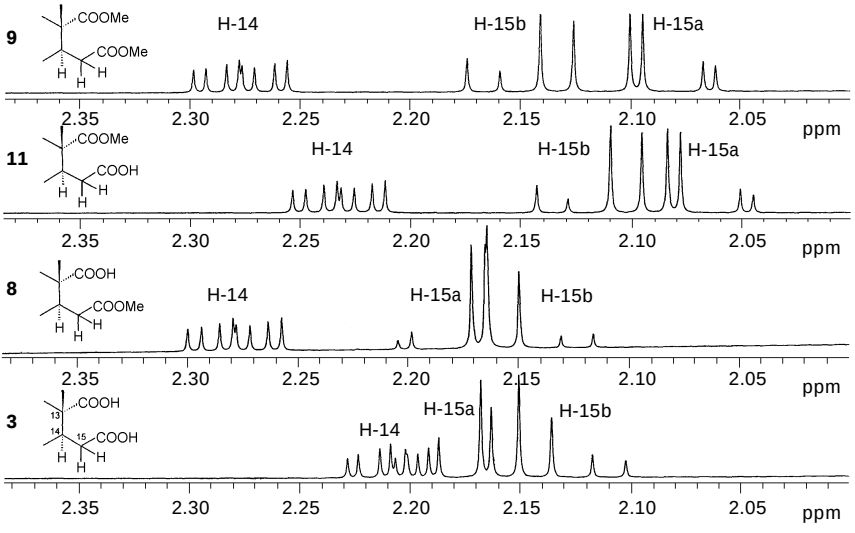


FIG. 1

Simulated proton subspectra of fragments –C(14)H–C(15)H_2–COOR in compounds **3**, **8**, **9** and **11** in $CDCl_3$–CD_3OD (9 : 1)

EXPERIMENTAL

Melting points were determined on a micro melting point apparatus Boetius (Germany) and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 50 °C/100 Pa. Optical rotations were measured in chloroform ($[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}), IR spectra of chloroform solutions were recorded on a Bruker IFS 88 spectrometer, wavenumbers are given in cm^{-1}. Proton NMR spectra of compounds **2**, **6**, **7**, **10**, **13** and **15** were measured on FT-NMR spectrometer Varian UNITY-200 (at 200 MHz) in $CDCl_3$ with tetramethylsilane as internal reference. Proton and carbon-13 NMR spectra of compounds **3**, **8**, **9**, **11** and **12** were acquired on Varian UNITY-500 (^{1}H at 500 MHz; ^{13}C at 125.7 MHz frequency) in $CDCl_3$–CD_3OD (9 : 1). Chemical shifts are given in ppm (δ-scale), coupling constants and width of multiplets in Hz. Unless otherwise stated, the data were interpreted as the first-order spectra. Thin-layer chromatography (TLC) was performed on silica gel (ICN Biochemicals), preparative TLC was carried out on 200 × 200 mm plates coated with an 0.7 mm thick layer of the same material. For column chromatography, silica gel 60–120 μm was used.

General Procedures for Hydrolysis and/or Oxidation

A) Substrate (0.13 mmol) and a magnetic stirrer were placed in a flask connected with a dropping funnel containing a solution of potassium hydroxide (1%) in methanol (6 ml, 1.07 mmol). The set was shortly evacuated and filled with nitrogen. This operation was repeated twice. The alkaline solution was then dripped into the flask containing the substrate and left at room temperature without stirring; the reaction was monitored by TLC. Usual work-up of the reaction mixture denotes diluting with brine (30 ml), acidifying with hydrochloric acid (10%, 0.5 ml), extraction with chloroform (3 × 15 ml), washing with brine (2 × 10 ml), drying over anhydrous sodium sulfate and evaporation of the solvent *in vacuo* to dryness.

