THIN-LAYER CHROMATOGRAPHY OF STEROLS

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Although thin-layer chromatography has only come into widespread use in the past four years, it has already gained recognition as a valuable tool in the analysis of steroids. Among the classes of steroids to which thin-layer and spread-layer chromatography have been applied are: estrogens[1-6], androgens[4-10] and other C_{19}-steroids[8,9,11,12], corticosteroids[4,9,12-16] and other C_{21}-steroids[4,5,7,9,11,12,14,16,17], cardenolides[18,19] and cardiac glycosides[19-21], etianic acid derivatives[4,13], bile acids and their esters[5,22-24], sterols[4,5,7,9,11,13,17,25,26] and cholesterol esters[4,5,7,27-31], cholestanones[13,32], sapogenins[4,5,9,33-38] and saponins[20], alkaloids[4,5,9,33,35,39], and aromatized steroids[40].

An examination of the above references reveals that separation by thin-layer chromatography is relatively easy where differences exist in the kind, number, position, or configuration of polar groups, but difficult in the absence of such differences. In certain cases a difference in substitution on a carbon atom adjacent to a polar group is sufficient to make separation possible (*e.g.*, progesterone and pregnane-3,20-dione[16]), and the resolution of A/B *cis–trans* isomers having a polar group in position 3 can be accomplished.

The scarcity of data concerning the influence on separability of structural differences remote from polar groups has led us to make a study of such effects. The eight 3β-sterols selected for this investigation differ only in Ring B and/or in the side chain and are of considerable biological interest.

EXPERIMENTAL

Except as described below, chromatograms were prepared and developed as in our previous papers[16,38].

Silica Gel G plates were used for all solvent systems except B, where Silica Gel G–Kieselguhr G (1:1)[38] was the adsorbent.

The composition of the solvent systems was as follows (minutes required for development in parentheses):

A: Cyclohexane–ethyl acetate–water, 600:400:1 (29).

B: Cyclohexane–heptane, 1:1 (25).

C: Cyclohexane–ethyl acetate–water, 1560:440:1 (34).

D: Isooctane–carbon tetrachloride, 19:1 (29).

Sterols were applied in 0.1 μg quantities.

RESULTS AND DISCUSSION

As previously reported[4,5,9,17], the mixtures of hydrocarbons with more polar solvents, commonly used for thin-layer chromatography, failed to separate 3β-sterols differing only by degree of unsaturation or number of carbon atoms in the side chain. Thus, a mixture of cholesterol, stigmasterol, β-sitosterol and desmosterol moved as a single spot in System A (see Fig. 1), and ergosterol and 7-dehydrocholesterol likewise failed to separate.

In view of our previous finding that isomers of sapogenins differing only in the configuration of a C-25 methyl group are resolved by mixtures of nonpolar solvents[38], solvent systems of this type were tested for the thin-layer chromatography of sterols. Mixtures of cyclohexane or isooctane with a series of solvents in descending order of polarity were examined, using cholestane and Δ^{16}-cholestene* as model compounds. While no separation was achieved with chloroform, toluene, or benzene, these hydrocarbons were resolved when carbon tetrachloride was used (System D, Fig. 1).

However, the four sterols differing in the side chain were not separated in systems containing carbon tetrachloride. A resolution of the pairs with saturated and unsaturated side chains β-sitosterol–stigmasterol and cholesterol–desmosterol was finally effected by a mixture of saturated hydrocarbons (System B, Fig. 1). Because

