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High-field NMR studies of 3β-tetrahydropyranyloxy steroids☆

Zsuzsanna Szendi[a], Péter Forgó[a,*], Katalin E. Kövér[b], Frederick Sweet[c]

[a]*Department of Organic Chemistry, József Attila University, Szeged, Hungary*
[b]*Department of Organic Chemistry, Kossuth Lajos University, Debrecen, Hungary*
[c]*Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO, USA*

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Abstract

Comprehensive NMR studies were carried out on 3β-hydroxy-pregnene and cholestene analogs, each containing a tetrahydropyranyl ether group at the 3-position. Two-dimensional NMR experiments (COSY, TOCSY, HSQC, and HSQC-TOCSY) permitted the complete assignments of both the ^{1}H and ^{13}C resonances of these derivatives in deuterated benzene or chloroform. The aromatic solvent-induced NMR signal shifts (ASIS) were also investigated. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

The 2-tetrahydropyranyl ether is the most frequently used blocking group for protecting hydroxyl functions while modifying the molecular structures of well-known steroids under strongly alkaline reaction conditions [1–8].

Introducing a tetrahydropyranyloxy (OTHP) group into a steroid molecule produces an additional asymmetric center and therefore a new pair of diastereoisomers. Diastereoisomerism associated with the 2-tetrahydropyranyl ether group is well established [1,9–13], and the NMR assignments are available for the simple 2-alkoxy- and 2-phenoxytetrahydropyrans from 3,4-dihydro-2H-pyran and the corresponding alcohols [14–17]. Similarly, the NMR spectra of the tetrahydropyranyl protecting group condensed with C-3 and C-17β in estrone and estradiol serving as models were comprehensively studied [18].

The tetrahydropyranyl group constituting a 2′R and 2′S diastereoisomeric mixture makes the complete configurational determination of new products difficult for validating stereochemistry by spectroscopic methods. In NMR spectroscopy, these diastereoisomeric pairs result in signal doubling. Chemical shift differences of signals belonging to different diastereoisomers are only a few Hertz, depending on the distance from the new chiral center, and exhibit solvent dependency. Nevertheless, very often, only the sharp singlets in the proton spectra due to the angular methyl signals at the C-18 and C-19 positions are used as sensitive indicators to differentiate the two diastereoisomers. When the 3β-position contains a tetrahydropyranyl ether group, the most sensitive signals in the carbon spectrum are those due to C-1, C-2, C-3, and C-4 in the steroid moiety.

The present report describes complete assignments for the ^{1}H and ^{13}C NMR spectra from (2′R,S) diastereoisomeric mixtures of steroid tetrahydropyranyl ethers of the well-known steroids: 3β-hydroxypregn-5-en-20-one 3-(2′-tetrahydropyranyl) ether **1**; 3β-hydroxypregna-5,16-dien-20-one 3-(2′-tetrahydropyranyl) ether **2**; (20R)-pregn-5-ene-3β,20-diol 3-(2′-tetrahydropyranyl) ether **3**; and also cholesterol analogs: 22,26-epoxy-(20R)-27-norcholesta-5,22-diene-3β,20-diol 3-tetrahydropyranyl ether **4** [11]; 3β,20-dihydroxy-(20S)-27-norcholest-5-en-25-one ethylene ketal 3-(2′-tetrahydropyranyl) ether **5**; 3β,20,25-trihydroxy-(20R)-26,27-bisnorcholest-5-en-22-one 3-(2′-tetrahydropyranyl) ether **6** [11]. This report also describes the $CDCl_3$ and C_6D_6 solvent influences on the chemical shifts of ^{1}H and ^{13}C in the mixtures of (2′R)- and (2′S)-tetrahydropyranyl ether diastereoisomers.

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* Corresponding author. Tel./fax: +36-62-54-42-00.
E-mail address: pforgo@sol.chem.u-szeged.hu (P. Forgó).

