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PAPER CHROMATOGRAPHIC CHARACTERISTICS OF SOME NEW 1-OXYGENATED STEROIDS

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SUMMARY

In an investigation of the paper chromatographic characteristics of a number of 1-oxygenated steroids, it has been shown that the contribution to polarity of the C-11 carbonyl group in 1,11-diketones is aberrantly large and that, contrary to the general rule, the equatorial member of most pairs of epimeric 1-ols is the more mobile. Various mechanisms have been proposed to account for these results.

INTRODUCTION

Recently we isolated a new 1-hydroxylated metabolite of cortisol, namely 1β, 3α,17α,20β,21-pentahydroxy-5β-pregnan-11-one and, in the course of its degradation, prepared a number of saturated and unsaturated 1-oxygenated 5β-pregnanes and androstanes both bearing and lacking carbonyl groups at C-11[1,2]. As illustrated in other publications from this laboratory[3–5], we made extensive use of partition paper and partition column chromatography to analyze the composition and to separate the components of reaction mixtures, to check the effectiveness of other fractionating procedures, and to verify the homogeniety of crystallized products. In addition to its general utility in this study, the method revealed aberrant paper chromatographic behavior in enough compounds to justify a more extended study, and to indicate the merits of correlating these results with certain unusual chemical properties of these compounds.

METHODS

With the exception of compound 29, the steroids used in this study were prepared previously[1,2,6] or are known substances. Compounds 30 and 31 were supplied by Drs. STEPHEN KRAYCHY, WALTER R. BENN and RAPHAEL PAPPO of G. D. Searle and Company. Compound 37 was prepared by incubating 5β-androstan-3α-ol-17-one with *Fusarium lini* (Bolley)[7] or, more successfully, with a *Penicillium* species (ATCC 11598). Its constitution was established by NMR spectroscopy.

Preparation of 5α-androstane-1β,3β-diol-17-one (compound 29)

To a solution of 16 mg of androst-5-ene-1β,3β-diol-17-one in ethanol–cyclohexane, 5% palladium on carbon was added and the suspension was agitated for 30 min at room temperature in a hydrogen atmosphere. The product was isolated in the usual way and twice crystallized from acetone–*n*-hexane. Constants: m.p. 199–199.5°, $[\alpha]_D$ + 72.9° (methanol). Its structure was established by sodium borohydride reduction to the known[8] 5α-androstane-1β,3β,17β-triol; the melting point of our product (220–222.5°) was found by Dr. BENN to be unaltered on admixture with the reference sample of 5α-androstane-1β,3β,17β-triol (m.p. 218.5–222°) prepared by Dr. PAPPO in the Searle laboratory. The infrared spectra of the two triols were identical.

As indicated in our cited publications, paper chromatography was carried out at 25 ± 1° after an equilibration period of not less than 8 h. Steroids were applied in the lowest discernible concentration. Detection methods included application of the Zimmermann reagent[9] (after *in situ* periodic acid oxidation in the case of 17,20,21-glycerols[10]), or dipping in 10% alcoholic phosphomolybdic acid followed by heating at 60–80°. The composition of systems referred to by number in Tables II–V appear in Table I. In those cases where we were interested in measuring the effects of individual

TABLE I

COMPOSITION OF PAPER CHROMATOGRAPHIC SYSTEMS

System No.	*Composition*[a]
1	Tol., 50; Iso., 150; AM, 150; HOH, 50 ml.
2	Tol., 170; Iso., 30; AM, 150; HOH, 50 ml.
3	Tol., 140; III-bu., 60; AM, 70; HOH, 80 ml.
4	EA, 30; Iso., 170; AM, 100; HOH, 100 ml.
5	EA, 75; Iso., 125; AM, 70; HOH, 130 ml.
6	Tol., 60; Iso., 140; AM, 160; HOH, 40 ml.
7	Bz., 5 ml, diluted to 25 ml with EA.
8	Tol., 140; Iso., 60; AM, 130; HOH, 70 ml.
9	Iso., 150; III-bu., 50; AM, 30; HOH, 170 ml.
10	EA, 50; Iso., 150; AM, 50; HOH, 150 ml.
11	IPE, 110; Hep., 90; AM, 140; HOH, 60 ml.
12	Tol., 105; EA, 95; AM, 120; HOH, 80 ml.
13	Tol., 145; III-bu., 55; AM, 70; HOH, 80 ml.
14	HOAc, 130; HOH, 70; Dodecane, 5 ml. Paper impregnated by drawing through a 15% (v/v) solution of dodecane in acetone. Immediate application of steroids and chromatography without prior equilibration.