TABLE III
Chemical shifts differences of selected carbon atoms and protons in compounds **3**, **8**, **9** and **11**

Compounds	C-12	C-13	C-14	C-15	H-16	H-12a/H12b	H-14	H-15a/H15b	H-18
					Effect of esterification at C(13)-COOH				
11–**3**	–0.19	0.36	–0.07	–0.32	–0.03	≈0/–0.13	0.03	–0.11/–0.02	≈0
9–**8**	–0.21	0.33	0.07	–0.21	≈0	–0.01/–0.13	≈0	–0.09/≈0	0.02
					Effect of esterification at C(15)-COOH				
8–**3**	0.09	≈0	–0.11	–0.46	–0.01	0.03/–0.10	0.07	≈0/0.02	–0.02
9–**11**	0.07	–0.03	0.03	–0.35	0.02	0.02/–0.10	0.04	0.02/0.03	≈0

B) Substrate (0.13 mmol) was added to a stirred solution of potassium hydroxide (1%) in methanol or 2-methylpropan-2-ol (6 ml, 1.07 mmol) in air. The stirring continued until the disappearance of the starting material, the mixture was worked up as above.

3β,16α-Dihydroxy-5α-androstan-17-one (**2**)

Following procedure *A*), diacetoxy ketone **1** (ref.[12], 50.0 mg, 0.13 mmol) was treated with the base in methanol (6 ml, 1.07 mmol) for 3 h. Product obtained (34 mg, 87%) was found identical with an authentic specimen of diol **2**, m.p. 182–184 °C (ref.[4] 183–185 °C). ^{1}H NMR: 0.83 s, 3 H (3 × H-19); 0.95 s, 3 H (3 × H-18); 3.60 m, 1 H, $W_{1/2}$ = 11 (H-3); 4.36 dd, 1 H, J = 2.4 and 7.6 (H-16).

Prolonged Autoxidation of Compound **1**

Following procedure *B*), diacetoxy ketone **1** (40.0 mg, 0.10 mmol) was stirred with the base in methanol (4.8 ml, 0.86 mmol) for 6 h. The mixture was allowed to stand for additional 72 h. Chromatography of the residue on a preparative plate in chloroform–methanol (9 : 1) afforded:

3β-Hydroxy-16,17-seco-5α-androstane-16,17-dioic acid (**3**), 3 mg (9%), m.p. 230–235 °C (ref.[13] 227–237 °C). For ^{1}H and ^{13}C NMR spectra see Tables I and II.

16-Methyl 3β-hydroxy-16,17-seco-5α-androstane-16,17-dioate (**8**), 20 mg (55%), m.p. 182–184 °C (ref.[9] 183–184 °C). For ^{1}H and ^{13}C NMR spectra see Tables I and II.

Dimethyl 3β-hydroxy-16,17-seco-5α-androstane-16,17-dioate (**9**), 4 mg (11%), m.p. 107–109 °C (ref.[14] 111–112 °C). IR: 3 610 (OH); 1 723 (C=O); 1 436 ($COOCH_3$); 1 237, 1 194 (C–O); 1 039 (C–OH). For ^{1}H and ^{13}C NMR spectra see Tables I and II.

17-Methyl 3β-hydroxy-16,17-seco-5α-androstane-16,17-dioic acid (**11**), 4 mg, (11%), m.p. 181–183 °C (ref.[15] 180.5–182.5 °C). For ^{1}H and ^{13}C NMR spectra see Tables I and II.

3β-Acetoxy-16,17-seco-5α-androstan-16,17-dioic Acid (**7**)

Following procedure *B*), diacetoxy ketone **1** (50.0 mg, 0.13 mmol) was stirred with potassium hydroxide in 2-methylpropan-2-ol (6 ml, 1.07 mmol) for 3 h. The product was separated by PLC in two components: diacid **3** (13 mg, 30%), identical with authentic sample[13], and compound **7** (27 mg, 55%), m.p. 227–230 °C (ref.[16] 229–231 °C). ^{1}H NMR: 0.79 s, 3 H (3 × H-19); 1.16 s, 3 H (3 × H-18); 2.02 s, 3 H (CH_3COO); 4.70 m, 1 H, $W_{1/2}$ = 11 (H-3).

3β,17β-Dihydroxy-5α-androstan-16-one (**10**)

Following procedure *A*), diacetoxy ketone **1** (50.0 mg, 0.13 mmol) was treated with potassium hydroxide in methanol (6 ml, 1.07 mmol) for 3 days. The rearranged product was crystallized from MeOH to give **10** (29 mg, 58%), $[\alpha]_D$ –135 (*c* 1.15, $CHCl_3$), m.p. 208–212 °C (ref.[17] $[\alpha]_D$ –149 (dioxane), m.p. 214–220 °C). ^{1}H NMR: 0.72 s, 3 H (3 × H-19); 0.84 s, 3 H (3 × H-18); 3.60 m, 1 H, $W_{1/2}$ = 11 (H-3); 3.73 s, 1 H (H-17).