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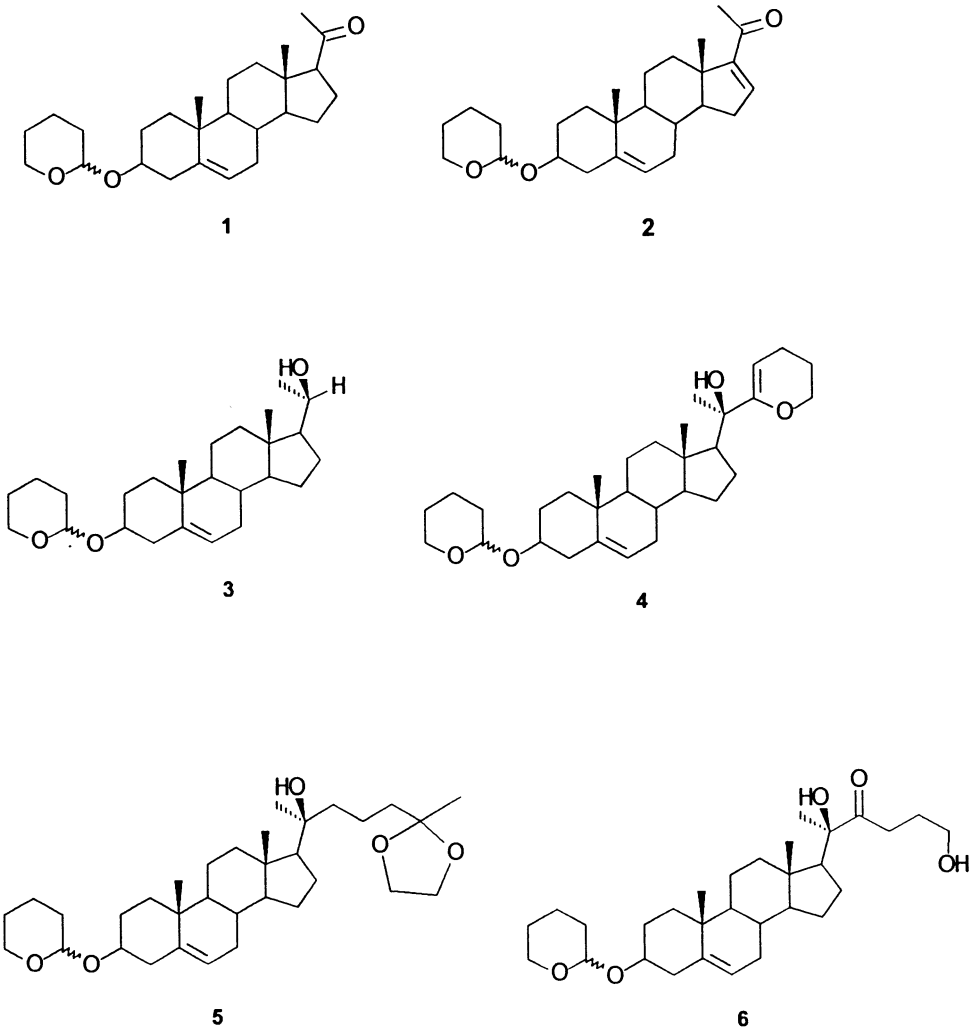

Scheme 1.

2. Experimental procedures

2.1. General

NMR spectra were recorded at room temperature on a Bruker AM-400 spectrometer using a 5-mm probehead (at 400.13 MHz and 100.62 MHz). Samples of compounds **1** to **6** were dissolved in $CDCl_3$ or C_6D_6. Two-dimensional proton-detected heteronuclear polarization transfer experiments were carried out on a Bruker AC-400 instrument, using 5-mm reverse constructed broad-band probe. An Aspect-3000 workstation was used for data processing. Chemical shift data are presented in ppm values and internal TMS was used as reference material.

All reagents and solvents were dried and fractionally distilled prior to their use. In the present study, the condensation of 3,4-[2H]-dihydropyran with 3β-hydroxysteroids was complete within 5 min. Compounds **3** and **5** were made from 3β-hydroxypregn-5-en-20-one 3-(2′-tetrahydropyranyl) ether (**1**). Preparations of compounds **4** and **6** are described in an earlier paper [11]. Melting points (mp) were measured in a Kofler block and are reported without corrections. The melting points varied with the ratios of the 2′R and 2′S diastereoisomers in the tetrahydropyranyl ether mixtures. Reactions were monitored on thin-layer chromatography plates (TLC) (Merck 5554 layer), eluted with methanol:benzene (1:9 v/v). The migrating components were detected under ultraviolet (UV) light (254 and 366 nm), and visualized by spraying the plates with concentrated H_2SO_4 and heating them at 120°C for 10 min. Infrared (IR) spectra were recorded with KBr pellets in a UNICAM SP 200 instrument.

*2.2. 3β-Hydroxypregn-5-en-20-one 3-(2′-tetrahydropyranyl) ether (**1**) [5]*

To pregnenolone (3.165 g, 0.01 mol) dissolved in 25 ml of dry CH_2Cl_2:benzene (40:10 v/v) were added 10 ml (0.1 mol) of 3,4-[2H]-dihydropyran (DHP) and 0.2 g *p*-toluenesulfonic acid hydrate. Within 5 min, all of the starting

