Notes

Secondary Deuterium Isotope Effects as a Sensitive Probe for Double Bond Participation. The Structure of the Cholesteryl Cation[1]

Marko Tarle, Stanko Borčić, and D. E. Sunko* [2]

Rugjer Bošković Institute, 41001 Zagreb, Croatia, Yugoslavia

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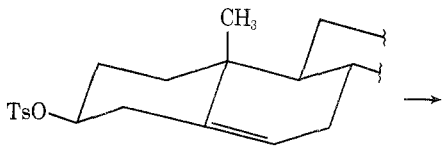
More than two decades ago Winstein concluded from the kinetic analysis of the solvolysis of cholesteryl *p*-toluenesulfonate (1) that "the relatively high rates of acetolysis and alcoholysis of 1 and other indication of high reactivity of Δ^5 materials show that the double bond furnishes a substantial driving force".[3]

Originally structure **2** has been proposed for the intermediate resonance-stabilized homoallylic cation and consequently for the transition state leading to it.[3,4a] Later[4b]



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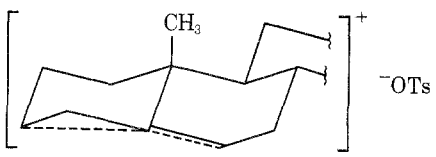

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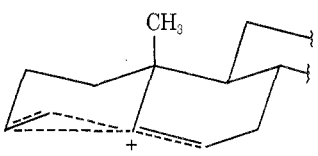
both symmetrical (**3**) and unsymmetrical (**4**) intermediates have been suggested and their relative merits discussed.[4c,d]



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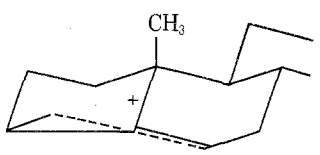

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In this paper we would like to present evidence in favor of the unsymmetrical structure **2**. Our conclusions are based on secondary deuterium isotope effect measurements which have been shown to be sensitive to neighboring group participation and thus can be successfully applied for the elucidation of transition state structures in anchimerically assisted reactions.[5,6] Specifically deuterated cholesteryl (**5–8**) and cholestanyl (**9, 10**)[6b] derivatives were prepared according to the procedures described in the Experimental Section and their solvolysis rates were measured. The kinetic data are given in Tables I and II. The α effects in both epimers are similar and rather small. Such low effects could be due either to an intervening SN2 reaction or to ion-pair return. In **6** a direct displacement by solvent at C-3 can be excluded on the basis of the observation that the methanolysis of *epi*-cholesteryl tosylate affords only rearranged substitution products, i.e., 4β-methoxycholest-5-ene and 6β-methoxycholest-4-ene and cholesta-3,5-diene as the elimination product.[7] There is also no evidence for a direct displacement in **5**, which leaves a significant ion-pair return[4b] as the alternative explanation for the low α effects in the solvolysis of both **5** and **6**.

The β effects in solvolysis of the anchimerically assisted tosylate **7** and the saturated analog **9** differ markedly. The negligible slightly inverse effect in **7** is in our opinion a consequence of double bond participation and bridging which counteracts the hyperconjugative electron release from the β C–D bond.[5,8] Also the geometry at C-3 which is oriented for overlap with C_5 is wrong for hyperconjugation with the C_4–H(D) bond.[4b] The only other case where such drastic reduction in magnitude of the usual value for β-D effects (1.15–1.20)[9] was observed is the solvolysis of 5-methoxy-2-pentyl-*1,1,1-d*$_3$ brosylate, a strongly n-participating system.[10–12]

In nonparticipating systems small or even inverse β effects have been observed only when the dihedral angle between the developing empty p orbital and the adjacent C–D bond is close to 90°.[14]

Participation of a double bond in ring B can also be considered as the reason for the observance of an inverse isotope effect in the solvolysis of **8**. Bridging between C-3 and C-5 (but not C-4) and the nucleophilic attack on C-6 are probably already well advanced in the transition state. The result is an overall force field increase in the originally sp^2 hybridized carbon atom 6, concurrent with a force field decrease on C-3 atom. In this respect the bridged ion **2** resembles a transition state in addition reactions to olefins, where substrates deuterated at the unsaturated carbon atom yield inverse effects.[15] This effect supports the idea of a significant ion-pair return[4b] with the nucleophilic attack by ethanol at C-6 in the tight ion as the rate-determining step.

