The Behavior of Sterols on Silica Surfaces and at Other Interfaces

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Abstract

The specific manner in which a molecule is oriented at an interface determines the reactions in which the molecule can participate. Sterols, being highly anisotropic molecules, present "ends" and "sides" to surfaces which differ greatly in their reactivity and interaction with the surface. Seen from the 3β-hydroxy end, as at an air-water interface, sterols are largely indistinguishable from one another in their behavior. On the other hand, at an organized interface, such as that of an absorbent, a great many differences in structure can be demonstrated. These include the distinction between nuclear and side-chain double bonds, the number and location of nuclear double bonds, the substituents in the side chain and the number of methyl groups in the molecule.

We have been interested in the degree of specificity which such interaction can display because of the obvious parallel to sterol-protein interactions of a structural or enzymatic nature. Through the synthesis of a variety of sterol structures possessing the desired arrangement of double bonds and numbers of methyl groups, we have established a reproducible correlation between adsorptive interaction and double bonds in the Δ^5, Δ^7, Δ^8, $\Delta^{5,7}$, and $\Delta^{7,9}$ positions, as well as for the saturated stanol. These correlations have been extended for the 4α, 4β, 4,4 dimethyl and 4,4,14α trimethyl series as well. The elucidation of the structure of macdougallin as a 14α methyl sterol in which the 4,4, gem dimethyl grouping was absent prompted a further study of the 14α methyl sterols, of which the Δ^7,Δ^8, $\Delta^{7,9}$ and unsaturated varieties have been prepared and tested. The presence of a 14α methyl group in the absence of other methyl groups confers a unique behavior on the molecule in that this series does not fit into the adsorptive scheme for the other sterols. Instead, the interaction of the double bond with the surface is almost completely independent of its position, suggesting that the molecule is "perched" on the 14α methyl group when adsorbed in the surface. This appears to be a highly significant feature in the orientation of the molecule, since the removal of this methyl group is usually regarded as an essential first step in the conversion of lanosterol to cholesterol.

Introduction

THE MAIN PURPOSE of this paper is to focus attention on some specific interactions of sterols with surfaces or interfaces; its relationship to the title of the symposium "The Metabolic Role of Lipids" may not be readily apparent and some preliminary explanations are perhaps warranted.

One may take either of two views of the metabolic roles of sterols. The first is simply that they are precursors whose true metabolic role is that of their derivatives; to wit: bile acids, adrenal steroids, estrogens, etc. This view would have it that a sterol is a molecule *in transitu*, to use the designation coined by Hickman (1). If we adopt this view, then we must admit that there is a great deal more sterol in the animal organism than is necessary to provide precursors for these compounds. On the other hand, instead of the "going *there*" role, there is the structural or "being *here*" role which has been described by Vandenheuvel (2) and others interested in the organization of lipids. This view prizes the ability of sterols to participate in the formation of unit membranes, rigid monolayers or mitochondrial lamellae. The planar characteristics of sterols enhance their van der Waals interactions along an axis perpendicular to the membrane surface, thereby conferring greater stability to planes parallel to the surface.

The second view has a satisfying nature as regards the properties of assembled membranes and lipoproteins; the first view, as regards a strictly catabolic property. An intermediate position may also be proposed: that the function (role) (behavior) of a sterol depends upon the surface on which it is found, and more specifically, it depends upon the surface energy of the interface. Of necessity, in the absence of a charged center in the sterol molecule, the primary forces acting on a molecule at such an interface are nonionic adsorptive energies. Consider the diagram in Figure 1, where three types of inorganic surfaces are shown, e.g., water, silica, and alumina, representing low, medium, and high energy surfaces. These have reaction capabilities for sterols ranging from simple orientation to chemical transformation. On the right hand side are listed the sterol properties that are involved in each of the reaction capabilities: "sideness," the anisotropic properties of sterols as opposed, for example to fatty acids; the location of functional groups within the molecule; and the chemical reactivity or number and types of groups.

For example, at a simple air/water interface 3β-hydroxy sterols are largely indistinguishable from one another on the basis of their properties (3). Their pressure:area isotherms give little or no hint of structural differences. Their strong intermolecular coherence in the presence of a low energy surface

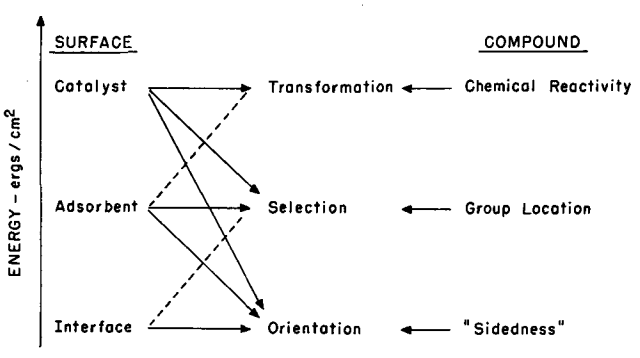

FIG. 1. Interactions of compounds with interfaces.