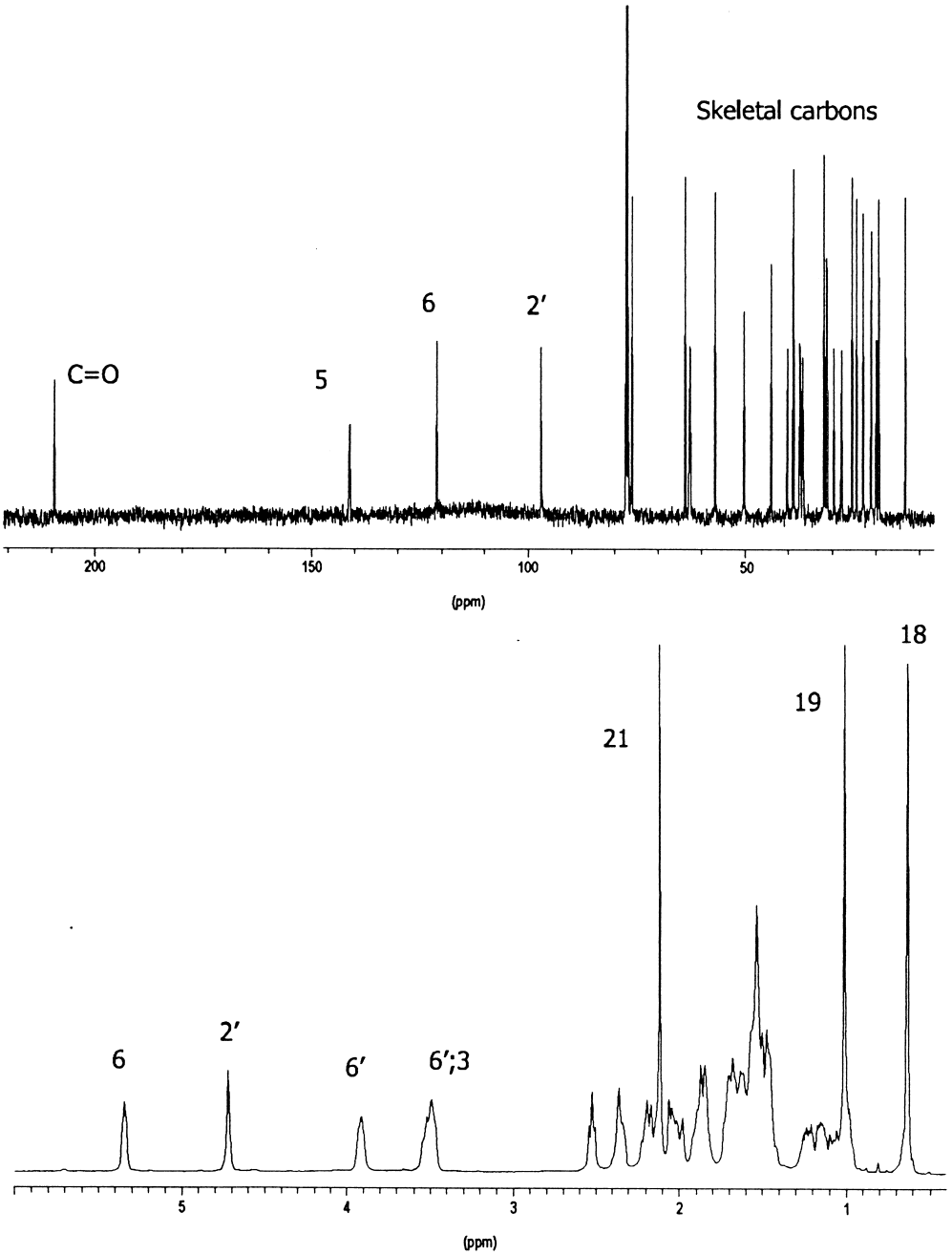

Fig. 1. ^{1}H (bottom) and ^{13}C (top) spectra of **1**.

material was completely converted into **1** in an exothermic reaction. The mixture was chilled with ice-water, neutralized with triethylamine, and then saturated with cold water. The product was extracted with benzene, and the organic layer was dried over $MgSO_4$. The combined extracts were filtered, and the solvent was removed under reduced pressure. The light yellow syrup was applied to the surface of a column packed with neutral, activated alumina and purified by chromatography. The steroidal tetrahydropyranyl ether was eluted with benzene, and the solvent was evaporated. The residue was crystallized by adding small amounts of benzene and methanol, to give white crystals in 73% yield (2.93 g, 7.3 mmol); mp: 120–127°C. Concentrating the mother liquor provided an additional 0.71 g (1.77 mmol, 17.7%) of **1**. The overall yield was 91%, R_f: 0.68.

Analysis calculated for $C_{26}H_{40}O_3$: C, 77.95; H, 10.06. Found: C, 77.82; H, 9.97.

2.3. 3β-Hydroxypregna-5,16-dien-20-one 3-(2′-tetrahydropyranyl) ether ***(2)***

Compound **2** was prepared similar to **1** from 9.5 g (0.03 mol) 3β-hydroxypregna-5,16-dien-20-one. Column chromatography with alumina was used for purification. The