[a] Tol. = toluene; Iso. = isooctane(2,2,4-trimethylpentane); AM = absolute methanol; III-bu. = *tert.*-butanol; EA = ethyl acetate; Bz. = benzene; HOAc = glacial acetic acid; IPE = isopropyl ether; Hep. = *n*-heptane.

functional groups on mobility, the results are expressed as ΔR_{Mg} values, a designation proposed by BUSH[11] based on the original ΔR_M concept of BATE-SMITH AND WESTALL[12].

RESULTS

Table II gives the ΔR_{Mg} values for the C-11 carbonyl group contribution in nine pairs of 5β-androstanes or androstenes and two pairs of 5β-pregnanes chromatographed

TABLE II

CONTRIBUTION OF C-11 CARBONYL GROUP IN 5β-ANDROSTANES, ANDROSTENES AND 5β-PREGNANES

Compound No.	Compound	Pair	System	R_F	ΔR_{Mg}	System	R_F	ΔR_{Mg}
1	5β-Androstane-3,17-dione	1	1	0.79	0.75	4	0.82	0.73
2	5β-Androstane-3,11,17-trione			0.40			0.46	
3	5β-Androstane-1,17-dione	2		0.87	1.46		0.89	1.54
4	5β-Androstane-1,11,17-trione			0.19			0.19	
5	5β-Androst-1-ene-3,17-dione	3		0.72	0.61		0.80	0.78
6	5β-Androst-1-ene-3,11,17-trione			0.39			0.40	
7	5β-Androst-2-ene-1,17-dione	4		0.77	1.48		0.83	1.75
8	5β-Androst-2-ene-1,11,17-trione			0.09			0.08	
9	Androst-4-ene-3,17-dione	5		0.60	0.72		0.66	0.82
10	Androst-4-ene-3,11,17-trione			0.22			0.22	
11	5β-Androstan-3α-ol-17-one	6	2	0.86	0.43	5	0.91	0.47
12	5β-Androstan-3α-ol-11,17-dione			0.70			0.77	
13	5β-Androstan-3α-ol-1,17-dione	7		0.65	0.87		0.71	1.26
14	5β-Androstan-3α-ol-1,11,17-trione			0.20			0.12	
15	5β-Androstane-1β,3α-diol-17-one	8		0.24	0.43		0.38	0.69
16	5β-Androstane-1β,3α-diol-11,17-dione			0.10			0.11	
17	5β-Androstane-3α,6α-diol-17-one	9		0.15	0.47		0.19	0.67
18	5β-Androstane-3α,6α-diol-11,17-dione			0.06			0.05	
19	5β-Pregnane-1β,3α,17α,20β,21-pentol	10	3	0.29	0.46			
20	5β-Pregnane-1β,3α,17α,20β,21-pentol-11-one			0.12				
21	5β-Pregnane-3α,17α,20β,21-tetrol-1-one	11		0.59	0.70			
22	5β-Pregnane-3α,17α,20β,21-tetrol-1,11-dione			0.22				

in a total of five systems. Examination of these data show that the ΔR_{Mg} values for pairs 2 and 4 are about twice as large as those for pairs 1,3 and 5 (systems 1 and 4), that the values for pair 7 are nearly twice those for pairs 6, 8 and 9 (systems 2 and 5), and that the value for pair 11 is similarly greater than that of pair 10 (system 3). When these results are related to the indicated structures of the steroids, it is evident that this marked enhancement of the effect of the C-11 carbonyl group is manifest only in those compounds (4, 8, 14 and 22) which also bear a carbonyl group at C-1.