The absence of a β isotope effect in **7** rules out significant bond weakening between C-4 and C-5 as implied by structures **3** and **4**. Also, as Winstein pointed out, the C-3 is secondary in **2**, while C-4 is primary in **4** rendering the latter less stable.[4b]

The results described in this paper seem to prefer, in harmony with arguments put forward by Story and Clark,[4d] the unsymmetrical structure **2** for the intermediate cation.

The slightly inverse isotope effect observed in the solvolysis of **10** is apparently of an inductive origin. Such remote inverse effects have been observed in the solvolysis of cyclopentyl-*3,3,4,4-d*$_4$ tosylate.[16]

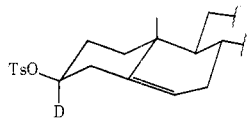
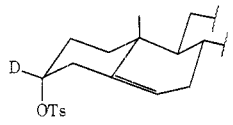
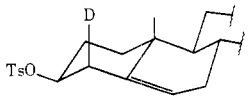
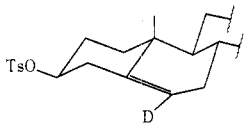
Experimental Section

General. Melting points (uncorrected) were taken on a Kofler apparatus. Proton magnetic resonance spectra were obtained using a Varian A-60A spectrophotometer in $CDCl_3$ solutions with internal tetramethylsilane. The ir spectra were recorded using potassium bromide pellets. The mass spectral data came from a Bell and Howell CEC-21-110C mass spectrometer. Optical rotations were determined with a Carl Zeiss polarimeter in chloroform solutions. Thin layer chromatography plates were prepared from silica gel G (Merck) and column chromatography was performed on silica gel (Merck). In the chromatograms of both types benzene–ethyl acetate (9:1 v/v) was used as solvent. Compounds and reaction mixtures were routinely checked by TLC prior to purification.

All chemicals were reagent grade. Spectrophotometric grade ethanol (Merck), 96%, v/v, was distilled twice prior to use as solvent in kinetic measurements.

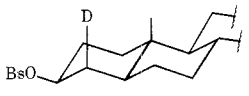
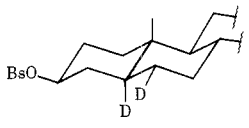
3β-Cholesteryl Tosylate (1). Commercial 3β-cholesterol was converted to 5α,6β-dibromocholesterol using the described procedure.[17] Oxidation of the crude product yielded 5α,6β-dibromocholesten-3-one[17] (64%): mp 71–74° (lit.[17] mp 73–75°); $[\alpha]_D$ −45.9° (lit.[17] $[\alpha]_D$ −47.0°). Debromination afforded cholesten-3-one[17]

Table I
Deuterium Isotope Effects in the Solvolysis of Cholesteryl Tosylates in 95% (v/v) Ethanol

Compd	Compd no.	Deuterium content, %	Temp, °C	k, sec^{-1} [a]	k_H/k_D
	5	99	50.2	$(2.400 \pm 0.016) \times 10^{-4}$ [b] $(2.120 \pm 0.010) \times 10^{-4}$	1.132 ± 0.008
	6	99	50.0	$(3.480 \pm 0.016) \times 10^{-5}$ [b] $(3.150 \pm 0.009) \times 10^{-5}$	1.104 ± 0.010
	7	91	50.0	$(2.150 \pm 0.020) \times 10^{-4}$ [b] $(2.173 \pm 0.020) \times 10^{-4}$	0.989 ± 0.018
	8	91	50.0	$(2.150 \pm 0.020) \times 10^{-4}$ [b] $(2.295 \pm 0.020) \times 10^{-4}$	0.937 ± 0.014

[a] Uncertainties are standard errors. [b] The values correspond to undeuterated compounds.