TABLE I

Relative Elution Volumes of Some Sterol Acetates

	REV[a]	Source or method of preparation
4,4,14 α trimethyl cholestane-3β-ol	0.709	Chanley, J. D., and T. Mezzetti, J. Org. Chem. *29*, 228 (1964).
4,4,14 α trimethyl cholest-8-ene-3β-ol	0.73	Prepared from crude "isocholesterol" purchased from Nutritional Biochemicals Corporation. Method of Johnston, J. D., F. Gautschi and K. Bloch, J. Biol. Chem. *224*, 185 (1957).
4,4,14 α trimethyl cholest-7-ene-3β-ol	0.83	Samples provided by J. L. Gaylor.
4,4,14 α trimethyl cholest-7,9-diene-3β-ol	0.804	Doree, C., and J. F. McGhie, J. Chem. Soc. 570 (1949).
4,4,14 α trimethyl cholest-8,24-diene-3β-ol	1.34	Prepared from crude "isocholesterol" purchased from Nutritional Biochemicals Corporation. Method of Johnston, J. D., F. Gautschi and K. Bloch, J. Biol. Chem. *224*, 185 (1957).
4,4,14 α trimethyl cholest-7,24-diene-3β-ol	1.48	Samples provided by J. L. Gaylor.
4,4,14 α trimethyl cholest-7,9,24-triene-3β-ol	1.66	Provided by C. H. Duncan, University of Louisville.
4,4 dimethyl cholestane-3β-ol	0.667	Atwater, N., J. Am. Chem. Soc. *82*, 2847 (1960).
4,4 dimethyl cholest-5-ene-3β-ol	0.640	Woodward, R. B., A. A. Patchett, D. H. R. Barton, D. A. J. Ives and R. B. Kelly, J. Am. Chem. Soc. *76*, 2852 (1954); see also Ref. 22.
4,4 dimethyl cholest-8(14)-ene-3β-ol	0.870	Gautschi, F., and K. Bloch, J. Biol. Chem. *233*, 1343 (1958).
4,4 dimethyl cholest-7-ene-3β-ol	0.956	Gautschi, F., and K. Bloch, J. Biol. Chem. *233*, 1343 (1958).
4 β methyl cholestane-3β-ol	0.750	Julia, S., and J. P. Lavaux, Bull. Soc. Chim. (France) 1223 (1963).
4 β methyl cholest-5-ene-3β-ol	0.794	Moriarity, R. M., and R. M. deSousa, J. Org. Chem. *28*, 3072 (1963). Julia, S., and J. P. Lavaux, Comptes Rendus *251*, 733 (1962).
4 α methyl cholestane-3β-ol	0.804	Mazur, Y., and F. Sondheimer, J. Am. Chem. Soc. *80*, 6296 (1958).
4 α methyl cholest-5-ene-3β-ol	0.901	Moriarity, R. M., and R. M. deSousa, J. Org. Chem. 28, 3072 (1963). Julia, S., and J. P. Lavaux, Comptes Rendus *254*, 3702 (1962).
4 α methyl cholest-7-ene-3β-ol	1.22	Neiderhiser, D. H., and W. W. Wells, Arch. Biochem. Biophys. *81*, 300 (1959); sample provided by W. W. Wells.
4 α methyl cholest-7,9-diene-3β-ol	1.435	Djerassi, C., G. W. Krakower, A. J. Lemin, L. H. Liu, J. S. Mills and R. Willotti, J. Am. Chem. Soc. *80*, 6284 (1958).
14 α methyl cholestane-3β-ol	0.918	Woodward, R. B., A. A. Patchett, D. H. R. Barton, D. A. J. Ives and R. B. Kelly, J. Am. Chem. Soc. *76*, 2852 (1954); see also Ref. 22. See Ref. 24.
14 α methyl cholest-8-ene-3β-ol	0.944	See Ref. 24.
14 α methyl cholest-7-ene-3β-ol	1.105	Woodward, R. B., A. A. Patchett, D. H. R. Barton, D. A. J. Ives and R. B. Kelly, J. Am. Chem. Soc. *76*, 2852 (1954); see also Ref. 23.
14 α methyl cholest-7,9-diene-3β-ol	1.077	See Ref. 24. See Ref. 21.
cholestane-3β-ol	0.860	Purchased from Nutritional Biochemicals Corporation.
cholest-5-ene-3β-ol	1.000	Purchased from Nutritional Biochemicals Corporation.
cholest-8-(14)-ene-3β-ol	1.206	Barton, D. H. R., and W. J. Rosenfelder, J. Chem. Soc. 2459 (1949).
cholest-7-ene-3β-ol	1.405	Fieser, L. F., and J. E. Herz, J. Am. Chem. Soc. *75*, 121 (1953).
cholest-3,5-diene-3β-ol	0.786	Cox, R. H., and E. Y. Spencer, Can. J. Chem. *29*, 398 (1951).
cholest-7,9-diene-3β-ol	1.640	Cornforth, J. W., I. Y. Gore and G. Popjak, Biochem. J. *65*, 94 (1957).
cholest-5,7-diene-3β-ol	1.77	Purchased from Nutritional Biochemicals Corporation.
cholest-7,14-diene-3β-ol	1.955	Fieser, L. F., and G. Ourisson, J. Am. Chem. Soc. *75*, 4404 (1953).
cholest-5,24-diene-3β-ol	1.98	Bergmann, W., and J. P. Dusza, J. Org. Chem. *23*, 459 (1958).
cholest-8,24-diene-3β-ol	2.42	Sample of this compound provided by O. N. Brievik of Fleischmann Laboratories.
sitosterol	1.04	Samples provided by E. R. Diller of Eli Lilly.
stigmasterol	1.09	Samples provided by E. R. Diller of Eli Lilly.
ergosterol	2.04	Purchased from Nutritional Biochemicals Corporation.

