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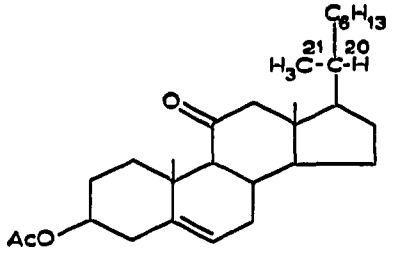
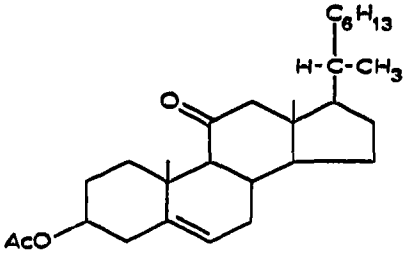
Thin-layer and gas–liquid chromatography of some 11-oxygenated cholesterols, 20-*iso*cholesterols, and related compounds

The 11-oxygenated cholesterols do not occur naturally, but were recently synthesized in the belief they will be of interest to those studying cholesterol metabolism or adrenal steroid biogenesis[1]. Since the synthetic method provides both the desired product, in this case 11-ketocholesterol acetate (3, Fig. 1), and its C-20 epimer (4), it was possible to prepare from them an additional six normal:20-*iso* pairs of stenols or stenones*. The aim of this paper is to record the contribution to polarity of a carbonyl or hydroxyl group at C-11 in both series, to note those structural features which influence separation of a given normal:20-*iso* pair, and to correlate these results with certain observations referred to in the original paper.



11-Ketocholesterol acetate (3) (20β-methyl)



11-Keto-20-*iso*cholesterol acetate (4) (20α-methyl)

Fig. 1. Structures of 11-ketocholesterol acetate (3) and its C-20 epimer (4).

Methods

Thin-layer chromatography (TLC) was carried out under conditions indicated in a recent paper[2]. Systems consisted of ethyl acetate–*iso*octane (2,2,4-trimethylpentane) mixtures. Each is indicated in the text by a number which corresponds to one of the following compositions [in each case the number is followed (in parenthesis) by that volume of ethyl acetate which, diluted to 25 ml with *iso*octane, comprises the system]: 1 (1.4), 2 (2.2), 3 (4), 4 (5.4), 5 (8), and 6 (0.93).

Gas–liquid chromatography (GLC) was performed on a Hewlett-Packard Model 5750 B instrument equipped with a hydrogen flame detector and employing a glass column, 4 ft. long and 4 mm I.D., packed with 2 % QF-1 (fluroalkyl silicone, Applied Science Laboratories, State College, Pa.) on Gas-Chrom Q. Operating temperatures were as follows: oven, 245°; injection port, 260°; flame, 260°. Helium was employed as the carrier gas at a flow rate of 40 ml per min.

Results

Table I presents data from four groups of compounds, each consisting of an 11-deoxy and a corresponding 11-oxygenated normal: 20-*iso* pair. The TLC results are given in terms of R_F values from which were derived expressions of the contribution to polarity (ΔR_M) (ref. 3) of the 11-oxygen function and an estimate, also cast

* See *Appendix* I.

TABLE I

TLC AND GLC OF 11-DEOXY AND 11-OXYGENATED CHOLESTEROL ACETATES AND CHOLESTENONES IN THE NORMAL AND 20 *iso* SERIES

Compound No.	*Trivial name*	*TLC*				*GLC*
		System No.	R_F[a]	ΔR_M (*11-oxygen function*)	ΔR_M ($n \rightarrow iso$)	*Retention time (min)*
1	Cholesterol acetate	1	0.28			1.64
2	20-*Iso*cholesterol acetate	1	0.29			1.50
3	11-Ketocholesterol acetate	1	0.12	+0.44		3.40
4	11-Keto-20-*iso*cholesterol acetate	1	0.15	+0.35	−0.14	3.02
1	Cholesterol acetate	2	0.34			
2	20-*Iso*cholesterol acetate	2	0.35			
5	11β-Hydroxycholesterol acetate	2	0.13	+0.53		3.04
6	11β-Hydroxy-20-*iso*cholesterol acetate	2	0.20	+0.32	−0.23	2.68
7	Cholestenone	3	0.20			2.96
8	20-*Iso*cholestenone	3	0.21			2.64
9	11-Ketocholestenone	3	0.11	+0.30		5.80
10	11-Keto-20-*iso*cholestenone	3	0.12	+0.28	−0.06	5.51
7	Cholestenone	4	0.28			
8	20-*Iso*cholestenone	4	0.29			
11	11β-Hydroxycholestenone	4	0.11	+0.48		5.40
12	11β-Hydroxy-20-*iso*cholestenone	4	0.17	+0.30	−0.22	4.80

[a] Averaged values from duplicate determinations except in case of compounds 1, 2, 7, and 8 which were chromatographed four times.

in ΔR_M terms, of the effect on mobility of substituting a 20-*iso* compound for its C-20 epimer.

