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Analytical methods based on transformations with hydrochloric acid

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Abstract: Ultraviolet spectrophotometric methods are described for α-ethyl benzhydrol derivatives, 3-trifluoromethyl-α-ethyl benzhydrol (flumecinol), 2,5-dimethyl-α-ethyl benzhydrol (RGH-3395) and an impurity of the latter, (1,4-di-(2,5-dimethyl-phenyl)-1,4-diphenyl-butan-1,4-diol). The methods are based on dehydrations catalysed by hydrochloric acid yielding unsaturated aromatic chromophores. The determination of 2-acetyl-3-phenyl-tetrahydro-1,2,4-oxadiazin-5-one (RGH-4615) is also based on treatment with hydrochloric acid; the chromophoric compound is a benzaldoxime derivative. The hydrochloric acid-catalysed transformation of ethynodiol diacetate to its 3,5-diene derivative enables the parent compound to be determined by gas chromatography.

Keywords: *Ultraviolet spectrophotometry; gas chromatography; α-ethyl benzhydrol derivatives; ethynodiol diacetate; hydrochloric acid transformations.*

Introduction

Transformation of organic compounds by stoichiometric reactions to various derivatives has been of interest in pharmaceutical analysis for half a century. The main aims of investigations in recent years have been to increase the volatility and/or thermal stability of compounds for gas chromatographic assay, and to enhance their UV absorption or fluorescence signals for more sensitive and selective detection in high-performance liquid chromatography (HPLC). In the latter field several multi-purpose derivatization reactions have been described for photometric or fluorimetric assay, for pre- or post-column derivatization for HPLC, and for *in situ* scanning of thin-layer chromatography (TLC) plates. Typical of this type are the dansylation reactions of various organic compounds, e.g. steroids [1–6]. Most derivatization reactions produce derivatives with higher molecular weights and more complicated structures than the original compounds. In contrast, this paper describes the reaction of pharmaceuticals with hydrochloric acid to catalyse dehydration, deacylation and rearrangement reactions. Although the derivatives thus formed are smaller and simpler molecules than the parent compounds, their conjugated bonding system results in strong UV absorption, to form the basis of selective and sensitive spectrophotometric methods. The use of the reaction as a derivatization tool in gas–liquid chromatography (GLC) will also be described.

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Experimental

Reagents and apparatus

All reagents and solvents in this study were of analytical reagent grade. Ultraviolet spectra were recorded using a model SP-1800 spectrophotometer (Pye-Unicam, Cambridge, UK). Absorbances were measured by means of a PMQ-2 Opton single beam spectrophotometer (Opton, Oberkochen, FRG). Nuclear magnetic resonance (NMR) spectra were scanned using a Varian EM-360 spectrometer (Varian, Walnut Creek, USA). Deuteromethanol was used as the solvent and deuterium chloride as the catalyst. The gas chromatographic studies were carried out using a model HP 7625 instrument (Hewlett–Packard, Avondale, USA), equipped with a flame ionization detector (FID) and a glass column (1.8 m × 4 mm), packed with 3% OV-101 on Chromosorb W HP (80/100 mesh). Nitrogen was used as eluent at 30 ml/min.

Materials

Ethynodiol diacetate was produced by the Chemical Works of Gedeon Richter Ltd., Budapest. All the other compounds were prepared in the research laboratories of this company and have been subjected to pharmacological and clinical investigation. Flumecinol (3-trifluoromethyl-α-ethyl benzhydrol) is an enzyme-inducing agent [7], RGH-3395 (2,5-dimethyl-α-ethyl benzhydrol) is a cholagogic drug [7], while RGH-4615 (2-acetyl-3-phenyl-tetrahydro-1,2,4-oxadiazin-5-one) is considered as a potential anti-epileptic drug [8].