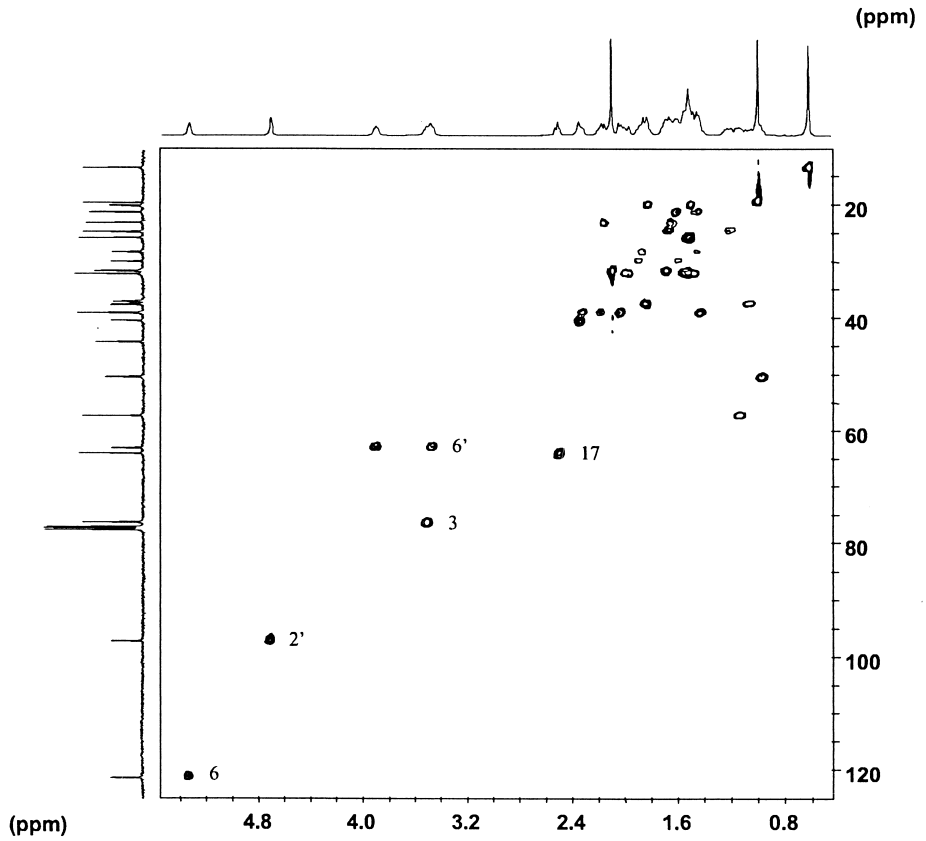

Fig. 2. Two-dimensional heteronuclear single quantum coherence (HSQC) spectrum of **1**.

tetrahydropyranyl ether was eluted with acetone:benzene (5:100 v/v). Fractions were collected, and the solvent was evaporated to give 6.6 g (16.55 mmol, 55.2%) of a white crystalline powder after recrystallizing this material from acetone; mp: 165–171°C, R_f: 0.66. An additional 2.19 g (5.49 mmol, 18.3%) **2** was obtained by evaporating the mother liquor. Overall yield 73.5% in crystalline form.

Anal. calculated for $C_{26}H_{38}O_3$: C, 78.34; H, 9.61. Found: C, 78.45; H, 9.59.

*2.4. (20R)-Pregn-5-ene-3β,20-diol 3-(2′-tetrahydropyranyl) ether **(3)***

Lithium-tri-*terc*-butoxyaluminum-hydride (0.01 mol) was dissolved in 20 ml of dry tetrahydrofuran and then heated for 5 min under reflux (N_2). The mixture was cooled to 2°C, then 2.0 g (5 mmol) of 3β-hydroxypregn-5-en-20-one 3-(2′-tetrahydropyranyl) ether (**1**) in 10 ml tetrahydrofuran were added. After stirring the resulting mixture at 0°C for 1.5 h, and at room temperature overnight, it was worked up by adding 3.0 g NH_4Cl in 40 ml ice-water and repeatedly extracting with benzene. The combined benzene extracts were washed with water, dried (Na_2SO_4), filtered, and then concentrated to dryness. Crystallization of the solid from acetone provided 1.64 g (4.07 mmol, 81.5%) of a white crystalline product. R_f: 0.58; mp: 127–134°C.

Anal. calculated for $C_{26}H_{42}O_3$: C, 77.56; H, 10.51. Found: C, 77.48; H, 10.36.

*2.5. 3β,20 Dihydroxy-(20S)-27-norcholest-5-en-25-one ethylene ketal 3-(2′-tetrahydropyranyl) ether **(5)***

Magnesium (0.73 g; 0.03 mol) was activated under N_2 with a few drops of ether containing one drop of CH_3I. The reaction flask was slightly warmed to evaporate the ether; then, 6.23 ml (0.04 mol) 5-chloropentan-2-one-ethylene ketal was slowly added in 20 ml tetrahydrofuran. A white solid precipitated, which dissolved after adding all of the reagent followed by 30 ml of anhydrous tetrahydrofuran. The reaction mixture was heated under reflux for 1.5 h. Then 8.0 g (0.02 mol) of 3β-hydroxypregn-5-en-20-one 3-(2′-tetrahydropyranyl) ether (**1**) in 25 ml tetrahydrofuran was added dropwise to the solution, and the mixture was heated under reflux for 4 h until the reaction was complete. Excess Grignard reagent was decomposed by adding to it a saturated NH_4Cl ice water solution, and stirring the resulting mixture at room temperature for 1 h. The product was extracted with three 50-ml portions of ether. The combined ethereal fractions were washed twice with 15 ml of saturated aqueous NH_4Cl, then with water and dried ($MgSO_4$). The product was purified by chromatography in a column of alumina, eluted with ether:hexane (1:4 v/v). The yield of the

