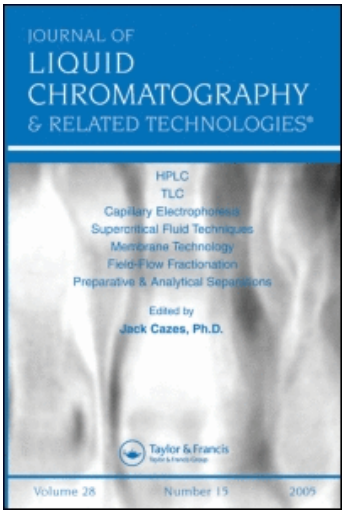

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High Performance Liquid Chromatography of Androgen Acetates

Jiann-Tsyh Lin[a]
[a] U. S. Department of Agriculture, Plant Physiology and Chemistry Research Unit, Western Regional Research Center, Agricultural Research Service, Berkeley, California, U.S.A.

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF ANDROGEN ACETATES

Jiann-Tsyh Lin
Plant Physiology and Chemistry Research Unit, Western Regional Research Center, Agricultural Research Service,
U. S. Department of Agriculture,
Berkeley, California 94710 (U.S.A.)

ABSTRACT

More than twenty $C_{19}O_2$ androgen acetates were chromatographed by both normal-phase (silica) and reversed-phase (C_{18}) HPLC. These two HPLC systems together with normal-phase and reversed-phase HPLC of free androgens have made the separation of various $C_{19}O_2$ androgen isomers possible. 3,17-Diacetoxyandrostane derivatives, in general, are more polar in normal-phase HPLC when the 3-acetoxyl group is equatorial than when it is axial. However, 3-acetoxyandrostan-17-one derivatives are more polar in normal-phase HPLC when the 3-acetoxyl group is axial than when it is equatorial. 17α-acetoxyandrostane derivatives in general are more polar than their 17β-analogs in both normal-phase and reversed-phase HPLC.

INTRODUCTION

High-performance liquid chromatography (HPLC) of androgens has been reviewed recently [1,2,3]. They were chromatographed as either free androgens or their derivatives. Androgen derivatives used were 2,4-dinitrophenylhydrazine derivatives[4], benzoates, p-nitrobenzoates[5], sulfates, glucuronides[6] and acetates[7,8]. Gorog and Herenyi[7] have separated epimers of ethynodiol diacetate by

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reversed-phase HPLC. Holder et al.[8] have used reversed-phase HPLC for the trace analysis of trenbolone acetate and trenbolone.

We have recently reported both normal-phase and reversed-phase HPLC of 69 underivatized free androgens including $C_{19}O$, $C_{19}O_2$ and $C_{19}O_3$[9] for the study of the metabolism of 4-[4-^{14}C] androstene-3,17-dione ($C_{19}O_2$). These two HPLC systems complement each other, but still are not good enough for the identification of the radioactive metabolites, because some androgens do not separate well in both systems. We have previously used both normal-phase and reversed-phase HPLC and co-crystallization to constant specific radioactivity to identify the reduction products of 4-[4-^{14}C]progesterone in pea plants[10]. That work required 10 mg of each reference compound for co-crystallization. In the study of the metabolism of 4-[4-^{14}C]androstene-3,17-dione in cucumber plants[11], we were limited by the scarcity of some androgens.

In this communication, both normal-phase and reversed-phase HPLC of androgen acetates are described. These two HPLC systems together with both normal-phase and reversed-phase HPLC of free androgens have made the identification of the radioactive metabolites of 4-[4-^{14}C]androstene-3,17-dione in cucumber plants possible without using co-crystallization[11]. Ten radioactive metabolites have been identified including the major metabolite, testosterone. This communication is limited to $C_{19}O_2$ androgens, because no significant radioactivities of $C_{19}O$ and $C_{19}O_3$ androgens metabo-

lized from 4-[4-^{14}C]androstene-3,17-dione were detected by the HPLC of free androgens in cucumber plants[11].

METHODS

The HPLC system consisted of a pump (Model 110A, Altex, Berkeley, California) with a high sensitivity pressure filter (Altex), a sample injector (Model 7125, Rheodyne, Cotati, California) with a loop volume of 1 ml, a variable wavelength UV-VIS detector (Model 155-30, Altex) and a recorder (Model 385, Linear, Irvine, California). The normal-phase column was a 25 cm x 0.46 cm stainless-steel chromatography tube, packed with Zorbax BP-SIL (silica; 7-8 μm; DuPont, Wilmington, Delaware). The reversed-phase column had the same dimensions, but it was packed with Zorbax BP-ODS (C_{18}; 7-8 μm; DuPont). The columns were packed in this laboratory[12].