[a] Cholesterol acetate equals 1.000.

leads to the formation of islands of vertically oriented molecules in which the only portion of the structure contacting the surface is the hydroxyl group (4). Under these circumstances, opportunities for selective processes at these interfaces are minimal; the sorts of reactions that might take place in close-packed arrays of this type are limited to esterification of the hydroxyl group and (possibly)) oxidation to ketones. These reactions would not be expected to show great specificity.

On the other hand, even on the alumina used for chromatographic separations the adsorption of a sterol may lead to transformations during the adsorbed state that are comparable to those on an enzyme surface. These include the hydrolysis of esters (5–7) both in a generalized and in a specific fashion (8), dehydration (9), removal of reactive groups (10), and isomerization of double bonds (11). The surface energy is the consequence of the type, number and distribution of functional groups on the surface; for alumina these are the hydrated alumina and alumina oxides, for silica they are the silanol and siloxane groups. If these surfaces seem dissimilar from enzyme surfaces and surface-catalyzed reactions totally irrelevant to enzyme function, one need only consider the induced enzyme: Δ5 steroid isomerase from *Pseudomonas testosteroni.* This enzyme, which has been isolated, crystallized and characterized by Talalay and his co-workers (12–15) promotes the isomerization of the Δ5,6 or Δ5(10) bond to the Δ4 position in keto steroids such as testosterone to form the α,β unsaturated ketone. This isomerization takes place in such a manner that the hydrogen atom removed at position 4 is used to reduce the 6 position without becoming available for exchange with the surrounding medium. This mechanism may or may not (16) be identical with the same isomerization on alumina reported by Reichstein and Shoppee (11) but it is of interest to this comparison that the functional groups of the enzyme appear to be four tyrosine residues, whose acidic hydroxyl groups suggest many analogies to the catalyst surface groups.

If then we must look at the surface on which sterols are found in order to draw conclusions about their function, there are two ways one can proceed. One way is to isolate the individual enzymes, determine the amino acid sequences, and reconstruct the surface of the enzyme. For obvious reasons, not the least of which is that many of the enzymes concerned with steroid transformations are particulate and probably not "soluble" in this sense, this approach has its limitations. The second method is to study the capabilities of simple surfaces such as catalysts and adsorbents to detect and recognize structural features of sterols and to act upon them. Thereby one may hope to obtain some idea of the minimal requirements for interactions of a specific nature. Prototypical studies of this nature have already been reported by Hodosan and co-workers (17,18).

One may, of course, regard the process in a converse manner; i.e., that the function of the sterol in interacting with the surface is to transform the *surface* through a configuration alteration or chemical modification such as reduction, oxidation, pro-

tonation, etc. The demonstration that even simple adsorbate-adsorbent interactions involve alteration of the adsorbent (through a reduction in the escaping tendency of the adsorbent surface) has been shown on theoretical grounds by Copeland and Young (35) and subsequently in experiments on the adsorption of water vapor on $BaSO_4$ by Wu and Copeland (36). Although the partition of enthalpy changes between adsorbate and adsorbent cannot be made with certainty in the chromatographic system used here, we have adopted the premise that this system reflects, in a low energy, non-transforming manner, the ultimate processes occuring at sterol-protein interfaces.