Table II
Deuterium Isotope Effects in the Solvolysis of 3β-Cholestanyl Brosylates in 95% (v/v) Ethanol

Compd	Compd no.	Deuterium content, %	Temp, °C	k, sec^{-1} [a]	k_H/k_D
	9	90	60.3	$(3.040 \pm 0.015) \times 10^{-5}$ [b] $(2.590 \pm 0.028) \times 10^{-5}$	1.174 ± 0.020[c]
	10	1.01	60.4	$(3.185 \pm 0.165) \times 10^{-5}$ [b] $(3.268 \pm 0.051) \times 10^{-5}$	0.974 ± 0.021

[a] Uncertainties are standard errors. [b] The values correspond to undeuterated compounds. [c] Isotope effect corrected to 100% deuterium content is 1.195.

(78%): mp 124–127° (lit.[17] mp 124–129°); $[\alpha]D$ −2.3° (lit.[17] $[\alpha]D$ −2.5°). Reduction of the ketone with lithium aluminum hydride gave a mixture of 3β-cholesterol and 3α-cholesterol[18] (97.6%) in the ratio 89.2:8.4 The epimers were separated and purified by column chromatography over silica gel:[19] 3β-cholesterol, mp 148–149° (lit.[19] mp 149–149.5°); $[\alpha]D$ −39.1°; NMR δ 2.5 (s, 1, –OH), 3.52 (m, 1, >CHOH), 5.35 (m, 1, vinyl); ir ν 3500 cm^{-1} (O–H); 3α-cholesterol, mp 140–141° (lit.[18] mp 140°); $[\alpha]D$ −32.2°; NMR δ 1.68 (s, 1, –OH), 4.05 (m, 1, >CHOH), 5.45 (m, 1, vinyl); ir ν 3500 cm^{-1} (O–H). 3β-Cholesteryl tosylate was prepared by a standard procedure[20] (56%): mp 129–131° (lit.[20] mp 131.7–132.6°); NMR δ 2.35 (s, 3, $-CH_3$), 4.26 (m, 1 >CHOTs), 5.22 (m, 1, vinyl), 7.20, 7.70 (d, d, 2, 2, C_6H_4). Data obtained from TLC confirmed the absence of the unreacted alcohol in the tosylate.

3α-Cholesteryl Tosylate (11). 3α-Cholesterol was converted to the corresponding tosylate using a previously described procedure[20] (54%): mp 94–95° (lit.[20] mp 96°); $[\alpha]D$ +7.2° (lit.[20] $[\alpha]D$ +6.5 ± 2°); NMR δ 2.32 (s, 3, $-CH_3$), 4.71 (m, 1, >CHOTs), 5.20 (m, 1, vinyl), 7.21, 7.72 (d, d, 2, 2, C_6H_4). The purity of the tosylate was confirmed, with respect to unreacted alcohol or olefinic by-products, by TLC analysis.

3β-Cholesteryl-3-d_1 Tosylate (5) and 3α-Cholesteryl-3-d_1 Tosylate (6). Monodeuterated tosylate esters **5** and **6** were prepared in the same manner as the unlabeled analogs[17–21] using lithium aluminum deuteride instead of $LiAlH_4$ in the reduction of cholesten-3-one. The ^1H NMR spectra of both deuterated esters did not show signals corresponding to the proton on C-3. Mass spectral data showed the presence of 0.99 D in both 3α-cholestanyl-3-d_1 and 3β-cholestanyl-3-d_1 tosylate.

