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GAS CHROMATOGRAPHIC SEPARATION OF ISOMERIC DIENESTROL DIACETATES AND ANALYSES OF INDUSTRIAL PRODUCTS

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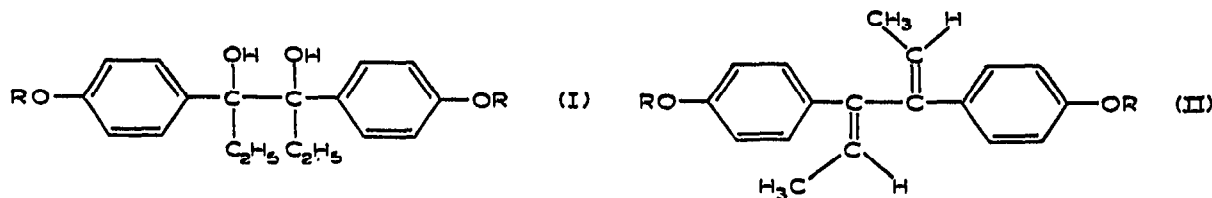
SUMMARY

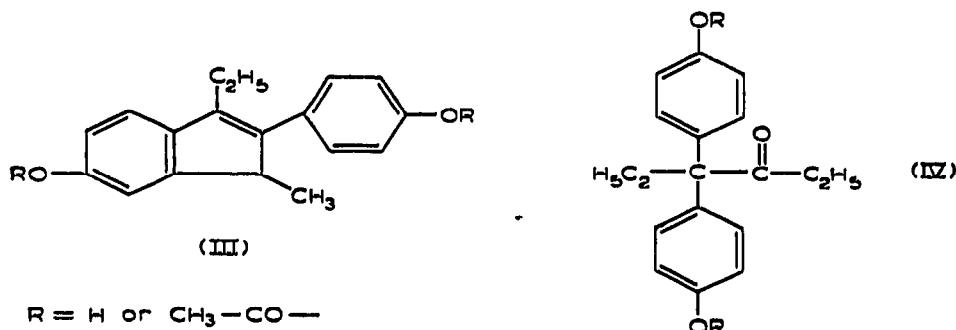
The three geometrical isomers of dienestrol diacetate may be determined quantitatively and simultaneously by gas-liquid chromatography using 3% SE-30 on Gas-Chrom Z as stationary phase; they can also be determined in the presence of indenestrol diacetate and pinacolone 4,4'-bis(*p*-hydroxyphenyl)-3-hexanone diacetate. The gas-liquid chromatographic method may be used in analyses of industrial residues and in purity determination of pharmaceutical grade dienestrol and dienestrol diacetate. An auxiliary thin-layer chromatographic procedure is also described.

INTRODUCTION

There are three possible geometrical isomers for 3,4-bis(*p*-hydroxyphenyl)-2,4-hexadiene (II) and its relative esters: the α -isomer (Dodd's compound), generally known as dienestrol, m.p. = 227-228° (diacetate = 119-120°); the β -isomer (or isodienestrol), m.p. = 184-185° (diacetate = 147-148°); and the γ -isomer, m.p. = 121-122° (diacetate = 104-105°). The relative estrogenic potencies, determined by tests on ovariectomized rats are: α -isomer 1; β -isomer 0.003 and γ -isomer 0.03.

These compounds have identical I.R. spectra in CCl₄. They may be reduced to mesohexestrol and are quantitatively transformed into indenestrol (III) by isomerization in ethanolic hydrogen chloride. They are obtained simultaneously, as diacetates, by the dehydrating action of an acetic anhydride-acetyl chloride mixture on 3,4-bis(*p*-hydroxyphenyl)-3,4-hexanediol (I), a pinacol-type compound. In addition indenestrol diacetate (III), the pinacolone 4,4'-bis(*p*-hydroxyphenyl)-3-hexanone (IV) and polymeric compounds may be obtained in this reaction, together with dienestrol diacetate (II) and its relative isomers.

R = H or CH₃-CO-



In practice, after separation of α -dienestrol diacetate as a pure crystalline powder, a mixture of the above mentioned products remains in the mother liquor. The three dienestrol isomers must be determined separately in order to study the composition of this mixture. An attempt to use column chromatography with silicic acid was unsuccessful, as reported by LANE AND SPIALTER¹.

However, we have achieved the separation of these closely related compounds by GLC and have determined indenestrol diacetate (III) and pinacolone diacetate (IV) at the same time. The method is useful in analyses of industrial products and in purity control of batches of dienestrol and dienestrol diacetate.

EXPERIMENTAL

In the present paper 3,4-bis(*p*-hydroxyphenyl)-3,4-hexanediol is abbreviated as pinacol (I) and 4,4'-bis(*p*-hydroxyphenyl)-3-hexanone as pinacolone (IV).

Materials

All the compounds used in the GLC analyses are diacetyl esters. Conversion of phenols to acetates was effected, when necessary, by refluxing with acetic anhydride and pyridine (2:1) for 30 min.