It has been of interest to us for the last few years to study the behavior of sterols on silica by chromatographic means; by determining their retention volumes under standard conditions. This value is a unidimensional measure of the selectivity displayed by comparatively simple surfaces for these compounds. It has already been shown, for example, that small pore diameter silica gels have the capability a) to distinguish the presence of a Δ24 double bond as opposed to a nuclear double bond (19), b) to establish the degree of group substitution about a side chain double bond, c) to separate most single and double bond isomers of nuclear double bonds and d) to distinguish between C_{27}, C_{28}, C_{29} and C_{30} sterols of the cholesterol-lanosterol variety (20). This report is concerned with further evidence of specificity as demonstrated by the behavior of sterols possessing the 14 α-methyl group and by the behavior of normal and conjugated diene sterol structures. Further, the specificity of the silica gel of small pore diameter has been compared with the specificity displayed by thin-layer chromatographic plates of silver nitrate impregnated silica gel. This comparison has shown some curious and unexpected similarities of silver nitrate impregnation to pore diameter effects in untreated silica gel.

Specificity for C_{30}, C_{29}, C_{28} and C_{27} Sterols

Table I lists the retention volumes of 34 sterol acetates, expressed in terms of the mobility of cholesterol acetate. Included in this series are 7 lanostane varieties, 4 C_{29} or 4,4-*gem*-dimethyl-compounds, 6 C_{28} sterols including both α and β configurations of the methyl group at position 4, 7 C_{27} varieties of double bond configuration, and 3 sterols of non-mammalian origin: sitosterol, stigmasterol and ergosterol. Within each series it is evident that double bond position influences the retention volume and that the number of methyl substituents is reflected in a reduction of the retention volume. The contribution of the 24 double bond within each series is large, usually resulting in a doubling of the sterol retention volume.

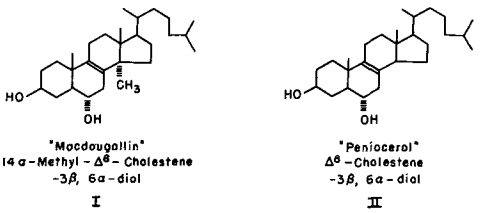

FIG. 3. Sterols of the cactus *Peniocerous macdougallii*.

The internal consistency of the retention volumes, exemplified by the additivity of group contributions, is shown in Figure 2. This orderly array indicates, for example, that there is a small increase in retention volume when the 14 α-methyl group is removed to produce a 4,4-dimethyl sterol from the lanosterol derivative. The effect is proportionate to the contribution of the double bonds in the nuclear rings, as is the effect when the transition from a 4,4-dimethyl to a 4 α-methyl, or from a 4 α-methyl group to the C_{27} variety is made.

Specificity of the 14 Alpha Methyl Group

Recently a new sterol, macdougallin, isolated from the cactus *Peniocereus macdougallii* was shown to have the structure of a 14 α-methyl cholest-8-ene-3β,6α-diol (Fig. 3)(2). The existence of a C_{28} sterol in which the 4 position was unsubstituted suggested that a study of the migration characteristics might provide an extension of the combinatorial vectors of Figure 2. On *a priori* grounds, it might be expected that the introduction of a 14 α-methyl group would have little influence on the migration characteristics of a C_{27} sterol, since the removal of the group had only a slight effect. Accordingly, 14 α-methyl cholest-7-ene-3β-ol was synthesized by a modification of the procedure of Woodward et al. (22,23)(Fig. 4). By the routes shown in Figure 5, the Δ7 sterol was converted to the Δ7,9, the Δ8 and the saturated sterol varieties (24) and the migration rate of each was determined. As seen in Table I, the introduction of the 14 α-methyl group reduced the retention volume of the Δ7 sterol (1.40); quite surprisingly its effect was much greater than the introduction of the same group at the 4 position. The 14 α-methyl Δ7,9 sterol proved to have a retention volume even *less* than that of the Δ7, indicating that the diene structure was blocked from interacting with the silica surface

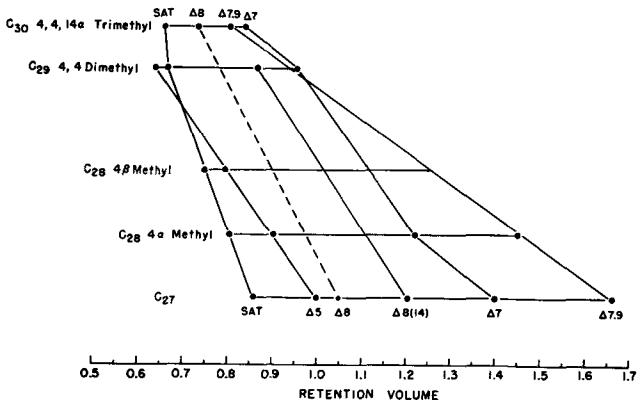

FIG. 2. Retention volume of sterol acetates on Code 12 silica gel columns eluted with 16% benzene/pentane.