Androgen acetates were made by acetylation of free androgen (1-5 mg) with 0.5 ml pyridine and 0.25 ml acetic anhydride (Acetylation Kit, Applied Science Laboratories, State College, Pennsylvania). The UV detector at 200 nm was used for androgen acetates, because the λ_{max} of 5α-androstane-3β,17β-diol is 200 nm. About 2 μg of the Δ^4- or Δ^5-androgen acetates and about 200 μg of the other androgen acetates were analyzed by HPLC. The chromatographic conditions are given in figure legend and Table 1.

RESULTS AND DISCUSSION

The results are summarized in Table 1. Systems 1 and 2, the normal-phase and reversed-phase HPLC of free androgens, have been

TABLE 1

HPLC Retention Times (min.) Of $C_{19}O_2$ Androgens
Hydroxyl groups are indicated by α and β, depending on orientation at C-3 and C-17. However, at C-5, α and β are used to designate the orientation of hydrogen. Keto groups are indicated by 0, and double bonds by Δ. Free androgens were chromatographed in Systems 1 and 2 (published previously[9]), acetates in all other systems. System 1, normal-phase column, hexane-ethanol (97:3); System 2, reversed-phase column, methanol-water (7:3); System 3, normal-phase column, hexane-ethanol (998:2); System 4, normal-phase column, hexane-ethanol (995:5); System 5, normal-phase column, hexane-ethanol (99:1); System 6 (see Fig. 1), reversed-phase column, methanol-water (9:1); System 7, reversed-phase column, methanol-water (8:2). Capacity factors, $k' = t-t_o/t_o$, t_o = 1.60 min (normal-phase), t_o = 1.24 min (reversed-phase).

No.	Substituents				Systems						
	3	4	5	17	1	2	3	4	5	6	7
1	0	-	β	0	7.5	15.5	-	-	-	-	-
2	0	-	Δ	0	8	17.5	-	-	-	-	-
3	0	-	α	0	8	17.75	-	-	-	-	-
4	0	-	β	α	8.5	23	-	17	7.5	-	11.5
5	β	-	β	0	10	14.25	-	12.5	-	-	12.5
6	0	-	α	α	10.25	18	-	31	9.5	-	12.5
7	α	-	α	0	12.5	16.25	-	14.5	-	-	14.25
8	β	-	Δ	0	12.5	11.25	-	11	-	-	12.75
9	0	-	α	β	12.5	16	-	15	-	-	14.75
10	0	-	β	β	12.5	15.75	-	16.25	-	-	13.25
11	β	-	α	0	13	14.75	-	11.5	-	-	15
12	α	-	β	0	13	16.25	-	10.5	-	-	12
13	β	-	β	β	16.5	11.75	15	-	-	9	-
14	α	-	α	β	18	16.75	12.5	-	-	9	-
15	β	-	β	α	19.25	16	15	-	-	7.75	-
16	0	Δ	-	0	20	11.5	-	-	-	-	-
17	β	-	Δ	β	20	10.25	19.5	5	-	9.25	-
18	β	-	α	β	20	13.5	22	-	-	10.5	-
19	β	Δ	-	β	20.25	9.75	20.75	-	-	8	-
20	α	-	α	α	20.25	33	14.75	-	-	8.25	-
21	β	-	Δ	α	20.5	11.25	23.5	5.5	-	7	-
22	β	-	α	α	20.5	14.5	20.75	-	-	7	-
23	0	Δ	-	α	21.5	12	-	-	18.5	-	9.75
24	α	-	β	α	24	32	21.25	-	-	7	-
25	α	-	β	β	24	15.5	13	-	-	9	-
26	0	Δ	-	β	25.75	10.75	-	-	16.75	-	9.75