Dienestrol diacetate, commercially available (Recordati, Milan) was used as a standard for the α -isomer. The β -isomer was prepared from diethylstilbestrol according to the procedure of EULER AND ADLER². Samples of the γ -isomer were isolated from mixtures of the isomers, as reported below. Indenestrol diacetate (m.p. = 117–118°) was prepared from dienestrol with hydrochloric acid according to the isomerization procedure, described by HAUSMAN AND WILDER-SMITH³.

Pinacolone diacetate (IV) (m.p. = 91–92°) was obtained from pinacol (I) by the use of sulphuric acid in an acetic acid–acetic anhydride medium⁴.

GLC separation procedure

A Fractovap GB gas chromatograph (Carlo Erba, Milan), equipped with a flame ionization detector and a U-shaped glass column, 2 m. long and 4 mm I.D., is used. The column is packed with 3% methyl silicone polymer SE-30 on Gas-Chrom Z 100–120 mesh (BDH). The carrier gas (nitrogen) has a flow rate of approximately 55 ml/min and the hydrogen and air flow are adjusted for maximum flame response. The column temperature is 230°, while that of flash heater and of detector is 280°.

Peaks with good shapes, having characteristic retention times are obtained

for the three isomers and for indenestrol diacetate. Against this, pinacolone (IV) diacetate has the same retention time as the β -isomer and cannot be separated. A chromatogram of an artificial mixture of standard compounds is shown in Fig. 1.

The isomerization procedure on a mixture of equal amounts of the standards was performed by passing dry hydrochloric acid gas through an alcoholic solution of the samples; a quantitative transformation of the dienestrol isomers in indenestrol diacetate takes place. The gas chromatogram is shown in Fig. 2.

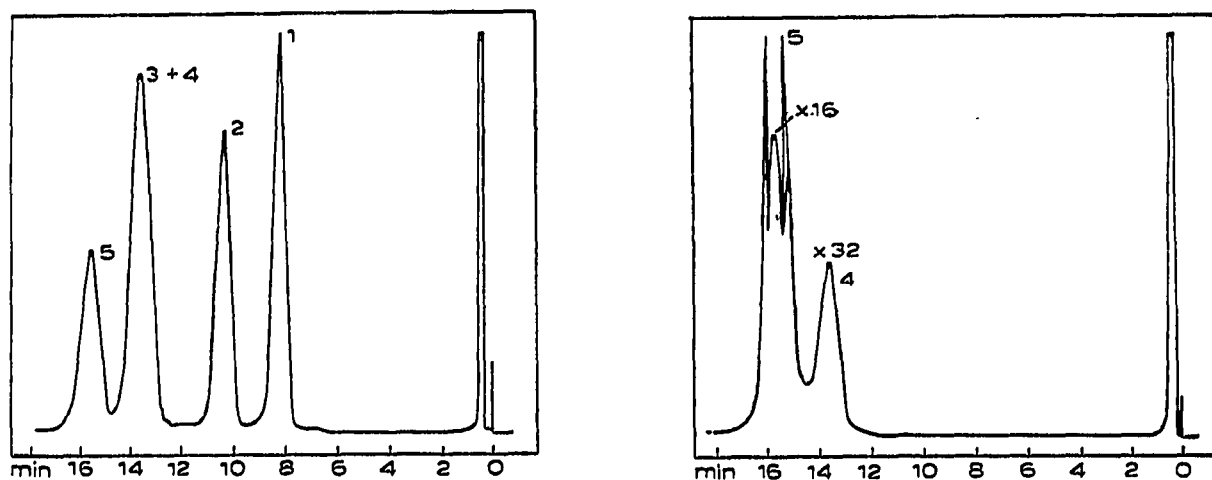


Fig. 1. Gas chromatogram of a standard mixture of (1) α -dienestrol diacetate; (2) γ -dienestrol diacetate; (3) β -dienestrol diacetate; (4) pinacolone (IV) diacetate; (5) indenestrol diacetate.

Fig. 2. Gas chromatogram of a standard mixture after isomerization. (4) pinacolone (IV) diacetate; (5) indenestrol diacetate.

The residue peak 4 in Fig. 2 is attributed to pinacolone (IV) diacetate, since it cannot be transformed into indenestrol. For a quantitative evaluation the areas of peaks are measured and the relative percentages calculated. A good relationship exists ($\pm 5\%$) between peak area and compound concentration. Pinacol (I), if present in the mixture, cannot be detected, as its retention time is the same as that of indenestrol diacetate. It may be separated by TLC.

TLC separation procedure

Plates prepared in the usual manner with Silica Gel GF 254 (Merck) are used (thickness = 0.3 mm). A 1% chloroform solution is prepared and a sample equivalent to 500 μg is applied, by means of a microsyringe, in a continuous band. The spots are detected with 0.5% vanillin solution in conc. sulphuric acid, or by means of U.V. (254 m μ). The TLC data for the compounds tested are summarized in Table I.