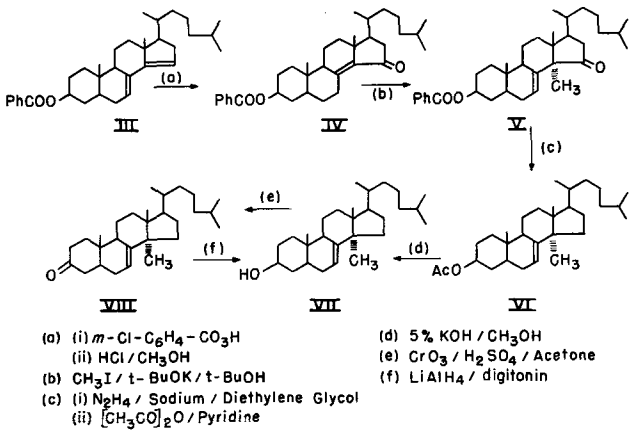

FIG. 4. Synthetic route to 14 α-methyl cholest-7-ene-3β-ol.

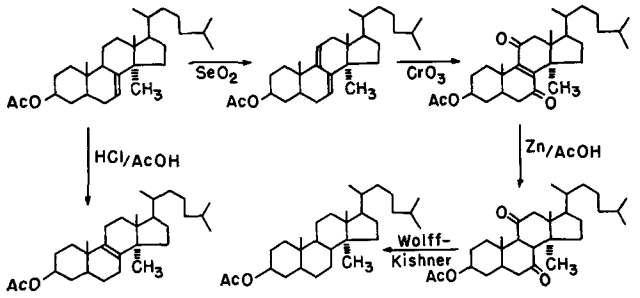

FIG. 5. Conversion of 14 α-methyl cholest-7-ene-3β-ol to the Δ8 isomer, the Δ7,9 isomer and the saturated stanol.

by the presence of the methyl group at position 14. The cholest-8-ene structure, whose double bond might be expected to contribute little to the adsorptive energy, proved to have a retention volume less than the previous two compounds. On the other hand, the retention of the 14 α-methyl stanol is *increased* (0.918 vs. 0.860). Figure 6 summarizes the shifts in migration rates in going from the C_{27} to C_{28} series by this methylation: in contrast to all other series tested, it is not possible to assign an "axis" for the 14 α-methyl group; in fact, as no other group does, this group confers a unique mobility on all sterols tested to date. Apparently the sterol molecule is "perched" on the 14 α-methyl group during its adsorption on the silica surface and the double bond location in the sterol nucleus is of little effect; none makes any appreciable contribution to this interaction.

One might argue that the removal of the 14 α-methyl group is an obligatory first step in the transformation of lanosterol to cholesterol in order that the adsorptive contribution of the nuclear double bond be "unmasked." The occurrence of peniocerol, the demethylated analogue of macdougallin, in the same cactus shows, however, that at least one enzyme system is capable of postponing this demethylation until the methyl groups at position 4 are removed. We have investigated the ability of rat liver homogenates to remove the 14 α-methyl group and have reported that this capability is also present (25) in animal tissue. In a more extensive study reported elsewhere (23) we have demonstrated that H^3-labeled 14 α-methyl cholest-7-ene-3β-ol can be converted by Bucher homogenates to cholesterol. This demonstration was achieved by isolating the product from the digitonin precipitate as the 5,6 dibromide, recrystallizing to constant specific activity and debrominating to obtain the radioactive cholesterol. The identity of the product was further demonstrated by chromatographing the acetate of the tritium-labeled product with cholesterol acetate-1-C^{14}. The chromatographic peak, shown in Figure 7, indicates complete

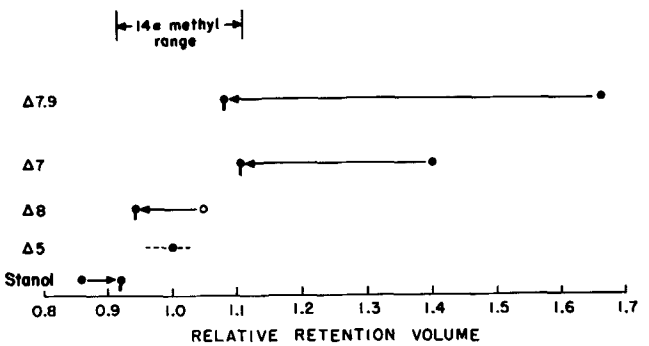

FIG. 6. The effect of introducing a 14 α-methyl group upon the retention volumes of 4 C_{27} sterols.