This effect of the 1,11-diketo system on mobility also was noted in the case of certain steroidal enol methyl ethers which were described in an earlier publication[2]. Structure assignments were made from their NMR spectra and there was a good correlation between the proposed structures and the observed constants. But as Table III illustrates, the relative mobilities, in system 6, of the 11-keto ethers (compound 24, mobile and compound 26, polar) are the reverse of those noted for the corresponding 11-deoxy ethers (compound 23, polar and compound 25, mobile). The basis

TABLE III

CONTRIBUTION OF THE C-11 CARBONYL GROUP IN STEROIDAL ENOL METHYL ETHERS

Compound No.	Compound	Pair	System	R_F	ΔR_{Mg}	System	R_F	ΔR_{Mg}
23	5β-Androst-1-ene-1-methoxy-3,17-dione	12	6	0.55	0.81	7	0.23	0.31
24	5β-Androst-1-ene-1-methoxy-3,11,17-trione			0.16			0.13	
25	5β-Androst-2-ene-3-methoxy-1,17-dione	13		0.70	1.30		0.42	0.33
26	5β-Androst-2-ene-3-methoxy-1,11,17-trione			0.10			0.25	

for this discrepancy became apparent when the mobilities of all four ethers were determined using adsorption (thin-layer, silica gel) chromatography. It will be noted (Table III, system 7) that the structure–mobility relationship is here a consistent one. TAMM[13] also noted, in the case of the chromatography on alumina of the enol methyl ethers derived from 5α-cholestane-1,3-dione, that the 3-methoxy derivative was the mobile member.

One of our objectives in the earlier and present study was to prepare pairs of epimeric 1-ols and to determine their relative mobilities in partitioning systems. Four such pairs, chiefly 17-ketosteroids and all in the 11-deoxy series, have been prepared to date. Their structures and R_F values in a total of six systems are given in Table IV. Each of the pairs of 17-ketones were chromatographed in four systems of widely

TABLE IV

R_F VALUES OF STEROIDAL EPIMERIC 1-OLS[a]

Compound No.	Compound	Pair	System	R_F	System	R_F	System	R_F	System	R_F
27	5β-Androstane-1α,3α-diol-17-one (e)	14	8	0.16	9	0.22	10	0.27	11	0.14
15	5β-Androstane-1β,3α-diol-17-one (a)			0.17		0.21		0.23		0.12
28	5α-Androstane-1α,3β-diol-17-one (a)	15		0.17		0.25		0.27		0.16
29	5α-Androstane-1β,3β-diol-17-one (e)			0.16		0.22		0.25		0.14
30	Androst-5-ene-1α,3β-diol-17-one (a)	16		0.12		0.13		0.13		0.10
31	Androst-5-ene-1β,3β-diol-17-one (e)			0.13		0.20		0.22		0.13
32	5β-Pregnane-1α,3α,17α,20β,21-pentol (e)	17	12	0.26	13	0.26				
19	5β-Pregnane-1β,3α,17α,20β,21-pentol (a)			0.23		0.24				

[a] The axial (a) or equatorial (e) configuration of the hydroxyl group at C-1 is indicated in parenthesis after the name of each compound.

varying composition, and the pregnane pair in two other, still different, systems. It is to be remarked that although the R_F differences in most cases are very small, such differences (as opposed to R_F values) could be demonstrated repeatedly.

In the case of the 5β-androstanes (pair 14), that member bearing an equatorial hydroxyl group at C-1 was the more mobile in three systems out of four. The greater mobility of the equatorial member also was evident with the 5β-pregnanes (pair 17) and with the androst-5-enes (pair 16). However, the reverse relationship holds for the 5α-androstanes (pair 15), where the member bearing an axially-oriented hydroxyl group at C-1 was the more mobile in all systems*.