Spectrophotometric determinations

The sample, containing about 0.01 g of flumecinol or RGH-3395, was dissolved in 20 ml 95% ethanol. To the solution 3 ml of 10 M hydrochloric acid were added; the mixture was refluxed for 30 min and diluted to 50 ml with 95% ethanol. A 5 ml aliquot of this stock solution was diluted to 100 ml with 95% ethanol. The absorbance of the resulting solution was determined at the wavelength of the absorbance maximum near 246 nm against a similarly treated reagent blank. Calculations were based on a reference standard treated concurrently. The amount of the impurity, 1,4-di-(2,5-dimethylphenyl)-1,4-diphenyl-butan-1,4-diol, in RGH-3395 was determined from the absorbance of the undiluted stock solution at 332 nm. RGH-4615 was determined similarly, except that 2 ml of 10 M hydrochloric acid was used.

Gas chromatographic determination of ethynodiol diacetate

A sample of 5 mg of ethynodiol diacetate was dissolved in 2 ml ethanol. A mixture of 1 ml of 10 M hydrochloric acid and 1 ml ethanol was added and the solution allowed to stand at room temperature for 15 min. Then 15 ml of water was added and the solution extracted twice with 2 ml portions of chloroform. Aliquots (2 μl) of the extract were injected into the gas chromatograph, with a column temperature of 230°C, the injector and FID temperatures being 250°C.

Results and Discussion

Treatment of organic compounds with strong mineral acids is one of the most frequently used methods in photometric analysis. In most cases, however, this treatment is restricted to the reversible transformation of organic bases to their protonated forms which often possess more favourable specrophotometric characteristics. In contrast,

concentrated hydrochloric acid sometimes transforms organic compounds to chromogenic or fluorogenic derivatives: the chemistry of many of the latter reactions has not been completely clarified. The methods described in this paper do not belong to either of these categories. The transformations described here are stoichiometric, irreversible reactions catalysed by moderate concentrations of hydrochloric acid.

The dehydration of flumecinol leading to 1-(3-trifluoromethyl-phenyl)-1-phenyl-1-propene takes place rapidly and quantitatively in boiling ethanol containing 1.5 M hydrochloric acid. The stability of the chromophore is excellent (Table 1). The

Table 1
Formation and stability of chromophoric derivatives: absorbance at various reaction times

Compound	Concentration (μg/ml)	Reaction time (min)				
		5	15	30	60	120
3-Trifluoromethyl-α-ethyl-benzhydrol (flumecinol)	9.81	0.204	0.430	0.485	0.486	0.485
2,5-Dimethyl-α-ethyl-benzhydrol (RGH-3395)	9.91	0.560	0.570	0.570	0.571	0.570
1,4-Di(2,5-dimethyl-phenyl)-1,4-diphenyl-butan-1,4-diol (impurity)	10.20	0.545	0.568	0.570	0.569	0.571
2-Acetyl-3-phenyl-tetrahydro-1,2,4-oxadiazin-5-one (RGH-4615)	8.02	0.317	0.508	0.540	0.542	0.541

quantitative nature of the reaction has been established by comparing the molar absorptivity obtained for the reaction mixture with that of the isolated reaction product (Table 2). The isolated benzenoid spectrum of flumecinol and the styrene-like spectrum of the reaction product are shown in Fig. 1. NMR investigation of the dehydration

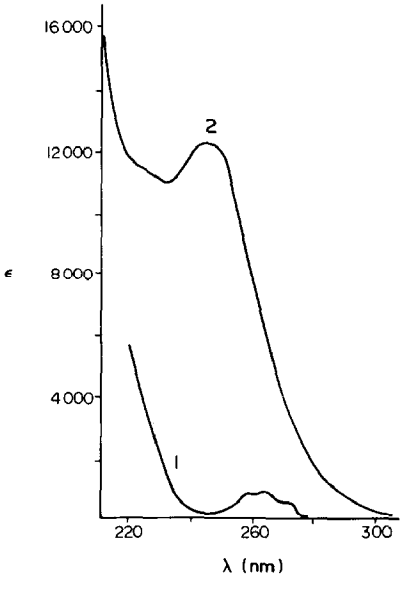


Figure 1
Ultraviolet spectra.
(1) Flumecinol (reference solvent: ethanol);
(2) Reaction mixture after dehydration of flumecinol (reference solvent: reagent blank — see text).