The isomers of dienestrol (and their diacetates) and indenestrol (and diacetate) cannot be separated. Pinacol (I) diacetate may be separated with mobile phases A and B (pinacol (I) with B and C). Pinacolone (IV) diacetate may be separated with mobile phases A and B (pinacolone (IV) with B).

TABLE I

R_F VALUES ON SILICA GEL GF 254 (MERCK)

Mobile phases: A = chloroform; B = chloroform-ethyl acetate (93:7); C = chloroform-ethyl acetate-methanol (85:15:5).

Compounds	Mobile phases			Color with 0.5% vanillin in conc. sulphuric acid
	A	B	C	
α -Dienestrol diacetate	0.47	0.94	1.0	Green
β -Dienestrol diacetate	0.39	0.92	1.0	Green
γ -Dienestrol diacetate	0.33	0.91	1.0	Green
Indenestrol diacetate	0.37	0.92	1.0	Green
Pinacolone (IV) diacetate	0.30	0.85	1.0	Pink
Pinacol (I) diacetate	0.15	0.30	0.82	Pink
α -Dienestrol	0.04	0.34	0.61	Bluish-green
β -Dienestrol	0.03	0.23	0.47	Bluish-green
γ -Dienestrol	0.09	0.28	0.48	Bluish-green
Indenestrol	0.05	0.34	0.60	Bluish-green
Pinacolone (IV)	0.01	0.20	0.49	Orange
Pinacol (I)	0.0	0.07	0.27	Orange

FIELDS OF APPLICATION

Industrial analyses of reaction mixtures

Pure α -dienestrol diacetate is formed following the reaction of an alcoholic solution of pinacol (I) with a mixture of acetic anhydride-acetyl chloride⁵.

The alcoholic mother liquor, on evaporation to dryness, gives a plastic resinous residue. 10 g of this residue are treated with 20 ml of 10% aqueous sodium hydroxide and 25 ml of cyclohexane and are warmed at 50° for 10 min. An insoluble substance, probably a polymeric compound, is obtained and the two layers are separated.

On cooling the cyclohexane phase to 25°, an oily film separates on the container walls. It may be isolated by decantation and dried (Sample A). The decanted liquor is then cooled to 17° and crystals separate (Sample B). These crystals, after two crystallizations from cyclohexane, gave pure γ -dienestrol diacetate (98.2% by GLC) which was used as a standard. (This product has a m.p. of 73–74° but when the melt is allowed to cool, it crystallizes to give a substance melting at 104–105°, as reported by LANE AND SPIALTER¹.) Finally the cyclohexane liquor is completely evaporated and a vitreous residue is obtained (Sample C).

The aqueous alkaline phase is acidified with conc. hydrochloric acid and extracted twice with 20 ml of chloroform. The chloroform extracts are evaporated and the residue is acetylated; 20 ml of 10% sodium hydroxide are added, and it is extracted twice with 20 ml of chloroform. These chloroform extracts are washed twice with the same volume of diluted hydrochloric acid and evaporated to dryness. The dense residue (Sample D) consists of the diacetates of the phenolic derivatives contained in the original sample.

Samples (A, B, C, D) are dissolved in chloroform, analyzed by GLC and TLC. Quantitative results obtained on analysing an industrial residue are reported in Table II. Neither indenestrol, nor pinacol (I) diacetates were found.

TABLE II

ANALYSIS OF AN INDUSTRIAL RESIDUE FROM α -DIENESTROL DIACETATE SYNTHESIS (10 g TESTED)

Materials	Quantity (g/10 g)	Relative percentages (from GLC analysis)			
		α -Dienestrol diacetate	β -Dienestrol diacetate	γ -Dienestrol diacetate	Pinacolone (IV) diacetate
Sample A	1.37	45	8	47	—
Sample B	1.76	4	2	94	—
Sample C	2.10	56	15	29	—
Sample D	2.60 (1.80)*	20	7	42	31

* (1.80) = weight of phenolic compounds equivalent to 2.60 g of acetylated materials obtained in the analytical procedure.

The results of these analyses give the complete composition of the residue. It consists of 18.6% α -dienestrol diacetate, 4.6% β -dienestrol diacetate, 29.0% γ -dienestrol diacetate, 3.6% α -dienestrol, 1.3% β -dienestrol, 7.6% γ -dienestrol and 5.6% pinacolone (IV). The residue was also found to contain 21.5% insoluble polymeric materials and 6.0% acetylating compounds (acetic anhydride, acetic acid, etc.).

Purity control of products for pharmaceutical use

The GLC and TLC methods described can be used for routine analysis of pharmaceutical grade dienestrol and dienestrol diacetate to check the absence of the β - and γ -isomers and thus ensure maximum estrogenic activity. The GLC and TLC methods are advantageous in comparison with unspecific official assays reported in Pharmacopoeias, based on acetylation or saponification⁶⁻⁸.

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