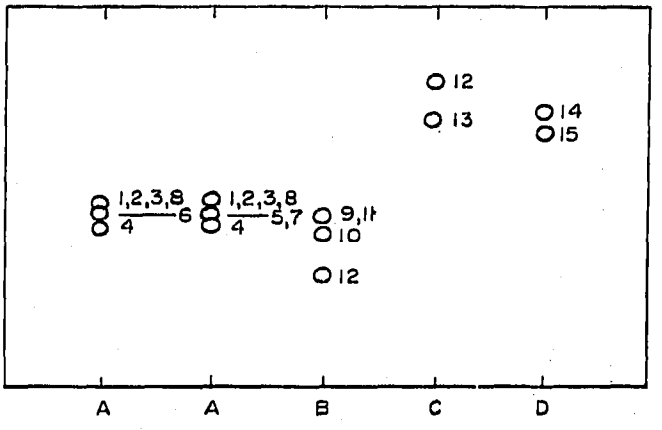

Fig. 1. Separation of sterols and sterol esters (for solvent systems A-D see text). (1) Cholesterol (Δ^5-cholesten-3β-ol); (2) Stigmasterol (24α-ethyl-$\Delta^{5,22}$-cholestadien-3β-ol); (3) β-Sitosterol (24α-ethyl-Δ^5-cholesten-3β-ol); (4) Δ^7-Cholesten-3β-ol; (5) Ergosterol (24β-methyl-$\Delta^{5,7,22}$-cholestatrien-3β-ol); (6) Cholestan-3β-ol; (7) 7-Dehydrocholesterol ($\Delta^{5,7}$-cholestadien-3β-ol); (8) Desmosterol ($\Delta^{5,24}$-cholestadien-3β-ol); (9) Cholesterol trifluoroacetate; (10) Stigmasterol trifluoroacetate; (11) β-Sitosterol trifluoroacetate; (12) Desmosterol trifluoroacetate; (13) Desmosterol acetate; (14) Cholestane; (15) Δ^{16}-Cholestene.

of the very low polarity of this system, appreciable mobilities were only obtained by chromatographing the sterols in the form of their trifluoroacetates on Kieselguhr G–Silica Gel G (1:1)[38]. A separation of cholesterol acetate and desmosterol acetate under similar conditions has recently been reported by MILLER, HAMILTON AND GOLDSMITH[41], who used glass paper impregnated with silicic acid as the adsorbent and isooctane as the developing solvent.

Even in System B an alkyl substituent in the side chain has no influence on mobility, as is shown by the failure of cholesterol trifluoroacetate and β-sitosterol

* Generously supplied by Dr. G. V. NAIR.

trifluoroacetate to separate. Thus, the large difference in mobilities between stigmasterol trifluoroacetate and demosterol trifluoroacetate can only be due to the difference in position of the double bond.

In contrast to sterols with different side chains, compounds differing in the degree and/or position of unsaturation in ring B were separable in polar systems (System A, Fig. 1). The greatest separation occurred between cholesterol and Δ^7-cholesten-3β-ol, but the latter sterol and cholestan-3β-ol, differing only by a double bond four carbon atoms removed from the hydroxyl group, were also separated, as were cholesterol and 7-dehydrocholesterol. It is interesting that 7-dehydrocholesterol, in spite of having one more double bond than Δ^7-cholesten-3β-ol, shows greater mobility. The difficulty of separating 5α- from corresponding Δ^5-steroids is again evident here, in the case of cholesterol and cholestan-3β-ol.

The trifluoroacetates of sterols 1–8 were not separable in polar systems. This is in agreement with our observations on sapogenin acetates[38]. However, as Fig. 1 shows, resolution of desmosterol acetate from its trifluoroacetate was possible in the polar system C. This suggests that while separations based on differences in the acidic portion of the ester are possible in both polar and nonpolar systems, only the latter are suitable for resolution on the basis of differences in the alcohol portion. The literature on the separation of cholesterol esters[4,5,7,27,28,31] also indicates that separations on the basis of differences in the acid portions are usually feasible.

The general applicability of the 50 % sulfuric acid spray was demonstrated by its ability to reveal even a saturated hydrocarbon, cholestane, in a concentration of 0.1 μg. A temperature of about 200° was necessary in this case, but for the sterols about 120° was usually sufficient.

Further work is needed before a systematic correlation of structural differences with separability is possible, but our results show that thin-layer chromatography is capable of rather subtle discriminations when the proper conditions are chosen. Our failure to separate sterols differing only by alkyl substituents in a saturated side chain may reflect a limitation of the method. Undoubtedly further improvements in the separation of the biologically important sterols differing in degree of unsaturation in ring B will be possible by experimenting with other systems, although no solvents of the type of System B could be found to give better resolution.

SUMMARY

3β-Sterols differing in unsaturation in ring B and in the side chain were separated by thin-layer chromatography. Differences in resolving power between polar and nonpolar systems were observed.

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