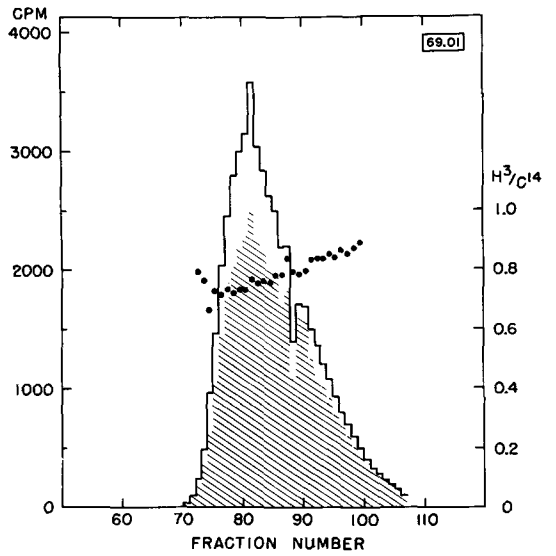

FIG. 7. The co-chromatography of cholesterol-2,4-H^3 acetate (derived by enzymatic conversion of 14 α-methyl cholest-7-ene-3β-ol-2,4-H^3 to cholesterol) with a reference standard of cholesterol acetate-C^{14}. Cross hatched area; H^3, outline: C^{14}, dots, H^3/C^{14}.

coincidence of the two labels and the consistent isotope ratio across the mixed peak except for the slope induced by the usual isotopic displacement (26) confirms the identity of the two substances.

Diene Structure and the Specificity of Silica Gel

In a previous report (20) it had been noted that the Δ5,7 diene structure of 7 dehydrocholesterol and of ergosterol appeared to be particularly susceptible to destruction, resulting in partial losses during chromatography. Subsequently, synthesis of 4,4-dimethyl cholest-5,7-diene-3β-ol and trial chromatography revealed that inevitably this compound was completely destroyed on the column. At that time, these were the only examples of conjugated dienes that had been tested, and it was of interest to determine whether conjugation per se was responsible for adsorptive destruction. In addition to two unconjugated dienes (Δ5,24 and Δ8,24), four conjugated diene structures have been tested: Δ3,5; Δ5,7; Δ7,9 and Δ7,14. The retention volumes for these dienes are shown in Table II and indicate a progressive increase with the distance of the double bonds from the oxygen function. That neither conjugation nor absolute adsorptive interaction is responsible for the destruction

TABLE II

Relative Elution Volumes and Recoveries of Diene Sterol Acetates

	REV	Recovered		
		[a]	[b]	[c]
4,4,14 α trimethyl cholest-7,9-diene-3β-ol	0.804	100		
4,4,14 α trimethyl cholest-8,24-diene-3β-ol	1.34	100		
4,4,14 α trimethyl cholest-7,24-diene-3β-ol	1.48	100		
4,4,14 α trimethyl cholest-7,9,24-triene-3β-ol	1.66	100		
4,4 dimethyl cholest-5,7-diene-3β-ol	?	0	0	0
cholest-3,5-diene-3β-ol	0.786	100		
cholest-7,9-diene-3β-ol	1.640	100		
cholest-5,7-diene-3β-ol	1.77	10	30	50
cholest-7,14-diene-3β-ol	1.955	100		
cholest-5,24-diene-3β-ol	1.98	100		
cholest-8,24-diene-3β-ol	2.42	100		
Ergosterol	2.04	10		

[a] Elution with 16% benzene in pentane.
[b] Column packed in 16% benzene/pentane with 0.1% pyridine, elution as in (1).
[c] Column packed in 16% benzene/pentane with 0.5% pyridine, elution as in (1).

of Δ5,7 dienes is demonstrated by the complete recovery of all of the other dienes including those (both conjugated and unconjugated) whose retention volumes are greater than 1.77. This leaves only the possibility of a homoannular diene structure being responsible for the specificity of interaction; unfortunately there are few other combinations of positions that could be utilized to test this assumption.

The destruction of Δ5,7 dienes by silica surfaces cannot, it would seem, be the result of excessively strong adsorptive sites. The gel used in these columns is subjected to controlled deactivation by water vapor before use to provide a uniform surface energy (29) and the recovery of other more strongly adsorbed species suggests that the destruction is due to specific sites present on the surface in low numbers, similar to those responsible for the polymerization activity of silica toward butadiene. The latter sites are thought to be acidic in nature and can be poisoned by ammonia or pyridine. Some treatment was carried out on the silica gel used in these columns by suspension of the gel in the usual solvent system of 16% benzene-pentane to which had been added either 0.1 or 0.5% pyridine. Elution carried out with pyridine-free solvents revealed that the gel had become partially deactivated as measured by the criterion of the retention volume for cholesterol acetate (which decreased). Simultaneously, the recovery of the test sample of cholest-5,7-diene-3β-ol acetate increased from 10 to 30% and to 50% with 0.5% pyridine respectively. Nevertheless, the affinity of these sites for 4,4-dimethyl-cholest-5,7,-diene-3β-ol remained so high that none of the compound was successfully eluted from the deactivated columns.