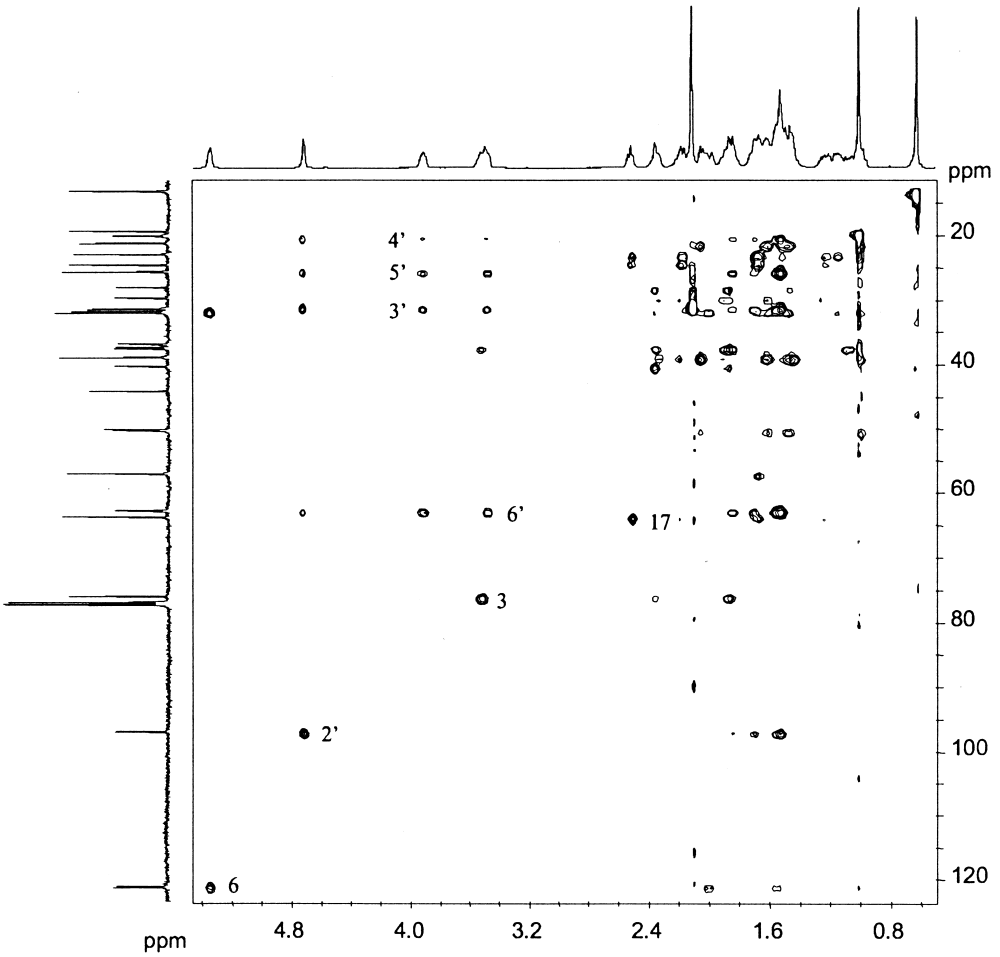

Fig. 3. Two-dimensional HSQC-TOCSY spectrum of **1**.

chromatographed product was 9.45 g (17.8 mmol, 89%) and after its recrystallization from acetone, 7.88 g (14.8 mmol, 74.2%) of the title product was obtained in pure crystalline form. R_f: 0.57, mp: 132–134°C.

Anal. calculated for $C_{33}H_{54}O_5$: C, 74.67; H, 10.25. Found: C, 74.73; H, 10.39.

3. Results and discussion

Earlier, we condensed 3,4-[2H]-dihydropyran or 2,3-dihydrofuran with pregnenolone, which provided a novel starting material for synthesis of a new series of 20-hydroxycholesterol analogs [11]. The C-3β hydroxyl in pregnenolone was protected by a tetrahydropyranyl group against the strongly alkaline reagents 6-(3,4-[2H]-dihydropyranyl)-lithium and 2-lithiodyhydrofuran. The synthesis produced excellent yields of the 20R and 20S isomers in each of the reactions. The major product (20R) was found to have the same configuration in its side-chain as cholesterol. The pure 20R isomer was readily isolated by fractional crystallization. But determination of the configuration at C-20 by NMR was complicated by the contribution to its spectrum of the 3-OTHP group and its C-2′ diastereoisomers. Many attempts by a variety of methods to separate the 2′R and 2′S diastereoisomers for resolving their NMR spectra were unsuccessful. However, the NMR spectra of the 3β-tetrahydropyranyloxy steroids in deuterated benzene yielded a resolvable doubling of the signals due to diastereoisomeric C-2′.

The ^{1}H spectrum arising from the steroid moiety in the 3-OTHP derivatives showed double signals for the hydrogens at the C-2 and C-4 positions. Also, small differences in chemical shifts were observed in the NMR spectra of the angular methyl groups. However, the only double signals in the ^{13}C NMR spectra were produced by the carbon atoms in the *steroid moiety*; and not from the tetrahydropyranyl group.

Analyses of the ^{1}H and ^{13}C NMR spectra from **4** and **6** in $CDCl_3$ shows mostly a single line series. However, recording the spectra of the compounds in deuterated benzene produces line separations. The ^{1}H and ^{13}C spectra of the starting material **1** similarly exhibited doubling of lines in deuterated benzene. This must be caused by diastereoisomerism associated with the OTHP groups.