Table 2
Spectroscopic data of the parent compounds and their chromophoric derivatives

Parent compound			Reaction product	Isolated product		Reaction mixture		Regression data†	
	λ_{max}(nm)	ϵ (l/mol cm)		λ_{max}(nm)	ϵ (l/mol cm)	λ_{max}(nm)	ϵ^* (l/mol cm)	z	m
3-Trifluoromethyl-α-ethyl-benzhydrol (flumecinol)	264	870	1-(3-trifluoromethyl-phenyl)-1-phenyl-1-propene	246	14 150	246	13 920±130	0.007	0.0496
2,5-Dimethyl-α-ethyl-benzhydrol (RGH-3395)	268	795	1-(2,5-dimethyl-phenyl)-1-phenyl-1-propene	247	13 970	247	13 790±100	0.005	0.0548
1,4-Di-(2,5-dimethyl-phenyl)-1,4-diphenyl-butan-1,4-diol (impurity)	269	1470	1,4-di-(2,5-dimethyl-phenyl)1,4-diphenyl-1,3-butadiene	–	–	332	24 500±120	0.004 0.013‡	0.0545 0.0537‡
2-Acetyl-3-phenyl-tetrahydro-1,2,4-oxadiazin-5-one (RGH-4615)	257	259	Benzaldehyde-carbox-amido-methoxime§	259	15 610	256	14 070±110	0.009	0.0646

* Mean ±SD (n = 8).
† Equation: Absorbance = z + m (concentration, μg/ml); correlation coefficient (p = 0.95): $r > 0.9995$ in all cases (n = 8).
‡ Calibration curve in the presence of 0.02 g/100 ml RGH-3395.
§ For details see Results and Discussion.

reaction mixture has revealed that the product is the mixture of the two geometrical isomers in a ratio of about 6:4, as shown by the splitting of the methyl doublet at δ 1.75 ppm. However, the difference between the UV spectra of the isomers is undoubtedly so small that this does not influence the reliability of the spectrophotometric assay. Consequently the recommended method has been successfully used for the determination of flumecinol in various dosage forms including oily solution and capsules.

The other benzhydrol derivative (RGH-3395) can be determined in an analogous manner on the basis of the dehydration reaction leading to 1-(2,5-dimethyl-phenyl)-1-phenyl-1-propene. In this case, in addition to the determination of the drug compound, the HCl-catalysed dehydration reaction offers the possibility of simultaneously determining the principal impurity of RGH-3395. Under the conditions for dehydration of RGH-3395, this impurity, 1,4-di-(2,5-dimethyl-phenyl)-1,4-diphenyl-butan-1,4-diol, loses two moles of water yielding the highly conjugated 1,4-di-(2,5-dimethyl-phenyl)-1,4-diphenyl-1,3-butadiene. The strong absorption of this derivative at 332 nm (Table 2) enables the impurity to be determined in RGH-3395. This method is highly selective since at the absorption maximum of the dehydrated impurity, RGH-3395 does not possess any appreciable absorption (Fig. 2). Typical results of a model experiment are the following: 1,4-di-(2,5-dimethyl-phenyl)-1,4-diphenyl-butan-1,4-diol; added, 0.51%; recovered, 0.54 ± 0.02%.

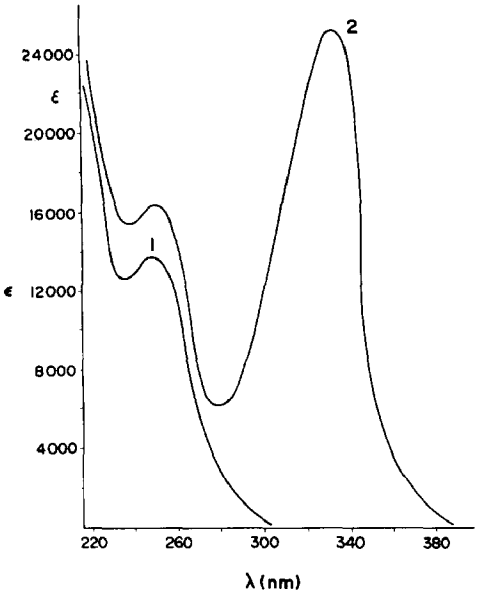


Figure 2
Ultraviolet spectra of the reaction mixtures after dehydration of:
(1) RGH 3395;
(2) 1,4-di-(2,5-dimethyl-phenyl)-1,4-diphenyl-butan-1,4-diol (reference solvents: reagents blanks — see text).