Specificity of Normal and $AgNO_3$ Impregnated TLC Plates for Sterol Acetates

A number of investigators, notably Morris (28), Copius-Peereboom and Beekes (29) and Ikan and Cuzinski (30) have shown that as with compounds in other analytical separations, the addition of silver nitrate to the plate will enhance the separation of saturated and unsaturated sterols. At the suggestion of Dr. Morris we have compared the migration rates (as Rs values), for a variety of the sterol structures available to us, on normal plates with those values obtained on plates of 2.5, 5, 10 and 15% argentation. The values are shown in Table III. In

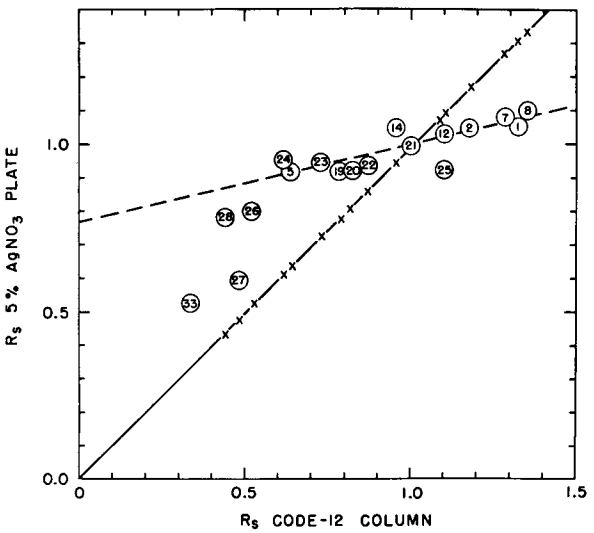

FIG. 8. Comparison of Rs values (cholestanol = 1.00) on 5% silver nitrate plates with Rs values on Code 12 silica columns. Numbers refer to compounds listed in Table III.

concurrence with Morris's findings, the optimum degree of argentation is certainly below 15% and probably lies between 2.5 and 5% for sterol acetates. But despite the obvious improvement over the untreated gel, the impregnation with silver nitrate does not confer the same degree of selectivity as a narrow por gel. A better appreciation of this comparison is shown in Figure 8 which contrasts the Rs (cholestanol equals 1.00 in both systems) for thin layer plates with 5% $AgNO_3$ with Code 12 silica gel columns for each of 18 sterols. If both systems displayed equal selectivity, the points would fall along the solid line at 45° to the x axis. A decreased slope indicates a lower selectivity of the plates vs. the column: a rough order of magnitude is provided by the intersection of the line with the left hand coordinate at a point about one quarter of the way down from the reference point, equivalent to a selectivity of one fourth that of the Code 12 silica. As previously noted by Morris, the greatest interaction of the silver nitrate plates is with the 5,7 diene system in 7-dehydrocholesterol and in ergosterol. These thin-layer chromatographic points are the only ones showing any close similarity to their counterpoints on the columns, and raises a curious specula-

TABLE III

Chromatographic Mobilities of Sterol Acetates on Thin-layer Plates With and Without $AgNO_3$ Impregnation

All mobilities are referred to the mobility of cholestanol acetate.

Number	Sterol acetate	Normal gel[a]	2.5% $AgNO_3$[b]	5% $AgNO_3$[b]	10% $AgNO_3$[c]	15% $AgNO_3$[c]
1	4,4,14 α methyl cholestane-3β-ol	0.98	1.01	1.07	1.04	10.1
2	4,4,14 α methyl cholest-8-ene-3β-ol	0.98	1.05	1.05	1.02	1.09
5	4,4,14 α methyl cholest-8,24-diene-3β-ol	0.98	0.96	0.93	0.94	1.00
7	4,4 dimethyl cholestane-3β-ol	0.99	1.04	1.09	1.05	1.04
8	4,4 dimethyl cholest-5-ene-3β-ol	1.04	1.04	1.10	1.04	1.04
12	4 β methyl cholest-5-ene-3β-ol	0.98	0.98	1.04	1.01	1.00
14	4 α methyl cholest-5-ene-3β-ol	1.00	0.97	1.04	0.99	0.99
19	14 α methyl cholest-7-ene-3β-ol	.....	0.95	0.93	0.94	0.98
20	14 α methyl cholest-7,9-diene-3β-ol	.....	0.94	0.93	0.90	0.99
21	cholestane-3β-ol	1.00	1.00	1.00	1.00	1.00
22	cholest-5-ene-3β-ol	0.96	0.99	0.94	0.97	0.97
23	cholest-8(14)-ene-3β-ol	0.94	0.97	0.95	0.97	1.00
24	cholest-7-ene-3β-ol	0.93	0.94	0.92	0.95	0.97
25	cholest-3,5-diene-3β-ol	.....	.....	0.92	.....	.....
26	cholest-7,9-diene-3β-ol	0.88	0.94	0.92	0.93	0.98
27	cholest-5,7-diene-3β-ol	0.89	0.67	0.60	0.64	0.67
28	cholest-7,14-diene-3β-ol	.....	.....	0.78	.....	.....
33	ergosterol	.....	.....	0.53	.....	.....