Table 1
Chemical shift values for ^{1}H-NMR from compounds **1** through **6** in $CDCl_3$ or C_6D_6

	1		2		3		4		5		6	
	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6
1	1.84; 1.05	1.72; 1.01	1.78; 1.00	1.65; 0.96	1.86; 1.08	1.74; 1.05	1.84; 1.03	1.73; 0.98	1.84; 1.05	1.74; 0.97 1.74; 1.02	1.85; 1.07	1.77; 1.03
2	1.88; 1.58 1.87; 1.45	2.13; 1.78 1.92; 1.57	1.84; 1.55 1.82; 1.40	1.86; 1.52 2.04; 1.69	1.89; 1.63 1.87; 1.48	2.10; 1.76 1.94; 1.54	1.88; 1.45	1.91; 1.55	1.87; 1.62 1.87; 1.48	2.05; 1.72 1.87; 1.52	1.89; 1.47	2.12; 1.78 1.93; 1.59
3	3.52	3.72	3.45	3.67	3.50	3.73	3.54	3.72	3.54	3.69	3.54	3.75
4	2.34 2.33; 2.18	2.70; 2.59 2.50; 2.38	2.28; 2.13 2.30	2.65; 2.57 2.48; 2.35	2.34; 2.19	2.46	2.35	2.71; 2.62 2.53; 2.40	2.36	2.56; 2.39	2.36 2.34; 2.20	2.69; 2.61 2.50; 2.39
6	5.32	5.33 5.37	5.28	5.33 5.38	5.35	5.40 5.43	5.35	5.42 5.39	5.35	5.41 5.39	5.33	5.39 5.42
7	1.97; 1.55	1.84; 1.45	1.95; 1.54	1.84; 1.52	1.97; 1.54	1.95; 1.56	1.96; 1.53	1.92; 1.52	1.97; 1.55	1.91; 1.50	1.95; 1.54	1.89; 1.53
8	1.44	1.30	1.61	1.54	1.50	1.50	1.46	1.47	1.50	1.40	1.52	1.47
9	0.96	0.85	0.95	0.97	0.97	0.95	0.92	0.91	0.90	0.87	0.95	0.94
11	1.60; 1.48	1.40; 1.25	1.53	1.48	1.53	1.48	1.46	1.44	1.51	1.40	1.55	1.44
12	2.03; 1.44	1.81; 1.08	2.34; 1.27	2.71; 1.38	2.08; 1.26	2.19; 1.23	2.06; 1.25	2.12; 1.22	2.10; 1.20	2.02; 1.04	2.17; 1.34	2.04; 1.12
14	1.13	0.82	1.36	1.26	1.04	0.92	1.03	0.94	0.98	0.84	1.00	0.83
15	1.67; 1.20	1.50; 1.08	2.23; 1.96	1.93; 1.70	1.68; 1.20	1.46; 0.99	1.59; 1.10	1.57; 1.11	1.60; 1.20	1.54	1.57; 1.16	1.46; 1.09
16	2.17; 1.60	2.33; 1.48	6.62	6.10	1.63; 1.14	1.53; 1.01	1.67; 1.57	1.83; 1.66	1.80; 1.62	1.82; 1.63	1.54; 1.17	1.62; 1.12
17	2.33	2.10	—	—	1.33	1.22	1.77	1.88	1.44	1.35	1.75	1.36
18	0.63	0.56*	0.87	0.94*	0.77	0.74*	0.83	0.97*	0.85	0.87*	0.89	1.02
19	1.01	0.91*	0.98	0.95*	1.01	1.00*	1.00	0.98*	1.00	0.97*	1.02	0.97*
20	—	—	—	—	3.72	3.54	—	—	—	—	—	—
21	2.13	1.83	2.18	1.96*	1.14	1.01	1.37	1.53	1.31	1.22	1.45	1.31
22	—	—	—	—	—	—	—	—	1.47; 1.31	1.44; 1.32	—	—
23	—	—	—	—	—	—	—	—	1.38	1.68; 1.53	3.66	3.32
24	—	—	—	—	—	—	—	—	1.58	1.67	1.87	1.69
25	—	—	—	—	—	—	—	—	—	—	2.66	2.38
26	—	—	—	—	—	—	—	—	1.26	1.33	—	—
2′	4.70	4.82	4.64	4.82; 4.79	4.70	4.84	4.71	4.73	4.72	4.83	4.71	4.84
3′	1.54	1.65	1.67; 1.49	1.64	1.73; 1.54	1.65	1.72; 1.55	1.64	1.70	1.62	1.71; 1.55	1.64; 1.44
4′	1.81; 1.30	1.83; 1.33	1.78; 1.48	1.84; 1.53	1.83; 1.53	1.81; 1.32	1.84; 1.52	1.84; 1.34	1.82; 1.53	1.79; 1.62	1.83; 1.53	1.82; 1.32
5′	1.52	1.34	1.48	1.39; 1.31	1.53	1.32	1.52	1.38; 1.30	1.53	1.31	1.54	1.38; 1.30
6′	3.89; 3.48	3.93; 3.44	3.82; 3.40	3.88; 3.39	3.90; 3.48	3.89; 3.42	3.92; 3.49	3.92; 3.42	3.90; 3.48	3.89; 3.43	3.90; 3.49	3.91; 3.41
2″	—	—	—	—	—	—	3.96	3.69	—	—	—	—
3″	—	—	—	—	—	—	1.76	1.44	—	—	—	—
4″	—	—	—	—	—	—	2.03	1.83	—	—	—	—
5″	—	—	—	—	—	—	4.75	4.71	—	—	—	—
$(CH_2O)_2$	—	—	—	—	—	—	—	—	3.94	3.61	—	—

* Chemical shift values labeled with * represent duplicate signals. However, the line separation is so small that the same chemical shifts can be given for both diastereoisomers.