In Table V we have assessed, in terms of ΔR_{Mg} values, the contribution to polarity of hydroxyl groups introduced at C-1 and at other positions in a number of 5α- and 5β-androstanes and androstenes. Two systems of contrasting composition

TABLE V

CONTRIBUTION OF HYDROXYL GROUPS IN 5β-ANDROSTANES, 5α-ANDROSTANES, AND ANDROST-5-ENES

Compound No.	*Compound*[a]	R_F *values*[b] *in system*				ΔR_{Mg} *in system*	
		9		*10*		*9*	*10*
27	5β-Androstane-1α,3α-diol-17-one (e)	0.85	0.22	0.88	0.27	1.30	1.31
15	5β-Androstane-1β,3α-diol-17-one (a)	0.85	0.21	0.88	0.23	1.34	1.41
28	5α-Androstane-1α,3β-diol-17-one (a)	0.86	0.25	0.88	0.27	1.27	1.31
29	5α-Androstane-1β,3β-diol-17-one (e)	0.86	0.22	0.88	0.25	1.35	1.36
30	Androst-5-ene-1α,3β-diol-17-one (a)	0.84	0.13	0.88	0.13	1.55	1.70
31	Androst-5-ene-1β,3β-diol-17-one (e)	0.84	0.20	0.88	0.22	1.33	1.43
33	5β-Androstane-3α,6α-diol-17-one (e)	0.88	0.12	0.88	0.07	1.74	1.92
34	5β-Androstane-3α,7β-diol-17-one (e)	0.88	0.14	0.88	0.15	1.65	1.62
35	5β-Androstane-3α,11α-diol-17-one (e)	0.88	0.33	0.88	0.27	1.17	1.31
36	5β-Androstane-3α,11β-diol-17-one (a)	0.88	0.51	0.88	0.55	0.85	0.78
37	5β-Androstane-3α,15α-diol-17-one (e')[c]	0.88	0.31	0.88	0.29	1.22	1.26

[a] The configuration of the hydroxyl group being examined is indicated in parenthesis after the name of the compound.

[b] In each case, values in the left column are derived from the appropriate stem compounds (5β-androstan-3α-ol-17-one, 5α-androstan-3β-ol-17-one, or androst-5-en-3β-ol-17-one), and those in the right column from the substituted stem compounds.

[c] Pseudoequatorial.

were used together with the appropriate 17-ketosteroids as compounds of reference.

These values are similar to those which we published earlier for a series derived from deoxycorticosterone[5] and, with the exception of those obtained for compound 36, are within the range for what may be termed "relatively unhindered" hydroxyl groups. We attach no particular significance to the small differences in ΔR_{Mg} values given by the two systems. As far as the 1-hydroxylated compounds are concerned, these results confirm those described in Table IV; in making these comparisons it should be recalled that R_F and ΔR_{Mg} values are inversely related.

* Recently we determined the relative mobilities of the epimeric 5α-androstan-1-ols[17] using a reversed-phase technique (system 14), and observed the same result: the axial member moved 104 mm from the origin in a running time of 24 h while its epimer moved 89 mm within the same time period.

DISCUSSION

Prior to considering explanations for the unusual polarity of the five 1,11-diketones (compounds 4, 8, 14, 22 and 26 in Tables II and III), it seems of interest to indicate other normal and abnormal properties of this class. JONES AND DIGIORGIO[14] have examined Dreiding models of compounds 4 and 8 (Table II and Fig. 1), and have determined their infrared spectra in chloroform and carbon disulfide solution. They reported that there was no more interaction between the C-1 and C-11 carbonyl groups