[a] Eluted with 4% ethyl acetate in pentane.
[b] Eluted with 10% ethyl acetate in pentane.
[c] Eluted with 15% ethyl acetate in pentane.

tion, to wit: is the consequence of impregnation on sterol separation explainable as a silver ion:double bond interaction or does it bear a closer resemblance to the pore diameter effect of unimpregnated small pore silica gels? A reduction in the average pore diameter by the introduction of a monolayer of silver ions might be expected to confer selectivity upon the adsorbent solely on geometrical grounds already known to enhance fractionation in silica gel.

Since the major aspects of selectivity on silica gel both with and without silver nitrate entail the number and location of double bonds in the adsorbate molecules, it would not seem possible to find a clear-cut means of comparing mechanisms of interaction on an unambiguous basis. One such possibility remains open, however, in the response to methyl groups, whose presence results in a decreased retention volume in narrow pore gels. By analogy, an increased Rf or Rs might be expected to result in thin-layer chromatography if silver nitrate impregnation affected the pore diameter of the adsorbent. Figure 9 shows the mobilities of 4 α-methyl, 4 β-methyl and 4,4-dimethyl cholesterol acetates vs cholesterol as a function of the per cent argentation. These values show an enhanced separation as the result of silver nitrate impregnation, having a maximum at 5% silver nitrate in the adsorbent. Since these values are based upon the retention volume for cholesterol acetate, they are corrected for any simple decrease in the adsorptive capacity of the gel as the result of impregnation. It might be argued, however, that the introduction of methyl groups produces a steric hindrance to the formation of a complex between the silver ions and the $\Delta 5$ bond and that the separation reflects a reduced affinity for the double bond. This possibility can be tested by comparing the mobilities of the saturated sterols: cholestanol, 4,4-dimethyl cholestanol and lanostanol. Figure 9 also shows that the methylated sterols show a similar enhancement of separation from cholestanol

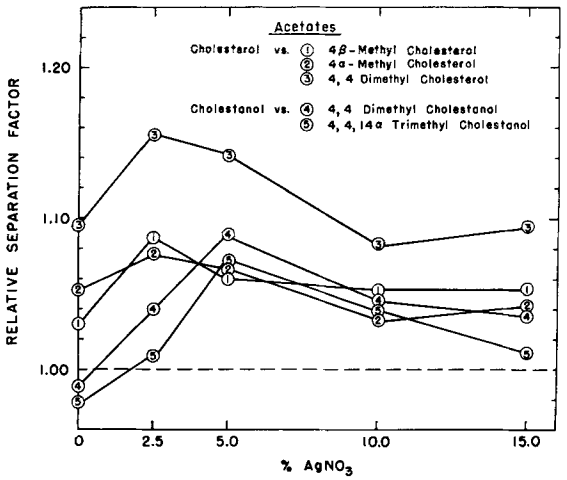

FIG. 9. Effect of argentation upon the separation of methyl sterol acetates from cholesterol or cholestanol acetate.

as the result of silver nitrate impregnation and that this enhancement is maximal between 2.5 and 5% silver nitrate. These results suggest to us that, if present, the silver-olefin complex is less effective in the thin-layer chromatography of sterols than are geometrical changes in the adsorbent pore diameter. Certainly the effect of the complex upon the separation characteristics of the plates that we have examined does not compare with the enhancement of separation of straight-chain olefin structures (31–34), nor have the separations reported by others for sterols on silver nitrate plates exceeded those we are accustomed to see on the narrow pore column.

In any event, fuller comparisons with silver nitrate-treated columns and with thin layer plates made with narrow pore gels should open more analytical opportunities. We can also foresee that they should provide us with a better picture of the behavior of sterols at interfaces.

ACKNOWLEDGMENT

Work supported by the United States Atomic Energy Commission.

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