The configurational assignment of (20R)-**4** and (20R)-**6** at C-20 was readily accomplished after removing the OTHP group under controlled acidic conditions when single isomers could be obtained. It could then be confirmed that the main cause of difficulties in establishing the configurations of the compounds by NMR was diastereoisomerism due to OTHP in (20R)-**4** and (20R)-**6**. Although each of these compounds was homogeneous with respect to C-20, they were diastereoisomeric mixtures due to C-2′ in the OTHP group. Subsequently, we isolated *both* 20R and 20S isomers in their 3β-OH and 3β-acetate forms.

An important objective of the present study was to derive complete assignments for the ^{1}H and ^{13}C signals of tetrahydropyranyloxy derivatives **1–6** (Scheme 1). Deriving the molecular structure of steroids from their NMR spectra is generally based on applying well-established, characteristic chemical shifts for ^{1}H-NMR signals associated, for example, with angular methyl groups or olefinic protons. Yet more than 70% of the ^{1}H signals typically exhibit resonances in a very narrow region (2.5 ppm) near 1.5 ppm (bottom spectrum in Fig. 1). The separations between signals for the steroid ^{13}C spectra are wider than for ^{1}H, but too often they are not wide enough to make unambiguous assignments (top spectrum in Fig. 1). Further complicating this problem, after condensing a tetrahydropyranyl protecting group with a 3β-hydroxysteroid, five additional carbon atoms and nine hydrogen atoms are introduced into the molecule. This renders the high-field NMR region that much more complex to resolve analytically. Within the tetrahydropyranyl group, the C-2′ hydrogen exhibits a chemical shift at around 4.7 ppm. The 6′ methylene protons resonate at 3.8 and 3.4 ppm. The chemical shifts due to ^{1}H

Table 2
Chemical shifts for ^{13}C-NMR from compounds **1** through **6** in $CDCl_3$ or C_6D_6

	1		2		3		4		5		6	
	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6
1	37.14	37.58	37.02	38.02	37.48	37.81	37.20	37.77	37.40	37.87	37.40	37.93
	37.39	37.78	37.27	37.83	37.24	37.61		37.57	37.15	37.67	37.15	37.72
2	27.88	28.54	27.91	30.78	29.69	30.37	27.98	30.40	29.64	30.42	29.60	30.42
	29.59	30.33	29.60	29.03	28.00	28.59		28.60	27.93	28.62	27.89	28.61
3	75.82	76.05	75.83	76.33	75.00	76.41	75.09	76.28	75.89	76.30	75.89	76.31
		76.21	75.91	75.98		76.19		76.08		76.17		76.15
4	38.65	39.36	38.75	41.61	40.27	41.15	40.23	41.12	40.19	41.11	40.15	41.11
	40.13	41.03	40.23	39.93	39.87	39.49		39.44	38.72	39.47	38.67	39.45
5	140.82	141.14	141.47	142.54	141.18	141.58	141.12	141.43	141.04	141.41	141.07	141.42
	141.00	141.31	141.64	142.37	140.88	141.41		141.26	140.86	141.25	140.88	141.26
6	121.12	121.48	120.77	121.53	121.41	121.63	121.40	121.73	121.41	121.68	121.28	121.63
	121.20		120.84		121.34				121.33	121.64	121.20	
7	31.79	32.14	31.53	32.47	31.91	32.46	31.78	32.23	31.77	32.24	31.71	32.23
8	31.74	31.99	30.15	30.92	31.77	32.10	31.37	31.68	31.27	31.68	31.24	31.63
9	49.95	50.39	50.52	51.42	50.22	50.69	51.16	50.59	50.06	50.55	50.19	50.68
	49.92								50.03			
10	36.75	37.02	36.90	37.71	36.75	37.18	36.76	37.15	36.76	37.02	36.75	37.12
	36.71	36.98	36.94	37.66		37.14		37.10	36.73	36.98	36.72	37.09
11	21.00	21.29	20.60	21.51	20.93	21.36	20.90	21.29	20.88	21.29	20.90	21.36
		21.33		21.47		21.32						
12	38.78	38.93	34.61	35.65	38.79	40.17	38.76	40.35	40.06	40.49	40.39	40.76
13	43.95	43.75	46.06	46.89	42.51	42.64	42.33	42.81	42.63	42.87	43.69	43.96
14	56.84	56.87	56.40	57.10	56.22	56.49	56.51	57.31	56.82	57.11	56.84	57.08
15	24.43	24.62	32.19	32.68	25.68	25.95	23.58	24.03	23.75	24.14	23.94	24.36
16	22.75	23.14	144.26	143.64	24.58	24.90	22.89	23.38	22.34	22.75	21.99	22.45
			144.31	143.57								
17	63.65	63.58	155.38	156.11	58.46	58.63	56.92	56.48	57.14	58.36	55.09	55.59
18	13.16	13.24	15.65	16.43	12.36	12.44	13.31	13.67	13.55	13.72	13.30	13.72
19	19.91	19.44	19.23	19.84	19.42	19.54	19.36	19.50	18.74	19.50	19.33	19.53
20	209.57	206.91	196.77	195.58	70.44	70.19	75.98	75.18	74.99	75.11	80.19	80.28
21	31.49	31.13	27.06	27.31	23.71	24.04	25.90	27.13	26.29	26.66	24.63	25.01
22	—	—	—	—	—	—	—	—	43.93	44.72	214.24	213.96
23	—	—	—	—	—	—	—	—	18.74	19.13	61.82	61.60
24	—	—	—	—	—	—	—	—	39.66	40.45	26.63	27.09
25	—	—	—	—	—	—	—	—	109.99	110.12	32.02	32.06
26									23.72	24.05	—	—
2′	96.73	96.63	96.83	97.21	96.85	96.54	96.95	96.60	96.84	96.66	96.90	96.76
	96.90	96.69		97.04	96.77		96.79		96.68	96.59	96.73	96.69
3′	31.19	31.58	31.23	32.07	31.29	31.59	31.25	31.59	31.21	31.52	31.18	31.62
4′	19.91	19.76	20.60	20.29	20.01	19.80	19.99	19.85	19.99	19.74	19.99	19.91
	20.00	19.85						19.78	19.92	19.68	19.90	19.82
5′	25.42	26.03	25.45	26.54	25.52	26.06	25.49	26.06	25.47	26.02	25.43	26.07
6′	62.72	61.88	62.73	62.38	62.76	61.85	62.76	61.82	62.74	61.91	62.82	62.04
	62.83	61.98	62.77	62.34				61.96	62.65	61.83	62.71	61.94
2″	—	—	—	—	—	—	66.21	66.21	—	—	—	—
3″	—	—	—	—	—	—	22.29	22.64	—	—	—	—
4″	—	—	—	—	—	—	19.99	20.28	—	—	—	—
5″	—	—	—	—	—	—	92.64	92.22	—	—	—	—
6″	—	—	—	—	—	—	159.11	160.33	—	—	—	—
$(CH_2O)_2$	—	—	—	—	—	—	—	—	64.53	64.65	—	—