In considering the contribution of the 11-oxygen function, it may be stated first that the ΔR_M values are similar to those observed for a variety of steroids when chromatographed by the thin-layer method*. It is to be noted that the value derived from a given normal compound exceeds in each case that provided by the 20-*iso* member of the pair. These invariable, if small, differences within pairs suggest that normal and 20-*iso* compounds exist as distinctly different, relatively stable, conformers.

20-*Iso* compounds are consistently more mobile than the corresponding normal compounds, but only marginally so in the 11-deoxy series (compare R_F values of compound 1 with 2, and 7 with 8 in this regard). Insertion of a carbonyl group at C-11 distinctly aids this separation (compare 3 with 4), but the effect is largely obliterated if, in addition, the Δ^4-3-keto arrangement is substituted for the Δ^5-3β-acetoxy system (pair 9, 10). The improvement in separation is nearly doubled by introducing an axially-oriented (β) hydroxyl group at C-11 (pair 5,6); this gain is largely uninfluenced by the change noted above (pair 11, 12). The mobility differences within the four pairs of epimers are given formal expression as $\Delta R_{M\ (n \rightarrow iso)}$ values**.

* See *Appendix* II.
** See *Appendix* III.

The GLC results are in close agreement with those obtained by TLC except in the case of the 11, 12 pair where the former method gave a notably better separation. All of the compounds, including those with relatively long retention times, gave single, sharp peaks. There was no evidence of dehydration of the 11β-hydroxyl group (in compounds 5, 6, 11, and 12) as judged by the absence of peaks derived from the corresponding unsaturated ($\Delta^{9,11}$) compounds.

TABLE II

TLC OF 11-DEOXY AND 11-OXYGENATED CHOLESTEROLS IN THE NORMAL AND 20-*iso* SERIES

Compound No.	*Trivial name*	*System No.*	R_F	ΔR_M ($n \rightarrow iso$)
13	Cholesterol	5	0.26	
14	20-*Iso*cholesterol	5	0.26	
15	11-Ketocholesterol	5	0.17	
16	11-Keto-20-*iso*cholesterol	5	0.18	−0.05
17	11β-Hydroxycholesterol	5	0.14	
18	11β-Hydroxy-20-*iso*cholesterol	5	0.16	−0.07
19	11α-Hydroxycholesterol	5	0.07	
20	11α-Hydroxy-20-*iso*cholesterol	5	0.10	−0.14

Table II gives R_F and $\Delta R_{M\,(n \rightarrow iso)}$ values obtained when the normal: 20-*iso* pairs, including the 11α-hydroxy pair, were chromatographed as the free compounds. In this case the 11-deoxy (13, 14) pair was not separated, and ΔR_M values for pairs 15, 16 and 17, 18 are smaller than noted in Table I. Moreover, the 11β-hydroxyl group is now only marginally more effective in promoting separation than a carbonyl group at this site. The effect of an equatorially-oriented (α) hydroxyl group at C-11 (pair 19, 20) is relatively marked, doubling the effect provided by its axially-oriented (β) counterpart.

Attempts to compare the free compounds by means of partition chromatography on paper were not very successful — the usual procedure gave mobilities too high to be useful even when very non-polar systems were employed, and a reversed-phase technique led to excessive streaking. But in both cases it could be seen that the mobility relationships qualitatively paralleled those observed using TLC.

Discussion

In considering these results, it is pertinent first to discuss a particular observation recorded in the earlier paper[1]. This is that 11-ketocholestenone (9) is a somewhat unstable substance, slowly yellowing in the crystalline state and generating what appear to be autoxidative products. It was also noted that its molecular rotation (M_D, +751) and the rotational increment (Δ) for the introduction of the C-11 carbonyl group (+414) are higher than we have observed for 11-ketones in this or other series. In contrast, the corresponding 20-*iso* compound (10) is perfectly stable. These facts suggest that the diketone 9 is conformationally distorted as a result of an inter-

action, as yet undefined, between the C-11 carbonyl group and the C-21 methyl group of the side-chain*.

We believe that the chromatographic results can be understood in terms of such an interaction. It is only necessary to assume that the interaction, and associated conformational distortion, have the effect of increasing the polarity (decreasing the mobility) specifically of the 11-oxygenated normal members. This would account both for the regular differences in $\Delta R_{M\,(11\text{-oxygen function})}$ values in normal:20-*iso* pairs (as already suggested), and for the effect of an oxygen function at C-11 on the separation of normal:20-*iso* pairs. We do not believe that what we ascribe to interaction is due to those conformational changes induced by the adsorbent itself, for the effect is also seen in paper chromatography where surface effects are minimal.

It was noted in the present work (Fig. 1 and associated discussion) that alterations in the molecule at sites other than C-11 influence $\Delta R_{M\,(n \rightarrow iso)}$ values. A similar effect of remote groups on normal:20-*iso* relationships was noted in the earlier work by determining the influence of such changes on optical rotation expressed as $M_{D\,(n \rightarrow iso)}$ increments. This is apparent in the following series (in each case the compound number is followed by the increment in parentheses): 15 (—108), 3 (−99), 17 (−74), 5 (−68), 9 (−38), and 11 (+11).