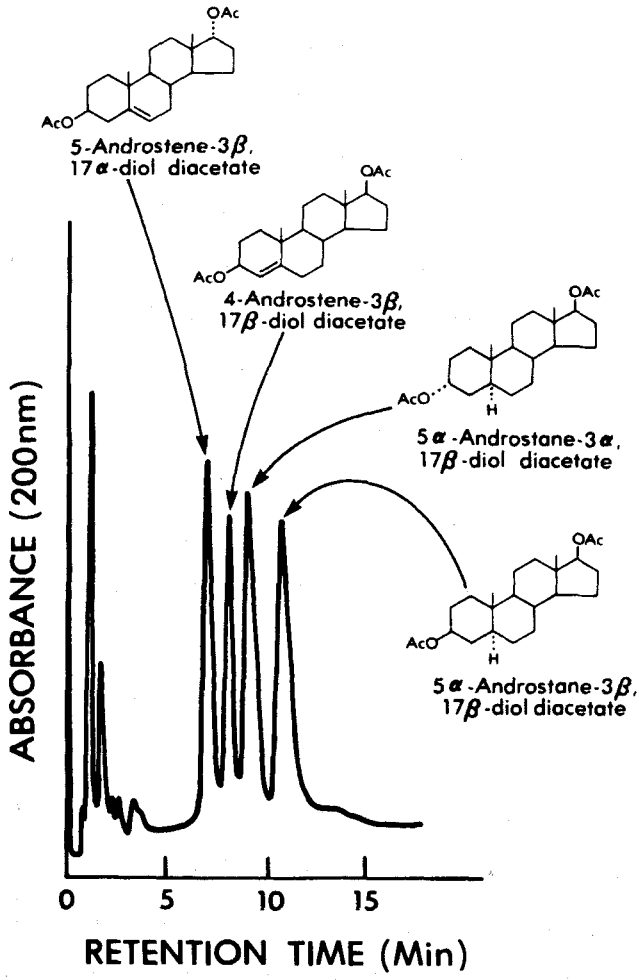

FIGURE 1. Reversed-phase chromatogram of androstane-3,17-diol diacetates. Between 1 μg (5-androstene-3β,17α-diol diacetate) and 30 μg (5α-androstane-3β,17β-diol diacetate) of diacetates, dissolved in 100 μl of methanol, were chromatographed on a column of Zorbax BP-ODS, 25 cm x 0.46 cm. Eluent, methanol-water (9:1); flow rate, 2 ml/min; pressure, 500 p.s.i.; UV detector, 200 nm, range 0.5; recorder, speed 12 cm/h, span 10 mv.

published[9]. The retention times (min) in Table 1 have never varied more than 5% except at System 3 where the eluent used contained very small amount of ethanol in hexane. The HPLC of androgen acetates (Systems 3-7, Table 1) showed that 17-acetoxy-4-androsten-3-ones (compounds 23 and 26, Table 1) are more polar than mono-acetoxy-monoketo-androstane derivatives (compounds 4-12) and that mono-acetoxy-monoketo-androstane derivatives are more polar than diacetoxyandrostane derivatives in both normal-phase and reversed-phase HPLC. Apparently, in these HPLC systems, a conjugated keto group is more polar than an isolated keto group and an isolated keto group is more polar than an acetoxyl group.

3,17-Diacetoxyandrostane derivatives, in general as shown in Table 2, are more polar in normal-phase HPLC when the 3-acetoxyl group is equatorial than when it is axial. This is similar to the results for 3-hydroxyandrostane derivatives[9]. However, 3-acetoxyandrostan-17-one derivatives, as shown in Table 2, are more polar in normal-phase HPLC when the 3-acetoxyl group is axial than when it is equatorial. Normal-phase HPLC, in general, separates pairs of equatorial 3-acetoxy- and axial 3-acetoxy-androstane derivatives better than reversed-phase HPLC. However, reversed-phase HPLC separates pairs of equatorial 3-hydroxy- and axial 3-hydroxy-androstane derivatives better than normal-phase HPLC except for the pairs of 5-epimers[9].

17α-Acetoxyandrostane derivatives, in general, are more polar than their 17β-analogs in both normal-phase and reversed-

TABLE 2

Relative Polarities Of Androgen Acetates
> Indicates that the first group of steroids is, as a rule, more polar than the second. N = normal-phase, R = reversed-phase, e = equatorial, a = axial, + indicates that the rule shown in each heading is obeyed, - indicates that it is violated, = indicates that analogues are inseparable. The superior HPLC system for each group of analogue separations is underlined. The compound numbers are the same as those in Table 1.

Comparison between 3-acetoxy(e) and 3-acetoxy(a)							
3α(e),5β>3α(a),5α		N	R	3β(e),5α>3α(a),5α		N	R
12	7	-	+	11	7	-	-
24	20	+	+	18	14	+	-
25	14	+	=	22	20	+	+
3β(e),5α>3β(a),5β		N	R	3α(e),5β>3β(a),5β		N	R
11	5	-	-	12	5	-	+
18	13	+	-	24	15	+	+
22	15	+	+	25	13	-	=
3β(e),Δ^5>3α(a),5α		N	R	3β(e),Δ^5>3β(a),5β		N	R
8	7	-	+	8	5	-	-
17	14	+	-	17	13	+	-
21	20	+	+	21	15	+	+
3β(e),Δ^4>3α(a),5α		N	R	3β(e),Δ^4>3β(a),5β		N	R
19	14	+	+	19	13	+	+

Comparison between 17α-acetoxy and 17β-acetoxy							
17α>17β		N	R	17α>17β		N	R
6	9	+	+	21	17	+	+
4	10	+	+	22	18	-	+
15	13	=	+	23	26	+	=
20	14	+	+	24	25	+	+

phase HPLC as shown in Table 2. However, 17α-hydroxyandrostane derivatives are less polar than their 17β-analogs in reversed-phase HPLC[9]. Androgen acetates inseparable by normal-phase HPLC can often be separated by reversed-phase HPLC and vice versa. These two HPLC systems together with normal-phase and reversed-phase HPLC of free androgens have made the separation of various $C_{19}O_2$ androgen isomers possible.

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