The mechanism of the formation of the chromophoric derivative is entirely different in the case of RGH-4615. The key step in this reaction is the acid-catalysed hydrolytic splitting of the *N*-acetyl moiety, followed by ring opening leading to the chromophoric benzaldoxime derivative. Figure 3 shows the spectra of the parent compound and its derivative. There is a minor difference between the wavelengths of the absorption maxima of the reaction mixture and the isolated carboxamide derivative (Table 2). TLC studies show that this primary reaction product is rapidly transformed to the free carboxylic acid and its ethyl ester. These changes are, however, remote from the chromophoric centre of the derivative and do not cause major changes in the spectrum.

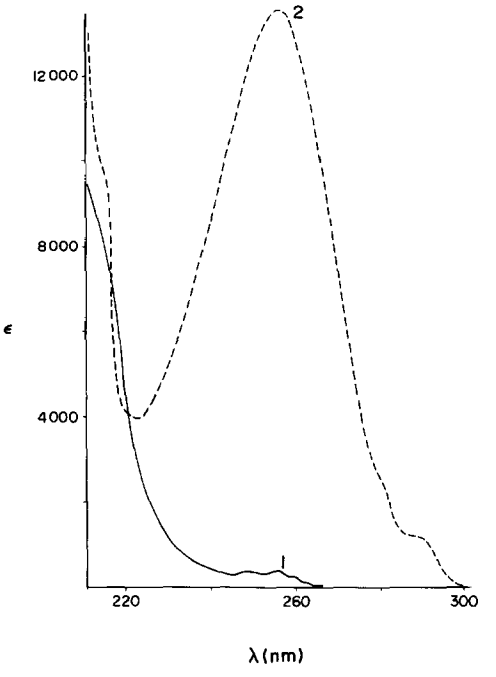
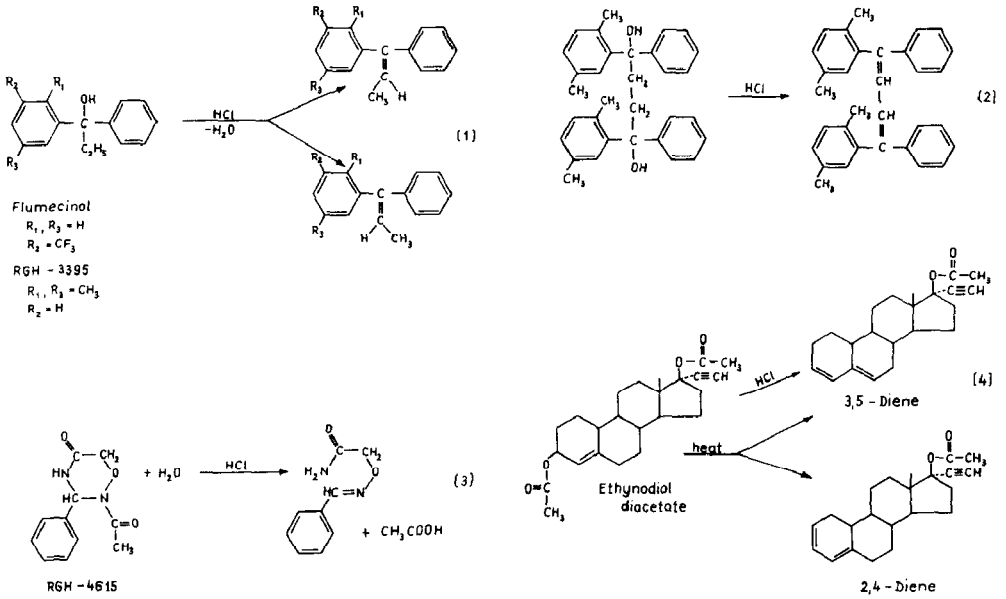


Figure 3
Ultraviolet spectra of:
(1) RGH 4615 (reference solvent: ethanol);
(2)Reaction mixture after treatment of RGH-4615 with hydrochloric acid (reference solvent: reagent blank — see text).