3β-Cholesteryl-4β-d_1 Tosylate (7). 3β-Cholesteryl benzoate (mp 146–147°, $[\alpha]D$ −13.7°) was converted by a standard procedure[22] to 4β-hydroxy-3β-cholesteryl benzoate (68%): mp 205–206° (lit.[22] mp 209–210°); $[\alpha]D$ −30.7° (lit.[22] $[\alpha]D$ −30.7°). The product was treated according to Young et al.[23] with tri-*n*-butylamine, gaseous HCl, and freshly distilled thionyl chloride, yielding (84%) 6β-chloro-Δ⁴-cholesten-3β-benzoate: mp 125–127° (lit.[23] mp 122–128°); $[\alpha]D$ −82.8° (lit.[23] $[\alpha]D$ −82.6°). Reduction of the latter with lithium aluminum deuteride gave 3β-cholesterol-4β-d_1[23] (88%): mp 147–149°; $[\alpha]D$ −39.4°. The ir spectrum showed an absorption band at 2140 cm^{-1}, characteristic for the axial C–D stretching.[23] The deuterium content as calculated from the mass spectrum was 0.91 atom D per molecule. 3β-Cholesteryl-4β-d_1 tosylate was prepared in the same manner as described for the unlabeled tosylate[20] (56%): mp 131–131.5°.

3β-Cholesteryl-6-*d* Tosylate (8). 3β-Cholesterol-6-d[24,25] was treated with tosyl chloride in the usual manner.[20] The crude **8** was recrystallized at low temperature from ether–pentane mixture (61%): mp 131–132°; NMR δ 2.32 (s, 3, $-CH_3$), 4.26 (m, 1, >CHOTs), 7.20, 7.70 (d, d, 2, 2, C_6H_4). The mass spectral data showed 0.91 atom D per molecule.

3β-Cholestanyl Brosylate (12). Catalytic hydrogenation of 3β-cholesterol over Adams catalyst in glacial acetic acid yielded partially acetylated 3β-cholestanol.[26] The crude product was purified by removal of the remaining cholesterol with concentrated H_2SO_4, hydrolysis with NaOH in EtOH, and filtration through an Al_2O_3 column (II/III) with CCl_4 as an eluent. After recrystallization from anhydrous EtOH 3β-cholestanol was obtained (69%): mp 142–144°; $[\alpha]D$ +23.3°; NMR δ 1.67 (s, 1, –OH), 3.65 (m, 1, >CHOH); ir ν 3500 cm^{-1} (O–H). 3β-Cholestanyl brosylate was prepared by treatment of the alcohol in dry pyridine with brosyl chlo-

ride at 0° for 2 days.[27] After the usual work-up procedure[27] the crude product was recrystallized from ether–ligroin mixture (82%): mp 119–121° (lit.[28] mp 120–122°); NMR δ 4.4 (m, 1, >CHOTs), 7.66 (m, 4, C_6H_4).

3β-Cholestanyl-4β-d_1 Brosylate (9). The mixture containing 3β-cholesterol-4β-d_1 and freshly distilled acetic anhydride was refluxed in dry pyridine for 20 hr.[29] After addition of ether (200 ml) the resulting solution was washed with water until neutral reaction. The organic layer was dried over $MgSO_4$ and filtered. Solvent was removed under reduced pressure and crude 3β-acetoxycholesterol-4β-d_1 was recrystallized from EtOH (91%): mp 113–115.5° (lit.[29] mp 114.5–116.5°); NMR δ 2.00 (s, 3, $-CH_3$), 4.70 (m, 1, >CHOAc).

Acetylated 3β-cholesterol-4β-d_1 was catalytically hydrogenated over Pt (Adams catalyst) in glacial acetic acid at 65° for 36 hr. This method does not lead to deuterium scrambling.[30] After the usual work-up the crude product was refluxed in an alkaline ethanol solution for 1 hr.[30] Purification and recrystallization from acetone yielded 3β-cholestanol-4β-d_1 (80%): mp 142–144°; $[\alpha]_D$ +23.4°; ir ν 2140 cm^{-1} (C–D).[23] The alcohol was converted to the corresponding brosylate **9** by the known procedure[27] (53%), mp 121–122°. Mass spectral data did not indicate deuterium scrambling. The deuterium content was determined as 0.91 atom D per molecule. The ir spectrum supported the presence of a 4β C–D bond in the substrate.[23]