in the C-3′, C-4′, and C-5′ methylene groups fall within the resonance signals due to analogous ring hydrogens in the steroid. The ^{13}C spectra of these compounds follow similar patterns. The carbons bonded to oxygen in the tetrahydropyranyl group have large chemical shifts. The signal for C-2′ is near 97 ppm and C-6′ is at 62 ppm. The three ring methylene carbons C-3′, C-4′, and C-5′ exhibit signals near those from steroid carbons in the high-field region. Often these signals cannot readily be identified.

Knowing that C-2′ and C-6′ have nuclei with lower field chemical shifts, we used these signals as starting points in polarization transfer experiments for identifying the tetrahydropyranyloxy group. Similarly, signals belonging to C-3 and C-6 nuclei can also be used to start polarization trans-

fers. The HSQC spectrum of **1** is shown in Fig. 2. The high-field correlations (C-6, C-2′, C-3, C-6′, and C-17) can easily be identified from this spectrum. However, low-field signals are in a crowded region at the upper right of this two-dimensional map. The HSQC-TOCSY experiment [19] (Fig. 3) provided relayed magnetization transfer inside the spin-system, thus allowing the complete and unambiguous assignments to be made for the NMR spectra.

Assignments for the ring methylene carbons in the OTHP group (C-3′, C-4′, and C-5′) were made by applying HSQC-TOCSY methods. Also, these types of experiments were useful in making the assignments for the 20-substitued side-chain. We found that doubled signals in both proton and carbon spectra for compounds **1** through **6** are associated with the C-2′ diastereoisomers of the OTHP group. The differences between the chemical shifts for the corresponding proton signals in two different isomers are only a few Hertz for H-2, H-4, H-6, and for H-18 and H-19 (the angular methyl groups). Where the line separation was exceedingly small, the doubled property is marked with an asterisk in Table 1. Similar differences were found in the carbon spectra at C-1, C-2, C-3, C-4, C-5, C-6, C-9, C-10, C-11, C-16, C-2′, C-4′, and C-6′ (in **2**). The magnitude of these differences is in the range from 10^{-2} to 10^{-1} ppm (Table 2).

We used two different solvents to obtain solvent-dependent structural information. Significant changes were observed in the chemical shifts (up to several tenths of a ppm) from the skeletal protons by changing the solvent from chloroform to benzene. Chemical shift changes appeared for nearly all of the steroid protons. Yet, the directions of these changes did not appear to follow a pattern. The signal due to H-16 in **2** exhibited the largest change associated with changing the solvents. The benzene-induced chemical shift change for H-16 is −0.52 ppm. The proton signals in the side-chain of **6** were fairly sensitive for solvent changes as were the protons at the steroid C-21 position. However, the signal from H-6 in the B-ring double bond of the steroids in the present study did not show any sensitivity to changes in solvents.

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