Thus the reliability of this spectrophotometric method is comparable with that of the methods for benzhydrol derivatives (Tables 1 and 2).

The spectrophotometric methods described for the four compounds discussed above have been successfully used for the solution of various practical problems, including analytical control of bulk materials and the assay of dosage forms. It is worth noting that in those cases where background absorption interferes strongly with the assay (as, for example, in animal feedstuffs), difference spectrophotometry can be used. In this case the reference cell contains the sample and hydrochloric acid, diluted to the same concentration as for the sample cell. However, the boiling step is omitted for the reference solution, so that the background interference is compensated by difference, since at room temperature and low hydrochloric acid concentration none of the reactions in Schemes (**1**)–(**3**) take place. A difference spectrophotometric method for the determination of ethynodiol diacetate in contraceptive pills has been described by one of the present authors [9]. This method is based on the formation of spectrophotometrically-active 3,5-diene derivatives, whose molar absorptivity is 19 200 at 236 nm [10].

In the present work this reaction is used in the gas chromatographic determination of ethynodiol diacetate as its derivative. As a consequence of its poor thermal stability, this synthetic gestogenic hormone cannot be studied directly by GLC [11]. Figure 4(a) shows the gas chromatogram of two identifiable peaks corresponding to the 2,4-diene and 3,5-diene respectively (Scheme **4**). An analogous phenomenon has been described for 4-androstene-3β, 17β-diol by Wotiz and Clark [12]. Since, in contrast to the thermal decomposition reaction, the acid-catalysed deacetylation leads exclusively to the 3,5-diene derivative, the gas chromatographic determination of ethynodiol diacetate can easily be carried out after preliminary treatment with hydrochloric acid (Fig. 4b).



The same principle has been successfully used in this laboratory for the thin-layer chromatographic detection and gas chromatographic determination of 17α-ethynyl-oestran-17-ol acetate, present as an impurity in 17α-ethynyl-4-oestren-17-ol acetate. The former is unaffected by hydrochloric acid and thus is easily separated from the 3,5-diene formed from the latter [13].

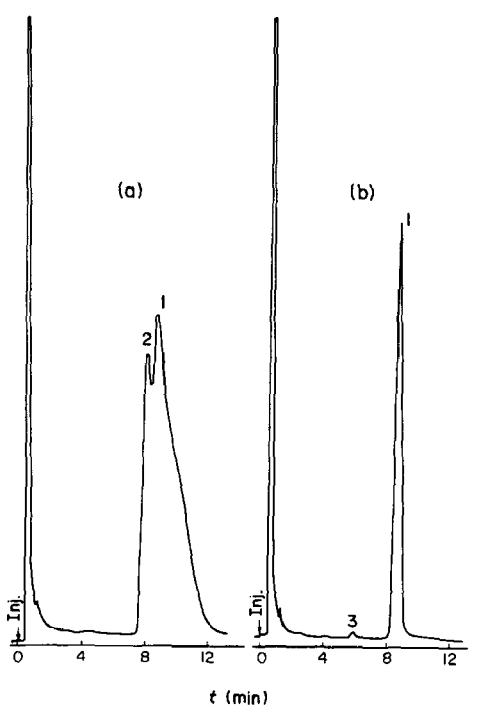


Figure 4
Gas chromatograms of ethynodiol diacetate:
(a) Chromatogram before treatment with hydrochloric acid;
(b) Chromatogram after treatment with hydrochloric acid.
Peaks:
(1) 17α-ethynyl-3,5-oestradien-17-ol acetate;
(2) 17α-ethynyl-2,4-oestradien-17-ol acetate;
(3) 17α-ethynyl-3,5-oestradien-17-ol.
For details see text.

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