Appendix

I. Compound number, trivial name, and formal name are related, in order, as follows:

1 cholesterol acetate, cholest-5-en-3β-ol acetate
2 20-*iso*cholesterol acetate, 20-*iso*cholest-5-en-3β-ol acetate
3 11-ketocholesterol acetate, 3β-acetoxycholest-5-en-11-one
4 11-keto-20-*iso*cholesterol acetate, 3β-acetoxy-20-*iso*cholest-5-en-11-one
5 11β-hydroxycholesterol acetate, cholest-5-ene-3β, 11β-diol 3-acetate
6 11β-hydroxy-20-*iso*cholesterol acetate, 20-*iso*cholest-5-ene-3β,11β-diol 3-acetate
7 cholestenone, cholest-4-en-3-one
8 20-*iso*cholestenone, 20-*iso*cholest-4-en-3-one
9 11-ketocholestenone, cholest-4-ene-3, 11-dione
10 11-keto-20-*iso*cholestenone, 20-*iso*cholest-4-ene-3, 11-dione
11 11β-hydroxycholestenone, 11β-hydroxycholest-4-en-3-one
12 11β-hydroxy-20-*iso*cholestenone, 11β-hydroxy-20-*iso*cholest-4-en-3-one
13 cholesterol, cholest-5-en-3β-ol
14 20-*iso*cholesterol, 20-*iso*cholest-5-en-3β-ol
15 11-ketocholesterol, 3β-hydroxycholest-5-en-11-one
16 11-keto-20-*iso*cholesterol, 3β-hydroxy-20-*iso*cholest-5-en-11-one
17 11β-hydroxycholesterol, cholest-5-ene-3β, 11β-diol
18 11β-hydroxy-20-*iso*cholesterol, 20-*iso*cholest-5-ene-3β,11β-diol
19 11α-hydroxycholesterol, cholest-5-ene-3β, 11α-diol
20 11α-hydroxy-20-*iso*cholesterol, 20-*iso*cholest-5-ene-3β,11α-diol.

The term "normal" refers, for present purposes, to compounds with the 20β-methyl configuration, that is to cholesterol itself and substances derived from it.

* See *Appendix* IV.

The established prefix "*iso*" denotes the 20α-methyl configuration in all cases, that is to 20-*iso*cholesterol and its products. In more general terms, cholesterol and 20-*iso*cholesterol may be regarded simply as C-20 epimers.

II. In order to assess the "true" contribution to polarity of a carbonyl or axially-oriented (β) hydroxyl group at C-11, a variety of 11-oxygenated and 11-deoxy pairs of steroids (rather than sterols) were chromatographed by the thin-layer method using ethyl acetate–*iso*octane systems. In the case of the carbonyl group, twenty-five determinations of a total of fourteen 11-keto:11-deoxy pairs gave an average ΔR_m value of 0.39, with extremes of 0.13 and 0.62. Seventeen analyses of a total of eight 11β-hydroxy:11-deoxy pairs gave an average value of 0.40, with extremes of 0.25 and 0.64. These variations, as well as small carbonyl: hydroxyl differences, seem characteristic of TLC, and contrast with the more regular results obtained with paper chromatography.

III. These differences underline the chief limitation of the synthesis, namely the column chromatographic separation of the normal:20-*iso* pair of products. Thus it is not possible to separate the 1,2 pair by this means. The 3,4 pair was separated but only under conditions bordering on the heroic [in the recorded example[1], a 5.1 g reaction product consisting chiefly of a 3,4 mixture was chromatographed on a silica gel column (Davison, grade 923), 80 × 1500 mm, prepared and developed with system 6. The flow rate was about 60 ml per h, and the total development time about one month. The efficiency of separation may be judged by the fact that the intermediate (mixture) band weighed just 50 mg]. Should 11β-hydroxycholesterol prove to be a useful compound, it would be advantageous to prepare it by reducing the 3,4 mixture (and re-acetylating) prior to chromatography since the 5,6 pair is relatively well separated. Evaluation of the effect of an 11α-hydroxyl group in the manner given in Table I requires preparation of the 3-acetates of the diols which we have not as yet achieved.

IV. It is clearly apparent from Dreiding models, and suggested in Fig. 1, that the C-21 methyl group in normal (20β methyl) compounds approaches the carbonyl group at C-11. Interaction in this case would tend to be favored, and stabilized, by the rearward disposition of the side-chain. In the 20-*iso* (20α-methyl) series, the methyl and carbonyl groups can approach only under circumstances where the side-chain is thrust toward the front (β) surface of the ring system. This is an unfavored arrangement due to conflict with the angular methyl groups.

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Department of Medicine, Jefferson Medical College, Philadelphia, Pa. 19107 (U.S.A.)

JOHN J. SCHNEIDER
LORRAINE J. HAEFFNER

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