3β-Cholestanyl-5α,6α-d_2 Brosylate (10). 3β-Acetoxycholesterol[29] was catalytically deuterated over Pt in AcOH-d_1 as previously described.[30] The reaction mixture was treated as usual,[30] yielding 3β-cholesterol-5α,6α-d_2 (91%): mp 142.5–144°; $[\alpha]_D$ +23.1°; ^1H NMR spectrum did not show the signal corresponding to a vinyl proton (δ 5.35). The deuterium distribution according to the mass spectrum was d_3, 3%; d_2, 96%; d_1, 1%; total 2.02 atoms D per molecule. The deuterated alcohol was converted to the brosylate **10** in a described manner[27] (67%), mp 120.5–122°.

Kinetic Measurements. The titrimetric rates were obtained using the automatic potentiometric titration method by means of a pH-stat, Radiometer, Copenhagen, SBR-2/TTT 11, maintaining a constant "pH setting" of 6.8. The substrate concentration was 1.5 mmol in all experiments. Six to eight solvolyses were performed for each sulfonate ester, alternating the measurement of the labeled and unlabeled derivative. The rate data were calculated from the standard integrated first-order law and evaluated using a nonlinear least-squares program. No trend was observed in the rate constants between 15 and 80% of the solvolysis completion.

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Registry No.—**1**, 1182-65-6; **2**, 56227-24-8; **5**, 55913-52-5; **6**, 55913-53-6; **7**, 55954-48-8; **8**, 55913-54-7; **9**, 55954-49-9; **10**, 55954-50-2; **11**, 3381-56-4; **12**, 35596-32-8; 3β-cholesterol, 57-88-5; 3α-cholesterol, 474-77-1; 3β-cholesteryl benzoate, 604-32-0; 3β-cholesterol-4β-d_1, 1973-68-8; 3β-cholesterol-6-d, 16374-87-1; 3β-cholestanol, 80-97-7; 3β-acetoxycholesterol-4β-d_1, 1973-64-4; 3β-cholestanol-4β-d_1, 55954-51-3; 3β-cholestanol-5α,6α-d_2, 55954-52-4.

References and Notes

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Configuration of 5-Cholestene Hydrochloride

Jane F. Griffin,[1a] M. G. Erman,[1a] William L. Duax,[1a] David S. Watt,[1b] and Francis A. Carey*[1b]

Medical Foundation of Buffalo, Buffalo, New York 14203, and Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901

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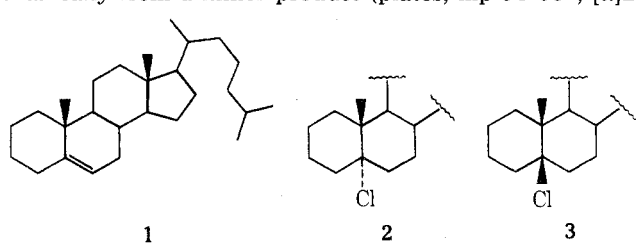
The addition of hydrogen chloride to 5-cholestene (**1**) was reported long before the constitution of the steroid nucleus was established.[2] The major product (prisms, mp 97°, $[\alpha]_D$ +4.7°) has been described as 5α-chlorocholestane (**2**), rather than the 5β isomer (**3**),[3] and reference has been made[4] to a structure determination using X-ray diffraction techniques.[5]

The structure of the major product ("Mauthner's hydrochloride") was, however, not solved. The crystallographic investigation was limited to the determination of the point group and dimensions of the unit cell. As pointed out by Bernal,[5] these data are not sufficient to be considered diagnostic as to the stereochemistry of the ring junction.

Subsequent experiments on the addition of hydrogen chloride to **1** substantiated Mauthner's observations but lacked rigorous proof of stereochemistry.[6] The major product (prisms, mp 96–97°, $[\alpha]_D$ +6.4°) was separated mechanically from a minor product (plates, mp 94–95°, $[\alpha]_D$



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