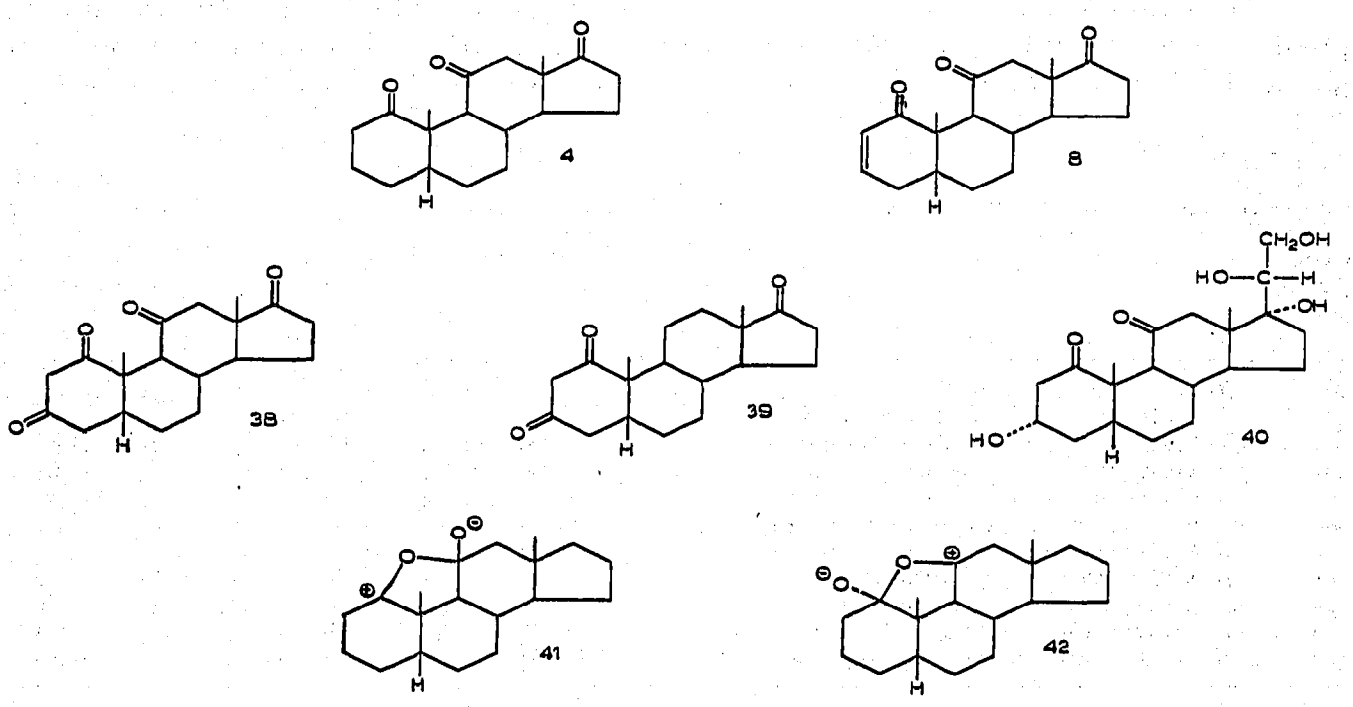

Fig. 1.

of these compounds, as judged by displacement of the C-11 band, than in 11,17-diketones generally. This is surprising since they noted that the two carbonyl groups of compound 4 are separated by only 2.8 Å (ring B chair conformation) while those of compound 8 are separated by 3.7 Å (ring B boat conformation). Since there was no marked absorption above 3000 cm^{-1}, it was concluded that neither steroid was appreciably enolized. In contrast, we have made two observations in this series which suggest the occurrence of marked physical interaction between the two carbonyl groups. The first is that the extinction coefficient, ε, in methanol of 5β-androstane-1,3,11,17-tetrone (compound 38 in Fig. 1) is only 6,400 or about one-half that of the corresponding 11-deoxy-β-diketone (compound 39, Fig. 1, ε = 12,650). The second observation is that 1,11-diketones, lacking carbonyl groups elsewhere, such as 3α,17α,20β,21-tetrahydroxy-5β-pregnane-1,11-dione (compound 40, Fig. 1), are very resistant to catalytic or metal hydride reduction, whereas the corresponding 11-deoxy-1-ketone readily is reduced to the equatorial alcohol. Both of these observations are discussed in detail in the earlier paper[2].