3β,17-Dihydroxy-16,17-seco-5α-androstano-16,17-lactone (**12**)

The borane–methyl sulfide complex (10 mol l^{-1}, 0.1 ml, 1 mmol) was added to a cold solution (0 °C) of 16-oic acid methyl ester **8** (9.0 mg, 0.03 mmol) in tetrahydrofuran (0.1 ml), stirred under nitrogen. After 30 min, ethyl acetate (3 ml) and potassium carbonate (66 mg,

0.48 mmol) in water (0.3 ml) were added. The organic layer was separated, washed with water, dried and stripped of the solvent, yielding lactone **12** (8.1 mg, 97%), m.p. 200–201 °C (ref.[18] 201 °C). For ^{1}H and ^{13}C NMR spectra see Tables I and II.

21-Hydroxy-5β-pregnane-3,20-dione (**6**)

The title compound was obtained from hemisuccinate **5** (ref.[19], 100 mg, 0.23 mmol) according to procedure *A*), *i.e.* by using a cold (0 °C) methanolic alkali solution (11 ml, 1.96 mmol) while standing for 2 h at room temperature. Compound **6** (70 mg, 91%) was identical with the authentic sample, m.p. 150–152 °C (ref.[20] records m.p. 152–154 °C). IR: 3 485, 3 380 (OH); 1 706 (C=O); 1 075 (C–OH). ^{1}H NMR: 0.66 s, 3 H (3 × H-18); 1.02 s, 3 H (3 × H-19); 2.69 t, 1 H, J = 14 (H-4α); 4.14 d and 4.23 d, 2 H, J = 20 (2 × H-21).

3-Oxo-5β-pregnane-17β-carboxylic Acid (**13**)

The title compound was obtained from hemisuccinate **5** (1.0 g, 2.3 mmol) according to procedure *B*) by 2 h stirring with alkali in methanol (50 ml, 8.92 mmol). The mixture was diluted with brine (150 ml) and hydrochloric acid (5%, 7 ml). A precipitate was filtered off, washed with brine (100 ml), dissolved in chloroform (50 ml) and dried over sodium sulfate. Removal of the solvent yielded crystalline acid **13** (866 mg, 91%), m.p. 248–251 °C (ref.[21] 246–249 °C). IR: 3 528 (OH); 3 085, 3 023, 2 747, 2 665, 2 637, 2 582 (OH, COOH dimer); 1 751 (C=O, COOH); 1 716 (C=O, COOH dimer); 1 704 (ketone). ^{1}H NMR: 0.75 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 2.69 t, 1 H, J = 14 (H-4α).

When the reaction was carried out in the presence of 1,4-benzoquinone (25 mg, 0.12 mmol), hemisuccinate **5** (100 mg, 0.23 mmol) afforded the same carboxylic acid **13** (86%).

Dimethyl 2,3-Seco-5α-cholestane-2,3-dioate (**15**)

The 5 day treatment (procedure *B*) of α-acetoxy ketone (**14**, ref.[7], 100 mg, 0.22 mmol) with alkali in 2-methylpropan-2-ol (11 ml, 1.96 mmol) afforded product which was esterified with diazomethane (105 mg, 2.5 mmol) in methanol (0.6 ml). Thin layer chromatography of the product afforded:

Dimethyl ester **15** (44 mg, 42%), m.p. 61–62 °C (ref.[7] 58–60 °C). IR: 1 729 (C=O); 1 195, 1 1180, 1 173 (C–O); 1 436 ($COOCH_3$). ^{1}H NMR: 0.64 s, 3 H (3 × H-18); 0.79 s, 3 H (3 × H-19); 0.84 d, 6 H, J = 6 (3 × H-26 and 3 × H-27); 0.91 d, 3 H, J = 6.0 (3 × H-21); 3.64 s and 3.66 s, 6 H (2 × OCH_3).

A mixture of partially oxidized products (43 mg).

Attempted Oxidation of Compounds **4**, **16** and **17**

Compounds **4** (ref.[22], 100 mg, 0.19 mmol), **16** (100 mg, 0.31 mmol) and **17** (100 mg, 0.35 mmol) were treated with potassium hydroxide in methanol in air (procedure *B*) for several days. Starting compounds were recovered unchanged.

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