We believe that the observed hyperpolarity of 1,11-diketones in partitioning

systems is a consequence of the proximity of the carbonyl groups. The effect of their close approach, as 1,4-diones in a *cisoid* relationship, is to depolarize both carbonyl groups and to reduce the positive charge on each carbon atom. It seems likely that the associated repulsive forces are sufficient to distort the conformation of the molecule with a consequent change in its distribution characteristics. In addition, the carbonyl group area could serve as a site for association with components of the stationary phase, or for the generation of an ionized species; both would serve to increase the solubility of the compound in the immobile phase and thus provide the observed result. Professor D. H. R. BARTON, in a personal communication, has suggested that the abnormal polarity of *cisoid* 1,4-diones might be explained by supposing an equilibrium between the classic structure and such polar structures as compounds 41 and 42 in Fig. 1. The infrared evidence against enolization does not prevent its occurrence under the conditions prevailing in chromatography, but it is to be remarked that these diketones do not behave chromatographically like enols. They do not streak in neutral systems and the capacity of such systems seems little altered on adding acetic acid. The observation that the mobility–structure relationship for the enol methyl ethers (Table III) is an irregular one in partitioning systems but regular on adsorption chromatography is in accord with the view[15] that the conformation adopted by a molecule adsorbed on a solid surface is not necessarily its preferred conformation in solution.

In considering the data of Tables IV and V, it is apparent from the small differences in R_F that the contribution to polarity of each epimer is very similar. In the 5β-androstane series, that member bearing an axially-oriented hydroxyl group at C-1 (compound 15) is less mobile in three systems out of four, thus providing an exception to the rule[16] which states that the axial member of an epimeric pair is the more mobile. Examination of a Dreiding model of compound 27 in the all-chair conformation (Fig. 2)

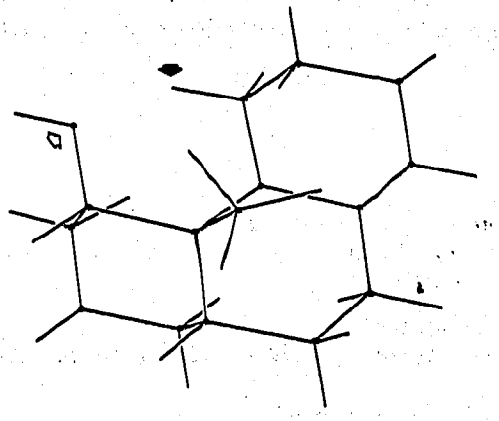

Fig. 2. Dreiding model of rings A (left), B and C of 5β-androstan-1α-ol, photographed from the front (β) face. Open arrow indicates oxygen atom of the equatorial (α) hydroxyl group at C-1; solid arrow points to the equatorial (α) hydrogen atom at C-11.

appears to offer an explanation: the distance between the oxygen atom of the equatorial hydroxyl group at C-1 and the 11α-hydrogen atom is very small, of the order of 1.6 Å. The resulting interaction would serve to reduce the activity of the hydroxyl group, presumably by limiting its association with components of the stationary phase.

The relative mobilities of the members of the 5β-pregnane pair (compounds 19

and 32) and of the androst-5-ene pair (compounds 30 and 31) also are anomalous and apparently for the same reason, namely the close approach, in compounds 31 and 32, of the equatorial hydroxyl group at C-1 and the 11α-hydrogen atom.

But the results obtained in the 5α-androstane series cannot be explained in these terms. Here, in accordance with the rule[16], the axial member (compound 28) is the more mobile even though a Dreiding display of its epimer (compound 29) shows that the equatorial hydroxyl group at C-1 closely approaches the 11α-hydrogen atom.

It is possible that conformational distortion, induced by the proximity of the oxygen function at C-1 and the 11α-hydrogen atom, may account for the observed differences in chromatographic behavior in, for example, the 5α-androstane and androst-5-ene pairs. While this factor may operate in all four cases, it might be supposed that it would have unequal effects in the 5α-androstane and androst-5-ene series because the double bond in the latter tends both to flatten rings A and B and to limit conformational mobility. It might be added that in those epimers not involved in C-1 : C-11 interaction, namely compounds 15, 19, 28 and 30, the axially-oriented hydroxyl group at C-1 appears to be relatively unhindered, sharing simple 1–3 interactions in all cases.

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